CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol. 8 (2)
2016

Technical University of Cluj Napoca
U.T.Press Publishing House

Carpathian Journal of Food Science and Technology

Print : ISSN 2066-6845
Online : ISSN 2344-5459
ISSN-L 2066-6845

Vol. 8, Nr.(2) 2016

UNIVERSITATEA TEHNICĂ DIN CLUJ-NAPOCA

Technical University of Cluj Napoca
U.T.Press Publishing House

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

ISSN-L 2066 -6845

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

Technical University of Cluj Napoca, Romania
U.T. Press Publishing House

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

CONTENT

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

USING OF DIRECT CONTACT MEMBRANE DISTILLATION FOR WASTEWATER TREATMENT OBTAINED AFTER WHEY PROCESSING

Yurii Zmievskii[1*], Ivanna Kyrychuk[1], Valerii Myronchuk[1]

[1]Department of Technological Equipment and Computer Design Technology, Faculty of Mechanical Engineering and Packaging Technology, National University of Food Technologies of the Ministry of Education and Science of Ukraine, Vladimirskaya str. 68, 01601, Kiev, Ukraine;
*Corresponding author: * yrazm@meta.ua*

Article history:
Received:
07 June 2015
Accepted in revised form:
06 May 2016

Keywords:
Membrane;
Direct contact membrane;
Distillation;
Whey;
Nanofiltration;
Wastewater;
Purification

ABSTRACT
This paper presents the results on the study of separation of model solutions of nanofiltration whey permeate by direct contact membrane distillation. The solute concentration varied from 0 to 450 g/L. The dependence of the change in water activity was determined for this range of concentrations. It would allow simulating the process of membrane distillation in future. There was a decrease in the selectivity of the membranes at a concentration of 300 g/dm^3 and above. It was most likely caused by the formation of a deposit layer on the membrane surface. A process flow diagram of the treatment of such wastewater was proposed. It consists of two stages: direct contact membrane distillation and electrodialysis. Using of the proposed technological scheme will allow us to reuse up to 92% of treated wastewater.

1. Introduction

The process of direct contact membrane distillation is based on the evaporation of the solvent through the hydrophobic porous membrane (Khayet and Matsuura, 2011), herewith "hot" and "cold" solutions are contacted from different sides of the membrane. Using of membrane distillation in water purification technologies makes it possible to obtain high-quality water, even if the initial solution contains components that are difficult to remove, such as arsenic, (Macedonio and Drioli, 2008), boron (Macedonio and Drioli, 2008, Hou et al., 2013), fluorine (Hou et al., 2010, Boubakri et al., 2014a), nitrate (Boubakri et al., 2014b), etc. An advantage of membrane distillation is also the opportunity to concentrate solutes to the limit of their solubility (Mariah et al., 2006, Hickenbottom and Cath, 2014). This is the reason why a number of technologies for processing of wastewater (Lu et al., 2014), groundwater (Hou et al., 2010) , seawater (Al-Obaidani, et al., 2008, Xu et al., 2006, Shirazi et al., 2014) etc. use membrane distillation.

The disadvantage of membrane distillation is the need to heat the feed solution to a temperature of 50-70° C. Therefore, to improve the economic indicators of the process, it is best to use in the presence of cheap sources of heat. One of these places can be a dairy plant with vacuum evaporation facilities where cooling water in condensers can be heated from 10-20° C to 45-55° C. This water can be reused after cooling in the cooling towers. If the wastewater is directed into the condensing apparatus instead of the pure water and is further concentrated (treated) by membrane distillation, it will be able to develop the high performance technology for utilization of excess heat and some quantity of wastewaters.

A promising target of such treatment can be nanofiltration whey permeate (Myronchuk et

al., 2013). Having the low temperature (8-20 ° C), it is not virtually treated and is discharged into drains as the wastewater (Kyrychuk et al., 2014).

Thus, about 65% of the water on the amount of the processed whey is lost. After the preliminary treatment this water can be reused in the dairy plant. Nanofiltration whey permeate contains about 4 g/L of solutes (about 2 g/L of lactose and about 2 g/L of salts) which after the appropriate separation and concentration, can be used for different technological processes (Zmievskii et al., 2014).

The aim of the present work was to study the process of direct contact membrane distillation during separation of model solutions of nanofiltration whey permeate. It will make possible to provide the dairy enterprises with an additional amount of technical water.

2. Materials and methods

The laboratory setup was composed of the membrane cell, two pumps and two heat-exchangers. The hydrophobic membrane MFFK-3 (ZAO STC "Vladipor", Russian Federation) with effective area of $4.8 \cdot 10^{-3}$ m^2 was settled in the cell horizontally making two chambers – the lower and the upper. The "hot" and "cold" solutions were directed into the lower and upper chamber respectively by the pump with circulation flow of 0.1 m/s. The height of the chambers was 2 mm. The turbulence promoters were installed inside the chambers. The temperature of the solutions was controlled by the mercurial thermometers with the accuracy of ±0.1 °C. The salts content was measured by a conductivity meter (HANNA Instruments DIST 1) with expansion bend. The water activity was determined using a portable device Aqualab (series 3, model TE, USA) with the accuracy of ±0.003. The water activity can also be calculated from the equation (1)

$$a_w = \frac{p}{p_0}, \qquad (1)$$

where p and p_0 are partial pressure of water vapour under the real solution and distillery water (Pa), respectively.

During filtration, permeate flow rate was measured by weighting the mass of coming out permeate. Thus the permeate flux J ($kg \cdot m^{-2} \cdot h^{-1}$) was calculated from the equation:

$$J = 3600 \frac{m}{S \cdot \tau}, \qquad (2)$$

where m is the mass of the permeate (kg), S is the membrane area (m^2), and τ is time (s).
The rejection R (%) was calculated as:

$$R = \left(1 - \frac{C_p}{C_r}\right) \cdot 100, \qquad (3)$$

where C_p and C_r are permeate and retentate concentrations (g/L), respectively.

Considering that permeate penetrating through the membrane comes to the upper chamber and is mixed with the cold solution (distillery water), the real value of the permeate concentration C_p was calculated as follows

$$C_p = \frac{\left(V_c + V_p\right) \cdot C_{ск} - V_c \cdot C_{сн}}{V_p} \qquad (4)$$

where V_p is the permeate volume (L), V_c is the volume of the cold solution (L), $C_{сн}$ and $C_{ск}$ are the solute concentrations in the cold solution before and after permeate samples were taken (g/L), respectively.

"Edible" lactose was used for preparation of model solutions of nanofiltration whey permeate, while all inorganic substances were of "chempure" qualification. The preparation of model solution involved the use of the following components: KCl (37.5%), NaCl (11.5%), $CaCl_2$ (1%), and lactose (50%).

3. Results and discussions

Pure water flux of MFFK-3 membrane was determined at the first stage of the study (Figure 1). The maximum temperature was 55 °C in the hot chamber. It is possible due to heating water to this temperature in the tube and shell condensers of vacuum evaporators.

From Figure 1, it can be seen that the pure water flux is higher at higher average temperatures when the difference between temperatures of "hot" and "cold" chambers is even. It can be explained by the nonlinear dependence of partial pressure of water vapor on temperature.

During filtration of model solutions of nanofiltration whey permeate, it was observed the drop of permeate flux in proportion to the increase of the solution concentration (Figure 2). To concentrate the solution up to the 450 g/L it is required to apply higher driving force. Thus, at the temperature difference of 15 °C (T_h=45 °C, T_c=30 °C) the solution was concentrated only up to 300 g/L, while at the temperature difference of 25 °C (T_h=55 °C, T_c=30 °C) it was concentrated up to 450 g/L. First of all the decrease of the permeate flux with the increase of solution concentration is probably associated with the reduction of the water activity. To confirm this supposition the water activity a_w was determined as the function of the solution concentration C (g/L) (Figure 3). The graph is nonlinear and can be described by a polynomial of the second degree to a high precision.

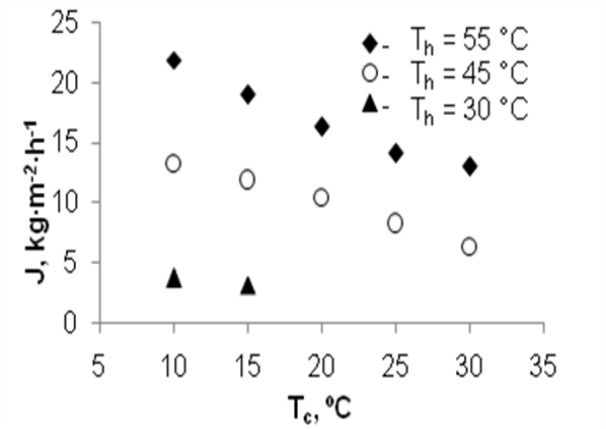

Figure 1. Pure water flux of MFFK-3 membrane vs. temperature in the "cold" chamber. T_h is temperature of the "hot" solution.

From figure 2, 3, it can be seen that at concentration of 300 g/L water activity decreased by just 11.1% while the permeate flux decreased from 35 to 77% as of initial value obtained during pyre water filtration. It indicates the need to consider the other factors such as membrane fouling, concentration polarization and heat polarization. It is obvious that the higher permeate flux is, the higher concentration and heat polarization are (Khayet and Matsuura, 2011). A brown deposit was observed on the membrane surface at the end of the run. For that reason the deep concentration is not always justified. For example, in paper (Hickenbottom and Cath, 2014) the deposition on membrane was observed at salt concentration of 250 g/L that resulted further in quality loss of permeate and decline of permeate flux. Authors (Hickenbottom and Cath, 2014) proposed several versions of reverse membrane distillation to reduce the membrane fouling. For the first one it was suggested to direct permeate into the concentration chamber and retentate into the chambers with permeate at regular intervals. The second one was the change in the solution temperature such as in the next chamber at regular times, i.e. the process was started inversely. Despite the positive effect of these measures it complicates the construction of the setup and increases time of its inefficient use. Thuswise, it is recommended to prevent scale or deposit formation on membrane surface. For this purpose the antiscalants may be used, as the special investigations showed (Sun *et al*., 2011).

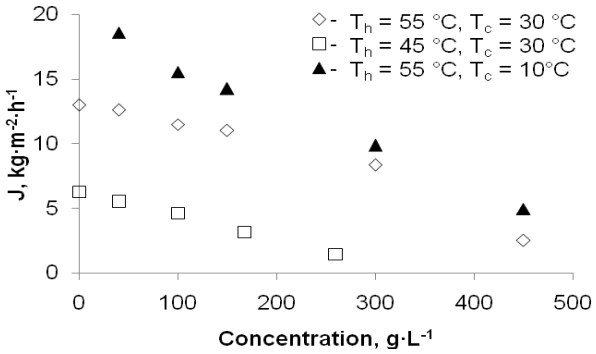

Figure 2. Permeate flux of MFFK-3 membrane vs. retentate concentration during filtration of nanofiltration whey permeate. T_h, T_c are

temperatures of the "hot" and "cold" solutions, respectively.

The water quality obtained after the wastewater treatment is of importance. Figure 5 shows the dependence of rejection and salt content in permeate on concentration of feed solution.

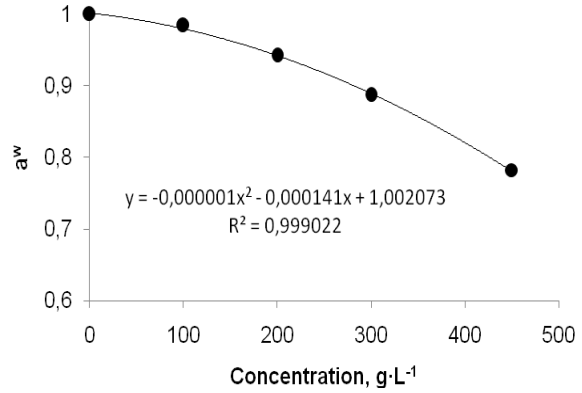

Figure 3. Water activity vs. solute concentration of the model solution of nanofiltration whey permeate.

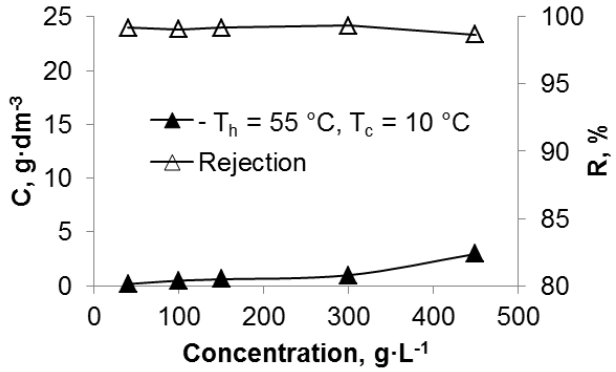

Figure 4.a.

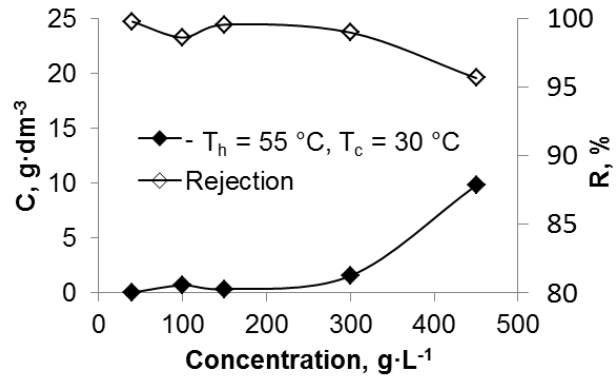

Figure 4.b.

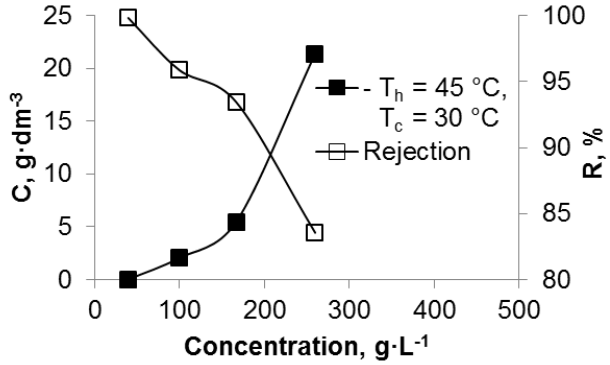

Figure 4. c.

Figure 4: a, b, c. Salt content in permeate and rejection of MFFK-3 membrane vs. solute concentration in the feed solution. T_h, T_c are temperatures of the "hot" and "cold" solutions, respectively.

The quality of the obtained water is essentially worsen during filtration of the solution with concentration higher than 300 g/L (Figure 4 a, b). It is probably associated with the scale formation on the membrane surface that resulted in the decrease of the selective masstransfer (Hickenbottom and Cath, 2014).

It is also can be seen from the figure 4 a-c, that the lower permeate flux is the more solutes permeate contains. It was supposed that MFFK-3 membranes have imperfections as hydrophilic pores, which are wetted by liquid. The diffusive or perhaps even the convective penetration of solutes is carried out through these pores. If we assume that these flows are constant and are functions only of the concentration difference, the quality of permeate will depend on the intensity of high selective mass transfer through the hydrophilic pores.

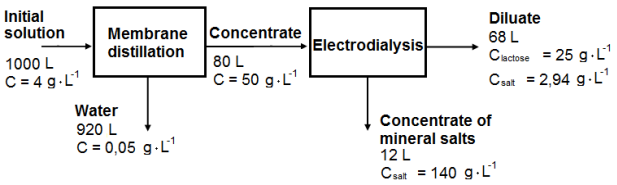

Figure 5. Process flow diagram of treatment of nanofiltration whey permeate, C – total solute concentration $C_{lactose}$, C_{salt} – lactose and salt concentration, respectively.

Summarizing the obtained results one must say that MFFK-3 membranes cannot be used for deep concentration of nanofiltration whey permeate. But they as well as process of membrane distillation can be used for pre-concentration of these wastewaters, e. g. up to 50 g/L. This solution can be directed further to electrodialysis (Zmievskii *et al*., 2014) to separate salts from lactose (Figure 5).

It will reduce the energy consumption at the stage of electrodialysis (Zmievskii *et al*., 2014) and will allow obtaining about 92 % of purified water.

Moreover 6.8% of lactose solution ($C_{lactose} \approx 25$ g/L) and 1.2% of salt concentrate containing mainly monovalent ions ($C_{salt} \approx 140$ g/L) on the amount of the treated wastewater are obtained after electodialysis. The possible reuse application of the received solutions after such two-stage treatment is lightened in paper (Zmievskii *et al*., 2014).

4.Conclusions

During filtration of model solutions of nanofiltration whey permeate, it was found that membrane distillation can concentrate the solutes from 4 to 450 g/L. However, the essential decrease of membrane rejection was observed at the concentration 300 g/L and higher. It is probably caused by deposit formation on the membrane surface.

The dependence of water activity on concentration in the range from 0 to 450 g/L was obtained for model solutions of nanofiltration whey permeate. It would allow simulating the process of membrane distillation in future.

The process flow diagram of two-stage treatment of nanofiltration whey permeate was proposed. It involves the use of direct contact membrane distillation for solutes concentration up to 50 g/L and electrodialysis for separation of salts from lactose. It allows obtaining approximately 92% of purified water on amount of treated wastewater.

5.References

Aleksandrov, A.A., Rivkin, S.L. (1975). Termodinamicheskie svojstva vody i vodjanogo para (Thermodynamic Properties of Water and Steam), Moscow: Energija. In russian.

Al-Obaidani, S., Curcio, E., Macedonio, F., Profio, G.D., Al-Hinai, H., Drioli, E. (2008). Potential of membrane distillation in seawater desalination: Thermal efficiency, sensitivity study and cost estimation *Journal of Membrane Science*, 323, 85-98.

Boubakri, A., Bouchrit, R., Hafiane, A., Al-Tahar, Bouguecha, S. (2014a). Fluoride removal from aqueous solution by direct contact membrane distillation: theoretical and experimental studies. *Environmental Science and Pollution Research*, 21(17), 10493-10501.

Boubakri, A., Hafiane, A., Al-Tahar, Bouguecha, S. (2014b) Nitrate removal from aqueous solution by direct contact membrane distillation using two different commercial membranes. *Desalination and Water Treatment*, DOI:10.1080/19443994.2014.981408.

Hickenbottom, K. L., Cath, T.Y. (2014). Sustainable operation of membrane distillation for enhancement of mineral recovery from hypersaline solutions. *Journal of Membrane Science*, 454, 426-435.

Hou, D., Dai, G., Wang, J., Fan, H., Luan, Z., Fu, C. (2013). Boron removal and desalination from seawater by PVDF flat-sheet membrane through direct contact membrane distillation. *Desalination*, 326, 115-124.

Hou, D., Wang, J., Zhao, C., Wang, B., Luan, Z., Sun, X. (2010). Fluoride removal from brackish groundwater by direct contact membrane distillation. *Journal of Environmental Sciences*, 22, P. 1860-1867.

Khayet, M., Matsuura, T. (2011) Membrane Distillation: Principles and Application, Amsterdam: Elsevier.

Kyrychuk, I., Zmievskii, Y., Myronchuk, V. (2014). Treatment of dairy effluent model solutions by nanofiltration and reverse osmosis. *Ukrainian Food Journal*, 3(2), 281-288.

Lu, J., Li, B., Wang, L., Wang, Y., Wang, S. (2014). Utilization of ammonia-containing wastewater by combining membrane absorption and vacuum membrane distillation. *Journal of Chemical Technology and Biotechnology*, 89(2), 312–321.

Macedonio, F., Drioli, E. (2008). Pressure-driven membrane operations and membrane distillation technology integration for water purification. *Desalination*, 223, 396-409.

Mariah, L., Buckley, C. A., Brouckaert, C. J., Curcio E., Drioli E., Jaganyi D., Ramjugernath D. (2006). Membrane distillation of concentrated brines—Role of water activities in the evaluation of driving force. *Journal of Membrane Science*, 280, 937-947.

Myronchuk, V. G., Grushevskaya, I. O., Kucheruk, D. D., Zmievskii, Yu. G. (2013). Experimental study of the effect of high pressure on the efficiency of whey nanofiltration process using an OPMN-P membrane. *Petroleum Chemistry*, 53(7), 439-443.

Shirazi, M. M. A., Kargari, A., Bastani, D., Fatehi L. (2014). Production of drinking water from seawater using membrane distillation (MD) alternative: direct contact MD and sweeping gas MD approaches. *Desalination and Water Treatment*, 52(13-15), 2372-2381.

Sun, X.-C., Wang, J., Hou, De-Y., Wang, B.-Q., Luan, Z.-K. (2011). Study on Concentration of Reverse Osmosis Concentrate by Membrane Distillation. *China Water & Wastewater*, 17, 22-25,30.

Xu, Y., B.-Ku, Zhu, Y.-Yi, Xu (2006). Pilot test of vacuum membrane distillation for seawater desalination on a ship. *Desalination*, 189, 165-169.

Zmievskii, Yu. G., Kirichuk, I. I., Mironchuk, V. G. (2014). Membrane treatment of wastewater obtained after the whey processing. *Journal of Water Chemistry and Technology*, 36(6), 309-316.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

SENSORY, MELTING AND TEXTURAL PROPERTIES OF FAT-REDUCED ICE CREAM INOCULATED WITH THERMOTOLERANT LACTIC ACID BACTERIA

Marina Fragoso[1], M. Lourdes Pérez-Chabela[2], Annel M. Hernández-Alcantara[2], Héctor B. Escalona-Buendía[2], Aurora Pintor[1], Alfonso Totosaus[1]*

[1] Food Science Lab & Pilot Plant, Tecnologico de Estudios Superiores de Ecatepec. Mexico
[2] Biotechnology Department, Universidad Autónoma Metropolitana Iztapalapa. Mexico
*Corresponding author: *alfonso.totosaus@gmail.com*

Article history:
Received:
07 January 2015
Accepted in revised form:
26 May 2016

Keywords:
Ice cream;
Lactic acid bacteria;
Sensory properties;
Melting;
Texture

ABSTRACT
A fat reduced ice cream with inulin as fat replacer was employed as prebiotic to inoculate thermotolerant probiotic lactic acid bacteria. Inoculation of thermotolerant lactic acid bacteria resulted in a softer and more adhesive ice cream texture, probably due to the exopolysaccharide production of the employed strain. In same manner, melting rate of inoculated samples was reduced, enhancing melting properties due lactic acid bacteria inoculation. Inoculated samples presented lower pH values and relatively higher titratable acid content due lactic acid growth during frozen storage. There was no presence of lactic acid bacteria in control samples. In sensory evaluation, although the growth and adaptation of thermotolerant lactic acid bacteria in ice cream, there was no difference between control and inoculated sample, in relation to texture and acceptation. In this view, thermotolerant probiotic lactic acid bacteria in fat reduced ice cream with inulin as fat replacer can be employed as a good alternative to produce symbiotic foods, with inulin as prebiotic ingredient.

1.Introduction

Ice cream is a complex food system with a disperse phase consist in three main structural components (air bubbles, ice crystals and emulsified fat globules) immersed in a continuous liquid phase (unfrozen water with dissolved sugar, proteins and hydrocolloids) (Marshall et al., 2003; Clarke, 2004). During ice cream process, air incorporation during frozen implies a great number of physical changes that are favored by the proteins and emulsifiers stabilizing both emulsion and foam formed. The capacity of the ingredients to interact with each other maintain the physical and sensory properties of the frozen ice cream base during and after the frozen process, cold-chain storage and finally, when ice cream is consumed (Pintor y Totosaus, 2013). Fat is a very important ingredient in the disperse phase. Fat is related to melting, air stabilization and ice crystals formation (Bolliger et al., 2000; Chung et al., 2003; Clarke, 2004; Granger et al., 2005; Goff, 1997). During frozen mixing dispersed and emulsified fat globules avoid coalescence forming a film around air bubbles, stabilizing the system (Chung et al., 2003). Fat interactions like partial coalescence of ice crystals or protein induced flocculation affect ice cream texture (Méndez-Velasco and Goff, 2012). Fat reduction can be

compensated with other ingredients. Pintor et al. (2014) replaced both butyric and vegetable fat in ice cream employing inulin.

Ice cream with probiotic has been proposed as a suitable vehicle for beneficial microorganisms' delivery if they remain viable during manufacture and frozen storage (Hekmat and McMahon, 1992; Cruz et al., 2009; Di Criscio et al., 2010; Mohammadi et al., 2011; Ayaz Javed and Nadeem, 2011). The technology to transform ice cream into a vehicle for probiotoic microorganisms must be well understood and studied simultaneously with the knowledge of the metabolism of the added cultures (Cruz et al., 2009). There are two plausible options to enhance probiotic survive during ice cream production. These are to protect bacteria by encapsulation or freeze dry, or to employ thermal shock proteins producing bacteria. Encapsulation has been proposed like an alternative to improve probiotic survival during ice cream manufacture and frozen storage (Mohammadi et al., 2011; Soukoulis et al., 2014), employing resistant starch as prebiotic (Homayouni et al., 2008) or alginate (Ahmadi et al., 2014; El-Sayed et al., 2015), or the use of freeze dry cultures (Nousia et al., 2011). Lactic acid bacteria with probiotic characteristics like adherence to epithelial cells, gastric conditions survive and pathogens inhibition (Ramirez-Chavarín et al., 2010, 2013) has been employed as started culture in cooked sausages (Pérez-Chabela et al., 2013; Diaz-Vela et al., 2015). When lactic acid bacteria survive thermal stress condition due the over-expression of thermal shock proteins, the adaptation to other stress condition is feasible. In this view, the objective of this work was to evaluate the use of a thermotolerant lactic acid bacteria to elaborate a fat reduce ice cream with inulin a prebiotic, in order to obtain a symbiotic ice cream.

2.Materials ad Methods

Ice cream elaboration

Fat-reduced ice cream was elaborated according the formulation described by Pintor et al. (2014). Solid ingredients like sugar (15% w/v), non-fat dry milk (8% w/v, DILAC, Cuautitlan Izcalli, Mexico), whey protein concentrate (4.0% w/v, DILAC, Cuautitlan Izcalli, Mexico), chicory inulin (3% w/v, Nano Nutrition, Naucalpan, México, Viscarin GP209 lambda carrageenan (0.25% w/v, FMC Biopolymers, Philadelphia), carboxymethylcellulose (0.25% w/v, FMC Biopolymers, Philadelphia), emulsifiers (sorbitan and glyceryl monostearates, 0.25% w/v, ARCY, Ecatepec, Mexico) were hydrated in water (ca. 58% v/v) at 60 °C, to disperse with a Oster homogenizer both butyric fat (7.0 % w/v, ARCY, Ecatepec, Mexico) and vegetal fat (3.5 % w/v, La Mixteca, Ecatepec, Mexico). The homogenized mixture was kept in refrigeration (2-4 °C) until processing.

A previously reported thermotolerant probiotic lactic acid bacteria, *Pediococcus pentosaceus* UAM22 (Ramirez-Chavarín et al., 2010, 2013) was reactivated in 10 mL Man, Rogosa, and Sharpe (MRS) broth, incubating at 37 °C for 24 h, until an optical density close to one (λ = 600 nm), containing approximately 10^8 CFU/mL. After centrifugation at 2,000 × g during 10 min, the cellular pellet was rinsed with distilled water and centrifuge at same condition again. The washed cellular pellet was dispersed in 5 mL of ice cream base and mixed with the rest of ice cream base before pasteurization. Control treatment was non-inoculated.

Ice cream base mixture was pasteurized at 70 °C during 30 min, ice cooled and stored overnight at 4 °C. Ice

cream was elaborated in a 2 quarters Frozen Ice Cream CIM-50RSA machine (Cuisinart, East Windsor), mixing during 30 min until obtain a uniform frozen paste. Ice cream was immediately distributed in 250 mL containers and kept in frozen storage at -23 °C until further analysis after at least 24 h.

2.1.Texture and melting properties

Ice cream textural properties were determined adapting the methodology reported by Soukoulis et al. (2008). Ice cream samples were tempered at room temperature for 10 min. before being penetrated with a 10 mm ∅ acrylic probe at a constant speed of 1 mm/s in a Brookfield LFRA 4500 texturometer (Brookfield Engineering Lab, Middleboro). From force-time curves, ice cream hardness (maximum force during penetration), penetration work (energy required to beak ice cream structure during penetration), and adhesiveness (negative force to separate the probe form sample after penetration) were reported.

Melting properties were determined removing the ice cream samples from their containers after 10 min of tempered at room temperature. Samples were placed in stainless steel mesh sieve size 14 (1.4 mm opening) registering the time for the first drop of melted ice cream. The weight of melted ice cream was recorded each 5 min during one h to calculate melting rate (weight change versus time, in g/min) (Soukoulis et al., 2008).

2.2.Titratable Acidity, pH and bacterial count

Lactic acid bacteria count was determined during ice cream frozen storage at 1, 6, 9, 13, 16 and 21 days. Ten g of both inoculated and control samples were diluted with 90 mL of a sterilized serological solution (0.85% NaCl). From this dilution, aliquot of 1 mL was diluted with 9 mL of same solution in order to be inoculated in bacteriological agar plates. Plates were incubated at 37 °C during 24 h, reporting CFU/g of ice cream (Akin et al., 2007).

The changes in acidity as a putative result of lactic acid bacteria growth in ice cream were determined measuring titratable acidity and pH of the samples. 20 mL of melted ice cream was mixed with 40 mL of distilled water adding six drops of phenolphthalein indicator solution. Samples were titrating with NaOH standard solution (0.1 N) while stirring constantly until a faint pink color appearance. Titration continues until a persistent pink coloration. Acidity in g of lactic acid per liter was reported. The pH was measured to melted ice cream samples (approx. 20 mL) with a Beckman Φ 500 Benchtop Meter (Beckman Coulter, Fullerton).

2.3.Sensory evaluation

The sensory evaluation was carried out in the Universidad Autonoma Metropolitana Iztapalapa Sensorial Laboratory facilities, in special individual booths. A total of 54 untrained panelists (students and staffs) were asked to participate in the sensory evaluation. In order to determinate the "affective status" or how well the ice cream inoculated with thermotolerant lactic acid bacteria is liked by consumers, an acceptance test was performed, comparing the inoculated samples with the non-inoculated control (Meilgaard et al., 1999) was carried out in two sessions, one for the expectation about the color, creaminess and acidity employing a 5 point hedonic scale (much more than expected/much less than expected). In another session, texture and taste acceptance were evaluated with a 7 point hedonic scale (dislike extremely/like extremely). Both ice cream samples (inoculated and non-

inoculated control) were presented directly from freezer (around -18 °C) in plastic containers (ca. 10 g), coded with three digit random numbers. Panelists were asked to rinse their mouth between samples. Ice creams samples had 13 days of frozen storage, time enough to allow lactic acid bacteria development.

2.4.Experimental design and data analysis

The effect of inoculate lactic acid bacteria in a fat reduced ice cream with inulin was determined according to the model:

$$yij = \mu + \alpha i + \beta j + \in ij \quad (1)$$

where yij represents the variable response for the i-th treatment (inoculated or non-inoculated) at the j-th time of storage (1, 6, 9, 13, 16 and 21 days); μ is the overall mean; αi and βj are the main effects of inoculation and storage time; and $\in ij$ is the residual or error terms assumed to be normally distributed with zero mean and variance σ^2 (Der and Everitt, 2002). Results were analyzed with the PROC ANOVA procedure in SAS Software v 8.0 (SAS System, Cary). Significant differences between means were determined by the Duncan means test.

For sensory evaluation, data of each analyzed attribute for both ice creams were compared using the t-test, PROC TTEST in SAS Software, to establish significantly ($P<0.05$) difference between the two ice cream samples.

3.Results and discussions

Ice cream texture and melting properties

The inoculation of thermotolerant lactic acid bacteria resulted in a significantly ($p<0.05$) softer texture, where ice cream hardness decreased significantly ($p<0.05$) during frozen storage. Same tendency was observed for penetration work, this is, control samples were significantly ($p<0.05$) harder than the inoculated ones. Energy to penetrate the ice cream also decreased significantly ($p<0.05$) during frozen storage. In contrast, ice cream tackiness was significantly ($p<0.05$) higher in inoculated samples, and this textural property increased significantly ($p<0.05$) during storage (Table 1).

The time to start ice cream melting, first drop, was not significantly ($p<0.05$) different for both ice cream samples, and the time for first drop decreased significantly ($p<0.05$) during storage. Melting rate was significantly ($p<0.05$) lower in inoculated samples. Ice cream melting rate decreased significantly ($p<0.05$) as well during storage time (Table 2).

The inoculation of thermotolerant lactic acid bacteria resulted in a softer and tackier texture. Although it has been reported that inulin improved texture and melting of probiotic ice cream (Akalin et al., 2008), in the ice cream formulations employed the only variable was the thermotolerant lactic acid bacteria inoculation. In this view, changes in ice cream texture must be attributed to thermotolerant lactic acid bacteria development at the experimental conditions employed. Pérez-Chabela et al. (2013) and Diaz-Vela et al. (2015) reported that this thermotolerant lactic acid strain, *P. pentosaceus* UAM22, produced exopolysaccharide in cooked meat batters, enhancing texture and moisture retention. *In situ* applications of exopolysaccharides are traditionally yogurt or kefir. Nonetheless, ropy cultures improved texture increasing viscosity in the cryo-concentrated serum phase during freezing, resulting in a more firm ice cream, replacing stabilizers (Goh et al., 2008).

Table 1. Ice cream textural properties during frozen storage

Time (days)	Hardness (N)		Penetration work (N s)		Tackiness (N)	
	Control	Inoculated	Control	Inoculated	Control	Inoculated
1	13.85±1.08 A, a	12.40±1.66 B, a	113.64±12.37 A, a	82.26±13.76 B, a	-4.28±0.63 B, d	-6.93±3.08 A, d
6	12.96±4.46 A, ab	11.75±1.29 B, ab	118.84±59.15 A, a	80.60±9.95 B, a	-4.64±2.54 B, c	-7.11±0.36 A, c
9	12.85±2.85 A, bc	11.55±3.06 B, bc	108.66±35.95 A, ab	75.21±7.96 B, ab	-5.01±1.33 B, bc	-7.47±0.71 A, bc
13	12.05±4.72 A, bc	11.23±0.88 B, bc	110.39±27.82 A, bc	75.13±10.30 B, bc	-5.85±0.00 B, b	-7.80±1.65 A, b
16	11.95±3.78 A, cd	10.75±0.72 B, cd	102.55±40.03 A, c	69.17±5.89 B, c	-6.12±1.12 B, a	-8.55±1.19 A, a
21	11.00±3.63 A, d	10.40±0.51 B, d	95.97±22.34 A, d	52.63±4.69 B, d	-6.99±2.66 B, a	-8.74±0.11 A, a

A, B Means with same letter are not significantly (P>0.05) different for control or inoculated sample.
a, b, c, d Means with same letter are not significantly (P>0.05) different for storage time.

Table 2. Ice cream melting properties during frozen storage

Time (days)	First drop (min)		Melting rate (g/min)	
	Control	Inoculated	Control	Inoculated
1	25.00±0.00 B, a	26.50±6.74 A, a	1.20±0.16 A, a	1.04±0.24 B, a
6	26.50±3.63 B, a	27.00±2.07 A, a	1.14±0.21 A, ab	0.98±0.17 B, b
9	25.10±0.00 B, b	26.00±2.07 A, b	0.98±0.18 A, bc	0.94±0.24 B, bc
13	25.00±2.07 B, b	25.00±0.00 A, b	0.92±0.04 A, bc	0.92±0.16 B, bc
16	22.00±2.07 B, bc	23.50±0.51 A, bc	0.86±0.18 A, c	0.82±0.13 B, c
21	21.50±1.55 B, c	23.60±3.11 A, c	0.83±0.11 A, c	0.81±0.10 B, c

A, B Means with same letter are not significantly (P>0.05) different for control or inoculated sample.
a, b, c, d Means with same letter are not significantly (P>0.05) different for storage time.

Table 3. Ice cream lactic acid bacteria (CFU), tritatable acidity and pH

Time (days)	Log CFU/mL		Tritatable acidity (lactic acid g/L)		pH	
	Control	Inoculated	Control	Inoculated	Control	Inoculated
1	0.0±0.0 B, c	7.28±0.53 A, c	2.30±0.67 B, d	2.69±0.72 A, d	6.10±0.23 A, c	6.14±0.08 B, c
6	0.0±0.0 B, b	8.23±0.10 A, b	2.32±0.65 B, d	2.58±0.83 A, d	6.24±0.11 A, ab	6.22±0.00 B, ab
9	0.0±0.0 B, a	8.60±0.01 A, a	2.93±0.77 B, b	3.56±0.63 A, b	6.28±0.07 A, a	6.21±0.03 B, a
13	0.0±0.0 B, a	8.65±0.51 A, a	3.09±1.18 B, a	3.92±0.76 A, a	6.27±0.09 A, b	6.15±0.22 B, b
16	0.0±0.0 B, b	8.26±0.05 A, b	2.58±0.39 B, c	2.69±0.72 A, c	6.34±0.04 A, ab	6.20±0.15 B, ab
21	0.0±0.0 B, bc	8.17±0.62 A, bc	2.52±0.68 B, c	2.90±0.50 A, c	6.41±0.08 A, b	6.13±0.04 B, b

A, B Means with same letter are not significantly (P>0.05) different for control or inoculated sample.
a, b, c, d Means with same letter are not significantly (P>0.05) different for storage time.

Table 4. Sensory analysis means scores for the expectation (color, creaminess and acidity) and acceptation (texture and taste) of ice cream

Treatment	Color*	Creaminess*	Acidity*	Texture**	Taste**
Control	3.09±0.83 a	2.48±1.21 a	3.85±0.89 a	3.24±1.31 a	3.36±1.25 a
Inoculated	3.29±0.98 a	2.20±1.07 a	3.83±0.82 a	3.31±1.22 a	3.42±1.61 a

a, b Means with same letter are not significantly (P>0.05) different for each sensory attribute
* Scores were based on a 5 points hedonic scale with 1 as “much less than expected” and 5 as “much more than expected”
** Scores were based on a 7 points hedonic scale with 1 as “dislike extremely” and 7 as “like extremely”

Cultures that produce exopolysaccharides increase cells thermal and physical shock presenting a thermotolerant capacity (Hong and Marshall, 2001). The production of exopolysaccharides affected as well the melting properties of inoculated ice cream. Non-inoculated ice cream melted faster than probiotic ice cream (Salem et al., 2005).

Lactic acid bacteria, titratable acidity and pH

As expected, lactic acid bacteria population was significantly ($p<0.05$) higher in inoculated ice cream samples. There was no detectable presence of lactic acid bacteria in control samples. Bacteria population increased significantly ($p<0.05$) during the first 13 days of storage, and then decline (that is why the sensory evaluation was performed at day 13). As consequence of lactic acid bacteria growth in ice cream, tritatable acidity was significantly ($p<0.05$) higher in inoculated samples, but the amount of titratable acid was not significantly ($p<0.05$) different during storage after the day one, with a tendency to decrease. In same manner, ice cream pH was significantly ($p<0.05$) higher in control samples, although with a significantly ($p<0.05$) tendency to increase as well during storage (Table 3).

The growth of inoculated thermotolerant lactic acid bacteria explains the changes in pH and titratable acidity. The decrease in bacterial counts is attributed to cell damage during ice cream manufacture (freezing plus mechanical stress, osmotic shock and air incorporation) (Akin et al., 2007; Magariños et al., 2007; Akalin et al., 2008; Turgut and Cakmakci, 2009; Ferraz et al., 2012). Cell viability depends on the strain, substrate, and final acidity (Mohammadi et al., 2011). Ice cream inoculated with *B. longum* and *B. lactis* reach a pH around 4.5 and resisted freezing process (Favaro-Trindade et al., 2006). Inulin in ice cream also improved bacteria survival. *L. acidophilus* and *B. lactic* growth in ice cream reached a pH of 5.9 (Akin et al., 2007). *L. casei* and *L. rhamnosus* growth in inoculated ice cream as well (Di Criscio et al. 2010).

As a consequence of lactic acid bacteria metabolism, inoculated ice cream had lower pH and higher acidity (Alamprese et al., 2005; Turgut and Cakmakci, 2009; Abghari et al., 2011; Nousia et al., 2011;) In ice cream elaborated with lactic acid bacteria, pH range was relatively high 6.24-6.42, due to ice cream buffer capacity (Salem et al., 2005). The pH variations in dairy products are influenced by the buffering capacity of milk soluble compounds like phosphate, calcium, citrate, caseins and whey proteins, and their distribution within the aqueous or solid phase of the product, where caseins and inorganic phosphate have maximum buffering capacity at pH between 5 and 6 (Salaün et al, 2005).

The no presence of lactic acid bacteria in control ice cream demonstrated the effectiveness of ice cream base thermal processing. In probiotic ice cream samples, Di Criscio et al. (2010) reported that undesirable microorganisms (enterobacteria, total and fecal coliforms) were detected at a very low numbers and not detectable. Although inulin improved lactic acid bacteria survive in ice cream (Akin, 2005; Akin et al., 2007), the thermotolerant capacity of the employed strain explains their prevalence during frozen storage. Abrupt decrease in temperature induces the over-expression of thermal shock proteins to the optimal adaptation to lower temperature (Hébraud and Potier, 1999). The temperature expressed cold-shock proteins are a response to cold shock and expressed when an exponentially

growing culture is shifted from its optimum growth temperature to a lower temperature (Phadtare 2004). Exposure of the microorganisms to temperatures of 4 °C during 24 h resulted in the cryotolerance, capacity to survive freezing conditions, of partially frozen mixture after ageing of ice cream (Abghari et al., 2011). Freezing temperatures do not negatively affect survival of lactic acid bacteria during ice cream storage (Başyiğit et al., 2006; Di Criscio et al., 2010).

Sensory test

Sensory evaluation revealed that color and acidity of inoculated ice cream were "just as expected", with no significantly ($p>0.05$) difference between inoculated and control samples. Ice cream creaminess resulted "less than expected", but with no significantly ($p>0.05$) difference between both ice cream samples as well. This means that the consumers expectation for an ice cream inoculated with lactic acid bacteria was as they expected, where no difference in color and acidity was detected. Consumers asserted that ice cream creaminess was "less than expected", but with no significantly ($p>0.05$) difference between inoculated and control samples (Table 4).

On other hand, the acceptation of texture and taste was in the range of "dislike moderately". Nonetheless, for both sensory attributes there was no significantly ($p>0.05$) difference detected by panelists (Table 4). Again, instrumental texture differences detected were not reflected in sensory appreciation of ice cream texture, although the acceptance was fairly accepted. In same manner, since the amount of lactic acid was not as high as in a fermented food like yogurt, the acceptance for taste had a tendency to dislike, but panelists didn't find difference between both inoculated and control samples.

Although the relatively higher amount of lactic acid bacteria in inoculated samples, the buffering capacity of ice cream formulation not allowed the concentration of acid and pH decrease during ice cream storage. In same manner, while instrumental texture and melting differences were detected, no change in sensory creaminess was appreciated. Inoculated ice cream presented relatively higher scores than non-inoculated ones, presenting a good acceptation (Salem et al., 2005; Favaro-Trindade et al., 2006; Nousia et al., 2011; Ferraz et al., 2012). Ice cream low acidity increased overall consumer acceptation (Hekmat and McMahon, 1992; Cruz et al., 2009). Microorganism addition along inulin had no effect on ice cream consistency or taste (El-Nagar et al. 2002; Akin et al. 2007; Di Criscio et al. 2010; Pandiyan et al, 2012). In general, during sensory evaluation the judges didn't find difference between both inoculated and non-inoculated ice cream samples. Ice cream color was "just as expected". Creaminess expectation was slightly lower, but acidity for the inoculated ice cream was above the expected. For texture and taste, both attributes was close to "neither like or dislike" in the ordinal hedonic scale proposed. The information provided to panelist about an ice cream containing lactic acid bacteria could provoke certain expectancy about texture and flavor, evaluated as creaminess and acidity. Lactic acid bacteria are associated to fermented foods as yogurt, or even yogurt ice cream, with an acid taste, but the overall expectancy was just as expected, although the acceptance was not over the favored or pleased acceptation. Probably the addition of fruit flavor could improve the taste acceptance.

4.Conclusions

The use of thermotolerant probiotic lactic acid bacteria in fat reduced ice cream with inulin as fat replacer and prebiotic demonstrated to be a good alternative to formulate symbiotic foods. Inoculation of lactic acid bacteria resulted in softer texture with longer melting rates. Although growth of lactic acid bacteria resulted in lower pH and more acidity, there was no difference in ice cream acceptation, even the expectation about an inoculated ice cream (associated to a sour flavor, related to ferment dairy products like yogurt). The capacity to survive thermal stress ensures that the lactic acid bacteria employed in this research survived ice cream process before and during frozen step and storage. The use of this strains is recommended to artisanal ice cream retail sold (average batch size 20 L with a shelf life between 10-15 days), since the time for consumption is shorter than industrial ice cream with higher volume of production.

5.References

Abghari, A., Sheikh-Zeinoddin, M., Soleimanian-Zad, S. (2011). Nonfermented ice cream as a carrier for *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. *International Journal of Food Science and Technology*, 46, 84-92.

Ahmadi, A., Milani, E., Madadlou, A., Ali, Mortazavi, S., Rezaei, Mokarram, R., Salarbashi D. (2014). Synbiotic yogurt-ice cream produced via incorporation of microencapsulated lactobacillus acidophilus (la-5) and fructooligosaccharide. *Journal of Food Science and Technology*, 51, 1568-1574.

Akalin, A.S., Erişir, D. (2008). Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream. *Journal of Food Science*, 73, M184-M188.

Akin, M.B., Akın, M.S., Kırmacı, Z. (2007). Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. *Food Chemistry*, 104, 93-99.

Akin, M.S. (2005). Effect of inulin and different sugars levels on viability of probiotic bacteria and the physical and sensory characteristics of probiotic fermented ice-cream. *Milchwissenschaft*, 60, 297-301.

Alamprese, C., Foschino, R., Rossi, M., Pompei, C., Corti, S. (2005). Effects of *Lactobacillus rhamnosus* GG addition in ice cream. *International Journal of Dairy Technology*, 58, 200-206.

Ayaz, Javed, M., Nadeem, M. (2011). Development of probiotics ice cream in Pakistan from buffalo milk by using *B. bifidum* and *L. acidophilus*. *Carpathian Journal of Food Science and Technology*, 3, 11-20.

Başyiğit, G., Kuleaşan, H., Karahan, A.G. (2006). Viability of human-derived probiotic lactobacilli in ice cream produced with sucrose and aspartame. *Journal of Industrial Microbiology and Biotechnology*, 33, 796-800.

Bolliger, S., Goff, D., Tharp, W. (2000). Correlation between colloidal properties of ice cream mix and ice cream. *International Dairy Journal*, 10, 303-309

Chung, S., Heymann, H., Grun, I. (2003). Temporal release of flavor compounds from low-fat and high-fat ice cream during eating. *Journal of Food Science*, 68, 2150-2156.

Clarke, C. (2004). The science of ice cream. Cambridge: Royal Society of Chemistry.

Cruz, A.G., Antunes, A.E.C., Sousa, A.L.O.P., Faria, J.A.F., Saad, S.M.I. (2009). Ice-cream as a probiotic food

carrier. *Food Research International*, 42, 1233-1239.

Der, G., Everitt, B.S. (2002). Handbook of statistical analyses using SAS. Boca Raton: Chapman & Hall/CRC, Boca Raton, pp. 101-116

Di, Criscio T., Fratianni, A., Mignogna R., Cinquanta L., Coppola R., Sorrentino E., Panfili G. (2010). Production of functional probiotic, prebiotic, and synbiotic ice creams. *Journal of Dairy Science*, 93, 4555-4564.

Díaz, Vela, J., Totosaus, A., Pérez-Chabela, M.L. (2015). Integration of agroindustrial by-products as functional food ingredients: cactus pear (*Opuntia ficus indica*) flour and pineapple (*Ananas comosus*) peel flour as fiber source in cooked sausages inoculated with lactic acid bacteria. *Journal of Food Processing and Preservation*. DOI: 10.1111/jfpp.12513.

El-Nagar, G., Clowes, G., Tudorică, C.M., Kuri V., Brennan, C.S. (2002). Rheological quality and stability of yog-ice cream with added inulin. *International Journal of Dairy Technology*, 55, 89-93.

El-Sayed, H.S., Slama, H.H., El-Sayed S.M. (2015). Production of symbiotic ice cream. *International Journal of Chemical Technology Research*, 7, 138-147.

Favaro-Trindade, C.S., Bernardi, S., Barbosa, Bodini, R., De Carvalho, Balieiro, J.C., De Almeida E. (2006). Sensory acceptability and stability of probiotic microorganisms and vitamin C in fermented acerola (*Malpighia emarginata* DC.) ice cream. *Journal of Food Science*, 71, S492-S495.

Ferraz, J.L., Cruz, A.G., Cadena, R.S., Freitas, M.Q., Pinto, U.M., Carvalho, C.C., Faria, J.A.F., Bolini H.M.A. (2012). Sensory acceptance and survival of probiotic bacteria in ice cream produced with different overrun levels. *Journal of Food Science*, 71, S24-S28.

Goff, D. (1997). Colloidal aspects of ice cream. *International Dairy Journal*, 7, 363-373.

Goh, K.K.T., Nair R.S., Matia-Merino L. (2008). Exploiting the functionality of lactic acid bacteria in ice cream. *Food Biophysics*, 3, 295-304.

Granger, C., Leger A., Barey P., Langendorff V., Cansell M. (2005). Influence of formulation on the structural networks in ice cream. *International Dairy Journal*, 15, 255-262.

Hébraud, M., Potier P. (1999). Cold shock response and low temperature adaptation in psychrotrophic bacteria. *Journal of Molecular Microbiology and Biotechnology*, 1, 211-219.

Hekmat, S., McMahon D. J. (1992). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. *Journal of Dairy Science*, 75, 1415-1422.

Homayouni, A., Azizi, A., Ehsani, M.R., Yarmand, M.S., Razavi, S.H. (2008). Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry*, 111, 50-55.

Hong, S.H., Marshall, R.T. (2001). Natural exopolysaccharides enhance survival of lactic acid bacteria in frozen dairy desserts. *Journal of Dairy Science*, 84, 1367-1374.

Magariños, H., Selaive, S., Costa, M., Flores, M., Pizarro, O. (2007). Viability of probiotic micro-organisms (*Lactobacillus acidophilus*La-5 and *Bifidobacterium animalis* subsp. lactis Bb-12) in ice cream. *International Journal of Dairy Technology*, 60, 128-134.

Marshall, T., Goff, D., Hartel, W. (2003). Ice Cream. Gaithersburgh: Aspen Publishers.

Meilgaard, M., Civille G.V., Carr, B.T. (1999). Sensory Evaluation Techniques (3rd edition). Boca Raton: CRC Press (chapter 12).

Méndez-Velasco, C., Goff, H.D. (2012). Fat structure in ice cream: A study on the types of fat interactions. *Food Hydrocolloids*, 29, 152-159

Mohammadi, R., Mortazavian, A.M., Khosrokhavar, R., Gomes, da Cruz A. (2011). Probiotic ice cream: viability of probiotic bacteria and sensory properties. *Annals of Microbiology*, 61, 411-424.

Nousia, F.G., Androulakis, P.I., Fletouris, D.J. (2011). Survival of *Lactobacillus acidophilus* LMGP-21381 in probiotic ice cream and its influence on sensory acceptability. *International Journal of Dairy Technology*, 64, 130-136.

Pandiyan, C., Annal, Villi, R., Kumaresan, G., Murugan, B., Gopalakrishnamurthy, T.R. (2012). Development of synbiotic ice cream incorporating *Lactobacillus acidophilus* and *Saccharomyces boulardii*. *International Food Research Journal*, 19, 1233-1239.

Pérez-Chabela, M.L., Díaz-Vela, J., Menéndez, C.V., Totosaus A. (2013). Improvement of moisture stability and textural properties of fat and salt reduced cooked sausages by inoculation of thermotolerant lactic acid bacteria. *International Journal of Food Properties*, 16, 1789-1808.

Phadtare, S. (2004). Recent developments in bacterial cold-shock response. *Current Issues in Molecular Biology*, 6, 125-136.

Pintor, A., Severiano-Pérez, P., Totosaus A. (2014). Optimization of fat-reduced ice cream formulation employing inulin as fat replacer via response surface methodology. *Food Science and Technology International*, 20, 489-500.

Pintor, A., Totosaus, A. (2013). Propiedades funcionales de sistemas lácteos congelados y su relación con la textura del helado. *Ciencia UAT*, 25, 56-61.

Ramirez-Chavarin, M.L., Wacher, C., Eslava-Campos, C.A., Perez-Chabela, M.L. (2013). Probiotic potential of thermotolerant lactic acid bacteria strains isolated from cooked meat products. *International Food Research Journal*, 20, 991-1000.

Ramírez-Chavarín, N.L., Wacher-Rodarte, C., Pérez-Chabela, M.L. (2010). Characterization and identification of thermotolerant lactic acid bacteria isolated from cooked sausages as bioprotective cultures. *Journal of Muscle Foods* 21, 585-596.

Salaün, F., Mietton, B., Gaucheron, F. (2005). Buffering capacity of dairy products. *International Dairy Journal*, 15, 95-109.

Salem, M.M.E., Fathi, F.A., Awad, R.A. (2005). Production of probiotic ice cream. *Polish Journal of Food and Nutrition Sciences*, 55, 267-271.

Soukoulis, C., Chandrinos, I., Tzia C. (2008). Study of the functionality of selected hydrocolloids and their blends with κ-carrageenan on storage quality of vanilla ice cream. *LWT-Food Science and Technology*, 41, 1816-1837.

Soukoulis, C., Fisk, I.D., Bohn, T. (2014). Ice cream as a vehicle for incorporating health-promoting ingredients: conceptualization and overview of quality and storage stability. *Comprehensive Reviews in Food Science and Food Safety*, 13, 627-655.

Turgut, T., Cakmakci, S. (2009). Investigation of the possible use of probiotics in ice cream manufacture. *International Journal of Dairy Technology*, 62, 444-451

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

NUTRITIONAL VALUE OF CHINCHILLA MEAT AND ITS AGROINDUSTRIAL DERIVATIVES

Angelica Fellenberg[1*], Alejandro Mac Cawley[2,3] and Ivan Peña[1]

[1]Animal Science Department, Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Macul, Santiago, Chile.
[2]Department of Industrial and Systems Engineering, Escuela de Ingeniería, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Macul, Santiago, Chile.
[3]Department of Agricultural Economics, Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Macul, Santiago, Chile.
*Corresponding author: * mafellen@uc.cl*

Article history:
Received:
05 February 2016
Accepted in revised form:
26 May 2016

Keywords:
Chilean chinchilla;
Chinchilla meat;
Chinchilla derivatives

ABSTRACT
The aim of this study is to provide new information increase the knowledge on Chinchilla *(Chinchilla laniger)* meat quality characteristics and compare it with other exotic meats. The chinchilla´s is a rodent raised in confinement for their fur and meat is considered as a byproduct of the process and is currently discarded. In this study we proceed to analyze the chinchilla raw meat by proximate analysis and compared its characteristic to other studies on exotic meats. Chinchilla raw meat was found to have less or similar crude protein, and fat than other rodents meats. Fatty acid profile is interesting because it had around 75% of monounsaturated and polyunsaturated fatty acids. Different agro-industrial preparations were made, analyzed chemically and tested by a sensory panel to obtain their organoleptic characteristics. Finally the meat was tested with a trained and non-trained tasting panel, the panel results indicate that the meat was accepted with good scores.

1. Introduction

The Chinchilla *(Chinchilla laniger)* has been hunted and bred for its light, fine and dense fur, which has a significant value for the trade. Its value has led to an irrational hunting that endangered this specie in the past. Nowadays, in Chile, wild *Chinchilla laniger* is protected by the government (Marinovic, 1990), which led to breeding performed under confinement ecological conditions because of its of major importance for the fur industry (Parker, 1982). Since the main objective in breeding Chinchilla is the fur, the meat is disposed without any use because of the lack of knowledge in its potential use for human consumption (Echalar et al., 1998). The nutritional value of the Chinchilla meat was first studied by Echalar et al., (1998), concluding that the meat has good food value and acceptability, no further studies have been conducted so far over this exotic meat. As Hoffman and Cawthorn (2013) indicates, there will be an increase in non-traditional meats and more research is required on the nutritional composition on the meats from alternate sources.

Chinchilla is native to the high and dry areas of Los Andes Mountains, Chile, Argentina, Perú and Bolivia (Adaro et al., 1999). The chinchilla belongs to the order of Rodentia and the suborder of Hystricognatha (Caviomorpha) (Spotorno et al., 2004 a). Chinchillas and mountain pampas viscachas

(*Lagidium* and *Lagostomus*) are in the C*hinchillidae* family (Spotorno et al., 2004 b). The genus chinchilla has two surviving species: *Ch. brevicaudata* (short tail chinchilla) that lives in the altiplanic area of Los Andes and *Ch. lanigera* (long tail chinchilla) that lives in the northern district of Chile known as Chile Chico.

In the work by Echalar et al., (1998) they determined the nutritional value of Chinchilla meat for three groups: raw, treated with dry heat and treated with wet heat. Also they compared the proximate composition with other meats such as: Vizcacha, cow, pig and chicken. They did not compared the proximal analysis to other rodents, such as Guinea Pig, commonly consumed in South America and did not performed an analysis of the fatty acid composition of the Chinchilla Finally, they analyzed the acceptance of the preparation using a panel of 30 trained judges. In their acceptance analysis they only asked the level of acceptance of the three treatments using a 3 level scale (like, indifferent and dislike).

Our goal is to complement the studies of Echalar et al., (1998) and Antonio et al., (2007) by extending the nutritional value assessment of the meat from chinchillas to the fatty acid composition. We compare its composition with other works published in non-traditional meats, such as: guinea pig (Kouakou et al., 2013), Capybara (Girardi et al., 2005), and Nutria (Cabrera et al., 2007). Finally we present other agro-industrial preparations such as: smoke-baked chinchilla meat, confit in vegetable oil and confit in lard; we present the results of acceptability performed with trained and untrained panel of consumers.

2. Materials and methods

Animals

Chinchillas used in this study were obtained from a commercial nursery located in Pirque (Central area of Chile). We used 66 chinchillas for raw meat analysis and for derivatives (Figure 1). In the case of raw meat analysis, fat and meat of eight animals were separated and analyzed in the laboratory of the Departamento de Ciencias Animales de la Facultad de Agronomía e Ingeniería Forestal de la Pontificia Universidad Católica de Chile.

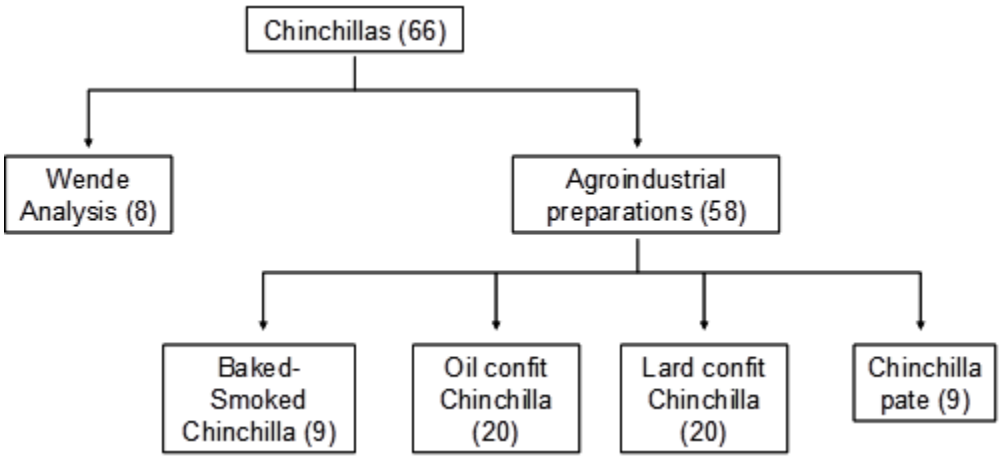

Figure 1. Used chinchillas.

Figure 2. A) Baked chinchilla; B) Oil confit of chinchilla; C) Lard confint of chinchilla

Chemical Analysis

Nutritional value of meat was determined by Weende or Proximate Analysis by obtaining dry matter (drying in a stove for 4 hours at 105 °C), protein (Kjeldahl method), fat (Soxhlet method) and carbohydrates (Crude Fiber and non nitrogen extract) (Methodenbuch 1998).

Fatty acid profile of the interstitial fat was determined by gas chromatography (Firestone, 1989) at Instituto de Nutrición y Tecnología de los Alimentos of Universidad de Chile.

Agroindustrial Preparations

Four different meat agroindustrial preparations of chinchilla were prepared:

Baked-Smoked chinchilla

A total of 9 carcasses (2.5 kg) were marinated in water (98%), ice (17%), Prinaham® (Sodium polyphosphate; sugar; salt; sodium erythorbate; sodium nitrite (1.8%)) (6%) and salt (9%) for 24 hours. After marinated they were sprayed with Pluscolor® (Salt; dextrose; sugar; sodium erythorbate; sodium ascorbate) (2 g/kg) and immersed in liquid smoke and water (1:2) for 30 seconds. Finally, they were baked at 120 ºC for 20 min, until inner meat reached 72 ºC. Then they were cooled at room temperature and refrigerated until evaluation (Figure 2A).

Chinchilla confit

Confit in vegetable oil. Twenty carcasses (5.2 kg) were processed to obtain legs and loins (2.47 kg). The legs and loins were washed, dressed with pepper and salted with 150 g of salt and 15 g/kg of Curaid® (Salt; sodium nitrite (6.0%); sodium nitrate (4.0%) for dry and cure and refrigerated for a period of 24 hours. Then they were taken out of the refrigerator and washed and weighed. In the next step the meat was sprayed with a roaster and confited in vegetable oil for eight minutes at 100-102 ºC, until the meat reached 72 ºC. Finally, the pieces (four legs and two loins) were set in glass jars with a capacity of 400 g and filled with new preheated oil at 100 ºC and the jars were sealed and placed upside down to sterilize the lid, and cooled at room temperature (Figure 2B).

Confit in lard. Twenty carcasses (5.3 kg) were processed to obtain legs and half a loin in one piece (2.9 kg). They were then washed, seasoned with pepper (2 g/kg) and salted with 150 g of salt and 15 g/kg of Curaid® for dry and cure and refrigerated for a period of 24 hours. Then they were taken out of the refrigerator and washed and weighed. In the next step the meat was sprayed with a roaster and confited in pork lard for seven minutes at 100-102 ºC, until the meat reached 70 ºC. Finally, they were set in plastic trays filled with new melted lard covering the pieces and cooled at room temperature until the lard solidified and refrigerated until evaluation (Figure 2C).

Sensory Analysis

Organoleptic quality of the product was analyzed through Scoring Method (15 cm non-structured scale), used by Serra et al., (2004) in a cattle meat quality, using 12 trained judges. The parameters evaluated were: appearance, color, aroma, texture, fattiness, consistency, saltiness, bitterness and flavor. The acceptability was evaluated with the Hedonic

Scale method with a non-structured assessment scale (between 0 – 15) with a 24 person non-trained panel.

Statistical Analysis

The statistical analysis was performed with SAS, based on the structure of probability associated with the randomization process. T student test was used to test the differences of the means.

3. Results and discussions

Table 1 shows nutritional composition of chinchilla meat.

Table 1. Nutritional contents of chinchilla meat (male and female)

	Males	Females	p
Weight (g)	461.8	449.8	0.693
Dry matter (DM)	25.9	26.6	0.748
Ether Extract (%)	6.0	6.1	0.974
Crude Protein (%)	18.7	19.5	0.153
Ash (%)	1.1	1.1	0.997
Fatty acid			
Saturated Fatty Acids			
C12:0	0.079	0.074	0.827
C14:0	2,029	1,171	0.390
C16:0	16,814	16,371	0.812
C18:0	3,203	3,066	0.786
C20:0	0.040	0.060	0.440
Monounsaturated Fatty Acids			
C14:1	0.116	0.142	0.522
C16:1	4,670	5,293	0.528
C18:1	29,201	27,966	0.514
C20:1 n9	0.263	0.266	0.926
Polyunsaturated Fatty Acids			
C18:2 n6	36,058	36,407	0.821
C18:3 n6	0.014	0.071	0.156
C18:3 n3	3,197	3,482	0.594
C20:2 n6	0.244	0.237	0.375
C20:3 n6	0.081	0.111	0.204
C20:3 n3	0.023	0.037	0.397
C20:4 n6	0.115	0.133	0.598
C22:5 n3	0.008	0.033	0.022
C22:6 n3	0.065	0.083	0.282

Chinchilla carcass weight was similar in male and female (p=0.6928), which was similar at Antonio et al., (2007) and a little bit different from that reported by Cabrera et al., (2007) in nutria meat (*Myocastor coypus*), where male carcass was heavier than female carcass when protein level in diet was 16% and was similar when protein level in diet were 19% or 22%.

In this study it was not evaluated protein diet intake, then it is needed more research to know if diet protein level is affecting chinchilla carcass weight. There were no differences in crude protein (CP), crude fiber and ash between male and female. Fatty acid profile of chinchilla meat was similar between male and female, except for docosapentaenoic acid (C22:5 n3) which was 4 times higher in female than male. In human health that is very interesting because docosapentaenoic acid belong to omega three family and plays an important role in brain formation and brain connections.

Two previous studies on Chinchilla meat nutritional value (Antonio et al., 2007; Echalar et al., 1998) have not been consistent in CP and fat content of chinchilla meat. Echalar et al., (1998), found that chinchilla meat had about 20% CP and 11.26% fat, while Antonio et al., (2007) showed that chinchilla meat had 16-18% CP and 20-30% fat. Our results are consistent with the results of (Echalar et al., 1998) with a 19%, but the fat content (6%) was below both previous studies. The differences can be attributed to the feed ingredients and other environmental considerations which might affect the fat content, for example genetic differences, since Echalar et al., (1998) research was performed in Argentina. More research is necessary to understand the differences in the fat content. Both studies did not report the fatty acid profile for chinchilla meat, to better understand the sources of the differences in the fat composition.

Crude protein content in similar rodent meats is between 18-24% (table 2). For the case of chinchilla meat, CP is around 19% and if it is compared with other meats it is slightly lower than the CP content in traditional meats (bovine, chicken, pork), but if it is compared with nontraditional meats (other rodents meats) protein content in chinchilla meat is definitely lower than those meats. Rodents may contribute to increase food security in some areas (Hardouin et al., 2003; Lammers et al., 2009) .

In fact, guinea pig (20.3% of CP) has been the meat source for the poorest people in Los Andes mountains for 3000 years. They are able to convert kitchen scraps and garden waste into meat (Hoffman and Cawthorn, 2013). In the other hand capybara and nutria are good sources of animal protein (21 – 22% CP) and they are raised in Latin America (Hoffman, 2008). Chinchilla meat has less CP than these rodents' meats, but it is important to bare in mind that the main objective in chinchilla industry is the fur, and meat is a by-product of this industry. From this point of view, chinchilla meat despite having lower protein content than others rodents meat, it could become an interesting source of protein. Fat content of chinchilla (ether extract) showed that this meat is leaner than bovine meat (table 3). That could be a positive aspect, because nowadays consumers are more concerned about fat contents and fat profiles of different foods, especially those of animal origin, since it is well known that fat and fatty acid profile are related with some kinds of diseases. Following the last idea, more important that fat level, is the fatty acid profile, because some types of fatty acids are healthier than others. Polyunsaturated fatty acids (PUFA) are healthier than saturated fatty acids (SFA).

Table 2. Nutrient composition in traditional and non traditional meats

	Species	Scientific name	n	Nutrient				Reference
				Moisture	Protein	Fat	Ash	
Traditional meat	Bovine	*Bos spp.*	3	67.0	19.22	9.8	0.9	Moreira el al., 2003
	Chicken	*Gallus gallus*		75.4	18.9	3.3	0.9	Hautrive et al., 2012
	Pork	*Sus domesticus*		75.0	21.3	1.3	1.1	Hautrive et al., 2012
Non traditional meat (rodent)	Chinchilla	*Chinchilla laniger*	8	73.7	19.1	6.1	1.1	
	Guinea pig	*Cavia porcellus*		70.6	20.3	7.8	0.8	Rosenfeld, 2008
	Viscacha	*Lagostomus maximus*		73.1	23.9	3.7		Arellano et al., 1993
	African giant rat	*Cricetomys gumbianus*		65.40	20.1	11.4	2.0	Oyarekua and Ketiku, 2010
	Nutria	*Myocastor coypus*	4	69.5	20.9	2.2	4.0	Cabrera et al., 2007
	Nutria	*Myocastor coypus*	42	75.7	22.1	1.3	1.0	Tulley et al., 2000
	Nutria	*Myocastor coypus*	5	73.8	21.0	1.6	-	Saadoun et al., 2006
	Capybara	*Hydrochoerus hydrocaeris*	13	75.6	22.0	1.8	1.1	Oda et al., 2004
	Capybara	*Hydrochoerus hydrocaeris*	7	76.2	22.3	1.0	1.1	Oda et al., 2004
	Capybara	*Hydrochoerus hydrocaeris*	18	74.4	20.9	1.8	1.2	Girardi et al., 2005

Table 3. Fatty acid composition (% of total fatty acids) of meat from different species.

Fatty acid	Species			
	Chinchilla	Guinea pig (a)	Nutria (b)	Capybara (c)
Lipid (g/100g)	6.1	7.8	1.8	1.8
Saturated				
12:0	0.08	-	-	-
14:0	1.60	1.59	3.60	2.00
15:0		0.29		
16:0	16.56	21.82	21.90	22.40
17:0	-	-	0.40	1.40
18:0	3.13	9.60	8.40	6.30
20:0	0.05	0.14	0.10	-
Total	***21.42***	***33.44***	***34.40***	***32.10***
Mono-unsaturated				
14:1	0.13	0.30	-	-
16:1	4.98	1.34	8.90	2.10
17:1	-	-	0.40	1.50
18:1	28.58	13.77	27.50	26.20
20:1	0.27	0.03	0.30	0.70
Total	***34.0***	***15.44***	***37.1***	***30.50***
Poly-unsaturated				
18:2	36.23	20.01	21.30	28.60
18:3 n6	0.04	0.24	-	2.70
18:3 n3	3.34	25.17	-	-
20:2	0.24	0.21	0.30	-
20:3 n6	0.10	0.19	-	0.10
20:3 n3	0.03	0.41	-	-
20:4	0.12	2.18	1.80	-
22:5	0.02	1.54	0.20	-
22:6	0.07	0.72	0.10	-
Total	***40.20***	***50.67***	***23.70***	***31.40***
S/MUFA	0.63	2.16	0.93	1.05
S/PUFA	0.53	0.65	1.45	1.02
MUFA/PUFA	0.85	0.30	1.57	0.97

a Kouakou et al., 2013, b Girardi et al., 2005, c Saadoun et al., 2006

Chinchilla meat had high content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), specifically the essential linoleic acid. When comparing chinchilla meat with others rodent meat (table 3), chinchilla meat has less SFA than guinea pig, nutria and capybara, similar monounsaturated fatty acid (MUFA) than nutria and capybara and more PUFA than nutria and capybara. Guinea pig has a little bit different fatty acid profile, because they have similar content of fat as chinchilla, but they have low content of MUFA (15.4%) and high content of PUFA (50.6%). Because rodents are non-ruminats, feed ingredients and fat source of the diet influence fat profile. If the diet is more unsaturated it is expected that meat fatty acid profile could be more unsaturated. More research is needed to learn about modify fatty acid profile through feeding handling.

Since the number of samples in the aforementioned studies (including ours) is small; we can only use them as descriptive studies contributing to the knowledge of different non-traditional meat.

As found by Echalar et al., (1998), chinchilla meat derivatives showed less water content than raw meat, probably because of dehydration and lower water holding capacity due to processing (Table 4). Derivatives showed increased protein content, probably due to a lower water holding capacity and because of the formation of a toast barrier which prevents loss of this nutrient (Cheftel et al., 1989).

Table 4. Nutritional value of chinchilla meat products

	Oil Confit	Lard Confit	Baked-Smoked Chinchilla
Water (%)	53.66	56.67	56.7
Fat (%)	8.67	11.34	16.48
Protein (N*6.25)	24.67	23.62	20.04
Minerals (%)	10.55	7.60	5.68
Non N extract (%)	2.45	0.77	1.10

Tasting trials with the different preparations of Chinchilla meat, showed that the baked-smoked chinchilla was perceived as a meat with a good color intensity, aroma and flavor and low bitterness (Table 5). The Confit was perceived as a meat with adequate aroma, flavor and fattiness, a little salty, with low bitterness and color intensity.

Table 5. Organoleptic characteristic of some derivates of chinchilla meat

Descriptor	Baked leg	Smoked loin	Oil Confit	Lard Confit
Appearence	11.0	7.7	7.7	8.5
Color Intensity	8.8	9.0	6.2	7.1
Aroma	8.9	9.9	7.1	7.3
Salty	10.1	8.0	9.9	10.8
Flavor	10.1	9.4	8.8	9.2
Bitterness	2.7	1.9	2.1	3.1
Fattyness	9.1	10.2	8.3	8.3
Hardness	7.7	9.1	--	--

The level of acceptance of Chinchilla meat was good (Figure 3) in accordance to what Echalar et al., (1998) found. Baked-smoked chinchilla (leg and loin) was the most preferred product (83%), followed by confit (62%). Baked leg showed a higher level of preference in the non-trained group rather than in the trained one. Opposite results were obtained for the case of loin evaluation. Vegetable oil and lard confit showed a good level of acceptability in both groups, with the latter being more preferred.

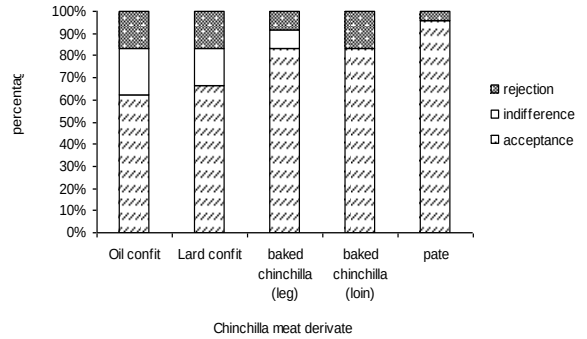

Figure 3. Percentage of acceptance, indifference and rejection of chinchilla meat derivates.

4. Conclusions

From our results we can indicate that Chinchilla meat is a good source of food for human consumption. In fact it can be recommended because: it is an interesting source of poly-unsaturated fatty acids, it has a high level of linoleic acid and a good level of protein

According to Hoffman and Cawthorn (2013) it is likely that there will be an increase in non-traditional meats consume in the future and more research is required on the nutritional component of the meat. Certainly, this study is adding more information about nutritional composition of Chinchilla meat

Finally, in this study tree types of derivatives were evaluated but it is necessary more research and trials to determine the more appropriate derivative of chinchilla meat. The acceptability of chinchilla meat derivatives was very good, however, this is not enough for commercialization and consumer approval. It is necessary to understand that chinchilla meat commercialization (like other exotic meats) is limited by its volume of production. This is the reason why the delicatessen market probably will be a good store for this kind of meat.

5. References

Adaro, L., Orostegui, P., Olivares, R., and Villanueva, S. (1999). Morphometric variations in male reproductor system in chinchilla in captivity through a year. *Av*ances Producción Animal (Chile), 24: 91-95.

Antonio, S. d. D., Velho, J. P., Carvalho, P. A., Backes, A. A., Sanchez, L. M. B., and Velho, I. M. P. H. (2007). Body composition prediction and net macroelements requirements (Chinchilla lanigera). Ciência e Agrotecnologia, 31: 548-553.

Cabrera, M., Del Puerto, M., Olivero, R., Otero, E., and Saadoun, A. (2007). Growth, yield of carcass and biochemical composition of meat and fat in nutria (*Myocastor coypus*) reared in an intensive

production system. Meat science, 76: 366-376.

Cheftel, J.-C., Lorient, D., and Cuq, J. L. (1989). *Proteinas alimentarias: bioquímica-propiedades funcionales-valor nuticional-modificaciones químicas*: Acribia.

Echalar, S. R., Jiménez, M. J. M., and Ramón, A. N. (1998). Valor nutritivo y aceptabilidad de la carne de chinchilla. Archivos latinoamericanos de nutricion 48: 77-81.

Firestone, D. (1989). Official Method Ce 1b-89. *Official Methods and Recommended Practices of the American Oil Chemists' Society*.

Girardi, F., Cardozo, R. M., de Souza, V. L., de Moraes, G. V., dos Santos, C. R., Visentainer, J. V., Zarab, R.F. and de Souza, N. E. (2005). Proximate composition and fatty acid profile of semi confined young capybara (*Hydrochoerus hydrochaeris hydrochaeris* L. 1766) meat. Journal of Food Composition and Analysis, 18: 647-654.

Hardouin, J., Thys, É., Joiris, V., and Fielding, D. (2003), Mini-livestock breeding with indigenous species in the tropics. Livestock Research for Rural Development, 15:30.

Hoffman, L. (2008). The yield and nutritional value of meat from African ungulates, camelidae, rodents, ratites and reptiles. Meat science, 80: 94-100.

Hoffman, L. C., and Cawthorn, D. (2013). Exotic protein sources to meet all needs. Meat science, 95: 764-771.

Kouakou, N., Grongnet, J.-F., Assidjo, N. E., Thys, E., Marnet, P.-G., Catheline, D., Legrand P., Kouba, M. (2013). Effect of a supplementation of *Euphorbia heterophylla* on nutritional meat quality of Guinea pig *Cavia porcellus* L.. Meat science, 93: 821-826.

Lammers, P. J., Carlson, S. L., Zdorkowski, G. A., and Honeyman, M. S. (2009). Reducing food insecurity in developing countries through meat production: the potential of the guinea pig (*Cavia porcellus*). Renewable Agriculture and Food Systems, 24: 155-162.

Marinovic, S. (1990). Como criar chinchillas. Nociones básicas. Chile Agrícola, 15:150-151.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

EFFECT OF TEMPERATURE, BACTERIAL PROPORTION AND INOCULUM SIZE ON THE FERMENTATION OF GOAT YOGURT WITH *BIFIDOBACTRIUM BIFIDUM*

Guowei Shu[*1], Ni Lei[1], He Chen[1], Chang Feng Wang[1], Hongchang Wan[2]

[1]*School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an, 710021, China*

[2]*Shaanxi Yatai Dairy Co., Ltd., Xianyang, 713701, China*

Corresponding author: [*] shuguowei@gmail.com

Article history:
Received:
28 December 2015
Accepted in revised form:
06 June 2016

Keywords:
goat yogurt;
inoculum size;
Bifidobactrium bifidum;
bacterial proportion

ABSTRACT

The effect of incubation temperature, bacterial proportion and inoculum size on goat milk fermented by probiotic culture containing *Bifidobactrium bifidum* was investigated by measuring the acidity, pH value, and viable counts during fermentation. Incubation temperature was 35℃, 37℃, 39℃, 41℃and 43℃, the ratio of *B. bifidum* to common starter cultures was 1:2, 1:1, 2:1, 3:1, 4:1, the inoculum size was 1%, 3%, 5%, 7% and 9%, respectively. The results showed that incubation temperature, bacterial proportion and inoculum size had significant impact on fermentation of goat milk. The optimum temperature was 39℃, the acidity, pH, the viable counts of *B. bifidum* and the total viable counts were 98.4^{0}T, 4.03, 7.60×10^{7}cfu/ml and 1.40×10^{9}cfu/ml, respectively. The optimum bacterial proportion of *B. bifidum* to common starter cultures was 4:1, the acidity, pH, the viable counts of *B. bifidum* and the total viable counts were 95^{0}T, 4.3, 7.40×10^{7}cfu/ml and 2.30×10^{9}cfu/ml respectively. The optimum inoculum size was 5%, the acidity, pH, the viable counts of *B. bifidum* and the total viable counts were 86^{0}T, 4.6, 1.61×10^{8}cfu/ml and 1.32×10^{9}cfu/ml respectively.

1. Introduction

Goat milk contains various nutrients needed by human body. Because of its peculiar taste and nutritional properties and its recognition as a healthy food, goat milk has received special attention by researchers and dairy industry. Some properties of goat's milk are known to be advantageous compared with those of cow's milk, such as higher tolerance by allergic children, which is related to the amount of and structural differences in whey proteins (*α-lactalbumin* and *β-lactalbumin*) and the high proportion of small fat globules (1.5 mm), which provide better digestibility (Albenzio and Santillo, 2011; Haenlein, 2004; Raynal-Ljutovac et al. 2005; Sheehan et al. 2009).In addition, the clotting behavior of goat milk was also different from cow milk. (Alloggio et al., 2000). In term of causing allergy, goat milk has been reported to have less allergenicity than cow milk (Sanz Ceballos et al., 2009).

Dairy foods are the main types of food matrices supplemented with probiotic bacteria and they have a positive reputation among consumers (Granato et al., 2010). Among the dairy products, yogurt/fermented milks have been the subject of several studies all over the world and different benefits for human health have been reported after their ingestion (Wang et al., 2012). According to FAO/WHO (2002), probiotics are live microorganisms which,

when administered in adequate amounts, confer health benefits on the host. Available evidence indicates that ingestion of probiotic bacteria may reduce the severity and frequency of diarrheal diseases and improve lactose digestibility among lactose-intolerant individuals (Mattila-Sandholm et al., 2002). Yogurts containing probiotics are claimed to provide several health benefits such as improve lactose utilization (De Vrese et al., 2001), prevent cancer (Rafter, 2003), maintain intestinal microflora balance (Mainville et al., 2005) and reduce serum cholesterol level (Baroutkoub et al., 2010). These therapeutic effects are the reason for which most probiotics are used in yogurts, fermented milks, ice creams and nutraceutical products (Cruz et al., 2010; De Oliveira and Jurkiewicz, 2009). Bifidobacteria are natural habitants of human gastrointestinal tract and can exert several beneficial effects to the host (Julio et al.,2010). They have been incorporated into a variety of food products,mainly daily, such as fermented milk and yogurts (Sanchez,de los et al.,2009)

In our previous study, the process of fermentation set-style goat yogurts was optimized by *S. thermophilus* and *L. bulgaricus* (Chen et al., 2010), the effect of inoculum and temperature on the fermentation of goat yogurt by *L. bulgaricus* and *S. thermophilus* (Shu et al., 2014) was investigated, The effect of the total inoculum size containing *L. acidophilus* or *L.casei* on he fermentation of goat milk was studied on the basis of *S. thermophilus* and *L.bulgaricus* as starter cultures (Chen et al., 2015). The purpose of this study was to study the effect of incubation temperature, bacterial proportion and inoculum size on the goat yogurt fermented by *B. bifidum* on the basis of *S. thermophilus* and *L. bulgaricus* as common starter cultures.

2. Materials and methods

Raw materials and reagents Fresh goat milk was purchased from local farmers (Xi'an Weiyang, China)，All chemicals used were of analytical grade unless otherwise specified.

Microorganism, *S. thermophilus, L. Bulgaricus and Bifidobactrium bifidum(BB)* were provided by School of Food and Biological Engineering, shaanxi university of science and technology, they were inoculated three successive times with rehydrated de Mann Rogosa Sharpe (MRS) broth (Haibo media, Qindao, China) for *L. bulgaricus* , MRS broth with 0.5% Cys-HCl for *B. bifidum* and M17 broth (Haibo media, Qindao, China) for *S. thermophilus* to obtain fresh culture. The activated, *B. bifidum* were inoculated respectively into sterilized goat milk at 5% inoculum size, mixed and cultivated at 42°C for *S. thermophilus and L. bulgaricus* (37°C for *B. bifidum*) until coagulation. They would be used for the production of goat yogurt containing *B. bifidum*.

Fermentation process of goat yogurt The fresh goat milk was pasteurized at 95℃ for 10 minutes, cooled, then goat milk was fermented with different temperature, different ratio of *B. bifidum* and common starter cultures or inoculated different inoculum size, The acidity, pH value, viable counts of *B. bifidum* and total viable bacteria were determined every other 1.5h, then gave a sensory evaluation after 12h.

Analysis method Plate coating method was used to determine the viable counts. The total viable counts were determinate by modified Tomato Juice medium, determination of *B. bifidum* by MRS agar containing 0.10% LiCl (Chen et al, 2011). The process was as follows: the agar medium packed in 250ml flask was sterilized, and15-20ml poured onto the plates in a clean bench after cooling to 50℃, 0.1ml aliquot bacteria dilutions were coated on it after coagulation, and then inoculated anaerobic 2-3d at 37℃, plates containing 30-300cfu/ml colonies were counted and the results expressed as colony-forming units per ml of sample.

The variation of pH was evaluated using a pH-meter （pHS-3c) at room temperature. Acidity of BB-goat milk was determined by sodium hydroxide titration and expressed in Jill Nieer degrees (^{0}T). The sensory evaluation of samples including color, smell, taste, texture

were organoleptically assessed by five panelists, who was trained on the basis of normal sensory acuity and consistency.

3. Results and discussions

Effect of temperature on the fermentation of BB-goat yogurt

The starter culture of 5% inoculum size was inoculated in the goat yogurt; the ratio of *B. bifidum* to common starter cultures was 1:1. Inocubation temperature was 35℃, 37℃, 39℃, 41℃ and 43℃ respectively. The results were shown in Figure1 and table 1.

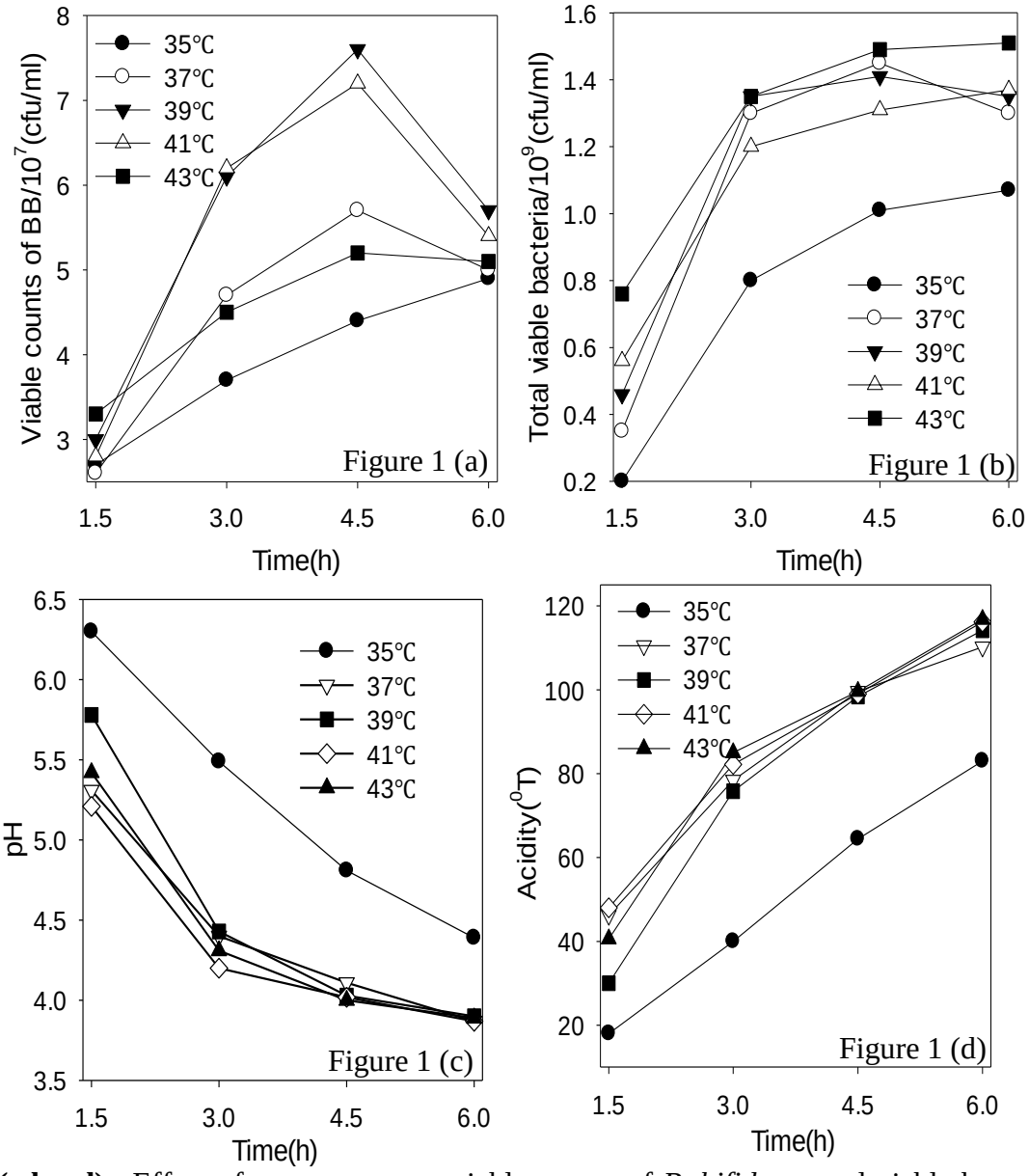

Figure 1(a,b,c,d). Effect of temperature on viable counts of *B. bifidum*, total viable bacteria, pH and acidity in BB-goat yogurt

Figure 1 (a) showed the viable counts of *B. bifidum* increased slowly at 35℃, 37℃and 43℃, but when the fermentation temperature was 39℃ and 43 ℃, the viable counts of *B. bifidum* increased rapidly within 4.5h,then presented a gradual decrease, that is because the acidity resistance of *B. bifidum* is not very well. Among then, the viable counts of *B. bifidum* at 39℃

reached the maximum, 7.60×10^7cfu/ml, the viable counts of *B. bifidum* at 35℃ was the lowest,4.40×10^7cfu/ml.

From figure 1 (b), each temperature of the total viable counts of goat yogurt had a fast growth within 3h, and increased slowly during 3-4.5h, then tended to be stable. Among then, the total viable counts of goat yogurt at 39℃, 41℃ and 43 ℃ were higher then that at other temperatures, the total viable counts of goat yogurt for 4.5h at 39 ℃, 41 ℃, 43 ℃ were 1.40×10^9cfu/ml,1.45×10^9cfu/ml and 1.49×10^9 cfu/ml respectively, that is because the common strains of goat yogurt was in the highest flight, the total viable counts of goat yogurt at 35℃ was the lowest, 1.01×10^9cfu/ml. From figure1(c) and 1(d), each temperature of the acidity and the total viable counts were basically same, that is because the *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* were important acid producing bacteria. Among then, the acidity and pH value of the goat yogurt at 39℃, 41℃ and 43 ℃ for 4.5h were 98.4^0T, 4.03, 99^0T, 4.02, 99.6^0T and 4.00 respectively, the acidity and pH value of goat yogurt at 35℃ for 4.5h were 64.4^0T and 4.81.

Table 1. Sensory evaluation of BB-goat yogurt under different incubation temperature

Temperature (℃)	Color	Smell	Taste	State	CE*
35	0.98	2.15	1.53	2.11	6.77
37	0.98	1.92	2.02	2.20	7.12
39	0.99	2.19	1.98	2.20	7.36
41	0.99	2.22	2.00	2.14	7.35
43	0.99	2.28	2.13	2.23	7.63

*: Comprehensive evaluation

From table 1, when the incubation temperature was 35℃-37℃ ,the temperature was too low and the coagulation time was long, the sour of goat yogurt was slight and can not cover the goaty flavor, while the sour and sweet of goat yogurt at 39℃-41℃ were moderate and the state was well.

Effect of bacterial proportion on the fermentation of BB-goat yogurt

The inoculum size of starter culture was 5%, the ratio of *B. bifidum* to common starter cultures were 1:2, 1:1, 2:1, 3:1 and 4:1, then gave a constant temperature fermentation at 39℃, the results were shown in figure 2 and table 2.

Based on the figure 2 (a), the viable counts of *B. bifidum* at the ratio of 1:2 increased slowly at the whole fermentation process, maybe because much acid and H_2O_2 produced by *L. bulgaricus* at the common starter culture were virulent to *B. bifidum*. At the ratio of 1:1, 2:1 and 3:1,the viable counts of *B. bifidum* increased slowly at the first 3h, and came to accelerate from 3h to 4.5h,then began to decreased. At the ratio of 4:1, the viable counts of *B. bifidum* increased fast at the initial stage of fermentation, reached the peak at 4.5h, then tended to be stable. Among then, the viable counts of *B. bifidum* at the ratio of 4:1 was the highest, 7.4×10^7cfu/ml, while at the ratio of 1:2,the viable counts of *B. bifidum* was the lowest, 4×10^7cfu/ml.

Figure 2(b) presented the variation tendency of the total viable counts at the different bacterial proportion were almost same, they were all increased rapidly at the first 4.5h,then started to decreased, among then, the total viable counts at the ratio of 4:1 reached the highest, 2.30×10^9cfu/ml, followed by the ratio of 1:2, 1:1, they were 1.99×10^9cfu/ml and 1.92×10^9cfu/ml, respectively, the relatively low total viable counts were presented at the ratio of 2:1 and 3:1, which were 1.74×10^9cfu/ml and 1.52×10^9cfu/ml respectively.

From figure2(c)and 2(d), the variation tendency of acidity and the total viable counts seemed to be same, among then, the acidity and pH value at the ratio of 4:1 for 4.5h were 95^0T and 4.3. when the ratio was 1:2 and 1:1,the acidity and pH for 4.5h were 94.6^0T, 4.26, 94.4^0T, 4.28, respectively, while the acidity and pH value at the ratio of 2:1 and 3:1 for 4.5h were 103.3^0T, 4.3, 96^0T and 4.28 respectively.

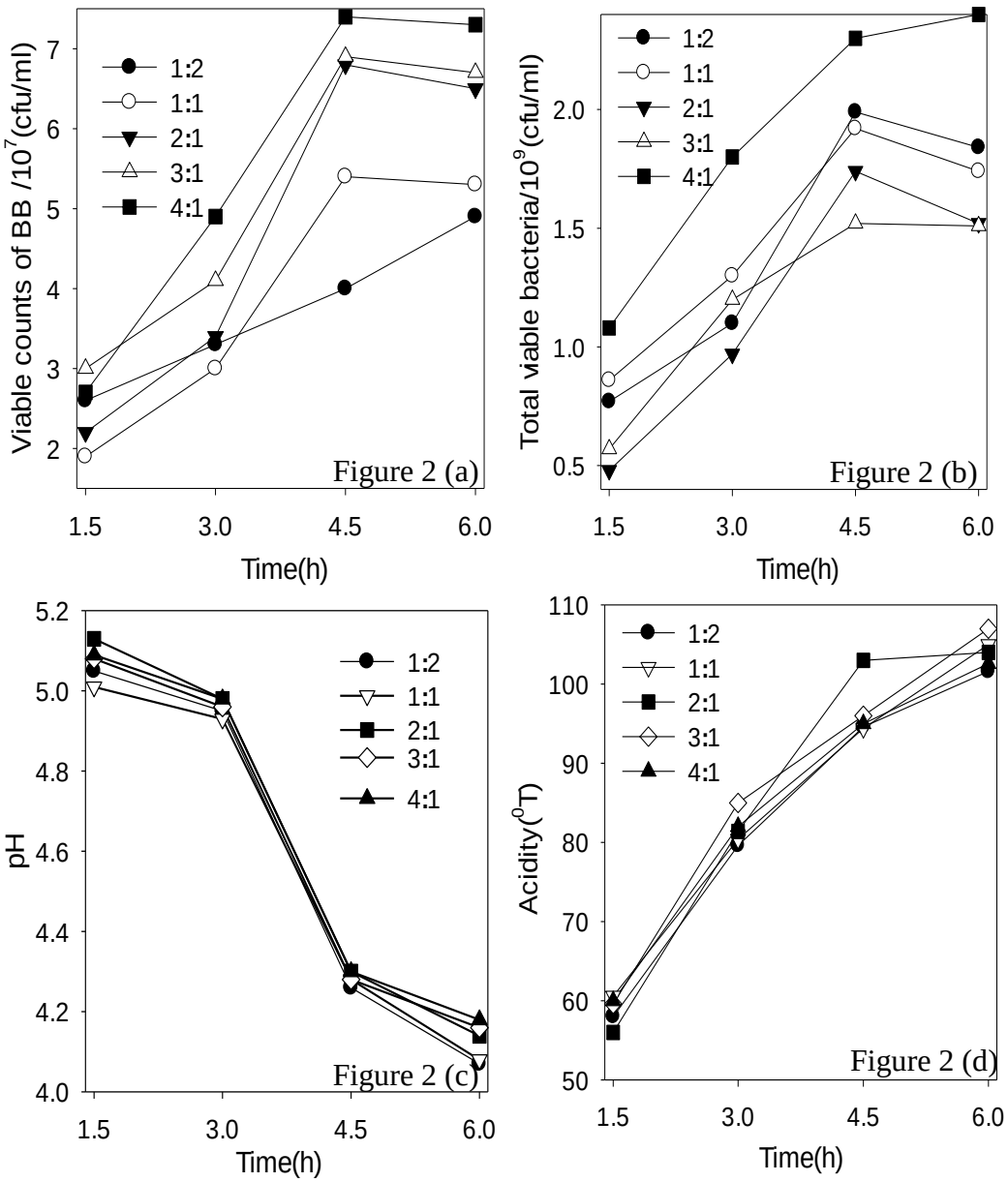

Figure 2(a,b,c,d). Effect of bacterial proportion on viable counts of *B. bifidum*, total viable bacteria, pH and acidity in BB-goat yogurt

Table 2. Sensory evaluation of BB-goat yogurt with different bacteria ratio

Inoculum size (%)	Color	Smell	Taste	State	CE*
1:2	0.98	2.28	1.80	2.33	7.38
1:1	0.98	2.28	1.98	2.35	7.59
2:1	0.98	2.30	2.17	2.29	7.73
3:1	0.98	2.23	2.16	2.31	7.67
4:1	0.98	2.32	2.16	2.32	7.75

*: Comprehensive evaluation

Table 2 presented the sensory evaluation of BB-goat yogurt, it can be suggested that each ratio had no obvious influence on the color, smell and the state of goat yogurt, but had a significant influence on the taste of goat yogurt, among then, the goat yogurt tasted sour at the ratio of 1:2, when the ratio was 1:1, 2:1, 3:1 and 4:1, the sour and sweet of goat yogurt were moderate and there was no goaty flavor.

Effect of inoculum size on the fermentation of BB-goat yogurt

The mixed liquid starter cultures of different inoculum size (1%, 3%, 5%, 7% and

9%) were inoculated in the fresh goat milk, the ratio of *B. bifidum* and common starter cultures was 4:1, and then gave constant temperature fermentation at 39℃, The results were shown in figure 3 and table 3.

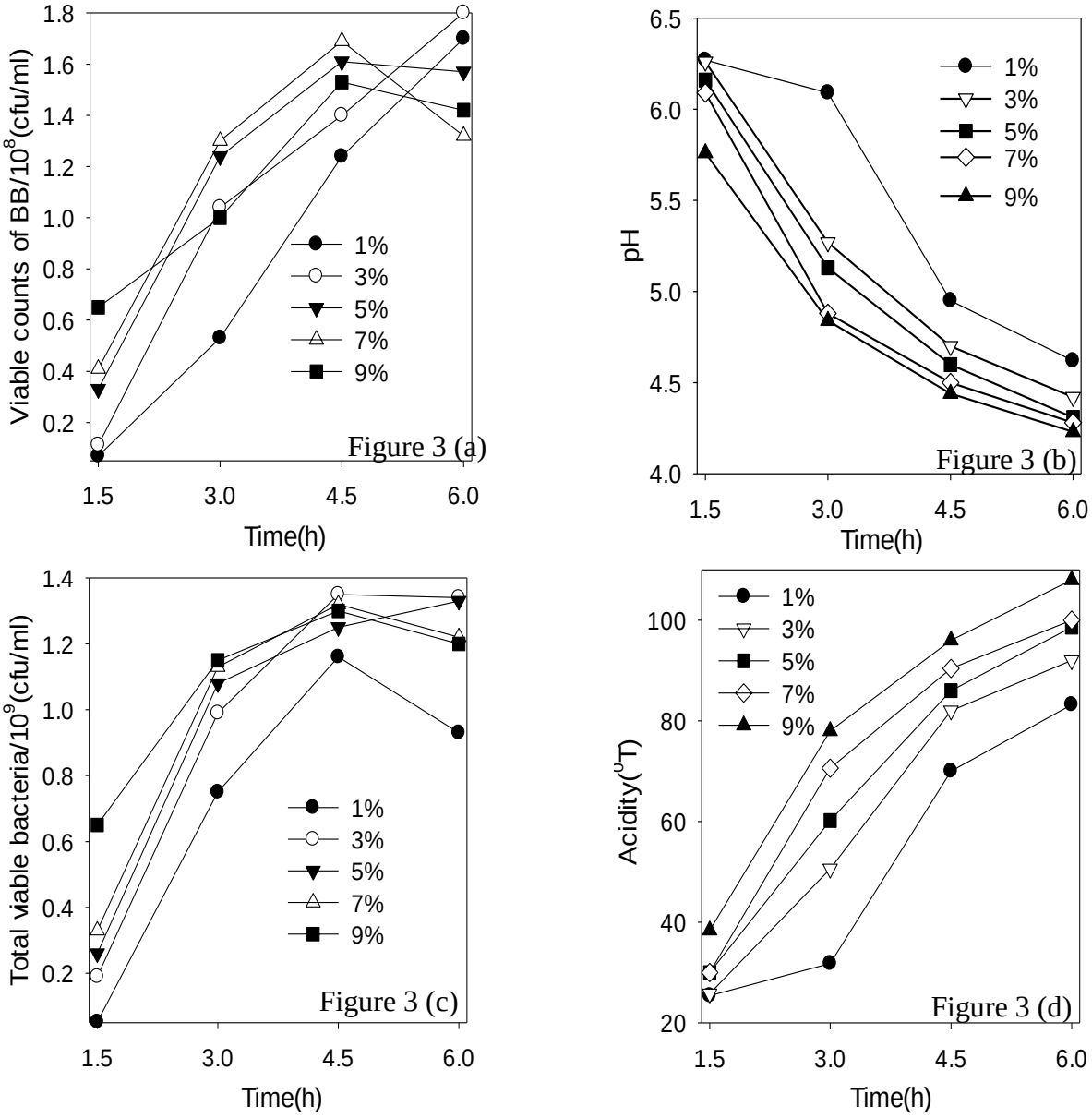

Figure 3 (a,b,c,d). Effect of inoculum size on viable counts of *B. bifidum*, total viable bacteria, pH and acidity in BB-goat yogurt

Figure 3(a) showed the viable counts of *B. bifidum* had an upward trend at the ratio of 1% and 3% at the whole fermentation progress, reached the maximum at 6h,when the inoculum size was 5%, 7% and 9%,the viable counts of *B. bifidum* increased fast at the initial stage of the fermentation progress, reached the peak at 4.5h,among then, the viable counts of *B. bifidum* at the ratio of 7% reached the highest, 1.69×10^8cfu/ml, followed by 5%, 1.61×10^8 cfu/ml, when the ratio was 1% and 9%, the viable counts of *B. bifidum* were low, 1.55×10^8cfu/ml, 1.53×10^8cfu/ml.

From figure 3(b), each inoculum size of the total viable counts increased fast within 3h, then began to slowed down from 3h to 4.5h, reached the peak at 4.5h, then tended to be stable. Among then, the total viable counts of goat yogurt at 7%

inoculum size was the highest, 1.35×10^9cfu/ml, followed by 5%, 1.32×10^9cfu/ml, while the total viable counts of goat yogurt at 1% was the lowest, 9.30×10^8cfu/ml.

From figure 3(c) and 3(d), the acidity of goat yogurt at 1% inoculum size had no obvious change within 3h, increased slowly during 3h-4.5h,then tended to be stable, the acidity at 3%, 5%, 7% and 9% had the same variation trend as the total viable counts at the same inoculum size. Among then, the acidity and pH value at 5% and 7% inoculum size for 4.5h were 86^0T, 4.6, 90.4^0T, and 4.5, respectively, while the acidity and pH value at 1% inoculum size for 6h were 70^0T and 4.62.

Table 3. Sensory evaluation of BB-goat yogurt with different inoculum size

Inoculum size (%)	Color	Smell	Taste	State	CE*
1:2	0.98	2.28	1.80	2.33	7.38
1:1	0.98	2.28	1.98	2.35	7.59
2:1	0.98	2.30	2.17	2.29	7.73
3:1	0.98	2.23	2.16	2.31	7.67
4:1	0.98	2.32	2.16	2.32	7.75

*: Comprehensive evaluation

From table 3, the inoculum size had no obvious influence on the color and state of goat yogurt, but had significant influence on the taste and smell of goat yogurt, among then, the goat yogurt at 1% and 3% inoculum size tasted not sour, and had slight goaty flavor and a little bit astringent and the coagulation was soft, the goat yogurt at 7% and 9% inoculum size was sour and had slight goaty flavor, when the inoculum size was 5%, the sour and sweet of goat yogurt was moderate and had no goaty flavor.

4. Conclusions

Based on the experiments, the temperature, Ratio of *B. bifidum* to common starter cultures, inoculum size had significant influence on the goat yogurt fermented by *B. bifidum*. The optimum temperature for BB-goat yogurt was 39℃, the acidity, pH value, the viable counts of *B. bifidum* and the total viable counts were 98.4^0T, 4.03, 7.60×10^7cfu/ml and 1.40×10^9 cfu/ml respectively and the score of sensory evaluation was 7.36.The optimum ratio of *B. bifidum* to common starter cultures was 4:1, the acidity, pH, viable count of *B. bifidum* and the total viable counts were 95^0T, 4.3, 7.40×10^7 cfu/ml and 2.30×10^9cfu/ml respectively and the score of sensory evaluation was 7.36.The optimum inoculum size was 5%, the acidity, pH value, viable counts of *B. bifidum* and the total viable counts were 86^0T,4.6,1.61×10^8cfu/ml, 1.32×10^9cfu/ml respectively, and the score of the sensory evaluation was 7.91.

5. References

Albenzio, M., & Santillo, A. (2011). Biochemical characteristics of ewe and goat milk: effect on the quality of dairy products. *Small Ruminant Research*, 101, 33-40.

Alloggio, V., Caponio, F, Pasqualone, A, and Gomes, T. (2000).Effect of heat treatment on the rennet clotting time of goat and cow milk. *Food Chemistry*, 70(1), 51-55.

Baroutkoub, A., Mehdi, R.Z., Beglarian, R., Hassan, J., Zahra, S., Mohammad, M.S. (2010). Effects of probiotic yoghurt consumption on the serum cholesterol levels in hypercholestromic cases in Shiraz, *Southern Iran. Science Resources*, Essays 5 (16), 2206–2209.

Chen He, Ji Liyuan, Shu Guowei, Wang Zhaowei, (2011). Effect of Lithium Chloride and Sodium Propionate on Growth of Selected Probiotics. *Key Engineering Materials*, 480-481,66-69.

Chen He, Wang Changfeng, Shu Guowei, (2010). Technological optimization of set-style goat yogurt fermentation. *Food science and technology*, 35(12), 71-74.

Chen He, Zhang Qian, Wan Hongchang, Shu Guowei and Li Hong, 2015. Effect of total inoculum size containing lactobacillus acidophilus or lactobacillus casei on fermentation of goat milk. *Advance Journal of Food Science and Technology*, 7(3),183-186.

Cruz, A. G., Walter, E. H. M., Cadena, R. S., Faria, J. A. F., Bolini, H. M. A., Pinheiro, H. P.,et al. (2010). Survival analysis methodology to predict the shelf-life of probioticflavored yogurt. *Food Research International*, 43, 1444–1448.

De Vrese, M., Steglman, A., Richter, B., Fenselau, S., Laue, C.,Scherezenmeir, J., (2001). Probiotics-compensation for lactase insufficiency. *The American Journal of Clinical Nutrition*. 73, 421–429.

De Oliveira, L. B., & Jurkiewicz, C. H. (2009). Influence of inulin and acacia gum on the viability of probiotic bacteria in symbiotic fermented milk. *Brazilian Journal of Food Technology*, 12, 138–144.

FAO/WHO (2002). Guidelines for the evaluation of probiotics in food, Joint FAO/WHO working group report on drafting guidelines for the evaluation of probiotics in food. London, ON, Canada, April 30th May 1st. ftp://ftp.fao.org/es/esn/food/ wgreport2.pdf Accessed August 4th, 2011.

Granato, D., Branco, G. F., Cruz, A. G., Faria, J. A. F., & Shah, N. P. (2010). Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety*, 9,455–470.

Haenlein, G. F. W. (2004). Goat milk in human nutrition. *Small Ruminant Research*,51, 155-163.

Julio, A., Patricia, A., Fabienne,B., Monique, Z., Marie-Christine,C.V., Marie-Jose, B. (2010). Proteomic comparison of the cytosolic proteins of three Bifidobacterium longum human isolates and B.longum NCC2705. *BMC Microbiology*, 10, 29.

Mainville, I., Arcand, Y., Farnworth, E.R., (2005). A dynamic model that simulates the human upper gastrointestinal tract for the study of probiotics. *International Journal of Food Microbiology*, 99 (3), 287–296.

Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R., & Saarela, M. (2002). Technological challenges for future probiotic foods. *International Dairy Journal*, 12, 173–182.

Rafter, J., (2003). Probiotics and colon cancer. *Best Practice & Research Clinical Gastroenterology*. 17, 849–859.

Raynal-Ljutovac, K., Gaborit, P., & Lauret, A. (2005). The relationship between quality criteria of goat milk, its technological properties and the quality of the final products. *Small Ruminant Research*, 60, 167e177.

Sanchez,B.,de los, Reyes-Gavilan, C.G., Margolles,A.,Gueimonde,M. (2009).Probiotic fermented milks:Present and future. *International journal of Dairy technology*, 62,472-483.

Sanz, Ceballos, L, Sanz, Sampelayo, M., Gil, Extremera, F., Rodriguez, Osorio, M. (2009) Evaluation of the allergenicity of goat milk, cow milk, and their lactosera in a guinea pig model. *Journal of Dairy Science*, 92(3), 837-846.

Sheehan, J. J., Drake, M. A., & Mcsweenwy, P. L. H. (2009). Effect of partial or total substitution of bovine for caprine milk on the compositional, volatile, nonvolatile and sensory characteristics of semi-hard cheeses. *International Dairy Journal*, 19, 498-509.

Shu, G., Li, C., Chen, H. and Wang C.(2014). Effect of inoculum and temperature on the fermentation of goat yogurt. *Advance Journal of Food Science and Technology*, 6, 1, 68-71

Wang, S., Zhu, H., Lu, C., Kang, Z., Luo, Y., Feng, L. (2012). Fermented milk supplemented with probiotics and prebiotics can effectively alter the intestinal microbiota and immunity of host animals. *Journal of Dairy Science*, 95, 4813–4822.

Acknowledgments

The project was partly supported by the Science and Technology Overall Planning for innovation Engineering project of Shaanxi Province (2016KTCL02-30), Shaanxi Innovation and Transformation Project of Agricultural Science and Technology (No.2016KTZDNY02-03) and Collaborative innovation project of Shaanxi Province (No. 2016XT-17).

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

A BOX-BEHNKEN EXPERIMENTAL DESIGN IN THE DEVELOPMENT OF OPTIMIZED MEDIUM FOR *STREPTOCOCCUS THERMOPHILUS*

He Chen[1]*, Chunju Bao[1], Chuanna Li[1], Hongchang Wan[2], Guowei Shu[1]

[1]School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an, 710021, China
[2]Shaanxi Yatai Dairy Co., Ltd., Xianyang, 713701, China
*Corresponding author: *chenhe419@gmail.com*

Article history:
Received:
28 December 2015
Accepted in revised form:
20 May 2016

Keywords:
Streptococcus thermophilus; medium optimization; Box-Behnken design; probiotics

ABSTRACT

Streptococcus thermophilus had been wildly used in food industries especially in milk productions. The objective of present work was to study the combine effects of soybean peptone, casein hydrolysate and glutamate on the viable counts of *Streptococcus thermophilus* in the medium and realize medium was cultured with high activity and density for bacteria. A Box-Behnken design was applied to perform the experiments and regression analysis. The viable counts of *Streptococcus thermophilus* can reach high at $(1.91\pm0.07)\times10^9$cfu/mL at the the optimum medium with soybean peptone 3%, casein hydrolysate 1%, glutamate 0.0015%, glucose 1%, K_2HPO_4 0.2%, Tomato juice 10%, Tween-80 0.05% and pH 6.8, respectively, which is consistent with the predicted values of the regression model. The results showed that the model used is feasible and adequate and it could provide theoretical basis for milk productions.

1. Introduction

Probiotics are a type of beneficial microorganisms and they had multiple functions like treat atopic dermatitis, balance intestinal environment, food allergy, cancer preventive, lactose intolerance, acute gastroenteritis, crohn's disease, change microbial community structure, et al. (Marco, et al., 2006; Million and Raoult, 2012; Tatsuya, et al., 2015). Probiotic bacteria had been widely applied in various industries like food, shrimp farms, and health production especially for dairy productions (Paulraj, et al., 2013). *Streptococcus thermophilus* (S. *thermophilus*) and *Lactobacillus bulgaricus* are the most common probiotics that used as yogurt starter in the dairy productions. They have a proto-cooperation interaction in yogurt and which combine metabolism with positive effects on the dairy product and other fermented products. (Pette and Lolkema, 1950; Angelov, et al., 2009). Furthermore,

Streptococcus thermophilus can significantly reduce the amount of serum total cholesterol and low density lipoprotein cholesterol (Akalin, et al., 1997). In addition, *Streptococcus thermophilus* can relieve the lactose intolerance and has the ability to inhibit tumor. In general, yogurt consumption was mainly lied on probiotic effects especially for these effects of number of live bacteria (Shihata and Shah, 2000). So the aim of the present study was to obtain the medium with the cultivation of high activity and high density for bacteria.

In our previous work, *Streptococcus thermophilus* which was suitable for the fermentation of goat milk have been screened out from commercial milk (Chen, et al. 2010). The effects of inoculum and temperature on the

fermentation of goat yogurt by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have been studied (Shu, et al., 2014). And optimum nitrogen sources, carbon sources/prebiotics and amino acids in the medium for *Streptococcus thermophilus* had been screened using Plackett-Burman design (Chen, et al., 2012; Chen, et al 2013). All of the research above provided a good theoretical and practical basis for the study of the present work. The objective of the present work was to apply a Box-Behnken design to study the effects of soybean peptone, casein hydrolysate and glutamate on the viable counts of *Streptococcus thermophilus* and determine the best composition of the medium.

2. Materials and methods

2.1. Materials

The strain used in this work, *Streptococcus thermophilus* was kindly provided by School of Food and Biological Engineering, Shaanxi University of Science and Technology. Soybean peptone, casein hydrolysate and glutamate were purchased from Sigma Chemical Co., USA. The chemicals used in the experiments were of analytical grade. The medium used for the cultivation for s*treptococcus thermophilus* were: glutamate 1g, peptone 0.75g, yeast 0.75g, potassium dihydrogen phosphate 0.2g, Tomato Juice 10mL, Twain-80 0.05mL and distilled water 90mL, and cultured at 118℃ for 15 min.

2.2. Preparation of starter

3-5% activated *Streptococcus thermophilus* power was inoculated into M17 medium and cultured at 42℃for 24 h. Microscopic judgment was conducted to make sure the viability of bacteria is stable until repeating experiments several times. It should pay attention that the bacteria be reactivated to maintain a high activity if it was not used after 7 days.

2.3. Analysis method

The pH of medium was directly measured through a pH-meter (pHS-3C Shanghai Precision Scientific Instrument Co., Ltd, Shanghai) at the room temperature and OD value was evaluated by ultraviolet spectrophotometer with version SP-756PC at the condition of 600nm. The viable counts of *Streptococcus thermophilus* were determined by plate coating method. Bacteria growth was determined by inoculating 2% bacteria into the optimum medium and cultured for 24h under anaerobic conditions. The pH and viable counts of samples were determined each 2h and each point were conducted in triplicate and average values were used.

2.4. Optimization of process parameters using response surface method

Three operational parameters (soybean peptone, casein hydrolysate and glutamate) important in the medium were researched using Box-Behnken design (Box and Behnken, 1960) with three levels as shown in Table 1, coded -1, 0 and 0 for low, middle and high concentrations (or values), respectively.

Table 1. The factors levels of Box-Behnken experimental design

Factors	Coded variable levels		
	-1	0	1
X_1 Soybean peptone (%)	2.4	3.0	3.4
X_2 Casein hydrolysate (%)	0.8	1.0	1.2
X_3 Glutamate (mg/L)	12	15	18

In order to obtain the optimal point, the relationship between independent variables and response value was fitted the second-order polynomial model and the polynomial equation represents in the following form:

$$Y=\beta_0+ \beta_1X_1+ \beta_2X_2+ \beta_3X_3+ \beta_{11}X_1^2+ \beta_{22}X_2^2+ \beta_{33}X_3^2 + \beta_{12}X_1X_2+ \beta_{13}X_1X_3+ \beta_{23}X_2X_3 \quad (1)$$

Where Y is the response value (viable counts of Streptococcus *thermophilus*), X_1, X_2 and X_3 are the independent variables corresponding to the concentration of soybean peptone, casein hydrolysate and glutamate, respectively. β_0 is the constant coefficient. β_1, β_2 and β_3 are liner coefficients. β_{11}, β_{22} and β_{33} are the quadratic coefficients. β_{12}, β_{13} and β_{23} are the interaction terms coefficients.

Statistical software SAS was used for the regression analysis of the experimental data

obtained. Analysis of variance (ANOVA) was employed to test the statistical significance of the regression equation coefficients by Box-Behnken design. The fitting of the second-order model equations was determined by the coefficient of determination (R^2) and a $p<0.05$ was considered statistically significant for all analysis.

3. Results and discussion

3.1. The results and analysis of the Box-Behnken design

The fifteen experimental results of the Box-Behnken design consisting of 12 trials plus 3-center points is summarized and listed in Table 2. X_1, X_2 and X_3 represent soybean peptone, casein hydrolysate and glutamate, respectively. The number of viable counts of *Streptococcus thermophilus* was represented by Y ($\times 10^9$ cfu/mL). The response values are OD, pH and Y, respectively. There is not a significant difference between OD and pH value as shown in Table 2. Hence, the major influence of medium was measured by Y.

The regression equation obtained according to the results of Box-Behnken experimental design and applying multiple regression analysis could be gotten as follows: $Y_1=1.953-0.031*X_1+0.016*X_2+0.072*X_3-0.230*(X_1)^2+0.042*X_1*X_2+0.295*X_1*X_3-0.115*(X_2)^2-0.125*X_2*X_3-0.178*(X_3)^2$ (2)

Table 2. The experimental design and results of Box-Behnken

Number	X_1	X_2	X_3	OD	pH	$Y/10^9$cfu/mL
1	-1	-1	0	0.920	4.09	1.61
2	-1	1	0	0.946	4.13	1.63
3	1	-1	0	0.946	4.17	1.50
4	1	1	0	0.943	4.17	1.69
5	0	-1	-1	0.929	4.15	1.50
6	0	-1	1	0.938	4.17	1.86
7	0	1	-1	0.943	4.16	1.71
8	0	1	1	0.947	4.17	1.57
9	-1	0	-1	0.889	4.13	1.80
10	1	0	-1	0.927	4.22	1.11
11	-1	0	1	0.021	4.16	1.39
12	1	0	1	0.918	4.22	1.88
13	0	0	0	0.894	4.25	2.04
14	0	0	0	0.913	4.27	1.93
15	0	0	0	0.899	4.25	1.89

ANOVA of the regression model was performed to see significance of the main effects and interaction effects of independent variables on viable counts of *Streptococcus thermophilus*. The liner coefficients of the regression equation for parameters X_1, X_3 are larger, which indicates that there is not a simple linear relationship between soybean peptone, glutamate on the viable counts of *Streptococcus thermophilus.* The negative effects of liner and quadratic coefficients of X_1 in the regression equation indicate that a decrease of viable counts occurs at a low level of parameter X_1. The interaction coefficient of X_1X_3 is bigger than others' showing that the interaction of soybean peptone and glutamate had a great influence on the growth of bacteria. It is also evident that the interaction effects of X_1X_3 is extremely significant with probability value (p=0.001) as shown in Table 3.

The influence of various factors on the response of the *Streptococcus thermophilus* was determined by F-test. The regression model was found to be very significant, as is

evident for F-test with a very low probability

The probability value for the lack-of-fit was found to be non-significant with high failure of a model to obtained data in the experimental value (p=0.004<0.01), shown in Table 3. The range at points which not containing in the regression was evaluated by Lack-of-fit test.

Table 3. The ANOVA of regression equation of Streptococcus *thermophilus*

Source	DF	SS	MS	F	Pr > F	Sig
X1	1	0.009	0.009	1.403	0.289	
X2	1	0.002	0.002	0.379	0.565	
X3	1	0.042	0.042	7.552	0.040	*
X1*X1	1	0.196	0.196	35.205	0.002	**
X1*X2	1	0.007	0.007	1.298	0.306	
X1*X3	1	0.348	0.348	62.514	0.001	***
X2*X2	1	0.049	0.049	8.833	0.031	*
X2*X3	1	0.063	0.063	11.224	0.020	*
X3*X3	1	0.117	0.117	20.990	0.006	**
model	9	0.789	0.088	15.742	0.004	**
Liner	3	0.052	0.017	3.111	0.127	
Quadratic	3	0.319	0.106	19.103	0.004	**
Cross	3	0.418	0.139	25.012	0.002	**
Error	5	0.028	0.006			
Lack of fit	3	0.016	0.005	0.872	0.574	
Pure error	2	0.012	0.006			
Total	14	0.817				

Note: *** P<0.001, extremely significant, ** P<0.01, very significant; * P<0.05, significant.
DF degree of freedom, SS sum of squares, MS mean square, F and Pr means F and P values, respectively.

Probability value was 0.574, which further tested that the quadratic model was statistical effective and feasible. Therefore, the optimum ratio of three factors can be obtained by using the regression equation. The determination coefficient R^2 and multiple correlation coefficients R^2 could measure the goodness of the regression model. The value of R^2 (0.9659) obtained from the present model indicating that the experimental and predicted values of the response had a good correlation. The adjusted R-squared can evaluate the amount of variation around the mean explained by the model adjusted for the number of terms. The value for adjusted R-squared (R^2_{adj}=90.46%) suggested the fitting degree of the regression equation and the experimental data is 90.46%, and the reliability is high. Furthermore, the F-value for first-order X_3 and quadratic X_1^2, X_3^2 are all relatively big which suggesting the there is not a simple liner relationship between the parameters X_1, X_3 and the response value. The effects of different independent variables on the viable counts of *Streptococcus thermophilus* at different levels are described in figure 1. The quadratic items of X_1^2 (P=0.002), X_2^2 (P=0.031) and X_3^2 (P=0.006) all are very significant. The viable counts of *Streptococcus thermophilus* increased first and then decreased sharply with the increase of soybean peptone

concentration, while the effects of casein hydrolysate on the viable counts of bacteria are relatively mild. When the concentration of casein hydrolysate was at a low level, the dependent variable increased slowly. The response value of Y decreased gradually when it reached its maximum value. Similarly, the response value of Y increased greatly and then decreased with the increase of parameter of glutamate. The rule was found out that the maximum value of Y occurs at inflection point of the three factors.

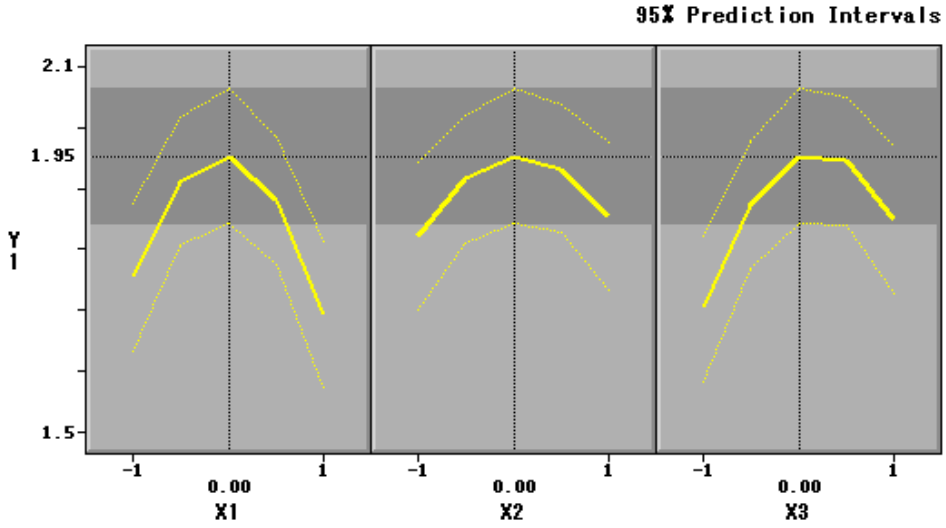

Figure 1. The trends of response value Y with factors

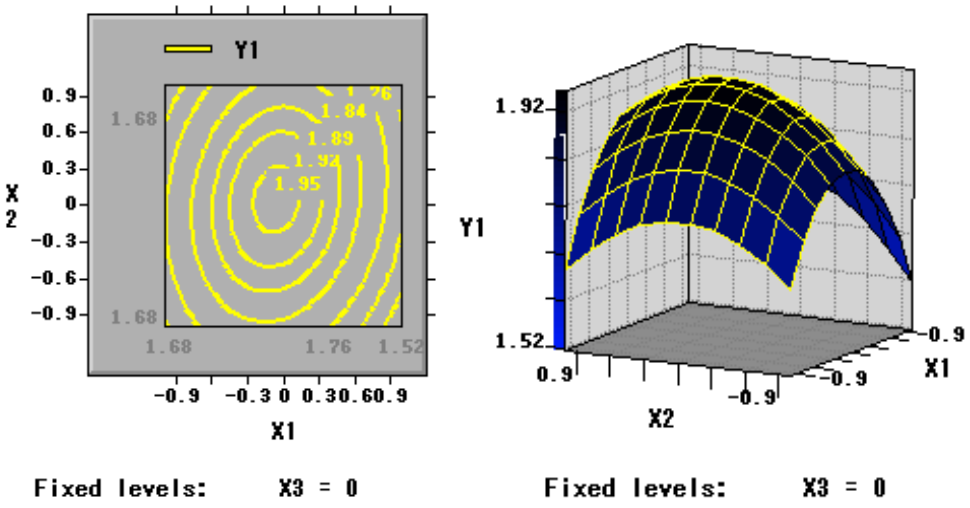

Figure 2. Response surface and contour plots of *S. thermophilus* as a function of X_1 and X_2

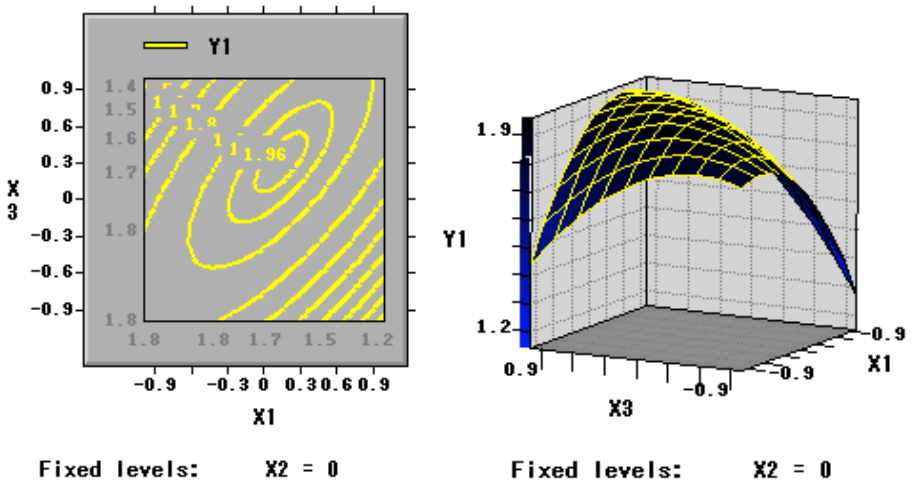

Figure 3. Response surface and contour plots of *S. thermophilus* as a function of X_1 and X_3

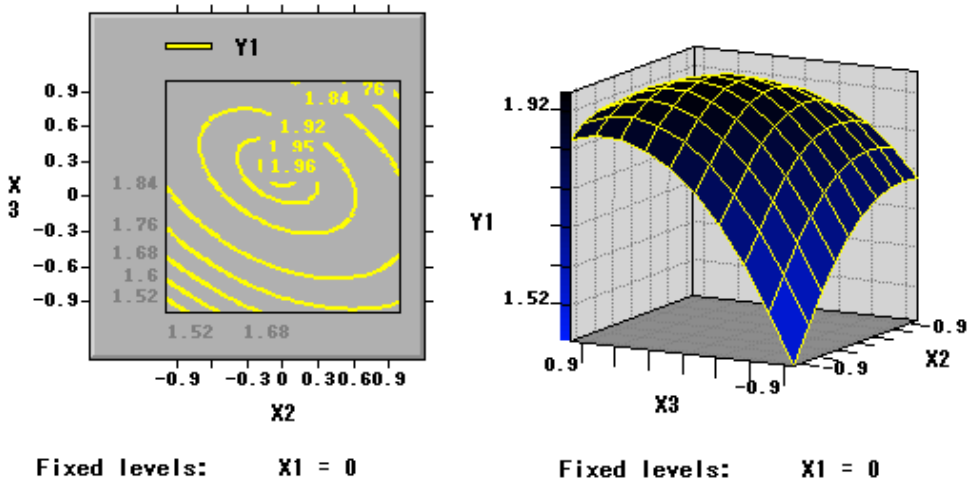

Figure 4. Response surface and contour plots of *S. thermophilus* as a function of X_2 and X_3

The aim of the present study was to track efficiently for the optimum medium for the growth of *Streptococcus thermophilus* which is the best and maximal for the bacteria. The best response range can be calculated by analyzing the plots according to the Box-Behnken design results. Response surface and contour plots was used to measure viable counts of bacteria for a pair of interactive variables including soybean peptone, casein hydrolysate and glutamate (Figures 2-4). The interaction effects of the factors on the response value are larger when the shape of the plots is oval, while the circular indicates that the interaction effects are light. One parameter is fixed to zero level, and other two parameters are investigated to study their interaction effects on the growth of *Streptococcus thermophilus.* As is shown in Fig.2, when the amount of glutamate was constant, the viable counts of *Streptococcus thermophilus* increased consistantly until reached it's maximal value and then decreased with the increase of the concentration of soybean peptone and casein hydrolysate. This is due to the high concentration of nitrogen source will inhibit the bacteria growth. The contour plots is not like oval indicating the effects of factors X_1 and X_2 is not significant, which further verified the analysis of the regression model.

Figures 3 showed that when the casein hydrolysate is constant, the viable counts of *Streptococcus thermophilus* increased first and then showed a downward trend with the

increase of soybean peptone and glutamate. This is because excess soybean peptone and glutamate are not conducive to the growth of bacteria. At the same time, the contour plots of *Streptococcus thermophilus* as a function of X_1 and X_3 have oval-shaped meaning that the two factors had a strong interaction effects on the viable counts of bacteria. This is consistent with the results of analysis of variance of the regression equation. Figures 4 described when the soybean peptone was at 3 g/100mL, the viable counts of bacteria was the same trend with the figure 3 described. Similarly, the interaction effects of casein hydrolysate and glutamate on the response value was relatively strong as it is shown in the contour plots of *Streptococcus thermophilus* as a function of X_2 and X_3, and the contour plots for it is oval.

Statistical software SAS was used to analysis the regression model and take the derivative for independent variables of X_1, X_2 and X_3 to obtain the maximal value of viable counts. The corresponding point of the code (0, 0, 0) for maximal value represent the level of the actual value, soybean peptone 3g/100mL, casein hydrolysis 1g/100mL and glutamate 1.5mg/100mL, respectively. The model predicts the viable counts of *Streptococcus thermophilus* was 1.95×10^9 cfu/mL under these optimum conditions. Three repeating experiments were conducted to test the predicted values in the optimum medium. The mean value of the three groups was $(1.91\pm0.07)\times10^9$ cfu/mL, which was very close to the predicted values. The results show that the mathematical model obtained by the response surface method can fit well with the experimental data.

3.2. Analysis of growth curve for Streptococcus thermophilus

The growth curve for *Streptococcus thermophilus* was determined to study the ability of growth, reproduction and acid producing for bacteria in the medium. The growth curve of *Streptococcus thermophilus* could evaluate if there is a difference in the medium optimized or not. Taking time as a horizontal coordinate, and pH, viable counts of bacteria as longitudinal coordinate, the growth curve plot of *Streptococcus thermophilus* was drawn as shown in Figures 5.

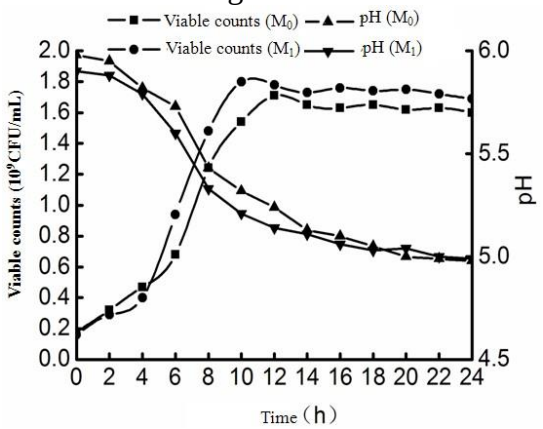

Figure 5. The growth curve and pH of Streptococcus thermophilus in the medium

As it shown in Figures 5, *Streptococcus thermophilus* began to enter the exponential growth period after the adjustment of beginning 4h. The bacteria started to grow, propagate and divide; the culture fluid became turbid; the number of viable bacteria increased and the pH value decreased significantly in this period. The bacteria entered a period of stability at 14-20h where the newly bred cells were roughly equal to the dying cells. While after 20 h, the cell began to enter a period of decline and the cell death rate is greater than the propagation rate which suggesting that environment is not conducive to bacterial growth. Besides, the whole cell populations showed a negative growth and viable counts of bacteria decreased significantly. At the same time, it can be seen that the viable counts of bacteria may reach 1.61×10^9 cfu/mL, 1.95×10^9 cfu/mL before and after the medium was optimized at 12h. The optimum medium for the bacteria is about 1.2 times higher than that of not optimized. The response surface method using to optimize the medium for *Streptococcus thermophilus* proved to be feasible and reliable for improving the viable counts.

3.3. Discussions

The articles about the effects of the composition and conditions of medium on the survival rate of the bacteria had been reported a

lot. Such as, Carvalho (Carvalho, et al., 2002) reported that the sugar, amino acids and sugar alcohols and other substances could improve the survival rate of *Lactobacillus bulgaricus* and *Lactobacillus plantarum* for the reason that small molecules had advantages of containing more than one hydrogen bond and ionizable groups, which can stop bacterial exposure to the medium by linking to the surface of the bacteria. And at pressure conditions such as low temperature, low pH and low water activity, the bacteria will easily died. While Beney (Beney and Gervais, 2001) found a different conclusion, the bacteria could form tolerance and accumulate the permeability of protective substances under pressure conditions, which can give a help of bacteria to establish a new balance of osmotic equilibrium and improve the viable counts of the bacteria. The strains used in the above research were different may caused the difference of the two conclusions. Furthermore, Carvalho (Carvalho, et al., 2003) study found that sugar (such as glucose, fructose, lactose, mannose, sucrose, et al.), sugar alcohols (sorbitol, inositol, et al.), non-reducing sugars (trehalose) was added in the medium, and it can play a protective role for bacteria during the freeze-drying process. Gao's (Gao, et al., 2008) study of enrichment medium for *Lacabacillus casei* Zhang had reached a conclusion that the optimum composition of the medium were: glucose 20.9g/L, soybean peptone 10.45g/L, yeast extract 10.45g/L, K_2HPO_4 3.5g/L, sodium citrate 2.35g/L, sodium acetate 14.6g/L, $MnSO_4 \cdot 5H_2O$ 54mg/L, $MgSO_4 \cdot 7H_2O$ 1.0g/L, $CuSO_4 \cdot 5H_2O$ 10mg/L, tween 80 1.0g/L, and the viable counts of *Lacabacillus casei* Zhang reached 4.78×10^9 cfu/mL, which was 10 times higher than MRS. Similarly, Zhang (Zhang and Yu, 2007) had screened and studied the culture medium for *Lactobacillus acidophilus*, and the viable counts could reach high at 2.2×10^9 cfu/mL. In our present study, soybean peptone, casein hydrolysate and glutamate were chosen as the enrichment factors, and the viable counts of *Streptococcus thermophilus* can reach at 1.95×10^9cfu/mL, which was 1.2 times than that of not been optimized (1.61×10^9cfu/mL).

4. Conclusions

An optimization of medium for improving the viable counts of *Streptococcus thermophilus* was conducted using Box-Behnken design experiment. ANOVA was used to test the predicted model. The viable counts of *Streptococcus thermophilus* can reach high at $(1.91\pm0.07)\times10^9$ cfu/mL at the the optimum conditions with soybean peptone 3%, casein hydrolysate 1%, glutamate 0.0015%, glucose 1%, K_2HPO_4 0.2% Tomato juice 10%, Twain-80 0.05% and pH 6.8, respectively. The predicted value (1.95×10^9 cfu/mL) is very close to the verification value confirming that regression model used for the experiments is useful. Moreover, the growth curve of the *Streptococcus thermophilus* had been analyzed in 24h before and after the medium optimized. And the viable counts of *Streptococcus thermophilus* can reach at 1.95×10^9 cfu/mL after it cultured for 12 h at the optimum medium which was 1.2 times than that values without optimization, besides, stable period of the bacteria take in advance. The optimum medium has obvious effects on the growth of bacteria, so the optimum medium for *Streptococcus thermophilus* using response surface methodology is proved to be feasible and effective.

5. References

Akalin, A.S., Gone, S. (1997). Influence of yegurt and acidephilus yegurt osrum cholesteml levels in mice. *Journal of Dairy Science*, 80, 2721-2725.

Angelov, M., Kostov, G., Simova, E., Beshkova, D., Koprinkova-Hristova, P. (2009). Proto-cooperation factors in yogurt starter cultures. *Revue de génie industriel*, 3, 4-12.

Box, G.E.P., Behnken, D.W. (1960). Some new three level design for study of quantitative variables. *Technometerics*, 2, 455-476.

Beney, L., Gervais, P. (2001). Influence of the fluidity of the membrane in response of

microorganisms to environmental stresses. *Applied Microbiology and Biotechnology*, 57(4), 34-42.

Carvalho, A.S., Silva, J., Teixeira, P. (2002). Effect of additives on survival of freeze-dried *Lactobacillus plantarum* and *Lactobacillus rhamnosus* during storage. *Biotechnology Letters*, 24(19), 1587-1591.

Carvalho, A.S., Silva, J., Teixeira, P.(2003). Protective effect of sorbitol and monosodium glutamate during storage of freeze-dried lactic acid bacteria. *LeLait*, 83(3), 203-210.

Chen, H., Wang, C.F., Shu, G.W., Peng, D., Zhang J.J. (2010). Technological optimization of set-style goat yogurt fermentation. *Food Science and Technology*, 35(12), 71-74.

Chen, H., Li, C.N., Shu, G.W., Wang, C.F. (2012). Screening of nitrogen sources in the medium for *Streptococcus thermophilus* using Plackett-Burman design. *Advanced Materials Research*, 531, 532-535.

Chen, H., Chen, S.W., Li C.N. (2013). Screening of carbon sources/prebiotics and amino acids in the medium for *Streptococcus thermophilus* using Plackett-Burman design. *Journal of Chemical and Pharmaceutical Research*, 5(12), 975-980.

Gao, P.F., Li, Y., Zhao, W.J., Chen, X., Cui, J.L. (2008). Study on the Optimization of Enrichment Medium of *Lactobacillus casei Zhang*. *Microbiology*,35(4), 623-628.

Marco, M.L., Pavan, S. and Kleerebezem, M. (2006). Towards understanding molecular modes of probiotic action. *Current opinion in biotechnology*, 17, 204-210.

Pette, M., Raoult, D. (2012). Publication biases in probiotics. *European Journal of Epidemiology*, (27), 885-886.

Pette, J.W., Lolkema, H., (1950). Yoghurt. I. Symbiosis and antibiosis in mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. *Netherlands Milk and Dairy Journey*, 4, 197-208.

Paulraj, Kanmani, R., Satish, Kumar, N., Atish, Kumar, N.(2013). Probiotics and Its Functionally Valuable Products—A Review. *Critical Reviews in Food Science and Nutrition*, 53, 641-658.

Shihata, A., Shah, N.P. (2000). Proteolytic profiles of yogurt and probiotic bacteria. *International Dairy Journal*, 10, 401-408.

Shu, G., Li, C., Chen, H., Wang, C. (2014). Effect of inoculum and temperature on the fermentation of goat yogurt. *Advance Journal of Food Science and Technology*, 6, 68-71.

Tatsuya, U., Jung-Hye, C., Hor-Gil, H. (2015). Changes in human gut microbiota influenced by probiotic fermented milk ingestion. *Journal Dairy Science*, 98(6), 3568-3576.

Zhang, Z., Yu P., (2007). Screen and study on culture medium of multiplying of *Lactobacillus acidophilus*. *Dairy Industry*, 35(6), 25-27.

Acknowledgements

The work was partly supported by the Scientific Research Program Funded by Shaanxi Provincial Education Department (No.15JF008), the Shaanxi Province overall innovative engineering project of science and technology (No.2013KTDZ03-01-03) and the Key Technology Project of Xianyang city (2014K01-15), China.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

STUDY OF DETERMINING THE TECHNOLOGICAL MODE IN THE FREEZE DRYING PROCESS OF ROYAL JELLY IN VIET NAM

Nguyen Tan Dzung[1]*

[1]Department of Food Technology, Faculty of Chemical and Food Technology, HCMC University of Technology and Education, No 01-Vo Van Ngan Street, Thu Duc District, Viet Nam
Corresponding author: tandzung072@yahoo.com.vn

Article history:
Received:
12 December 2015
Accepted in revised form:
15 April 2016

Keywords:
Royal jelly;
Royal jelly product;
Freeze dried royal jelly;
Freeze drying technology royal jelly;
Optimization freeze-drying of royal jelly.

ABSTRACT
The aim of this study was to find the optimal technological mode for the freeze drying of Royal jelly. By the experimental method, the multi-objective optimization problem to describe the freeze drying process of Royal jelly was built. Anh by the restricted area method, solving the multi-objective optimization problem were found out the technological mode of the freeze drying process of Royal jelly as follow: the optimal temperature of freeze drying chamber was Z_1^{opt} = 20.58^{0}C, the optimal pressure of the freeze drying chamber was Z_2^{opt} = 0.411mmHg and the time of the freeze drying of Royal jelly Z_3^{opt} = 18.283h. Corresponding to these optimal factors, the energy consumption for 1 kg final product reached the minimum value of y_{1P}^R = 6.32kWh/kg, the residual water content in Royal jelly reached the minimum value of y_{2P}^R = 4.19% under 4.5% (< 4.5%), the loss of protein, carbohydrate, lipid, mineral salts, 10-HDA, vitamin B_5, free fatty acids and viscosity reached the minimum value of y_{3P}^R = 1.77%, y_{4P}^R = 1.83%, y_{5P}^R = 2.07%, y_{6P}^R = 2.36%, y_{7P}^R = 0.78%, y_{8P}^R = 2.47%, y_{9P}^R = 2.67%, y_{10P}^R = 3.58%.

1. Introduction

It is common knowledge that Royal jelly is rare natural product, it contains a lot of important nutritional substances for human's health such as protein, lipid, carbohydrate, mineral salts, free fatty acids, free amino acids, vitamins and enzymes. In addition, Royal jelly contains bioactive compounds that have extremely good effect on human's health such as 10-HDA (Anna et al., 2009). Protein components inside Royal jelly contain hardly all essential amino acids for humans, including 29 amino acids and derivatives. Besides, protein inside of Royal jelly also contains some enzymes such as protease, glucose oxidase, phosphatase, cholinesterase and an insulin-like substance (Antinelli et al., 2003), these enzymes is essential for biochemical processes in human's body. Carbohydrate components inside Royal jelly are mainly fructose, glucose and sucrose. But ratio of fructose in carbohydrate components is higher than ratio of glucose and sucrose. In addition, other sugars present in much lower quantities are maltose, trehalose, melibiose, ribose and erlose (Lercker et al., 1992; Gaëlle and Hervé, 2012), they are types of bioactive carbohydrate that have very good effect on human. Lipid components inside Royal jelly has low rate, but it contains bioactive compounds rare in the nature, free fatty acids and fatty acids inside lipid components have unusual and uncommon structures, they are mostly short carbon chain (from 8 to 10 carbon atoms) of hydroxyl fatty acids or dicarboxylic acids. These free fatty acids are most of the recorded biological properties of Royal jelly (Schmidt et al., 1992)

such as 10-HDA (10-hydroxy-2-decenoic acid) and its isomer. Besides, the lipid inside Royal jelly contains some neutral lipids including: sterols (cholesterol, esgosterol) and an unsaponifiable fraction of hydrocarbons similar to beeswax extracts (Lercker et al., 1992; Hattori et al., 2007). In addition, Royal jelly contains a lot of mineral salts, the total ash in Royal jelly has low rate about 2 to 3 % of dry weight. The major mineral salts are, in descending order: K, Ca, Na, Zn, Fe, Cu and Mn, with a strong prevalence of potassium (Andreas Stocker et al., 2005). Besides, Royal jelly is extremely rich in water-soluble vitamins and fat-soluble vitamins (Schmidt and Buchmann, 1986). For the water-soluble vitamins include B_1, B_2, B_3 (PP), B_5, B_6, B_c and H. For the fat-soluble vitamins include A, D, E, K (Benfenati et al., 1992). Therefore, it can be seen that Royal jelly supply complete mineral salts and vitamins for humans (Lercker et al., 1992, Benfenati et al., 1992).

Figure 1. The Royal jelly product in Viet Nam

According to the results of Mohamed, F. R., et al. (2012), it shown that the most important nutritional compounds of Royal jelly including protein, carbohydrate and lipid have balanced rate between essential amino acids with different amino acids, between fructose, glucose with different sugars, between unsaturated fatty acids with saturated fatty acids that are very rare in nature. This nature product is very good and very perfect for growing up and development processing of human's health. According to Antinelli, J. F., et al. (2003); Anna G.S. (2009); Isidorov et al., (2011). Royal jelly was also called as a natural pharmaceutical product, it has the ability of anti-aging; prevent the formation of free radicals from biochemical reactions in human's body. It can help prevent the heart disease or heart disorder, psychophysiological disorder, nervous disorder, digestive disorder, cancer and many other diseases. It has the capable of restoring and protecting human's skin as well as increase energy and restores human's health. In addition, it can cure women of gynaecological disease. According to analytical data of Dzung (2013), the important chemical composition of Royal jelly in Viet Nam contains many different substances but their main constituents are water, protein, carbohydrate (sugars), lipids and mineral salts, impurity, 10-HDA, vitamin B_5 and free fatty acids. It can be seen in Table 1.

From Table 1, it is obvious that Royal jelly is rich in nutritional components. It is an advantageous environment in order that microorganism grows up and develops. If Royal jelly is not preserved, it will be easily decomposed or hydrolyzed and oxidized, it will be no longer value of use (Isidorov et al., 2011; Hiroyuki M. et al., 2012). Therefore, Royal jelly needs to be preserved in suitable environment in order to be prolonged use. Currently, there are two methods that often use to preserve Royal jelly; those are the freezing method and the freeze drying method. The final products have always a very good quality when they are made by these two technologies (Mohamed, F. R., et al., 2012). But the freezing method, Royal jelly after freezing process must be preserved in environment that has suitable temperature about -20^0C to -18^0C and the range of this temperature must be maintained during use time and export time. As a result, it makes to increase the expenditure of preservation process of Royal jelly. For the freeze drying method are used the most popular. The Royal jelly after the freeze drying placed in nylon bags, vacumn seaming is preserved in usual environment of 25^0C. Therefore, it will be not lost the expenditure for preservation process (Dzung et al., 2014; 2015).

Table 1. The chemical composition of Royal jelly in Viet Nam

No	Substance	The ratio of initial material weight	The ratio of dry weight
1	Water	59.2%	-
2	Proteins	14.26%	34.95%
3	Carbohydrate (sugars)	15.95%	39.09%
4	Lipids	4.00%	9.80%
5	Minerals	1.10%	2.70%
6	Impurity (wax, …)	2.39%	5.86%
7	10-HDA	3.1 %	7.60 %
8	Vitamin B_5	4.05 mg/100g	9.93 mg/100g
9	Free fatty acids	558.95 mg/100g	1370 mg/100g
Thermophysical parameter of Royal jelly in Viet Nam			
10	Viscosity at 25^0C	11817.9 cP	-

However, the problem posed here is how to determine the technological mode for the freeze drying process of Royal jelly in order that Royal jelly after freeze drying have the best quality, the residual water content of Royal jelly is under 4.5% (Dzung et al., 2015), the energy consumption of 1 kg final product reaches the minimum value. This is a question that had not any research to mention for a long time ago. To answer this problem, in this study the multi-objective optimization problem describing about the relationship between objective functions, including: y_1 (kWh/kg) – the energy consumption of 1 kg final product of Royal jelly after freeze drying; y_2 (%) – the residual water content of Royal jelly after freeze drying; y_3 (%) – the loss of total protein; y_4 (%) – the loss of carbohydrate; y_5 (%) – the loss of lipid; y_6 (%) – the loss of mineral salts; y_7 (%) – the loss of 10-HDA; y_8 (%) – the loss of vitamin B_5; y_9 (%) – the loss of free fatty acids and y_{10} (%) – the loss of viscosity of Royal jelly after freeze drying; with technological factors, including: Z_1 (^{0}C) – the temperature of freeze drying chamber, Z_2 (mmHg) – the pressure of freeze drying chamber; Z_3 (h) – the time of freeze drying process is built by experimental method. The multi-objective optimization problem is expressed as follow: Finding $Z^{opt} = \{Z_1^{opt}, Z_2^{opt}, Z_3^{opt}\} \in \Omega_Z$ in order that $y_{jmin} = f(Z_1^{opt}, Z_2^{opt}, Z_3^{opt}) = \min\{f_j(Z_1, Z_2, Z_3)\}$. After that by the restricted area method, solving this multi-objective optimization problem is determined the technological mode for the freezing process of Royal jelly, (Dzung, 2012).

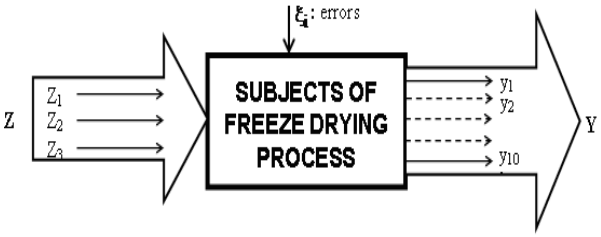

Figure 2. Diagram of subjects of freezing process

2. Materials and methods

2.1. Materials

The Royal jelly is harvested from bees's nest to grow up at Bao Loc area in Lam Dong province of Viet Nam. It is the pure natural product and does not mix any chemical composition. It is very thick solution, has pale yellow and sour. The basic composition of Royal jelly is presented in Table 1, (Dzung, 2013; 2014; 2015).

The Royal jelly is poured into trays, they are made by glass. According to results of Dzung (2015), it shown that Royal jelly layer in glass trays at optimal technological mode of the freezing process of Royal jelly have depth of 12.93mm.

Before carrying out the freeze drying process of Royal jelly, Royal jelly is frozen at the optimal technological mode: the temperature of freezing environment was -

40.46^0C; the time of freezing process was 1.63h; the thickness of Royal jelly in the tray was 12.93mm. Corresponding to these technological parameters, the freezing temperature of Royal jelly was -18.33^0C and water inside Royal jelly was completely crystallized $\omega = 1$ or $\omega = 100\%$, (Dzung, 2014; 2015).

2.2. Apparatus

Equipments used to research the technological mode for the freeze drying process of Royal jelly are listed (Dzung, 2012a & b, 2013; 2014; 2015):

- Equipments used to determine weigh of Royal jelly by Satoriusbasic Type BA310S: range scale (0 ÷ 350)g, error: ± 0.1g = ± 0.0001 kg.
- The Freeze Drying System DS-3 (Fig 3a) and The Refrigeration System DL-3 (Fig 3b) that were controlled automatically by computer. It could reduce the temperature of environment to $(-50 \div -45)^0$C. The temperature, pressure and time profile of freeze drying process are measured by computer.

Figure 3a. The freeze drying system DS-3 with the auto-freezing $(-50 \div -45)^0$C

- Kjeldahl, Soxhlet, GC-MS, HPLC, Viscometer and other equipment were used to analyse and determine protein, carbohydrate, lipid, ash, 10-HDA, free fatty acids, vitamins and viscosity of Royal jelly during freeze drying process.

Figure 3b. The Refrigeration system DL-3 with the auto freezing $(-45 \div -40)^0$C

2.3. Methods

Using in this study to include some method as follow (Dzung, 2012; 2013; 2014; 2015):

- Determining the temperature (Z_1, ^{0}C), pressure (Z_2, mmHg) of freeze drying chamber and the time of freezing process (Z_3, h) of Royal jelly by the automatic measure and control system on computer of the Freeze Drying System DS-3.

- Determining the energy consumption (y_1, kWh/kg final product) of 1 kg Royal jelly after freeze drying process by the equation (1), (Holman J. , 1992; Figura et al., 2007).

$$y_1 = \frac{P.\tau}{G} = \frac{U.I.\tau.\cos\varphi}{G}, \text{ kWh/kg} \qquad (1)$$

Where: G (kg) – weight of the final product; U (V) – number of Voltmeter; I (A) – number of Amperemeter; τ (s) – second; $\cos\varphi$ – power factor.

- Determining the residual water content of the final product of Royal jelly after freeze drying (y_2, %) by the mass sensor controlled by computer, (Dzung, 2012a & b).

$$y_2 = 100 - \frac{G_0}{G_e}(100 - W_0) \qquad (2)$$

Where: G_0 (kg) – weight of the initial material of Royal jelly used for freeze drying; G_e (kg) – weight of the final product of Royal jelly after freeze drying; W_0 (%) – the residual water content of the initial material of Royal jelly.

- Determining the total protein inside Royal jelly before and after freeze drying by the Kjeldahl method (FAO, 14/7, 1986).
- Determining the total carbohydrate inside Royal jelly before and after freeze drying by the hight - performance anion - exchange chromatography method (TCVN 4594:1988).
- Determining the lipid inside Royal jelly before and after freeze drying by the Soxhlet method (FAO, 14/7, 1986).
- Determining ash inside Royal jelly before and after freeze drying by the AOAC 2000 (971.14) method (FAO, 14/7, 1986) as follow: Royal jelly samples were burned into ash by heat. Then, ash was determined by weight method.
- Determining the 10-HDA inside Royal jelly before and after freeze drying by the Gas Chromatography method (GC-ISO/CD 5509:94).
- Determining vitamin B_5 inside Royal jelly before and after freeze drying by the HPLC/UV (High performance liquid chromatography/UV-vis) method (TK. AOAC 985.33).
- Determining free fatty acids inside Royal jelly before and after freeze drying by the AOCS (1997) method (TCVN 6127-2010).
- Determining viscosity of Royal jelly before and after freeze drying by viscometer of Brookfield. Royal jelly after freeze drying is soaked into water in order to determine viscosity, the weight of soaked water is equal weight of separate water from freeze drying process.
- Determining the loss of total protein; the loss of carbohydrate; the loss of lipid; the loss of mineral salts; the loss of 10-HDA; the loss of vitamin B_5; the loss of free fatty acids and the loss of viscosity of the final product (y_j, %) by equation (2), with $j = 3 \div 10$.

$$y_j = \frac{m_1 - m_2}{m_1}100\% = \frac{\Delta m}{m_1}100\%; \quad j = 3 \div 10 \tag{3}$$

Where: the total protein, carbohydrate, lipid, mineral salts, 10-HDA, free fatty acids, free amino acids, vitamin and and viscosity of the material initial and after freeze drying respectively m_1 (%) and m_2 (%) were calculated according to weight of dry matter. If it is viscosity, unit of m_1 and m_2 will be cP (mPa.S = 10^{-3} Pa.S). In the fact that the final product of Royal jelly after freeze drying achieves the best quality means $y_{jmin} = 0$. In the fact, $y_j > 0$.

- Using quadratic orthogonal experimental planning method (Dzung, 2012a; 2015) to build the mathematical model about relationships between y_j ($j = 1 \div 10$) and technological factors effect on the freezing process (Z_1, Z_2, Z_3). These mathematical models of y_j ($j = 1 \div 10$) were written as follow (Dzung, 2012a; 2015):

$$y_j = b_0 + \sum_{u=1}^{k} b_u x_u + \sum_{u \neq i; u=1}^{k} b_{ui} x_u x_i + \sum_{u=1}^{k} b_{uu}\left(x_u^2 - \lambda\right) \tag{4}$$

These variables x_1, x_2, x_3 were coded by variables of Z_1, Z_2, Z_3 presented as follow:

$$x_i = (Z_i - Z_i^0)/\Delta Z_i;\ Z_i = x_i.\Delta Z_i + Z_i^0 \tag{5}$$

Where: $Z_i^0 = (Z_i^{max} + Z_i^{min})/2$;

$$\Delta Z_i = (Z_i^{max} - Z_i^{min})/2; \tag{6}$$

$$Z_i^{min} \leq Z_i \leq Z_i^{max};\ i = 1 \text{ to } 3$$

The experimental number is determined:

$$N = n_k + n_* + n_0 = 2^k + 2k + n_0 = 18 \quad (7)$$

With: $k = 3$; $n_k = 2^k = 2^3 = 8$;

$n_* = 2k = 2x3 = 6$; $n_0 = 4$

The value of the star point:

$$\alpha = \sqrt{\sqrt{N.2^{(k-2)}} - 2^{(k-1)}} = 1.414 \quad (8)$$

The condition of the orthogonal matrix:

$$\lambda = \frac{1}{N}\left(2^k + 2\alpha^2\right) = 2/3 \quad (9)$$

- Using the mathematical tools to solve the multi-objective optimization problem to determine the technological mode for the freeze drying process of Royal jelly.

3. Results and discussions

3.1. Develop the mathematical models of the freeze drying process of Royal jelly

The constituent objective functions of the freeze drying process including: y_1 (kWh/kg) – the energy consumption of 1 kg final product of Royal jelly after freeze drying; y_2 (%) – the residual water content of Royal jelly after freeze drying; y_3 (%) – the loss of total protein; y_4 (%) – the loss of carbohydrate; y_5 (%) – the loss of lipid; y_6 (%) – the loss of mineral salts; y_7 (%) – the loss of 10-HDA; y_8 (%) – the loss of vitamin B_5; y_9 (%) – the loss of free fatty acids and y_{10} (%) – the loss of viscosity of final product of Royal jelly of freeze drying process depended on the technological factors, including: temperature of freeze drying chamber (Z_1, ^{0}C), pressure of freeze drying chamber (Z_2, mmHg), time of freeze drying (Z_3, h).

Therefore, these constituent objective functions were determined by the experimental planning method with the quadratic orthogonal experimental matrix ($k = 3$, $n_0 = 4$). In addition, the experimental factors were established by conditions of the technological freeze drying (Dzung, 2012a; 2013; 2015), they were summarized in Table 2.

Table 2. The technological factors levels design

Parameters		Z_1, (^{0}C)	Z_2, (mmHg)	Z_3, (h)
Le-vels	- α (-1.414)	17.93	0.008	17.172
	Low (-1)	20	0.137	18
	Central (0)	25	0.447	20
	High (+1)	30	0.758	22
	+ α (1.414)	32.07	0.886	22.828
Deviation ΔZ_i		5	0.3105	2

The experiments were carried out with all of the factor levels in Table 2 to determine the value of the objective functions that describe relationships between the energy consumption of 1 kg final product of freeze drying Royal jelly; the residual water content of final product of Royal jelly after freeze drying; the loss of total protein; the loss of carbohydrate; the loss of lipid; the loss of mineral salts; the loss of 10-HDA; the loss of free fatty acids; the loss of vitamin and the loss of viscosity of the final product of Royal jelly after freeze drying and technological factors, (Dzung, 2012a; 2015). The results were summarized in Table 3a, 3b, 3c and 3d.

Table 3a. The orthogonal experimental matrix level 2 (k = 3, n_0 = 4)

N		x_0	x_1	x_2	x_3
2^k	1	1	1	1	1
	2	1	-1	1	1
	3	1	1	-1	1
	4	1	-1	-1	1
	5	1	1	1	-1
	6	1	-1	1	-1
	7	1	1	-1	-1
	8	1	-1	-1	-1
2k	9	1	1.414	0	0
	10	1	-1.414	0	0
	11	1	0	1.414	0
	12	1	0	-1.414	0
	13	1	0	0	1.414
	14	1	0	0	-1.414
n_0	15	1	0	0	0
	16	1	0	0	0
	17	1	0	0	0
	18	1	0	0	0

Table 3b. The orthogonal experimental matrix level 2 (k = 3, n_0 = 4)

x_1x_2	x_1x_3	x_2x_3	$x_1^2 - \lambda$	$x_2^2 - \lambda$	$x_3^2 - \lambda$	y_1
1	1	1	0.333	0.333	0.333	7.85
-1	-1	1	0.333	0.333	0.333	7.62
-1	1	-1	0.333	0.333	0.333	8.18
1	-1	-1	0.333	0.333	0.333	8.02
1	-1	-1	0.333	0.333	0.333	6.41
-1	1	-1	0.333	0.333	0.333	6.36
-1	-1	1	0.333	0.333	0.333	6.37
1	1	1	0.333	0.333	0.333	6.29
0	0	0	1.333	-0.667	-0.667	7.18
0	0	0	1.333	-0.667	-0.667	6.78
0	0	0	-0.667	1.333	-0.667	6.70
0	0	0	-0.667	1.333	-0.667	7.68
0	0	0	-0.667	-0.667	1.333	9.20
0	0	0	-0.667	-0.667	1.333	5.98
0	0	0	-0.667	-0.667	-0.667	7.00
0	0	0	-0.667	-0.667	-0.667	7.05
0	0	0	-0.667	-0.667	-0.667	7.22
0	0	0	-0.667	-0.667	-0.667	7.06

Table 3d. The orthogonal experimental matrix level 2 (k = 3, n_0 = 4)

N		y_2	y_3	y_4	y_5
2^k	1	2.94	4.03	3.25	3.06
	2	3.58	3.89	2.92	3.47
	3	2.59	4.12	2.66	2.96
	4	3.49	3.72	2.12	2.65
	5	4.11	2.83	2.58	2.45
	6	4.45	2.63	1.97	2.24
	7	4.19	2.98	2.35	2.14
	8	4.51	1.69	1.97	2.04
2k	9	3.08	3.20	3.02	3.16
	10	3.83	2.32	2.17	2.96
	11	3.80	3.00	2.84	2.65
	12	3.02	2.63	2.05	2.55
	13	2.57	4.23	2.84	3.27
	14	4.73	1.32	2.07	2.14
n_0	15	3.34	2.09	1.87	1.94
	16	3.18	2.32	2.00	1.84
	17	3.25	2.23	1.82	1.63
	18	3.17	2.52	1.94	1.73

Table 3c. The orthogonal experimental matrix level 2 (k = 3, n_0 = 4)

y_6	y_7	y_8	y_9	y_{10}
2.26	2.89	4.13	4.01	18.13
2.11	1.71	3.32	3.94	5.19
3.70	2.50	3.93	3.72	9.89
3.41	1.58	3.22	2.92	8.65
2.96	2.24	3.42	3.21	10.26
2.52	0.79	2.82	2.99	5.15
3.26	1.05	2.48	2.85	2.84
2.78	0.92	2.21	2.70	5.69
2.30	2.11	4.93	4.74	13.27
2.00	1.32	2.72	2.48	6.18
1.96	1.71	2.11	3.14	13.14
3.67	1.84	2.01	2.63	9.97
3.22	1.97	4.63	4.16	16.19
2.48	1.45	2.82	1.82	6.76
2.41	1.05	2.42	3.65	3.54
2.33	0.79	2.52	3.80	1.36
2.41	0.92	2.62	3.50	1.57
2.52	1.18	2.32	3.43	3.50

The mathematical model of regression equations (y_j, j = 1 to 10) from Eq. (10) to Eq. (19) were obtained after processing the experimental data, calculating the coefficients, testing the significance of the coefficients by the Student criterion, and testing the regression equations for the fitness of the experimental results by Fisher criterion (Dzung, 2012a, Dzung, 2013; Dzung, 2015).

Results received were the mathematical models as follow:

- *The energy consumption of 1 kg final product of Royal jelly after freezing process*:

$$y_1 = f_1(x_1, x_2, x_3) = 7.125 + 0.091x_1 - 0.166x_2 + 0.899x_3 - 0.104x_2x_3 - 0.123x_1^2 + 0.18x_3^2 \quad (10)$$

- *The residual water content of final product of Royal jelly after freezing process*:

$$y_2 = f_2(x_1, x_2, x_3) = 3.216 - 0.271x_1 + 0.116x_2 - 0.643x_3 - 0.111x_1x_3 + 0.139x_1^2 + 0.118x_2^2 + 0.237x_3^2 \quad (11)$$

- *The loss of total protein of final product of Royal jelly after freezing process*:

$$y_3 = f_3(x_1, x_2, x_3) = 2.245 + 0.274x_1 + 0.814x_3 + 0.303x_1^2 + 0.332x_2^2 + 0.311x_3^2 \quad (12)$$

- *The loss of carbohydrate of final product of Royal jelly after freezing process*:

$$y_4 = f_4(x_1, x_2, x_3) = 1.978 + 0.225x_1 + 0.228x_2 + 0.263x_3 + 0.144x_2x_3 + 0.239x_1^2 + 0.162x_2^2 + 0.169x_3^2 \quad (13)$$

- *The loss of lipid of final product of Royal jelly after freezing process*:

$$y_5 = f_5(x_1, x_2, x_3) = 1.934 + 0.131x_2 + 0.404x_3 + 0.417x_1^2 + 0.186x_2^2 + 0.237x_3^2 \quad (14)$$

- *The loss of mineral salts of final product of Royal jelly after freezing process*:

$$y_6 = f_6(x_1, x_2, x_3) = 2.328 + 0.149x_1 - 0.475x_2 + 0.084x_3 - 0.273x_2x_3 + 0.257x_2^2 + 0.276x_3^2 \quad (15)$$

- *The loss of 10-HDA of final product of Royal jelly after freezing process*:

$$y_7 = f_7(x_1, x_2, x_3) = 1.075 + 0.401x_1 + 0.369x_3 + 0.198x_1x_2 + 0.231x_1^2 + 0.262x_2^2 + 0.231x_3^2 \quad (16)$$

- *The loss of vitamin B_5 of final product of Royal jelly after freezing process*:

$$y_8 = f_2(x_1, x_2, x_3) = 2.556 + 0.46x_1 + 0.166x_2 + 0.519x_3 - 0.156x_2x_3 + 0.552x_1^2 - 0.335x_2^2 + 0.501x_3^2 \quad (17)$$

- *The loss of free fatty acids of final product of Royal jelly after freezing process*:

$$y_9 = f_{10}(x_1, x_2, x_3) = 3.606 + 0.37x_1 + 0.224x_2 + 0.512x_3 - 0.244x_2^2 - 0.189x_3^2 \quad (18)$$

- *The loss of viscosity of final product of Royal jelly after freezing process*:

$$y_{10} = f_{11}(x_1, x_2, x_3) = 4.896 + 2.205x_1 + 1.346x_2 + 2.605x_3 + 2.457x_1x_2 + 1.488x_1x_3 + 2.235x_2^2 + 2.194x_3^2 \quad (19)$$

3.2. Building one-objective optimization problems for the freeze drying process of Royal jelly

It was obvious that all objective functions (y_j, j = 1 to 10) for the freeze drying process of Royal jelly depended on the technological parameters (x_i, i = 1 to 3). If every objective function was individually surveyed, these one-objective functions along with the technological parameters would constitute the one-objective optimization problems. Because all the one-objective functions were to find the minimal value, the one-objective optimization problems were restated as follow (Dzung, 2012a, 2013 and 2015): Finding in common the test $x^{jopt} = (x_1^{jopt}, x_2^{jopt}, x_3^{jopt}) \in \Omega_x = \{-1.414 \leq x_1, x_2, x_3 \leq 1.414\}$ in order that:

$$\begin{cases} y_j = f_{j\min}\left(x_1^{jopt}, x_2^{jopt}, x_3^{jopt}\right) \\ \qquad = \min f_j\left(x_1, x_2, x_3\right) \\ j = 1 \div 10;\ \forall x \in \Omega_x = \\ \quad = \left\{-1.414 \le x_1, x_2, x_3 \le 1.414\right\}; \end{cases} \quad (20)$$

3.3. Building multi-objective optimization problems for freeze drying process of Royal jelly

The establishment of the technological mode during freeze drying process of Royal jelly was based on factors including: economic, technicality and quality of the final product, (Dzung, 2012a). The final product of Royal jelly after freeze drying has the best quality, the energy consumption of 1 kg final product reached the minimum value and the residual water content of the final product reached the minimum value but under 4.5%. In addition, the final product of Royal jelly after freeze drying must satisfy all conditions of economic and technical criterion as well as quality criterion for technology of the freeze drying (Dzung, 2012a). For example:

- *$y_1 < C_1 = 6.5$ kWh/kg, if the energy consumption of 1 kg product is over 6kWh ($y_1 > C_1 = 6.5$), it will increase the product price and difficult commercialization.*
- *$y_2 < C_2 = 4.5\%$, if the residual water content of the final product is over 4.5% ($y_2 > C_2 = 4.5\%$), the microorganisms will be capable to grow, develop and damage products.*
- *$y_j < C_j = 3.0\%$ with $j = 3 \div 9$, if the loss of total protein; the loss of carbohydrate; the loss of lipid; the loss of mineral salts; the loss of 10-HDA; the loss of free fatty acids and the loss of vitamin are over 3.0% ($y_j > C_j = 3.0\%$; $j = 3 \div 9$), the final product of Royal jelly after the freeze drying will reduce quality.*
- *$y_{10} < C_{10} = 5.0\%$, if the loss of viscosity of the final product is over 5.0% ($y_{10} > C_{10} = 5.0\%$), the protein inside Royal jelly after freeze drying will be denatured, not be able to recover the original its quality. It make to reduce ability to link between water and protein, this is cause to reduce viscosity of Royal jelly after freeze drying and reverted to the original state. Finally, it makes to reduce quality of final product.*

It is obvious that the multi-objective optimization problem during the freeze drying of Royal jelly appeared in this case. The technological parameters (x_1, x_2 and x_3) of the freeze drying process of Royal jelly have simultaneously influenced on eleven objective functions (y_j, $j = 1 \div 10$) with the identified domain $\Omega_x = \{-1.414 \le x_1, x_2, x_3 \le 1.414\}$. Therefore, the mathematical model of eleven-objective optimization problem to determine the technological mode of the freeze drying process of Royal jelly was restated as follow: Finding in common the root $x = (x_1^{opt}, x_2^{opt}, x_3^{opt}) \in \Omega_x = \{-1.414 \le x_1, x_2, x_3 \le 1.414\}$ in order that (Dzung, 2012a & 2015):

$$\begin{cases} y_j = f_{j\min}\left(x_1^{opt}, x_2^{opt}, x_3^{opt}\right) \\ \qquad = \min f_j\left(x_1, x_2, x_3\right) \\ \forall x \in \Omega_x = \\ \quad = \left\{-1.414 \le x_1, x_2, x_3 \le 1.414\right\} \\ y_j < C_j; \quad j = 1 \div 10 \end{cases} \quad (21)$$

3.4. Solving one-objective optimization problems for freeze drying process of Royal jelly

According to the results of Dzung et al (2012a; 2015), if all the one-objective optimization problems (20) have the same roots: $(x_1^{jopt}, x_2^{jopt}, x_3^{jopt}) = (x_1^{kopt}, x_2^{kopt}, x_3^{kopt})$ with $k \ne j$, these roots called are utopian roots and also roots of multi-objective optimization problem (21). The optimal plan of utopian roots called is utopian plan. If the utopian roots and the utopian plan do not exist, multi-objective optimization problem (21) will be solved to

find the optimal Pareto roots and the optimal Pareto plan.

Therefore, solving one-objective optimization problems (20) were found to achieve: $y_{jmin} = \min f_j(x_1, x_2, x_3)$, $j = 1 \div 10$, with the identified domain $\Omega_x = \{-1.414 \le x_1, x_2, x_3 \le 1.414\}$. By using the meshing method programmed in Matlab R2008a software, the results of the optimal parameters of every objective function from (10) to (19) limited in the experimental domain were summarized in Table 4, (Dzung, 2012a; 2015):

Table 4. Minimum roots of each one-objective optimization problems

j	y_{jmin}	$x_1^{j\,opt}$	$x_2^{j\,opt}$	$x_3^{j\,opt}$
1	5.812	-1.414	1.414	-1.414
2	2.425	1.414	-0.492	1.414
3	1.651	-0.452	0.000	-1.309
4	1.782	-0.533	-0.441	-0.590
5	1.739	0.000	-0.352	-0.852
6	1.863	-1.414	1.182	0.447
7	0.720	-1.036	0.391	-0.799
8	1.740	-0.482	-1.000	-0.564
9	1.738	-1.307	-1.150	-1.112
10	1.243	-1.414	0.476	-0.114

In the Table 4, it is obvious that the utopian point was indentified: $f^{UT} = (f_{1min}, f_{2min}, f_{3min}, f_{4min}, f_{5min}, f_{6min}, f_{7min}, f_{8min}, f_{9min}, f_{10min}) = (5.812, 2.425, 1.651, 1.782, 1.739, 1.863, 0.720, 1.740, 1.738, 1.243)$. However, the utopian root and the utopian plan did not exist, because of $x^{jopt} = (x_1^{jopt}, x_2^{jopt}, x_3^{jopt}) \ne x^{kopt} = (x_1^{kopt}, x_2^{kopt}, x_3^{kopt})$ with $j, k = 1 \div 10$, $j \ne k$ (Dzung, 2011, 2012a, 2012c & 2015).

From results of solving one-objective optimization problems (21), it was obvious that the utopian root and utopian plan do not exist. Therefore, multi-objective optimization problems (21) need to have to be solved to find the optimal Pareto root and the optimal Pareto plan in order that optimal Pareto effect $y_P^R = (y_{1P}^R, y_{2P}^R, y_{3P}^R, y_{4P}^R, y_{5P}^R, y_{6P}^R, y_{7P}^R, y_{8P}^R, y_{9P}^R, y_{10P}^R)$ closest to the utopian point f^{UT} and the furthest from the restricted area $\{y_j \ge C_j\}$.

3.5. Solving multi-objective optimization problems for freeze drying process of Royal jelly

The purpose of the experiment was to reach the targets of the freeze drying process which were expressed by 10 regression equations from (10) to (19), but the tests satisfying all function values ($y_{1min}, y_{2min}, y_{3min}, y_{4min}, y_{5min}, y_{6min}, y_{7min}, y_{8min}, y_{9min}, y_{10min}$) could not be found. Hence, the idea of the multi-objective optimization problem (22) was to find the optimal Pareto root for $y_P^R = (y_{1P}^R, y_{2P}^R, y_{3P}^R, y_{4P}^R, y_{5P}^R, y_{6P}^R, y_{7P}^R, y_{8P}^R, y_{9P}^R, y_{10P}^R)$ closest to the utopian point and the furthest from the restricted area, but $y_j = y_j(x) = f_j(x_1, x_2, x_3)$, with $j = 1 \div 10$ must satisfy technological conditions with requirements in $\{y_j < C_j\}$, (Dzung, 2011, 2012a & 2015).

By the restricted area method, solving the multi-objective optimization problem of the freeze drying (21) as the followings: Establishing the R*-objective combination function $R^*(y_1, y_2, y_3, y_4, y_5, y_6, y_7, y_8, y_9, y_{10}) = R^*(x_1, x_2, x_3) = R^*(x)$ as follow, (Dzung, 2012a, 2012c & 2015):

$$\begin{cases} R^*(x) = R^*(x_1, x_2, x_3) \\ \quad = \sqrt[10]{\prod_{j=1}^{10} r_j(x_1, x_2, x_3)} = \sqrt[10]{\prod_{j=1}^{10} r_j(x)} \\ \Omega_x = \{-1.414 \le x_1, x_2, x_3 \le 1.414\}; \\ x = (x_1, x_2, x_3) \end{cases} \quad (22)$$

Where: $r_j(x) = \left(\dfrac{C_j - y_j(x)}{C_j - y_{jmin}} \right)$

when $y_j(x) < C_j$ (23)

$r_j(x) = 0$ when $y_j(x) \ge C_j$ (24)

$r_1(x) = (6.5 - y_1(x))/(6.5 - 5.812)$

when $y_1(x) < 6.5$

$r_1(x) = 0$ when $y_1(x) \ge 6.5$

$r_2(x) = (4.5 - y_2(x))/(4.5 - 2.425)$

when $y_2(x) < 4.5$

$r_2(x) = 0$ when $y_2(x) \ge 4.5$

$r_3(x) = (3.0 - y_3(x))/(3.0 - 1.651)$

when $y_3(x) < 3.0$

$r_3(x) = 0$ when $y_3(x) \geq 3.0$

$r_4(x) = (3.0 - y_4(x))/(3.0 - 1.782)$

when $y_4(x) < 3.0$

$r_4(x) = 0$ when $y_4(x) \geq 3.0$

$r_5(x) = (3.0 - y_5(x))/(3.0 - 1.739)$

when $y_5(x) < 3.0$

$r_5(x) = 0$ when $y_5(x) \geq 3.0$

$r_6(x) = (3.0 - y_6(x))/(3.0 - 1.863)$

when $y_6(x) < 3.0$

$r_6(x) = 0$ when $y_6(x) \geq 3.0$

$r_7(x) = (3.0 - y_7(x))/(3.0 - 0.720)$

when $y_7(x) < 3.0$

$r_7(x) = 0$ when $y_7(x) \geq 3.0$

$r_8(x) = (3.0 - y_8(x))/(3.0 - 1.740)$

when $y_8(x) < 3.0$

$r_8(x) = 0$ when $y_8(x) \geq 3.0$

$r_9(x) = (3.0 - y_9(x))/(3.0 - 1.738)$

when $y_9(x) < 3.0$

$r_9(x) = 0$ when $y_9(x) \geq 3.0$

$r_{10}(x) = (5.0 - y_{10}(x))/(5.0 - 1.243)$

when $y_{10}(x) < 5.0$

$r_{10}(x) = 0$ when $y_{10}(x) \geq 5.0$

From (23), it was obvious that if $y_j(x) \rightarrow y_{j\,min}$ and $\forall y_j(x) < C_j$, $r_j(x) \rightarrow r_{jmax} = 1$. By choosing R*(x) as the objective function, the m-objective optimization problem is restated as: Find $x^R = (x_1^R, x_2^R, x_3^R) \in \Omega_x$ in order that R*(x) reaches the maximum value (Dzung, 2012a & 2015).

$$\begin{cases} R^*_{max} = R^*(x^R) = R^*(x_1^R, x_2^R, x_3^R) \\ = \max\left\{R^*(x)\right\} = \max\left\{10\sqrt{\left[\prod_{j=1}^{10} r_j(x)\right]}\right\} \\ \Omega_x = \left\{-1.414 \leq x_1,\ x_2,\ x_3 \leq 1.414\right\}; \\ x = (x_1, x_2, x_3) \end{cases} \quad (25)$$

From (23), it can be seen: $0 \leq R^*(x^R) \leq 1$. If $R^*(x^R) = 1$, $x^R = x^{UT}$ – the utopian root. If $R^*(x^R) = 0$, one of the values of $y_j(x)$ violates $\{y_j < C_j\}$, which means that $y_j(x)$ belongs to the restricted area $C = \{y_j \geq C_j\}$, (Dzung, 2012a).

The eleven-objective optimization problem needed to indentify $x^R = (x_1^R, x_2^R, x_3^R) \in \Omega_x$ in order that $R^*(x_1^R, x_2^R, x_3^R) = Max\{R^*(x_1, x_2, x_3)\}$. The maximum value of (25) was determined by the meshing method programmed in Matlab R2008a software (Dzung, 2012a):

$$R^*(x)_{max} = Max\{R^*(x_1, x_2, x_3)\} = R^*(x_1^R, x_2^R, x_3^R) = 0.476$$

Where: $x_1^R = -0.883$;
$x_2^R = -0.115$;
$x_3^R = -0.859$;

Then, transforming into real variables:

$Z_1^{opt} = 20.58^0C$;
$Z_2^{opt} = 0.411mmHg$;
$Z_3^{opt} = 18.283h$

Substituting x_1^R, x_2^R, x_3^R into these equations from (10) to (19), the results were obtained as:

$y_{1P}^R = 6.32$; $y_{2P}^R = 4.19$;
$y_{3P}^R = 1.77$; $y_{4P}^R = 1.83$;
$y_{5P}^R = 2.07$; $y_{6P}^R = 2.36$;
$y_{7P}^R = 0.78$; $y_{8P}^R = 2.47$;
$y_{9P}^R = 2.67$; $y_{10P}^R = 3.58$;

Where: $x^R = (x_1^R, x_2^R, x_3^R)$ called optimal Pareto root and $f_p^R = y_P^R = (y_{1P}^R, y_{2P}^R, y_{3P}^R, y_{4P}^R, y_{5P}^R, y_{6P}^R, y_{7P}^R, y_{8P}^R, y_{9P}^R, y_{10P}^R)$ called the optimal Pareto effect.

For this reason, through the calculation from the experimental models from Eq. (10) to Eq. (19), technological parameters of the freeze drying process which satisfied the maximum R*-Optimal combination criterion were determined as: temperature of freeze drying chamber was $Z_1^{opt} = 20.58^0C$, pressure of freeze drying chamber was $Z_2^{opt} = 0.411mmHg$, time of freeze drying was $Z_3^{opt} = 18.283h$. Corresponding to: the energy consumption of 1 kg final product was $y_{1P}^R = 6.32$ kWh/kg; the

residual water content of the product was y_{2P}^R = 4.19% (< 4.5%); the loss of total protein was y_{3P}^R = 1.77%; the loss of carbohydrate was y_{4P}^R = 1.83%; the loss of lipid was y_{5P}^R = 2.07%; the loss of mineral salts was y_{6P}^R = 2.36%; the loss of 10-HDA was y_{7P}^R = 0.78%; the loss of vitamin B_5 was y_{8P}^R = 2.47%; the loss of free fatty acids was y_{9P}^R = 2.67%; the loss of viscosity was y_{10P}^R = 3.58%. Compared with the experimental results from the Table 3a & Table 3b, these results above were suitable and satisfying with the objectives of the problem.

3.6. Experiment to test the optimal Pareto root of multi-objective optimization problem

Carrying out the freeze drying process of Royal jelly at the optimal Pareto root: temperature of freeze drying chamber of Z_1^{opt} = 20.58^{0}C, pressure of freeze drying chamber of Z_2^{opt} = 0.411mmHg, and time of freeze drying Z_3^{opt} = 18.283 hours, the experimental results were determined as: energy consumption of 1 kg final product was y_1 = 6.34 kWh/kg; the residual water content of the product was y_2 = 4.17% (< 4.5%); the loss of total protein was y_3 = 1.80%; the loss of carbohydrate was y_4 = 1.79%; the loss of lipid was y_5 = 2.05%; the loss of mineral salts was y_6 = 2.34%; the loss of 10-HDA was y_7 = 0.79%; the loss of vitamin B5 was y_8 = 2.45%; the loss of free fatty acids was y_9 = 2.69%; the loss of viscosity was y_{10} = 3.61%.

Consequently, it was very noticeable that the results from the optimization problems of the freeze drying process had the approximation to the experimental results. When the pressure of freeze drying chamber was fixed: x_2 = -0.115, respectively Z_2 = 0.411 mmHg, the relationship between y_1, y_2, y_3, y_4, y_5 y_6, y_7, y_8, y_9 and y_{10} combination function with 2 variables x_1, x_3 was performed geometrically in 3D (Figures 4, 5, 6, 7, 8, 9, 10, 11, 12, 13). When x_1 was fixed with constant values, the variation of x_3 was shown in Figures 14, 15, 16, 17, 18, 19, 20, 21, 22, 23.

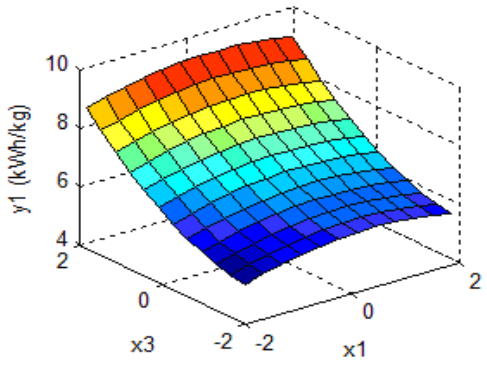

Fig 4. The energy consumption of 1kg final product y1 (kWh/kg)

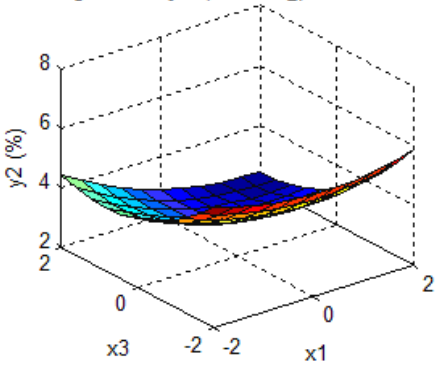

Fig 5. The residual water content of final product y2 (%)

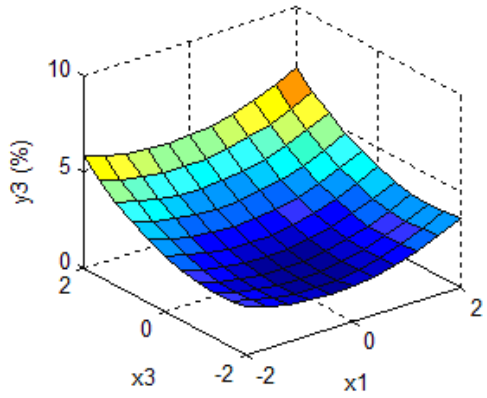

Fig 6. The loss of total protein of final product y3 (%)

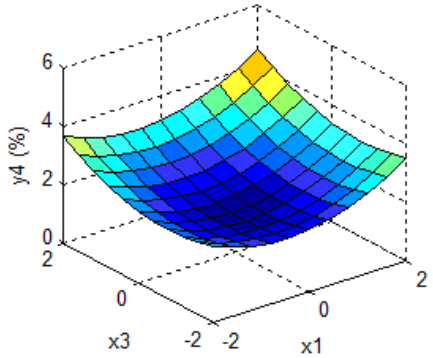

Fig 7. The loss of carbohydrate of final product y4 (%)

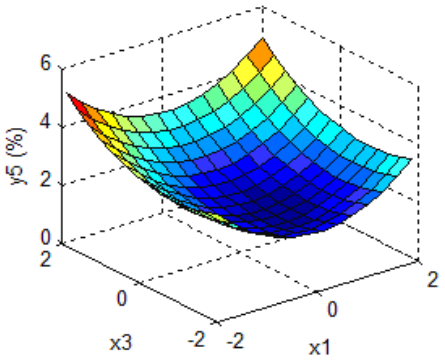

Fig 8. The loss of lipid of final product y5 (%)

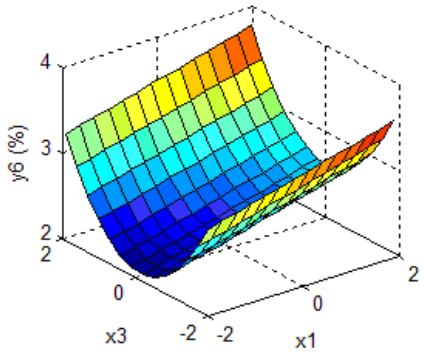

Fig 9. The loss of mineral salts of final product y6 (%)

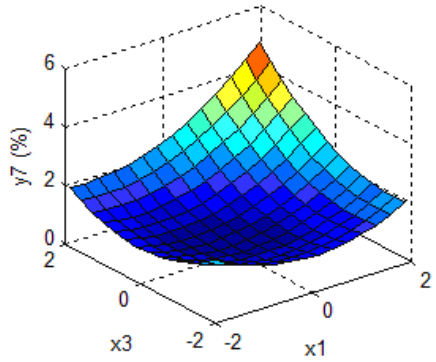

Fig 10. The loss of 10-HDA of final product y7 (%)

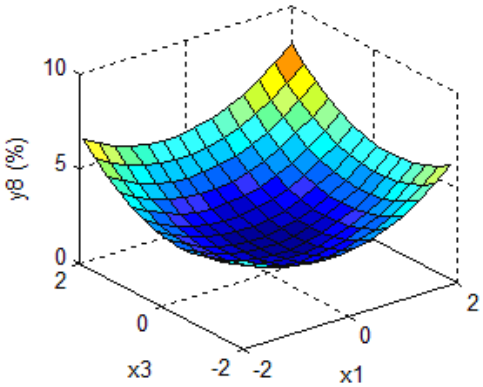

Fig 11. The loss of vitamin B5 of final product y8 (%)

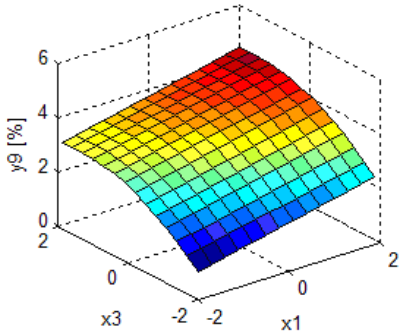

Fig 12. The loss of free fatty acids of final product y9 (%)

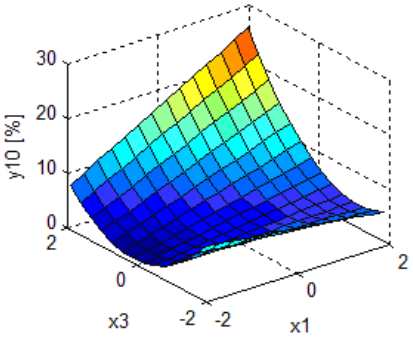

Fig 13. The loss of viscosity of final product y10 (%)

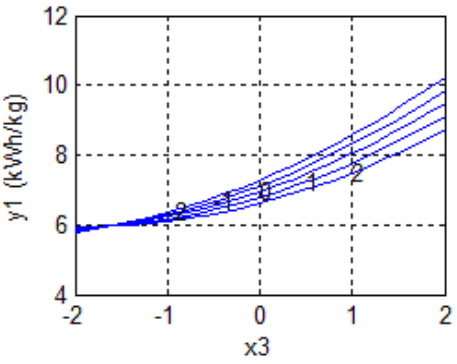

Fig 14. The energy consumption of final product y1 (kWh/kg)

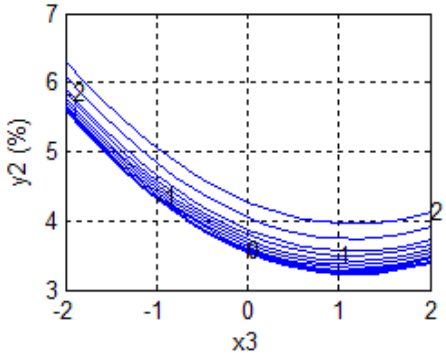

Fig 15. The residual water content of final product y2 (%)

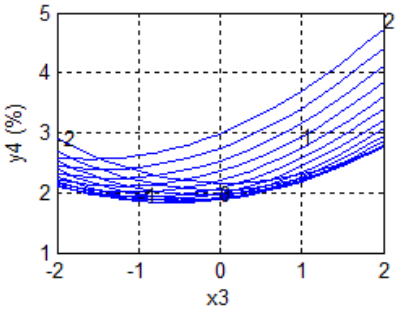

Fig 17. The loss of carbohydrate of final product y4 (%)

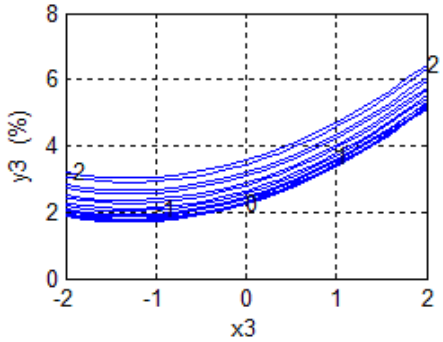

Fig 16. The loss of total protein of final product y3 (%)

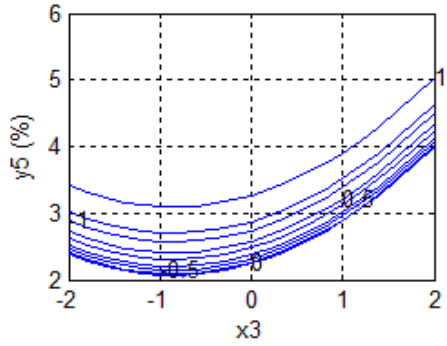

Fig 18. The loss of lipid of final product y5 (%)

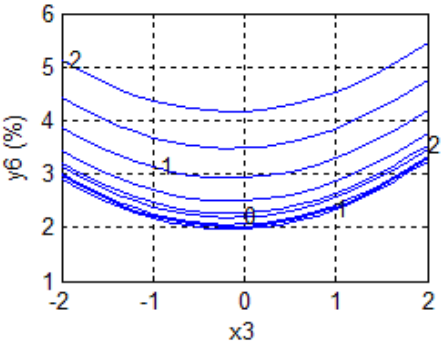

Fig 19. The loss of mineral salts of final product y6 (%)

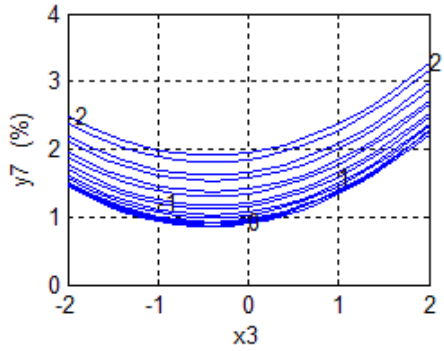

Fig 20. The loss of 10-HDA of final product y7 (%)

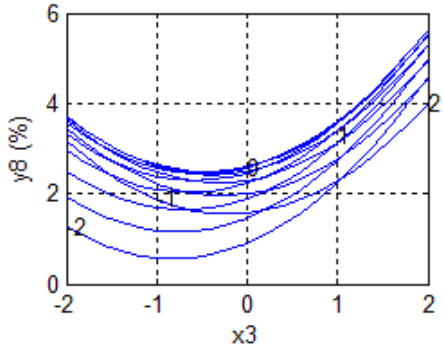

Fig 21. The loss of vitamin B5 of final product y8 (%)

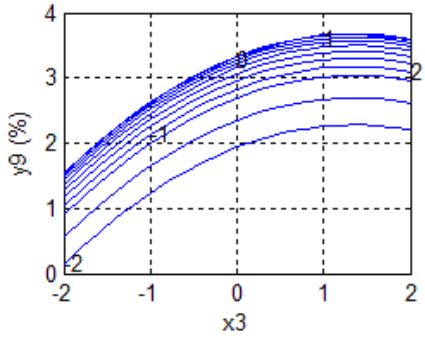

Fig 22. The loss of free fatty acids of final product y9 (%)

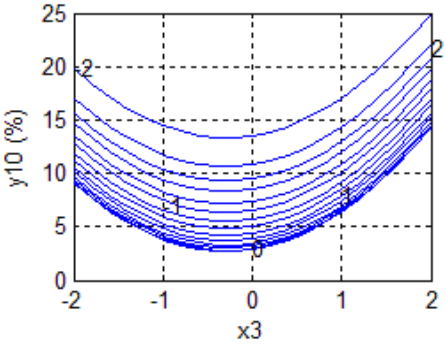

Fig 23. The loss of viscosity of final product y10 (%)

All Figures on above was obvious that objective functions were varied by effect factors during the freeze drying process of Royal jelly. This varying objective functions were completely suitable with experimental results. Therefore, it proved that relationships between objective functions with effect factors very well described for the freeze drying process of Royal jelly.

3.7. Determining technological mode of freezing process of Royal jelly

From results on above, it allowed to set up the technological mode during the freeze drying process of Royal jelly in Table 5 as follow:

Table 5. The technological mode of the freeze drying process of Royal jelly

No	Technological Parameters	Symbol and unit	Value
1	The temperature of the freezing environment	$Z_1 = T_{\infty}$, (^{0}C)	20.58
2	The time of freezing process	Z_2 = P, (mmHg)	0.411
3	the thickness of Royal jelly in the tray	$Z_3 = \tau$, (h)	18.283
The standards of final product of Royal jelly after freeze drying			
4	The energy consumption of 1 kg final product	y_{1P}^R, (kWh/kg)	6.32
5	The residual water content of final product	y_{2P}^R, (%)	4.19
6	The loss of total protein of final product	y_{3P}^R, (%)	1.77
7	The loss of carbohydrate of final product	y_{4P}^R, (%)	1.83
8	The loss of lipid of final product	y_{5P}^R, (%)	2.07
9	The loss of mineral salts of final product	y_{6P}^R, (%)	2.36
10	The loss of 10-HDA of final product	y_{7P}^R, (%)	0.78
11	The loss of vitamin B5 of final product	y_{8P}^R, (%)	2.47
12	The loss of free fatty acids of final product	Y_{9P}^R, (%)	2.67
13	The loss of viscosity of final product	y_{10P}^R, (%)	3.58

From Table 5, it was obvious when Royal jelly was carried out at the optimal technological mode of freeze drying process, the quality of Royal jelly after freeze drying had very good as the same quality of Royal jelly before freeze drying. In the fact that has not any drying method can create product to have good quality as the same product of the freeze drying process. Therefore, the technological mode of freeze drying process of Royal jelly was found out on above, it can be completely applied for Royal jelly preservation in order to be prolonged use time and export time (Dzung, 2012a).

4. Conclusions

The mathematical models (10) to (19) which were established from the experiments quite well described the relationship between the temperature of freeze drying chamber; the pressure of freeze drying chamber; the time of freeze drying process of Royal jelly with the energy consumption of 1 kg final product of freeze drying Royal jelly; the residual water content of final product of Royal jelly after freeze drying; the loss of total protein; the loss of carbohydrate; the loss of lipid; the loss of mineral salts; the loss of 10-HDA; the loss of vitamin B_5; the loss of free fatty acids and the loss of viscosity of final product of Royal jelly (Dzung, 2012a).

The system of equation (21) was the multi-objective optimization problems of the freeze drying process of Royal jelly. This mathematical model was suitably used for calculating and setting up the technological mode of the freeze drying process of Royal jelly (Dzung, 2012a).

Solving the multi-objective optimization problems (21) determined the technological

mode of the freeze drying process of Royal jelly (Dzung, 2012a). The results were presented in Table 5.

5. References

Andreas, S., et al. (2005). Trace and mineral elements in royal jelly and homeostatic effects, *Journal of Trace Elements in Medicine and Biology* 19, 183–189.

Anna, G.S. (2009). Quality and standardisation of Royal Jelly, *Journal of ApiProduct and ApiMedical Science* 1(1), p 1-6. DOI: 10.3896/IBRA.4.1.01.04.

Antinelli, J. F., et al. (2003). "Evaluation of (E)-10-hydroxydec-2- enoic acid as a freshness parameter for royal jelly". *Food Chemistry,* 80, 85-89.

Benfenati et al. (1986); Lercker et al., (1984 and 1992); Schmidt and Buchmann, (1992). Book "value-added products from beekeeping, chapter 6, Royal jelly", FAO.

Dzung N.T., et al. (2015). Study Technological Factors Effect on the Loss of Protein, Carbohydrate and Lipid inside Royal Jelly in the Freeze Drying Process. *Current Research Journal of Biological Sciences*, 7(2): 22-30.

Dzung N.T. (2013). Study technological factors effect on the loss of 10-HDA (Bioactive compound) inside Royal Jelly in the freeze drying process, *Jokull Journal* (Iceland), Vol 63, No 9, Section 3, Sep 2013, pp 30-40.

Dzung N.T, (2014). Building the Method and the Mathematical Model to Determine the Rate of Freezing Water inside Royal Jelly in the Freezing Process. *Research Journal of Applied Sciences*, Engineering and Technology, 7(2): 403-412.

Dzung, N.T. (2011). Application of Multi-Objective Optimization by The Utopian Point Method to Determining the Technological Mode of *Gac* Oil Extraction, *International Journal of Chemical Engineering and Applications*, Vol.3, No.1.

Dzung, N.T. (2012a). Optimization the Freezing Process of Penaeus Monodon To Determine Technological Mode of Freezing for Using in the Freeze Drying, *Canadian Journal on Chemical Engineering & Technology*, Vol. 3, No. 3, April 2012.

Dzung, N.T. (2012c), Optimization The Freeze Drying Process of Penaeus Monodon to Determine The Technological Mode, *International Journal of Chemical Engineering and Application*, Vol.3, No.3, June 2012, p.187-194.

Dzung, N.T., et al. (2012b). Building The Method To Determine The Rate of Freezing Water of Penaeus Monodon, *Carpathian Journal of Food Science & Technology*; ,4(2), p.28.

Figura L.O., Teixeira A.A. (2007). Food Physics: Physical properties Measurement and Application, Germany, 554.

Gaëlle, D., Hervé, C. (2012). Sugar composition of French royal jelly for comparison with commercial and artificial sugar samples. *Food Chemistry,* 134 (2012), 1025–1029.

Hattori, N. et al., (2007). "Royal jelly and its unique fatty acid, 10-hydroxy-trans-2-decenoic acid, promote neurogenesis by neural stem/progenitor cells in vitro". *Biomedical research (Tokyo, Japan)* 28 (5), 261–266. PMID 18000339.

Hiroyuki M., et al. (2012). Effect of royal jelly ingestion for six months on healthy volunteers, *Nutrition Journal*, 11, 77.

Holman J. (1992). Heat Transfer, McGraw – Hill, New York.

Isidorov, V.A., et al. (2011). Determination of royal jelly acids in honey, Food Chemistry 124 (2011), 387–391.

Mohamed, F. R., et al. (2012). Bioactive compounds and health-promoting properties of royal jelly: A review, *Journal of functional foods*, 4 (2012), 39–52.

Acknowledgments

The authors thank Head of Lab Food Engineering and Technology, Department of Food Technology, Faculty of Chemical and Food Technology, HCMC University of Technology and Education, Viet Nam, for help with experiments carrying out.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

EFFECTS OF DIFFERENT GUMS ON THE SOME PROPERTIES OF FRIED BEEF PATTIES

Osman Kilincceker[1]*, Mustafa Tahsin Yilmaz[2]

[1]University of Adiyaman, Technical Sciences Vocational School, Department of Food Processing, , Adiyaman, Turkey
[2]Yildiz Technical University, Chemical and Metallurgical Engineering Faculty, Department of Food Engineering, 34210, Istanbul, Turkey.
*Corresponding author: *okilincceker@adiyaman.edu.tr*

Article history:
Received:
03 March 2016
Accepted in revised form:
29 May 2016

Keywords:
Beef patty;
guar gum;
xanthan gum;
gum Arabic;
frying

ABSTRACT
The aim of the study was to determine the effects of treatments with different gums on fried beef patties. Meat patties were prepared with three different formulations (0.5, 1 and 1.5%) for each of guar gum, xanthan gum, and gum Arabic. Some physical, chemical, and sensorial properties of fried samples were evaluated. As a result, guar gum and xanthan gam showed better effect on yield and diameter reduction. Gum Arabic increased L and b values of fried meat patties whereas guar more increased the moisture retention than others. However, gum Arabic had higher performance on the sensorial properties of beef patties. It was also observed that high levels of gums had much better results as an ingredient in fried patties compared with the other treatments. According to results, it was determined that especially 1.5% level of guar and all level of gum Arabic can be recommended for beef patties.

1. Introduction

Application of various techniques in daily eating foods, including meat products, with decreased levels of fat, cholesterol, and calories, have become a trend for various health reasons. Reduction of fat by using modern technologies would apparently be the most efficient method for producing meat products. In addition there are many technological problems like excessive soft or firm texture, cooking loss and shrinkage are considered by consumers. Regulation of meat product composition with non-meat ingredients can lead to decrease fat value and to improve texture of the final food. The use of these ingredients may increase production yield, sensory properties, and shelf life (Colmenero et al., 1996; Demirci et al., 2014).

Some researchers have reported that different hydrocolloids can increase friction and binding properties among particles and decrease the activity of water in meat products. They can enhance the structure of cooked food decrease texture problems and moisture loss (Ulu, 2004; Modi et al., 2009; Ibrahim et al., 2011; Tabarestani and Tehrani, 2014).

Consequently, some non-meat ingredients have been examined as adjuncts to enhance the quality of meat products during manufacturing (Giese, 1992; Mansour and Khalil 1997; Caprioli et al., 2009; Özen et al., 2011). Especially, when adding different gums at various concentrations in meat batter indicated that the product quality can also be changed (Ulu, 2006; Lopes et al., 2015). Their high water-binding ability is the major functional property of food product. This property affects the texture, enhancement of colour and sensory properties of meat products. Moreover, they are non-allergic and have good mechanical properties and they have wide applications in food production as thickening, stabilizing, gelling, and emulsifying agents (Ulu, 2006; Kilincceker et al., 2009).

It has been found that the addition of gums in meat patty formulations improves the cooking characteristics and also decreases mass transfer and diameter reduction, which results in high yield and improves the thickness of products after frying or cooking (Ulu, 2006; Demirci et al., 2014).

However, compared to the studies on using of different gums in meat patties, studies of other hydrocolloids are many. Thus, the objective of this study was to evaluate the effects of guar gum, xanthan gum and gum Arabic on some frying properties of meat patties and to provide different alternative to consumer.

2. Materials and methods

2.1. Materials

In this study, guar gum, xanthan gum, and gum Arabic were purchased from Kimbiotek Co. (Istanbul, Turkey). Lean beef meat and beef back fat were obtained from local meat seller in Adıyaman. Corn oil was used as frying medium (Yudum, Yudum Co., Balıkesir, Turkey). A mini fryer (Arzum, AR 247) that has a thermostatic heat control was used for carrying out deep frying operations.

2.2. Preparation of meat patties

Meat and fat were cut into 2-3 cm^3 and kept at -18 ^oC until the meat patty production. Then, they were thawed at +4 ^oC and minced using mincing machine, separately. The minced meat was used to prepare the meat batter according to following receipt:

Meat batter was consisted of 87.5% mince, 10% fat, 1.5% salt, 0.5% red pepper, and 0.5% black pepper.

Then, nine different treatments were prepared by adding 0.5%, 1%, and 1.5% gums to the formulation and kneaded and kept at +4 ^oC for up to 45 min. Control did not contain gum. Batters were re-kneaded and each 27 g of batter was shaped with silicone moulds into 14 mm thick and 48 mm diameter circular-shaped patties. Then, patties was used to perform of some frying characteristics at 180 ^oC during 2 min.

2.3. Determination of the frying yield and diameter reduction

Frying yield and diameter reductions after frying were obtained as follows:

$$\text{Frying yield (\%)} = \frac{\text{fried patty weight}}{\text{raw patty weigt}} \text{ x } 100 \quad (1)$$

$$\text{Diameter reduction (\%)} = \frac{\text{raw patty diameter} - \text{fried patty diameter}}{\text{raw patty diameter}} \text{ x } 100 \quad (2)$$

2.4. Determination of the moisture, fat, and moisture retention

Moisture contents of raw and fried samples were determined by oven air method at 105±2 ^oC and fat contents were determined by using soxhlet extraction method (AOAC, 2002). Moisture retention was calculated according to following equation.

$$\text{Moisture retention (\%)} = \frac{\text{moisture in fried patty (\%)}}{\text{moisture in raw patty (\%)}} \text{ x frying yield} \quad (3)$$

2.5. Instrumental colour analysis

Surface colour values of meat patties were measured by using a portable colorimeter (Minolta CR-400, Osaka, Japan) after frying processes for fried samples. The instrument was standardised against a white standardisation plate before each measurement. The colour was maintained according to CIELAB systems as *L* (lightness), *a* (redness) and *b* (yellowness) values, as described by Dogan (2006). Four meat patties were used for the analysis of each treatment. Four measurements were taken for each sample.

2.6. Sensory analysis

The fried patties were coded after 5 min and served in a random order. Ten semi-trained judges assessed the sensory properties using a hedonic scale for the appearance, colour, odour, flavour, and texture scores. The average score of these parameters be deemed to the overall acceptability. Different values in the scale indicated the following reactions: 1: extreme dislike, 2: very much dislike, 3: moderate dislike, 4: slight dislike, 5: neutral, 6:

like slightly, 7: like moderately, 8: like very much, 9: like extremely (Gokalp et al., 1999).

2.7. Statistical analysis

Conventional statistical methods were used to calculate means. The measurements were repeated twice with three replications. Collected data were subjected to statistical analysis using JMP version (JMP version 9.0.2 (SAS Institute, Inc., Cary, USA). Analysis of variance (ANOVA) was used to evaluate effect of gum type and gum concentration on some physical, chemical and sensory properties. Least Significant Differences (LSD) test was used to determine if the effects of factors on the studied parameters were significant ($P < 0.05$).

3. Results and discussions

The frying yields and diameter reductions are shown in table 1. Generally, gums had similar effects on yield and diameter reductions at the low levels. However, guar and xanthan caused higher yield than gum Arabic at high levels. In addition, xanthan gum decreased the diameter reductions of samples at high levels. Addition of gum increased frying yields whereas decreased diameter reductions compared to control. The highest frying yields were calculated as 74.58% and 74.38% in samples with 1.5% guar and xanthan gum. The lowest diameter reductions were in sample with 1% and 1.5% levels of xanthan gum (14.74% and 13.86%). Colour values were presented in table 1. According to results, gum Arabic caused high *L* values on meat patties. However, it had similar results to the *L* values on control. *L* values increased with more gum addition. The highest *L* values were on control and all of samples with gum Arabic (in the range of 26.13-28.04). *a* values of samples were generally similar on samples with gums that are lower than control. However, it was low at 0.5% level of xanthan (4.80). Control, 0.5%, and 1.5% gum Arabic had higher *a* values than other levels in fried samples. Control, xanthan, and gum Arabic increased the *b* values compared to guar gum whereas addition of gum did not affect this colour value. 1% and 1.5% levels of gum Arabic had higher *b* values as 8.32 and 8.31 than other treatments (Table 1).

Table 1. Effect of gum type and concentration on yield, diameter reduction, and colour values of fried beef patties

	Gum type	**Gum concentration**			
		Control (0%)	0.5%	1%	1.5%
Yield (%)	Guar	68.31^{aBC}	66.36^{aC}	70.84^{bB}	74.58^{aA}
	Xanthan	68.31^{aB}	67.19^{aB}	73.93^{aA}	74.38^{aA}
	Gum Arabic	68.31^{aBC}	66.15^{aA}	67.24^{cA}	66.26^{bA}
Diameter Reduction (%)	Guar	20.92^{aA}	18.51^{aA}	19.71^{aA}	16.88^{abA}
	Xanthan	20.92^{aA}	21.63^{aA}	14.74^{bB}	13.86^{bB}
	Gum Arabic	20.92^{aA}	17.70^{aC}	18.54^{abBC}	19.48^{aB}
L	Guar	26.13^{aA}	23.64^{bC}	23.77^{aC}	24.65^{abB}
	Xanthan	26.13^{aA}	20.20^{cC}	22.51^{aBC}	24.37^{bAB}
	Gum Arabic	26.13^{aA}	27.36^{aA}	27.25^{aA}	28.04^{aA}
a	Guar	6.57^{aA}	5.66^{aB}	5.83^{aB}	5.82^{aB}
	Xanthan	6.57^{aA}	4.80^{bC}	5.86^{aB}	5.71^{aB}
	Gum Arabic	6.57^{aA}	6.08^{aAB}	5.57^{aB}	6.11^{aAB}
b	Guar	6.95^{aA}	6.44^{bA}	6.49^{bA}	6.98^{aA}
	Xanthan	6.95^{aA}	6.71^{abA}	7.41^{abA}	7.67^{aA}
	Gum Arabic	6.95^{aA}	7.81^{aA}	8.32^{aA}	8.31^{aA}

$^{a-c}$ Within each column, different superscript lowercase letters show differences between the gum types within each concentrate ($P < 0.05$); $^{A-C}$ Within each row, different superscript uppercase letters show differences between the concentrations within each gum ($P < 0.05$).

Moisture and fat rations of raw and fried patties were shown in table 2. Also, moisture retentions that calculated from these were presented in this table. Control and gum Arabic increased the moisture rations of raw samples whereas addition of gum decreased this value. The highest moisture values for raw samples were determined in sample with control and 0.5% gum Arabic as 64.50% and 64.70%. Gum Arabic more decreased the fat rations in raw sample than with guar and xanthan gum. Especially, samples with levels of 0.5% and 1.5% gum Arabic had lower fat rations than other samples as 12.46% and 12.36%. Generally, addition of gum decreased the fat rations in raw meat patties. As in raw sample, moisture rations of fried samples with gum Arabic and control generally higher than other fried patties. However, moisture increased at 1.5% level of guar gum in patties (56.25%). Addition of gum increased the moisture rations in fried sample with guar and gum Arabic whereas caused fluctuation change in with xanthan gum. Guar gum and gum Arabic more decreased the fat rations in fried meat patties. Also, addition of gum decreased fat rations in these samples. The lowest fats were determined in sample with 1.5% guar and 0.5% gum Arabic as 11.31% and 12.32%, respectively. Moisture retentions affected from types and addition of gums. Especially, guar and xanthan caused good results in fried samples. Generally, moisture retention increased with more gum addition in fried samples. This values for 1.5% levels of guar gum and xanthan gum in meat patties were determined to be higher compared with other treatments (Table 2).

Table 2. Effect of gum type and concentration on moisture, fat, and moisture retention values of fried beef patties (%)

		Gum type	**Gum concentration**			
			Control (0%)	0.5%	1%	1.5%
Raw samples	Moisture	Guar	64.50^{aA}	63.72^{bA}	63.63^{aA}	61.47^{aA}
		Xanthan	64.50^{aA}	64.08^{bA}	63.78^{aA}	60.58^{aB}
		Gum Arabic	64.50^{aA}	64.70^{aA}	62.20^{aB}	61.41^{aB}
	Fat	Guar	14.37^{aA}	14.56^{aA}	14.25^{aA}	13.77^{aA}
		Xanthan	14.37^{aA}	13.64^{abAB}	12.95^{aB}	13.32^{aAB}
		Gum Arabic	14.37^{aA}	12.46^{bB}	13.70^{aA}	12.36^{aB}
Fried samples	Moisture	Guar	54.76^{aB}	52.53^{bC}	54.73^{bB}	56.25^{aA}
		Xanthan	54.76^{aA}	48.65^{cC}	53.19^{cAB}	52.57^{bB}
		Gum Arabic	54.76^{aB}	56.50^{aA}	56.54^{aA}	55.79^{aAB}
	Fat	Guar	13.73^{aAB}	14.68^{aA}	12.48^{bBC}	11.31^{bC}
		Xanthan	13.73^{aB}	16.27^{aA}	16.78^{aA}	16.98^{aA}
		Gum Arabic	13.73^{aA}	12.32^{bB}	12.77^{bAB}	12.86^{bAB}
Moisture retention		Guar	58.00^{aBC}	54.71^{abC}	60.95^{aB}	68.24^{aA}
		Xanthan	58.00^{aB}	51.02^{bC}	61.65^{aAB}	64.56^{abA}
		Gum Arabic	58.00^{aA}	57.77^{aA}	61.13^{aA}	60.21^{bA}

$^{a-c}$ Within each column, different superscript lowercase letters show differences between the gum types within each concentrate ($P < 0.05$); $^{A-C}$ Within each row, different superscript uppercase letters show differences between the concentrations within each gum ($P < 0.05$).

Data in table 3 indicate that fried meat patties formulated with gums were affected from types and addition of gum for appearance, colour, texture, and overall acceptability whereas they were not affected for odour. In addition, taste scores were not affected from the addition of gums. Control and samples with gum Arabic had higher appearance than other treatments. Addition of gum increased the appearance values in sample with gum Arabic. Generally, addition of guar and xanthan decreased this value compared to control (Table 3). Control and all level of gum Arabic caused higher appearance scores than other samples (in the range of 7.00-7.90). Colour scores on control and samples with guar and gum Arabic were high. Addition

of gum did not affect this value for guar and gum Arabic whereas increased for xanthan. 1.5% guar, and 0.5, 1, and 1.5% gum Arabic caused the higher colour scores than other treatments (in the range of 7.20-7.50). Taste scores increased with guar gum and gum Arabic whereas addition of gum did not affect this value. The highest values were in sample with 1.5% guar (7.00) and all level of gum Arabic (in range of 7.00-7.80). Textures of fried patties with guar gum, gum Arabic and control were better than xanthan whereas this value did not affected from addition of gum. All level of guar, gum Arabic, and control were similar, statistically (in range of 6.30-7.40). Generally, overall acceptability values were higher in samples with guar, gum Arabic and control than xanthan. Addition of gum did not affect the overall acceptability for sample with guar and gum Arabic. The highest results were in sample with 1.5% guar, all level of gum Arabic, and control as 6.86, 6.88, 7.28, 7.34, and 6.48 (Table 3).

Table 3. Effect of gum type and concentration on sensory properties of fried beef patties

	Gum type	**Gum concentration**			
		Control (0%)	0.5%	1%	1.5%
Appearance	Guar	7.00^{aA}	6.50^{aB}	6.00^{bC}	6.40^{bBC}
	Xanthan	7.00^{aA}	3.30^{bB}	3.40^{cB}	3.80^{cB}
	Gum Arabic	7.00^{aB}	7.30^{aB}	7.90^{aA}	7.80^{aA}
Colour	Guar	6.60^{aA}	6.20^{aA}	6.50^{aA}	7.20^{abA}
	Xanthan	6.60^{aA}	4.60^{bC}	5.10^{bBC}	5.80^{bAB}
	Gum Arabic	6.60^{aA}	7.20^{aA}	7.50^{aA}	7.40^{aA}
Odour	Guar	5.70^{aA}	5.30^{aA}	6.40^{aA}	6.30^{aA}
	Xanthan	5.70^{aA}	4.80^{aA}	4.90^{aA}	4.80^{aA}
	Gum Arabic	5.70^{aA}	6.00^{aA}	6.60^{aA}	6.50^{aA}
Taste	Guar	5.80^{aA}	6.60^{abA}	5.80^{bA}	7.00^{aA}
	Xanthan	5.80^{aA}	5.40^{bA}	5.20^{bA}	4.40^{bA}
	Gum Arabic	5.80^{aA}	7.00^{aA}	7.70^{aA}	7.80^{aA}
Texture	Guar	7.3^{aA}	6.40^{aA}	6.30^{abA}	7.40^{aA}
	Xanthan	7.3^{aA}	4.60^{bB}	4.90^{bB}	4.80^{bB}
	Gum Arabic	7.3^{aA}	6.90^{aA}	6.70^{aA}	7.20^{aA}
Overall acceptability	Guar	6.48^{aA}	6.20^{aA}	6.20^{bA}	6.86^{aA}
	Xanthan	6.48^{aA}	4.54^{bB}	4.70^{cB}	4.72^{bB}
	Gum Arabic	6.48^{aA}	6.88^{aA}	7.28^{aA}	7.34^{aA}

$^{a-c}$ Within each column, different superscript lowercase letters show differences between the gum types within each concentrate ($P < 0.05$); $^{A-C}$ Within each row, different superscript uppercase letters show differences between the concentrations within each gum ($P < 0.05$).

Discussions

The frying yield and diameter reduction are important factors for manufacturers. They are results of the deneration of meat protein. They affect the economic profit and packaging system. Gelatinization and water holding capacity of gums affect them (Ulu, 2006; Demirci et al., 20014). Modi et al. (2009) determined that carrageenan gum increase frying yields and decrease diameter reductions of meat kofte. Also, they found that addition of carrageenan more increase the frying yields and decrease diameter reductions. They said that this could be due to the binding property of carrageenan which caused to form complex with water and protein. Thus, they decrease the mass transfer and retain the shape of kofte. In our study, guar gum and xanthan gum shown similar effect in this study. Gibis et al. (2015) found that moisture loss decrease with increasing addition of microcrystalline cellulose from 0.5% to 3% in fried beef patties. In another study, beef burgers were produced with *Aloe vera* in the range of 0-5% and their properties were determined. Consequently,

Aloe vera acts as a hydrocolloid and decreased cooking losses and diameter reductions of burgers. Also, it determined that increase of concentration of *Aloe vera* contributed to decrease of cooking loss and diameter reductions. They reported that increasing of yield and diameter resulted from the high water holding capacity and moisture retention of *Aloe vera* during cooking (Soltanizadeh and Ghiasi-Esfahani, 2015). Colour values are important factors in consumer choice. They can be affected by the ingredients in patties and the frying process. As in our study, Demirci et al. (2014) reported that *L* values of meatballs affected from gum types and increased with the gum addition in formulations. Also, Yasarlar et al. (2007) said that *L* values of cooked samples resulted differently in cooked meatballs with various cereal bran and increased with more bran addition on samples. Khalil (2000) determined that low-fat patties formulated with starch/water combinations had lower red colours compared with the control. Yasarlar et al. (2007) found that *a* value of cooked meatballs affected from type of cereal bran and decreased with more bran addition. Demirci et al. (2014) reported that meat ball redness decreased and yellowness increased with more gum addition in cooked samples whereas *a* and *b* values changed depending on gum types. In addition, similar findings were also reported by Lin and Huang (2003) and Yılmaz and Dağlıoğlu (2003) also.

Moisture ration of low level of gum Arabic in raw meat patties was higher than other and this can be connected with water binding of it. The level of moisture in the raw samples decreased when the level of xanthan and gum Arabic increased in meat patties. Accordingly, at 0.5% level of gum Arabic and addition of xanthan and gum Arabic decreased the fat rations in raw samples compared to control. Similar findings have also been previously reported for various types of meat products such as patties (Khalil, 2000; Yilmaz and Dağlıoğlu, 2003; Yasarlar et al., 2007; Demirci et al., 2014).

In fried patties, lower moisture values of samples with xanthan were due to more friable structure than with guar and gum Arabic that was also observed by the panellist during work. Demirci et al. (2014) also found that a reduction in the moisture of cooked meatballs formulated with xanthan gum compared to guar, carrageenan, and locust bean gums. Especially, addition of guar gum increased the moisture rations in fried samples whereas samples with gum Arabic did not affected from addition gum. As the raw patties, this resulted from water binding ability of guar and gum Arabic. The gums form a firm matrix and prevent the migration of moisture from fried food and the penetration of fat in it during frying. Also, fat can be partly replaced by water and non-meat ingredients such as the gums (Demirci et al., 2014). Guar and gum Arabic had lower fat values because of high moisture rations in fried samples. Especially, high level of these gums prevented the moisture loss of fried patties and decreased fat absorption. Friable structure of samples with xanthan also increased moisture loss and fat absorption inside it during frying. Similarly, Yilmaz (2004) reported that the most effective method in lowering calorie content is decreasing fat content in meat products. In addition, he found that addition of rye bran at the level of 20% in meat balls resulted in a significant reduction in fat content compared with 5%, 10%, and 15% levels. Our results agree with those reported by Mansour and Khalil (1997) and Soltanizadeh and Ghiasi-Esfahani (2015), who reported significantly decreased fat contents for beef burgers with added dietary fibre and *Aloe Vera* in different rations to reduce the fat content, respectively.

Sensory properties have also effect on the attractiveness of foods and consumer preference such as colour attributes. Thus, they should be determined in the new product. Control and all of samples with gum Arabic had higher appearance scores than other patties. This can be connected with *L* values of samples that were higher than with guar and xanthan. As the appearance, colours can be also affected from *L* values of the patties. Increasing of lightness provided a bright colour formation and raised preferential ability of the product visually. Decrease of appearance and colour with addition of guar gum may be resulted from decrease of *L* and *a* values on these patties. They created a dark colour on meat patties. Increasing of taste

scores in sample with 1.5% guar and all of samples with gum Arabic may be resulted with fat values of these patties. Generally, fat rations of these samples lower than other fried samples. Consequently, low rations of fat reduced the formation of a heavy fatty taste which was noted by panellist. Textures of control and sample with guar and gum Arabic were very good compared to samples with xanthan. Textures of these samples may be improved with moisture ration of fried patties. Especially, 1.5% guar and all of samples with gum Arabic contained higher moisture compared to other patties. Moisture of these samples caused to form juicy and softer structure and increased texture scores. Decreasing of texture with addition of xanthan connected with their friable structure. Similar results were determined by Demirci et al. (2014) for meatballs containing different gums, Ibrahim et al. (2011) for chicken burger containing maltodextrin and potato starch and Yilmaz (2005) for low-fat meatballs prepared with wheat bran. In addition, Mansour and Khalil (1997) reported that the addition of wheat fibres can be used to make acceptable and desirable low fat beef burgers. The overall acceptability values supported the sensory properties of meat patties and high sensory scores caused the increasing of this value. Yilmaz (2004), and Mansour and Khalil (1997) also found similar results in their studies.

4. Conclusions

The study shows that the use of gums can enhance the quality of beef meat patties during frying. As a result, the performance of gums on patties increased with the addition of their more amounts. Especially, 1.5% guar and all levels (0.5, 1, and 1.5%) of gum Arabic are suitable alternative to produce good quality meat patties with better sensory acceptability for frying processes. Therefore, the addition of these gums and the specified levels in meat patties are more advantageous than other treatments during manufacturing.

5. References

AOAC, (2002). *Offical methods of analysis* (17th ed.). Association of Official Analytical Chemists, Washington.

Caprioli, I., Q'Sullivan, M., Monahan, F.J. (2009). Use of sodium caseinat/glycerol edible films to reduce lipid oxidation in sliced turkey meat. *European Food Research Technology*, 228(3), 433-440.

Colmenero, E.L., Barreto, G., Fernandez, P., Carballo, J. (1996). Frozen storage of bologna sausages as a function of fat content and levels of added starch and egg white. *Meat Science*, 42(3), 325-332.

Demirci, Z.O., Yılmaz, I., Demirci, A.Ş. (2014). Effects of xanthan, guar, carrageenan and locust bean gum addition on physical, chemical and sensory properties of meatballs. *Journal of Food Science and Technology*, 51(1), 936-942.

Dogan, I.S. (2006). Factors affecting wafer sheet quality**.** *International Journal of Food Science and Technology*, 41(5), 569-576.

Gibis, M, Schuh V, Weiss, J. (2015). Effects of carboxymethyl cellulose (CMC) and microcrystalline cellulose (MCC) as fat replacers on the microstructure and sensory characteristics of fried beef patties. *Food Hydrocolloids*, 45(2), 236-246.

Giese, J. (1992). Developing low-fat meat products. Food Technology 46(4), 100-108.

Gokalp, H. Y., Kaya, M., Tulek, Y., Zorba, O. (1999). Laboratory application guide and quality control in meat and meat products (In Turkish). Atatürk Üniversitesi Ziraat Fakültesi, Yay No: 318, Erzurum, Turkey.

Ibrahim, M.A., Salama, M.F., Hussein, A.A. (2011). Production of low-fat chicken burger. *Australian Journal of Basic and Applied Science*, 5(12), 3149-3154.

Khalil, A.H. (2000). Quality characteristics of low-fat beef patties formulated with modified corn starch and water. *Food Chemistry*, 68(1), 61-68.

Kilincceker, O., I.S. Dogan, Kucukoner, E., (2009). Effect of edible coatings on the quality of frozen fish fillets. *LWT- Food Science and Technology*, 42(4), 868-873.

Lopes, B.M., Lessa, V.L., Silva, B.M., Filho, M.A., Schnitzler, E., Lacerda, L.G. (2015). Xanthan gum: properties, production conditions, quality and economic

perspective. *Journal of Food and Nutrition Research*, 54(3), 185-194.

Lin, K.W., Huang, H.Y. (2003). Konjac/gellan gum mixed gels improve the quality of reduced fat frankfurters. *Meat Science*, 65(2), 749-755.

Mansour, E.H. and Khalil, A.H. (1997). Characteristics of low-fat beef burger as influenced by various types of wheat fibers. *Food Research International*, 30(3), 199-205.

Modi, V.K., Yashoda, K.P., Naveen, S.K. (2009). Effect of carrageenan and oat flour on quality characteristics of meat *kofta*. *International Journal of Food Properties*, 12(1), 228-242.

Özen, B.Ö., Eren, M., Pala, A., Özmen, İ., Soyer, A. (2011). Effect of plant extracts on lipid oxidation during frozen storage of minced fish muscle. *International Journal of Food Science and Technology*, 46(4), 724-731.

Soltanizadeh, N., Ghiasi-Esfahani, H. 2015. Qualitative improvement of low meat beef burger using *Aloe vera*. *Meat Science*, 99(1), 75-80.

Tabarestani, H.S., Tehrani, M.M. (2014). Optimization of physicochemical properties of low-fat hamburger formulation using blend of soy flour, split-pea flour and wheat starch as part of fat replacer system. *Journal of Food Processing and Preservation*, 38(1), 278-288.

Ulu, H. (2004). Effect of wheat flour, whey protein concentrate and soya protein isolate on oxidative process and textural properties of cooked meatballs. *Food Chemistry*, 87(4), 523-529.

Ulu, H. (2006). Effects of carrageenan and guar gum on the cooking and textural properties of low fat meatballs. *Food Chemistry*, 95(4), 600-605.

Yasarlar, E.E., Daglioglu, O., Yilmaz, I. (2007). Effects of cereal bran addition on chemical composition, cooking characteristics and sensory properties of Turkish meatballs. *Asian Journal of Chemistry*, 19(3), 2353-2361.

Yilmaz, I. (2004). Effects of rye bran addition on fatty acid composition and quality characteristics of low-fat meatballs. *Meat Science*, 67(2), 245-249.

Yilmaz, I., (2005). Physicochemical and sensory characteristics of low fat meatballs with added wheat bran. *Journal of Food Engineering*, 69(3), 369-373.

Yılmaz, I., Dağlıoğlu, O. (2003). The effect of replacing fat with oat bran on fatty acid composition and physicochemical properties of meatballs. *Meat Science*, 65(2), 819-823.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

STRUCTURE PROPERTIES OF STIRRED YOGHURT MADE WITH TRANSGLUTAMINASE AND AMARANTH

Shleikin A.G. [1], Zipaev D.V. [2], .Zhilinskaya N.T.[1], Barakova N.V. [1], Danilov N.P. [1]*, Argymbaeva A.E. [1]

[1]ITMO University, Institute of Refrigeration and Biotechnologies, Department of Chemistry and Molecular Biology, St. Petersburg, Russia

[2]Samara State Technological University, Department of Food Technology, Samara, Russia

*Corresponding author: *danilovn2005@ya.ru*

Article history:
Received:
13 February 2016
Accepted in revised form:
06 May 2016

Keywords:
Stirred yoghurt;
transglutaminase;
protein cross-linking;
amaranth

ABSTRACT

The aim of this work was to investigate structure changes in stirred yoghurt manufacture. Stirred yoghurts were made from UHT milk subjected to heat treatment at 90 °C for 5 min. After cooling to 40 °C, amaranth flour and transglutaminase (TG) were added and mixed. Lyophilized concentrated starter culture was added in amount of 1 %. Milk was fermented until pH 4.6 – 4.7. Amaranth flour was added to sour milk as protein additive for nutritive value increasing and structure modification. TG enzyme well-known for its binding ability was added to catalyze protein network formation. It was shown viscosity decreases when amaranth flour concentration increases. Use of TG allows increasing yoghurt viscosity and therefore preventing the negative effect of polysaccharides containing in amaranth flour on the viscosity. TG contributes to a smoother yoghurt surface formation. TG binding effect was confirmed electrophoretically. All samples were judged as organoleptically acceptable. The data obtained indicate the possibility of applying amaranth flour and TG for stirred yoghurt manufacture.

1.Introduction

There are various additives for dairy products functionality. It was studied influence of fruit components on polyphenols and antioxidant activity (Oliveira et al., 2015; Chouchouli et. al, 2013) and on rheological properties (El-Said et al., 2014) of yoghurts. It is reported (Sun-Waterhouse et al., 2013) blackcurrant polyphenols can be added to drinking yoghurt pre- or post-fermentation resulting in higher yoghurt viscosity.

Viscosity is a quality attribute of drinking yoghurts. To alter rheological properties of final product we suggest using milk fortification with plant proteins concomitantly with TG enzyme action to form protein network in yoghurt manufacture (Argymbaeva et al., 2015; Shleikin et al., 2015). Earlier, we have also conducted research aimed at processing of set yogurt with amaranth and transglutaminase (Shleikin and Danilov, 2015). Also we showed possibility of TG utilization for whey protein – gluten combining products manufacture to prevent gluten toxity for celiac patients nutrition (Shleikin et al., 2011).TG (EC 2.3.2.13) is an enzyme widely used in food applications due to ability to cross-link different proteins and impact on products rheological properties (Gaspar and de Góes-Favoni, 2015). TG addition improves quality attributes both set and stirred yoghurt (Cancinoa et al., 2006). TG affects significantly on the viscosity of stirred yoghurt, especially in the low-shear rate region and at small deformations (Jaros et al., 2007). It is stated TG catalyzed formation of casein intramicellar cross-links (Mounsey et al. 2005; O'Sullivan et al. 2002; Vasbinder et al. 2003). It was noted higher viscosity and degree of protein polymerization if use TG for stirred

yoghurts (Tabari, 2010). Methods of TG utilization in stirred yoghurt production and their impact on yoghurts shear stress are shown in study (Iličić et al., 2008).

Thermal treatment of milk (95°C for 5 min) allows denaturing the whey proteins and inducing interactions between the κ-casein, β-lactoglobulin and α-lactalbumin, therefore the hydrophilic properties of the coagulum and the stability of the yoghurt gel increase (Tamime and Robinson, 1988). In stirred yoghurt, milk is fermented in a tank, the yoghurt gel being broken up during the stirring, cooling and packaging stages. The rheological properties of stirred yoghurt may be changed due to several factors. These have physical nature related with total solid content, milk composition and type of starter culture, or processing conditions-related, such as homogenisation, thermal pre-treatment of the milk and post-incubation stages including: stirring, pumping, cooling and packaging (Afonso and Maia, 1999).

Food fortification is one of the most important processes for improvement of the nutrients quality and quantity in food. Yoghurt can be fortified with vitamins, calcium, iron, fibers e t.c. (Hadi et al., 2015). We suggest to fortify stirred yoghurt with amaranth flour addition. Amaranth is technically a pseudo-cereal. Amaranth seeds are gluten-free foodstuff important for celiac disease patients nutrition (Mlakar et al., 2009). Amaranth is gaining popularity due to its nutritional excellence and agronomic features. Amaranth contains high protein amounts, up to 35% more than rice, oats and wheat flour. In addition, the protein present in amaranth flour is complete and it contains all the vital amino acids in contrast with other protein sources (Chauhan et al., 2015). Amaranthus seeds were added to bio-yoghurt to increase the levels of starch and dietary fibre (Sady et al., 2005). In addition, we suppose to study milk and amaranth protein binding in our experiments; therefore, amaranth should be added prior to fermentation simultaneously with TG.

2.Materials and methods

UHT milk (Pyatigorsk dairy plant, Pyatigorsk, Russia) with protein content of 2.8 % and fat content of 3.2 % was used. Yoghurt starter culture was AiBi 22.11 R3 (lyophilized concentrated starter culture of Streptococcus thermophilus, Lactobacillus bulgaricus – $5.0*10^{10}$ CFU/g) obtained from Green Lines Ltd, Krasnogorsk, Russia. TGase preparation was presented by "Next Ingredients" company (Moscow, Russia) had the activity of 100 U/g. Amaranth flour (Russian Olive Ltd, Voronezh, Russia) with high protein concentration (30 % of protein, 10 % of fats, 40 % of carbohydrates) was used as vegetable additive.

2.1. Yoghurt preparation

The milk was heated to 90 °C, kept at this temperature for 5 min, then cooled to fermentation temperature of 40 °C, was poured into the samples. Amaranth flour was added in the amount of 1 %, 2 %, 3 % and stirred until the formation of homogeneous mass. Then TG was added into the samples in an amount of 0.5 U/g, 1 U/g, 2 U/g and stirred for 5 min. Starter culture was added in an amount of 1 % and stirred. The prepared samples were put into thermostat at 40 °C for 7 hours until the pH 4.6 to 4.7. The finished product was cooled to 4 °C. Samples were numbered accordingly to table 1.

Table 1. Yoghurt samples labeling.

Amaranth flour concentration, % w/v	TG concentration, U/g			
	0	0.5	1	2
0	1	2	3	4
1	5	6	7	8
2	9	10	11	12
3	13	14	15	16

2.2. Viscosity measurement

The yoghurt samples were heated to a temperature of 25 °C. Viscosity measurements were carried out on a rotational viscometer Rheotest RN4.1 (Rheotest Medingen GmbH, Medingen, Germany) within 30 s, a shear gradient of 100 s^{-1}, used spindle S1. Viscosity – time curves were received and analyzed using "Rheo-42" and "Excel" software.

2.3. Texture Profile Analysis

The yoghurt samples of 0.1 L were cooled to 4 °C. Texture analysis was performed using a TA-XT Plus texture analyzer with a load cell of 5 kg (Stable Micro Systems Ltd., UK). The samples were subjected to a compression test to construct the Texture Profile Analysis graphs, using 5 mm Cylinder Probe. The parameters were as follows: pretest speed of 3 mm/s; test-speed of 1 mm/s and post-test speed of 3 mm/s; distance of 20 mm; temperature of 4 °C; and force of 1 g.

Force–time (g–s) curves were recorded and analyzed using the Texture Exponent Application (Stable Micro Systems Ltd, UK) and softness was calculated. The results were calculated as the average of three measurements taking into account the standard deviation.

2.4. The study of the microstructure of the yoghurt surface

A few drops of yoghurt samples were applied on an object glass, rubbed to a thin layer. The structure changes were estimated with the MC100 microscope (Micros HgmbH, St. Veit/Glan, Austria). Magnification 40x and 100x was used.

2.5. Electrophoretic studies

To yoghurt sample of 100 µl Tris/HCl buffer of 100 µl with pH = 8.7 was added. Then SDS (3 % solution) was added in amount of 150 µl and prepared solution was left for 30 min. at room temperature. Then distilled water of 650 µl was added and mixture was held at 100 °C for 5 min. Bromophenol blue 1 % solution was added in amount of 100 µl. Ready solution of 3 µl was applied into the wells on the gel surface. Gel was made as follows: 5 ml of 0.2 M Tris/HCl buffer mixed with 5 ml of 12.5 % acrylamide solution. TEMED of 14 µl was added. The reaction mixture was purged with argon. SDS was added 10 mg per 10 ml of solution, potassium persulfate in amount of 0.2 ml of solution with a concentration of 7 mg/ml. The resulting solution was applied to the openings between the plates for electrophoresis. The formation of a gel was observed. Electrophoresis was carried out on the Flat Bed Apparatus FBE-3000 (Pharmacia, Uppsala, Sweden). The apparatus was filled with 450 ml of 0.1 M Tris/HCl buffer containing 0.1% SDS. The electric field intensity was 30 V/cm. The voltage U = 300 V, current I = 100 mA, power W = 40 watts. Gels were stained with 0.2 % Coomassie R-250 in mixture of 7.5 % v/v of acetic acid, 25 % v/v of methanol and 67.5 % v/v of water. Gels were photographed and quantified using ImageJ (National Institutes of Health, Bethesda, Maryland, USA) software.

2.6. Sensory analysis

Sensory analysis was performed by a panel of 5 trained assessors. The samples were served at the 12 °C in 50 g of cups. Mean scores for each attributes were used for comparison of the samples. Whey separation ("syneresis"), "color," "firmness", "flavor intensity", "distribution of ingredients", "acidity", and "sweetness," were rated using a 10-point scale with the end values labeled as "weak" and "strong" where 10 was as higher point. The sensory sets of data were subjected to a one-way ANOVA using Minitab 16 (Minitab Ltd., Coventry, UK) software, to establish whether the sensory scores differ. The means were compared using Fisher's least significant difference test and the statistical significance was determined at $P < 0.05$.

3.Results and discussion

The effect of additives on the viscosity of yoghurt

The yoghurt samples were prepared in accordance with p. 2.1. Viscosity analysis was conducted in accordance with p.2.2. The viscosity of yoghurts with the addition of amaranth flour, without the addition of TG, depending on the time of rotation of the rotor is presented in Figure 1.

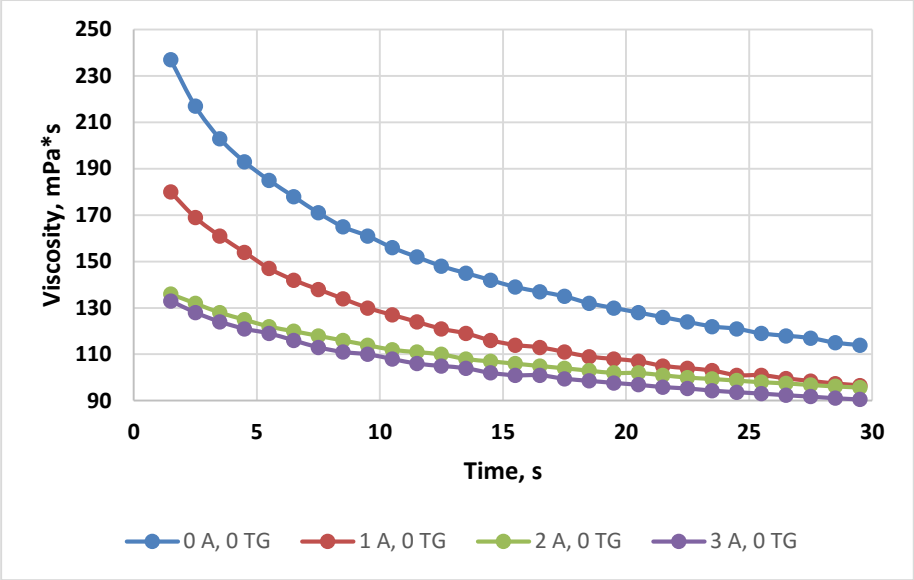

Figure 1. The viscosity of yoghurts with the addition of amaranth flour, without the addition of TG, depending on the time of rotation of the rotor

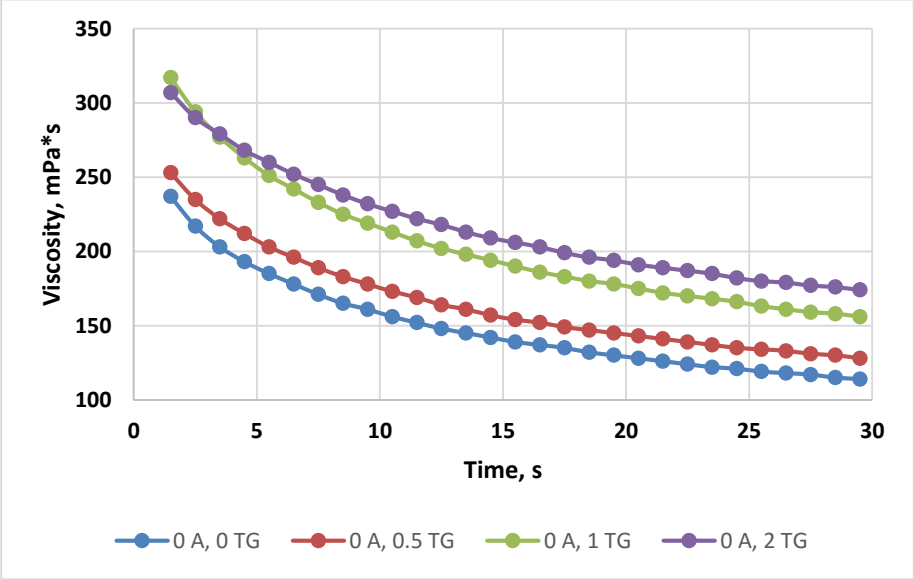

Figure 2. The viscosity of yoghurts with the addition of TG, without the addition of amaranth flour, depending on the time of rotation of the rotor.

Figure 1 shows the decrease of viscosity curves in the plane graph with increasing concentration of amaranth flour. Thus, the increase in the concentration of amaranth flour leads to a decrease of the viscosity of the finished product. In Figure 2 was shown the dependence of the viscosity of yoghurts with the addition of TG, without the addition of amaranth flour, from time of rotation of the rotor.

It can be seen from Figure 2 that the increase in TG concentration shifts the viscosity curves in the upper part of the graph. The use of TG, therefore, allows to increase the viscosity of the final product. The dependence of the viscosity of the yoghurts on the concentration of amaranth flour is presented in Figure 3.

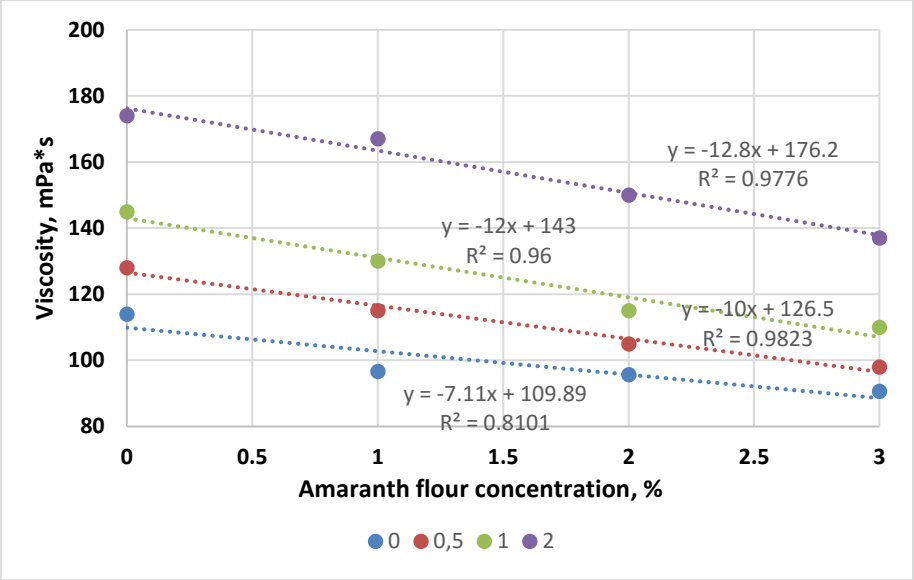

Figure 3. The viscosity of yoghurts with the addition of TG (see legend 0, 0.5, 1, 2 U/g) depending on the concentration of amaranth flour.

As can be seen from Figure 3, the use of amaranth flour reduces the viscosity of yoghurt. The dependencies are described by linear equations of the form $y = kx + b$, where k is the angle of inclination of a straight line, characterizes the rate of change of the dependent variable. As can be seen from the graphs Figure 3, the rate of the yoghurt viscosity change depending on the concentration of amaranth flour ranges from -7 to -12 mPa*s/% at concentrations of amaranth flour 0 – 3% w/v.

The dependence of the viscosity of the yoghurts on the concentration of TG is presented in Figure 4.

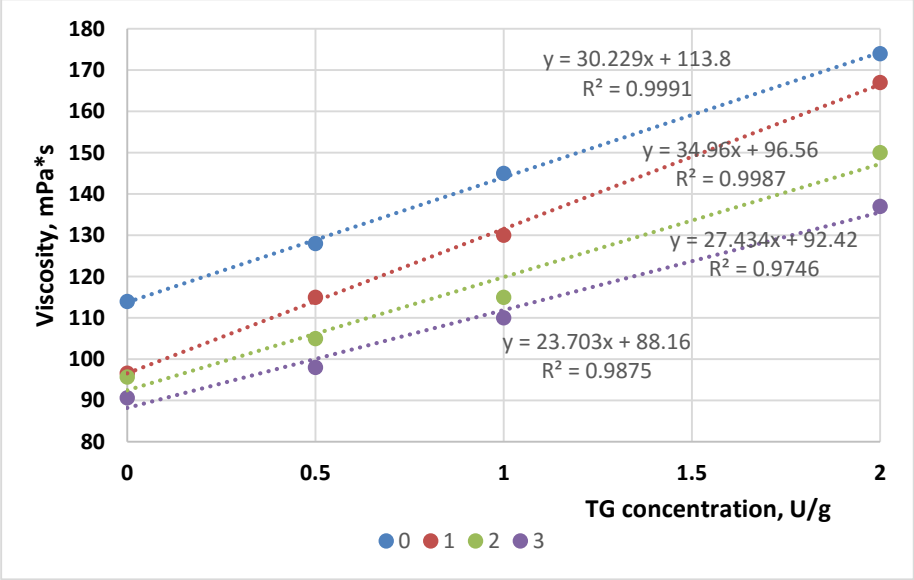

Figure 4. The viscosity of yoghurts with the addition of amaranth flour (see legend 0, 1, 2, 3 % w/v) depending on the concentration of TG.

As can be seen from Figure 4, the use of TG increases the viscosity of yoghurt. The dependencies are described by linear equations of the form $y = kx + b$. As can be seen from the graphs Figure 4, the rate of the yoghurt viscosity change depending on the concentration of TG is between 23 to 30 mPa*s/(U/g) in TG concentration range 0 – 2 U/g. Analyzing the data of Figure 3 and 4, it

can be concluded that among the studied samples use of TG gives a higher positive effect on the viscosity of yoghurt due to the protein binding effect. In comparison with this, the negative influence of amaranth flour, associated with the thinning effect of ballast substances of flour, – mainly polysaccharides – was less. In other words, the use of TG allows to override the negative effect of amaranth flour on the viscosity of the final product and get the yoghurt of the required viscous parameters.

The effect of additives on the textural properties of yoghurt

Prepared samples were analyzed in accordance with p. 2.3. The data obtained are presented in table. 2.

Table 2. The dependence of the strength of the yoghurts on the concentration of amaranth flour and TG.

Amaranth flour concentr., %	TG concentration, U/g			
	0	0,5	1	2
0	5.30±0.22	5.15±0.21	5.08±0.24	5.31±0.20
1	4.76±0.18	5.39±0.20	4.97±0.15	5.03±0.18
2	3.82±0.41	4.99±0.21	5.29±0.13	5.76±0.25
3	5.03±0.19	4.83±0.21	5.40±0.22	5.50±0.25

As can be seen from table 2, the addition of TG causes a more pronounced change in the strength of the yoghurt when using amaranth flour in concentrations of 2 % and 3 %. In the control sample without amaranth flour and the yoghurt sample with amaranth flour at a concentration of 1%, this change is virtually nonexistent. The obtained data demonstrate the success of applying TG to increase the strength of yoghurt with amaranth flour in concentrations of 2 % and 3 %.

The effect of additives on the microstructure of yoghurt

Preparation of samples of the yoghurt and the analysis were carried out in accordance with p. 2.4. The data obtained are presented in Figure 5.

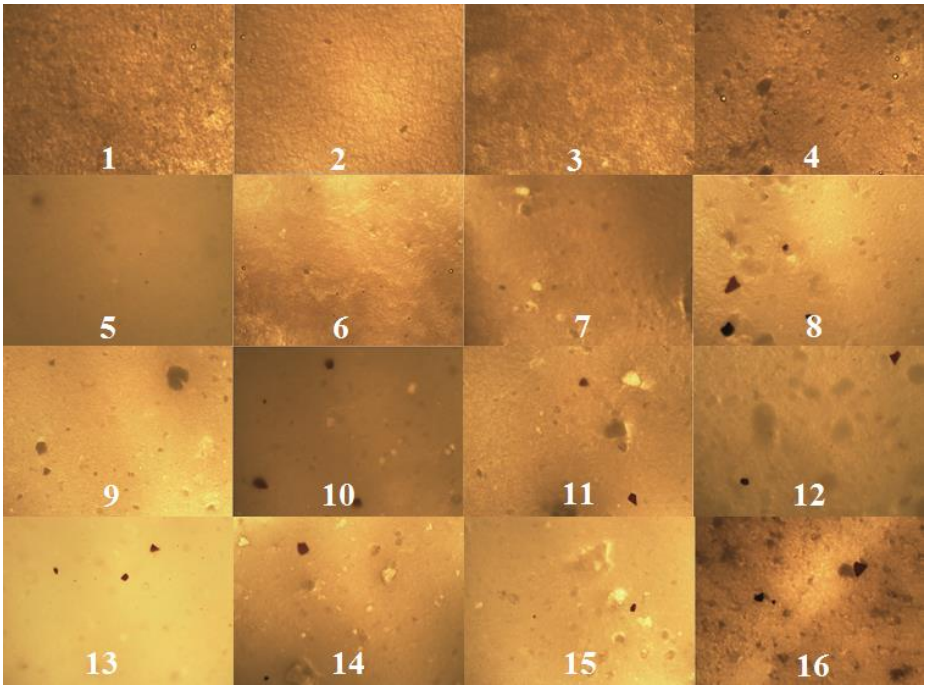

Figure 5. Photomicrographs of yoghurt samples with 40x magnification (see table 1 for yoghurt samples 1 – 16 notation).

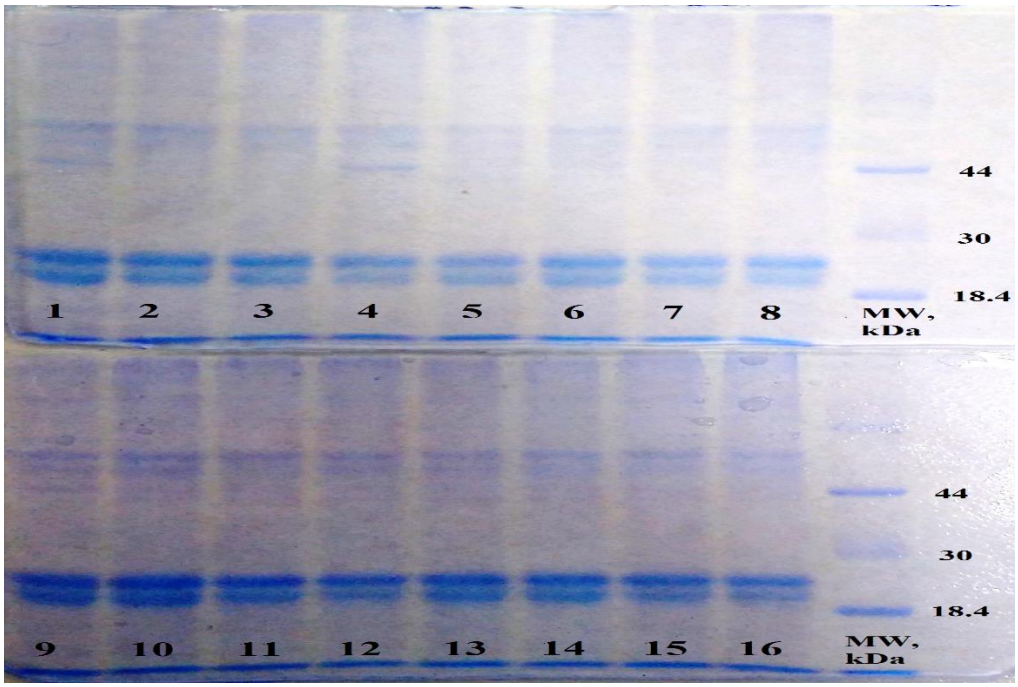

Figure 6. Electrophoregrams of yoghurt samples (see table 1 for yoghurt samples 1 – 16 notation).

3.4. The results of electrophoresis of yoghurt

Electrophoretic studies were carried out in accordance with p. 2.5. The data are presented in Figure 6.

In Figure 6 bands corresponding to casein (20 – 26 kDa) present. The bands corresponding to serum proteins (14 – 18 kDa), are not visible, which indicates the denaturation resulting from thermal treatment of milk prior to fermentation. The data peaks replotted were represented in Figure 7.

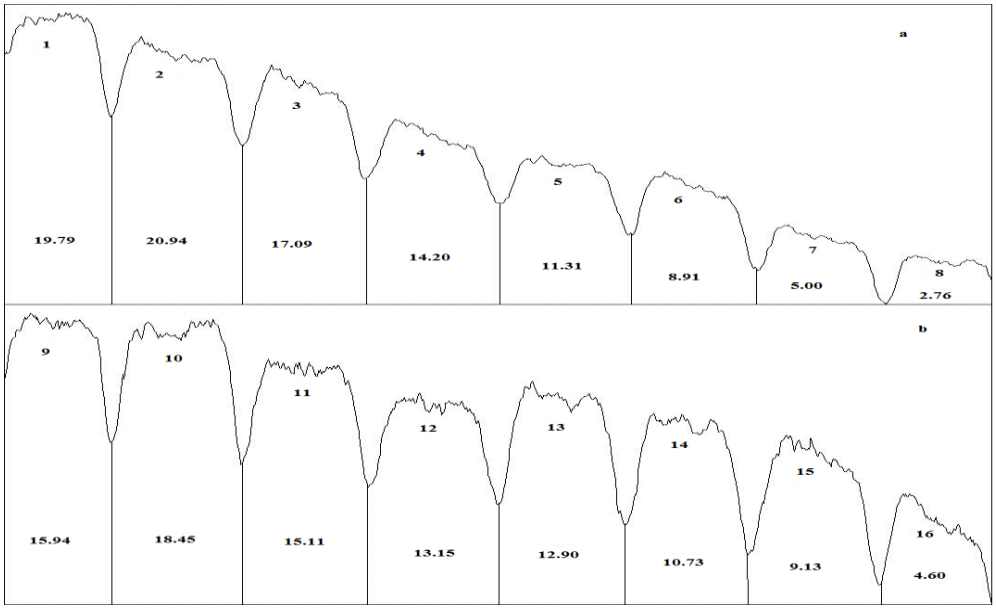

Figure 7. Replotted band peaks percentage of SDS-PAGE analysis of yoghurt samples (see table 1 for yoghurt samples 1 – 16 notation)

It can be seen lower bands intensity when use TG compared to control (bands 3, 4 compared to 1, bands 6 –8 compared to 5, bands 11, 12 compared to 9 and bands 14 – 16 compared to 13), which indicates binding effect.

3.5. Sensory evaluation

Sensory analysis was conducted accordingly to p. 2.6. Scores are presented in table 3.

Table 3. Sensory characteristics of yoghurt samples.

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Syneresis	8.5^{c}	9.5^{a}	9.5^{a}	9.5^{a}	8.4^{c}	9.5^{a}	9.4^{a}	9.3^{a}	8.2^{c}	9.4^{a}	9.3^{a}	9.3^{a}	8.0^{c}	9.3^{a}	9.3^{a}	9.2^{a}
Color	9.4^{a}	9.5^{a}	9.5^{a}	9.3^{a}	9.5^{a}	9.4^{a}	9.4^{a}	9.3^{a}	9.4^{a}	9.5^{a}	9.4^{a}	9.3^{a}	9.3^{a}	9.3^{a}	9.3^{a}	9.2^{a}
Firmness	8.5^{c}	9.5^{a}	9.6^{a}	9.5^{a}	8.4^{c}	9.5^{a}	9.5^{a}	9.4^{a}	8.3^{c}	9.4^{a}	9.5^{a}	9.5^{a}	8.2^{c}	9.3^{a}	9.4^{a}	9.4^{a}
Flavor	9.0^{b}	9.6^{a}	9.5^{a}	9.4^{a}	9.0^{b}	9.5^{a}	9.4^{a}	9.4^{a}	8.9^{b}	9.4^{a}	9.4^{a}	9.4^{a}	8.8^{b}	9.3^{a}	9.3^{a}	9.2^{a}
Distribution of ingredients	9.4^{a}	9.4^{a}	9.3^{a}	9.2^{a}	9.3^{a}	9.3^{a}	9.2^{a}	9.2^{a}	9.2^{a}	9.2^{a}	9.2^{a}	9.1^{a}	9.1^{a}	9.1^{a}	9.1^{a}	9.1^{a}
Acidity	9.5^{a}	9.6^{a}	9.5^{a}	9.5^{a}	9.4^{a}	9.5^{a}	9.4^{a}	9.4^{a}	9.4^{a}	9.4^{a}	9.4^{a}	9.3^{a}	9.3^{a}	9.4^{a}	9.3^{a}	9.2^{a}
Sweetness	9.6^{a}	9.5^{a}	9.5^{a}	9.4^{a}	9.5^{a}	9.5^{a}	9.4^{a}	9.4^{a}	9.4^{a}	9.4^{a}	9.3^{a}	9.3^{a}	9.3^{a}	9.3^{a}	9.2^{a}	9.2^{a}

a,b,c Means with different letter differ significantly($P < 0.05$)

All samples were judged as acceptable. No significant changes were found in “color”, “acidity”, “distribution of ingredients”, or “sweetness” for either type of yogurt. Control sample and sample with amaranth and without TG were got almost the same scores at all characteristics. The scores for “syneresis”, “firmness”, and “flavor intensity” showed significant differences for control, samples with amaranth, and samples with TG. Yoghurts with TG were got the best scores at these characteristics.

4. Conclusions

Amaranth flour and TG were added in sour milk concomitantly with starter. The influence of these additives on stirred yoghurt structure was investigated. Rheological measurements show viscosity depends on additives concentration linearly on equation $y = kx + b$,wherein k is the rate of viscosity change. k is between -7…-12 range if use amaranth flour in the investigated range of concentrations 0 – 3 % w/v. The use of TG contributes to yoghurt viscosity increasing: k = 23…30 in the investigated range of TG concentrations 0 – 2 U/g. Thus, use of TG allows overlapping the negative effect of amaranth polysaccharides on yoghurt viscosity and can be recommended in yoghurt with amaranth addition manufacture. Texture measurements show more pronounced effect of TG on yoghurt gel strengthening if use amaranth flour in 2 % and 3 % concentrations compared to control and 1 %. It also gives good opportunity to use TG in combined yoghurt production. Results of optical microscopy shows positive effect of TG in 0.5 U/g and 1 U/g concentrations on the yoghurt surface, it becomes smoother than control. Exceeding of these optimal concentrations leads to protein aggregates formation that break structure homogeneity. Results of electrophoresis clearly indicate TG binding effect: caseins bands become less bright. Whey protein bands are not observed due to whey protein denaturation during milk heat treatment. All yoghurt samples were judged as organoleptically acceptable, moreover samples with TG were got the best values for “syneresis”, “firmness”, and “flavor intensity”. The data obtained indicate the possibility of applying amaranth flour and TG for stirred yoghurt manufacture.

5.References

Afonso, I.M., Maia, J.M.(1999) Rheological monitoring of structure evolution and development in stirred yoghurt. *Journal of Food Engineering*, Vol. 42, Is. 4, December 1999, Pp 183–190.

Argymbaeva, A.E., Danilov, N.P., Shleikin, A.G. (2015) The use of tranglutaminase for stirred yoghurt production with a first grade amaranth flour addition. 29th EFFoST International Conference Proceedings. 29th EFFoST International Conference Food Science Research and Innovation: Delivering sustainable

solutions to the global economy and society, 10-12 November 2015, Athens, Greece.

Cancinoa, B., Fuentesa, P., Kulozik, U., Bönisch, M.(2006) Effect of the protein addition on the structure of set style and stirred yoghurt with and without the use of transglutaminase. *Desalination*, Vol. 200, Is. 1–3, 20 November 2006, Pp. 531-532.

Chauhan, A., Saxena, D.C. Singh, S.. Total dietary fibre and antioxidant activity of gluten free cookies made from raw and germinated amaranth (*Amaranthus spp*.) flour. *LWT - Food Science and Technology*. In press, available online 11 April 2015.

Chouchouli,V., Kalogeropoulos,N., Konteles, S.J., Karvela. E., Makris, D.P., Karathanos V. T.(2013), Fortification of yoghurts with grape (*Vitis vinifera*) seed extracts. *LWT - Food Science and Technology***,** Vol. 53, Is. 2, October 2013, Pp. 522–529.

El-Said, M.M., Haggag, H.F., El-Din, H.M.F., Gad, A.S., Farahat, A.M. (2014), Antioxidant activities and physical properties of stirred yoghurt fortified with pomegranate peel extracts. *Annals of Agricultural Sciences*, Vol. 59, Is. 2, December 2014, P. 207–212.

Gaspar, A.L.C., de Góes-Favoni, S.P.(2015) Action of microbial transglutaminase (MTGase) in the modification of food proteins: A review. *Food Chemistry*, Vol. 171, 15 March 2015, Pp. 315–322.

Hadi, H.G., Hadi, E.M., Gholamreza, M., Amin, H.M.(2015) Scientific and Technical Aspects of Yogurt Fortification: a Review. *Food Science and Human Wellness*. In press, available online 30 March 2015.

Iličić, M.D., M.Đ. Carić, S.D. Milanović, L.P. Dokić, Đurić, M.S., G.S. Bošnjak, Duraković, K.G. (2008), Viscosity changes of probiotic yoghurt with transglutaminase during storage. *Acta periodica technologica*, Is. 39, 2008, Pp. 11-19.

Jaros, D., Heidig, C., Rohm, H. (2007) Enzymatic modification through microbial transglutaminase enhances the viscosity of stirred yogurt. Journal of Texture Studies, Vol. 38, Is. 2, April 2007, P. 179–198.

Mlakar, G.S.,Turinek, M.,Jakop, M., Bavec, M., Bavec, F.(2009), Nutrition value and use of grain amaranth potential future application in bread making. *Agricultura*, Vol. 6, 2009, P. 43-53.

Mounsey, J.S., O'Kennedy, B.T., Kelly, P.M. (2005) Influence of transglutaminase treatment on properties of micellar casein and products made therefrom. *Lait*, Vol. 85, 2005, P. 405–418.

Oliveira, A., Alexandre, E.M.C., Coelho, M., Lopes, C., Almeida, D.P.F., Pintado, M. (2015) Incorporation of strawberries preparation in yoghurt: Impact on phytochemicals and milk proteins. *Food Chemistry*, Vol. 171, 15 March 2015, Pp. 370–378.

O'Sullivan, M.M., Kelly, A.L. Fox, P.F. (2002), Influence of transglutaminase treatment on some physico-chemical properties of milk. *Journal of Dairy Research*, Vol. 69, Is. 03, August 2002, Pp. 433-442.

Sady, M., Grega, T., Najgebauer, D., Domagala, J., Faber, B.(2005) Nutritive value of bio-yoghurts with amaranthus seeds and oat grains additives. *Biotechnology in Animal Husbandry*, Vol. 21 (5-6), Pp. 245-249.

Shleikin, A.G. and Danilov, N.P.(2015), Set yoghurt production with an amaranth infusion and transglutaminase. *Ciencia e Tecnica Vitivinicola*, Vol. 30, Is.7, 2015, P. 74 – 96.

Shleikin, A.G., Danilov, N.P., Argymbaeva, A.E.(2015), Transglutaminase treatment of milk with amaranth added: effect on the textural properties of set yoghurt. Proceedings of FABE 2015 2nd International Conference on Food and Biosystems Engineering, 28 – 31 May 2015, Mikonos, Greece.

Shleikin, A.G., Danilov, N.P. Ternovskoy, G.V.(2011), Modification of food products properties by use of transglutaminase. *Procedia Food Science***,** Vol. 1, 2011, P. 1568–1572**,** 11th International Congress on Engineering and Food (ICEF11).

Sun-Waterhouse, D., Zhou, J., Wadhwa, S.S. (2013), Drinking yoghurts with berry polyphenols added before and after fermentation. *Food Control*, Vol. 32, Is. 2, August 2013, P. 450–460.

Tabari, M.(2010) An empirical study of transglutaminase cross-linking in probiotic stirred yoghurt. *Journal of Biotechnology*, Vol. 150, Supplement, November 2010, P. 335–336.

Tamime, A.Y., Robinson, R.K. Fermented milks and their future trends. Part II. Technological aspects. Journal of Dairy Research, Vol. 55, 1988, P. 281-307.

Vasbinder, A.J., Rollema, H.S., Bot, A., de Kruif C.G.(2003), Gelation mechanism of milk as influenced by temperature and pH; studied by the use of transglutaminase cross-linked casein micelles. *Journal of Dairy Science***,** Vol. 86, Is. 5, May 2003, P. 1556–1563.

Acknowledgements

The reported study was funded by RFBR according to the research project No. 15-34-50741 мol_nr.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

INHIBITION IMPACTS OF NATURAL CLINOPTILOLITE ON BIOGENIC AMINES PRODUCTION BY COMMON FOOD-BORNE PATHOGENS IN ARGININE DECARBOXYLASE BROTH

Abdelkader Bensid[1*], Saadet Gökdogan[2], Fatih Özogul[2]

[1]HASAQ Laboratory, High National Veterinary School, BP 161, El Harrach, 16000 Algiers, Algeria;
[2]Department of Seafood Processing Technology, Faculty of Fisheries, Cukurova University, 01330, Adana, Turkey;
*Corresponding author: *bensidvet@laposte.net*

Article history:
Received:
30 January 2016
Accepted in revised form:
30 May 2016

Keywords:
Food-borne pathogens;
Ammonia;
Agmatine;
Biogenic amines;
Clinoptilolite;
HPLC

ABSTRACT

The effect of natural clinoptilolite (CLINOPT) on ammonia (AMN) and biogenic amines (BAs) production by different food borne-pathogens (FBPs) was studied in arginine decarboxylase broth (ADB) using HPLC. All tested bacteria were found to have an ability to produce ammonia and BAs in ADB. It was demonstrated that ammonia and biogenic amine production could be significantly influenced by adding CLINOPT ($P<0.05$). Both concentrations of CLINOPT (1 and 5%) had a clear inhibition effect only on putrescine (PUT) formation by both Gram negative and positive FBPs. On the other hand, CLINOPT resulted in strong increases in biogenic amines production by *Enterococcus faecalis*, although remarkable decreases were observed for biogenic amines by *Salmonella paratyphi* A in the presence of CLINOPT. Consequently, it can be concluded that the effect of CLINOPT on AMN and BAs production varied depending on not only FBPs, but also CLINOPT concentrations used.

1. Introduction

Biogenic amines (BAs) are basic nitrogenous compounds, of low molecular weight, formed in food by the decarboxylation of amino acids or by the amination and transamination of aldehydes and ketones as a result of microbial, vegetable and animal metabolic processes (Silla-Santos, 1996). According to their chemical structure, they can be classified in three categories which are aromatic amines (e.g., histamine, tyramine, β-phenylalanine, and tryptamine), aliphatic diamines (e.g., putrescine and cadaverine), and aliphatic polyamines (e.g., agmatine, spermidine, and spermine) (Smith, 1980). Biogenic amines occur naturally in small concentrations in most living organisms, in which they have strong physiological effects and play an important role in the stabilisation of membranes, and likely also in the regulation of nucleic acid function and protein synthesis (Halász et al., 1994). However, when excessive amount of biogenic amines was ingested with food, or when the natural mechanisms for their catabolism are inhibited, they can have toxicological effects on humans' health, such as blood pressure changes, headache, nausea, respiratory disorder, cardiac palpitation and even anaphylactic shock (Kalac and Krausová, 2005).

BAs accumulation in foods requires the availability of free amino acids, the presence of microorganisms capable of producing decarboxylases, and favourable environmental conditions (such as slightly acid pH and

anaerobiosis) for bacterial growth and decarboxylase activity (Buňková et al., 2010). Microbial strains with high proteolytic enzyme activity potentially increase the risk of biogenic amines formation in food and food products by increasing the availability of free amino acids. On the other hand, many bacterial genera are involved in biogenic amine toxicity, such as *Bacillus*, *Citrobacter*, *Clostridium*, *Escherichia*, *Klebsiella*, *Photobacterium*, *Proteus*, *Pseudomonas*, *Shigella*, and the some lactic acid bacteria (Mohamed et al., 2009). It was reported that *Staphylococcus aureus* strains were histamine-producer bacteria in soy broth supplemented with 1.0% L-histidine (Chang et al., 2008). *Escherichia coli* and *Listeria monocytogenes* were shown to have the highest decarboxylase activity in tyrosine containing medium, with consequent production of tyramine (von Beutling, 1993), while *Klebsiella pneumoniae* has been reported to be the most prolific histamine producer (Emborg and Dalgaard, 2006). Middlebrooks et al. (1988) stated that *Aeromonas hydrophila* isolated from refrigerated mackerel has the ability to produce histamine, and *Enterococcus faecalis* accumulates tyramine (Bover-Cid and Holzapfel, 1999). Agmatine can be produced by a broad range of microorganisms, including *Enterococcus faecalis* and *Pseudomonas aeruginosa* (Sakakibara and Yangisava, 2003; Griswold et al., 2006). Kuley and Özogul (2011) reported that *S. paratyphi* A had an ability to produce significant amounts of BAs in vitro conditions.

Various methods for reducing BAs accumulation in foodstuffs including storage in low temperature, appropriate packaging and the incorporation of synthetic or naturally occurring preservatives (Kuley et al., 2005; Özogul et al., 2011; Kim et al., 2011) have been used. Among the bio-preservatives studies, clinoptilolite (CLINOPT) has increasingly gained the interest of researchers and food processors. Clinoptilolite is defined as a crystalline, hydrated alumino-silicate of alkali and alkaline earth cations having an infinite three-dimensional structure. Both its physical and chemical properties to act as ion exchangers, catalysts and adsorbents have led to a wide range of industrial and agricultural applications (Mumpton, 1999). Natural CLINOPT has been previously used as effective adsorbent of ammonia and biogenic amine accumulation, particularly histamine and tyramine from the sardine fillets (Kuley et al., 2012). CLINOPT has capacity to adsorb mycotoxins and also excess moisture, ammonia and radionuclides (Djordjevic et al., 2003). Significant research efforts are being conducted regarding the use of CLINOPT for antimicrobial packaging of food products. In fact, CLINOPT is used as active antimicrobial agents when incorporated into the food contact material, which then exerts its action on the surface of the food to inhibit the growth of Gram-negative and Gram-positive bacteria, molds, and yeasts (Pehlivan et al., 2005). There is no information about the relationship between clinoptilolite and food-borne pathogenic bacteria in terms of formation of ammonia and BAs in arginine decarboxylase broth (ADB). Therefore, the purpose of the present study was to investigate the effect of natural clinoptilolite at different doses on ammonia and BAs production by common food-borne pathogens in arginine decarboxylase broth (ADB).

2. Materials and methods

2.1. Bacterial strains

Staphylococcus aureus (ATCC29213), *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC700603), *Enterococcus faecalis* (ATCC29212), *Pseudomonas aeruginosa* (ATCC27853), *Listeria monocytogenes* (ATCC7677) were acquired from The American Type Culture Collection (Rockville, Md., U.S.A.). *Aeromonas hydrophila* (NCIMB1135) and *Salmonella paratyphi* A (NCTC13) were obtained from National Collections of Industrial Food and Marine Bacteria (Aberdeen, UK) and National Collection of Type Cultures (London, UK), respectively.

2.2. Culture media and bacterial extraction

The production of biogenic amines by all strains used in this work was monitored using arginine decarboxylase broth (ADB). The ADB contained, in g/L of distilled water : 2 g peptone, 1 g Lab-Lemco powder (Oxoid CM0017, Hampshire, England), 5 g NaCl (Merck 1.06404. 1000, Darmstadt, Germany), 8.02 g L-arginine (Sigma, Steinheim, Germany) and 5 mg pyridoxal HCl (Sigma P9130, Steinheim, Germany). The pH was adjusted according to their optimum growth pH with 1 M KOH (Riedel-de Haen 06005, Seelze, Germany) or 6% trichloroacetic acid (Riedel-de Haen 27242, Seelze, Germany). Clinoptilolite was added to the ADB with the concentration of 1 and 5% (w/v). After that arginine decarboxylase broth (prepared with/without clinoptilolite) was pipetted in 10 mL bottles and then autoclaved at 121°C in 15 min prior to use.

Nutrient broth (Merck 1.05443.0500, Darmstadt, Germany) was used for propagation of FBP cultures. Total of 100 µL of each food-borne pathogen culture was inoculated into 10 mL of Nutrient broth. Pathogen bacterial strains were incubated at 30°C for 2 or 3 days, which after 0.5 mL of these bacterial cultures was removed and put into the ADB to allow these bacteria to produce biogenic amines.

For extraction of the bacterial strains, 5 mL of the ADB containing pathogen bacterial strains was removed to separate bottles and then 2 mL sulphosalicylic acid (6%) was added. The samples were centrifuged at 3000 ***g*** for 10 min and then filtered through a filter paper (125 mm, Schleicher & Schuell, Dassel, Germany). After that, 4 mL of bacterial supernatant from each bacterial strain was taken for derivatisation in order to analyse in high-performance liquid chromatography (HPLC). Each experiment was carried out in quadruplicate.

2.3. Chemical reagents

L-arginine and all BA standards were purchased from Sigma-Aldrich (Munich, Germany). The mobile phase consisted of acetonitrile and HPLC grade water for amine analyses.

The used BA standards were: histamine dihydrochloride, tyramine hydrochloride, typtamine hydrochloride, putrescine dihydrochloride, 2-phenylethylamine hydrochloride, cadaverine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, 5-hydroxytryptamine (serotonin), 3-hydroxytyramine hydrochloride (dopamine), agmatine sulphate, trimethylamine hydrochloride and ammonium chloride. The final concentration of free base for each amine was 10 mg/mL solution. A series of dilutions (0, 0.005, 0.05, 0.5 and 5 mg/mL) were prepared from the standard stock solution and used to obtain the standard curve.

2.4. Derivatisation of bacterial extraction

A stock solution was prepared by dissolving 2% benzoyl chloride in acetonitrile to enhance the reaction with amines. For derivatisation of standard amine and ammonia solutions, 100 µL was taken (4 mL for extracted bacterial cultures) from each free base standard solution (10 mg/mL). Sodium hydroxide (1 mL of 2 M) was added, followed by 1 mL of 2% benzoyl chloride (dissolved in acetonitrile) and the solution mixed on a vortex mixer for 1 min. The reaction mixture was left at room temperature for 5 min and then centrifuged for 10 min. After that, the benzoylation was stopped by adding 2 mL of saturated sodium chloride solution and the solution extracted twice with 2 mL of diethyl ether. The upper organic layer was transferred into a clean tube after mixing. Afterwards, the organic layer was evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 mL of acetonitrile and 10 µL aliquots were injected into the HPLC.

2.5. Analytical method

Biogenic amines analysis was done using the method of Özogul (2004) and measured in milligram amines per litre broth. The confirmation of biogenic amines production was accomplished using a rapid HPLC method

with a reversed phase column by using a gradient elution program. The same analytic method was used for ammonia and trimethylamine separation.

2.6. HPLC apparatus and column

The apparatus for HPLC was a Shimadzu (Shimadzu, Kyoto, Japan) equipped with a SPD-M20A diode array detector and two binary gradient pumps (Shimadzu LC-10AT), auto sampler (SIL 20AC), column oven (CTO-20AC), and a communication bus module (CBM-20A) with valve unit FCV-11AL. For the biogenic amine analyses, the column was Columbus C18, 150 × 4.6 mm (Phenomenex, Macclesfield, Cheshire, UK).

2.7. Statistical analysis

All statistical analyses were performed using the Statistical software, SPSS Version 15.0 for windows (SPSS Inc., Chicago, IL, USA). Contents of biogenic amines in different samples were expressed as mean values accompanied by the standard deviation of means (mean of at least four determinations for each sample). The Duncan's test was used for mean comparison when a significant variation was found by the ANOVA test. The significance of results was at $P<0.05$.

3. Results and discussions

3.1. The effect of clinoptilolite on biogenic amines production by Gram negative FBPs

The effect of clinoptilolite (CLINOPT) on ammonia (AMN) and biogenic amines production by Gram negative FBPs in ADB is shown in Table 1 and Figure 1.

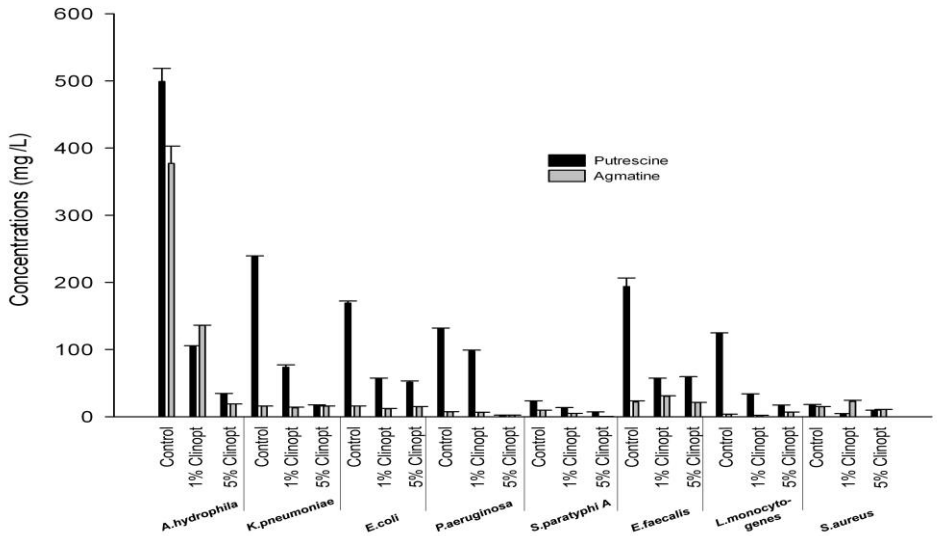

Figure 1. Putrescine and agmatine production by food-borne pathogens in the presence or absence of clinoptilolite (CLINOPT) at different doses in arginine decarboxylase broth

AMN production by Gram negative FBPs was very high (>1250.46 mg/L), indicating arginine degradation into AMN through arginine. L-arginine was present in the broth at concentration high (8020 mg/L) enough to produce that level of AMN. Significant reduction ($P<0.05$) on AMN production by *K. pneumoniae*, *P. aeruginosa* and *S. paratyphi* A was observed in the presence of CLINOPT. Others authors have already reported that CLINOPT had capacity to adsorb mycotoxins and also excess moisture, ammonia and radionuclide (Djordjević et al., 2003). We also observed that the removal efficiency of

ammonia by both CLINOPT concentration (1 and 5%) increases with increasing amount of CLINOPT. This effect can be attributed to an increased surface area and number of adsorption sites of CLINOPT (Nemr et al., 2009). However, in this study, the presence of CLINOPT in the ADB resulted in significantly higher AMN production by *A. hydrophila* and *E. coli*. This might be due to the accumulation of higher ammonia in ADB by these bacteria in the presence of CLINOPT, and the decrease of ammonium adsorption by the CLINOPT, which reached the saturation point (Wu et al., 2008).

Table 1. Ammonia and biogenic amines production by gram negative foodborne pathogens treated with clinoptilolite at different doses in ADB.

Microorganism	Clinoptilolite [g L^{-1}]	AMN	PUT	CAD	SPD	TRPT	PHEN	SPN
		[mg L^{-1}]						
Aeromonas hydrophila	0	1250.46 ± 24.43 ^{b}	499.16±19.51^{a}	4.55±0.09^{b}	27.10±0.15^{a}	0.00±0.00^{b}	23.81±0.91^{a}	0.00±0.00^{b}
	10	1523.46±106.98^{a}	105.33±0.47^{b}	47.74±0.33^{a}	2.70±0.01^{b}	3.96±0.22^{a}	6.78±0.36^{c}	0.00±0.00^{b}
	50	1671.89±25.11^{a}	34.10±0.56^{c}	0.36±0.01^{c}	0.00±0.00^{c}	0.00±0.00^{b}	11.87±0.59^{b}	1.28±0.12^{a}
Klebsiella pneumoniae	0	1454.16±30.81^{a}	237.80±1.61^{a}	1.64±0.01^{b}	17.88±0.20^{b}	0.00±0.00^{b}	24.78±0.66^{a}	0.00±0.00^{b}
	10	1089.63±22.67^{b}	73.57±3.49^{b}	42.90±1.02^{a}	45.77±0.30^{a}	0.00±0.00^{b}	13.12±0.10^{b}	0.00±0.00^{b}
	50	713.17±0.24^{c}	17.33±0.24^{c}	0.66±0.01^{b}	0.00±0.00^{c}	1.55±0.07^{a}	11.24±0.09^{c}	67.96±2.77^{a}
Escherichia coli	0	1293.41±65.45^{c}	169.13±2.99^{a}	13.15±0.48^{a}	6.36±0.55^{b}	0.00±0.00^{b}	22.58±0.73^{b}	0.00±0.00^{c}
	10	1928.76±15.27^{b}	56.78±0.49^{b}	4.75±0.21^{b}	39.32±0.22^{a}	0.00±0.00^{b}	18.82±1.07^{c}	17.24±0.08^{b}
	50	2454.04±30.33^{a}	51.18±2.09^{b}	1.27±0.04^{c}	1.29±0.06^{c}	6.58±0.27^{a}	25.30±0.38^{a}	40.45±4.39^{a}
Pseudomonas aeruginosa	0	1861.23±53.50^{a}	131.85±0.21^{a}	0.67±0.06^{a}	8.55±0.78^{b}	0.00±0.00^{b}	7.44±0.31^{b}	1.69±0.02^{b}
	10	1488.35±34.95^{b}	97.45±1.67^{b}	0.57±0.05^{a}	24.09±0.01^{a}	11.72±0.11^{a}	10.31±0.20^{a}	13.59±0.13^{a}
	50	506.57±42.31^{c}	2.18±0.05^{c}	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{b}	3.04±0.18^{c}	0.00±0.00^{c}
Salmonella paratyphi A	0	2074.84±57.08^{a}	23.47±0.18^{a}	0.93±0.01^{a}	2.63±0.04^{a}	0.00±0.00	9.51±0.23^{a}	43.30±0.05^{a}
	10	1028.57±14.09^{b}	13.67±0.03^{b}	0.35±0.00^{b}	0.00±0.00^{b}	0.00±0.00	2.95±0.02^{c}	0.81±0.01^{b}
	50	937.82±8.53^{b}	7.03±0.04^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00	4.10±0.03b	0.00±0.00^{c}

AMN: ammonia; PUT: putrescine; CAD: cadaverine; SPD: spermidine; TRPT: tryptamine; PHEN: 2-Phenyl-ethylamine; SPN: spermine. Values are expressed as mean ± standard deviation, n = 4. a–c: indicate significant differences ($P<0.05$) in a row among groups. ADB: Arginine Decarboxylase Broth.

Table 1. Ammonia and biogenic amines production by gram negative foodborne pathogens treated with clinoptilolite at different doses in ADB (Continued).

Microorganism	Clinoptilolite [g L^{-1}]	HIS	SER	TYR	TMA	DOP	AGM
		[mg L^{-1}]					
Aeromonas hydrophila	0	1.40±0.09^{b}	120.79±10.69^{a}	17.00±0.14^{a}	3.86±0.02^{a}	435.31±24.48^{a}	377.25±25.76^{a}
	10	4.10±0.39^{a}	1.10±0.04^{b}	0.91±0.01^{c}	0.37±0.03^{b}	38.32±0.06^{b}	135.68±0.50^{b}
	50	3.74±0.02^{a}	0.93±0.01^{b}	4.66±0.14^{b}	0.41±0.00^{b}	40.23±0.03^{b}	18.88±0.05^{c}
Klebsiella pneumoniae	0	0.76±0.03^{b}	4.40±0.38^{b}	12.42±0.41^{a}	1.11±0.06^{a}	53.53±0.37^{b}	15.72±0.18^{a}
	10	1.35±0.12^{a}	18.71±0.45^{a}	1.45±0.05^{c}	0.16±0.01^{b}	106.64±0.55^{a}	13.00±1.07^{b}
	50	1.59±0.05^{a}	1.36±0.03^{c}	3.63±0.20^{b}	0.11±0.01^{b}	42.59±2.95^{c}	15.63±0.15^{a}
Escherichia coli	0	4.40±0.26^{b}	53.68±0.94^{a}	10.81±0.14^{c}	30.29±0.32^{c}	90.11±1.46^{c}	15.83±0.14^{a}
	10	5.74±0.21^{a}	24.63±0.81^{c}	17.34±0.43^{b}	158.61±0.36^{b}	164.17±1.50^{b}	11.64±0.50^{b}
	50	4.18±0.06^{b}	43.48±0.67^{b}	29.15±0.43^{a}	178.34±0.27^{a}	360.99±9.72^{a}	14.79±0.36^{a}
Pseudomonas aeruginosa	0	1.76±0.10^{a}	32.75±0.07^{b}	1.49±0.01^{b}	1.08±0.06^{b}	62.69±1.35^{b}	7.06±0.54^{a}
	10	1.59±0.10^{a}	111.13±1.35^{a}	8.30±0.10^{a}	1.99±0.10^{a}	120.74±0.21^{a}	6.35±0.34^{a}
	50	0.00±0.00^{b}	0.15±0.00^{c}	1.14±0.05^{c}	0.14±0.00^{c}	10.03±0.34^{c}	2.46±0.08^{b}
Salmonella paratyphi A	0	1.65±0.02^{a}	31.72±1.16^{a}	5.62±0.35^{a}	2.38±0.52^{a}	60.62±0.31^{a}	9.61±0.18^{a}
	10	0.29±0.41^{b}	1.25±0.08^{b}	2.03±0.16^{b}	0.37±0.02^{b}	9.46±(0.81) ^{c}	4.87±0.16^{b}
	50	0.34±0.00^{b}	1.83±0.05^{b}	1.54±0.13^{b}	0.13±0.01^{b}	36.42±1.13^{b}	0.47±0.03^{c}

HIS: histamine; SER: serotonin; TYR: tyramine; TMA: trimethylamine; DOP: dopamine; AGM: agmatine.
Values are expressed as mean ± standard deviation, n = 4. a–c: indicate significant differences ($P<0.05$) in a row among groups. ADB: Arginine Decarboxylase Broth.

The main amines formed by Gram negative FBPs were putrescine (PUT), followed by dopamine (DOP). The used Gram negative FBPs were seemed to convert agmatine (AGM) into PUT. It has been reported that PUT can be formed from L-arginine by arginine decarboxylase (ADC) via AGM, which is converted directly to PUT by the enzyme

agmatinase, while AGM is first hydrolysed by AGM deiminase into N-carbamoylputrescine and AMN, and PUT is formed by removal of the ureido group from N carbamoylputrescine by the enzyme N-carbamoylputrescine amidohydrolase (Wunderlichová et al., 2012). The addition of CLINOPT significantly inhibited PUT production by Gram negative bacteria ($P<0.05$) (Fig. 1). Treated group with 5% CLINOPT was more effective to suppress PUT formation than treated group with 1% CLINOPT. The CLINOPT concentration was found to have a significant effect on the formation of PUT.

In the present study, *E. coli* produced considerably higher cadaverine (CAD) (13.15 mg/L) than the other tested bacteria. Similar findings are reported also by other researchers (Durlu-Özkaya et al., 2001), who also found that *E. coli* EC03 isolated from meat products was the most important CAD producer with a production level of 454.8 mg/L in brain-heart infusion medium. The effectiveness of dose used was strain-dependent. The addition of CLINOPT had an inhibitor effect on CAD production by *E. coli*, *P. aeruginosa* and *S. paratyphi* A. However, the use of CLINOPT at dose of 1% resulted in significant increases in CAD accumulation by *A. hydrophila* and *K. pneumoniae*, while CLINOPT at dose of 5% suppressed CAD production by Gram negative bacteria. Spermidine (SPD) production ranged from 2.63 mg/L for *S. paratyphi* A to 27.10 mg/L for *A. hydrophila*. The effect of CLINOPT on SPD production was dependent on bacterial strains. The use of CLINOPT significantly suppressed SPD accumulation by *A. hydrophila* and *S. paratyphi* A, although CLINOPT addition stimulated SPD production by *K. pneumoniae*, *E. coli* and *P. aeruginosa*.

Tryptamine (TRPT) was not produced in the absence of CLINOPT by Gram negative bacteria. The use of CLINOPT significantly stimulated TRPT production by *A. hydrophila*, *K. pneumoniae*, *E. coli* and *P. aeruginosa* whilst *S. paratyphi* A had no ability to produce TRPT in ADB. Production of 2-Phenyl-ethylamine (PHEN) by *E. coli* was low (22.58 mg/L). Durlu-Özkaya et al. (2001) reported production of small amounts of PHEN from *E. coli* (<30 mg/L). CLINOPT decreased PHEN accumulation by Gram negative FBPs, except for *E. coli* and *P. aeruginosa*. Spermine (SPN) was produced only by *S. paratyphi* A and *P. aeruginosa*, while *A. hydrophila*, *K. pneumoniae* and *E. coli* had not an ability to produce SPN. However, Özogul and Özogul (2007) found that *K. pneumoniae* (673) accumulated more than 32 mg/L SPN in ADB, formed from PUT and SPD. The CLINOPT application increased SPN production by Gram negative FBPs except for *S. paratyphi* A.

Histamine (HIS) production by *K. pneumoniae* was 0.76 mg/L, which is lower than that produced by the other tested Gram-negative bacteria. Low production of HIS by *K. pneumoniae* may be attributed to HIS degradation by diamine oxidase (DAO) (Dapkevicius, 2000), which has been detected in several types of bacteria including *Klebsiella* spp. (Ienistea, 1971). CLINOPT had no effect on HIS production by *E. coli*, whereas significant reduction on HIS production by *P. aeruginosa* and *S. paratyphi* A was observed in the presence of CLINOPT, but the CLINOPT application increased HIS production by *A. hydrophila* and *K. pneumoniae*. Serotonin (SER) production was the highest for *A. hydrophila* (120.79 mg/L). Among the Gram negative FBPs, *K. pneumoniae* was characterized as the lowest SER producer, followed by *S. paratyphi* A and *P. aeruginosa*. Both of CLINOPT doses used reduced SER accumulation by *A. hydrophila*, *E. coli* and *S. paratyphi* A, whereas significant increases were observed for SER production by *K. pneumoniae* and *P. aeruginosa* at dose of 1% CLINOPT, but CLINOPT at dose of 5% suppressed SER production by Gram negative FBPs.

Tyramine (TYR) was generally formed at low levels (1.49–17 mg/L). *P. aeruginosa* synthesized lower amounts of TYR (<2 mg/L) than the other tested bacteria. However, it was found that *Pseudomona*s spp. is usually TYR producer bacteria (Georgnaras et al., 1995; Silla-

Santos, 1996). The use of CLINOPT significantly suppressed TYR accumulation by *A. hydrophila*, *K. pneumoniae* and *S. paratyphi* A, although CLINOPT addition stimulated TYR production by *E. coli* and *P. aeruginosa*. There were significant differences in trimethylamine (TMA) content among the control and the treated groups ($P<0.05$). The addition of CLINOPT in the ADB reduced TMA accumulation by *A. hydrophila*, *K. pneumoniae* and *S. paratyphi* A. However, the presence of CLINOPT in the ADB resulted in significantly higher TMA production by *E. coli*, but CLINOPT at dose of 5% suppressed TMA accumulation by *P. aeruginosa*.

Dopamine (DOP) production by *A. hydrophila* was 435.31 mg/L, whereas the other Gram negative bacteria produced lower than 91 mg/L. The addition of CLINOPT had an inhibitor effect on DOP production by *A. hydrophila* and *S. paratyphi* A, whilst significant increases on DOP production by *K. pneumoniae*, *E. coli* and *P. aeruginosa* was found in the presence of 1% or 5% CLINOPT in the medium, but CLINOPT at dose of 5% suppressed DOP production by *P. aeruginosa*. AGM is produced directly from arginine by the reaction of arginine decarboxylase that had been secreted from lactic acid bacteria and nitric acid-reducing bacteria (Umezu et al., 1977; Inaba et al., 2004). In this work, there were significant differences between *A. hydrophila* and the other Gram negative bacteria in term of AGM production (Fig. 1). *A. hydrophila* produced considerably higher AGM (377.25 mg/L) than the other tested bacteria. *E. coli* produced low amount of AGM (15.83 mg/L). Low in vitro production of AGM (7.20 ± 0.81 mg/L) by *E. coli* was also reported by Buňková et al. (2009). CLINOPT had no effect on AGM production by *K. pneumoniae* and *E. coli*, whereas significant reduction on AGM production by *A. hydrophila*, *P. aeruginosa* and *S. paratyphi* A was observed in the presence of CLINOPT.

3.2. The effect of clinoptilolite on biogenic amines production by Gram positive FBPs

The influence of CLINOPT on AMN and biogenic amines production by Gram positive FBPs in ADB was given in Table 2. In this study, stronger AMN accumulation in ADB was observed for *L. monocytogenes* (1532.26 mg/L), *E. faecalis* (1122.27 mg/L) and *S. aureus* (896.86 mg/L). The respective AMN production by *L. monocytogenes*, *E. faecalis* and *S. aureus* were found as 1552.22, 2321.43 and 2620.19 mg/L in ADB (Özogul, 2011), whilst Gökdogan et al. (2012) found lower AMN production in histidine decarboxylase broth (52.29, 125.49, 78.89 vs. mg/L). CLINOPT inhibited significantly ($P<0.05$) AMN accumulation by *S. aureus*, although the use of CLINOPT at dose of 1% resulted in higher AMN production by *L. monocytogenes* and *E. faecalis*.

All strains produced various amounts of PUT (Fig. 1). Highest amount of PUT was accumulated by *E. faecalis* (193.79 mg/L), while the lowest amount of that was produced by *S. aureus* (18.12 mg/L). Similar findings are reported also by other researchers (Ladero et al., 2012; Llácer et al., 2007), who found that *E. faecalis* has been identified as PUT producer. They also found that *E. faecalis*, independent of their origin, produced PUT from AGM indicating that PUT production is a general, species-level characteristic of *E. faecalis*. The use of CLINOPT resulted in a lower PUT accumulation by Gram positive FBPs. CAD production by Gram positive bacteria was below 5 mg/L (Table 2).

Table 2. Ammonia and biogenic amines production by gram positive foodborne pathogens treated with clinoptilolite at different doses in ADB.

Microorganism	Clinoptilolite [g L^{-1}]	AMN	PUT	CAD	SPD	TRPT	PHEN	SPN
		[mg L^{-1}]						
Enterococcus faecalis	0	1122.27±19.77^{c}	193.79±12.67^{a}	4.43±0.09^{a}	9.41±0.01^{b}	0.00±0.00^{b}	7.03±0.61^{b}	0.58±0.04^{c}
	10	1706.37±2.49^{a}	57.25±0.06^{b}	3.37±0.18^{b}	28.29±0.03^{a}	7.30±0.30^{a}	10.45±0.13^{a}	18.94±1.33^{a}
	50	1409.37±30.13^{b}	58.14±1.38^{b}	2.88±0.16^{c}	2.90±0.29^{c}	0.32±0.02^{b}	11.57±0.42^{a}	6.41±0.13^{b}
Listeria monocytogenes	0	1532.26±7.90^{b}	124.40±0.29^{a}	2.61±0.16^{b}	12.61±0.16^{a}	3.22±0.11^{a}	10.89±0.21^{b}	86.04±4.30^{a}
	10	1666.34±55.02^{a}	33.76±0.16^{b}	0.41±0.02^{c}	6.86±0.37^{b}	0.00±0.00^{b}	6.90±0.22^{c}	2.78±0.12^{b}
	50	783.18±38.18^{c}	16.05±1.32^{c}	22.61±0.05^{a}	1.89±0.01^{c}	0.17±0.01^{b}	13.86±0.08^{a}	2.01±0.01^{b}
Staphylococcus aureus	0	896.86±14.06^{a}	18.12±0.14^{a}	2.51±0.03^{a}	9.04±0.00^{a}	0.00±0.00	2.68±0.10^{b}	0.00±0.00^{a}
	10	568.46±1.90^{b}	4.41±0.03^{c}	2.31±0.02^{b}	1.32±0.03^{c}	0.00±0.00	3.61±0.07^{a}	0.52±0.02^{a}
	50	511.74±1.26^{c}	9.39±0.20^{b}	0.85±0.01^{c}	4.80±0.28^{b}	0.00±0.00	1.83±0.14^{c}	0.59±0.83^{a}

AMN: ammonia; PUT: putrescine; CAD: cadaverine; SPD: spermidine; TRPT: tryptamine; PHEN: 2-Phenyl-ethylamine; SPN: spermine. Values are expressed as mean ± standard deviation, n = 4. a–c: indicate significant differences (P<0.05) in a row among groups. ADB: Arginine Decarboxylase Broth.

Table 2. Ammonia and biogenic amines production by gram positive foodborne pathogens treated with clinoptilolite at different doses in ADB (Continued).

Microorganism	Clinoptilolite [g L^{-1}]	HIS	SER	TYR	TMA	DOP	AGM
		[mg L^{-1}]					
Enterococcus faecalis	0	0.44±0.02^{b}	13.94±0.09^{c}	7.30±0.34^{c}	0.15±0.01^{c}	16.93±0.21^{c}	21.77±1.65^{b}
	10	12.32±0.18^{a}	85.65±3.40^{a}	15.62±0.17^{a}	536.06±7.91^{a}	382.33±10.44^{a}	30.32±0.70^{a}
	50	1.70±1.46^{b}	45.58±2.38^{b}	10.76±0.30^{b}	28.93±0.06^{b}	55.68±1.55^{b}	21.14±0.03^{b}
Listeria monocytogenes	0	1.49±0.12^{c}	114.69±4.44^{a}	12.22±0.20^{b}	160.39±0.41^{a}	232.74±0.81^{a}	3.54±0.33^{b}
	10	15.06±0.09^{a}	2.43±0.11^{b}	13.84±0.59^{a}	0.36±0.02^{b}	124.46±0.15^{b}	1.77±0.14^{c}
	50	3.80±0.29^{b}	2.29±0.04^{b}	6.27±0.13^{c}	0.54±0.01^{b}	8.34±0.08^{c}	6.49±0.38^{a}
Staphylococcus aureus	0	0.00±0.00^{b}	5.09±0.01^{a}	2.77±0.13^{b}	4.87±0.25^{a}	85.71±5.83^{a}	14.58±0.37^{b}
	10	0.57±0.03^{a}	1.34±0.05^{b}	3.27±0.08^{a}	0.08±0.02^{c}	8.09±0.02^{b}	22.54±1.92^{a}
	50	0.00±0.00^{b}	0.95±0.07^{c}	1.93±0.02^{c}	2.06±0.07^{b}	17.96±0.64^{b}	10.68±0.32^{c}

HIS: histamine; SER: serotonin; TYR: tyramine; TMA: trimethylamine; DOP: dopamine; AGM: agmatine.
Values are expressed as mean ± standard deviation, n = 4. a–c: indicate significant differences (P<0.05) in a row among groups. ADB: Arginine Decarboxylase Broth.

The effect of CLINOPT on CAD production was also strain-dependent. CLINOPT had generally significant effect on reducing CAD accumulation by *E. faecalis* and *S. aureus*, although application of CLINOPT at dose of 5% resulted in higher CAD accumulation by *L. monocytogenes*.

The production of SPD by Gram negative FBPs was above the 9 mg/L. *L. monocytogenes* produced medium amounts of SPD (12.61 mg/L). However, Geornaras et al. (1995) found that *Listeria* strains had not an ability to produce BAs. The use of 1% CLINOPT caused significant reduction on SPD formation by *L. monocytogenes* and *S. aureus*, whereas significant increases in this amine were observed with 1% CLINOPT by *E. faecalis*. TRPT production by *L. monocytogenes* was suppressed by use of CLINOPT, but *S. aureus* did not form TRPT. The presence of 1% CLINOPT in broth resulted seven-fold higher TRPT production by *E. faecalis*, whereas *E. faecalis* did not have the ability to produce TRPT in the absence of CLINOPT in ADB. There was a stimulation of SPN for *E. faecalis* by the use of 1% or 5% CLINOPT while CLINOPT suppressed SPN accumulation by *L. monocytogenes*.

HIS production by Gram-positive FBPs was below 2 mg/L whilst *S. aureus* had no ability to produce HIS in ADB. Chang et al. (2008) reported that *S. aureus* isolated from swordfish fillets implicated in a food poisoning was weak histamine-former and produced only between 12.7 ppm and 33.0 ppm of histamine in trypticase soy broth supplemented with 1.0% L-histidine, while, Komprda et al. (2010) identified *E. faecalis* as the most prolific HIS producer within *Enterococcus* isolates from dry fermented sausages, which was not in

agreement with our own experimental results. PHE may result from phenylalanine decarboxylation by various species of *Enterococci*, *Cocci* Gram positive and *Lactobacillus* (Ansonera et al., 2002; Bover-Cid et al., 2001). In this study, we observed that a higher PHEN production was related to a higher increase in TYR. These results are in agreement with a study reporting that PHEN generally occurs when a high amount of TYR is present because microorganisms also have moderate capacity to decarboxylate phenylalanine (Joosten, 1987). The use of CLINOPT also showed stimulation effect on PHEN and HIS production by Gram positive bacteria. Similar results were reported by Gökdogan et al. (2012), who found that PHEN and HIS production was stimulated for Gram positive FBPs by the use of 1% or 5% CLINOPT concentrations.

CLINOPT showed a stronger inhibitor effect on SER formation by *L. monocytogenes*, although CLINOPT addition stimulated SER production by *E. faecalis*. TYR production by Gram positive FBPs was ranged from 2 to 13 mg/L. TYR formation by *E. faecalis* was above the 7 mg/L. Özogul (2011) found little amount of TYR production (2.52 mg/L) by Gram positive bacteria in histidine decarboxylase broth. However, Pircher et al. (2007) reported that *E. faecalis* formed significant concentrations of TYR (100 to 1000 mg/L) in dry fermented sausages. Engesser et al. (1990) also reported that TYR was produced in highest concentration by *E. faecalis* under experimental conditions. The highest TMA production was obtained by *L. monocytogenes* (160.39 mg/L), followed by *S. aureus* (4.87 mg/L) and lowest with *E. faecalis* (0.15 mg/L). By the presence of CLINOPT in ADB, significant reduction in TMA accumulation was observed by *L. monocytogenes* and *S. aureus*, though the use of CLINOPT increased TMA formation by *E. faecalis*.

The highest DOP accumulation was found for *L. monocytogenes* (232.74 mg/L), which is not in agreement with result of Özogul (2011) reporting that the *E. faecalis* produced significantly higher DOP than *L. monocytogenes* and *S. aureus*. Significant reductions by CLINOPT application were also observed for DOP by *L. monocytogenes* and *S. aureus*. However, the presence of 1% CLINOPT in the ADB resulted in significantly higher DOP production by *E. faecalis*. Gram positive FBPs showed a low AGM production in ADB (Fig. 1). Özogul and Özogul (2007) found that the highest AGM production by *K. pneumoniae* and *E. faecalis* were in lysine decarboxylase broth and in tyrosine decarboxylase broth, respectively. They demonstrated that bacteria could vary in their relative importance as biogenic amine producers in different decarboxylase broths. Others authors reported that the availability of precursors is not a fundamental problem in BAs accumulation, and the presence of specific amino acids may not be indicative of specific peptidase activities but represents the results of an equilibrium between amino acid liberation and metabolization (Lanciotti et al., 2007). AGM production was stimulated for *E. faecalis* and *S. aureus* by the use of 1% CLINOPT, and for *L. monocytogenes* use of 5% CLINOPT dose.

4. Conclusions

All tested bacteria had an ability to produce ammonia and biogenic amines in ADB. The study results showed that CLINOPT had a clear inhibition effect only on PUT accumulation in ADB by both Gram negative and positive FBPs. On the other hand, the results demonstrate significant variations on biogenic amine production in the presence of CLINOPT. These results confirm that the effect of CLINOPT on AMN and biogenic amines was strain dependent and differed for specific amine. More in-depth research is needed to determine how CLINOPT affects decarboxylase activity in food products and how this can ultimately affect the formation of biogenic amines both quantitatively and qualitatively.

5. References

Ansonera, D., Montel, M.C., Rokka, M., Talon, R., Eerola, S., Rizzo, A., Raemaekers, M., Demeyer, D. (2002). Analysis of biogenic amines in northern and southern European sausages and role of flora in amine production. *Meat Science*, 61, 141-147.

Bover-Cid, S., Holzapfel, W.H. (1999). Improved screening procedure for biogenic amine production by lactic acid bacteria. *International Journal of Food Microbiology*, 53, 33-41.

Bover-Cid, S., Hugas, M., Izquierdo-Pulido, M., Vidal-Carou, M.C. (2001). Amino acid-decarboxylase activity of bacteria isolated from fermented pork sausages. *International Journal of Food Microbiology*, 66, 185-189.

Buňková, L., Buňka, F., Hlobilová, M., Vaňátková, Z., Nováková, D., Dráb, V. (2009). Tyramine production of technological important strains of *Lactobacillus*, *Lactococcus* and *Streptococcus*. *European Food Research and Technology*, 229, 533-538.

Buňková, L., Buňka, F., Klčovská, P., Mrkvička, V., Doležalová, M., Kráčmar, S. (2010). Formation of biogenic amines by Gram-negative bacteria isolated from poultry skin. *Food Chemistry*, 121, 203-06.

Chang, S.C., Kung, H.F., Chen, H.C., Lin, C.S., Tsai, Y.H. (2008). Determination of histamine and bacterial isolation in swordfish fillets (*Xiphias gladius*) implicated in a food borne poisoning. *Food Control*, 19, 16-21.

Dapkevicius, M.L.N.E., Nout, M.J.R., Rombouts, F.M., Houben, J.H., Wymenga, W. (2000). Biogenic amine formation and degradation by potential fish silage starter microorganisms. *International Journal of Food Microbiology*, 57, 107-114

Djordjević, N., Adamović, M., Grubić, G., Koljaić, V., Bočarov-Stančić, A. (2003). Influence of min-azel plus on biochemical, microbiological and mycotoxicological parameters of lucerne silage. *Journal of Agricultural Science*, 48, 171-78.

Durlu-Özkaya, F., Ayhan, K., Vural, N. (2001). Biogenic amine produced by *Enterobacteriaceae* isolated from meat products. *Meat Science*, 58, 163-166.

Emborg, J., Dalgaard, P. (2006). Formation of histamine and biogenic amines in cold-smoked tuna: An investigation of psychrotolerant bacteria from samples implicated in cases of histamine fish poisoning. *Journal of Food Protection*, 69, 897-906.

Engesser, D., Hammes, W.P., Holzapfel, W.H. (1990). Production of biogenic amines in semi-synthetic media by lactic acid bacteria (including strains used in food fermentations). *Food Biotechnology*, 4, 478-482.

Geornaras, I., Dykes, G.A., Holy, A. (1995). Biogenic amine formation by poultry associated spoilage and pathogenic bacteria. *Letters in Applied Microbiology*, 21, 164-168.

Gökdogan, S., Özogul, Y., Kuley, E., Özogul, F., Kacar, C., Ucar, Y. (2012). The Influences of Natural Zeolite (cliptinolite) on Ammonia and biogenic amine formation by foodborne pathogen. *Journal of Food Science*, 77, 452-457.

Griswold, A.R., Jameson-Lee, M., Burne, R.A. (2006). Regulation and physiologic significance of the agmatine deiminase system of *Streptococcus mutans* UA159. *Journal of Bacteriology*, 188, 834-841.

Halász, A., Barath, A., Simon-Sarkadi, L., Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. *Trends in Food Science and Technology*, 5, 42-48.

Ienistea, C. (1971). Bacterial production and destruction of histamine in foods, and food poisoning caused by histamine. *Die Nahrung*, 15, 109-113.

Inaba, Y., Tokishita, S., Hamada-Sato, N., Kobayashi, T., Imada, C., Yamagata, H., Watanabe, E. (2004). Development of agmatine sensor using the combination of putrescine oxidase and agmatinase for squid

freshness. *Biosensors and Bioelectronics*, 20, 833-840.

Joosten, H.M.L.J. (1987). Conditions allowing the formation of biogenic amines in cheese: 3. Factors influencing the amounts formed. *Netherlands Milk and Dairy Journal*, 41, 329-357.

Kalac, P., Krausová, P. (2005). A review of dietary polyamines: Formation, implications for growth and health and occurrence in foods. *Food Chemistry*, 90, 219-230.

Kim, J.H., Kim, D., Park, P., Kang, H.I., Ryu, E.K., Kim, S.M (2011). Effects of storage temperature and time on the biogenic amine content and microflora in Korean turbid rice wine, Makgeolli. *Food Chemistry*, 128, 87-92.

Komprda, T., Sladkova, P., Petirova, E., Dohnal, V., Burdychova, R. (2010). Tyrosine- and histidine decarboxylase positive lactic acid bacteria and *enterococci* in dry fermented sausages. *Meat Science*, 86, 870-7.

Kuley, E., Özogul, F. (2011). Synergistic and antagonistic effect of lactic acid bacteria on tyramine production by foodborne pathogenic bacteria in tyrosine decarboxylase broth. *Food Chemistry*, 127, 1163-68.

Kuley, E., Özogul, F., Özogul, Y. (2005). Effects of aluminium foil and cling film on biogenic amines and nucleotide degradation products in gutted sea bream stored at 2±1 °C. *European Food Research Technology*, 221, 582-91.

Kuley, E., Özogul, F., Durmus, M., Gokdogan, S., Kacar, C., Özogul, Y., Ucar, Y. (2012). The impact of applying natural clinoptilolite (zeolite) on the chemical, sensory and microbiological changes of vacuum packed sardine fillets. *International Journal of Food Science and Technology*, 47, 1977-1985.

Ladero, V., Fernández, M., Calles-Enríquez, M., Sánchez-Llana, E., Cañedo, E., Martín, M. C., Alvarez, M.A. (2012). Is the production of the biogenic amines tyramine and putrescine a species-level trait in *enterococci*? *Food Microbiology*, 30, 132-138.

Lanciotti, R., Patrignani, F., Iucci, L., Guerzoni, M.E., Suzzi, G., Belletti, N., Gardini, F. (2007). Effects of milk high pressure homogenization on biogenic amine accumulation during ripening of ovine and bovine Italian cheeses. *Food Chemistry*, 104, 693-701

Llácer, J.L., Polo, L.M., Tavárez, S., Alarcón, B., Hilario, R., Rubio, V. (2007). The gene cluster for agmatine catabolism of *Enterococcus faecalis*: study of recombinant putrescine transcarbamylase and agmatine deiminase and a snapshot of agmatine deiminase catalyzing its reaction. *Journal of Bacteriology*, 189, 1254-1265.

Middlebrooks, B., Toom, P., Douglas, W., Harrison, R., Mcdowell, S. (1988). Effects of storage time and temperature on the microflora and amine development in Spanish mackerel (*Scomberomurus maculatus*). *Journal of Food Science*, 53, 1024-1029.

Mohamed, R., Livia, S.S., Hassan, S., Soher, E.S., Ahmed-Adel, E.B. (2009). Changes in free amino acids and biogenic amines of egyptian salted-fermented fish (Feeseekh) during ripening and storage. *Food Chemistry*, 115, 635-638.

Mumpton, F.A. (1999). La roca magica: Uses of natural zeolites in agriculture and industry. *Proceedings of the National Academy of Sciences*, 96, 3463-3470.

Nemr, A.E., Abdelwahab, O., El-Sikaily, A., Khaled A. (2009). Removal of direct blue-86 from aqueous solution by new activated carbon developed from orange peel. *Journal of Hazardous Materials*, 161, 102-10.

Özogul, F. (2004). Production of biogenic amines by *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei* using a rapid HPLC method. *European Food Research Technology*, 219, 465-69.

Özogul, F. (2011). Effects of specific lactic acid bacteria species on biogenic amine

production by foodborne pathogen. *International Journal of Food Science and Technology*, 46, 478-84.

Özogul, F., Kuley, E., Kenar, F. (2011). Effects of rosemary and sage tea extract on biogenic amines formation of sardine (*Sardina pilchardus*) fillets. *International Journal of Food Science and Technology*, 46, 761-766.

Özogul, F., Özogul, Y. (2007). The ability of biogenic amines and ammonia production by single bacterial cultures. *European Food Research Technology*, 225, 385-94.

Pehlivan, H., Balkose, D., Ulku, S., Tihminlioglu, F. (2005). Characterization of pure and silver exchanged natural zeolite filled polypropylene composite films. *Composites Science and Technology*, 65, 2049-2058.

Pircher, A., Bauer, F., Paulsen, P. (2007). Formation of cadaverine, histamine, putrescine and tyramine by bacteria isolated from meat, fermented sausages and cheeses. *European Food Research and Technology*, 226, 225-231.

Sakakibara, Y., Yanagisawa, H. (2003). Agmatine deiminase from cucumber seedlings is a mono-specific enzyme: purification and characteristics. *Protein Expression and Purification*, 30, 88-93.

Silla-Santos, M.H. (1996). Biogenic amines: Their importance in foods. *International Journal of Food Microbiology*, 29, 213-231.

Smith, T.A. (1980). Amines in food. *Food Chemistry*, 6, 169-200.

Umezu, M., Shibata, A., Maeda, M. (1977). Production of amines by nitrate reducing-bacteria and *lactobacilli* for sake brewing. *Hakkokogaku*, 55, 68-74.

von Beutling, D. (1993). Studies on the formation of tyramine by microbes with food hygienic relevance. *Archiv für Lebensmittelhygiene*, 44, 83-87.

Wu, Z., An, Y., Wang, Z., Yang, S., Chen, H., Zhu, Z. Mai, S. (2008). Study on zeolite enhanced contact–adsorption regeneration–stabilization process for nitrogen removal. *Journal of Hazardous Materials*, 156, 317-26.

Wunderlichová, L., Buňková, L., Koutný, M., Buňka, F. (2012). The possibilities of detection of putrescine production in gram negative bacteria. *Journal of Microbiology, Biotechnology and Food Sciences*, 1, 848-854.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

EFFECT OF POST-MORTEM STORAGE PRIOR TO SALTING ON QUALITY OF SALTED SHRIMP PASTE (*KAPI*) PRODUCED FROM *MACROBRACHIUM LANCHESTERI*

Jaksuma Pongsetkul[1], Soottawat Benjakul[1*], Punnanee Sumpavapol[1], Kazufumi Osako[2] and Nandhsha Faithong[1]

[1]Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, Thailand, 90112;
[2]Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 5-7 Konan 4, Minato-ku, Tokyo 108-8477, Japan;
*Corresponding author:*soottawat.b@psu.ac.th*

Article history:
Received:
02 May 2016
Accepted in revised form:
30 May 2016

Keywords:
Shrimp
Macrobrachium lanchesteri
Kapi
Salted shrimp paste
Antioxidant properties

ABSTRACT

Effect of post-mortem storage time of shrimp (*Macrobrachium lanchesteri*) on quality of shrimp and the resulting *Kapi*, salted shrimp paste, was investigated. Shrimp underwent deterioration when stored at room temperature (28-30°C) up to 18 h as indicated by the increases in pH, total volatile base (TVB), trimethylamine (TMA) contents, thiobarbituric acid reactive substances (TBARS) and total viable count (TVC). Protein degradation was more pronounced as evidenced by the decrease in band intensity of myosin heavy chain with coincidental increase in TCA soluble peptides. Post-mortem storage time of shrimp prior to salting had impact on the quality of resulting *Kapi*. With increasing storage time, *Kapi* became browner with higher antioxidative activity. Also, volatile compounds including aldehydes, ketones, alcohols and pyrazines increased continuously. The highest and lowest overall likeness scores were obtained for *Kapi* prepared from shrimp stored for 6 and 18 h, respectively. Therefore, post-mortem storage time of shrimp used as raw material had the marked influence on quality of resulting *Kapi*.

1. Introduction

Kapi, a Thai traditional fermented shrimp paste, is widely consumed as a condiment. In general, small shrimp or krill have been used as the main raw materials to produce *Kapi*. Shrimp or krill tissues undergo enzymatic breakdown during the fermentation and bacterial action assists in proteolysis and flavour development (Hajeb and Jinap, 2015). Peptides in salted shrimp paste possessed bioactive activities, especially antioxidant activities (Peralta et al., 2008; Faithong and Benjakul, 2012; Pongsetkul et al., 2014; Pongsetkul et al., 2015)

Kapi is made by mixing shrimp or krill with salt at a ratio of 3-5:1, followed by sun-drying. Sun-dried salted shrimp ithoroughly ground before being compacted in a container, usually earthen jar and allowed to ferment for at least 1 month or longer until the typical aroma is developed (Pongsetkul et al., 2014). Salting and drying processes increase the shelf-life and flavour intensity of the product. Putrefactive microorganisms are inhibited by salt at concentrations above 6 to 8% (Phithakpol, 1993). The delay in salting might contribute to the differences in quality of resulting *Kapi.*

However, the planktonous shrimp or krill (*Mesopodopsis orientalis*), which is the traditional raw material for *Kapi* production, have dropped by 3% per year since 1990

(Meland and Willassen, 2007). Therefore, the alternative raw material for *Kapi* production has been searched. Small shrimp (*Macrobrachium lanchesteri*) is generally by-products from commercial fishing and it usually founds in southern part of Thailand throughout the year. This species seem to be a potential alternative source for making *Kapi* because of its availability and low price. Generally, species of shrimp, the quantity of salt used, and the treatment of raw materials prior to fermentation, can be varied, leading to the different characteristics, especially flavour and taste (Phithakpol, 1993).

Nevertheless, no information regarding the characteristics and quality of *Kapi* from shrimp (*M. lanchesteri*) as influenced by post-mortem storage before salting has been reported. Thus, the aim of this study was to investigate the effect of different post-mortem storage time of shrimp (*M. lanchesteri*) prior to salting on the characteristics and some

2. Materials and methods

2.1. Sample collection

Live shrimp (*M. lanchesteri*) were purchased from a village in The-Pha, Songkhla, Thailand. Shrimp were transported in ice with a shrimp/ice ratio of 1: 2 (w/w) in a polystyrene container to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand, within approximately 2 h. Whole shrimp had 80.04% moisture, 16.31% protein, 0.58% lipid, 2.26% ash and 0.81% carbohydrate as determined by AOAC method (AOAC, 2000).

2.2. Quality changes of shrimp during post-mortem storage

Upon arrival, shrimp were placed in the basket and stored at room temperature (28-30°C). Shrimp were periodically taken at 0, 3, 6, 9, 12, 15 and 18 h. The collected samples were pooled and blended using a blender (National, Tokyo, Japan) prior to analysis.

2.2.1. Chemical analysis

2.2.1.1. pH

The pH was measured according to the method of Nirmal and Benjakul (2009).

2.2.1.2. Total volatile base (TVB) and trimethylamine (TMA) contents

TVB and TMA contents were determined using the Conway micro-diffusion method (Conway and Byrne, 1936). The amounts of TVB and TMA were calculated and expressed as mg N/ g sample.

2.2.1.3. Thiobarbituric acid reactive substances (TBARS)

TBARS were determined as described by Nirmal and Benjakul (2009) with some modifications. A standard curve was prepared using 1,1,3,3-tetramethoxy propane at concentrations ranging from 0 to 2 ppm. TBARS value was calculated and expressed as mg malonaldehyde/ kg sample.

2.2.1.4. TCA-soluble peptide content

Oligopeptide content of samples was determined according to the method of Sriket et al. (2012). Soluble oligopeptide content in the supernatant was measured according to the Lowry method (Lowry et al., 1951) and expressed as μmol tyrosine equivalent/ g sample.

2.2.1.5. Protein patterns

Protein patterns were determined by SDS-PAGE using 4% stacking gel and 10% running gel according to the method of Laemmli (1970). High and low molecular weight protein markers (GE Healthcare UK Limited, Buckinghamshire, UK) were used to estimate the molecular weight of proteins.

2.2.2. Microbiological analysis

Shrimp (25 g) were transferred into a stomacher bag containing 225 ml of peptone water. Blending was performed in a Stomacher 400 Lab Blender (Seward Ltd., Worthing, UK) at high speed for 3 min. Peptone water was used for diluting the samples. Thereafter, the sample diluted in serial 10-fold steps was used for analysis by the spread plate technique on plate count agar (PCA-Merck). The plates were incubated at 35°C for 48 h. Total viable count

(TVC) was recorded and expressed as log CFU/ g sample (BAM, 2001).

2.3. Effect of post-mortem storage time on characteristics and properties of *Kapi*

2.3.1. Preparation of Kapi

Shrimp with different post-mortem storage times (0, 6, 12 and 18 h) were used as raw material. Shrimp were mixed with salt at a ratio of 5:1 (w/w) and transferred into the basket and covered with cheesecloth. Salted shrimp were made as per the method of Pongsetkul et al. (2015). After 30 days, the obtained *Kapi* samples were taken for analyses.

2.3.2. Characterization of Kapi

2.3.2.1. pH

pH of samples was determined as previously described.

2.3.2.2. Formal, ammonia and amino nitrogen contents

Formal, ammonia and amino nitrogen contents were determined by the titration method as described by Pongsetkul et al. (2014). The results were calculated and expressed as mg N/ g sample.

2.3.2.3. Colour

Colour of samples was determined using a colourimeter (ColourFlex, Hunter Lab Reston, VA, USA) and reported in the CIE system. L^* (lightness), a^* (redness/ greenness), and b^* (yellowness/blueness) were recorded. Additionally, ΔE^* (total difference of colour) and ΔC^* (the difference in chroma) were calculated.

2.3.2.4. Browning index, Maillard reaction product and antioxidative activity

- Preparation of water extract

Water extract was prepared according to the method of Peralta et al. (2008) with a slight modification. *Kapi* (2 g) was mixed with 50 ml of distilled water. The mixtures were homogenised at a speed of 10,000 ×g for 2 min. The homogenates were then subjected to centrifugation at 13,000 ×g for 15 min at room temperature. The supernatant was collected. The pellet was re-extracted in the same manner. The supernatants were combined and adjusted to 50 ml using distilled water. These extracts were then analysed as follows:

- Measurement of browning intensity

The browning intensity of the extract was measured as per the method of Pongsetkul et al. (2014). Appropriate dilution was made using distilled water and the absorbance was measured at 420 nm using the UV-1601 spectrometer (Shimadzu, Kyoto, Japan).

- Measurement of Maillard reaction products

Fluorescent intermediate products from Maillard reaction in the extract were determined as described by Pongsetkul et al. (2014). A_{280} and A_{295} of the extracts were determined also determined.

2.3.2.5. Antioxidative activities

Water extracts prepared as mentioned above were determined for antioxidative activities. Prior to assay, the extracts were approximately diluted using distilled water.

- DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method of Faithong and Benjakul (2012) with a slight modification. The standard curve was prepared using Trolox in the range of 10-60 μM. The activity was expressed as μmol Trolox equivalents (TE)/ g sample.

- ABTS radical scavenging activity

ABTS radical scavenging activity was determined as described by Pongsetkul et al. (2014) with a slight modification. A Trolox standard curve (50-600 μM) was prepared and ABTS radical scavenging activity was expressed as μmol Trolox equivalents (TE)/ g sample.

- Ferric reducing antioxidant power (FRAP)

FRAP was evaluated by the method of Faithong and Benjakul (2012). The standard curve was prepared using Trolox ranging from 50 to 600 μM. The activity was expressed as μmol Trolox equivalents (TE)/ g sample.

2.3.2.6. Volatile compounds

Volatile compounds of *Kapi* samples were determined using a solid-phase microextraction gas chromatography mass spectrometry (SPME

GC-MS) following the method of Pongsetkul et al. (2014). Volatile compounds were presented in term of abundance.

2.3.2.7. Sensory properties

Kapi samples were evaluated by 50 untrained panellists, who consumed *Kapi* regularly. The samples were cut to obtain a thickness of 1 cm. The sample (2×2 cm^2) was wrapped with aluminum foil. Thereafter, it was heated in a hot air oven at 60°C for 30 min. Panellists were instructed to rinse their mouths with water or cucumber between different samples. The panellists were asked to assess samples for appearance, colour, odour, flavour, texture and overall-liking using a 9-point hedonic scale (Mellgard et al., 2007).

2.4. Statistical analysis

All experiments were conducted in triplicate. Statistical analysis was performed using one-way analysis of variance (ANOVA). Mean comparison was carried out using Duncan's multiple range test (Steel et al., 1980). SPSS statistic program (Version 10.0) (SPSS, 1.2, 1998) was used for data analysis.

3. Results and discussions

3.1. Quality changes of shrimp during post-mortem storage

3.1.1. Changes in pH

A slight increase in pH of shrimp (*M. lanchesteri*) from 7.07 to 7.42 was observed as the post-mortem time increased (Fig.1A). The increase of pH might be associated with the production of volatile basic components, such as ammonia, trimethylamine, etc. by some spoilage bacteria (Pongsetkul et al., 2014). However, the different buffering capacity of muscle proteins from different species plausibly contributed to varying rate of pH changes (Riebroy et al., 2008). In the present study, shrimp were stored at room temperature, in which the deterioration could take place to a faster rate, compared with the storage in ice or refrigerated condition. After capture, shrimp were placed in container without icing before off-loading. This could induce the spoilage, particularly with the longer storage or handling time.

3.1.2. Changes in total volatile base (TVB) and trimethylamine (TMA) contents

Fig.1B and Fig.1C show the changes in TVB and TMA contents of shrimp during 18 h of post-mortem storage at room temperature. TVB and TMA contents of fresh shrimp (0 h) was 6.99 mg N/ g sample and 4.70 mg N/ g sample, respectively. As the post-mortem time increased, both TVB and TMA content continuously increased up to the end of storage (18 h) (P<0.05). After 18 h, the highest TVB (81.55 mg N/ g sample) and TMA content (14.49 mg N/ g sample) were obtained. This was more likely the results of the deterioration of nitrogenous compounds. Trimethylamine oxide (TMAO), a non-volatile and non-odouriferous compound, could be reduced to trimethylamine (TMA) as mediated by spoilage microorganisms (Dissaraphong et al., 2006). In general, the increases in both TVB and TMA contents were in accordance with the increase in pH (Fig.1A). The result suggested that the spoilage caused by bacteria occurred to a higher extent in shrimp, particularly when the storage time increased.

3.1.3. Changes in thiobarbituric acid reactive substances (TBARS)

Changes in TBARS value of shrimp during 18 h of post-mortem storage at room temperature are presented in Fig.1D. TBARS value of fresh shrimp was 0.60 mg MDA/ kg sample. It was increased when post-mortem time increased (P<0.05) and the highest TBARS value (0.97 mg MDA/ kg sample) was found in shrimp with post-mortem time of 18 h (P<0.05). The result suggested that lipid oxidation took place during the extended storage. Shrimp or krill lipids have been known to contain high content of polyunsaturated fatty acid (PUFA) (Takeungwongtrakul et al., 2012). Those PUFA are prone to oxidation as indicated by the presence of TBARS in the samples. Furthermore, autolysis caused by endogenous proteases might lead to the

disruption of the organelles, thereby facilitating the release of pro-oxidants as well as reactants (Dissaraphong et al., 2006). This led to the enhanced lipid oxidation in the sample.

3.1.4. Changes in TCA-soluble peptide

TCA-soluble peptide content of fresh shrimp (0 h) was 63.75 mmol/ g sample (Fig.1E). It was suggested that protein of shrimp rapidly degraded into small peptide or free amino acids after death, particularly during transportation. As the post-mortem time increased, a continuous increase in oligopeptides was observed (P<0.05). After 18 h, TCA-soluble peptide content of shrimp was 77.26 mmol/ g sample. Whole shrimp contained the cephalothorax, where hepatopancreas and other digestive organs were located. Hepatopancreas of Pacific white shrimp was rich in trypsin (Senphan and Benjakul, 2014). Therefore, degradation caused by both indigenous and microbial proteases could be enhanced when shrimp were stored at room temperature for an extended time as evidenced by the higher TCA-soluble peptide contents.

3.1.5. Protein patterns

Fig.2 shows protein patterns of whole shrimp with various post-mortem storage times. Fresh shrimp contained myosin heavy chain (MHC) as the most dominant protein. The band intensity of MHC was gradually decreased when post-mortem storage time increased. MHC still remained at 10-15%, compared to that found in fresh shrimp (0 h), after storage time for 18 h. Sriket et al. (2012) reported that MHC is susceptible to proteolytic degradation than other muscle proteins such as actin, troponin and tropomyosin. For actin (MW of 45 kDa), it was found at lower extent, compared with MHC. It was noted that band intensity of actin remained constant throughout 18 h of storage. Additionally, proteins or peptides with MW about 100 kDa continuously decreased, whilst proteins or peptides with MW about 20 kDa increased with increasing storage time. The result suggested that proteins in shrimp underwent degradation drastically during post-mortem storage. Shrimp cephalothorax containing hepatopancreas has been known to be the major source of proteases, mainly serine protease and metalloprotease (Sriket et al., 2012). Those indigenous proteases along with bacterial proteases played a role in hydrolysis of proteins in shrimp. The formation of low molecular weight peptides was in accordance with the increasing TCA-soluble peptide contents (Fig.1E).

3.1.6 Change in total viable count (TVC)

Fresh shrimp showed initially TVC of 3.22 log colony forming units (CFU)/ g sample (Fig.1F). Microbiological counts of shrimp increased as post-mortem time increased (P<0.05). After 18 h of storage at room temperature, the highest TVC (5.75 log CFU/ g sample) was found. In general, TVC increased markedly when the sample was stored at room temperature, in which mesophiles could grow rapidly. Vanderzant et al. (1973) reported that warm water marine shrimp often showed total aerobic counts of 10^6 CFU/ g sample when captured, but after cleaning process and storage at low temperature, microbial count can be lowered. Those microorganisms might contribute to the final quality of the resulting *Kapi* when used as raw material.

3.2. Effect of post-mortem storage time on characteristics and properties of *Kapi*

3.2.1. pH

pHs of *Kapi* produced from shrimp with different post-mortem storage times are shown in Table1. pH of all samples was in ranges of 7.28-7.54. There were no differences in pH (P>0.05) amongst *Kapi* samples produced from shrimp stored for up to 12 h. Nevertheless, *Kapi* prepared from shrimp stored for 18 h had the highest pH (7.54) (P<0.05). Higher pH of *Kapi* correlated with the increasing pH of shrimp used as raw material, particularly those having the longer post-mortem storage time. This was also in accordance with increasing TVB content in raw material (Fig.1B).

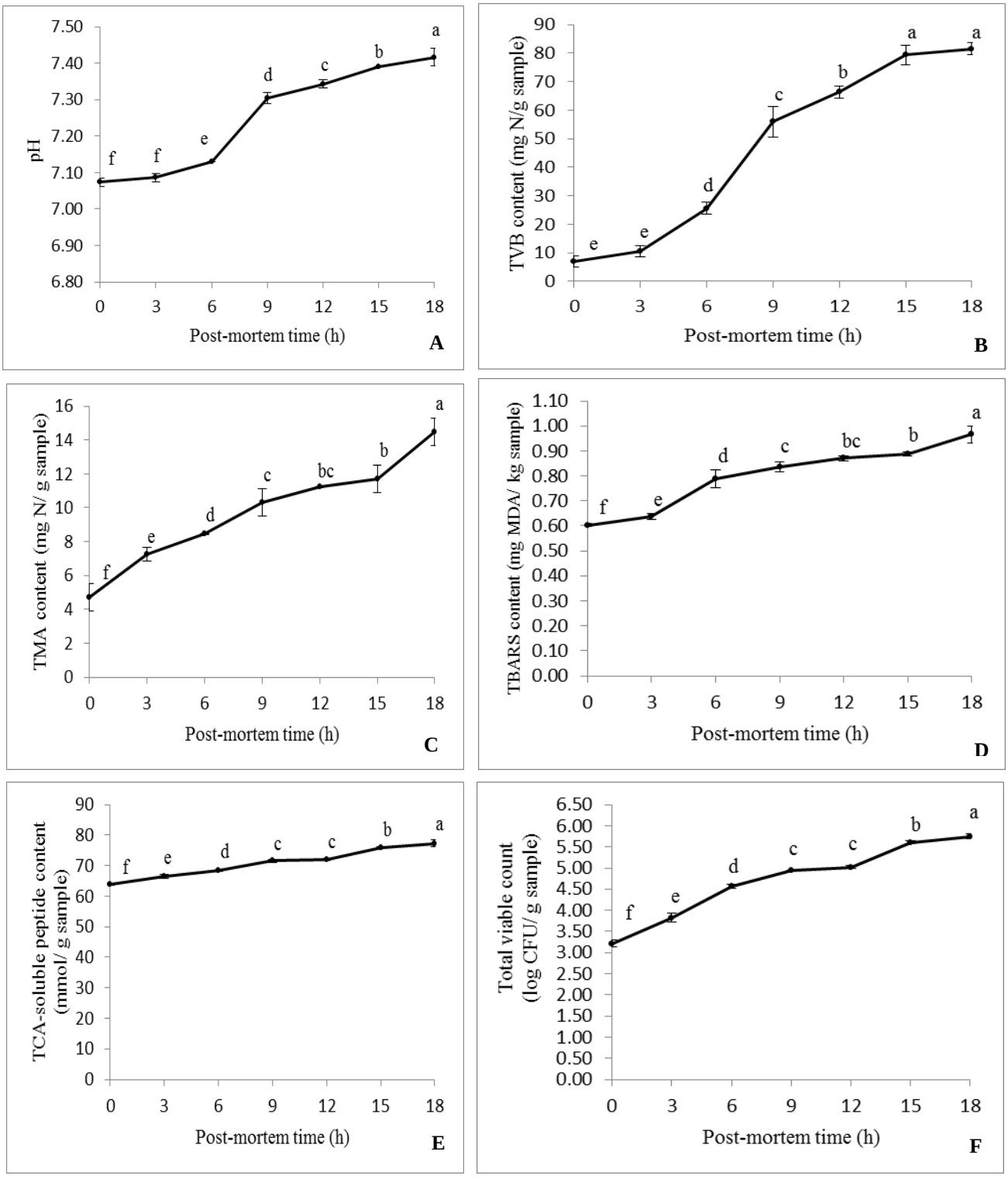

Figure 1. pH (A), total volatile base (TVB) (B), trimethylamine (TMA) contents (C), thiobarbituric acid reactive substances (TBARS) (D), TCA-soluble peptide contents (E) and total viable count (TVC) (F) of shrimp (*M. lanchesteri*) during post-mortem storage at room temperature. Different lowercase letters on the bars indicate the significant difference (P<0.05).

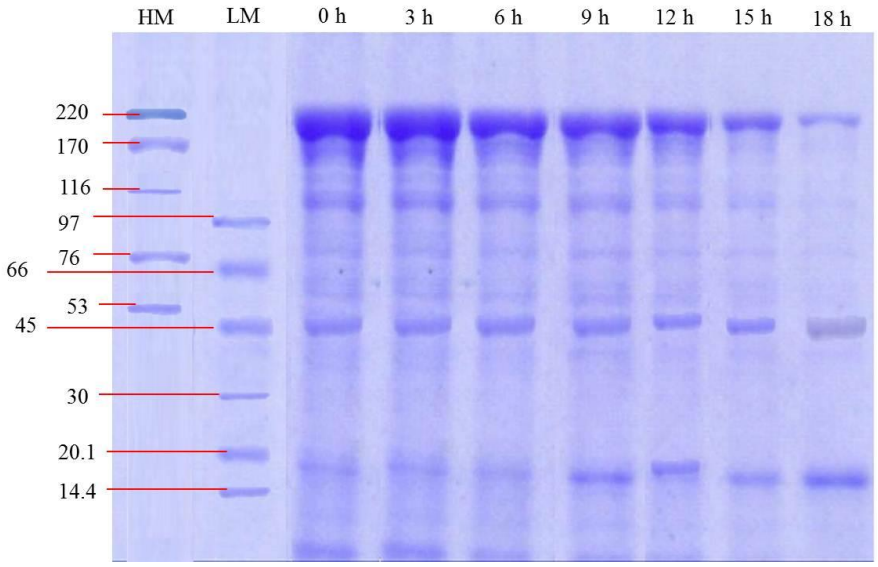

Figure 2. Protein patterns of shrimp (*M. lanchesteri*) during post-mortem storage at room temperature, HM: high molecular weight marker, LM: low molecular weight marker.

Table 1. pH, colour, browning intensity and antioxidative properties of *Kapi* produced from shrimp with different post-mortem times.

Properties	Post-mortem time			
	0 h	6 h	12 h	18 h
pH	7.28±0.53^{b}	7.31±0.10^{b}	7.30±0.35^{b}	7.54±0.46^{a}
*L**	49.76±0.30^{a}	45.95±1.01^{b}	42.94±0.46^{c}	41.76±1.07^{d}
*a**	6.53±0.27^{c}	6.89±0.30^{b}	7.67±0.18^{a}	7.67±0.93^{a}
*b**	13.54±0.49^{a}	11.88±0.29^{b}	11.41±0.17^{c}	9.36±0.45^{d}
Δ*E**	46.32±0.41^{c}	49.58±0.91^{b}	52.50±0.43^{a}	53.25±1.14^{a}
Δ*C**	14.12±0.53^{a}	12.82±0.22^{b}	12.83±0.06^{b}	11.19±0.50^{c}
A_{280}	0.64±0.02^{c}	1.04±0.07^{a}	0.92±0.05^{b}	1.06±0.12^{a}
A_{295}	0.78±0.01^{c}	0.77±0.19^{d}	0.84±0.11^{b}	0.87±0.06^{a}
Browning intensity (A_{420})	0.15±0.03^{d}	0.22±0.04^{c}	0.35±0.02^{b}	0.56±0.01^{a}
Fluorescence intensity	392.67±8.47^{b}	399.34±6.66^{b}	397.22±6.76^{b}	416.85±1.90^{a}
DPPH radical scavenging activity (μmol TE/ g sample)	1.05±0.19^{d}	1.65±0.19^{c}	2.14±0.14^{b}	2.38±0.19^{a}
ABTS radical scavenging activity (μmol TE/ g sample)	10.22±0.13^{d}	12.06±0.17^{c}	13.69±0.13^{b}	15.62±0.21^{a}
FRAP (μmol TE/ g sample)	7.59±0.31^{d}	10.61±0.25^{c}	11.85±0.18^{b}	12.89±0.24^{a}

Mean ± SD from triplicate determinations. Values in parentheses indicate the content expressed, based on dry weight. Different lowercase superscripts in the same row indicate the significant difference (P<0.05).

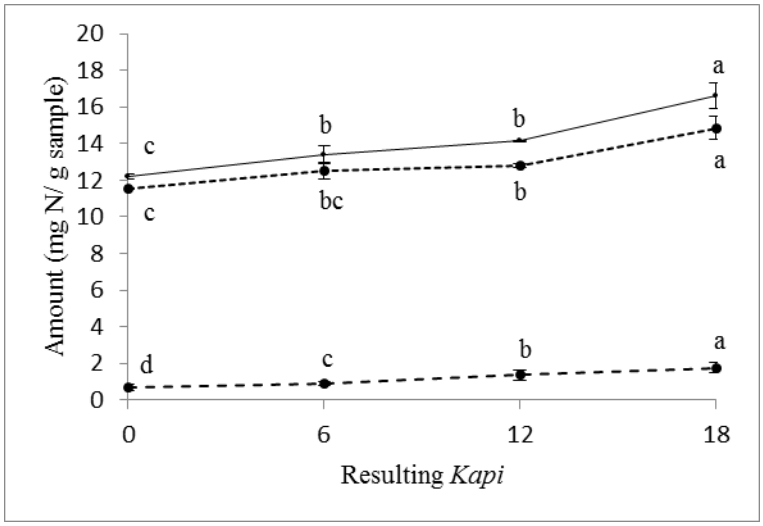

Figure 3. Formal nitrogen content (____), ammonia nitrogen content (.....) and amino nitrogen content (_ _ _ _) of K*api* produced from shrimp with different post-mortem times. Different lowercase letters on the bars within the same parameter indicate the significant difference (P<0.05). Bars represent the standard deviation (n = 3).

3.2.2. Nitrogen content

Formal, ammonia and amino nitrogen contents of resulting *Kapi* are depicted in Fig.3. Formal nitrogen content increased from 12.21 to 16.61 mg N/ g sample as post-mortem time of shrimp used as raw material increased from 0 to 18 h. In general, formal nitrogen content has been used to measure the degree of protein hydrolysis (Faithong and Benjakul, 2012). The results indicated that shrimp having the longer post-mortem storage time yielded *Kapi* with the greater degradation of protein as evidenced by greater formal nitrogen content. The varying degradation of proteins might contribute to characteristics of *Kapi* differently.

Ammonia nitrogen content of all *Kapi* samples increased when the post-mortem time of shrimp used as raw material increased (P<0.05) as shown in Fig.3. *Kapi* produced from shrimp with 18 h of post-mortem time showed the highest ammonia nitrogen content (1.75 mg N/ g sample. The ammonia nitrogen content indicates the breakdown of soluble protein and peptides into free amino acid and volatile nitrogen (Faithong and Benjakul, 2012). Higher degradation of protein might favour the subsequent deamination of proteins, as indicated by higher ammonia nitrogen content. Amino nitrogen contents in *Kapi* using shrimp having various post-mortem times as raw material are depicted in Fig.3. Amino nitrogen content represents the amount of primary amino group of the sample. An increase in amino nitrogen content is related to the degradation of polypeptide (Pongsetkul et al., 2014). A similar trend was found to that of formal and ammonia nitrogen contents. The results suggested that the longer post-mortem storage times of raw material resulted in considerable increases in free amino acids. Those free amino acids might serve as the nutrient for microorganisms. Also they could contribute to the taste or flavour of *Kapi.*

3.2.3. Colour

Kapi produced from shrimp with different post-mortem storage times had differences in colour as shown in Table1. L^* (lightness), a^* (redness), b^* (yellowness), ΔE^* (total difference in colour) and ΔC^* (difference in chroma) were in the range of 41.76-59.76,

6.53-7.67, 9.36-13.54, 46.32-53.25 and 11.19-14.12, respectively. *L**, *b** and Δ*C**-value of *Kapi* decreased when the post-mortem time of shrimp used as raw material increased, whereas *a**, Δ*E**-value of *Kapi* sample slightly increased with increasing post-mortem times of shrimp prior to salting ($P<0.05$). The results indicated that *Kapi* became darker when unfresh shrimp were used as raw material. *Kapi* produced from shrimp with 18 h of post-mortem time had the highest *a**-value, suggesting that the pronounced autolysis might cause the increased release of carotenoids from carotenoprotein during extended post-mortem times of shrimp. Astaxanthin, has a red-orange in colour, especially when it was separated from protein moiety (Faithong and Benjakul, 2012). With the delay in salting, biochemical change, especially enzymatic reactions e.g. polyphenoloxidase (PPO) occurred (Nirmal and Benjakul, 2009). This more likely contributed to the darker colour of resulting *Kapi*

3.2.4. Browning and Maillard reaction product

Browning intensity and Maillard reaction product of water extract of *Kapi* produced from shrimp with different post-mortem storage times are shown in Table1. Browning intensity (A_{420}) of resulting *Kapi* was observed when there was the delay in salting of shrimp ($P<0.05$). During extended post-mortem times, enzymatic browning reactions induced by polyphenoloxidase (PPO) occurred (Pongsetkul et al., 2014). PPO has been known to induce the hydroxylation of phenols with subsequent polymerisation, in which melanin is formed (Nirmal and Benjakul, 2009). Therefore, prolonged post-mortem times prior to salting might result in substantial increases in browning intensity. This coincided with the decreases in *L**-value (Table1).

A_{280} and A_{295} of resulting *Kapi* slightly increased when the post-mortem time of shrimp used as raw material increased ($P<0.05$). Generally, A_{280} and A_{295} have been used to determine the formation of non-fluorescent intermediate compounds of the Maillard reaction (Ajandouz et al., 2001). With larger amount of free amino group in shrimp stored for a longer time, the Maillard reaction could take place during fermentation of 30 days to a higher degree. This was evidenced by higher A_{280} and A_{295}.

No differences in fluorescence intensity among *Kapi* produced from shrimp stored for 0, 6 and 12 h ($P>0.05$). Nevertheless, the increase in fluorescence intensity was noticeable in *Kapi* produced from 18 h-stored shrimp. Fluorescence intensity has been used to monitor the occurrence of intermediate products, which subsequently undergo polymerisation to form the brown pigments (Ajandouz et al., 2001). With higher Maillard reaction intermediates, *Kapi* produced from shrimp with 18 h of post-mortem time could further undergo browning reaction to a higher extent. As a result, *Kapi* was browner in colour.

3.2.5. Antioxidative activities

Antioxidant activities of water extract of *Kapi* produced from shrimp with different post-mortem times as tested by DPPH, ABTS radical scavenging activity and FRAP are presented in Table1. DPPH, ABTS radical scavenging activity and FRAP of water extracts of *Kapi* were in range of 1.05-2.38, 10.22-15.62 and 7.59-12.89 μmol TE/ g sample, respectively.

ABTS radical scavenging assay has been used to determine both hydrophilic and lipophilic antioxidants. FRAP is generally used to measure the capacity of a substance in reducing TPTZ-Fe(III) complex to TPTZ-Fe(II) complex (Sun and Tanumihardjo, 2007).

Overall, DPPH, ABTS radical scavenging activity and FRAP of water extracts of *Kapi* increased when the post-mortem time of shrimp used as raw material for *Kapi* production increased ($P<0.05$). Water extract of *Kapi* produced from shrimp with 18 h of post-mortem time showed the highest DPPH, ABTS radical scavenging activity and FRAP. On the other hand, the extract of *Kapi* produced by fresh shrimp (0 h) had the lowest activities.

During the extended post-mortem storage prior to salting, low molecular weight peptides and amino acids could be generated to a higher

extent. Protein hydrolysis or degradation during fermentation step also led to the formation of substantial active peptides which could interact with free radical and terminate the chain reaction of auto-oxidation.

This correlated with pronounced degradation of proteins in shrimp stored for a longer time before salting (Fig.2). The extracts from salted shrimp paste have been reported to possess antioxidant activities (Pongsetkul et al., 2015). Furthermore, Maillard reaction products might be partially involved in antioxidant activity. Maillard reaction products were reported to have antioxidative activity (Benjakul et al., 2005). Maillard reaction products were also increased in *Kapi* prepared from unfresh (Table1). Therefore, antioxidative activity of *Kapi* was governed by post-mortem time of shrimp prior to salting.

3.2.6. Volatile compounds

Volatile compounds in *Kapi* produced from shrimp with different post-mortem times analysed by SPME GC-MS are shown in Table2. Thirty-six volatile compounds were isolated and identified. Those compounds could be classified as aldehydes (5), ketones (6), alcohols (9), nitrogen-containing compounds (7), hydrocarbons (4) and others (5). Lipid-derived components such as aldehydes, alcohols as well as nitrogen-containing compounds were the major volatile compounds in *Kapi.*

3-methyl-butanal, pentanal, 4-heptanal, hexanal and benzaldehyde were the prevalent aldehydes found in all *Kapi* samples. *Kapi* with varying abundance of aldehydes might influence the flavour acceptability differently. In general, the presence of these compounds is related with lipid oxidation, which was more likely generated during storage or fermentation (Dissaraphong et al., 2006). Shrimp contained high amounts of ω-3 fatty acids, which were highly susceptible to lipid oxidation (Takeungwongtrakul et al., 2012). *Kapi* produced from shrimp stored for 18 h before salting showed higher abundance in all aldehydes, except 4-hexanal.

This was coincidental with the highest TBARS value of shrimp used as raw material stored for 18 h (Fig.1D). Furthermore, Steinhaus and Schieberle (2007) reported that branched short chain aldehydes or aromatic aldehydes plausibly resulted from deamination of amino acids. This was also in agreement with the highest amino nitrogen content found in this sample (Fig.3). Ketones were also found in *Kapi* including 2-heptanone, 1-(2-pyridinyl)-ethanone, 3,5-Octadien-2-one, etc. The higher abundance of most ketone compounds was obtained in *Kapi* produced from shrimp with 18 h of post-mortem time. This confirmed that delayed post-mortem salting allowed more lipid oxidation in raw material to take place. Those products were still presented in resulting *Kapi.* However, such compounds with low concentrations and high odour threshold values might not significantly contribute to flavour of salted shrimp paste (Cha and Cadwallader, 1995). Normal and branched alcohols, which were quite low in abundance, also detected in *Kapi*. 3-methyl, 1-butanol was found at higher abundance than others. This alcohol was increased in *Kapi* prepared from shrimp with increasing post-mortem times. However, Cha and Cadwallader (1995) reported that alcohols might not have a paramount impact on *Kapi* flavour because of their high flavour thresholds. All resulting *Kapi* consisted of 7 nitrogen-containing compounds, which were all pyrazine derivatives. 2-ethyl-5-methyl-pyrazine was dominant in all samples, followed by 2,5-dimethyl-pyrazine and 3-ethyl-2,5-dimethyl-pyrazine. Jaffres et al. (2011) reported that pyrazines derivatives associated with meaty flavour of shrimp sauce. Abundance of most pyrazine derivatives of resulting *Kapi* increased when the post-mortem time of shrimp used as raw material increased (P<0.05). Pyrazines were reported to be formed by Maillard reaction through strecker degradations from various nitrogen sources such as amino acids (Cha and Cadwallader, 1995).

Table 2. Volatile compounds of *Kapi* produced from shrimp with different post-mortem times.

Volatile compounds	Peak area (Abundance) × 10^6			
	0 h	6 h	12 h	18 h
Aldehydes				
3-methyl-butanal	215.22	206.11	411.12	732.51
Pentanal	294.58	199.76	408.81	466.25
4-heptanal	117.22	132.99	130.19	98.66
Hexanal	54.51	501.11	511.19	526.63
Benzaldehyde	162.8	203.42	452.61	688.22
Ketones				
1-phenyl-ethanone	128.71	134.34	205.55	205.13
1-(2-pyridinyl)-ethanone	237.93	304.49	314.51	505.82
1-(2-aminophenyl)-ethanone	150.54	68.88	102.25	111.33
2-Heptanone	597.82	413.22	499.65	404.52
2-Octanone	265.60	244.12	300.64	305.77
3,5-Octadien-2-one	117.14	332.26	304.55	502.65
Alcohols				
Benzenemethanol	42.95	35.55	69.32	167.83
2-Butoxy-ethanol	56.14	181.22	555.92	608.73
3-methyl, 1-butanol	299.19	625.55	632.72	820.24
1-Pentanol	225.61	203.44	144.41	28.32
1-Penten-3-ol	188.13	55.67	59.11	9.88
1-Hexanol	404.33	133.92	129.29	266.51
2-ethyl-hexanol	122.97	166.92	208.82	277.01
1-Octen-3-ol	171.89	204.45	229.33	301.99
Octa-1,5-dien-3-ol	57.04		106.66	169.72
Nitrogen-containing compounds				
Methyl-pyrazine	214.34	506.66	511.23	495.95
Trimethyl-pyrazine	252.17	345.52	304.41	406.77
2,5-dimethyl-pyrazine	898.28	933.49	925.67	1055.61
2,6-dimethyl-pyrazine	90.42	215.62	307.66	313.49
2-ethyl-5-methyl-pyrazine	1524.77	1866.17	1908.22	1855.52
2-ethyl-2,5-dimethyl-pyrazine	155.54	260.08	477.71	503.31
3-ethyl-2,5-dimethyl-pyrazine	644.91	601.98	802.22	899.13
Hydrocarbon				
2,6,10,14-tetramethyl-pentadecane	21.53		19.22	111.19
2-Undecyne		13.31	455.63	109.92
Hexadecane			61.63	13.34
Cyclododecane	23.98	50.66	45.61	77.84
Others				
2-methyl-propanoic acid	68.42	56.33		23.18
Octanoic acid	126.10	15.03		102.31
Methyl-ester-octadecanoic acid		19.44	18.49	
Phenol	261.08	505.55	635.48	904.41
1H-Indole	711.41	805.92	8908.23	9181.46

ND: non-detectable * Value in the parenthesis represent the abundance of compound in each sample.

This was in accordance with increasing browning intensity when post-mortem time of shrimp was extended. Thus, different freshness of raw material more likely had the influence on meaty flavour, as well as browning colour to some degrees.

Four hydrocarbon compounds were found in *Kapi*. Only cyclododecane was detected in all *Kapi. Kapi* produced from shrimp with 18 h of post-mortem time showed the higher abundance than others. Alkanes and alkenes are mainly formed from lipid autooxidation of fatty acids released from triglycerides (Latorre-Moratalla et al., 2011).

Additionally, all samples contained phenol and indole and *Kapi* produced from shrimp with 18 h of post-mortem times had the highest abundance than other samples. Cha and Cadwallader (1995) reported that phenol give an undesirable aroma in seafoods, whilst Lakshmanan et al. (2002) reported that indole is the degradation product from tryptophan and has been used as the index for shrimp spoilage. The higher abundance of indole in *Kapi* correlated well with the increased spoilage of shrimp as indicated by increased TVB, TMA as well as TVC.

Therefore, several reactions might be involved in formation of volatiles. Lipid hydrolysis and autooxidation, proteolysis and transformation of amino acids to several compounds could be generated during *Kapi* production. *Kapi* produced from shrimp with different post-mortem times showed varying volatile compounds. Thus, post-mortem times of shrimp used as raw material might have a great influence on the volatile composition which impact on the final flavour and odour of resulting *Kapi*.

Table 3. Likeness score of *Kapi* produced from shrimp with different post-mortem times.

Attributes	Post-mortem time			
	0 h	6 h	12 h	18 h
Appearance	6.86±0.77^{a}	6.81±0.93^{a}	6.92±0.87^{a}	6.95±0.74^{a}
Colour	6.90±0.83^{a}	7.05±0.92^{a}	7.24±0.83^{a}	7.14±0.91^{a}
Odour	6.90±0.97^{ab}	7.38±1.02^{a}	6.52±0.93^{b}	5.81±1.08^{c}
Texture	6.95±0.80^{a}	7.00±0.95^{a}	7.05±0.97^{a}	6.57±0.68^{a}
Flavour	6.67±1.08^{ab}	7.10±0.70^{a}	6.38±0.74^{b}	5.48±1.02^{c}
Overall	6.57±0.68^{b}	7.29±0.96^{a}	6.19±1.27^{bc}	5.86±1.02^{c}

Values are given as mean ± SD (n = 3).
Score are based on a 9-point hedonic scale (1: Dislike extremely, 5: Neither like nor dislike, 9: Like extremely).
Different lowercase superscripts within the same row indicate the significant differences (P<0.05).

3.2.7. Sensory properties

Likeness scores of *Kapi* produced from shrimp with different post-mortem times are shown in Table3. Amongst all samples, appearance, colour and texture likeness scores were not different (P>0.05). However, *Kapi* produced from shrimp with 18 h of post-mortem time showed the lowest odour and flavour likeness score (P<0.05). The result suggested that this sample might have undesirable volatiles, which were associated with deterioration products, particularly formed during the extended post-mortem storage prior to salting. This was in agreement with the highest abundance of aldehydes, ketones, phenol along with indole found in this sample. Furthermore, *Kapi* produced from shrimp stored for 18 h prior to salting also had the lowest overall likeness score (P<0.05). Different tastes or flavors were possibly caused by differences in volatile compounds (Table2), governed by freshness of raw material used. It was noted that *Kapi* prepared from shrimp stored for 6 h prior to salting showed the

highest overall likeness score (P<0.05). This might be caused by partial decomposition of nucleotides, in which some derivatives, especially inosine monophosphate (IMP) was formed. IMP has been known to have the umami taste (Hajeb and jinap, 2015). Non-significantly higher score for flavour likeness was also found in this sample. Based on overall likeness score, shrimp should not be stored more than 12 h before salting.

4. Conclusions

Small shrimp (*M. lanchesteri*) could be used as an alternative raw material for *Kapi* production. Protein degradation as well as lipid oxidation proceeded during the extended storage. Freshness of shrimp affected *Kapi* characteristics, in which *Kapi* became darker but had higher antioxidative activity as the raw material was unfresh. The delay in salting of shrimp should not exceed 12 h, when the resulting *Kapi* still had the sensorial property equivalent to that prepared from fresh shrimp.

5. References

Ajandouz, E.H., Tchiakpe, L.S., Ore, F.D. et al. (2001). Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Journal of Food Science*, 66 (7), 926-931.

AOAC. (2000). Official methods of analysis. Washington, DC: Association of Official Analytical Chemists.

BAM. (2001). Aerobic plate count. In J. Bryce (Ed.), Bacteriological analytical manual. (pp. 53-67), New York: U.S. Food and Drug administration, E-con publishing.

Benjakul, S., Visessanguan, W., Phongkanpai, V. et al. (2005). Antioxidative activity of caramelisation products and their preventive effect on lipid oxidation in fish mince. *Food Chemistry*, 90 (2), 231-239.

Cha, Y.J., Cadwallader, K.R. (1995). Volatile compounds in salt-fermented fish and shrimp pastes. *Journal of Food Science*, 60 (1), 19-27.

Conway, E.J., Nyrne, A. (1936). An absorption apparatus for the micro-determination of certain volatile substances I: The micro-determination of ammonia. *Biochemical Journal*, 27 (2), 419-429.

Dissaraphong, S., Benjakul, S., Visessanguan, W. et al. (2006). The influence of storage conditions of tuna viscera before fermentation on the chemical, physical and microbiological changes in fish sauce during fermentation. *Bioresource Technology*, 97 (16), 2032-2040.

Faithong, N., Benjakul, S. (2012). Changes in antioxidant activities and physico chemical properties of *Kapi*, a fermented shrimp paste, during fermentation. *Journal of Food Science and Technology*, 51 (10), 2463-2471.

Hajeb, P., Jinap, S. (2015). Umami taste components and their sources in asian foods. *Critical Reviews in Food Science and Nutrition*, 55 (6), 778-791.

Jaffres, E., Lalanne, V., Mace, S. et al. (2011). Sensory characteristics of spoilage and volatile compounds associated with bacteria isolated from cooked and peeled tropical shrimps using SPME-GC-MS analysis. *International Journal of Food Microbiology*, 147 (3), 195-202.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Food Microbiology*, 22 (7), 680-685.

Lakshmanan, R., Shakila, J.R., Jeyasekaran, G. (2002). Survival of amine-forming bacteria during the ice storage of fish and shrimp. *Food Microbiology*, 19 (6), 617-625.

Latorre-Moratalla, M.L., Bosch-Fusté, L., Bover-Cid, S. et al. (2011). Contribution of enterococci to the volatile profile of slightly-fermented. *LWT - Food Science and Technology*, 44 (1), 145-152.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. et al. (1951). Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry*, 193 (1), 265-275.

Meland, K., Willassen, E. (2007). The disunity of "*Mysidacea*" (Crustacea). *Molecular Phylogenetics and Evolution*, 44 (3), 1083-1104.

Mellgard, M.C., Civille G.V., Carr, B.T. (2007). Sensory evalution of Food: Principles and Practices. In M.C. Mellgard, (Ed.), Sensory Evaluation Techniques. (pp. 82-88), New York: CRC Press.

Nirmal, N.P., Benjakul, S. (2009). Effect of ferulic acid on inhibition of polyphenoloxidase and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. *Food Chemistry*, 116 (1), 323-331.

Peralta, E.M., Hatate, H., Kawabe, D. et al. (2008). Improving antioxidant activity and nutritional components of Philippine salt-fermented shrimp paste through prolonged fermentation. *Food Chemistry*, 111 (1), 72-77.

Phithakpol, B. (1993). Fish fermentation technology in Thailand. In K.H. Steinkraus, P.J. Reilly, (Eds.), Fish Fermentation Technology. (pp. 155-166), United Nation University Press.

Pongsetkul, J., Benjakul, S., Sumpavapol. et al. (2014). Chemical composition and physical properties of salted shrimp paste (*Kapi*) produced in Thailand. *International Aquatic Research*, 6 (3), 155-166.

Pongsetkul, J., Benjakul, S., Sumpavapol. et al. (2015). Properties of salted shrimp paste (*Kapi*) from *Acetes vulgaris* as affected be post-mortem storage prior to salting. *Journal of Food processing and preservation*, DOI: 10.1111/jfpp.12643.

Riebroy, S., Benjakul, S., and Visessanguan, W. 2008. Properties and acceptability of Som-fug, a Thai fermented fish mince, inoculated with lactic acid bacteria starters. *LWT-Food Science and Technology*, 41 (4), 569-580.

Senphan, T., Benjakul, S. (2014). Use of the combined phase partitioning systems for recovery of proteases from hepatopancreas of Pacific white shrimp. *Separation and Purification Technology*, 129 (1), 57-63.

Sriket, C., Benjakul, S., Visessanguan, W. et al. (2012). Low molecular weight trypsin from hepatopancreas of freshwater prawn (*Macrobrachium rosenbergii*): Characteristics and biochemical properties. *Food Chemistry*, 134 (1), 351-358.

Steel, R.G.D., Torrie, J.H., Dickey, D.A. 1980. In R.G.D. Steel (Ed.), Principle and procedure of statistics. (pp. 457-490), New York: McGraw-Hill.

Steinhaus, P., Schieberle, P. (2007). Characterization of the key aroma compounds in soy sauce using approaches of molecular sensory science. *Journal of Agricultural and Food Chemistry*, 55 (15), 6262-6269.

Sun, T., Tanumihardjo, S.A. (2007). An integrated approach to evaluate food antioxidant capacity. *Journal of Food Science*, 72 (9), 159-165.

Takeungwongtrakul, S., Benjakul, S., H-Kittikun, A. (2012). Lipids from cephalothorax and hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*): Compositions and deterioration as affected by iced storage. *Food Chemistry*, 134 (4), 2066-2074.

Vanderzant, C., Cobb, B.F., Thompson, C.A. et al. (1973). Microbial flora, chemical characteristics and shelf life of four species of pond reared shrimp. *Milk and Food Technology*, 36 (4), 443-449.

Acknowledgments

This research was supported by Prince of Songkla University and the Grant-in-Aid for dissertation from Graduate School, Prince of Songkla University, Thailand. The TRF Distinguished Research Professor Grant was also acknowledged for the financial support.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

CLASSIFICATION IDENTIFICATION OF ABALONE FLAVORING LIQUIDS BASED ON METAL SENSOR ARRAY

Xinglu Zhang[1,2,3], Yan Lv[1,2,3*], Huihui Wang[1,2,3], Xueheng Tao[1,2,3] and Xu Zhang[1,2,3]

[1]Dalian Polytechnic University, Dalian 116034, P.R China;
[2]National Engineering Research Center of Seafood;
[3]Key Laboratory for Seafood Processing Technology and Equipment of Liaoning Province;
*Corresponding author: * lvyan@dlpu.edu.cn*

Article history:
Received:
13 February 2016
Accepted in revised form:
24 April 2016

Keywords:
Abalone flavoring liquids;
Sensor arrays;
Principal component analysis;
Probabilistic Neural Network

ABSTRACT
In order to identify abalone flavoring liquids with different tastes and guarantee uniformity quality of instant abalone with the same taste, a sensor array composed of seven metal electrodes is constituted to test the taste quality of the abalone flavoring liquids. Five solutions which represented five basic tastes like salty, fresh, sour, bitter and sweet are tested by the sensor array to identify its identification ability in basic tastes. One-way analysis of variance, principal component analysis and probabilistic neural network algorithm are used to evaluate the recognition effect of this sensor array on basic tastes. The result shows that this sensor array has excellent identification ability on basic tastes. Then the sensor array is used to identify abalone flavoring liquids with five different tastes. The principal component analysis is used to achieve dimensionality reduction of measured data, and three principal components employed as the input neurons of the probabilistic neural network. The result shows that this sensor array has a good performance on abalone flavoring liquid identification and the correct identification rate is 92%.

1. Introduction

With the expansion of the seafood consumption market, the instant abalone product is accepted by more and more consumers due to its unique flavor, convenient to eat, resistant to storage and nutrient retain comprehensively (Liu et al., 2013). The taste quality of instant abalone product is mainly determined by abalone flavoring liquid adding in precooking process. At present, the taste quality of the abalone flavoring liquid is mainly evaluated by two methods: manual tasting and traditional physical and chemical test. However, manual tasting is based on the experience of the valuator, so the result has some disadvantages of strong subjectivity and poor repeatability. On the other hand, the traditional physical and chemical testing method can only be used to analyze the content of certain components.

The comprehensive information resulted from the interactions between different tasty substances can't be reflected by the result (Liu, 2012).

Relatively comprehensive and objective results can obtain when using a sensor array for gustatory analysis in the food industry. In recent years, sensor arrays are widely used in food industry. In foreign countries, Ghosh et al used five kinds of precious metal (Au, Ir, Pd, Pt, Cd) to constitute a sensor array, quickly determined the content of thea rubigin and thea flavin in black tea and at last estimated it

(Ghosh et al, 2012). Apetrei and his partners used a screen-printed electrode modified with polypyrrole, qualitatively and quantitatively analyzed the content of phenolic compound in virgin olive oil (Apetrei, 2013). At home, Muyan Zhou et al used 6 electrodes (Pt, Au, Pd, W, Ti and Ag) to constitute a sensor array, distinguished rice wine in different type, region, brand, vintage (Zhou et al, 2012 and Zhou et al, 2013). Zhenbo Wei et al constituted a sensor array with Au, Ag, Pt and Pd electrodes to detect the quality index of yogurt (Wei et al, 2013).

At present, researches of abalone products are focus on the processing technology, the taste quality of them catches less attention. Therefore, this paper proposes a method to constitute a sensor array with metal electrodes to identify the basic taste substances and present its identification ability to basic tastes. After that this sensor array is used to identify abalone flavoring liquids with different tastes, in order to effectively control the taste and guarantee uniformity quality of the products.

2. Materials and methods

2.1. Experiment equipment and test parameters

CHI620B electrochemical workstation and multi-channel selection instrument, Shanghai Chenhua Instrument Co., Ltd.; Nine metal disk electrodes with a diameter of 2mm (Ag, Au, Pd, Pt, W, Ti, Ni, Zn, Al) are used as working electrode, Two 1 × 10 mm platinum wire electrodes are used as reference electrode and auxiliary electrode respectively, this three types of electrode constitute a sensor array, Tianjin Aida Hengsheng Technology Co., Ltd.; HJ-2B multihead magnetic heating stirrer, Changzhou Guohua Electric Co., Ltd.; KQ-2200DB ultrasonic cleaning machine, Kunshan Ultrasonic Cleaning Equipment Co., Ltd.; JA1003B electronic balance, Shanghai Yueping Scientific Instrument Co., Ltd.; A computer is used for data collection and analysis.

The detecting system used in this experiment is composed of a sensor array, a data acquisition device and a computer. The output weak signals from the sensor array are collected by an electrochemical workstation, and then are sent to the computer to process. Thus a complete taste detecting system is constituted.

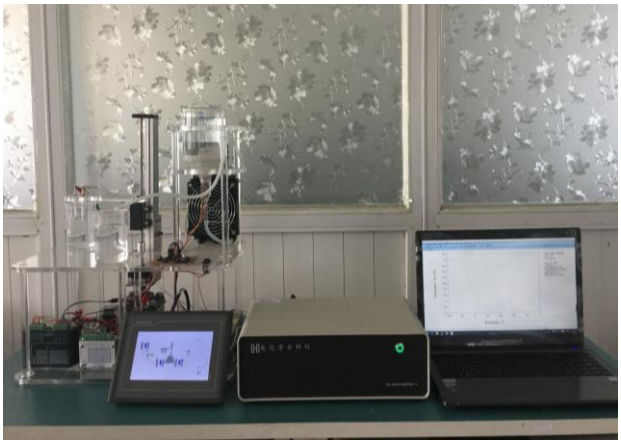

Figure 1. Detecting system

The electrode should be polished with a polishing cloth and aluminum oxide polishing powder when used for the first time, and it will be polished by polishing powder from coarse to fine, until the surface is smooth as a mirror. After that the electrode would be cleaned by ultrasonic with ethanol and deionized water and drew with filter papers. The working electrode would be polished with a polishing cloth without polishing powder in each measure. The auxiliary electrode and the reference electrode should be cleaned with deionized water and dried with filter papers(Tian, 2007).

The experiment is carried out under the condition of room temperature is 23 ℃. The reaction surface of working electrode immerse in sample 10mm deep. Cyclic voltammetry is used as the detecting method, its initial voltage is -0.8V, low voltage is -0.8V, high voltage is 0.6V, terminative voltage is 0.6V, scanning rate is 0.1v/s, sampling interval is 0.001V, data collecting frequency is 50Hz and sensitivity is e^{-5}～e^{-3} (Men et al., 2013).

2.2. Materials and reagents

Drugs represented basic tastes are: sucrose represents sweet taste, sodium chloride represents salty taste, lemon acid represents

sour taste, monosodium glutamate represents fresh taste, magnesium sulfate represents bitter taste. Drugs are analytical reagent made from Da Mao chemical reagent factory. Deionized water is used to prepare solutions with the concentration are 1%. Each sample is 80ml.

In order to maintain the original taste of seafood, the component of abalone flavoring liquid is simple. It mainly contains salt, monosodium glutamate, bone soup and other accessories. Manufacturers usually adjust the recipe according to the demands of customers. In this experiment, abalone flavoring liquids with five different tastes are used to test the identification ability of the sensor array. The recipe list of abalone flavoring liquid with five different tastes is shown in tab1. Condiments are dissolved in 100ml deionized water according to recipe list to make testing samples. All samples are prepared 20 minutes before the experiment and placed at the environment of 23 ℃.

Table 1. Recipe list of abalone flavoring liquid with 5 different tastes

Content	Salt	Monosodium Glutamate	Vinegar	Sugar
1 Light	1g	0.5g	0	0.5g
2 Sweet and fresh	1g	2g	0	3g
3 Salt and fresh	3g	2g	0	0
4 Sour and sweet	1g	0.5g	2ml	3g
5 Sour and fresh	1g	2g	2g	0

Note: the brand and kind of condiments: salt is natural salt made from China National Salt Industry Corporation, monosodium glutamate is made from Henan Lotus Gourmet Powder Co. Ltd, vinegar is white vinegar made from Shanghai DingFeng brewing food Co. Ltd., sugar is white granulated sugar made from Guangzhou Overseas Chinese sugar Co. Ltd.

2.3. Data processing method

One-way analysis of variance (One-Way ANOVA) is used to distinguish which group has a significant difference among the dependent variable groups, that is to do multiple comparison of mean value.

Principal Component Analysis (PCA) is a kind of data dimension reduction method, which is used to convert high dimensional data into a low one, and keep the characteristics of the original data as much as possible (Lu et al., 2013). The new variables of PCA are known as the principal components, which can be used to describe the sample space. PCA is executed by Statistical Product and Service Solutions (SPSS) software.

Probabilistic Neural Network (PNN) is a parallel algorithm developed from Bayesian criteria, and the Bayesian criteria are based on non-parameter estimation of probability density function (Liu, J.J et al., 2012). The PNN is executed by Matlab software.

In order to evaluate the identification result of each sample, correct rate is defined as:

$$C_R=(n-N_w)/n\times 100\% \quad (1)$$

In equation (1), C_R is the correct rate of identification; n is the number of samples; N_w is the misclassification number of samples.

3. Results and discussions

3.1.Basic tastes identification

3.1.1. A sensor response to basic tastes

Detecting accuracy of the system is decided by response performances of the sensor, which are based on stability of the sensor to the same sample and distinguish ability of the sensor to the different sample (Zeng et al., 2013). Five duplicate samples are prepared for each basic taste. A response current peak for potential excitation signals is used as the eigenvalue of a basic taste sample. The standard deviation (STDEV) of response signal to the same taste is calculated for each sensor. Except Al and Zn electrodes, the STDEV values of other electrodes are all below 0.5%. It means that the stability of them is very good. The STDEV values of Al and Zn electrodes are about 5%. It means that they are instable. Therefore Al and Zn electrodes are removed from the sensor array. The response of each sensor to different

taste substances has some difference. The method of least significant difference (LSD) for multiple comparisons is used to analyze measured data of seven electrodes (Ag, Au, Pd, Pt, Ni, Ti and W) that have a good stability. In this way, the distinguish ability of sensor for basic tastes can be recognized. The result shows that Ni electrode can commendably distinguish five kinds of basic tastes, and Ag, Pt and W electrodes can clearly distinguish salty, sweet and bitter tastes, Au electrode has a good identification ability for salty, sweet and fresh tastes, Pd electrode can distinguish sour, sweet and fresh tastes significantly, Ti electrode can only identify sweet taste. So it is difficult to make a distinct identification based on a single sensor, and it requires a comprehensive consideration of the impact of each sensor. Seven sensors (Ag, Au, Pd, Pt, Ni, Ti and W) are used to constitute a sensor array. Measured data of the sensor array are used for further analysis by means of PCA.

3.1.2. Principal component analysis of basic tastes

Three duplicate samples are prepared for each basic taste. Each sample is detected for 5 times. The response current peak of the sensor is taken as the measured data of the sample, and takes the average of 5 times measured data as the eigenvalue of a sample. That is obtaining 15 groups of 7 dimensional data. Bartlett spherical degree is used to test these data, the F value is less than 0.001, and the KMO testing coefficient is 0.695, greater than 0.5. That is to say that the data are reliable and valid. They can be used for PCA.

Fig.2 shows the results of PCA of basic taste substances. It intuitively presents relative position of the 15 groups of 7 dimensional data in 3 dimensional spaces. In the space of pc1＞0, only contains salty and sweet taste substances, and the rest taste substances are in the space of pc1＜0. Fresh taste substance is in the second quadrant of the plane consisted of pc1 and pc2, sour and bitter taste substances are in the third quadrant, close to each other. So the first three principal components should be retained for completely represents the sample characteristics. The variance contribution rates of the first three principal components are 49.54%, 29.081% and 15.412% respectively, and the cumulative contribution rate is 93.997%, which means the original information can be retained more than 93.997%.

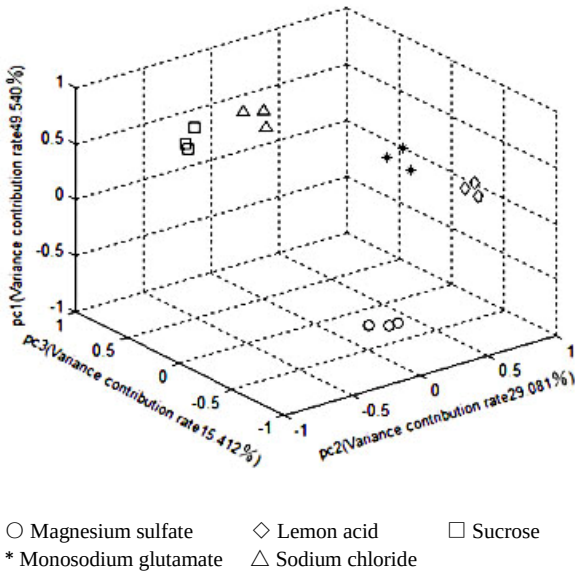

Figure 2. PCA results of basic taste substances

3.1.3. PNN construction of basic tastes

Five kinds of basic taste substances are tested in experiment. The response current peak of sensor is taken as the measured value of the sample. Three duplicate samples are prepared for each basic taste. Each sample is detected for 5 times. That is obtaining 75 groups of 7 dimensional data. 50 groups are chosen as the training set and the other 25 groups are used as the testing set. The training set is used to construct a probabilistic neural network and the testing set is used to test it. The input neurons are 3, which are the first three principal components resulted from PCA of 75 groups data of basic taste samples. The output neurons are 5, which are represented five kinds of basic tastes. The experiment verifies that the optimal smoothing parameter (spread) is 1.5. After network training, the data groups in testing set are correctly classified in their categories respectively. It means that the correct

identification rate of PNN is 100%. This sensor array has excellent identification ability for basic taste substances, so it can be used for identification of abalone flavoring liquid.

3.2. Identification of abalone flavoring liquid

3.2.1. PCA of abalone flavoring liquid

Abalone flavoring liquids with five different tastes are prepared according to the recipe list shown in tab.1. A sensor array consisted of seven kinds of electrodes is used to test these five kinds of abalone flavoring liquid. Three duplicate samples are prepared for each abalone flavoring liquid. Each sample is detected for 5 times. The response current peak of sensor is taken as the measured value of the sample, and takes the average of 5 times measured data as the eigenvalue of a sample. That is obtaining 15 groups of 7 dimensional data. These data are analyzed by means of PCA, and the first three principal components are retained. The variance contribution rates of the first three principal components are 45.877%, 32.569% and 12.861% respectively, and the cumulative contribution rate is 91.307%.

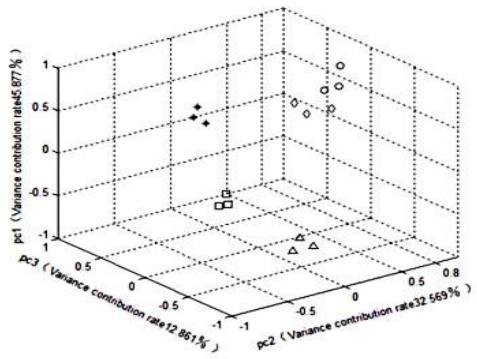

Figure 3. PCA results of abalone flavoring liquid in five different tastes

The distribution of the first three principal components in three dimensional spaces is shown in Fig.3. Each point represents a sample. There are three points in the figure to represent a same sample of abalone flavoring liquid. The points represented a same taste sample are close to each other. The points represented different taste samples have some distance. It suggests that these five different tastes abalone flavoring liquid can be distinguished commendably by the sensor array.

3.2.2. PNN Classified identification of abalone flavoring liquid

In order to identify the abalone flavoring liquids with five different tastes by PNN, the response current peak of sensor is taken as the measured value of the sample. Three duplicate samples are prepared for each tastes of abalone flavoring liquid. Each sample is detected for 5 times. That is obtaining 75 groups of 7 dimensional data. 50 groups are chosen as the training set and the other 25 groups are used as the testing set. The training set is used to construct a probabilistic neural network and the testing set is used to test it .The input neurons are 3, which are the first three principal components resulted from PCA of 75 groups data of abalone flavoring liquid samples. The output neurons are 5, which are represented five different tastes of abalone flavoring liquid. Other parameters are the same as the above mentioned PNN used for basic tastes recognition. After network training, the data groups in testing set are correctly classified in their categories except for one group of light taste and one group of salt and fresh taste. The correct identification rate of PNN is 92%. The result shows that the sensor array has excellent identification ability for 5 different tastes of abalone flavoring liquid.

4. Conclusions

(1) STDEV and One-way ANOVA are used to analyze the detecting signals of metal electrodes for basic taste substances. The metal electrodes which do not have good stability and distinguish ability are removed from the sensor array. The selected sensor array is used for identification of basic taste substances. PCA and PNN algorithm are used to analyze the measured data. The result shows that the sensor array has excellent identification ability for basic taste substances.

(2) The sensor array is used for classification identification of five different tastes of abalone flavoring liquid. The first three principal components are retained. The cumulative contribution rate of the first three principal components is 91.307%. The distribution of the first three principal components in three dimensional spaces can be represented intuitively by a three-dimensional diagram of PCA. The retained first three principal components are used as the input neurons of PNN. The output neurons are 5, which represents the five different tastes of abalone flavoring liquid. The constructed PNN is used to identify the abalone flavoring liquids. The correct identification rate of PNN is 92%.

5. References

Liu, Y., Wang, J., Sun, J.F., et al. (2013).The utilization situation and development tendency of marine shellfish resources in China. *Modern Food Science and Technology*, 29(3), 673-677.(in Chinese)

Liu, M. (2012).The application research of intelligent artificial taste analysis methods on food quality inspection of several kinds. *Doctor's degree thesis of Zhe Jiang University*. (in Chinese with English abstract)

Ghosh, A., Tudu, B., Tamuly, P., et al. (2012). Prediction of theaflavin and thearubigin content in black tea using a voltammetric electronic tongue. *Chemometrics and Intelligent Laboratory Systems*, 116,57-66.

Apetrei, I.M., Apetrei, C.(2013).Voltammetric electronic tongue for the quantification of total polyphenol content in olive oils. *Food Research International*, 54(2), 2075-2082.

Zhou, M.Y., Hu, X.H., Xu, J., et al. (2012). Research on sensory evaluation indicators and quantitatively predict methods of smartongue on yellow rice wine taste. *Liquor-Making Science and Technology*, 11, 39-45. (in Chinese)

Zhou, M.Y., Chen, F.R., Wu, X.H., et al. (2013).Determination of flavor components of Shaoxing yellow rice applying with electronic tongue technique. *Liquor-Making Science and Technology*, 3, 58-60. (in Chinese)

Wei, Z.B., Wang, J., Jin, W. (2013).Evaluation of varieties of set yogurts and their physical properties using a voltammetric electronic tongue based on various potential waveforms. *Sensors and Actuators B: Chemical*, 177,684-694.

Tian, S.Y. (2007).Construction and application of multiple frequency pulse electronic tongue system. *Master's degree thesis of Zhejiang Gongshang University*. (in Chinese with English abstract)

Men, H., Ning, K., Zhang, Y.P., et al. (2013). Classification of green tea using a differential pulse voltammetric electronic tongue based on FCM-SVM. *China Agricultural Mechanization Teansactions*, 34(5), 201-205.

Lu, X.Q., Chen, J., Zhou, X.B. (2013). Chemometrics methods.*Beijing: Science and Technology Publishing House.*

Liu, J.J., Sun, Y.H., Xie, G.P., et al. (2012).Classified identification of corn juice beverage based on the taste sensor array. *Agricultural Engineering Teansactions*, 28(24), 265-271. (in Chinese)

Zeng, X.S.H., Deng, D.W., Shi, L., et al. (2013).Sensor Array for Discrimination between Raw Milk and Deteriorated Milk. *Food Science*, 34(12), 208-212.(in Chinese)

Acknowledgments

This study is funded by Open Foundation of National Engineering Research Center of Seafood (2012FU125X03), Open Foundation of Key University Science and Technology Platform of Liaoning Province (No.2011-191) and Public Science and Technology Research Funds Projects of Ocean (201505029).

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

STUDY ON ROUTE OPTIMIZATION OF COLD CHAIN LOGISTICS OF FRESH FOOD

Jian Chai[1,2*]

[1]School for Humanities and Social Science, BeiHang University, Beijing, 100191, China
[2]Taiyuan University of Science and Technology, Taiyuan, 030024, China
*Corresponding author:*chaijian2002@126.com*

Article history:
Received:
4 March 2016
Accepted in revised form:
11 May 2016

Keywords:
Optimization;
Genetic algorithm;
Cool chain logistics;
Fresh food

ABSTRACT
Take the distribution of the national express refrigerated transport company as a example to verify the basic model of cold chain logistics distribution routes. If the transportation cost and the number of customers are consistent in per unit mileage, the longer service mileage from distribution center to the customers, the greater total transportation cost. Therefore, the total cost of vehicle transport is proportional to service mileage. The greater spoilage costs per product caused by the accumulation of mileage and the lapse of time, the higher total damage costs. The higher unit price, the greater total damage costs of fresh products caused by transport time and car door opening. Hence, the damage cost is proportional to the transport time and the unit price of goods. Divide customer points according to the regions of customer points and the limit load capacity of 11t vehicles based on good road condition, cluster analysis of results, respectively use the standard genetic algorithm and the improved genetic algorithm to calculate under the same control parameters, compare them and obtain the results. The results show that the improved genetic algorithm is superior to the standard genetic algorithm in the calculation of the speed and efficiency.

1. Introduction

Logistics distribution is a set of logistics activities include classifying and allocating goods in the logistics center in accordance with the requirements of the customer orders, and delivering prescribed goods to the consignees promptly. Distribution is the last link of customer service; its position is very prominent. Optimizing logistic distribution routing problem, also called Vehicle Routing Problem (VRP), which is one of the hot topics in the current researches of logistics system. It refers to the proper organization of vehicle routing between the delivery points and the receiving points under some constraint conditions such as customers' quantity demanded for goods, vehicles bearing capacity, shipping time, delivery time and travelled mileage, to achieve the goal of the shortest distance, least cost, delivery on time, used vehicle as less as possible. Many experts at home and abroad have proved that the genetic algorithm (GA) has great advantage in solving the VRP.

2. Materials and methods

2.1. component of Vehicle Routing Problem

The main components of Vehicle Routing Problem include goods, distribution center (or logistics center), customers and vehicles.

(1) Good

The goods is the object of distribution. We can take each demand goods as a batch of individual goods. Each batch of goods includes following properties: name, weight, volume, packaging, required delivery time and place, whether can partial distribution or not(Wang,

2012). The goods in cold chain logistics include the aforementioned processed foods, fresh fruits and vegetables and other agricultural products, as well as a part of drugs and other special products that need refrigerated transport(Montanari, 2008).

(2) Distribution center

Distribution center is engaged in disposing goods which consists of consolidation, processing, picking, allocation and organization of the delivery in order to achieve the modern distribution facilities for supply or sale(Palacio and Nuin, 2009). In a distribution system, distribution centers can appropriately adjust their number according to the distribution network problems (Hoang and Alvarez, 2012). If some distributions have a lot networks and their coverage is quite extensive, we often adopt multilevel distribution center to realize the delivery: the first level distribution center sends goods to the next level distribution center; under multiple two levels distribution centers, the research on how to arrange the distribution between different distribution centers relates to the optimizing distribution problem. (Li and Nien, 2010).

(3) Consumer

Customers can also be referred to users, including the retail stores, consumer point, etc. The number of goods required by a single customer may exceeds or less than the allowable loading capacity of a certain vehicle in a logistics distribution. In the above case, when the total demand for goods exceeds the total transport capacity, we need to deliver goods by several times or several vehicles; when the demand is less than the allowable loading capacity of a vehicle, we should carry out cargo stowage if possible (Wu and Zhao, 2013). The time of goods demanded by a customer is the required delivery time and it can be divided into the following several situations: no time window constraint; required during a specified period of time, namely complete delivery within the time window; have time window constraint, but may not comply if we take given punishment(Lan and Xue, 2013).

(4) Vehicles

Vehicle is the carrier of goods, its attributes include: type, loading capacity, size, purchase price, service life, the maximum stroke of distribution, vehicle parking location before and after the completion of the task, etc(Xu and Gong, 2016). Cold chain vehicle is the main distribution equipment for cold chain logistics system; the allocation of vehicle plays a decisive role in maintaining freshness of fresh food, improving transport efficiency, saving energy and reducing transportation costs; it not only affects the enterprise's economic benefit, but also related to the benefits of the whole society.

In order to play the distribution's role to achieve the distribution efficiency, the distribution process including: formulate a distribution plan, issue the distribution plan, confirm requisite amount according to the distribution plan, distribution points deliver goods to the warehouse, allot goods, packaging, finance department issue a specific task distribution, shipping and deliver, as shown in Figure 1.

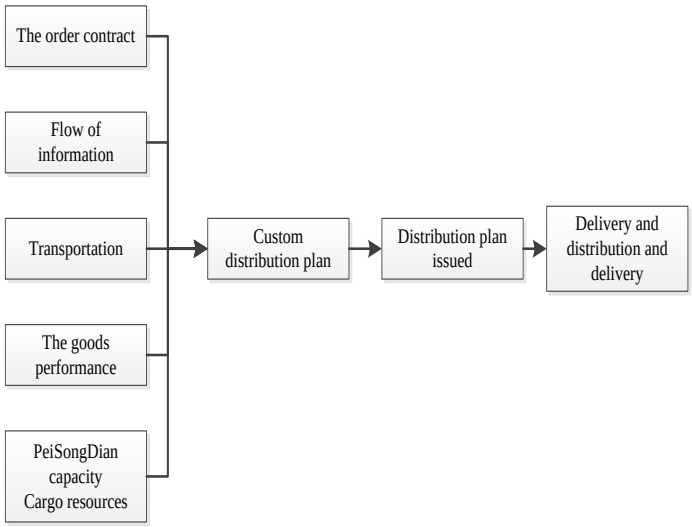

Figure 1. The working process of the vehicle distribution

2.2. Establishment of distribution mode

(1) Hypothesis on distribution problem

Model of cold chain logistics distribution is a distribution model where one DePot to many customers, the type of fresh food in distribution is single, also meets the following conditions:

①One-way flow of goods, that is, pure delivery;

②There are K vehicles, each vehicle has a certain load capacity constraint, but the total capacity of all vehicles is greater than a single customer demand on the transport route;

③Each customer's demand is known, the required goods can only be completed by a vehicle, and all customers have to get service;

④Set distribution centers in the starting point and the end point of each route, that is, all vehicles must complete the task within the specified time and return to the distribution center;

⑤Each customer has a designated service time window; Delivery must be carried out within this time range;

⑥If it belongs to multiple targets, the transportation cost is minimum, the transportation mileage and the total waiting time of all customers is shortest;

⑦The optimal distribution routes between distribution center and the customer, as well as between any two customers have been calculated by the logistics distribution route optimization system;

⑧Do not consider the situation of vehicle congestion, regard the road traffic is smooth, no rush hour.

(2) Establishment of distribution model

The transportation cost of distribution vehicle is comprised of 2 parts, fixed cost and variable cost. The fixed cost is constant, which is not directly related to the number of customers and transportation mileage. Generally, it includes vehicle depreciation expense, ancillary equipment, and fixed assets which is related to transportation, such as the driver's wages. If there are m vehicles, the fixed cost per vehicle is c_k, then the total fixed cost is $\sum_{k=1}^{m} c_k$. The variable cost of distribution vehicles includes fuel consumption, maintenance and other costs; the variable cost of vehicles is proportional to the traveled miles. For the general vehicle transportation costs of distribution, we adopt the formula (1) to calculate.

$$Z1 = \sum_{k=1}^{m} c_k + \sum_{i=0}^{n}\sum_{j=0}^{n}\sum_{k=1}^{m} c_{ijk} x_{ijk} \quad (1)$$

s.t.

$$\sum_{i=0}^{n} g_i y_{ik} \le q, k = 1, 2, \cdots, m \quad (2)$$

$$\sum_{k=1}^{m} yik = \begin{cases} 1 & i = 1, 2, \cdots, m \\ m & i = 0 \end{cases} \quad (3)$$

$$\sum_{i=0}^{n} x_{ijk} = y_{jk}, j = 0, 1, \cdots n, ; k = 1, 2, \cdots m \quad (4)$$

$$\sum_{i=0}^{n} x_{ijk} = y_{ik}, \mathrm{i} = 0, 1, \cdots n, \quad (5)$$

$$x_{ijk} = 0 \quad or \quad 1 \quad i, j = 0, 1, \cdots n; k = 1, 2 \cdots, m \quad (6)$$

$$y_{ik} = 0 \quad or \quad 1 \quad i, j = 0, 1, \cdots n; k = 1, 2 \cdots, m \quad (7)$$

(3) The damage cost in the process of delivery

Fresh food belongs to perishable food, there are many factors affecting the spoilage of fresh food. It is assumed that the fresh food can be maintained in fixed temperature in the delivery, then the decay of fresh food in distribution only relate to the delivery time without considering other influencing factors(Lan, 2012). On the other hand, the back door of vehicle will be opened due to customer service; it will increase the addled speed of fresh food. With the different opening frequency and length of time, the quality of fresh food will be affected in varying degrees. So we analyze the fresh food spoilage into two kind situations: One damage is caused by fresh food spoilage due to the accumulation of time in the delivery; the other damage is caused by customer service. When the back door of vehicle is opened, the hot air

from outside flow into and the cold air from inside flow out, which increases the temperature in vehicle, then the damage of goods is caused by rapid decline in quality of fresh products. The two parts of the cost can use formula (8) to calculate.

$$Z_2 = p\sum_{k=1}^{m}\sum_{j=0}^{n}\lambda_{jk}\left(a_1 zi_j + a_2\beta_j\right) \tag{8}$$

When the outside temperature is relatively high, the energy cost of the refrigerator vehicle is also higher; the real-time change of the temperature will affect the energy cost of the refrigerated vehicle during the distribution of fresh products. In the aspect of energy cost calculation, the energy cost in the distribution process is proportional to the temperature difference between the inside and outside of the car. Set the temperature difference between the inside and outside of the car at a given time asΔh (t), a is the energy cost at per temperature difference and per unit time, the total energy cost is:

$$\sum_{k=1}^{m}\left[\int_{t_1}^{t_2} a\Delta h(t)dt\right] \tag{9}$$

Amend the original model based on this one.

(4) The penalty cost when exceed the customer delivery time

$$\begin{aligned} &s0 = 0 \\ &x_{ijk} = 1 \Rightarrow s_i + t_i + t_{ij} = s_j \\ &i, j = 0,1,\cdots n, i \neq j \\ &E \le s_j \le T_j,\ j = 0,1,\cdots, n \\ &t_i = \max\left\{E_i - s_i, 0\right\} \end{aligned} \tag{10}$$

The following can be expressed as the penalty cost of the time window during the distribution of fresh goods:

$$Z_3 = d\sum_{j=0}^{n}\max(E_j - s_j, 0) + e\sum_{j=0}^{n}\max\left(s_j - T_j, 0\right) \tag{11}$$

(5) Optimization model of distribution

For the genetic algorithm, usually the evaluation function is only one. Under the circumstance of uniform target unit and dimension, the multi object model is processed directly by using the linear weighting method, which the simply add several targets into a single objective. Therefore, the optimization model of cold chain logistics distribution is as follows:

$$\begin{aligned} \min Z = &\sum_{k=1}^{m} C_k + \sum_{i=0}^{n}\sum_{j=0}^{n}\sum_{k=0}^{m} c_{ijk}x_{ijk} + p\sum_{k=1}^{m}\lambda_{jk}\left(a_1 z_{ij} + a_2\beta_j\right) + \sum_{k=1}^{m}\left[\int_{x_1}^{x_2} a\Delta h(x)dx\right] \\ &+ d\sum_{j=1}^{n}\max\left(E_j - s_j, 0\right) + e\sum_{j=1}^{n}\max\left(s_j - T_j, 0\right) \end{aligned} \tag{12}$$

s.t.

$$\begin{aligned} &\sum_{i=0}^{n} g_j y_{ik} \le q, k = i, 2, \cdots m \\ &\sum_{i=0}^{n} x_{ij} = 1,\ j = 0,1,\cdots n \\ &\sum_{j=0}^{n}\sum_{i=0}^{n} x_{ij} = n \\ &\sum_{k=1}^{m} y_{jk} = 1,\ j = 0,1,\cdots n \\ &E_j \le s_j \le T_j,\ j = 0,1,\cdots n \end{aligned} \tag{13}$$

Among them: The formula (13-1) indicates that the cargo capacity per vehicle does not exceed its maximum allowable cargo capacity q.

The formula (13-2) indicates that each customer must be serviced only once, no duplicate service;

The formula (13-3) indicates that all customers are served, not missing any of them.

The formula (13-4) indicates that each customer is serviced by only one car.

The formula (13-5) indicates that the service time of the customer shall be within the acceptable range of the customer.

2.3. Solution methods for vehicle routing model in cold chain logistics

Genetic algorithm is a kind of "generation and detection" iterative search algorithm. It takes all individuals in the population as operand, and every individual is the solution to correspondence problem. There are three main operations: selection, crossover and mutation. Apply Matlab7.0, compile the computer program which is suitable for the algorithm model, complete the solving process on the distribution problem, and obtain the optimal path, to achieve the guidance of the actual problem.

Structure the receiving points for fresh perishable goods as the chromosomes in the solution vector of distribution route optimization problem by using the natural number coding method. Make the solution vector in mathematical model into a chromosome which length is m+1 to express a feasible solution (feasible distribution route), that is, the first vehicle sets out from "0",after the completion of the tasks "i11,i12,i13, …i1t", it backs to" 0 ", which form a sub path 1; The second vehicle resets out from " 0 " to complete the tasks"i21,i22,i23, …,i2t"which are not visited before, then it also backs to "0" to form the sub path 2; Repeat in this way until all the tasks are completed.

For example, the distribution routes of chromosome 014502306780 are as follows:

Sub path 1: distribution center 0→customer 1 → customer 4→ customer 5 → distribution center 0.

Sub path 2: distribution center 0 → customer 2 →customer 3 → distribution center 0.

Sub path 3: distribution center 0→customer 6→ customer 7→ customer 8 → distribution center 0.

These sub paths' chromosome structures are ordered, if task 1and 4 exchange position in sub path 1, then the objective function value is changed; but the chromosome structures between sub paths are disordered, if the sub paths 1 and 2 exchange position will not affect the objective function value.

3. Results and discussions

3.1. Overview of living example

In order to verify and improve the effectiveness of genetic algorithm, we take the distribution process of Fuzhou national express refrigerated transport co., LTD for the customers in the urban area as an example. The number of distribution center is "0", which conducts distribution services for 30 customer points in the urban area. According to the information provided by the third-party cold chain logistics distribution business, the goods delivered to the supermarket are some fresh vegetables which are selling every day like cabbages, Chinese cabbages, tomatoes and potatoes, they can't be tainted by other odor, and their time limit of preservation is 12 hours. When the outside temperature exceeds 20 degrees, the temperature of the refrigerator car should be controlled within 2-15 degrees; when the outside temperature is lower than 20, the refrigerator car should deliver goods in normal temperature.

The coordinate value of each distribution point (employ the Beijing coordinate system), time window constraints and service time, and the quantity demanded in each distribution point, the delivery distances which are calculated by the cold chain logistics distribution optimization system see the attached sheet. The carrying capacities of transport vehicles which are equipped by company are respectively ISUZU III refrigerator cars with 3 t, 5 t, 8 t, refrigerator cars with 11 t mainly see Table 1. All sections are not forbidden segments, and the average running speed of the vehicles in the process of distribution is 35 km/h, the unit price has consulted market real price, as shown in table 5. Arrange the distribution routes reasonably to fully meet the time window constraints of the distribution points.

Table 1. The demand of different PeiSongDian distribution distance

Number	X coordinates	Y coordinates	Demand	The first to accept service time	Accept the latest service time	Service time
0	13271.603	2 896.715	0	6：15	17:00	0
1	13270.702	2 998.124	2	7：00	9:45	30
2	13270.466	2 900.727	2.5	7：00	10:00	45
3	13269.094	2 899.413	1	6：45	14:00	15
4	13254.365	2 900.727	3	6：45	12:00	30
5	13265.366	2 899.418	2	8：00	15:00	15
7	13271.607	2 896.715	2	7：00	17:30	15
7	13270.702	2 898.861	2	6：45	18:00	15
8	13270.466	2 900.727	1	6：15	14:00	30
9	13269.093	2 899.313	1.5	6：30	14:30	30
10	13254.365	2 900.727	3	7：00	13:30	20
11	13265.367	2 999.418	2	6：45	14:00	30
12	13271.603	2 896.715	2	6：15	15:40	30
13	13270.702	2 898.251	1	7：00	14:00	30
14	13270.446	2 900.727	1	7：30	17:30	30
15	13269.093	2 869.413	3	6：45	18:00	15
16	13254.365	2 900.723	2	6：15	11:00	15
17	13265.358	2 899.418	4	7：00	12:00	15
18	13271.603	2 839.415	2.5	8：30	18:00	20
19	13270.702	2 900.727	2.5	6：45	14:30	30
20	13870.466	2 899.418	2	6：15	14:00	30
21	13269.093	2 897.715	2	8：00	14:00	30
22	13554.365	2 428.861	2	7：00	17:00	45
23	13245.366	2 899.413	3	6：45	14:00	15
24	13271.603	2 911.727	3	6：15	14:30	15
25	13225.702	2 899.418	1	7：00	18:00	45
26	13270.466	2 896.715	1	7：00	13:00	30
27	13219.093	2 898.861	4	6：45	14:00	30
28	13254.365	2 899.418	2	7：00	14:00	25
29	13275.366	2 896.715	2.5	6：45	12:00	35
30	13265.366	2 878.861	1	6：00	14:00	15

Table 2. Cluster analysis results

The vehicle number	The customer points	The customer number	Bearing capacity of the vehicle
1	21,24,27,22,28	5	8
2	25,29	2	9.5
3	9,23,26,30	5	8.5
4	14,15,18,20	1	9
5	7,10,11,13	4	8
6	3,4,6,12,19	3	9
7	1,2,5,8,16,17	2	10

Table 3. The delivery of improved genetic algorithm for time window to check

Vehicle	The customer number	Client access order	Distribution range (km)	Distribution range (km)	Delay time (m)	Algorithm to calculate time (s)
1	5	0-27-22-28-24-21-0	15.477	0	0	2.275
2	2	0-25-24-0	27.095	0	0	1.769
3	3	0-23-25-30-9-0	17.822	0	0	2.288
4	2	0-21-14-15-18-0	13.498	0	0	2.703

5	2	0-5-11-14-10-0	16.526	0	0	3.688
6	3	0-4-3-6-12-19-0	14.658	0	0	3.131
7	4	0-1-3-8-14-17-5-0	12.522	0	0	3.219

Table 4. Delivery time window to check the standard genetic algorithm

Vehicle	The customer number	Client access order	Distribution range (km)	Distribution range (km)	Delay time (m)	Algorithm to calculate time (s)
1	5	0-27-22-28-24-21-0	13.477	0	0	2.247
2	2	0-25-24-0	24.025	0	0	1.769
3	3	0-23-25-30-9-0	12.827	0	0	2.388
4	2	0-21-14-15-18-0	13.494	0	0	2.503
5	2	0-5-11-14-10-0	15.226	0	0	3.621
6	3	0-4-3-6-12-19-0	14.658	0	0	3.231
7	4	0-1-3-8-14-17-5-0	12.523	0	0	3.219

3.2. Example analysis

Under the good road condition, adopt large trucks as far as possible to carry out a joint distribution for improving the full load rate and reducing operating costs. According to the specific requirements of the distribution of vegetable products, select the truck with 11 t as a distribution vehicle to solve the problem.

According to the data in Table 1, divide customer points according to the regions of customer points and the limit load capacity of 11t vehicles based on good road condition. The results of cluster analysis see Table 2 (each serial number represent the vehicle number of each transportation route).

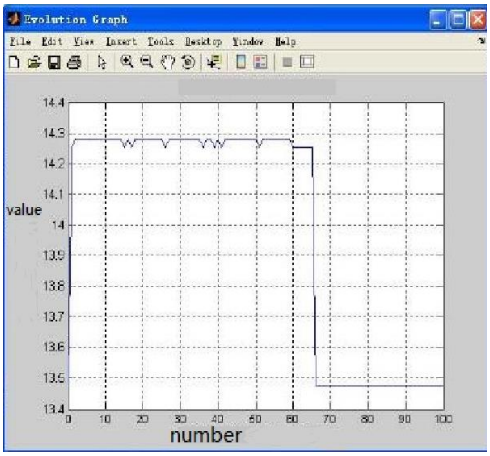

Figure 2. The improved genetic algorithm for the calculation process

Set the specified population number n = 100, the maximum evolution algebra C=100, crossover probability Pc=0.9, mutation probability Pm=0.02 by using the proposed improved genetic algorithm. To achieve rapid search and get the optimal distribution routes with the help of Matlab 7.0.

Figure 2 is the improved genetic algorithm for the calculation process of the first routes; Figure 3 is the standard calculation process of genetic algorithm.

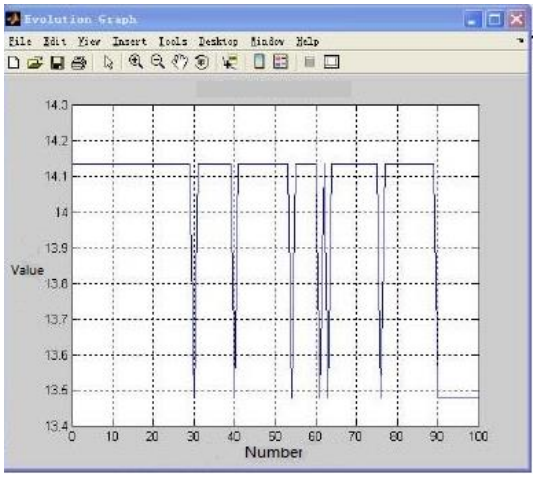

Figure 3. The standard calculation process of genetic algorithm

The distribution time of the above 30 distribution points are within the time window, so there is no penalty cost. The distribution routes in Table 5 appear the violation of the restrictions of time window. Therefore, there should be a corresponding penalty cost, we can see that the improved genetic algorithm has significantly improved in computing speed and efficiency when compared with the standard genetic algorithm.

Check each distribution task in list 3, the distribution time of the above 30 distribution points are within the time window, so there is no penalty cost. The distribution routes on Table 4 appear the violation of the restrictions of time window. Therefore, there should be a corresponding penalty cost. Contact Table 2, we can see that the improved genetic algorithm has significantly improved in computing speed and efficiency when compared with the standard genetic algorithm.

3.3. Results Analysis

According to the results of the comprehensive calculation, we can know that the total cost of the distribution by the improved genetic algorithm is 6 192.2 RMB, and the total cost by standard genetic algorithm is 7105.8 RMB, in which the transport cost is the largest cost of the project, which accounts for about 58.5%-68.4%.

The total sum of energy cost and damage cost accounts for about 20.06%-23.02% of the total cost of distribution. If delivery goods in the normal temperature, the variable cost of transportation accounts for about 22.04%-24.57% of the total cost of the distribution, and the fixed cost accounts for 49.98%-57.62%. Therefore, in the distribution of fresh products, only considering the energy costs and the damage costs can reflect the meaning of cold chain logistics distribution. At the same time, the damage costs in cold chain logistics is significantly lower than that in the normal temperature logistics; it helps to reduce the cost of fresh food distribution, and proves the practical value of the research.

Compare the total distribution cost of the improved genetic algorithm with that of the standard genetic algorithm, the optimal distribution routes which are obtained by using improved genetic algorithm can not only satisfy the time window constraints between chain stores and vehicle capacity constraints, but also in computing speed and efficiency. Standard genetic algorithm only considers the capacity constraints of distribution vehicles, and does not take the time window constraints of the chain stores into account, which increases the penalty cost. Therefore, the improved genetic algorithm adopted in this research is better than the standard genetic algorithm in solving the optimization problem of cold chain goods distribution routes.

4.Conclusions

Take the distribution of the national express refrigerated transport company as an example to verify the basic model of cold chain logistics distribution routes. If the transportation cost and the number of customers are consistent in per unit mileage, the longer service mileage from distribution center to the customers, the greater total transportation cost. Therefore, the total cost of vehicle transport is proportional to service mileage. The greater spoilage costs per product caused by the accumulation of mileage and the lapse of time, the higher total damage costs. The higher unit price, the greater total damage costs of fresh products caused by transport time and car door opening. Hence, the damage cost is proportional to the transport time and the unit price of goods

Divide customer points according to the regions of customer points and the limit load capacity of 11t vehicles based on good road condition, cluster analysis of results, respectively use the standard genetic algorithm and the improved genetic algorithm to calculate under the same control parameters, compare them and obtain the results. The results show that the improved genetic algorithm is superior to the standard genetic algorithm in the calculation of the speed and efficiency.

5.References

Hoang, H.M., Alvarez, G. (2012). Combined deterministic and stochastic approaches for modeling the evolution of food products along the cold chain. *Part I: Methodology. International Journal of Refrigeration*, 35(4), 907-914.

Lan, H. (2012). Decision-making on food cold chain collaborative distribution models.

Advances in Information Sciences and Service Sciences, 4(23), 660-666.

Li, C.M., Nien, C.C. (2010). Development of wireless sensor module and network for temperature monitoring in cold chain logistics. *Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)*, 43(6), 1707-1711.

Lan, H., Xue, H. (2013). Classification and application on food cold chain collaborative distribution models. *LISS 2012 - Proceedings of 2nd International Conference on Logistics Informatics and Service Science*, 2(12), 123-128.

Montanari, R. (2008). Cold chain tracking: a managerial perspective. *Trends in Food Science and Technology*, 19(8), 425-431.

Palacio, F., Nuin, M.Z. (2009). RFID smart tag for traceability and cold chain monitoring of foods: Demonstration in an intercontinental fresh fish logistic chain. *Journal of Food Engineering*, 93(4), 394-399.

Xu, Y., Gong, B. (2016). A feasibility research on the mechanism of cold chain business in Deppon logistics, *International Journal of Technology*, 9(3),157-168.

Wu, L.Z., Zhao, Y. (2013). Cold chain logistics temperature monitoring system based on internet of things technology. *Applied Mechanics and Materials*, 4(16), 1969-1973.

Wang, X. (2012). Experimental study of response characteristics of gas sensors for monitoring table grape cold-chain logistics. *Nongye Jixie Xuebao/Transactions of the Chinese Society for Agricultural Machinery*, 47(1), 240-246.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

INDIVIDUAL ATHLETE DIETARY AND NUTRITION KAB METHODS RESEARCH

Ming Tang[1*]

[1]School of Basic Teaching, Jiangsu Food & Pharmaceutical Science College, Huaian, 223000, P. R. China
*Corresponding author: *mingtang_ty@126.com*

Article history:
Received:
25 February 2016
Accepted in revised form:
5 May 2016

Keywords:
Athletes;
Nutrition education;
Dietary behaviors;
Logistic regression;
Nutrition KAB

ABSTRACT
In this paper, the integrated use of expert interviews, literature review, questionnaires, mathematical statistics, methods and means was discussed. On the part of nutrition education and nutrition status, college athletes KAB investigation was analysis. Athletes lack of proper nutrition concept, low nutrition knowledge, which exist more errors. There is eat animal meat that is nutritional supplements, it can enhance physical fitness. Before the game gluttony assault nutritional supplement, it can improve physical fitness. Some knowledge of science or even less than ordinary people. Survey results showed poor results that athletes nutrition education method is not standardized. 43.8% think athletes sports performance nutrition awareness of no effect, 40.6% of the athletes are not interested in nutrition knowledge, the data indicate a great need for athletes exhibition effective nutrition education and established the correct concept of nutrition.

1. Introduction

Athlete nutrition has two basic characteristics: first, its unique characteristics of vocational training and competition, leading to energy and nutritional needs of athletes, which is different from the general population. The second is due to the different sports are engaged, resulting in a certain sport athlete nutrition items group and special features(Brown, 2012;Roseman, 2011). Characteristics determine the athlete nutrition, which is not a general pattern in the general population nutrition "quality" and "quantity" on the simple increase or decrease, but it has a lot of own characteristics (Jitomir, 2008; Zhang, 2015; Qu, 2015; Stevens, 1999). Athlete's athletic ability, the skill ability, physical ability, tactical ability, intelligence and other mental capacities, of which the relationship between physical ability and dietary most closely (Volpe, 2007). Physical ability is the human body morphology, functional capacity, organic combination of athletic ability and coordination of comprehensive ability to interact formed an important part of sports ability. Scientific training, nutrition reasonable diet, good competitive state and psychological quality of athletes achieved outstanding competition results of an important guarantee(Herzman-Harari, 2013).

Athletes are a special group, always to deal with the increasingly fierce various types of athletic tasks. Under the premise of prohibiting the use of stimulants to maintain a strong physical and intense athletic resume as soon as possible after regeneration to fight another day, a great need for reasonable and effective nutrition interventions (Wagenmakers, 2015; Justin, 2013). Players eliminate fatigue after training means doping-free nutritional

supplement the diet than before is an important part of the best state of motion arise (Griffin, 2016). At present, China's athletes diet and long-term existence of protein, fat and carbohydrate intake disorders, these three pyrogen metabolism in the body is using each other, mutual restraint, in a dynamic equilibrium, so they must have an appropriate ratio, in order to have conducive to the normal physiological function and increase metabolism, and improve exercise capacity (Wessels, 2016). Too fat for energy, low carbohydrate, and with the increasing problem of economic development, which is no doubt the health of athletes and athletic ability are very unfavorable, especially adolescent athletes is an important period of growth and development, it is a reasonable and balanced diet the material basis for growth and development, but also for lay a solid foundation movement (Wroblesk, 2010; Swinbourne, 2015; Sedeaud, 2014; Walle, 2007). Studies have shown that nutrition for disease occurrence, development and prognosis have a great impact. With economic development and living standards improve, more and more people pay attention to proper nutrition, eager to gain more knowledge of nutrition (Cadzow, 2015). However, in real life, there are still a lot of malnutrition and nutrition problems, serious harm to human health, its main causes is the lack of proper nutrition knowledge, can not reasonably choose and match food caused. Students are in the shape of growth period, there is great plasticity, health education for college students to correct poor eating behavior will play a multiplier effect (Helzberg, 2010). Especially medical students, because of their special nature of work undertaken after graduation, improve their nutritional knowledge is essential capabilities and a reasonable choice of food.

Nutrition education is by changing people's eating behavior and achieve a change in the nutritional status of the purpose of planned behavior. As a convenience, economic interventions, governments and nutritionists have been one of the main effective means of improving people's nutritional status. Nutrition education as a "bridge" that allows players more effectively and quickly understood that the application of various benefit their nutritional knowledge. In this paper, nutrition education and nutrition portion of professional athletes and college athletes know the knowledge, Attitude, Behavior status of a preliminary investigation, to carry out teaching nutrition to provide scientific ideas to keep the sports system and reference.

2. Materials and methods

2.1. Athlete's nutrition cognitive status

As can be seen in Table 1, two groups of athletes majority (68.1%, 55.2%), "do not know" and "well aware" of the athletes were only 5.4 %, 9.8%. Situation two are basically the same, no significant difference (P> 0.05). Since most athletes completely unknown or just little understanding, meal tower five-layer structure of the food, so there are a great misunderstanding on food, that an increase in nutrition is to eat more meat and protein, while ignoring the meal pagoda bottom of whole grains and fruits and vegetables of the second layer, resulting in unreasonable athletes diet, protein, fat and carbohydrates three pyrogen imbalance (Adams, 2008; Gibala, 2016).

Table 1. The realization to nutritional knowledge of the athletes

Item	Province Team			P. E. College	
	Familiar	Little	Blind	Familiar	Little
Dietary guidelines	4.8%	27%	68.2%	9.1%	36.9%
Poisoned food	10.2%	50.1%	38.1%	7.9%	64%
Overeating	17.2%	63.5%	19.5%	12.4%	75.9%

Good nutrition knowledge can guide the selection of athletes scientific and reasonable balanced diet and a healthy lifestyle, the formation of physical fitness, enhance physical fitness, in order to improve training and competition results. Nutrition KAB connotations are interrelated and influence each other. Provincial team Athletes 25.9% believe that very effect, 66.5% think that some action, and that there is no effect of 7.6%, the institute Athletes 48.5% believe that very effect, 49.5% think that some action. Only 2.1% believe there is no memory effect.

Medical students understand the current level of understanding and knowledge of nutritional eating behavior, provide a reference for future conduct nutrition health education. According to Health Education Knowledge - - line model theory, the process of health behavior change is a change of attitude by the knowledge to behavior, and only have a certain knowledge and a good attitude to education, be possible to achieve this transformation. Nutrition and nutrition knowledge attitude and behavior is positively related to improve the nutritional knowledge can affect nutrition attitude, and then guide them to take the correct eating behavior, correct nutrition attitude, help to improve the nutritional health of the entire population.

2.2. Statistical analysis

Based nutrition KAB questionnaire model and the specific circumstances of the students design their own questionnaires, pre-survey, to discuss revisions, including an analysis of the project and checked for the project. Unified training for investigators, who pass as an official investigators. Investigation by the method according to the class cluster sampling, the questionnaire valid numbers through questionnaires filled out by the ombudsman after the respondents to explain himself, after questionnaires were collected by investigators for review, excluding invalid questionnaires.

Establish Epidata database, after checking data entered into the computer, use SPSS12.0 to organize and analyze data. The data were the variables descriptive analysis, $\chi 2$ test and logistic regression analysis. With $\alpha = 0.05$ as the test standard.

In regression problems if the response y is binary in nature, it actually becomes classification, the so-called binary classification. To make the problem more intuitive analysis of some argument assumes y is 0 or 1. Logistic regression is an effective way to solve this binary classification problem. The law is also under some assumed probability model launch. First examine the function:

$$h_w(x) = g\left(w^T x\right) = \frac{1}{1+e^{-w^T x}} \quad (1)$$

In it:

$$g(s) = \frac{1}{1+e^{-z}} \quad (2)$$

Called Logistic function or sigmoid function. Assume that a given x; conditions under w, y = probability of 1 obey Bernoulli distribution, and can be expressed as:

$$p(y=1|x,w) = h_w(x) \quad (3)$$

$$p(y=0|x,w) = 1-h_w(x) \quad (4)$$

Above two equations can be combined into a compact form:

$$p(y|x,w) = h_w(x)^y \left(1-h_w(x)\right)^{1-y} \quad (5)$$

Wherein y∈ {0,1}. M in the number of samples for independent case, sample data likelihood function is:

$$\begin{aligned} L(w) &= p(Y|X,w) \\ &= \prod_{i=1}^{n} p\left(y^{(i)}\left|x^{(i)},w\right.\right) \\ &= \prod_{i=1}^{n} \left(h_w\left(x^{(i)}\right)\right)^{y^{(i)}} \left(1-h_w\left(x^{(i)}\right)\right)^{1-y^{(i)}} \end{aligned} \quad (6)$$

Another easy way to express the value of the function y Release index is {1,1} when:

$$J(w) = -\sum_{i=1}^{n} \frac{1+y_i}{2} \log p_i + \frac{1-y_i}{2} \log\left(1-p_i\right) \quad (7)$$

To make the process clear and concise derivation, we only consider the case of a single variable x is x, and without loss of generality, the results can be generalized to the case of vector x.

Linear regression model assumes that the probability of:

$$p(x,\theta) \sim N(\mu,\sigma^2) \quad (8)$$

In binary logistic regression, it is assumed probability model:

$$p(x,\theta) \sim \text{Bernoulli}(\phi) \quad (9)$$

The fact that these models can be generalized into a class of model families, known as generalized linear models.

3. Results and discussions

3.1. Drinks drunk in training rehydration

Figure 1 shows that the majority of athletes (72%) choose rehydration aspect of water, purified water, mineral water, into the rehydration misunderstanding, did not understand the purpose of not only the movement of fluid replenishment, but also to supplement the loss of movement, fatigue useful energy source. Only 12 % of the athletes: do choose the fruit and vegetable drinks. Athletes in training lost large amounts of water and electrolytes, rehydration for athletes to restore volume and penetration Kivu, supplementary energy source, eliminating fatigue is very important. Cola and other carbonated beverages containing CO_2, could easily lead to stomach discomfort, and the inorganic salt content of cola towel may not be able to meet replenish lost salts motion needs, not suitable for use as a post-exercise rehydration.

Drinking water and other materials as a supplementary energy of little significance, and fruit and vegetable drinks rich in vitamins, carbohydrates, trace elements, is a complement both the vertical movement of water and salts lost, but also to supplement the energy consumption of drinks. Therefore, athletes should be reasonable nutrition education rehydration. In training, the race to promote a scientific and reasonable formula supplemented sports drinks.

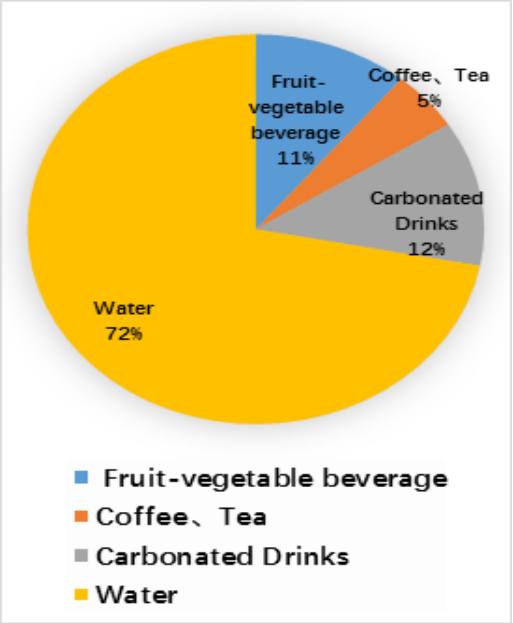

Figure 1. Drinks drunk in training rehydration

Universal acceptance of nutritional knowledge of a good attitude, in the 603 survey respondents, most athletes believe that nutrition is important, 88.7% of the athletes are willing to change their eating habits to their health, 94.7% of athletes want to make their diet more in line with nutritional requirements. 78.4% of the athletes against smoking, 90.7% of the athletes never smoked; 52.9% of the athletes against drinking and 62.5% of the athletes never drinking. 60.7% of the athletes say that physical exercise is the best way to lose weight.

3.2. Nutritional supplements inappropriate drawbacks

Improper anti-nutritional supplements can harm the body. Athletes' heat comes mainly from within the egg, fat, carbohydrates. Since these three metabolism in the body with relative valley, phase watt constraints in dynamic equilibrium, so they must have an appropriate ratio, to improve and benefit the normal physiological function, exercise capacity and increase metabolism. Disproportionate influenced not only physiological function, but also reduced exercise capacity and cause certain diseases. Such as: too much fat is not only to increase metabolic oxygen consumption, but also

impede the metabolism of carbohydrates, acidic metabolites increased, affecting exercise capacity. Long-term intake of too much fat can lead to coronary heart disease.

Multi-body exercise in a hypoxic state, a high intake of fat and carbohydrates and less fat is not easy to be fully oxidized to produce bodies, blood accumulation of acidic substances, pH values decrease, prompting athletes fatigue, reduced exercise capacity. At the same time elevated blood lipids, blood viscosity tone, blood flow slowed down, the body more oxygen, absorb too much cholesterol, can easily lead to coronary heart disease. Prisoners should be appropriate for this limit fat intake, and depending on the item for energy characteristics, heat the fat ratio is controlled at about 25%, while increasing carbohydrate intake.

3.3. Athlete's physical

Based on interviews understood that during the two most high school athletes namely began receiving special training, mainly in the sports school time with coaches, teammates life, some athletes through sports talented students into the provincial team selection procedures or universities, with a small number of athletes in the experience general school students like learning, examination into the college.

Ideal physique is the body in full, based on the potential of innate endowment, acquired by actively cultivate, so that the body morphological structure, physiological function and psychological state of the environment, ability to adapt to the overall development, with relatively good physical condition.

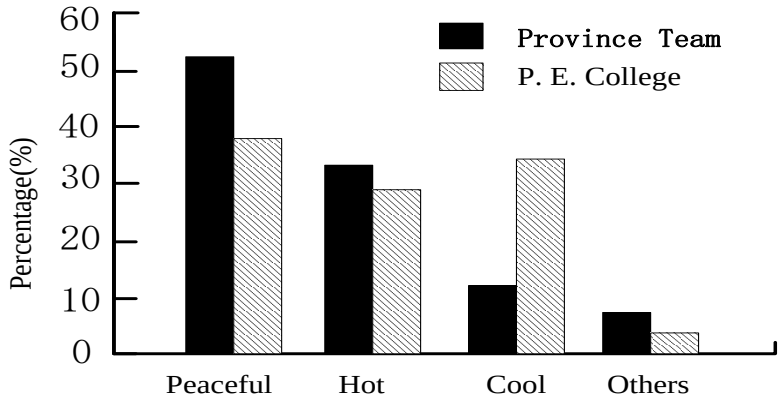

Figure 2. Compare two athletes physique type

Figure 2 show two sets of data, the provincial team group level and type accounted for 50.3%, compared with a significant difference (P <0.01) Partial cold type. Since the street team group training, sports-based, supplemented by theoretical study, reflecting the movement of the body to promote the quality of health.

The survey also found that the way athletes nutrition knowledge of newspapers and books in descending order of 83.4%, TV 54.9%, 44.6% and other schools, school classroom education only ranked No. 3. Classification of eating behavior score (Y) long on logistic regression analysis showed that: the attitude of nutritional knowledge (X1), nutrition knowledge score (X2), father's education are protective factors of eating behavior, indicating knowledge about nutrition deeper level, the better the attitude of acceptance of nutritional knowledge, the higher the father's education level, the more healthy eating behaviors. Colleges and universities are education, life and job skills to master an important place in education and health plans to play a larger role in place. Therefore, the school should play to their strengths in education, increase nutrition knowledge and education efforts through the classroom, combined with newspapers, books, television, blackboard newspaper and other forms, there are plans to athletes preach health-related knowledge,

training athletes good health awareness improve the ability to adapt and self-care ability, to develop good habits, to guide the behavior of athletes out of the diet misunderstanding.

3.4. Athletes taking nutritional supplements

To accommodate the large amount of specialized training and high-strength, balanced diet alone can not meet a lot of energy substances and other nutrients consumed by professional training, must be functional sports nutrition supplements to help athletes improve athletic endurance and stamina and recovery ability to help athletes under more pressure training and additional training to adapt to greater stress, create a better athletic performance. Nutritional supplements improve Athletes huge role in promoting, it has gradually attracted the attention of people in the field of exercise science.

Figure 3 shows that the group most provincial team athletes recognize that the appropriate use of nutritional supplements can be beneficial to training and competition, taking the rate of 4%; the institute group took 40.2% rate compared very significant difference between two groups (P <0.01). Institute group athletes did not participate in athletic competition, so 59.8% of the athletes nutritional supplements not know the place.

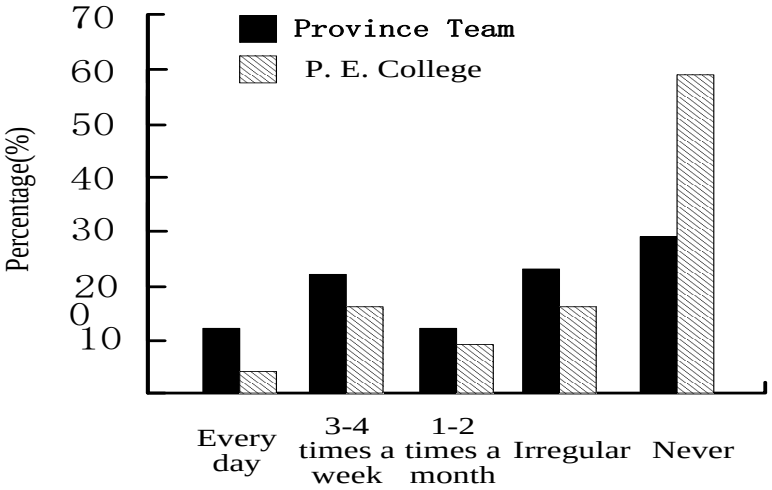

Figure 3. Athlete taking nutritional supplements

3.5. Nutrition K, A, B scores and total scores

K Score: nutrition knowledge score; A point: knowledge of nutrition and healthy attitude score; B point: reasonable dietary behavior score) (Table 2).

Table 2. Result of K, A, B score and total score

Item	K	A	B	Total
Full Score	40.00	10.00	21.00	71.00
Mean Score	21.73	7.87	11.17	40.78
STD	6.62	1.54	3.20	8.58

Nutrition knowledge at the middle level (low accounted for 56.1%, moderate share of 36.2%); poor nutrition behavior at level (low accounted for 63.7%, moderate share of 30.3%; but showed better nutritional attitudes (score of 66%); out of lower middle (low 59.0%) , which was shown in Table 3.

Table 3. Distribution of K,A,B score and Total score

Level	K(ratio)	A(ratio)	B(ratio)
<60	338(56.1)	45(8.0)	388(63.7)
60-80	316(36.2)	188(28.5)	156(30.8)
>80	44(7.8)	420(66.0)	32(5.2)

By K, A, B method, it found that the majority of players to score a reasonable diet, vitamins and trace elements in food sources, diet and disease (such as diabetes, hypertension, high cholesterol) relationship, Formaldehyde, clenbuterol, phytochemicals alerted rate relatively low. Athletes surveyed general lack of nutritional knowledge. Nutritional behavior scores are lower, but most athletes are interested in nutrition knowledge, nutrition attitude is better, indicating a strong desire for knowledge athletes, hoping to get more nutrition knowledge to change their bad eating habits, improve their quality of life. Instructions to carry out knowledge of nutrition education in athletes is necessary, urgent and possible. From the nutritional point of view, the gap is appropriate to eat two meals a higher nutritional value of fruit snacks, milk, it can compensate for the lack of meals, the body is beneficial. Conversely, if a large number of meals to eat some high energy content and low content of other nutrients snacks, such as chocolate candy, puffed food, instant noodles, will affect the appetite dinner time, resulting in inadequate food intake dinner. So snack time snack type, amount of snacks to make a rational choice.

Survey data show that the health of athletes were majority (73.0%, 72.7%), common symptoms of sub-health state: colds, insomnia, bad stomach. Account for a minority. Description This two athletes most in good health. Athletes by interview survey found that healthy athletes training, the law of life, dietary diversification, pay more attention to the therapeutic application of health knowledge.

3.6. Multivariate logistic regression analysis

The eating behavior score divided by the median high and low two kinds of eating behavior score classification (Y) long on logistic regression analysis showed that: the attitude of nutritional knowledge (X1), nutrition knowledge score (X2) father cultural grade level (X3) is closely related to eating behavior (Table 4).

Table 4. Logistic regression of correlated factors of dietary behavior

Variable	B	S.E.	Wald Value	P Value	Exp(B)
Constant	-1.219	0.344	12.527	0.000	0.296
Attitude	0.828	0.201	17.026	0.000	1.437
Knowledge	0.044	0.014	9.848	0.002	1.045
Education level	0.432	0.182	5.634	0.018	1.540

With economic development and people's living standards improve, unhealthy lifestyle has become a hazard to people's health an important factor, especially with nutrition and diet-related cardiovascular disease has topped the cause of death, the people in order to adapt to the fast pace of work and the pursuit of quality of life, reduction and control of chronic diseases and reduce costs of medical expenses, eager to get medical nutrition knowledge to reduce disease and promote health. Clients are people of all communities, their nutritional knowledge, attitude and behavior, not only affect their own growth and development and health, but also

directly affect the health of the communities they serve people, affecting the patient's recovery and rehabilitation. Therefore, improving medical students' knowledge of nutrition class, correct nutrition attitudes, changing dietary behavior, proper arrangements for meals, will be beneficial to its own population and health services.

4. Conclusions

Athletes physical health, physical fitness can form strong than short-term, long-term need for a reasonable and balanced diet. In order to allow the athletes come out of nutrition misunderstanding, we establish the correct concept of nutrition as soon as possible. It should be widely carried out in the athletes early groups, efficient sports nutrition knowledge and improve athletes overall understanding of nutrition science from the breadth depth. Players only through correct nutrition taught in various forms to understand the relevant knowledge of nutrition, and establish a positive, correct beliefs and attitudes, will it be possible to integrate the formation of the active feel good for their own healthy eating behavior. In this paper, the integrated use of expert interviews, literature review, questionnaires, mathematical statistics, methods and means, on the part of nutrition education and nutrition status of college athletes KAB investigation and analysis.

5. References

Adams, J.R. (2008). The Relationship Between Cardiovascular Risk Factors and Body Habitus Variables in Division I Collegiate Football Players, Miami University, 2008.

Brown, B., Noonan, C., Harris, K.J. (2012). Developing and piloting the Journey to Native Youth health program in Northern Plains Indian communities, *The Diabetes Educator*, 39(1), 73-122.

Cadzow, R.B., Chambers, M.K., Sandell, A.M.D. (2015). School-Based Obesity Intervention Associated with Three Year Decrease in Student Weight Status in a Low-Income School District, *Journal of community health*, 40(4), 709-713.

Gibala, M.J., Little, J.P., Van Essen, M. (2006). Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance, *The Journal of physiology*, 575(3), 901-911.

Griffin, J.R., Maxwell, T.M., Griffin L. (2016). The prevalence and consequences of obesity in athletes, *Current Orthopaedic Practice*, 27(2), 129-134.

Helzberg, J.H., Waeckerle, J.F, Camilo J. (2010). Comparison of cardiovascular and metabolic risk factors in professional baseball players versus professional football players, *The American journal of cardiology*, 106(5), 664-667.

Herzman-Harari, S., Constantini, N., Mann, G. (2013). Nutrition knowledge, attitudes, and behaviors of Israeli female combat recruits participating in a nutrition education program, *Military medicine*, 178(5), 517-522.

Jitomir, J., Willoughby D.S.(2008). Leucine for retention of lean mass on a hypocaloric diet, *Journal of medicinal food*, 11(4), 606-609.

Justin, M. (2013). Attitudes and Prevalence of Evidence-based Practice in Undergraduate Athletic Training Education Programs, *Journal of Athletic Enhancement*, 2:2. doi:10.4172/2324-9080.1000111

Qu, F. (2015). Simple analysis of potential immune regulation effect of Cucurbitacin E on professional athletes engaged in high intensity training, *Carpathian Journal of Food Science and Technology*, 8(1), 123-133.

Roseman, M.G., Riddell, M.C., Haynes J.N. (2011). A content analysis of kindergarten-12th grade school-based nutrition interventions: taking advantage of past learning, *Journal of nutrition education and behavior*, 43(1), 2-18.

Sedeaud, A., Marc A., Schipman, J. (2014). Secular trend: morphology and performance, *Journal of sports sciences*, 32(12), 1146-1154.

Stevens, J, Cornell C.E., Story, M. (1999). Development of a questionnaire to assess knowledge, attitudes, and behaviors in

American Indian children, *The American journal of clinical nutrition*, 69(4), 773s-781s.

Swinbourne, R., Gill, N., Vaile, J. (2015). Prevalence of poor sleep quality, sleepiness and obstructive sleep apnoea risk factors in athletes, *European journal of sport science*, 2015, 1-9.

Volpe, S.L., Lowe, N.M., Woodhouse, L.R. (2007). Effect of maximal exercise on the short-term kinetics of zinc metabolism in sedentary men, *British journal of sports medicine*, 41(3), 156-161.

Wagenmakers, A.J.M., Strauss, J.A., Shepherd, S.O. (2015). Increased muscle blood supply and transendothelial nutrient and insulin transport induced by food intake and exercise: effect of obesity and ageing, *The Journal of physiol*ogy, 11(1), 138-139.

Walle, T. (2007). Methylation of dietary flavones greatly improves their hepatic metabolic stability and intestinal absorption, *Molecular pharmaceutics*, 4(6), 826-832.

Wessels, A.G., Kluge H., Hirche, F. (2016). High Leucine Diets Stimulate Cerebral Branched-Chain Amino Acid Degradation and Modify Serotonin and Ketone Body Concentrations in a Pig Model, *Plos one*, 11(3), e0150376.

Wrobleski, M.M. (2010). The challenge of teen nutrition: An ecological view of socio-cognitive influences on urban, African-American adolescent diet quality, University of Maryland College Park, 2010.

Zhang, Z. (2015). Influence of Physical Ability Fast Recovery of Athletes Based on Movement Food Nutrition, *The Open Cybernetics & Systemics Journal*, 9(1), 1756-1761.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

RESEARCH ON FOOD COLD CHAIN LOGISTICS SYSTEM COLLABORATION

Xiaoping Wang[1]*

[1]*Business College, Inner Mongolia University of Finance and Economics, Hohhot, Inner Mongolia, China.*
*Corresponding author:*sophia188@126.com*

Article history:
Received:
06 March 2016
Accepted in revised form:
29 May 2016

Keywords:
Food cool chain logistics;
Collaboration system;
Coordinate analysis;
Collaborative process

ABSTRACT
Logistics system aims to meet customer needs in all aspects of logistics services, provides professional and efficient logistics services, to reduce the costs and improve customer satisfaction. The improvement of the overall efficiency of the logistics system cannot be achieved rely on a single enterprise, it need the collaboration between the main enterprises in logistics system, so logistics collaboration has become an important trend in the modern logistics operation. This paper focuses on the analysis of the connotation of food cold chain logistics system collaboration in order to provide the theoretical basis for the implementation of the food cold chain logistics system collaboration and specific and operational ideas for the realization of the collaborative target of food cold chain logistics system: it clear defines the goals and principles of collaboration. Finally, it analyzes the content of collaboration and collaborative process.

1. Introduction

Logistics system aims to meet customer needs in all aspects of logistics services, provides professional and efficient logistics services, to reduce the costs and improve customer satisfaction. The improvement of the overall efficiency of the logistics system cannot be achieved rely on a single enterprise, it need the collaboration between the main enterprises in logistics system, so logistics collaboration has become an important trend in the modern logistics operation (Liu et al., 2011).

Logistics collaboration has crucial strategic significance for the operation of logistics system. To see it simply, the collaboration of logistics system refers to the formulation of corresponding logistics plan and implementation strategy by each main enterprise in logistics system based on the overall objectives of the system (Zhang and Chen,2014). They jointly set up the logistics system through mutual cooperation in the actual operation of the process, and aim to improve the level of logistics service, reduce logistics costs, enhance logistics efficiency (Qi and Tian,2011).

After the clear definitions of food cold chain logistics and logistics collaboration, this paper puts forward the meaning of food cold chain logistics collaboration (Zheng et al.,2013). Food cold chain logistics system collaboration means that in the flow process of the entity from supplying place to receiving place, the main enterprises in food cold chain logistics system through mutual cooperation, information and resource sharing to achieve the seamless joint in all aspects of food cold chain logistics in order to maintain food in required temperature environment which is necessary to maintain its quality (Qiu and Zhang,2009), to realize the goals of food security, the improvement of the efficiency of food logistics and reduction of logistics cost.

This paper focuses on the analysis of the connotation of food cold chain logistics system collaboration in order to provide the theoretical basis for the implementation of the food cold

chain logistics system collaboration and specific and operational ideas for the realization of the collaborative target of food cold chain logistics system: it clear defines the goals and principles of collaboration. Finally, it analyzes the content of collaboration and collaborative process. As shown in figure 1.

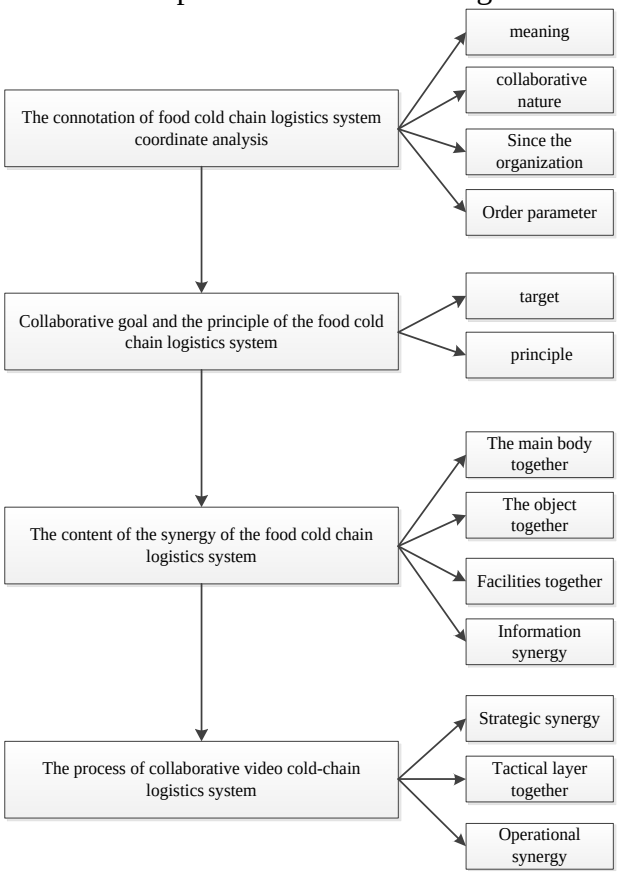

Figure 1. The Content of collaborative video cold-chain logistics system

2. Materials and methods

2.1. The goal of Food cold chain logistics system collaborative

Food cold chain logistics system collaboration achieves the seamless connection of food cold chain logistics in the actual operation process by mutual cooperation and information and resource sharing. In view of this, it can be concluded that the food cold chain logistics collaboration has three objectives, namely, safety, efficiency and cost. See Figure 2.

(l) The safety objective of the food cold chain logistics collaboration

On the one hand, the food cold chain logistics reaches a higher standard of food logistics safety by the standardization of food logistics operation, so as to guarantee the safety in the process of logistics. On the other hand, the food cold chain logistics must conducted effective supervision to ensure the timely detection of problems and reduce the hazards to a minimum. This can be realized from two aspects. One side is to establish strict inspection to conduct effective inspection of food; another side is to implement temperature monitoring in the process of logistics to prevent harmful effects due to temperature change (Li et al.,2015).

(2) The efficiency objective of the food cold chain logistics collaboration

For various kinds of fresh and perishable food, the less time from production place to consumption place, the more able to ensure the freshness and quality, which requires the food cold chain logistics system with high efficiency. There are two factors to determine the efficiency of food cold chain logistics: one is the efficiency of the logistics between subjects in different system, another is the efficiency of the transfer between subjects in the logistics system(Hu et al.,2015). The main factor that determines the efficiency of the logistics between subjects in different system is the efficiency of the transport system, and the main factor that determines the efficiency of the transfer between subjects in the logistics system is the collaborative degree of each subjects. The objectives of food cold chain logistics collaboration are to improve the collaborative degree of each subject (Wang et al.,2009), reduce the waste of time in handover process at different nodes, and enhance the logistics efficiency between different subjects through mutual cooperation and information and resource sharing to ultimately increase the efficiency of the whole food cold chain logistics system (Hang and Wang,2014).

(3) The cost objective of the food cold chain logistics collaboration

As a subsystem of social economic system, food cold chain logistics system regards the economic efficiency as an important goal. The above has been stated that the operating costs of food cold chain logistics system are higher than that of the general logistics system. This is because the operation of food cold chain logistics needs to be equipped with a dedicated refrigeration facilities supported by logistics networks. In terms of facilities, the biggest difference between the cold chain logistics and the normal temperature logistics is that the cold chain logistics need refrigerated trucks, insulation vehicles and cold storage. These facilities not only cost a lot, but also consume high energy in the operation process, which resulting in high investment and operating costs. To a certain extent, resource sharing can reduce the investment of enterprise's resources and the operation cost of cold chain logistics(Li,2010). Secondly, the food cold chain logistics collaboration can shorten or eliminate the invalid link in the logistics process and reduce the logistics time. And the food in the process of logistics needs refrigerated transport vehicles, which have higher refrigeration costs in transit, so we can shorten the logistics time to reduce the logistics cost.

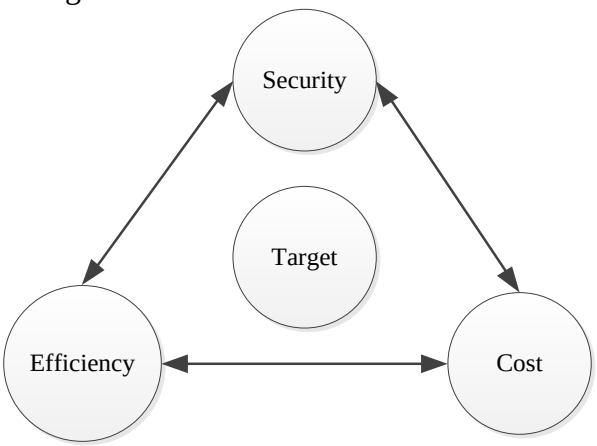

Figure 2. The food cold chain logistics system goal together

2.2. Content analysis of the food cold chain logistics collaboratio

Food cold chain logistics system can be divided into three types of elements: subject, object, facilities. Correspondingly, collaborative object can be classified as subject collaboration, object collaboration and facilities collaboration; in the collaborative process of various elements, information collaboration plays an important role, so the food cold chain logistics collaboration can fall into the subject collaboration, object collaboration, facilities collaboration and information collaboration from the aspect of content. The following are the details of the food cold chain logistics system collaboration, see Figure 3.

2.3. The subject collaboration in food cold chain logistics system

The subjects of food cold chain logistics system refer to the members in food cold chain logistics including the suppliers of food raw materials, the manufacturers of food processing, the retailers of food wholesale, the providers of food logistics. This paper expounds the collaboration between the subjects in food cold chain logistics from two aspects: the nature and the number of the collaborative subject.

(1) Classify according to the nature of collaborative subject in food cold chain logistics system

From the point of the nature of collaborative subject in food cold chain logistics system, the collaboration in food cold chain logistics includes horizontal collaboration and vertical collaboration.

① The subject horizontal collaboration in food cold chain logistics

In general, the food cold chain includes the suppliers of food raw materials, the manufacturers of food processing, the retailers of food wholesale, the providers of food logistics and other members; these members are also the main enterprises of food cold chain logistics. The vertical collaboration between subjects in food cold chain logistics means the collaboration between the same type enterprises

in different food cold chain logistics systems, such as the suppliers' collaboration between different food raw materials, manufacturers' collaboration between various food processing and the providers' collaboration between different food cold chain logistics, as shown in Figure 4.

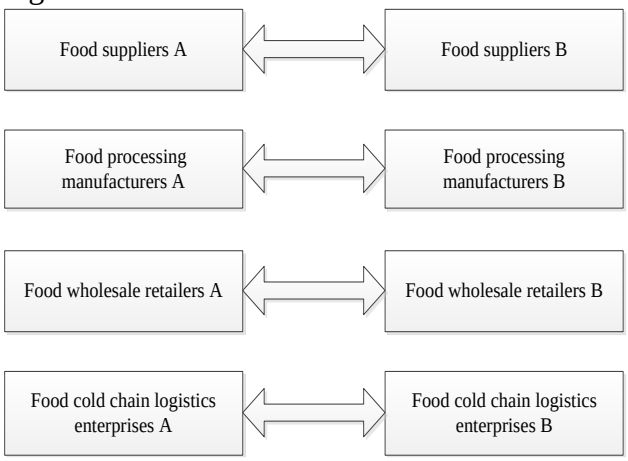

Figure 3. Food cold chain logistics main body horizontal coordination

The suppliers' collaboration between different food raw materials refers to the cooperation between the suppliers of food raw material in order to obtain the collaborative effect.

Such as the cooperation between the suppliers who belong to same type of food enterprises in order to solve the problem of insufficient resource of refrigeration storage and reduce the costs and risks of investment.

The suppliers of food raw material in different food cold chain can achieve the goal of enhancing their competitive power through the horizontal collaboration to improve both competitiveness of the food cold chain.

②The subject vertical collaboration in food cold chain logistics

For the food cold chain logistics system, the collaboration forms between the subjects are as follows: the collaboration between food suppliers and food processing manufacturers, the collaboration between food manufacturers and retailers, etc., as shown in Figure 5

There are two forms in the collaboration between food suppliers and food processing manufacturers: one is under the powerful food suppliers, in order to strengthen the function of sales or achieve the efficiency of logistics business, food suppliers play the main role in the logistics, or they carry out the distribution with high frequency and small volume for food processing manufacturers by the use of their information network. Another is that the food suppliers' power are relatively weak, but the food processing manufacturers' strength are strong, the logistics activities processed intensively by food processing manufacturers.

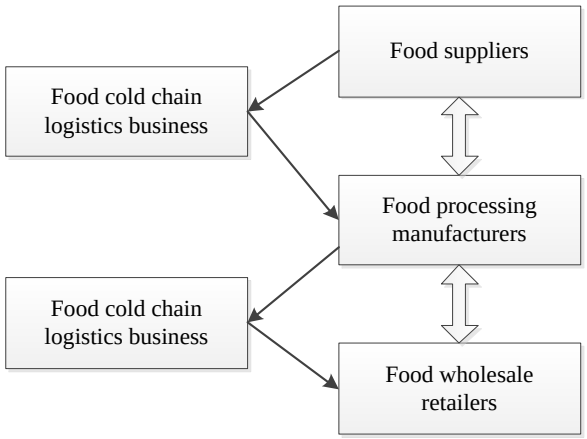

Figure 4. Food cold-chain logistics vertical coordination

(2) Classify according to the range of collaborative subject in food cold chain logistics system

The collaboration between subjects in the food cold chain logistics refers to the collaboration between the various sectors in the same enterprise to achieve their own collaborative effect. That is, after the implementation of the overall collaboration between the various departments within the enterprise, the overall efficiency of the enterprise is beyond the sum efficiency of the various departments of the enterprise.

Take the food cold chain logistics enterprise as an example, the internal collaboration means that the departments within the enterprise like business department,

warehousing department, transportation department and customer service department realize collaboration of every department in terms of personnel, equipment, institutions and organizations to achieve the common goal of improving the business efficiency and benefit.

The collaboration between these departments not only helps to achieve the seamless convergence of enterprise internal business process, but also improves the efficiency of enterprise operation.

The implementation of subject internal collaboration is the basis for food cold chain to achieve collaboration; only each subject straightens out their business process, realizes the efficient and smooth operation in all business processes and related organizations and personnel.

2.4. Object collaboration in food cold chain logistics system

The object of food cold chain logistics system is food. Compared with the objects of the general logistics, the food's characteristics and its requirements for food cold chain logistics can be explained from two aspects: the temperature and the time, the following will discuss object collaboration in food cold chain logistics system from these two aspects.

(1) Temperature collaboration in food cold chain logistics system

Temperature is the most typical factor that reflects the difference between food and other logistics objects.

Food, especially the food that needs to be frozen and refrigerated, should be ensured its safety and quality, reduce the loss, and the logistics process of these foods must be in a specific temperature range.

Different types of food have different requirements on the temperature, which can be divided into four categories: cooled food, frozen food, iced fresh food and ultralow temperature food. Different kinds of food must be stored in a special low temperature environment to ensure its quality, prevent food safety problems caused by its deterioration, and reduce losses. The characteristics of the food are exactly why the operation of food cold chain logistics system is more difficult than the general logistics system. But there is a broken link in the operation process of food cold chain logistics. Seamless connection between the subjects in the food cold chain is not realized which often leads to temperature fluctuations of food in the process of transport or storage, and brings about higher loss rates and food safety issues.

Table 1 The coordinated development of food cold chain logistics system process

	Organization	**Resources**	**Information**
Strategic synergy	Construction of cooperative organization Collaborative maintenance and continuous improvement of the organization	Software resource synergy Hardware resource synergy	Determine the information system strategy Determine the information sharing mechanism
Tactical layer together	Subjects to the enterprises determine the content The main body enterprise internal organization adjustment	Software resources tactical coordination layer Hardware resources tactical coordination layer	Share the order information Information sharing distribution plan Shared inventory information
Operational synergy	The application of information technology Operation standardization and normalization		

(2) Time collaboration in food cold chain logistics system

In addition to the temperature, another characteristic of the food is reflected in its special requirement of time. The general goods outside the food also have higher demands for the time in the process of logistics, which mainly to improve the efficiency of logistics and reduce the cost of logistics. The shorter the time used in the logistics process, the higher the logistics efficiency, the lower the corresponding logistics costs. For food, its requirements for reducing the logistics time are not only reflected in the reduction of logistics costs, but also in the special requirements of the time, such as the restriction of shelf life.

Beyond the restriction of time, we cannot guarantee the food's quality, taste, character, which may resulting in food safety issues. Therefore, in order to shorten the time of food logistics and improve the efficiency of food logistics, the time collaboration in food cold chain logistics system needs to be realized.

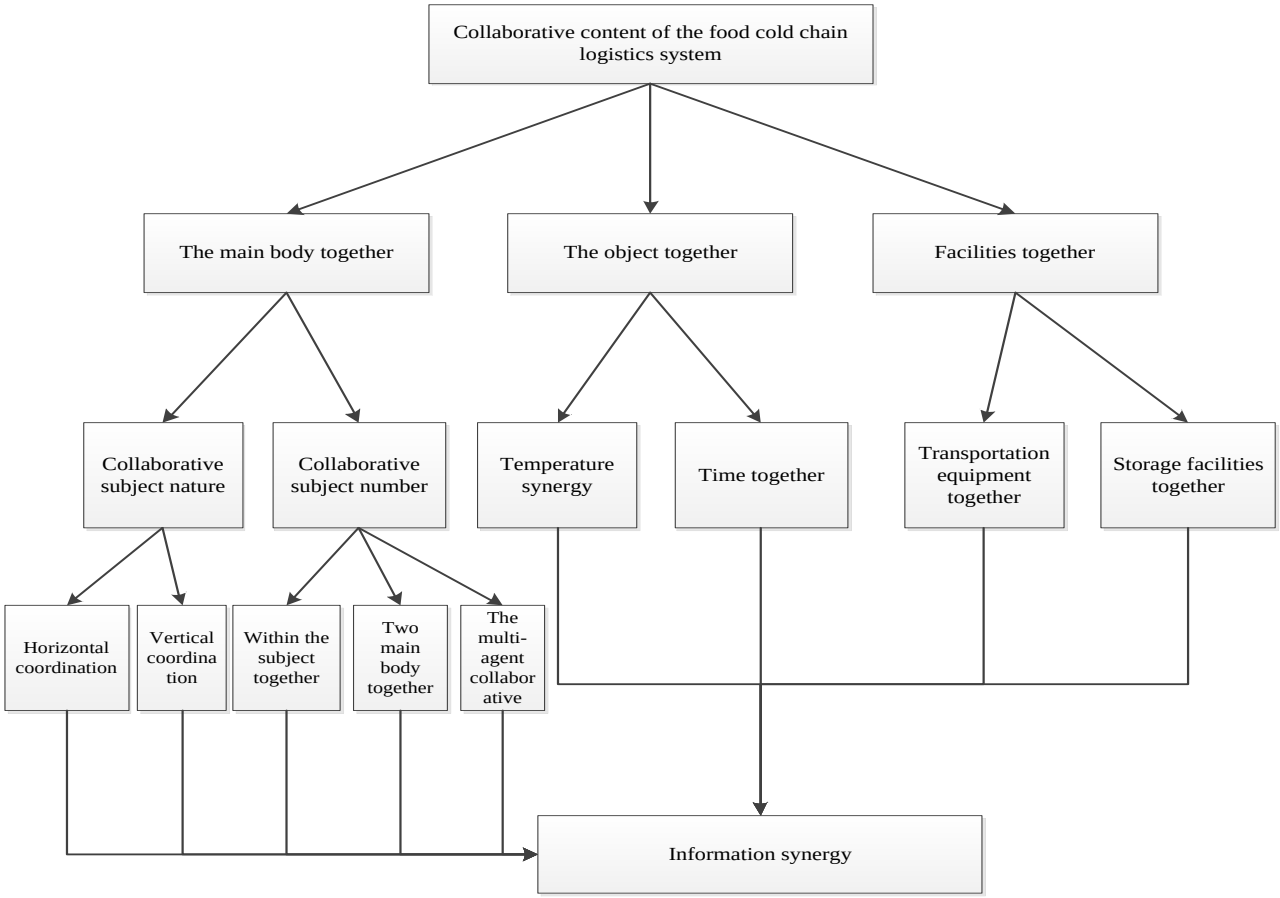

Figure 5. The classification of food cold chain logistics system coordination

2.5. Facilities collaboration in food cold chain logistics system

Facilities collaboration in food cold chain logistics system refers to a consensus reached between subjects on transport equipment purchase, maintenance, use and other aspects so as to improve equipment utilization rate. The main transport equipment used in cold chain logistics activities are: refrigerated vehicles, railway refrigerated vehicles, refrigerated ships, refrigerated containers, refrigerated aircraft, etc. In order to meet the requirements of food safety, transport equipment should satisfy the relevant demands. For example, non-toxic, harmless, no extraneous odor, no pollution, and meets the relevant requirements of food hygiene. The carriage body should be equipped with automatic temperature recording device to record the temperature inside the carriage body. The equipment also should be regularly inspected and maintenance, and immediately stop using if find any abnormalities of equipment, and timely maintain the equipment.

3. Results and discussions

The strategic collaboration of food cold chain logistics system carry out qualitative and quantitative analysis of the whole food cold chain based on conceptual model and collaborative management theory; the research

content includes the management factors and mechanism of logistics system collaboration. The performance of the s strategic collaboration is beyond the limit of tolerance for each other in the past, but to bear responsibility for each other, to pay and to harvest. The tactical collaboration mainly includes the logistics collaborative strategy between upstream and downstream enterprise in food cold chain with a direct relationship between supply and demand; tactical collaboration integrate business processes between enterprises, which tightens business joint of each link and smooth the flow. Operational collaboration is the key and foundation for supply chain to realize collaboration, which mainly study on how to realize synchronization operation and information collaboration in supply chain; it integrates the information between partners or members tightly, realizes the real-time flow and sharing of information, which makes the partners have a better collaboration, and quickly response to changes and needs of customers and partners.

The collaboration process of food cold chain logistics system starts from the strategic collaboration to carry on the tactical collaboration based on the operation collaboration; the results of operation collaboration will affect the tactical collaboration. In the same way, the tactical collaboration will also impact the strategic collaboration. As shown in figure 7.

This paper analyzes the collaboration process of food cold chain logistics from three aspects: the organization (subject), resources and information according to the characteristics and elements of food cold chain logistics system, as well as the above content of the food cold chain logistics system collaboration, as shown in Table 1.

3.1. Strategic collaboration in food cold chain logistics

As mentioned before, the information is an important method and means to ensure the realization of cooperation; the collaboration can be guaranteed only when the matched information strategy is established after analyzing the collaborative strategy on the organization and resource in food cold chain logistics system. Therefore, the first step in information strategy is to plan the goals of system's information construction and construct the framework under this goal based on the strategic targets of food cold chain logistics system. We should note that in determining the goals of information construction of the food cold chain logistics information system, the situation of members' information construction in the organization also need to be taken into account, such as the adopted information system, the modules included in the information system. Combining the actual situation with the goals of system information construction to make the information construction goals in line with the actual situation of the system, and also make the construction of the information system easy to be promoted among the members. Besides, the contents of the information system construction which depend on practical situations of the organization members and the resources collaboration will determine which modules,

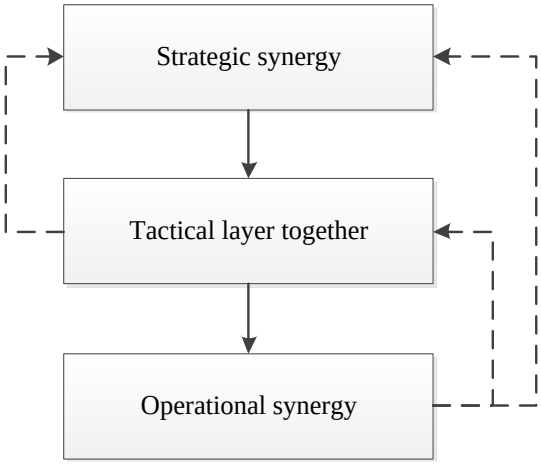

Figure 6. The collaborative process food cold-chain logistics system

what function should be included in the information system or what type of information sharing need subjects to realize for satisfying the organization and resource needs.

3.2. Tactical collaboration in food cold chain logistics

The production of enterprises will affect the supply of downstream enterprises.

In food cold chain logistics system, the downstream enterprises determine their inventory and production according to the production of upstream suppliers. Similarly, production of downstream enterprises also decided his demand to the supplier, thus they will affect the supplier's inventory and production plan. Hence, manufacturers can use the supplier's production and distribution plan to improve their planning standard, suppliers can also provide reliable supplies for the manufacturer based on the manufacturer's production plan. Moreover, exorbitant inventory is considered to be an important factor which influences the performance of food cold chain logistics system. Through the sharing of each subject's inventory information in food cold chain logistics system can greatly reduce the safety stock level of the whole logistics system and increase the competitiveness of the logistics system. For example, manufacturers timely adjust production through the understanding of the distributor's inventory information, etc.

3.3. Operational collaboration in food cold chain logistics

In order to guarantee no error happened in the various processes of the food cold chain logistics operation, we need to formulate appropriate operating standards in the actual operation process. In order to cover the various processes of the logistics operation, the formulated standards of food cold chain logistics operation need to include temperature records, tracking and monitoring, cargo inspection, cargo transportation and cargo warehousing and other fields.

At the operational level, the use of information technology makes all kinds of information can flow smoothly between each main enterprise; furthermore, the use of these techniques also can improve logistics efficiency; operating standards and norms formulation make the strategic and tactical measures implemented in accordance with the norms of operation. The combination of these two aspects makes the strategic collaboration and tactical collaboration of food cold chain logistics achieved at the operational level.5.

In addition to the temperature, another characteristic of the food is reflected in its special requirement of time. The general goods outside the food also have higher demands for the time in the process of logistics, which mainly to improve the efficiency of logistics and reduce the cost of logistics. The shorter the time used in the logistics process, the higher the logistics efficiency, the lower the corresponding logistics costs. For food, its requirements for reducing the logistics time are not only reflected in the reduction of logistics costs, but also in the special requirements of the time, such as the restriction of shelf life. Beyond the restriction of time, we cannot guarantee the food's quality, taste, character, which may resulting in food safety issues. Therefore, in order to shorten the time of food logistics and improve the efficiency of food logistics, the time collaboration in food cold chain logistics system needs to be realized.

4. Conclusions

The content of food cold chain logistics system is divided into subject collaboration, object collaboration, facilities collaboration and information collaboration. This paper expounds the subject collaboration in food cold chain logistics from two aspects: the nature and the number of the collaborative subject. The natures of the collaborative subjects in food cold chain logistics system include horizontal collaboration and vertical collaboration. The numbers of the collaborative subjects in food cold chain logistics system include internal collaboration of a single subject, collaboration between the two subjects and the collaboration among multiple subjects. The object collaboration in food cold chain logistics system includes temperature collaboration and time collaboration. Facilities collaboration in food cold chain logistics system includes transport equipment collaboration and

warehousing facilities collaboration. In addition, it divides the collaborative process into strategic collaboration, tactical collaboration and operational collaboration. It also analyzes the collaboration process of food cold chain logistics from three aspects: the organization, resources and information according to the characteristics and collaboration content.

5.References

Hang, Y., Wang, M. (2014). Prediction and analysis of fresh food cold chain logistics demand, 2014 International Conference on Mechatronics, *Electronic, Industrial and Control Engineering. MEIC 2014*, 1686-1689.

Hu, Z., ang, Y., Chen, Y.(2015). Modeling and optimization of food cold-chain intelligent logistics distribution network. *Advance Journal of Food Science and Technology*, 7(8), 573-578.

Li, J. (2010). Issues of food-related cold-chain logistics management in China. *2010 International Conference on Logistics Systems and Intelligent Management*, 8(3), 1319-1322.

Li, J., Zhao, W., Zhao, F.(2015). On the working mechanism of cold chain logistics in food industry based on O2P theory. *Advance Journal of Food Science and Technology*, 9(11), 849-853.

Liu, Q., Zhao, D., Shen, X. (2011). Evaluation on regional agri-food cold chain logistics competitiveness, *Journal of Applied Sciences*. 13(14), 2670-2675.

Qi, L., Tian, D. (2011). Sensing data compression method based on SPC for agri-food cold-chain logistics. *Nongye Jixie Xuebao/Transactions of the Chinese Society of Agricultural Machinery*, 42(10), 129-134.

Qiu, Q., Zhang, Z. (2009). Application research of cross docking logistics in food cold-chain logistics, 2009 International Conference on Information Management, *Innovation Management and Industrial Engineering*. ICIII 2009, 3(9), 236-240.

Wang, Y., Ma, X., Sun, Y. (2009). Present situation and development strategies of food cold-chain logistics in China, *4th Asian Conference on Refrigeration and Air-Conditioning*. 12(3), 263-266.

Zhang, Y.J., Chen, E.X. (2014). Comprehensive monitoring system of fresh food cold chain logistics. *Applied Mechanics and Materials*, 602(2), 2340-2343.

Zheng, W., Li, Z., Tang, Y.(2013). Development and application of time-temperature indicators used on food during the cold chain logistics. *Packaging Technology and Science*, 26(1), 80-90.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

ATHLETES FATIGUE RECOVERY AND SPORTS NUTRITION ANALYSIS BASED ON SPORTS NUTRITION AND THE LOAD ADJUSTMENT METHOD

Jun Zhuang[1*], Qingcheng Huang[2]

[1] *Department of physical Education, University Huaiyin Institute of Technology, Jiang Su, Huaian, 223003, P. R. China*
[2] *Department of physical Education, University Huaiyin Institute of Technology, Jiang Su, Huaian, 223003, P. R. China*
Coresponding author: [*]hygxytyjxb@sina.com

Article history:
Received:
17 March 2016
Accepted in revised form:
17 May 2016

Keywords:
Immunoglobulins;
Nutrition interventions;
Whey protein;
Sports training

ABSTRACT
This paper focuses on the relationship between exercise training, nutrition and immune function, and discusses the role of nutritional interventions to improve physical function. Movement of athletes immune suppression in order to promote further research in this field. The load adjustment is limited to the recovery of sports nutrition and training to adjust the load down to creatine kinase, blood urea nitrogen recovery index values are valid, but there was no significant difference. The large amount of exercise training and competition will make the Hb value decreased, BUN, CK rises, before the game to add protein powder and continuous glutamine, skeletal muscle cells can promote the synthesis of proteins, significantly improved body rowers function and promote recovery from fatigue after exercise load. Glutamine creatine powder and whey protein powder and sugar FDP viability as a nutritional supplement, from two months before to two weeks after the continuous application to help improve immune system function rowers and improve the quality of training and competition results.

1. Introduction

Sports training is a training-fatigue-recovery-training-fatigue and then recover, and finally the body ultra-compensation process (Rodriguez, 2009; Hausswirth, 2014; Kreider, 2003). In this cycle, training, fatigue and recover a part of one less, no fatigue training is meaningless training, also did not resume training is meaningless or even harmful training. Improve motor function level athletes in training - fatigue - Recovery cycle of circulation gradually increased(Van, 2006). So now resume training after the sports world increasingly valued in the international recovery team some well-known sports and training has been seen as equally important position, give the relevant sports nutrition and adjust the training load is restored after training the two most important means(Halson, 2014; Chaouachi, 2009).

Numerous studies show that when athletes large amount of exercise training or competition may cause suppression of immune function, so that the body of an increased risk of infection, particularly upper respiratory tract infection(Halson, 2014). Many factors can affect exercise-induced suppression, such as physical, psychological and environmental stress(Bettonviel, 2015; Mountjoy, 2014).Also, nutritional factors on immune function also play an important role(Zhang, 2015; Jeukendrup, 2014; Lewi, 2015; Hansen, 2016). A large number of epidemiological evidence and clinical data suggest that nutritional deficiencies can alter the body's immune system, increasing the risk of infection, which

affects the exercise capacity of athletes(Killer, 2015).

Exercise also decreased immune function long plagued sports coaches, athletes a thorny issue (Wehbe, 2015). The development and use of domestic and foreign sports nutrition supplements has made some achievements in sports practice also played a role, but these research and development work is relatively fragmented, difficult to solve all the problems from training to competition(Thomas, 2016). Rowing is a kind of high-intensity aerobic capacities, push forward vessels sailed into the competitive nature of sports projects under reasonable action technology(Bengtsson, 2013). Before training is an important part of the whole training cycle, its purpose is to adjust the state of the athletes, it is in the best competitive level in the game. Therefore, the Athletes before and after high intensity training or competition rational nutrition intervention, by immunological tests, and test some blood biochemical indices combined with subjective fatigue and training load athletes to analyze the reasonableness of the validation exercise program implementation, understanding functional status and degree of recovery of athletes, and the athletes next sports training arrangements, performance tuning and medical supervision have important guiding significance. This paper focuses on the relationship between exercise training, nutrition and immune function, and discusses the role of nutritional interventions to improve physical function and movement of athletes immune suppression in order to promote further research in this field.

2. Materials and methods

2.1. Relationship between exercise and immune function and disease

Usually exercise to increase body resistance is seen as an effective way to continue to adapt to environmental changes both inside and outside the body (Medina, 2014). In numerous studies on the integration of data analysis can be seen on the basis of a certain intensity of exercise or physical activity can enhance immune function, reduce the chance of infection and cancer; and excess or depletion of training and competition can lead to immune suppression and suffering increased risk of disease (Bieuzen, 2013). That exercise between immune function and disease infection and presented a linear relationship, we propose inverted J hypothesis. Figure 1 more clarity on the relationship between them is expressed, this theory certainly has been a lot of epidemiological data. But the change caused by the movement of immune function if the root causes of disease is susceptible rise remains to be further confirmed.

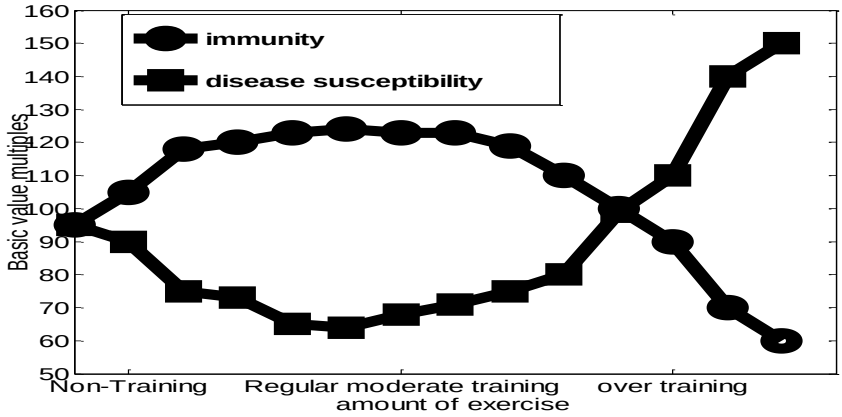

Figure 1. Inverted J hypothesis

2.2. Analysis of basal metabolic rate

Tomb base metabolic rate refers to the human body in a sober, supine, fasting and energy metabolic rate at 20 degrees Celsius. First law of thermodynamics has a profound impact on the application of biological organisms regard. First law of thermodynamics can be written as: open a total energy of the system is equal to the heat input increment ΔQ system plus the work force made to the system.

$$\Delta E = \Delta Q + \Delta W \quad (1)$$

According to this formula each star changes, we can describe the relationship between the energy of the whole body. Whether the individual is a rest or exercise, always keep the food stored chemical energy into other forms of energy required to maintain the body various organs, tissue and cell function, called catabolic processes in the body during the break the internal energy continue to decrease, AE, eight E is negative. To compensate for decomposition consume energy metabolism, you must eat food. Part of the catabolism of energy for the body to an external system to do work, and partly converted into heat .Q spread in vitro and W with the relationship between the time rate of change between the seven following formula:

$$\left[\frac{\Delta E}{\Delta t}\right] = \left[\frac{\Delta Q}{\Delta t}\right] - \left[\frac{\Delta W}{\Delta t}\right] \quad (2)$$

Measured by the respiratory oxygen consumption rate, the other is also known that consume a liter, if generated approximately 48 kcal of energy with oxygen, then the average catabolic rate can be written as:

$$\left[\frac{\Delta E}{\Delta t}\right] kcal / s = 4.8 \left[\frac{\Delta Q}{\Delta t}\right] L / s \quad (3)$$

2.3. Subjects and methods

We selected 16 rowers. City games were the 2007 and 2008 National Youth Championships athletes, including six athletes was the 2006 Provincial Games champion, the other two is the sixth City Games open single-stage double champion. All subjects volunteered to participate in the test, based on past medical history and a thorough physical examination to determine the subject and no history of cardiopulmonary exercise performance have not been taking drugs.

2.4. Experimental method

The 16 female rowers were randomly divided into experimental and control group 8. In the two months before the Qing Jin to two weeks after the implementation of sports nutrition interventions. Nutritional supplement program: ①Kang Bite glutamine capsules (Gln) - once a day, every 5; ②Kang Bite whey protein powder: once a day, each 30g, dissolved in warm water or milk taken: ③Kang Bite pure muscle acid powder (Cr): twice a day, 1--2 hours after half an hour before exercise and sports various; FDP vitality sugar: twice a day, two hours before training, immediately after training and the service once every four. Follow this nutrition intervention program, nutritional supplements given only to the experimental group, no difference in the athletes during the day, according to the experimental training program normal training, the experimental group and control group training program.

Data tests were conducted on the University Hospital of Wuhan Institute of Physical Roche INTEGRA 400 PLUS biochemical analyzer, CK and BUN kit provided by the Changchun City Department of Biological Technology Co., immunoassay kit provided by the Shanghai Fuxing Changzheng Medical Limited.

2.5. Sample collection and processing

The experiment was around women rowing team in preparing for the National Youth Championships conducted. Sampling time are in the great cycle training next morning 6-7 points, players awake in fasting venous blood 2.5ml sterile conditions in a quiet, anticoagulant tube save a 4 oC. Specimen: whole blood samples obtained after 20ul hemoglobin test, all remaining sample was centrifuged in serum, placed in -20 oC refrigerator spare to detect IgA, IgG, IgMCK, BUN. All subjects during three experiments to test the indicators, baseline: implementation of

nutrition interventions before (positive surgical winter training period); before Value: National Youth Championship the week before: the value of game: After the match resumed two weeks.

3. Results and discussions

3.1. The overall analysis of the experimental results

Rowers must not only have good explosiveness also requires good physical fitness. Athletes in the consumption of substances in the body is very large, two subjects per day minimum training sports teams. Athletes in training every day calories consumed by projections of about 20000J during normal training observation period, ordinary people 's daily life need about 10 times the heat. Training in energy consumption and in vivo in vivo synthesis of the material necessary to reduce substance may be the main cause of fatigue that results from functional index value point of view, after a long period of heavy load training will always be accompanied by a reduction in hemoglobin, red blood cells reduce hematocrit, decreased serum testosterone values such phenomena occur, and reduce these substances in the body that would cause a decline in production and athletic ability of fatigue, so make sugar, blood, provide high-quality protein, and promote material synthesis in vivo, to remove the body sports nutrition accumulation of various functions metabolites become major sports nutrition to promote physical recovery, the main measures issued corresponding function sports nutrition will become this observation after recovery from fatigue of athlete

Table 1. The indicator comparison of the athletes before recovery and intervention

Items	Guangdong provincial judo team			Shenzhen city judo team		
	Before recovery	1 week after intervention	difference	Before recovery	1 week after intervention	difference
HGB(g/L)	138±13	146±16	8±3	135±13	147±17	12±6
HCT(%)	38.2±2.5	41.0±2.2	3.0±0.1	37.0±2.1	42.0±2.2	5.0±1
Testostrone (nmol/L)	14.8±2.0	16.7±2.8	1.9±0.8	15.5±2.1	19.1±3.7	4.3±1.7
CK(U/L)	8.9±2.05	6.52±1.45	2.42±0.60	9.8±2.62	6.70±1.53	3.12±1.08
BUN (mmol/L)	8.7±2.3	7.1±1.9	1.6±0.4	8.5±2.0	6.6±1.5	1.9±0.5

Games lost time increase iron in the body, long-distance running parenteral iron loss will increase. During the observation period, Rowers training lasted fifty-six hours a day, the loss of iron in the body will be more consumption and shorter than normal and the movement of people, materials necessary for the synthesis of hemoglobin is iron in hemoglobin composed of four subunits each subunit has a prosthetic group containing iron, iron reduction will directly affect the synthesis of hemoglobin. After the analysis of various iron supplements, and blood Alzheimer fly sheet formulation more scientific, there are iron fumarate, folic acid, selenium, zinc, hemoglobin powder, vitamins, etc., they are synthetic hemoglobin and red blood cells essential substances, such as folic acid without it, differentiation and maturation of red blood cells cannot. Glucose on erythrocyte membrane has a protective effect, fructose as well as anti-lipid peroxidation, can stabilize the cell membrane to a certain extent, the absence of additional red blood cells can be funded for glycogen storage, it must always be sufficient

to absorb from the blood sugar in order to maintain functional activities, and therefore sugar on red cell life activities is very important.

Exercise is a lot of blood flow to skeletal muscle and other organs of the movement, will reduce the other body tissues and organs demand for sugar, and prolonged high load training may also lead to depletion of sugar, make red blood cells and other non-movement organ and tissue glucose deprivation the phenomenon is further exacerbated, increasing the impact on the red blood cell functional activity, it could lead to further reduction in the number of red blood cells, and therefore carbohydrate supplement may be one of a method to prevent the reduction of red blood cells. Waite sugar pump is a kind of complex sugars, dubbed by the fructose, the polysaccharides, oligosaccharides, and other sugars, easily absorbed by the body. Therefore, in this observation by carbohydrate supplement on erythrocyte membrane protection and reduction of red blood cell damage, and blood through the sheet and whey protein to provide raw materials for the synthesis of red blood cells to help speed up recovery and hemoglobin, hematocrit and the like. Bucks essence osmotic pump is the main component velvet active factor, the laser power saponin is a plant extract, both of which promote the synthesis of testosterone in the human body have a certain role, so as to enhance the concentration of testosterone, and therefore selected as the present observation complement testosterone supplements.

This observation athletes in hemoglobin, hematocrit, testosterone drops to normal after more than individuals taking these blood sports nutrition category, test results showed that the hemoglobin, hematocrit, testosterone has a certain improvement, difference before and after their recovery was significantly sex. Players hemoglobin, hematocrit, testosterone decline after more than normal individuals, not to take the appropriate sports nutrition, but reduce the intensity and amount of training load, the test results also show that hemoglobin, hematocrit, testosterone has increased, but the difference. It was not significant. Players in reducing the training load, the consumption of substances in the body also greatly reduced, and three meals a day diet can also provide the synthesis of hemoglobin, hematocrit, testosterone substances, so hemoglobin, hematocrit, testosterone values recovery is also possible. Hemoglobin decline caused training is of the sports anemia, anemia and exercise non-pathological, at no drug treatment, after a period of time generally may be restored to its original level. Reduce training load hemoglobin, hematocrit, testosterone relative recovery sports nutrition supplements not having the corresponding functions rise, probably when necessary supplements synthetic material timeliness and relevance of sports nutrition supplements is far less than the corresponding functions, so simply reducing the burden of training the body to adjust to relatively slow recovery, so the sports nutrition supplement related to hemoglobin, hematocrit, testosterone recovery faster more efficient more.

3.2. Blood index

As can be seen from Table 2, the experimental group and the control group in the late winter training hemoglobin index nutritional intervention values were within the normal range in both groups Hb values are not significantly different; the experimental and control groups. Before the game before Hb values were increased compared to the value of nutrition intervention, and there was significant difference ($P<0.05$) (Figure 2a). Fatty mean experimental group was significantly higher than the same period index ($P<0.05$) (Figure 2b); experimental group and the control group after the game Hb values were decreased compared to the value of nutrition intervention, and there is a significant difference ($P<0.05$), Hb and the average of the experimental group the control group compared to the same period index was a significant difference ($P < 0.01$).

Table 2. The effect on Hemoglobin BUN and CK level of nutrition intervention

	Group Type	HGB(g/L)	BUN(mmol/L)	CK(U/L)
Before Intervention	Exp group	143.0±0.90	8.12±0.44	148.22±0.22
	Control	131.0±0.45	7.21±0.23	146.64±0.17
Before Match	Exp group	158.7±0.87	7.16±0.59	109.26±0.24
	Control	154.0±0.87	7.01±0.13	92.33±0.13
After Match	Exp group	121.0±0.48	9.33±0.52	186.22±0.26
	Control	108.4±0.21	9.526±0.50	189.55±0.19

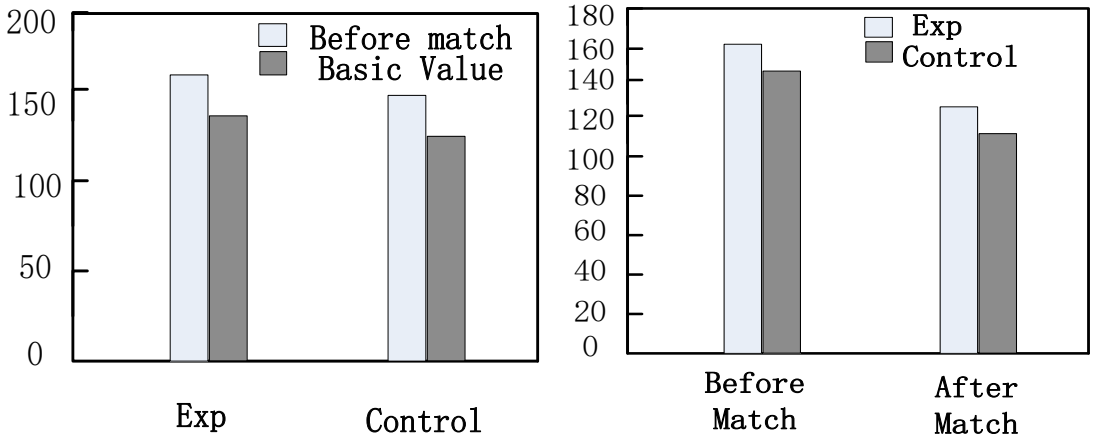

Figure 2. (a) Before the experimental and control groups after the game hemoglobin comparison between the left; (b) the experimental and control groups before Hb values and intervention values comparison chart

3.3. Immunoglobulin

As can be seen from Table 3, the entire test observation period, the immunoglobulin index lgA experimental group and control group were decreased compared to the value of the previous value before lgG and nutrition interventions, and there is a significant difference (P <0.05), before lgM values and nutrition interventions compared to the value also declined, but there was no significant difference. immunoglobulin before each index in the experimental group was not significantly decreased, compared with the same period in the control group were significantly different (P <0.05). After the match value lgh, lgG recovered to the level before the intervention, the experimental group after the game lgG values higher than the previous nutritional intervention and there is a significant difference (P <0.05), the experimental and control groups after the game IgM.

Nutrition interventions before the average value ratio decreased. and there was a significant difference (P <0.05), the experimental group after the game IgA, IgG value compared with the same period in the control group were significantly different (P0.05), IgM value compared with the same period in the control group, there was a significant difference (P <0.01).

Table 3. The variation of immune globulin level of experiment group and control

	Group Type	IgA(g/L)	IgG(mmol/L)	IgM(g/L)
Before Intervention	Exp group	2.01±0.42	8.34±1.06	3.48±1.15
	Control	2.00±0.40	8.14±0.39	2.87±1.20
Before Match	Exp group	1.87±0.39	7.72±0.28	3.34±0.89
	Control	1.68±0.68	7.19±0.72	2.86±0.94
After Match	Exp group	2.03±0.52	8.66±0.64	3.13±1.02
	Control	1.75±0.38	8.17±1.01	2.39±0.91

Sampler using a micro pipette at a concentration of 1.0 mg/ten kinds of stimulants hydrochloride standard ml solution of each of 0, 10, 50, 100 μl in 10 mlPyrex test tube, add 5ml H2O, with 1.4 backward approach to gas chromatographic analysis, The content of Y to X relative area plotted to obtain the regression equation shown in Table 4.

Table 4. The regression equations and correlation coefficients of 10 stimulants

stimulants	Regression equation	Correlation coeffient
Heptaminol	Y=0.0553x-0.0297	r=1
Methylamphetamine	Y=0.2168x-0.0531	r=1
Fenfluramine	Y=0.1017x-0.0631	r=0.9999
Cathine	Y=0.2157x-0.1625	r=0.9998
Ephedrine	Y=0.1972x-0.1993	r=0.9998
Amfepramone	Y=0.1114x-0.2102	r=0.9953
MDMA	Y=0.1450x-0.0484	r=1
Caffeine	Y=0.1296x-0.0482	r=1
Pipradol	Y=0.1242x-0.0026	r=0.9997
Strynchine	Y=0.0743x-0.0625	r=0.9997

3.4. Discussion and analysis

3.4.1. Nutritional intervention on athletes hemoglobin

In addition to nutrition and hemoglobin affected by general factors, but also affected by season training, training methods and techniques. Recent studies have shown that, Hb fluctuates with the large amount of exercise training in athletes during heavy initial training Hb decline, which is due to the large amount of exercise training accelerated red blood cell destruction, hemoglobin free out of the red blood cells involved in muscle protein synthesis and red blood cell, red blood cell destruction and hemoglobin decline is a reaction to the large amount of exercise training early. After a phase of training athletes to exercise gradually adapt, improve the functional state of athletes, Hb content will rise, then the athletes good performance status, race generally better results. Hb values in this study the training and control groups in each stage before the change is consistent with this conclusion, indicating that before the training program will be reasonable, the athletes did not appear tired.

The initial value of the nutrition intervention before the test, the experimental and control groups were within the normal range, there is no significant difference, indicating that the two groups of athletes for winter training load to stimulate better adaptability, Hb resume soon after the end of winter training normal level.

3.4.2. Nutritional intervention on athletes

Blood urea nitrogen BUN is catabolic end products of protein and amino acids and other substances, is the human body protein metabolism assessment index. Under normal physiological conditions, urea production and excretion in dynamic equilibrium, blood urea concentration is relatively stable. When normal quiet BUN value 1.7-7mmol/L, athletes quiet high blood urea concentration can be reached 5.5-7.0mmol / L, because of the influence of the training, body protein metabolism. BUN is an important indicator of assessment of training load and recovery of functional status, exercise load changes and relationship BUN load strength compared closely, when the greater load, BUN increased, the more obvious the next morning day also slow recovery. Under the same load conditions, the body's adaptability to load the worse after exercise is to generate the more BUN, quick recovery training status is good, slow recovery is poor. When the body adapt to environmental changes, BUN levels will rise, but the high level of training athletes reaction ridicule small scratch. BUN morning training period variation can be divided into three types: (1) BUN content in a slight increase in the normal range training period, indicating that exercise is not big enough or athlete training to improve the level; (2) training period began to rise, and then gradually recovered to near normal levels, indicating that a large enough amount of exercise, the body to produce adaptive response; (3) the training period BUN daily increased, indicating that excessive exercise, or after a period of training, the body has not been restored and training, body suited.

3.4.3. Nutritional intervention on athletes creatine kinase

Serum CK is an effective indicator of skeletal muscle load assessment. Exercise stress can cause CK values have increased, and serum CK activity increased with the magnitude of the relationship between physical activity very closely, elevated serum CK activity is not only related to the length of time span, and with the motion intensity. Under normal circumstances, muscle cells intact structure, function J under often makes CK rarely revealing the cell membrane, when strenuous exercise machine from CK cells poured into the blood, so serum CK activity in muscle cells can reflect the degree of adaptation for sports training. From the perspective of energy metabolism, muscle response to training stimuli generated more obvious, the other can understand in the case of excess muscle cells for energy, CK from the number of muscle cells into the blood whether the reduction. Therefore, according to 'serum CK parameters to adjust the intensity of training is scientific, serum CK measurement can provide important information for the coaches to understand the function of muscle training to adapt to the state level and athletes to ensure scientific training, can truly reflect the changes in serum CK muscle cell adaptation for sports training.

Most studies show that strenuous exercise serum CK activity increased significantly, exercise ultimate strength of CK activity can be increased to 500~ 1000 U/L, after exercise serum CK activity has increased in delayed characteristics, usually after exercise 16 -24d, reached the peak value. After the training of athletes in serum CK activity recovered rapidly, usually within 24 hours to return to normal, if you want a few days to return to normal levels, indicating that athletes may fatigue symptoms.

3.4.4. Nutritional intervention on athletes' immunoglobulin

Immune globulin is produced by the B lymphocytes. Present in the body serum, tears, saliva and other secretions of a class of glycoproteins. Antibodies can occur with a specific antigen immunoglobulin response, with many important functions, the most important is the ability to bind to the pathogen surface antigens, stimulating other immune cell differentiation and activation. Human-specific antigen depending on the molecular structure and stable region of their heavy chains, the immunoglobulin is divided into five categories,

namely Igh, IgM, IgG, IgE, IgD. In exercise immunology used in many of the former three, namely Igh, IgM, IgG, IgG which is the main component of serum immunoglobulins, most antibacterial antibiotics and antiviral IgG antibodies belong to, it is hot infection play the main force role: secretory Igh mucosal defense is the main material of the body of infection, the cells around it local immune system, protects against bacteria, fungi, viruses, and respiratory and gastrointestinal infections; IgM in the blood to prevent bacteria disease plays an important role.

Immunoglobulin IgA index value measured before nutritional intervention, the experimental group and the control group, IgG and IgM between two groups did not show significant differences. IgA youth tournament before the value of the experimental group and the control group, before IgG and IgM values in comparison with the intervention were significantly decreased ($P < 0.05$), showed that the high intensity training before reducing serum IgA, IgG, and IgM concentration, consistent with results of previous studies. Experimental group before immunoglobulins indicators IgA, IgG and IgM were no significant decline in the average, and significantly lower than those in the control group ($P < 0.05$), suggesting that, two months before the start of exogenous oral supplement Valley glutamine in the gut was effectively absorbed in part by the direct use of intestinal cells, intestinal cells can reduce plasma glutamine intake; the other part is absorbed into the bloodstream, increase plasma glutamine levels. Plus adding that creatine powder, whey protein powder and sugar FDP vitality, promote the repair protein athletes, accelerating resynthesis of energy, to reduce the degree of suppression of immune index, suppression of the immune shorten time, improve the B lymphocytes Immune Function.

4. Conclusions

Sports nutrition in the recovery after training hemoglobin, hematocrit, testosterone and other indicators to be significantly better than the load adjustment is limited to the recovery of sports nutrition and training to adjust the load down to creatine kinase. Blood urea nitrogen recovery index values are valid, but there was no significant difference. Before the large amount of exercise training and competition will make the Hb value decreased, BUN, CK rises, before the game to add protein powder and continuous glutamine, skeletal muscle cells can promote the synthesis of proteins, significantly improved body rowers function and promote recovery from fatigue after exercise load. Glutamine, creatine powder and whey protein powder and sugar FDP viability as a nutritional supplement, from two months before to two weeks after the continuous application to help improve immune system rowers and improve the quality of training and competition results.

5. References

Bengtsson, H., Ekstrand, J., Hägglund, M..(2013). Muscle injury rates in professional football increase with fixture congestion: an 11-year follow-up of the UEFA Champions League injury study, *British journal of sports medicine*, 47(12): 743-747.

Bettonviel, A.E.O., Brinkmans, N.Y.J., Russcher, K.(2015). Nutritional Status and Daytime Pattern of Protein Intake on Match, Post-Match, Rest and Training Days in Senior Professional and Youth Elite Soccer Players, *International Journal of Sport Nutrition and Exercise Metabolism,* 26(3), 285-293.

Bieuzen, F., Borne, R., Toussaint, J.F.(2013). Positive effect of specific low-frequency electrical stimulation during short-term recovery on subsequent high-intensity exercise, *Applied Physiology, Nutrition, and Metabolism*, 39(2), 202-210.

Chaouachi, A., Coutts, A.J., Chamari, K.(2009). Effect of Ramadan intermittent fasting on aerobic and anaerobic performance and perception of fatigue in male elite judo athletes, *The Journal of Strength & Conditioning Research*,23(9), 2702-2709.

Halson, S.L.(2014). Monitoring training load to understand fatigue in athletes, *Sports Medicine*, 44(2), 139-147.

Hansen, M., Bangsbo, J., Jensen, J.(2016). Protein intake during training sessions has no effect on performance and recovery during a strenuous training camp for elite cyclists, *Journal of the International Society of Sports Nutrition*, 13(1), 1.

Hausswirth, C., Louis, J., Aubry A.(2014). Evidence of disturbed sleep and increased illness in overreached endurance athletes, *Medicine and science in sports and exercise*, 2014, 19-27.

Jeukendrup A.(2014). A step towards personalized sports nutrition: carbohydrate intake during exercise, Sports Medicine, 44(1): 25-33.

Killer, S.C., Svendsen, I.S., Jeukendrup, A.E.(2015). Evidence of disturbed sleep and mood state in well-trained athletes during short-term intensified training with and without a high carbohydrate nutritional intervention, *Journal of Sports Sciences*, 2015, 1-9.

Kreider, R.B. (2003). Effects of creatine supplementation on performance and training adaptations, *Molecular and cellular biochemistry*, 244(1-2), 89-94.

Medina, D., Lizarraga, A., Drobnick, F..(2014). Injury prevention and nutrition in football, *Sports Science Exchange*, 27(132): 1-5.

Mountjoy, M., Sundgot-Borgen, J., Burke L.(2014). The IOC consensus statement: beyond the female athlete triad—Relative Energy Deficiency in Sport (RED-S), *British journal of sports medicine*, 48(7), 491-497.

Rodriguez, N.R., DiMarco, N.M., Langley S.(2009). Nutrition and athletic performance, *Medicine and science in sports and exercise*, 41(3), 709-731.

Thomas, D.T., Erdman, K.A., Burke, L.M.(2016). Position of the academy of nutrition and dietetics, dietitians of canada, and the american college of sports medicine: Nutrition and athletic performance, *Journal of the Academy of Nutrition and Dietetics*, 116(3), 501-528.

Van, E.M., Gibala, M.J.(2006). Failure of protein to improve time trial performance when added to a sports drink, *Medicine and Science in Sports and Exercise*, 38(8), 1476-1483.

Wehbe, G., Gabbett, T., Dwyer, D. (2015). Monitoring neuromuscular fatigue in team-sport athletes using a cycle-ergometer test, *International Journal of Sports Physiology & Performance*, 10(3), 292-297.

Zhang, Z. (2015). Influence of Physical Ability Fast Recovery of Athletes Based on Movement Food Nutrition, *The Open Cybernetics & Systemics Journal*, 9(1), 28-36.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

EMPIRICAL STUDY ON CHINA DAIRY INDUSTRIAL CLUSTER AND INFLUENCE FACTORS-BASED ON PROVINCIAL PANEL DATA SPATIAL ECONOMETRIC ANALYSIS

Zhiming Zhang[1*]

[1]*Capital University of Economics and Business, Beijing, China*
*Corresponding author: *cgfwzx@126.com*

Article history:
Received:
22 February 2016
Accepted in revised form:
29 May 2016

Keywords:
Dairy industry;
Industry cluster;
Spatial econometric model;
Spatial dependency;
Influence factors

ABSTRACT

The paper explores and analyzes China's dairy industrial cluster and influence factors by the employments of spatial lag model and a spatial error model related to the fixed effect and random effect and with spatial panel data of 29 provinces from the year of 2009 to the year of 2013 as samples. In conclusion, China's provincial dairy industry has strong spatial dependency and a positive spatial correlation; the degree of industrial cluster has a positive correlation with resource endowment, external economic conditions, industrial profitability as well as governmental supports, but has a negative correlation with economic foundation; and the labor cost does not play an obvious role in industrial cluster.

1. Introduction

With the development of dairy industry, many dairy enterprises have overcome the geo-spatial limit. It is gradually obvious for the dairy spatial cluster. Dairy enterprises quantity has increased to 658 from only a few at the beginning. And these dairy enterprises' geographical occurrence is mainly in north of China, and northwest of China. According to the statistics from National Bureau of Statistics, the dairy products output of the year 2013 grew by 5.15% over the same period of 2012 to 26,980,300 tons. And the liquid milk output grew by 7.01% over the same period of 2012 to 23,559,700 tons. The turnover of the dairy enterprises with scales rose by 14.16% over the same period of 2012 to RMB 283.159 billion. With the fast development of dairy industry, how is the correlation of China dairy industrial cluster spatial considering the industrial cluster spatial spillovers? What are the influence factors of cluster? By using spatial econometric analysis, the paper is try to construct China dairy industrial spatial econometric model to do empirical analysis on the influence factors, and to interpret the spatial spillover and heterogeneity of cluster, so as to provide theoretical reference for the government to make reasonable dairy industrial development policies. The research on the dairy industrial cluster and influence factors are in its beginning stage both at home and abroad and most of the researches are on the dairy industrial cluster measurement. Foreign scholars did more research on industrial cluster. As for the basic theory researches, new classic trading school believe the endowment of natural resources, labor, technology and others influenced the industrial cluster(Ohlin B, 1968). New economic geography school put the external scale economy into the industrial cluster analysis, and thinks a industrial cluster will be promoted due to the existence of external scale economy (Henderson JV, 1974; Fujita M.A, 1988). Some of the foreign

scholars did researches on a series of economic problems by using the spatial econometric model related theories. For instance, Lesage (1999) found that China provincial economy growth exists obvious spatial cluster ways by using spatial econometric model research. Meanwhile, the economy growth will influence the economy growth of other countries. It proved the spillovers of economy growth (Easterly, 1988). Tschoegl (2000) believes that external scales economy will promote the finance institutions to choose the certain areas. This area will be more advantageous when many finance institutions clustered here. Dairy industry cluster and influence factors are researched in foreign countries less than China.

Some of Chinese scholars did a series of researches on dairy industrial cluster. On the one hand, it is the research on the provincial dairy industrial cluster in all over China. China dairy clustered situation and spatial layout are the two important aspects which are influencing economic achievement and industrial competition. China dairy industry cluster degree and spatial cluster degree are not high (Hua, 2007). China dairy industry is clustered mainly in northeast China, Inner Mongolia, and north China. At the same time industry cluster showed marked enhancement in the dairy industry growth (Cheng, 2012). On the other hand, it is the research on some provinces dairy industrial cluster. Inner Mongolia dairy industry, livestock producing ability, returns to scale, industry investment location choice are the decisive factors of industry cluster. Livestock producing ability, industry cluster degree, the scale of original industry fixed assets are the decisive factors of industry investment location choice (Li, 2008). It also analyzed the development trends of Inner Mongolia animal products industry cluster (Li and An, 2008). Chinese scholars introduced spatial econometric model into the research on industry cluster, for example, on textiles industry, finance industry and others (Niu and Jiang, 2011; Ren et al., 2010).

The above researching results promoted the development of dairy industry cluster theory. However, the researches on China dairy industry cluster didn't take the correlation between the industry cluster and spatial geography location into consideration, neglected the space dimension heterogeneity, and used few econometric models in the empirical research on dairy industry cluster. There is significant difference among the different provinces. The traditional regression model can't effectively estimate the spillovers of the dairy industry cluster. Therefore, the paper put the spatial econometric model into the research on China 29 provinces dairy industry cluster influence factors, based on the provincial panel data, to explore the deep cause of dairy industry cluster.

2. Materials and methods

Theory hypothesis

Hypothesis 1: China dairy industry has spatial dependency and spatial spillovers.

Industry cluster refers to the same industry is highly concentrated in a certain geography location, and the industry capital clusters gradually in the spatial range. Since 19th century, the economists Alfred Marshall, Weber, Porter, Hoover have done deep analysis on industry cluster. Chinese scholars Zhang Xueliang, Zhao Liangshi, Sun Qinggang, Liu Hedong, Niu Honglei, Ren Yinghua respectively found that China infrastructure, utilization ratio of water resource, energy intensity, regional innovation, textile industry cluster, finance industry cluster all have the spatial dependency and spatial spillovers. There are few studies on China dairy industry cluster. The large scaled dairy enterprises like Mengniu, Yili, Bright, Firmus, Longdan, Wandersun have spatial geography proximity. Therefore, the paper makes the hypothesis that China dairy industry cluster has spatial dependency and spatial spillovers.

Hypothesis 2: Resources endowment namely the availability of raw materials is the

core factor of influencing dairy industry cluster, and is promoting effectively the dairy industry cluster.

New classic trading theory holds the opinion that the natural resources endowment advantage decides the industry location cluster [1]. For the industry that relies heavily on the raw materials, resources endowment is the key factor of industry location selecting, and is the foundation of the industry cluster process. Dairy industry needs raw milk as the raw materials. Raw milk is with short fresh-keeping time, and is perishable. Dairy industry would treat the raw milk availability as the first important factor to choose the industry location. Therefore, the paper put forward the hypothesis that the availability of raw materials is the key factor of influencing dairy industry cluster.

Hypothesis 3: Labor cost will significantly influence the dairy industry cluster. There is negative correlation between labor cost and dairy industry cluster.

New classic trading theory believes that except the natural resources endowment, labor force, technology, and other external resources endowment are the important factors of influencing industry cluster. Wang (2010) found that many industries clustered in coastal region caused a lot of middle east China countryside labor force transferring to the east China. Labor transfer will further promote the industry cluster. As the "soft production factor", labor force is the important factor of improving manufacturing industry competition. The cheap labor force is the cost advantage of local industry development, and promotes industry cluster. Dairy industry belongs to manufacturing industry. The availability of labor force and labor force cost will influence the dairy industry cluster. Generally, the less of the labor cost, the more attractive it will be for the manufacturing industry cluster.

Hypothesis 4: There is positive correlation between industry scale levels and dairy industry cluster.

The external economic theory, which was put forward by economist Marshall in 1890, and developed and perfected by scholar Krugman, believes that larger scaled industry is more efficient in manufacturing than the smaller scaled industry. The expansion of industry scale will cause the increase of the industry profit, thus the same industry and its supporting department will cluster in one or several places. Represented by Henderson [2] and Fujita [3], the new economic geography school thinks that the average cost decreased due to the increasing return to scale, and it increased the competition in further expanding scales, and it promoted the industry highly clustered. The current scale level of dairy industry will decide whether it will form the external scale economy. The larger scale level, the more helpful it will be for the dairy industry clustering in this place.

Hypothesis 5: There are highly positive correlation between government supporting degree and dairy industry cluster.

The development of the new economy geography school injected new energy to industry cluster theory. Baldwin (1999) believes that the government powerful protecting measures will help to increase local industry profit, attract capital, accelerate the capital accumulation, and promote the industry cluster. The study by Lanaspa et al., found the district where the government with higher efficiency is more attractive for the industry cluster by research. Dairy industry cluster can't get rid of the government's role. The local economic development protecting measures taken by government will be more helpful for the dairy industry cluster.

Hypothesis 6: There are positive correlation between economic basis and dairy industry cluster.

Dairy industry is part of the national economy. The district economic development level will affect this district industry development. In better economic based, and more developed districts, there will be better infrastructures, better investment development

environment, perfect rules guarantee, higher consuming level, and easier for the dairy industry clustering. In the poor economy based, and undeveloped districts, the infrastructure, investment environment and rules guarantee situations are worse, and it is not helpful for the dairy industry clustering. Therefore, the paper put forward the hypothesis that there are positive correlation between economic basis and dairy industry cluster.

Hypothesis 7: The regional industry profitability has positive influence on dairy industry cluster.

Represented by August Losch, the location theory of economic school believes that the internal competition decreases the cost and increases the demand. The concentrated demands will promote the manufacturing concentration. Industry clustered enterprises efficiency will be improved forced by the fierce competition, and the cost will be decreased. In this way it will meet the various marketing demands, and gain higher profit. Therefore, industry cluster region's profitability is stronger than others. Regional industry profitability will be very attractive for the industrial enterprises. The differences of the regional industry profitability will influence the dairy industry cluster.

Moreover, industry cluster theory considers that public transport infrastructure, and degrees of opening up to the world are the important factors of influencing industry cluster. But after experiencing a fast period of China transport infrastructure construction, it has basically formed the grades roadway, railway, high-speed rail, airlines and others multi-dimensional transportation system. Dairy industry, which is different from capital-intensive large scaled manufacturers, has weaker demand for transportation, so the transportation infrastructure situation will not be considered as the factor of influencing dairy industry cluster. China dairy industry enterprises are mainly domestic enterprises and the foreign countries enterprises of joint-stock. It is seldom for the foreign investors to build a factory to produce. And it has little influence on dairy industry cluster. Therefore, we don't take foreign investors into consideration in the influence factors of industry cluster.

Variables Selection

- Industry cluster degree: it uses the location quotient LQ to measure China dairy industry cluster degree. LQ, which was put forward by Haggett, is used to measure a certain region factors' space distribution situation. By calculating China different provincial dairy industry LQ, we could conclude that the dairy industry is relatively concentrated in which provinces. The accumulating formula is:
- $$LQ_{ij} = \frac{m_{ij} / gdp_{ij}}{M_j / GDP_j} \quad (1)$$
- m_{ij} refers to the jth year's dairy industry products sales turnover in the i province. The gdp_{ij} refers to the jth year's dairy industry products gross products in the i province. M_j is the jth year's dairy industry products sales turnover all over China. The larger is the LQ, the higher degree of dairy industry cluster will be. If LQ is more than one, we could believe that the dairy industry is highly clustered in this province.
- Resources endowment namely the availability of raw materials: the milk yield of the province could be the symbol to measure the dairy industry raw material availability. It will reflect the dairy industry resources endowment situation of every province. Represented in MY (milk yield).
- Labor force cost: the dairy industry employees' average wages is the best symbol of dairy industry labor force cost. But there are no unified standards of the employees' average wages in the dairy statistics annual, so the average wages of urban workers in every province institution will reflect dairy industry labor force cost. Represented in AWUW(average wages of urban workers)
- The current scale level: generally speaking, the larger is the industry assets scale, the

more output it will have. As it is difficult to get the existing assets statistics of every province dairy industry, and the relationship between assets scales and output ability, the paper selects the dairy products value to reflect the existing scale level of every province. Represented in DPV (dairy products value).

- The government support: the government revenue rate of GDP could reflect the government supporting degree in local marketing protection and support. The higher is the rate, more motivated government will be to support related industry development in this province. Represented by GRR (government revenue rate).
- The economic basis: there are many indexes of the region's economic basis. We usually use Per Capita GDP, gross GDP, government revenue etc. As the higher is the Per Capita GDP, the stronger the consuming capacity will be, the Per Capita GDP reflects the region's existing economic developed level. Represented in PCGDP (per capita GDP).
- Regional Industry profitability: the profit of dairy industry of every province could reflect the dairy industry profitability of every province. The higher is the profit rate, the stronger the profitability will be. We use the dairy industry profit rate divided by products sales revenue approximately reflects profitability. Represented by PM (profit margin).

Table 1. The influence factors of dairy industry cluster

Influence factors	Measuring index	Symbols abbreviation	Forecast Positive or Negative
Resource endowment	Milk Yield	MY	Positive
Labor force cost	Average wages of urban workers	AWUW	Negative
External scaled economy	Dairy products sales value	DPV	Positive
Government supporting degree	Government revenue rate	GRR	Positive
Economic basis	Per Capita GDP	PCGDP	Positive
Regional industry profitability	Profit Margin	PM	Positive

Basic Models Setting

- Based on the mentioned above hypothesis and related measuring index selecting, we set the liner model as below:

$$LQ_{ij} = \beta_0 + \beta_1 MY_{ij} + \beta_2 AWUM_{ij} + \beta_3 DPV_{ij} + \beta_4 GRR_{ij} + \beta_5 PCGDP_{ij} + \beta_6 PM_{ij} + \varepsilon_m \quad (2)$$

β_0 is constant; β_k is regression coefficient, k =1,2…6; i=1,2,3…29 represents the 29 provinces of China; j=1,2,3,4,5 represents the five years panel data from 2009 to 2103, ε is stochastic error.

- When selecting the samples, the study selects total 29 provinces without Hong Kong, Macau, Hainan, and Tibetan, as there is no neighbor of Hainan province, lack of some statistics of Tibetan. For the sake of the statistics availability and integrity, the statistics are mainly from 2010-2014 *Statistical Yearbook of China*, *Milk Yearbook of Chin*, and *China National Bureau* of *Statistics* official website. The data analysis is mainly done by advances spatial econometric scheme.

3. Results and discussions

Spatial Econometric Model Setting

In the traditional statistics theory, suppose the observation values are independent from each other, reviewing the spatial statistics, spatial econometric theory thinks that there are a few independent observation values. There is spatial interaction among observation values, namely there is spatial dependency and spatial autocorrelation among the regional economic geography statistics. Based on spatial econometric model, dairy industry cluster influence factors spatial econometric analysis should test if there is spatial autocorrelation in dependent variable. If there is, we should construct spatial autoregressive model and error models to spatial econometric estimation test of dairy industry influence factors.

(1) Dairy industry cluster spatial autocorrelation inspection

Global Spatial Autocorrelation will generally depict the provincial dairy industry distribution from provincial space. It is the important way to inspect if the industry cluster degree is high, and if the neighbor space point cluster is relevant. This paper inspects there is spatial relation among dairy industry clusters by using spatial autocorrelation index Moran's I. The formula is:

$$Moran'sI = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} W_{ij}(LQ_i - \overline{LQ})(LQ_j - \overline{LQ})}{S^2 \sum_{i=1}^{n}\sum_{j=1}^{n} W_{ij}} \quad (3)$$

And

$S^2 = \frac{1}{n}\sum_{i=1}^{n}(LQ_i - \overline{LQ})$; $\overline{LQ} = \frac{1}{n}\sum_{i=1}^{n} LQ_i$; LQ_i refers to the dairy industry cluster LQ coefficient of prince i, i=1,2,3…29; W_{ij} is nearby spatial weight matrix in binary, and it could recognize the neighborhood relation among different spaces. This paper uses weight matrix set in the neighborhood distance, namely if two provinces are neighbors, the W_{ij} is 1, if not W_{ij} is 0. The formula is as below:

$$W_{ij} = \begin{cases} 0 & \text{0 means province i and province j are not neighbors} \\ 1 & \text{1 means province i and province j are neighbors} \\ 0 & \text{0 means i=j} \end{cases}$$

And i=1,2,3…29,j=1,2,3…29, when i=j, Wij is 0, namely weight matrix diagonal line element is 0, $Moran'sI$ index is between -1 and 1. If $Moran'sI$ index is more than 0, there is positive spatial autocorrelation in dairy industry clusters. If $Moran'sI$ index is less than 0, there is negative spatial autocorrelation in dairy industry clusters. By drawing spatial correlation coefficient $Moran'sI$ scatter plot, China 29 provinces dairy industry cluster distribution is divided into four quadrants spatial dependency. The first quadrant HH (high-high), high dairy industry cluster degree province is surrounded by high cluster degree provinces; the second quadrant LH (low-high), the low dairy industry cluster degree province is surrounded by high cluster degree provinces; the third quadrant LL (low-low) the low dairy industry cluster province is surrounded by low cluster degree provinces; the fourth quadrant HL (high-low) the high dairy industry cluster province is surrounded by low cluster degree provinces.

According to $Moran'sI$, we can use the Normal Distribution Assumption to inspect if there is spatial autocorrelation among the 29 provinces. That is to calculate the $Moran'sI$ standard Z value under the condition of normal distribution assumption. The formula is as below:

$$Z(\mathrm{d}) = \frac{Moran'sI - E(Moran'sI)}{\sqrt{\mathrm{var}(Moran'sI)}} \quad (4)$$

The expected value

$$E(Moran'sI) = -\frac{1}{n-1}, \quad (5)$$

Variance

$$\mathrm{var}(Moran'sI) = \frac{n^2 W_1 + nW_2 + 3W_0^2}{W_0^2(n^2-1)} - E(Moran'sI) \quad (6)$$

If Z values are all more than the boundary 1.96 or 1.65 with the confidence level 0.01 or 0.05, there will be positive correlation in China dairy industry cluster, and there is significant spatial dependency.

(2). Spatial econometric model

There are two reasons for causing spatial autocorrelation. One is there is objective relation in neighborhood regions. The other is there is space error in selecting sample statistics, reflected in spatial autoregressive model error and dependent variables lags. Therefore, there are two spatial

econometric models: Spatial Lag Model, SLM, and Spatial Error Model, SEM.

- Spatial Lag Model, SLM

SLM is mainly used the province dairy industry cluster influence factors' influencing situation on dairy industry of neighborhood provinces, namely studying if there are spillovers of this variable in provinces. The expression is:

$$Y = \rho WY + X\beta + \varepsilon \quad (7)$$

Y is dependent variable, X is independent variable matrix, W is spatial weight matrix, WY is spatial autoregressive dependent variable, ρ is spatial auto-regression coefficient, ε is constant error. According to the above formula, dairy industry cluster influence factors spatial regression model is set as below:

$$LQ = \rho(E_T \otimes W)LQ + \beta_1 MY + \beta_2 AWUM + \beta_3 DPV + \beta_4 GRR + \beta_5 PCGDP + \beta_6 PM + \varepsilon \quad (8)$$

$(E_T \otimes W)$ is the kronecker products of matrix, E_T is T-order Matrices , W is spatial weight matrix, the parameter $\beta_i, i = 1,2...6$ reflects the dairy industry cluster influence factors' influence on cluster degree. Spatial regression variable $(E_T \otimes W)LQ_{ij}$ reflects the spatial distance influence on regional dairy industry cluster, and it is an endogenous variable.

- Spatial Error Model, SEM

There is the difference among different regions, we should use spatial error model to estimate. The formula of spatial error model is:

$$Y = X\beta + \varepsilon \quad (9)$$

$$\varepsilon = \lambda W\varepsilon + \mu = (E_n - \lambda W)^{-1}\mu \quad (10)$$

λ is spatial coefficient to measure spatial dependency, ε is random error, μ represents the random error vector matched the normal distributions, E_n is n-order matrix. According to the expression above, dairy industry cluster influence factor spatial error model set as below:

$$LQ = \beta_1 MY + \beta_2 AWUM + \beta_3 DPV + \beta_4 GRR + \beta_5 PCGDP + \beta_6 PM + \varepsilon + \mu \quad (11)$$

Parameter $\beta_i, i = 1,2...6$ reflects all the dairy industry cluster influence factors' influence on cluster degree, μ represents the random error vector matched the normal distributions, $\varepsilon = (E_n - \lambda W)^{-1}\mu$ 。

In the spatial econometric model, the estimate of traditional least square estimate variable coefficient is biased or even not effective. Spatial econometric model estimation usually uses the general least square estimate or maximum likelihood estimation. As for spatial lag and spatial error model, we adopt the method of maximum likelihood estimation.

Spatial Lag Model or Spatial Error Model, when we judge which model is more suitable to choose; generally we choose it according to the goodness-of-fit R2. The higher is R2, the higher goodness-of-fit will be, the better the fitting effect will be. What's more, we can choose according to Log likelihood (LogL), Likelihood Ratio (LR), Akaike information criterion (AIC), Schwartz criterion (SC).

The larger is LogL, the smaller AIC, SC are, the higher is the goodness-of-fit; the smaller LogL is, the larger AIC, SC are, the lower is the goodness-of-fit. We could taking R2, LogL, AIC, SC vales into consideration when we choose spatial lag model and spatial error model.

Discussions of empirical results analysis

(1)Dairy industry cluster spatial autocorrelation inspection.

According to LQ expression (1), China provinces dairy industry LQ coefficients shows in Table 2. Reviewing from regions, China dairy industry heavy cluster is mainly located in north China, northeast China, and northwest China. While south China, southwest China, and east China is with light cluster. Reviewing from time levels, from 2009 to 2013,dairy industry cluster degree generally increased fast in northwest China, especially in Ningxia and Shaanxi Province; but the traditional dairy industry provinces Inner Mongolia and Heilongjiang industry cluster degree decreased significantly from 2009 to 2013. In 2009, the top six provinces with heavy dairy industry cluster degrees are: Inner Mongolia, Heilongjiang, Ningxia, Shaanxi, Shanghai, and Hebei province. In 2013, the top six provinces with heavy dairy industry cluster degrees are: Heilongjiang, Ningxia, Inner Mongolia, Shaanxi, Hebei, and Shanghai. There is no change of the top six provinces, but there is a sequence change. Inner Mongolia dairy industry cluster degree decreased to the third rank from the first place; Heilongjiang increased to the first rank from the second; Ningxia increased from the third to the second; Shanghai decreased to the six; Hebei increased to the fifth from the sixth. However, reviewing from the cluster degree values, it is decreasing in Heilongjiang, Inner Mongolia and Shanghai; it is increasing in Ningxia, Shaanxi and Hebei province. Generally reviewing from 2009 to 2013, China most provinces dairy industry cluster degree were increasing, a few provinces cluster degree decreased differently. The traditional dairy industry clustered regions continued dairy industry, and there is no obvious new industry cluster. But the cluster degree absolute value is increasing and decreasing. The quick increasing of Ningxia, Shaanxi and Hebei dairy industry cluster degree injected new energy to China dairy industry development.

Table 2. Every province dairy industry LQ coefficient

	Province	2009	2010	2011	2012	2013
North	Beijing	1.078415	1.034069	1.069968	1.077882	1.102943
	Tianjin	0.502999	0.618738	0.865858	0.713553	1.365149
	Hebei	1.503711	1.567345	1.656046	1.74751	1.785628
	Shanxi	0.750314	0.937223	0.783025	0.784102	0.758432
	Inner Mongolia	7.202618	6.522545	5.909151	4.824749	4.710306
Northeast	Liaoning	1.243544	1.19077	1.194555	1.073878	1.059847
	Jilin	0.246448	0.340034	0.479164	0.484211	0.4691
	Heilongjiang	6.83371	6.664661	6.219887	5.221178	5.184054
East	Shanghai	1.510664	1.565186	1.503468	1.720428	1.478702
	Jiangsu	0.218232	0.217845	0.260504	0.352624	0.369237
	Zhejiang	0.22644	0.188895	0.230163	0.294521	0.284968
	Anhui	0.751748	0.81895	0.733367	0.839488	0.803823
	Fujian	0.234334	0.207023	0.109558	0.130401	0.134807
	Jiangxi	0.515701	0.474673	0.410789	0.47635	0.493416
	Shandong	1.037763	1.075496	1.072848	1.296846	1.189423
South	Henan	0.497506	0.546818	0.711102	0.790279	0.836593
	Hubei	0.463306	0.469348	0.470466	0.466516	0.500674
	Hunan	0.743311	0.524612	0.520196	0.365889	0.402325
	Guangdong	0.50279	0.544303	0.565706	0.597154	0.62086
	Guangxi	0.199281	0.304914	0.343655	0.370322	0.433459
Southwest	Chongqing	0.309863	0.475355	0.488744	0.398242	0.545348
	Sichuan	0.384578	0.502431	0.538048	0.512992	0.540088
	Guizhou	0.367003	0.448483	0.510066	0.187972	0.172577
	Yunnan	0.429736	0.445402	0.465339	0.634735	0.621552
Northwest	Shaanxi	1.744979	1.736334	1.886317	2.030003	2.144147

	Gansu	0.42323	0.529859	0.476545	0.805404	0.839343
	Qinghai	0.820136	1.376558	0.604174	0.906551	0.822348
	Ningxia	2.147102	1.772315	2.263251	4.400071	4.8391
	Xinjiang	1.018264	1.113483	0.975052	1.041966	0.899895
	Province	2009	2010	2011	2012	2013
North	Beijing	1.078415	1.034069	1.069968	1.077882	1.102943
	Tianjin	0.502999	0.618738	0.865858	0.713553	1.365149
	Hebei	1.503711	1.567345	1.656046	1.74751	1.785628
	Shanxi	0.750314	0.937223	0.783025	0.784102	0.758432
	Inner Mongolia	7.202618	6.522545	5.909151	4.824749	4.710306
Northeast	Liaoning	1.243544	1.19077	1.194555	1.073878	1.059847
	Jilin	0.246448	0.340034	0.479164	0.484211	0.4691
	Heilongjiang	6.83371	6.664661	6.219887	5.221178	5.184054
East	Shanghai	1.510664	1.565186	1.503468	1.720428	1.478702
	Jiangsu	0.218232	0.217845	0.260504	0.352624	0.369237
	Zhejiang	0.22644	0.188895	0.230163	0.294521	0.284968
	Anhui	0.751748	0.81895	0.733367	0.839488	0.803823
	Fujian	0.234334	0.207023	0.109558	0.130401	0.134807
	Jiangxi	0.515701	0.474673	0.410789	0.47635	0.493416
	Shandong	1.037763	1.075496	1.072848	1.296846	1.189423
South	Henan	0.497506	0.546818	0.711102	0.790279	0.836593
	Hubei	0.463306	0.469348	0.470466	0.466516	0.500674
	Hunan	0.743311	0.524612	0.520196	0.365889	0.402325
	Guangdong	0.50279	0.544303	0.565706	0.597154	0.62086
	Guangxi	0.199281	0.304914	0.343655	0.370322	0.433459
Southwest	Chongqing	0.309863	0.475355	0.488744	0.398242	0.545348
	Sichuan	0.384578	0.502431	0.538048	0.512992	0.540088
	Guizhou	0.367003	0.448483	0.510066	0.187972	0.172577
	Yunnan	0.429736	0.445402	0.465339	0.634735	0.621552
Northwest	Shaanxi	1.744979	1.736334	1.886317	2.030003	2.144147
	Gansu	0.42323	0.529859	0.476545	0.805404	0.839343
	Qinghai	0.820136	1.376558	0.604174	0.906551	0.822348
	Ningxia	2.147102	1.772315	2.263251	4.400071	4.8391
	Xinjiang	1.018264	1.113483	0.975052	1.041966	0.899895

We can calculate China 29 provinces general $Moran'sI$, E(I), Z, sd(I), and P value from 2009 to 2013 by using formulas(3) (4) (5) (6), as shows in Table 3. From 2009 to 2013, the $Moran'sI$ P values are all less than 0.05, Z value is all more than the boundary 1.96 with the confidence level 0.05. $Moran'sI$ value increased to 0.336 in 2013 from 0.273 in 2009. It shows that there are obviously positive spatial spillovers in China 29 provinces dairy industry cluster, and there is spatial dependency among the 29 provinces. The dairy industry spatial distribution is not independent; instead, the provinces with higher cluster degrees are neighbors, the provinces with lower cluster degrees are neighbors. The spatial cluster is very obvious. To further analyze dairy industry spatial cluster characteristics, the paper made the $Moran'sI$ scatterplot, shows as Graph 1. This $Moran'sI$ and Table 4 shows most of our provinces are distributed in the first quadrant and the third quadrant. The first quadrant represents the higher cluster degree province are surrounded by other higher cluster degree provinces, including Ningxia, Shaanxi, Inner Mongolia, Heilongjiang, Hebei, Tianjin total six provinces, which accounts for 20.6% of the whole provinces, mainly locates in northwest and north China.

Table 3. China 29 provinces dairy industry cluster $Moran'sI$ index value

Year	Morna's I	E(I)	sd(I)	z	P-value
2009	0.273	-0.036	0.101	3.067	0.002
2010	0.270	-0.036	0.101	3.028	0.002
2011	0.308	-0.036	0.103	3.334	0.001
2012	0.331	-0.036	0.113	3.254	0.001
2013	0.336	-0.036	0.113	3.297	0.001

Note: Hainan, who doesn't have neighbors, was not studied; Hong Kong, Macau, Taiwan, and Tibet

Autonomous Region, whose data is not available, were not studied.

The second quadrant refers to the lower cluster degrees provinces are surrounded by higher cluster degree provinces, including Beijing, Shaanxi, Liaoning, Jilin, Gansu total five provinces, mainly locates in north and northeast China; The third quadrant refers to the lower cluster degrees provinces are surrounded by lower cluster degree provinces, including Jiangsu, Zhejiang, Anhui, Fujian, Jiangxi, Shandong, Henan, Hubei, Hunan, Guangdong, Guangxi, Chongqing, Sichuan, Guizhou, Yunnan, Qinghai, and Xinjiang total 17 provinces, accounts for 58.62% of the whole provinces, mainly locates in east and south and southwest China; The fourth quadrant refers to the higher cluster degrees provinces are surrounded by lower cluster degree provinces, only with Shanghai in the fourth quadrant, accounts for 3.45% of the whole provinces. The above analysis shows: one is that there is spatial cluster phenomenon in China dairy industry cluster; the other is that there is spatial dependency and heterogeneity in industry cluster, it proves the Hypothesis 1. Therefore, we should analyze dairy industry influence factors from spatial econometric perspective.

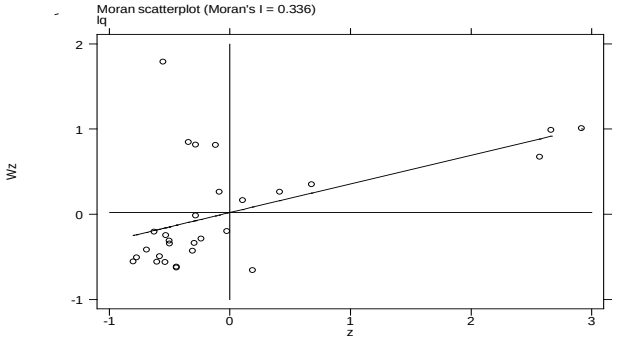

Figure 1. China dairy industry cluster $Moran'sI$ scatter plot

Dairy industry spatial econometric model estimation

The spatial correlation inspection result has showed there is obvious spatial autocorrelation in China 29 provinces dairy industry cluster. Traditional least square regression estimation result is biased. Therefore, the paper adopts the SLM and SEM under the fixed effects and random effects, and estimates and inspects the dairy industry cluster influence factors with China 29 provinces spatial panel data basis. It will choose the best model and explain according the inspection results. To compare easily, first it makes the ordinary least square regression. The results show in Table 5. The least square estimation general goodness-of-fit R^2 is 0.7143. Resources endowment, external economy, government policies environment regression coefficient are positive, and the distribution passed the 5% and 1% confidence level inspection. It shows that there is positive correlation between these three variables and dairy industry cluster. While the economy basis coefficient is negative and passed the 5% confidence level inspection, this is possibly because of the neglecting of spatial autocorrelation. Therefore, it needs spatial econometric model to estimate and inspect dairy industry cluster.

Table 4. Different quadrants provinces distribution

Quadrant No.	Spatial Relevant ways	Province
Quadrant I	HH(high-high)	Ningxia, Shaanxi, Inner Mongolia, Heilongjiang, Hebei, Tianjin
Quadrant II	LH(low-high)	Beijing, Shanxi, Liaoning, Jilin, Gansu
Quadrant III	LL(low-low)	Jiangsu, Zhejiang, Anhui, Fujian, Jiangxi, Shandong, Henan, Hubei, Hunan, Guangdong, Guangxi, Chongqing, Sichuan, Guizhou, Yunnan, Qinghai, and Xinjiang
Quadrant Ⅳ	HL(high-low)	Shanghai

The parameter estimation of fixed effects and random effects spatial lag model and spatial error model used maximum likelihood

estimation method. The result shows as in Table 6. In the spatial error model result shows, the P value of CHI^2 in the Hausman testing is 0.9613. It rejects the original hypothesis and adopts the fixed effects. Meanwhile, the AIC and SC value of fixed effects are much more less than random effects.

Table 5. Least Square Estimation OLS

Variable	Coefficient ρ	Standard Deviation σ	t statistic value	P value
MY	3.39e-07**	6.78e-08	5.00	0.000
AWUW	6.73e-06	0.0000144	0.47	0.642
DPV	0.0073747***	0.0017541	4.20	0.000
GRR	10.57208***	4.029894	2.62	0.010
PCGDP	-0.0000173**	7.10e-06	-2.44	0.016
PM	-1.211589	2.304065	-0.53	0.600
cons	-0.4989081	0.3605385	-1.38	0.169
R2	0.7143			
AIC	352.927			
SC	373.7641			

Note: ***, **, * represents respectively 1%, 5% and 10% confidence level significant test 。

Therefore, fixed effects spatial error model is better than the random effects spatial error model. But the fixed and random effect of spatial lag model parameter λ doesn't pass the 10% confidence level significance testing. Therefore, the spatial error model is not goodness-of-fit for the whole model. The spatial lag model results shows the P value of CHI^2 in the Hausman testing is 0.973. It rejects the original hypothesis and adopts the fixed effects. Meanwhile, the AIC and SC value of fixed effects are much more less than random effects, and the R^2 of the fixed effects is 0.8062, and it is more than the R^2 of random effects. That is to say, the fixed effects spatial lag model is with the best goodness-of-fit., and the fixed effects spatial lag model parameter ρ passed the 10% confidence level significance testing. Compared with the above mentioned five spatial econometric models, the paper chooses the best goodness-of-fit fixed effects spatial lag model, and defines the parameter estimated economy.

Table 6. Dairy industry cluster spatial lag model and spatial error model estimation results

Variable	Spatial Error Model, SEM				Spatial Lag Model, SLM			
	Fixed effects	P value	Random effects	P value	Fixed effects	P value	Random effects	P value
MY	3.43e-07***	0.000	3.50e-07***	0.000	3.40e-07***	0.000	3.28e-07***	0.000
AWUW	8.76e-06	0.529	-8.86e-07	0.942	0.0000112	0.390	-5.90e-07	0.960
DPV	0.0075375***	0.000	0.0070135***	0.000	0.0075642***	0.000	0.0069709***	0.000
GRR	16.70575***	0.001	15.31339***	0.001	15.89509***	0.001	15.03425***	0.001
PCGDP	-0.0000356***	0.005	-0.0000219**	0.023	-0.0000373***	0.002	-0.000022**	0.017
PM	2.705108**	0.033	2.157213	0.119	2.629744**	0.036	2.232159*	0.102
cons			-0.677795*	0.086			-0.7964253**	0.041
σ^2	0.0908785***	0.000	0.1146391***	0.000	0.0909542***	0.000		
σ							0.1159495***	0.000
ρ					0.1147027*	0.101	0.1354406	0.155
λ	0.0496378	0.722	0.0914267	0.513				
R2	0.6979		0.7294		0.8062		0.7464	
LogL	-31.9151		-94.6815		-31.9479		-93.9300	
AIC	77.83026		207.363		76.89588		205.86	
SC	98.6674		234.1536		97.73302		232.6506	
Hausman	Prob>chi2 = 0.9613				Prob>chi2 = 0.9735			

Note: Same as Table 5.

Results Analysis

Hypothesis 1 is supported. The Table 6 results that fixed effects spatial lag model ρ passed the 10% confidence level significance testing. It means China dairy industry cluster is becoming gradually obvious, and there is spatial autocorrelation and spatial spillovers. The paper will analyze dairy industry cluster influence factors one by one.

Hypothesis 2 is supported. Fixed effects spatial lag model MY coefficient is 3.43e-7, and it passed the 1% confidence level significant testing. It has positive correlation with dairy industry cluster. It is one of the factors influencing dairy industry cluster. But due to the improvement of transportation infrastructure and quality keeping and fresh-keeping technology, raw milk transporting and cool-keeping are no the key problems that dairy industry enterprises are concerning.

Hypothesis 2 is rejected. Fixed effects spatial lag model labor cost AWUW coefficient estimation doesn't pass the 10% confidence level significance testing. That is to say the labor cost will not significantly influence China dairy industry cluster. It is incompatible with the materials endowment theory of Hypothesis 1. The reasons are: one is dairy industry clustered provinces Heilongjiang, Inner Mongolia, Shaanxi, Ningxia natural conditions, location, infrastructure aspects are behind the east coastal regions. Thus it will somehow offset the labor cost advantage. The other one is the existing of snowballing effects. Dairy industry once clustered in the provinces, it will develop very well and self-improved well. And it will further improve cluster, and finally it forms the snowballing effects, and weakens the cheap labor cost's influence on dairy industry cluster.

Hypothesis 4 is supported. Fixed effects spatial lag model existing scales level DPV coefficient is 7.5375e-3, it passed 1% confidence level significance testing, and it has positive correlation with dairy industry cluster. This means the dairy industry external economic effect truly exists in Hypothesis 4. When the dairy industry is cluster in a province, it will promote the dairy cluster with the whole scale increasing and general cost decreasing. Namely when the existing scale level increase one by unit, dairy industry cluster degree will increase 7.537e-3 by unit.

Hypothesis 5 is supported. Fixed effects spatial lag model government supporting degree GRR coefficient is 15.89509, and it passed 1% confidence level significance testing, and it has obvious positive correlation with dairy industry cluster. It proves that the government supporting degree provides good developing environment for the whole industry, and it will further exist industry cluster in the region hypothesis. Meanwhile it means government is playing important role in industry developing and industry clustering. When the coefficient is 15.89509, it is namely when government revenue percentage for GDP increase by 1%, cluster degree coefficient will increase by 0.1589509.

Hypothesis 6 is incompatible. Fixed effects spatial lag model economic basis PCGDP coefficient is -3.73e-5, and it passed 1% confidence level significance testing. It has negative correlation with dairy industry cluster. So it is incompatible with hypothesis 6. That is to say provincial economic development will not positively influence dairy industry cluster. On the contrary, it is negative correlated. When Per Capita GDP increases 1 by unit, cluster degree will decrease 3.73e-5 by unit. The reason is that the Per Capita GDP of east China economy developed provinces is much higher than the middle and west China. However, factory construction, production managing, enterprise managing, employees wages costs are also much higher than middle and west China. Meanwhile, we have to think about the raw milk availability, that most of the dairy industry enterprises are located mainly in the undeveloped provinces like Inner Mongolia, Heilongjiang, Ningxia, Shaanxi province. Therefore, there comes the result of negative correlation between economic development and diary industry cluster.

Hypothesis 7 is supported. Fixed effects spatial lag model regional profitability PM coefficient is 2.629744, and it passed the 5% confidence level significance testing. It has positive correlation with dairy industry cluster. It proves Hypothesis 7 that the higher is the dairy industry profit margin rate, the more attractive for the enterprise it will be.

4. Conclusions

The paper finally choose fixed effects spatial lag model to explain China diary industry cluster influence factors by comparing ordinary least square estimation (OLS), and constructing fixed effects and random effects spatial lag model(SLM) and spatial error model (SEM), and come to the conclusion below:

There is positive correlation among China dairy industry cluster. There is strong spatial dependency and positive spatial spillovers in different provinces, namely, the neighborhood provinces' dairy industry development will promote this province dairy industry development and cluster. Therefore, it will enhance China dairy industry develop in a healthy and fast way by communicating and cooperating more with neighborhood provinces, and promoting regions rational flow of talents and capitals

The results of the most of industry cluster studies with time dimensions are similar. There is significant correlation among the resources endowment, external scope economy, industry profitability and China dairy industry cluster. According to the self-raw milk availability characteristics, and the existing scale level, different provinces takes different diary industry developing strategies. It is playing significant practical role in promoting diary industry cluster development and improving industry branded enterprise' competition. There is significant positive correlation between government supporting degree and dairy industry cluster. Government maintaining good economic running environment is the catalyst and the guarantee of the dairy industry cluster. Based on every province's different dairy industry developing levels, the province will push dairy industry further development with government and marketing joint efforts. Different from the hypothesis, labor force is no the significant factor of influencing dairy industry cluster. The dairy industry clustered provinces are not getting benefit from the labor force cost. Incompatible with the hypothesis, there is negative correlation between the economic basis and dairy industry cluster. Compared with developed provinces, dairy industry tends to cluster in the undeveloped provinces. Middle and west China should seize this opportunity to promote it's dairy industry cluster, and activate the economic development.

While, there is broad space to improve the paper. On the one hand, the diary industry cluster influence factors spatial econometric model constructed in the paper could be expanded, for instance, technology, chance factors could be introduced into the model to be further observed and studied. On the other hand, the penal data yeas span is limited in this paper, without enough consideration of time dimension characteristics, and the class weights setting without consideration of economic distance. If the paper uses long time span dynamic spatial panel econometric model, the conclusion will be more purposeful and persuasive.

5.References

Baldwin, R.E. (1999). Agglomeration and endogenous capital, *European economic review*, 43, 253-280.

Cheng, Xiaoping. (2012). China dairy industry spatial cluster and the influence on industry growing, *Journal of Wuhan University of Technology (Social Scientific Edition)*, 05, 688-693.

Easterly, W, Levine, R. (1998). Troubles with the neighbors: Africa's problem, Africa's opportunity, *Journal of African economics*, 7(1),120-142.

Fujita, M.A. (1988). Monopolistic competition model of spatial agglomeration: differentiated product approach, *Regional science and urban economics*, 18(1), 87-124.

Henderson, J.V. (1974). The sizes and types of cities, *American economic review*, 64(4), 640-656.

Hua, Junguo, Zhu, Xiangrong, Yin, Chenwen. (2007). The analysis of China dairy cluster situation and spatial distribution, *China rural economy*, 02, 49-54+80.

Lanaspa, L.F, Pueyo, F. & Sanz F. (2001). The public sector and core-peripthery models, Urban studies, 38,1639-1649.

Lesage, J P.A (1999). Spatial econometric examination of China's economic growth, *Geographic information sciences*, 5(2),143-153.

Li, Li. (2008). Industry investment location selecting and industry cluster—take Inner Mongolia Animal Products Industry as an example, *China rural economy*, 01,12-22.

Li, Li, An, Yufa. (2008). Regional production specialization and industry cluster — analysis of Inner Mongolia animal products industry cluster trends. *Agricultural economic issues* ,05,44-49.

Liu, Hedong. (2013). Empirical study of regional creation inside overflow, outside overflow, and spatial spillover effect, *Science research management*, 01, 28-36.

Niu, Honglei, Jiang, Keshen. (2011). The panel data analysis of China textile Industry cluster Distribution layout and influence factors, *Application of statistics and management*, 04, 571-584.

Ohlin, B. (1968). Interregional and International Trade. Cambridge: Harvard University Press, (89-92).

Ren, Yinghua, Xu, Ling, You, Wanhai. (2010) Financial cluster influence factors and application, *The journal of quantitative technical economics*, 05,104-115.

Sun, Qinggang, Guo, Ju'e, Shi, Bo. (2013). China Provincial Energy Intensity Spatial Spillover Analysis, *China's population, resources and environment*, 11, 137-143.

Tschoegl, Adrian E. (2000). International banking Centers, geography, and foreign banks, financial markets, institutions & instruments, 9 (1), 1-321.

Wang, Yongpei, Yuan, Pinghong. (2010). Wages difference, labor force flowing, and industry clustering—based on new economic geography explanation and empirical testing, *Finance and economics*,03,59-66.

Zhang, Xueliang. (2012). Does China communication infrastructure promote regional economy growth—the communication infrastructure spatial spillovers effect, *Social science of China*, 03,60-77; 206.

Zhao, Liangshi, Sun Caizhi, Zheng Defeng. (2014). China Inter-provincial water resource utilization efficiency and spatial spillover effect measure, *Geography journal*, 01,121-133.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

A NEW PARTICLE SWARM OPTIMIZATION MATHEURISTIC SOLUTION TO EMERGENCY FOOD DISTRIBUTION

Dali Jiang[1], Lei Qi[1*], Longbang Ma[1], Zijun Wang[1], Hua Yan[1], Yuanwen Chen[1]

[1] Department of Logistics, Logistical Engineering University, 401311 Chongqing, China;
*Corresponding author: *qilei198743@163.com*

Article history:
Received:
05 March 2016
Accepted in revised form:
23 May 2016

Keywords:
Emergency Food Distribution;
Particle Swarm Optimization;
Algorithm;
Poisson-Process;
Multi-level Network

ABSTRACT
The research of emergency food distribution and decision models mostly focus on deterministic models and exact algorithms. Some studies have been done on the multi-level distribution network and matheuristic algorithm. In this paper, random process theory is adopted to establish emergency food distribution and decision model for multi-level network. By analyzing the characteristics of the model, a modified discrete particle swarm optimization matheuristic algorithm (MBPSO) is proposed to solve the problem. In MBPSO, appropriate degradation mechanism and parallel global search structure are designed. Through an instance, MBPSO has a capability of global optimum search and fast convergence property for hybrid integer programming model with the multi-constrained and weighted single objective.

1. Introduction

Emergency food distribution has been studied in extensive literature mainly focus on single-level network, deterministic models and planning algorithm, less research has been done on uncertainty in the emergency environment, multi-level distribution network and intelligent matheuristic algorithm.

R. Ji and Z. Xiao-lei formulate the problem through an integer programming model and a Lagrangian heuristic algorithm is developed to solve the problem (Ji and Xiao lei, 2014). Toyoglu et al., provide an ammunition distribution algorithm to a three-layer commodity-flow location routing formulation that distributes multiple products (Toyogluet et al., 2011). Naval warfare is studied in this literature (Gue, 2003), mainly concentrate on logistics center location, emergency food presets and emergency food distribution optimization problem. I. O. Pierskalla and W. P. propose a three-index formulations for solving the problem of locating regional blood banks to serve hospitals (Pierskalla and P., 1979). in literature (Perl and Daskin, 1985) for designing the division's distribution system, in consideration of the system number, size, and locations of central depots.

Above these are some research achievements on distribution of emergency food. Most of them concentrate on the deterministic models and exact algorithm. Few of them focus on mataheuristic and matheuristic algorithm.

The authors propose also a matheuristic which aims at alternatively solving emergency food distribution design problem with estimated distribution amount, using exact methods, and determining the routing decisions and transportation, using heuristic procedures (Prodhon and Prins, 2014).

The main differences of our approach with other proposals existing in the literature

are that, in this paper, Poisson-Process is adopted to establish random risk of emergency food distribution (one of the three indexes). Meanwhile, an improved discrete particle swarm optimization (MBPSO) matheuristic algorithm is proposed to solve the problem.

The paper is organized as follows. In Section 2 we formulate the problems of emergency food distribution. and propose modified discrete particle swarm optimization algorithm (MBPSO). In Section 3, through an instance discussing different performance of three algorithms. Finally, Section 4 contains some conclusions and future research development.

2. Materials and methods

Emergency food distribution for Multi-level network is generally composed of three layers, supply points, transit points (depots) and demand points. As shown in Figure 1.

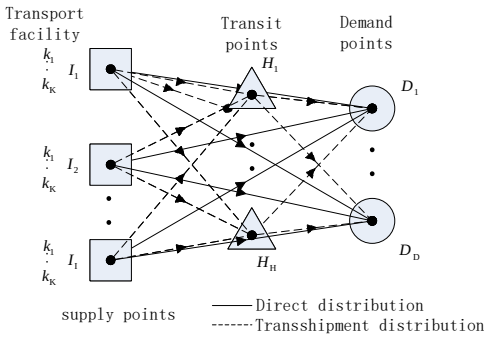

Figure 1. Three layers distribution network

In order to illustrate the model exactly, we first declare some symbol explanations.

T	Set of total time of distribution.	h
x_{id}	Set of amount of emergency supplies to be distributed to demand point d at supply point i ,where $i \in I$, $d \in D$.	t
dis_{id}	Set of distance between supply point i and demand point d ,where $d \in D$.	km
dis_{ih}	Set of distance between supply point i and transit point h ,where $i \in I$, $h \in H$.	km
dis_{hd}	Set of distance between transit point h and demand point d ,where $h \in H$, $d \in D$.	km
v_k	Set of velocity of transport facility k ,where $k \in K$.	km/h
t_{zz}	Set of transshipment time.	h
C	Set of total cost of distribution.	
c_{inv}^k	Set of fixed cost of transport facility k .	
c_v^k	Set of variable cost of transport facility k , where $k \in K$.	
c_{zz}	Set of the transshipment cost.	
p	Set of probability of being found by enemy in delivery paths.	
S	Set of random risk from enemy in delivery paths.	

Greek Symbols

λ_{id}	Set of Poisson intensity between supply point i and demand point d , where $i \in I$, $d \in D$.
λ_{ih}	Set of Poisson intensity between supply point i and logistics transit point h , where $i \in I$, $h \in H$.
λ_{hd}	Set of Poisson intensity between transit point h and demand point d , Where $h \in H$, $d \in D$.
$\lambda_{i?d}$	1,if transport facility does not get through any transit point;0 otherwise, where $i \in I$, $d \in D$.
$\lambda_{i?k}$	1,if transport facility k will be chosen,0 otherwise, on condition that $\lambda_{i?d} = 1$. where $i \in I$, $k \in K$.
$\lambda_{i?h}$	1,if the transit point h be chosen,0 otherwise. where $i \in I$, $h \in H$.
$\lambda_{ih?k}$	1,if transport facility k will be chosen,0 otherwise，on condition that $\lambda_{i?h} = 1$,from supply point i to logistics transit point h , where $i \in I$, $h \in H$, $k \in K$.
$\lambda_{hd?k}$	1,if transport facility k will be chosen,0 otherwise，on condition that $\lambda_{i?h} = 1$, logistics transit point h to demand point d , where, $d \in D$ $h \in H$, $k \in K$.

Subscripts

i	supply point. $i \in I$
h	transit point. $h \in H$
d	demand point. $d \in D$
k	transport facility. $k \in K$
v	velocity

inv invariable
? choose which one

Transport time and cost is divided into direct part and transshipment part. In every route in multi-level network, different transport facility will spend different time and cost, but only allowed to choose one of transport facilities in one route. If route pass through a transit point, it will produce transshipment time and transshipment cost, which associated to emergency food amount. The larger amount of emergency food will expend the more time and cost. The total time and total cost of emergency food distribution is shown in equation (1) and equation (2).

$$T=\begin{cases}\sum_{d\in D}\sum_{i\in I}\sum_{k\in K}\lambda_{i?d}\lambda_{i?k}\left(\frac{dis_{id}}{v_k}\right) & \lambda_{i?d}=1\\ \sum_{d\in D}\sum_{i\in I}\sum_{h\in H}(1-\lambda_{i?d})\lambda_{i?h}\begin{pmatrix}\sum_{k\in K}\lambda_{ih?k}\left(\frac{dis_{ih}}{v_k}\right)+\cdots\\ x_{id}t_{zz}+\sum_{k\in K}\lambda_{hd?k}\left(\frac{dis_{hd}}{v_k}\right)\end{pmatrix} & \lambda_{i?d}=0\end{cases} \quad (1)$$

$$C=\begin{cases}\sum_{d\in D}\sum_{i\in I}\sum_{k\in K}\lambda_{i?d}\lambda_{i?k}\left(c_{inv}^{k}+c_{v}^{k}x_{id}dis_{id}\right) & \lambda_{i?d}=1\\ \sum_{d\in D}\sum_{i\in I}\sum_{h\in H}(1-\lambda_{i?d})\lambda_{i?h}\begin{pmatrix}\sum_{k\in K}\lambda_{ih?k}\left(c_{inv}^{k}+c_{v}^{k}x_{id}dis_{ih}\right)+\cdots\\ x_{id}c_{zz}+\cdots\\ \sum_{k\in K}\lambda_{hd?k}\left(c_{inv}^{k}+c_{v}^{k}x_{id}dis_{hd}\right)\end{pmatrix} & \lambda_{i?d}=0\end{cases} \quad (1)$$

$\{N(t),t\geq 0\}$ means a random number of destroy from uncontrollable factor in environment in multi-level network during period $[0,t)$ .Assume that $\{N(t),t\geq 0\}$ Obey strength λ for the Poisson Process, where the strength λ is mean to random number of destroy from uncertain environment to one of routes in unit time. In once destroy process, the probability of our emergency food being destroy is denoted by p , where $0<p<1$, and the event of environment destroying emergency food within each time interval is mutual independent.

So we know that $\{Y(t),t\geq 0\}$ belongs to Compound Poisson Process which obey the strength λp .In order to investigate the random risk degree in transport process. We have to investigate some characteristic functions of this Poisson Process. In this paper, we choose the mean function as a target to evaluate the random risk degree of emergency food distribution.

Note that $\{Y(t),t\geq 0\}$ obey strength λp in Compound Poisson Process, so the mean function of $\{Y(t),t\geq 0\}$ is shown in equation (3).

$$m_N(t)=E\left[Y(t)\right]=\gamma pt \quad (2)$$

Through equation (3) we can conclude that the expectation value of Compound Poisson Process is proportional to Poisson intensity, subsystems probability and duration time. Based on the above deduction, the random risk of emergency food distribution in multi-level network is shown below.

$$S=\begin{cases}\sum_{d\in D}\sum_{i\in I}\sum_{k\in K}\lambda_{i?d}\lambda_{i?k}\left(\frac{dis_{id}}{v_k}\right)\lambda_{id}p & \lambda_{i?d}=1\\ \sum_{d\in D}\sum_{i\in I}\sum_{h\in H}(1-\lambda_{i?d})\lambda_{i?h}\begin{pmatrix}\sum_{k\in K}\lambda_{ih?k}\left(\frac{dis_{ih}}{v_k}\right)\lambda_{ih}p+\cdots\\ \sum_{k\in K}\lambda_{hd?k}\left(\frac{dis_{hd}}{v_k}\right)\lambda_{hd}p\end{pmatrix} & \lambda_{i?d}=0\end{cases} \quad (4)$$

The objective function is shown in equation (5). It is consisted of time, cost and random risk these three indexes, on which we will put corresponding weight factor to cater to different needs in different scenario in section 3, we use uniformitarian process for objective function before evaluating its fitness. Some constraints are shown below.

$$\min \quad Z=\alpha T+\beta C+\gamma S \quad (3)$$

$$S.T. \quad \sum_{h\in H}\lambda_{i?h}=1 \quad \forall h\in H \ \forall i\in I \quad (4)$$

$$\sum_{k\in K}\lambda_{id?k}=1 \quad \forall i\in I \ \forall d\in D \tag{7}$$

$$\sum_{k\in K}\lambda_{ih?k}=1 \quad \forall i\in I \ \forall h\in H \tag{8}$$

$$\sum_{k\in K}\lambda_{hd?k}=1 \quad \forall h\in H \ \forall d\in D \tag{9}$$

$$\sum_{i=1}^{I}x_{id}=x_d \quad \forall i\in I \ \forall d\in D \tag{10}$$

$$\sum_{d=1}^{D}x_{id}\le X_i \quad \forall i\in I \ \forall d\in D \tag{11}$$

Constraints (6) describes that we can choose only one transit point. Constraints (7) demonstrates that we can choose only one transport facility, on condition that on condition that $\lambda_{i?d}=1$. Constraints (8) ensure that we can choose only one transportation from supply point i to transit point h , on condition that $\lambda_{i?d}=0$.

Constraints (9) ensure that we can choose only one transportation from supply point h to demand point d , on condition that $\lambda_{i?d}=0$ .Constraints (10) guarantee that the emergency food amount of all supply points I to be distributed should be equal to the amount of demand point d .Constraints (11) exhibits that the total emergency food amount to be distributed in arbitrary supply point i should be less than their own inventory.

In recent years, many scholars proposed a variety of approach like heuristic algorithm, bionic intelligent algorithm, algorithm combined with the constraint condition etc, to investigate the problem (Mezura-Montes et al., 2010).But existing approach cannot solve our model. So, a new matheuristic algorithm will be proposed in this paper. In next section we will introduce a modified matheuristic algorithm to solve this problem.

Improved Discrete Particle Swarm Optimization **:** The particle swarm optimization algorithm (PSO) is proposed by Kennedy and Eberhart (Kennedy and Eberhart, 1995). In PSO, a potential solution for a problem is considered as a bird, which is called a particle, flies through a D-dimensional space and adjusts its position according to its own experience and other particles'. In PSO, a particle is represented by its position vector p and its velocity vector v. In time step t, particle i calculates its new velocity then updates its position according to equation (12) and equation (13), respectively.

$$v_i^{'d}=\omega v_i^d+c_1r_1\left(p_i^d-x_i^d\right)+c_2r_2\left(p_g^d-x_i^d\right) \tag{5}$$

$$x_i^{'d}=x_i^d+v_i^{'d} \tag{6}$$

where ω is the inertial weight, and c_1 and c_2 are positive acceleration coefficients used to scale the contribution of self-cognitive and social-sharing components, $v_i^{'d}$ is the current speed value. v_i^d is the last speed value. respectively. p_i^d is the best position that particle i has been experienced in d dimensions. p_g^d is the best position found by all particles I in d dimensions. r_1 and r_2 are uniform random variables in range.

Standard PSO algorithm is suitable to continuously problem. In order to make the PSO algorithm more adaptive to solve discrete optimization problems, J. Kennedy and K. C. Eberhart, introduce a Binary-Particle Swarm Optimization (BPSO), which is more suitable for solving the problem of discrete.

BPSO algorithm inherits the velocity updating equation of the standard PSO algorithm. Firstly ,utilize equation (12) to update the velocity value, then, SIGMOID function is used to convert velocity value into the probability of binary digit to get value 1.The process is shown below.

$$s\left(v_i^d\right)=\frac{1}{1+\exp\left(-v_i^d\right)} \quad (7)$$

$$x^{d}_{i}=\begin{cases}1 & if\ \ rand()\le s\left(v_i^d\right)\\ 0 & otherwise\end{cases} \quad (8)$$

Where $rand()$ is uniform random variables in range [0,1]. It is necessary to set a maximum velocity $v_{\max}$ to limit the range of v_i^d, denoted by $v_i^d\in[-v_{\max},v_{\max}]$.

The standard BPSO algorithm with fast convergence speed, but due to its following features, The particle population is easy to fall into local extremum. In order to overcome the deficiencies, we improved the standard BPSO optimization algorithm, making BPSO algorithm more efficiently and accurately search the global optimuml solution.

Solution Structure： It is can be seen from Figure 2., The structure of the solution is divided into two parts ,the first part is linear programming part. Which represents the amount of emergency food to be distributed. The second part is heuristic part, which means combination of route and means of transport. Also, solving process is divided into two parts .Firstly, combination of transport means and routes is generated by MBPSO algorithm, then linear programming is used to find the best fitness of objective function.

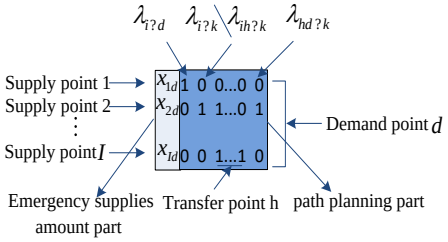

Figure 2. solution structure

MBPSO Implement Steps： Step1 Population initialization. In the problem definition domain, initializing population position and velocity value randomly, and calculating its fitness.

Step 2 Stop judging. Stop and exit, if the algorithm meet stop condition. Otherwise, continue.

Step3 velocity and position update. equation (12), (14), (15) are used to update the velocity and position of populations and calculating new populations' fitness.

Step4 Parallel algorithm structure. At every predetermined sampling point, another parallel global search mechanism will be triggered in sampling period. Some separate population will be randomly initialized to get global optimum which denoted by Gbest1. In sampling period, if Gbest1 is better than Gbest, replace it.

Step5 Population degradation mechanism. At every predetermined sampling point. In roulette random way, with a certain probability substitute one of select particles' best solution it experienced (Pbest) for current global optimum (Gbest). Meanwhile, Storing Gbest. When sampling period is over, if there is no other better optimal value updated, then give the last stored Gbest back to the current optimal value.

Step 6 Evaluate fitness, Turn to Step 2.

3. Results and discussions

In order to verify the validity and practicability of the model and modified discrete particle swarm optimization algorithm (MBPSO), Constructing a three level emergency food distribution network, consists of 4 supply points, 3 transit point, 5 demand point and two kinds of transport facility. α=0.5、β=0.2、$\gamma=0.3$is the weight of time, cost and random risk respectively. Transit costs and Transit time is that $c_{zz}=20\$/t$ and $t_{zz}=0.3h/t$ .Other parameters will be random initialized.

Parameters Combination Experiment： MBPSO algorithm performance is sensitive

to parameters setting of c_1 , c_2 and w , which affects the convergence speed, accuracy and other properties. Therefore, combination value of c_1 , c_2 and w is to be investigated firstly.

(1) parameters combination experiment

w is the inertial weight, and c_1 and c_2 are positive acceleration coefficients used to scale the contribution of self-cognitive and social-sharing components. There have been lots of research on PSO algorithm parameters analysis, but without unified conclusion. For different question need to retest and reset the parameters.

According to the literature, parameter combinations test is designed for six groups, the result is shown in Figure 3 and Table.7

Table 1. MBPSO performance comparison with different parameter combinations

	C1	C2	W	Mean value
				*E+05
(a)	0	2	0.9	2.6422
(b)	2	0	0.9	2.6862
(c)	0	0	0.9	2.7542
(d)	2	2	0	2.7247
(e)	2	2	0.9	**2.5658**
(f)	2	2	0.9~0.1	**2.6365**

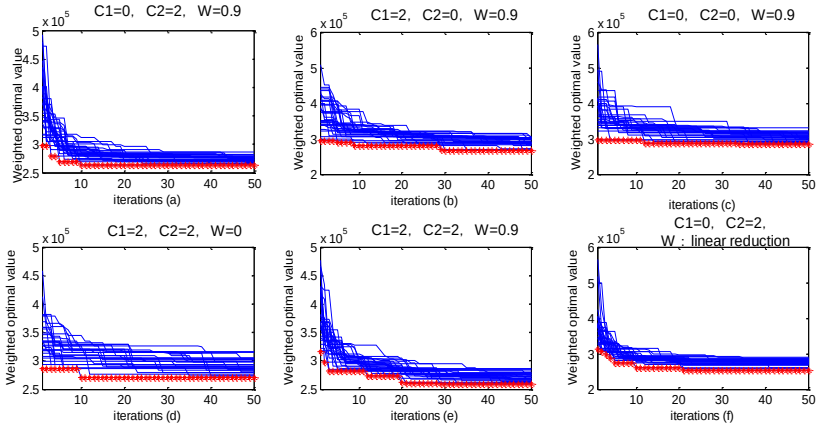

Figure 3. MBPSO particles convergence track under different parameter combinations

We can see that group (a) is a Social Model , because it's parameter c1=0,which presents the group (a) does not have the cognitive part. Similarly. group (b) is cognitive model. group (c) has neither society nor cognitive part, just has inertial weight. group (d) has both society and cognitive part, without inertial weight. **Eroare! Fără sursă de referință.** shows that the group(e) is the best on convergence accuracy. According to the literature, (Kennedy and Eberhart, 1997)In group(f) , the inertial weight decreases linearly. It makes the algorithm running in the early stages can be carried out large-scale global search, and later with a strong local search ability.

(2) Algorithms comparison

In order to verify the effectiveness of the algorithm comparison among MBPSO, BPSO and HCA was taken to be done In terms of calculation accuracy and convergence.

As shown in Figure 4 and

Table **2**. The comparison among the HCA, BPSO and MBPSO in terms of calculation accuracy under different running time .As can be seen from sub-graph (a) in Figure 4, where MBPSO algorithm is

running under short time, and its performance is mediocre. The calculation accuracy trajectory of the three algorithms are close. The reason is because MBPSO algorithm does not take full advantage of degeneration mechanism and parallel global search function, it's structure is similar to BPSO algorithm, so its performance is not the best. whereas, with running a longer time, MBPSO algorithm degradation mechanisms and parallel global search function comes into play. It makes all particles follow Gbest in local search, but also can rushed out of the local extremum restrictions to take global optimization. It is also can be seen from sub-graph (b,c,d), with running a longer time, the calculation accuracy trajectory of MBPSO algorithm starts better than the other two algorithms. Also we can conclude from variance image in Figure 4, with running a longer time, the stability of MBPSO is becoming better gradually.

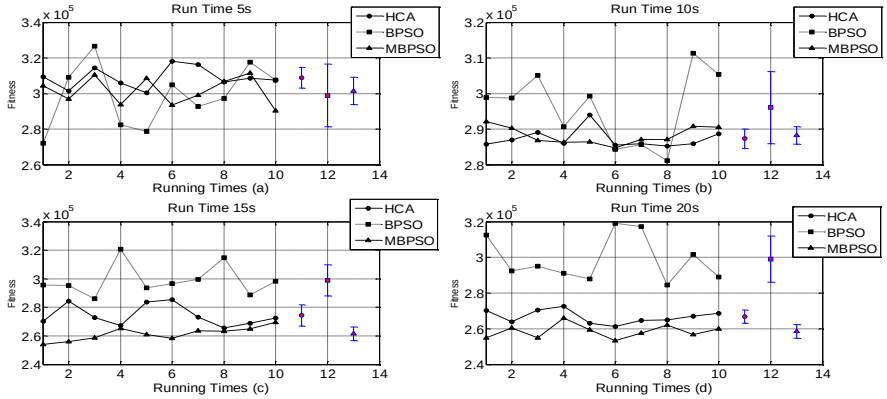

Figure 4. Algorithms' calculation accuracy comparison

Table 2. Mean value comparison of Algorithm calculation in 10 times run

run time	Algorithms	Mean value	run time	Algorithms	Mean value
		*E+05			*E+05
5s	HCA	3.0888	10s	HCA	**2.873**
	BPSO	**2.9887**		BPSO	2.9605
	MBPSO	3.0151		MBPSO	2.8822
15s	HCA	2.7439	20s	HCA	2.6671
	BPSO	2.9893		BPSO	2.99
	MBPSO	**2.6148**		MBPSO	**2.5854**

4. Conclusions

On the premise of time and cost, Adopting Poisson-Process to establish emergency food distribution and decision model in multi-level network. By analyzing the characteristics of the model, On the basis of the standard discrete particle swarm optimization algorithm (BPSO), algorithm structure of appropriate degradation mechanism and parallel global search is designed.

In order to verify the effectiveness of the algorithm, contrasting (Mdified Binary-Particle Swarm Optimizaion, MBPSO) with (Standard Binary-Particle Swarm Optimizaion, BPSO) and (Hill Climbing Algorithm, HCA), In terms of accuracy and convergence. The results show that (MBPSO) is of a global optimum and a fast convergence property for multiple constrained multi-objective integer programming model. But in the case of the running time is short, the

algorithm performance is almost the same with the other two algorithms.

In summary, there is a general and practical meaning for emergency food distribution and decision models in Multi-level network. Providing a new way for multi-constrained and multi-objective high dimensional optimization combination problem.

5. References

Gue, K. R. (2003). A dynamic distribution model for combat logistics, *Computers & Operations Research*, 30(01), 367-381.

Ji, R., Xiao-lei, Z. (2014). Logistic Supply Network Design in Battlefield Uncertain Environment with Enemy Attack Consideration, *Fire Control & Command Control*, 39 (6), 126-130.

Kennedy, J., Eberhart, R. C. (1995). Particle swarm optimization, in Proc. IEEE Int. Conf. Neural Netw., 4, 1942-1948.

Kennedy, J., Eberhart, R. C. (1997). A discrete binary version of the particle swarm algorithm, Paper presented at the Systems, Man, and Cybernetics, Computational Cybernetics and Simulation.

Mezura-Montes, E., Coello-Coello, C. A. (2011). Constraint-handling in nature-inspired numerical optimization: Past, present and future, *Swarm and Evolutionary Computation*, 1(4), 173-194.

Perl, J., Daskin, M. S. (1985). A Warehouse Location-Routing Problem, Transportation Research Part B Methodological, 19b(5), 381-396.

Pierskalla, I. O., P., W. (1979). A Transportation Location-Allocation Model for Regional Blood Banking, Iie Transactions, 11(2), 86-95.

Prodhon, C., Prins, C. (2014). A survey of recent research on location-routing problems, *European Journal of Operational Research*, 238(1), 1-17.

Singh, H. K., Ray, T., Smith, W. (2010). C-PSA: Constrained Pareto simulated annealing for constrained multi-objective optimization, *Information Sciences*, 180(13), 2499-2513.

Toyoglu, H., Karasan, O. E., Kara, B. Y. (2011). Distribution network design on the battlefield, *Naval Research Logistics*, 58(3), 188-209.

Acknowledgments

This work was supported by National Natural Science Foundation of China (71401172).The authors would like to thank Yan Hua for excellent technical support and Professor DaLi Jiang for critically reviewing the manuscript.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

PHARMACOKINETICS OF MANIDIPINE IN HEALTH VOLUNTEERS

Xin Xie[1,*], Xiuwei Fang[2]

[1]*Department of Cooking and Food, Henan Polytechnic College, No.210, Pingan Road, Zhengzhou, Henan province, 450046, China;*

[2] *Department of Food Chemical Engineering, Henan Quality Polytechnic, Middle of the Yaodian Street, Pingdingshan, Henan province, 467000, China.*

*Corresponding author: *656271158@qq.com*

Article history:
Received:
07 January 2016
Accepted in revised form:
19 May 2016

Keywords:
Manidipine
HPLC-MS
Health volunteers
Pharmacokinetics

ABSTRACT

To establelish a HPLC-MS method for determining the concentrations of *Manidipine* in human plasma and to evaluate its pharmacokinetic characteristics. A Venusil XBP-C_8column column was used to separate *Manidipine* in plasma with a mobile phase of a mixture of 5mM ammonium acetate（0.5%acetic acid）- methanol - methy1 cyanide ((15：40：45, V/V/V) at a flow rate of 0.6ml.min^{-1}. Atmospheric pressure electronic spray ionization (AP-ESI) and ion mass spectral (m/z) of 611.4 were selected to quantify Manidipine, and 441.1 for *Nimodipine* (internal standard). The linear range of the standard curve of *Manidipine* was 0.25-50ng.ml^{-1}, and the determination limit was 0.25ng.ml^{-1}. The extraction recoveries were more than 49.85%, intra-day and inter-day RSD were less than 7.44%. The *Manidipine* plasma concentrations were determined after single and multiple dose and its pharmacokinetic parameters were calculated. The method is sensitive, fast and accurate. It is suiTablele for therapeutic *Manidipine* monitoring and its pharmacokinetic studies.

1. Introduction

Manidipine is calcium antagonist of dihydropyridine class and had no effects on the heart, will not affect the heart rate, has the function of atherosclerosis in some degree, has a protective effect of kidneys, does not affect the sympathetic nervous system, less adverse reaction, for hypertension merger in patients with type Ⅱ diabetes or glucose tolerance to reduce and elderly patients have good antihypertensive effect (Benchawan and Pornsak, 2016). *Manidipine* is unsTablele in plasma or exposed to light and the routine analytical method cannot meet the requirements of its pharmacokinetic studies in human body (Cheer and McclEllan, 2005; Naylere and Panagiotopoulos, 2001). A HPLC-MS method for determining the blood concentration of *Manidipine* with *Nimodipine* as internal standard was developed and report (Vitor et al., 2015). It is sensitivity, specialty and precision, and suiTablele for *Manidipine* therapy drug monitoring and pharmacokinetic studies (Fogari et al., 1999).

2. Materials and methods

2.1. Instruments and reagents

The HP1100LC-MS system was used to separate and detect *Manidipine* in human plasma, METTLER TOLEDO AX-205 electronic balance， XW-80A eddy mixer, PROINO high speed centrifuge, PK514BP ultrasonic cleaner were supplied by American Agilent Company, Mettler-Toledo Instrument (Shanghai) Co. Ltd, Shanghai Jingke Company,

American Kendro Laboratory Products and Germany Bandel Company, respectively.

Manidipine Hydrochloride Tablele of 10mg were offered by Jinan Limin Pharmaceutical Factory. Chinese Drug and Biological Products Quality Control institute provided the internal standards (IS) of *Manidipine* and *Nimodipine*. Methanol, methyl cyanide and ethyl acetate were all chromatographic pure grade.

2.2. Conditions for mass spectra and chromatogram

AP-ESI positive ion mode was used with atmospheric pressure of 50 psig and protective air of N_2 at a flow rate of 10L.min^{-1}, capillary voltage of 4000V，drying air temperature of 350℃. The selected iron monitoring (SIM) was used as ion collecting mode. The ion mass spectral (m/z) of 611.4 (M+1) was selected to quantify *Manidipine* and 441.1 (M+Na) for *Nimodipine.* Debris voltage 140 v and 120 v respectively. The separation was carried out with a mobile phase of a mixture of 5mM.l-1 ammonium acetate （0.5%acetic acid）-methanol- methyl cyanide ((15 : 40 : 45, V/V/V) at a flow rate of 0.6mL.min^{-1} and a sTablele phase of A Venusil XBP-C_8 column (150mm×4.6mm，5μm), 50μL of purified sample was injected (NguyenLan et al., 2016).

2.3. Method of pretreatment

The stock solutions of *Manidipine* and *Nimodipine* at the concentration of 0.1 mg.mL^{-1} and 0.12mg.mL^{-1} respectively were dissolved under methanol, kept from light during all course. A liquor of 1.0mL plasma of sample plus 50μL of IS was alkalinized by adding 0.1mL of 2 mol.mL^{-1} Sodium Hydroxide and 5mL of ethyl acetate。Then it was vortex-mixed 3 min, centrifuged at 4000 r·min^{-1} for 5 min .The water phase was discarded and 4mL of organic phase was moved to a clean glass tube and dried under Nitrogen in a 40 ℃ water bath. The residue was reconstituted with 0.1mL of mobile phase and 50μL of it was injected for analysis.

2.4. Corroboration of methods

Under above conditions, the retention time of IS and *Manidipine* were 6.7 and 4.5 min, respectively. The blank plasma, blank plasma spiked with IS plus *Manidipine*, volunteer samples spiked with IS, the SIM chromatograms and mass spectra of *Manidipine* were show in Fig 1 and Fig 2.

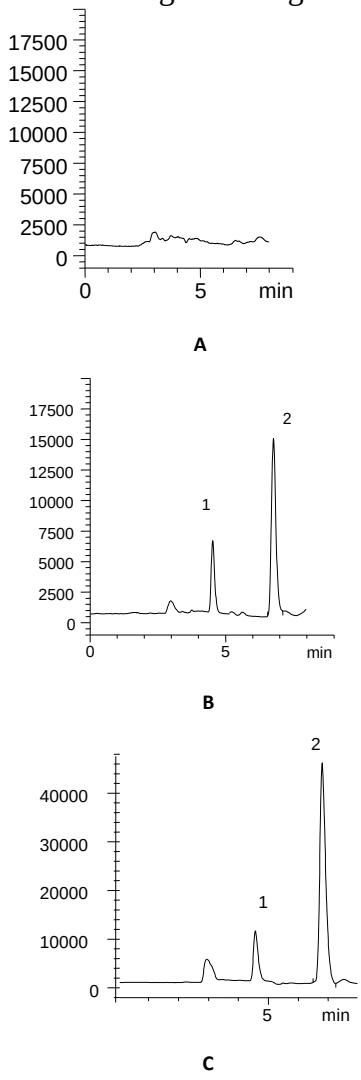

Figure 1. Chromatograms for the determination of *Manidipine* by HPLC

A: blank plasma；B: blank plasma plus *Manidipine* and internal standard; C: plasma sample of volunteer plus internal

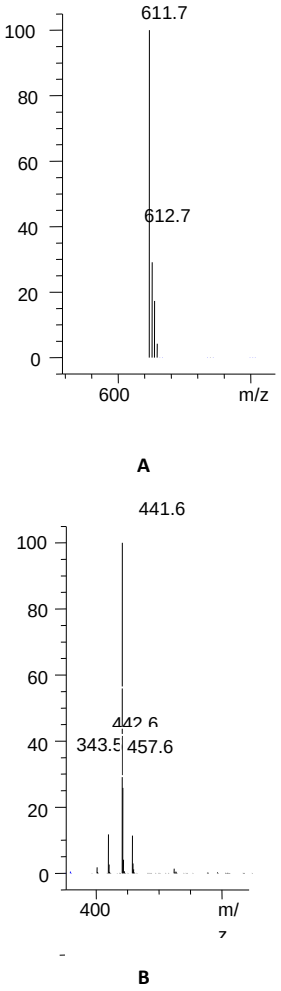

Figure 2. Mass spectra of control *Manidipine* and internal standard
A: *Manidipine*; B: internal standard

1.0 mL blank plasma was added in each glass tube which contained *Manidipine* at concentrations of 0, 0.25, 0.5, 1, 5, 10, 25 and 50 ng·mL^{-1} after dried with Nitrogen, purified, injected and analyzed. The regressive equation was as follows: Y=0.07481+20.29X, r= 0.99744, the limit of quantity (LOQ) is 0.25 ng·mL^{-1}.

0.5, 5 and 25 ng·mL^{-1} of *Manidipine* were spiked in blank plasma and analyzed at above conditions. The recovery rate, intra-day and inter-day RSD were calculated (Table1). After the samples which contained *Manidipine* at concentrations of 0.5, 5 and 25 ng·mL^{-1} were stored at -20℃ for 24 hours and 7 days, evaluated the affection of frost thawing and period of storage upon stableility of *Manidipine* (Table 2)

Table 1. Recovery rate, intra-day and inter-day relative standard deviation (RSD) of *Manidipine*(n=5)

Concentration /ng·ml-1	Recovery rate /%	Intra-day RSD /%	Inter-day RSD /%
0.5	49.92	4.15	3.08
5	49.85	3.21	7.76
25	50.50	7.44	2.41

Table 2. the data of sTableility test ($\bar{x}$ ±SD, n=5)

Concentration /ng.ml-1	Before frost thawing /ng.ml-1	frost thawing once /ng.ml-1	frost thawing twice /ng.ml-1
0.5	0.49±0.02	0.49±0.04	0.50±0.03
5	4.88±0.25	5.28±0.18	5.17±0.28
25	26.22±0.97	25.92±1.55	26.90±1.66

2.5. Subject and design

Twelve healthy volunteers were participated in this study after physical examination and laboratory screening. They were asked to avoid all prescription for at least 10 days before the study. Those who had a history of drug or alcohol abuse or allergy to the components of *Manidpine* Tablelets or capsules and those who had concomitant drug therapy were excluded. All subjects gave their written informed consent at the beginning of the study and being explained the nature of the drug and purpose of this study (Lifen et al., 2011).

A single dose of 10mg *Manidipine* normal Tablelet were given at am 7:00 after having a standard meal and 5mL blood samples were obtained before and 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0h after the administration of *Manidipine* normal Tablelet preparations. The blood samples were centrifuged and plasma were collected and stored at -20℃ for analysis (Lixin et al., 2014). After one-week washout period, a single dose of 20mg were given and experimentation was repeated.

The third week, each subject was orally taken 10mg *Manidipine* Tablelet at am 7:00 after having a standard meal for 7 days. Blood sample were collected from the 5th to 7th day before administration, and the 7th day after administration as same as those with single dose.

3. Results and discussions

3.1. Plasma concentrations of *Manidipine* in each group

The average plasma concentrations of manidipine after a sing and multiple doses administration were show in Table 3. The time-concentrations curves were show in Fig 3 and Fig 4.

Table 3. Mean plasma concentrations of *Manidipine* after a sing doses (10mg and 20mg) and multiple doses administration ($\bar{x}$ ±SD, n=12, ng·mL^{-1})

Time/h	Single dose of 10mg	Single dose of 20mg	multiple doses of 10mg
-48	-	-	0.358±0.137
-24	-	-	0.348±0.068
0	-	-	0.374±0.133
0.25	0.425±0.432	1.002±1.391	1.095±0.509
0.5	2.009±3.052	3.245±3.433	2.193±1.063
1.0	4.435±4.624	8.384±6.171	3.720±1.587
1.5	6.163±4.189	13.162±8.166	5.621±2.785
2.0	6.079±3.443	10.464±4.356	6.836±4.732
2.5	4.464±2.627	7.056±3.341	6.223±4.107
3. 0	3.185±1.706	5.276±2.394	4.430±2.702
4.0	2.232±0.988	3.407±1.552	3.301±1.839
6.0	1.547±0.508	2.220±0.794	2.153±0.820
8.0	0.965±0.378	1.430±0.437	1.339±0.469
12.0	0.554±0.146	0.722±0.153	0.805±0.259
24.0	0.311±0.068	0.368±0.087	0.335±0.095

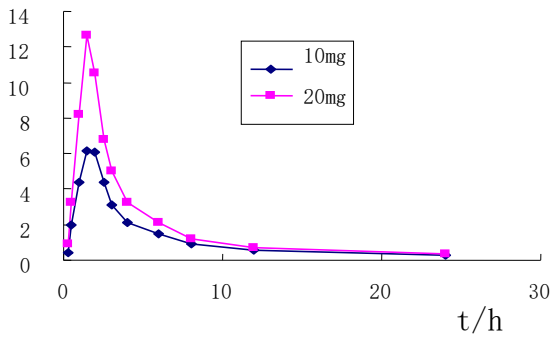

Figure 3. Mean time-concentration curves after a single dose of Manidipine

3.2. Pharmacokinetic parameters of manidipine in each group

The mean pharmacokinetic parameters of *Manidipine* in each group after a single and multiple doses administration were shown in Table 4 and Table 5.

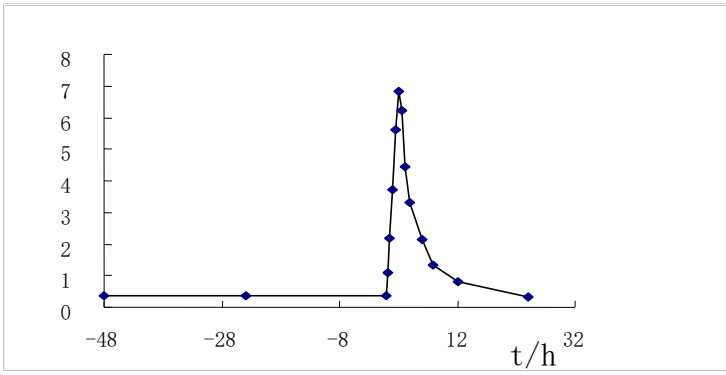

Figure 4. Mean time-concentration curves after multiple doses of Manidipine

Table 4. Pharmacokinetic parameters after a single dose of *Manidipine* ($\bar{x}$ ±SD, n=12)

parameters	10mg	20mg
$t_{1/2}$/h	6.67±2.83	6.89±2.66
C_{max}/ng·mL^{-1}	7.22±4.42	14.26±7.54
T_{max}/h	1.67±0.33	1.71±0.26
AUC_{0-24}/ng·mL^{-1}· h	29.42±12.92	46.69±18.19
$AUC_{0-\infty}$/ng·mL^{-1}·h	31.77±12.55	49.96±17.74

Table 5. Pharmacokinetic parameters after multiple doses of *Manidipine*($\bar{x}$±SD, n=12)

parameters	Coax/ng·mL^{-1}	Cmin/ng·mL^{-1}	Cav/ng·mL^{-1}
10mg	7.88±4.36	0.37±0.13	1.56±0.64
parameters	DF/%	AUCssng·h·mLl^{-1}	
10mg	4.60±0.88	37.39±15.34	

4. Conclusions

Manidipine is unsTablele in plasma or exposed in light. The blood concentrations of Manidipine is only 0.25-25 ng·mL^{-1}. The routine HPLC-UV method can't meet the requirement of pharmacokinetic studies and therapeutically monitoring of *Manidipine* in human body (NguyenLan et al., 2016). HPLC-MS has dual functions of both separation and detection and a strong anti-interference ability. The separation was carried out with a mobile phase of a mixture of 5mM·L^{-1} ammonium acetate（0.5%acetic acid）-methanol- methyl cyanide ((15 : 40 : 45, V/V/V) at a flow rate of 0.6ml·min-1 and a sTablele phase of A Venusil XBP-C_8 column (150mm×4.6mm, 5μm), 50μL of purified sample was injected. The ion mass spectral (m/z) of 611.4 (M+1) was selected to quantify *Manidipine* and 441.1 (M+Na) for *Nimodipine*. The period of analysis was only about 8 min. The determination of concentrations of *Manidipine* in human plasma by HPLC-MS method is sensitive and accurate (Tsukasa et al., 2006). In the process of experiment there was no interference of endogenous substances and there was no ion effect. The pharmacokinetic model of Manidipine belongs to two compartment model. So it can be used in the low limit detection for pharmacokinetic studies and therapeutically monitoring of *Manidipine* (Han and Fu Hong, 2006).

After taking *Manidipine* orally, the time to peak concentrations is about 1 to 2 hours, the half-life is about 6 hours, antihypertensive effect last 24 hours. It has a good peak/valley ratio of blood concentrations when taking *Manidipine* orally once a day (Francisco et al., 2011). When taking *Manidipine* orally after the meal, the peak concentration in plasma is 1.3 times and AUC is 1.6 times than those of an empty stomach, the time to peak concentration has no change (Saruta and Suzuki, 1999). Clearance in the body relate to the dose, AUC of taking 20 mg is 1.6 times than that of taking 10 mg (Zanchetti et al., 2001). *Manidipine* did not accumulate in plasma and the pharmacokinetic characteristics shows not significant difference between male and female volunteers.

Manidipine is new medicine chemicals with 3.1 classes. The test plan approved by the ethics committee and informed consent was signed before test.

5. References

Cheer, M., McclEllan, K.(2005). Manidipine, a review of its use in hypertension, *Drugs*, 61(12), 1777-1799.

Benchawan, C., Pornsak, S.(2016). Effect of cooling technique on physicochemical properties of ternary solid dispersion of manidipine hydrochloride prepared by melting method, *Asian Journal of Pharmaceutical Sciences*, 11 (1), 325-328.

Fogari, R., Ogari, R., Zoppi, A., Mugellni, A., et al.(1999). Effect of low dose manidipine on ambulatory blood pressure in very elderly hypertensives, *Cardiovasc Drugs Ther*, 13(3),243-248.

Francisco,J., Martinez, M., Macias-Batista, A., Cristina,C., Rodriguez-Rosas, H., Soriano-Perera, P., Pedrianes-Martin, P.,(2011). Effects of Manidipine and its Combination with an ACE Inhibitor on Insulin Sensitivity and MeTableolic, Inflammatory and Prothrombotic Markers in Hypertensive Patients with MeTableolic Syndrome, *Clinical Drug Investigation*, 31 (3), 201-212.

Han, J., Fu Hong, Y. (2006). Determination of rimantadine hydrochloride in compound rimantadine hydrochloride capsules by

capillary gas chromatography, *Sepu*, 23(6). 683.

Lifen, Z., Wangping, Z., Donghong, L., Xignqian,Y. et al.(2011). Research advance on the ultrasound assisted extraction of food functional components, *Journal of Chinese Institute of Food Science and Technology*, 11 (3), 128-132.

Lixin, C., Changhong, J., Chunle, Y.(2014). Study on the extraction technology of flavonoids from corn cob by response surface methodology, *Science and Technology of Food Industry*, 35(2), 259-263.

Naylere. G., Panagiotopoulos. S.,(2001). The antiatherosclerotic effect of the calcium antagonists and their implications in hypertension, *Am HeartJ*, 125(2 pt2),626-629.

NguyenLan, H., NguyenHuu, H., SungYong, H., JeWon, P.(2016). Determination of Manidipine in Human Plasma by HPLC-MS/MS and Its Application to A Bioequivalence Study, *Current Pharmaceutical Analysis*, 12 (2), 152-156.

Saruta, T., Suzuki, H.(1999). Efficacy of manidipine in the treatment of hypertensive with renal impairment, a muticenterial, *Am Heart J*, 125(2pt 2),630.

Tsukasa, U., Tadashi, O., Shigeru, M., Kazunobu, S.,(2006). Effect of grapefruit juice on the disposition of manidipine enantiomers in healthy subjects, *British Journal of Clinical Pharmacology*, 61 (5), 185-189.

Vítor, T., Maximiliano, S., Gustavo, K., Juliana, A., Nadia, V.,(2015). Delapril and Manidipine Main Degradation Products, LC-UV and LC-ESI-MS Evaluations, Decay Kinetic, and In Vitro Cytotoxicity Studies, *Journal of Liquid Chromatography & Related Technologies*, 38 (13), 1333-1342.

Zanchetti, A., Omboni, S., La Commare, P., De Cesaris, R., Palatini, P.,(2001). Efficacy, tolerability, and impact on quality of life of long-term treatment with manidipine or amlodipine in patients with essential hypertension, *Journal of Cardiovascular Pharmacology*, 38 (4), 642-50.

Acknowledgments

The work presented in this paper was supported by the Science and Technology Research Projects of Henan China (Grants No.152102310130) and the Scientific Research Fund Project of Henan Polytechnic college of China (Grants No. 2015-HZK-05).

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

EFFECT OF PHENOLIC COMPOUNDS ON ANTIOXIDANT ACTIVITY IN 8 BLUEBERRY (*VACCINUM SPP.*) JUICES

Lingli Zhang[1], Pengxiang Yue[1], Jun Jiang[1], Jisheng Fan[2] and Xueling Gao[1]*

[1]School of Tea & Food Science, Anhui Agricultural University, 130 West Changjiang Rd., Hefei 230000, China
[2]Anhui Huiwang Foodstuff Co., Ltd, 17 jiuhuashan Rd., Hefei 230000, China
*Correspondence author:*gaogao606@hotmail.com*

Article history:
Received:
25 April 2016
Accepted in revised form
25 May 2016

Keywords:
Blueberry juices;
Phenolic compounds;
antioxidant activity;
correlation analysis.

ABSTRACT

Phenolic compounds of juices from 8 blueberry cultivars, Baldwin, Gardenblue, Anna, O'Neal, Misty, Bluecrop, Elliott and Brigitta, were evaluated. Antioxidant capacity (by DPPH, FRAP, and reducing power assays) of 8 juices were also investigated. Eight blueberry juices contained phenolics levels of 597.23–1417.45 mg gallic acid equivalents/L $_{juice}$, flavonoids levels of 102.80–546.25 mg rutin equivalents/L $_{juice}$ and anthocyanins levels of 138.60–492.62 mg cyanidin-3-glucoside equivalents/L $_{juice}$, respectively. Among these juices, the juice from 'Gardenblue' cultivar possessed the highest content of phenolics, flavonoids, and anthocyanins. Variance analysis showed significant differences ($P < 0.05$) in phenolic compounds (phenolics, flavonoids, anthocyanins) and antioxidant activities among 8 blueberry juices. Correlation analyses revealed phenolics, flavonoids, and anthocyanins were distinctly responsible for antioxidant capacity. A strong correlations was found between flavonoids and antioxidant capacity of 8 cultivars (DPPH, $r = 0.95$; FRAP, $r = 0.97$; reducing power, $r = 0.89$).

1.Introduction

Blueberry (genus Vaccinium, family Ericaceae) originates from North America and Europe (Rimando et al., 2004). Blueberries not only contain essential nutrients but also possess abundant phenolic compounds, such as anthocyanins, flavonoids and chlorogenicacids (Prior et al., 1998; Manach et al., 2005; Giovanelli et al., 2013). The phenolic compounds can protect organisms against oxidative stress induced by free radicals (Manach et al., 2005) and exhibit a wide range of biological properties, including cardioprotective, anti-inflammatory, and anticarcinogenic properties (Nohynek et al., 2006; Tsuda, 2012).

Blueberry fruits are commonly consumed in fresh or processed food, such as juice, wine, jam and so on (Anna and Grzegorz, 2015; Nindo et al., 2005). Processed blueberry juice not only increases the berries' commercial life but also allows wider consumer access to this diet-enriching food. Blueberry juice with higher amounts of phenolic compounds and stronger antioxidant capacity has increased nutritional and health benefits for the consumer (Nindo et al., 2005; Rossi et al., 2003). However, processing method and blueberry cultivars vary greatly in their phenolic compound content as well as antioxidant capacity (Prior et al., 1998; Sapers et al., 1984; Rodrigues et al., 2011;

Wang et al., 2012a; Wang et al., 2012b; Xin et al., 2015). Previous researches mainly focused on phenolic compound content and antioxidant capacity of different varieties blueberry fruits (Giovanelli and Buratti (2009); You et al., 2011; Howard et al., 2003), only a few data concern the differences of bioactive compounds from bluberry juices.

Because of blueberry's unique nutrition, blueberry species have been successfully propagated and cultivated in China since 1989. With the increase in production, growers require additional options, and processing into blueberry juice is an effective way to maximize usage and markets. The aim of this work was to investigate the bioactive compositions of juices from 8 blueberry (*Vaccinium spp.*) cultivars and further study the effect of bioactive composition on antioxidant activity

2.Materials and methods

2.1.Preparation of blueberry juices

Blueberries of uniform size and at physiological maturity were handpicked in the morning from a commercial blueberry plantation located in Hefei (31˚52'N, 117˚ 17'E) in central-eastern China. Eight cultivars, namely Baldwin, Gardenblue, Anna, O'Neal, Misty, Bluecrop, Elliott, and Brigitta, were harvested from June 11, 2014 to August 15, 2014, respectively. All fruits were collected and stored at -20 ºC until used.

Frozen blueberries were thawed and crushed into mashes, respectively. The mash was macerated with pectinase (Laffort, Sydney, Austrilia) at a concentration of 0.07 g/kg $_{fruit}$ for 2 h at room temperature, then squeezed with 200 mesh of silk cloth and centrifuged at 3100 × g for 15 min to obtain blueberry juice.

2.2.Chemicals

Folin–Ciocalteu reagent and gallic acid were obtained from Sinopharm Chemical Reagent (Shanghai, China). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 4, 6-Tris (2-pyridyl)-1, 3, 5-triazine (TPTZ) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Other general chemicals with analytical grade were obtained from local suppliers.

2.3.Chemical analyses

Total soluble solids (expressed as °Brix) were measured on a digital refractometer (TD-45, TOP Instrument Co., Ltd., Zhejiang, China) at 25± 1℃ and results were expressed in °Brix. The titratable acidity and total sugars were also determined AOAC methods (1980).

2.4.Total phenolic content (TPC)

The TPC was determined according to Folin–Ciocalteu method (Sanchez-Patan et al., 2015). The absorbance of the sample was determined at 765 nm. The results were expressed as mg gallic acid equivalents (GAE) per one liter of juice (mg GAE/L $_{juice}$).

2.5.Total flavonoid content (TFC)

The TFC were measured using a colorimetric assay adapted from Mahmood et al. (2012).The absorbance was measured at 510 nm. The results were expressed as mg rutin equivalent (RE) per one liter of juice (mg RE/L $_{juice}$).

2.6.Total anthocyanin content (TAC)

The TAC was estimated using the pH differential method. Aliquots of each sample were diluted with pH 1.0 or pH 4.5 buffers to the same dilution. The absorbance was measured at 510 nm and 700 nm in both pH 1.0 and pH 4.5 buffers. The TAC was calculated by using Eq. (1).

$$TAC = \frac{(A \times MW \times DF \times V_e \times 1000)}{\varepsilon \times 1 \times M} \quad (1)$$

Where *A* is the difference in absorbance between pH 1.0 and 4.5, MW is the molecular weight of cyanidin-3-glucoside (449 g/mol); *DF* is the dilution factor; *Ve* is the extract volume, ε is the molar extinction coefficient of cyanidin-3-glucoside (29,600), and *M* is the mass of the blueberry extracted.

The results were expressed as mg cyanidin-3-glucoside (C3G) equivalents per one liter of juice (mg C3G/L $_{juice}$).

2.7.Free radical scavenging capacity (DPPH)

The DPPH free radical-scavenging capacity was estimated by adding 2.95 microliters of 0.1 mM DPPH methanolic solution to 50 μL of the sample extracts. The solution was thoroughly mixed and in dark for 30 min. The absorbance was measured at 517 nm. The results were expressed in mg VC equivalent antioxidative capacity per one liter of juice (mg VC/L $_{juice}$).

2.8. Ferric reducing antioxidant power (FRAP) assay

FRAP reagent was prepared fresh daily in acetate buffer (adjusted pH to 3.6 by acetic acid) by mixing TPTZ solution (10 mM in 40 mM HCl) and 20 mM iron chloride solution in the proportion of 10:1:1, respectively. Each sample (90 μL) was mixed with 3.0 mL of the FRAP reagent and incubated for 10 min at 37 °C. The absorbance was read at 593 nm. The results were expressed as mmol ferrous ion per one liter of juice (mmol Fe^{2+}/ L $_{juice}$).

2.9. Reducing power assay (RP)

The RP was determined by adding diluted samples (1 mL) into phosphate buffer (2.5 mL 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated (50 °C, 20 min). Five mL of 10 % trichloroacetic acid was added to the mixture before centrifugation for 10 min at 2400 × g. A 2.5 mL aliquot of the supernatant was mixed with ultrapure water (2.5 mL) and 0.5 mL of 0.1% $FeCl_3$. The absorbance was read at 700 nm after standing for 2 min; the final result was expressed as mg VC equivalent per one liter of juice (mg VC/L $_{juice}$).

2.10.Statistical analysis

Data were expressed as the means ± the standard deviation (SD) of triplicate determinations. Mean differences were determined by one-way ANOVA followed by Duncan's range test using Prism™ v6.0 software. The differences were considered significant when $P < 0.05$ and are denoted by different letters. Linear regression plots were generated and correlations between antioxidant activities and phenolic, flavonoid and anthocyanin contents were computed as Pearson's correlation coefficient (*r*) using Prism™ v6.0 software.

3.Results and discussions

3.1.Quality attribute of blueberry juices

The quality and acceptability of juice are related to its total soluble solids (TSS) content, acidity and ratio of total soluble solids to acidity. Six quality attributes of juices from 8 common blueberry cultivars, namely pH, titratable acidity (TA), total soluble solids (TSS), total sugars (TS), and the ratio of total soluble solids / titratable acidity (TSS/TA), were measured (Table 1).

As shown in Table 1, eight blueberry juices contained TSS levels of 10.1 °Brix (Bluecrop)–14.8 °Brix (Anna), TA levels of 2.45 (Anna)–18.58 g/L $_{juice}$ (Elliott) and TSS/TA levels of 0.66 (Elliott)–6.04(Anna),

respectively (Table 1). TS varied from 86.33 (Bluecrop) to 137.66 g/ L $_{juice}$ (Anna). The pH values ranged from 2.59 (Elliott) to 3.60 (Anna).

Table 1. Titratable acidity (TA), pH, total soluble solids (TSS), total sugars (TS), and the ratio of total soluble solids / titratable acidity (TSS/TA) in the juices pressed from eight blueberry cultivars of *Vaccinium* species

Cultivars	Quality attributes				
	pH	TA (g/L $_{juice}$)	TSS (°Brix)	TS (g/L $_{juice}$)	TSS/TA
Anna	3.60±0.06^{h}	2.45±0.12^{a}	14.8±0.2^{e}	137.66±0.43^{g}	6.04
O'Neal	3.46±0.13^{gh}	3.61±0.18^{b}	10.8±0.4^{ab}	107.14±0.18^{c}	2.99
Misty	3.07±0.12^{ef}	6.72±0.31^{cd}	10.7±0.5^{ab}	102.04±0.45^{b}	1.50
Bluecrop	2.74±0.10^{abc}	12.22±0.56^{e}	10.1±0.2^{a}	86.33±0.74^{a}	0.83
Elliott	2.59±0.12^{a}	18.58±0.21^{g}	12.3±0.3^{d}	117.56±0.29^{e}	0.66
Brigitta	2.60±0.13^{ab}	14.21±0.71^{f}	11.4±0.5^{bc}	107.14±0.35^{c}	0.80
Baldwin	2.87±0.13^{cd}	7.03±0.33^{d}	12.0±0.6^{cd}	117.10±0.33^{d}	1.71
Gardenblue	2.96±0.06^{de}	6.57±0.32^{cd}	14.6±0.3^{e}	136.36±0.87^{f}	2.22

Significance testing among the different samples was performed by one-way ANOVA followed by Duncan's range test. Different superscripts between rows represent significant differences between samples ($P < 0.05$).

Table 2. The phenolic content (TPC), flavonoid content (TFC), and anthocyanin content (TAC), the antioxidant capacity (DPPH, FRAP, and RP), in the juices pressed from eight blueberry cultivars of *Vaccinium* species.

Cultivars	Phenolics				Antioxidants		
	TPC (mg/L $_{juice}$)	TFC (mg/L $_{juice}$)	TAC (mg/L $_{juice}$)		DPPH (mg/L $_{juice}$)	FRAP (mmol/L $_{juice}$)	RP (mg/L $_{juice}$)
Anna	1180.21±6.88^{f}	199.75±1.23^{f}	138.60±0.12^{a}		49.72±0.31^{e}	20.41±0.70^{c}	6.37±0.08^{f}
O'Neal	647.87±4.12^{b}	131.72±0.27^{d}	269.27±2.08^{f}		34.74±1.62^{c}	12.13±0.16^{a}	3.74±0.05^{d}
Misty	852.55±6.89^{d}	267.28±0.97^{g}	197.88±1.02^{e}		44.24±0.90^{d}	20.21±0.75^{c}	5.49±0.06^{e}
Bluecrop	645.11±3.43^{b}	102.80±0.72^{a}	166.15±1.16^{b}		26.68±0.57^{b}	11.61±0.48^{a}	3.19±0.01^{c}
Elliott	738.72±5.74^{c}	113.16±0.89^{b}	193.90±3.79^{d}		24.04±0.12^{a}	12.06±0.15^{a}	2.72±0.04^{a}
Brigitta	597.23±5.02^{a}	138.23±1.02^{e}	180.77±2.33^{c}		26.48±1.17^{b}	11.72±0.57^{a}	2.92±0.04^{b}
Baldwin	975.96±6.82^{e}	115.28±0.67^{c}	330.64±0.83^{g}		33.22±0.45^{c}	15.48±0.56^{b}	2.73±0.03^{a}
Gardenblue	1417.45±10.11^{g}	546.25±2.37^{h}	492.62±3.33^{i}		76.59±1.49^{f}	33.97±0.23^{d}	8.02±0.10^{g}

Significance testing among the different samples was performed by one-way ANOVA followed by Duncan's range test. Different superscripts between rows represent significant differences between samples ($P < 0.05$).

Sweet taste positively correlated with TSS and TS, sour correlated with titratable acidity (TA), and negatively correlated with pH and TSS/TA ratio (Bett-Garber et al., 2015). 'Anna' was less acidic (TA: 2.45 g/L $_{juice}$) and sweeter taste (TSS:14.8%;TS:137.66g/L$_{juice}$), consequently, 'Anna' cultivar had more advantageous TSS/TA ratio (6.04). While 'Elliott' was more acidic with a TA value of 18.58 g/L $_{juice}$ and had lower TSS/TA ratio (0.66). Sapers et al. (1984) also noted that 'Elliott' had the higher TA and lower TSS/TA of 11 highbush cultivars.

3.2.Phenolic compounds and antioxidant capacity

Phenolic compounds (phenolics, flavonoids, anthocyanins) and antioxidant capacity were shown in Table 2. Eight blueberry juices contained total phenolic content (TPC) of 597.23–1417.45 mg GAE /L $_{juice}$, total flavonoid content (TFC) of 102.80–546.25 mg RE/L $_{juice}$ and total anthocyanin content (TAC) of 138.60–492.62 mg C3G/L $_{juice}$, respectively (Table 2). TPC in 8 blueberry juices varies widely. TFC and TAC had significant differences ($P < 0.05$) among 8 cultivars. 'Gardenblue' possessed all the highest content of TPC, TFC and TAC in these cultivars.The free radical scavenging capacity (DPPH), ferric reducing antioxidant power (FRAP) and reducing power (RP) were also investigated. DPPH, FRAP and RP were 24.04–76.59 mg VC/L $_{juice}$, 11.61–33.97 mmol Fe^{2+}/L $_{juice}$ and 2.72–8.02 mg VC/L $_{juice}$, respectively (Table 2). There were obvious differences in DPPH among 8 cultivars (P <0.05). 'Gardenblue' displayed the highest scavenging effect (76.59 mg VC/L $_{juice}$) in these cultivars, followed by 'Anna' and 'Misty', respectively. Similar to the DPPH value, the highest FRAP (33.97 mmol Fe^{2+}/L) and the highest RP (8.02 mg/L) were found in 'Gardenblue' cultivar, followed by the 'Anna' and 'Misty' cultivars. Jessica *et al.* (2013) research showed that anthocyanin contents of blueberry fruits were primarily influenced by cultivar. Connor *et al.* (2002) and Rodrigues *et al.* (2011) reported total phenolic and anthocyanin contents presented significant differences among blueberry cultivars. Lee *et al. (*2004) and Gunduz *et al.,* (2015) stated the variability in total phenolic content, total anthocyanin content and antioxidant activity in various *Vaccinium* species. Wang *et al.* (2012b) found considerable variation was in flavonoid content and antioxidant activity among 42 blueberry (*Vaccinium spp.*) cultivars. Cultivars play a more important role in influencing total phenolics, total anthocyanins, total flavonols and oxygen radical-absorbing capacity in blueberries (Howard *et al.,* 2003). Koca and Karadeniz (2009) also reported that FRAP values varied from 7.41 to 57.92 μmol/g for six lowbush and four highbush blueberry fruits. It has been argued that the antioxidant activity of a product cannot be reasonably validated by a single method due to the complex nature of phytochemicals and their interactions, so it was important to use multiple assay systems measuring different indices (Pérez-Jiménez *et al.,* 2008).

3.3.Correlations between phenolic compounds and antioxidant activity in blueberries

Blueberry phenolics were reported as constituents, which are responsible for their high radical scavenging capacity (Giovanelli *et al.,* 2009; Wang *et al.,* 2008). In order to evaulate the antioxidant potential of 8 blueberry juices in terms of their polyphenol contents, linear regression plots were generated and the Pearson correlation coefficients were calculated (Figure 1).A very good correlation was noted between the RP values and the TPC (r = 0.84). Nevertheless, DPPH assay and FRAP assay showed striking correlations with TPC (DPPH, r = 0.90; FRAP, r = 0.92). Ramful *et al.* (2011) reported that TPC of the pulp extracts also correlated strongly with the antioxidant activities using the FRAP assays. TFC showed strong correlations with DPPH values (r = 0.95), with FRAP values (r = 0.97) and with RP values (r = 0.89). Considerable variation was found in flavonoid content, antioxidant activity, and their contribution to total antioxidant activity among 42 blueberry cultivars reported by Wang *et al.* (2012b).

A good correlation was obtained between FRAP assay values and TAC (r = 0.71). However, a moderate correlation was noted between TAC and antioxidant capacity of the cultivars (DPPH, r = 0.69; RP, r = 0.46).

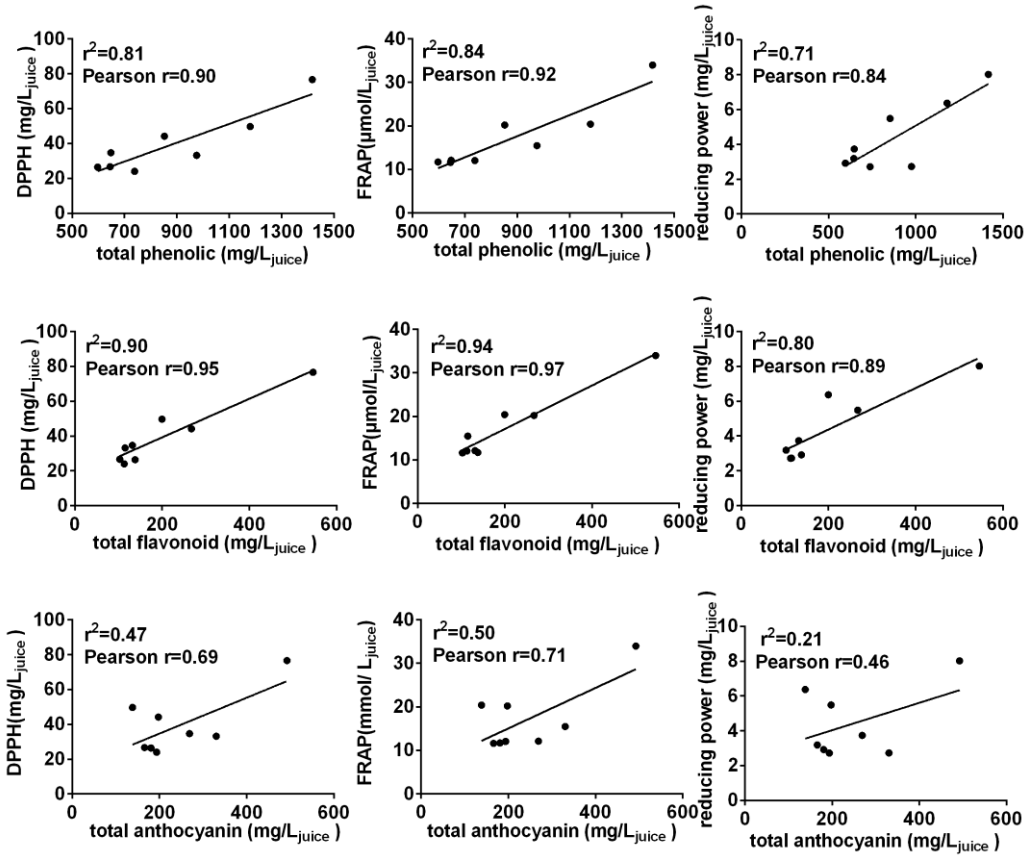

Figure 1. Linear regression plots and Pearson 's correlation coefficients of DPPH, FRAP, and reducing power values with respect to phenolics, flavonoids, and anthocyanins, of the juices pressed from eight blueberry cultivars of *Vaccinium* species

In our work, total phenolic and flavonoid had higher antioxidant potential than total anthocyanin (Figure 1). Vanessa *et al.* (2014) found the total flavonoid content was highly and positively correlated to the total monomeric anthocyanin content in berry fruits. Castrejon *et al.* (2008) reported anthocyanins in mature blueberries have lower antioxidant potential than other phenolic compounds. Giovanelli and Buratti (2009) pointed out the antioxidant activity was more related to the total phenolic rather than to the anthocyanin. The same type of linear correlation between antioxidant activities and phenolic contents had been found in fruit juices (Gardner *et al.*, 2000).

4.Conclusions

The present study demonstrated that the juices from 8 blueberry cultivars possessed significantly different phenolic contents and antioxidant activities. Correlation analyses revealed that the phenolics and flavonoids were distinctly responsible for the antioxidant capacity, and total flavonoids showed strong correlations with DPPH, FRAP and reducing power assays (DPPH, r = 0.95; FRAP, r = 0.97; reducing power r = 0.89). Among 8

blueberry juices, the cultivar 'Gardenblue' possessed the highest content of phenolic compounds (phenolics, flavonoids, and anthocyanins), corresponding to the highest value of antioxidant activity (DPPH, FRAP and Reducing power).

5.References

Anna, M., and Grzegorz, L. (2015). Bioactive Compounds of Blueberries: Post-Harvest Factors Influencing the Nutritional Value of Products. *International journal of molecular sciences*, 16 (8), 18642-18663.

Association of Official Analytical Chemists (1980). Official methods of analysis of the Association of Official Analytical Chemists (13th ed.) Washington, DC.

Bett-Garber, K.L., Lea, J.M., Watson, M.A., Grimm, C.C., Lloyd, S.W., Beaulieu, J.C., Stein-Chisholm, R.E., Andrzejewski, B.P., and Marshall, D.A. (2015). Flavor of Fresh Blueberry Juice and the Comparison to Amount of Sugars, Acids, Anthocyanidins, and Physicochemical Measurements. *Journal of Food Science,* 80 (4), S818-S827.

Castrejon, A.D.R., Elchholz, I., Rohn, S., Kroh, L.W., and Huyskens-Keil, S. (2008) .Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum L.*) during fruit maturation and ripening. *Food Chemistry.*, 109 (3), 564-572.

Connor, A.M., Luby, J.J., Tong, C.B.S., Finn, C.E., and Hancock, J.F. (2002). Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. *Journal of the American Society for Horticultural Science*, 127 (1), 89–97.

Gardner, P.T., White, T.A.C., McPhail, D.B., and Duthie,G.G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry,* 68 (4), 471-474.

Giovanelli, G., and Buratti, S. (2009). Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. *Food Chemistry,* 112 (4), 903-908.

Giovanelli, G., Brambilla, A., and Sinelli, N. (2013). Effects of osmo-air dehydration treatments on chemical, antioxidant and morphological characteristics of blueberries. *LWT – Food Science and Technology,* 54 (2), 577-584.

Gunduz, K., Serce, S., and Hancock, J.F. (2015). Variation among highbush and rabbiteye cultivars of blueberry for fruit quality and phytochemical characteristics. *Journal of Food Composition and Analysis*, 38, 69-79.

Howard, L.R., Clark, J.R., and Brownmiller, C. (2003). Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *Journal of the Science of Food and Agriculture*, 83 (12), 1238–1247.

Jessica, S., David, S., and Duncan, H. (2013). Blueberry estimated harvest from seven new cultivars: Fruit and anthocyanins. *Food Chemistry,* 139 (1-4), 44–50.

Koca, I., and Karadeniz, B. (2009). Antioxidant properties of blackberry and blueberry fruits grown in the Black Sea Region of Turkey. *Scientia Horticulturae*, 121 (4), 447-450.

Lee, J., Finn, C.E., and Wrolstad, R.E. (2004b). Comparison of anthocyanin pigment and other phenolic compounds of Vaccinium membranaceum and Vaccinium ovatum native to the Pacific Northwest of North America. *Journal of Agricultural and Food Chemistry,* 52, 7039–7044.

Mahmood, T., Anwar, F., Abbas, M., and Saari, N. (2012). Effect of Maturity on Phenolics (Phenolic Acids and Flavonoids) Profile of Strawberry Cultivars and Mulberry Species from Pakistan. *International Journal of Molecular Sciences*, 13 (4), 4591-4607.

Manach, C., Williamson, G., Morand, C., Scalbert, A., and Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition*, 81(1), 230S-242S.

Nindo, C. I., Tang, J., Powers, J. R., and Singh, P. (2005). Viscosity of blueberry and raspberry juices for processing applications. *Journal of Food Engineering*, 69 (3), 343–350.

Nohynek, L. J., Alakomi, H. L., Kahkonen, M. P., Heinonen, M., Helander, K.M., Oksman-Caldentey, K.M., and Puupponen-Pimia, R.H. (2006). Berry phenolics: Antimicrobial properties and mechanisms of action against severe human pathogens. *Nutrition and Cancer*, 54 (1), 18-32.

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz- Rubio, M., Serrano, J., Goñi, I., and Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food research international*, 41 (3), 274-285.

Prior, R.L., Cao, G.H., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., and Krewer, G. (1998). Antioxidant Capacity As Influenced by Total Phenolic and Anthocyanin Content, Maturity, and Variety of *Vaccinium* Species. *Journalof Agriculturaland Food Chemistry*, 46 (7), 2686-2693.

Ramful, D., Tarnus, E., Aruoma, O.I., Bourdon, E., and Bahorun, T. (2011). Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. *Food research international*, 44 (7), 2088-2099.

Rimando, A.M., Kalt, W., Magee, J.B., Dewey, J., and Balling, J.R. (2004). Resveratrol, pterostilbene, and piceatannol in vaccinium berries. *Journal of Agricultural and Food Chemistry*, 52 (15), 4713-4719.

Rodrigues, E., Poerner, N., Rockenbach, I.I., Gonzaga, L.V., Mendes, C.R., and Fett, R. (2011). Phenolic compounds and antioxidant activity of blueberry cultivars grown in Brazil. *Ciencia e Tecnologia de Alimentos*, 31 (4), 911-917.

Rossi, M., Giussani, E., Morelli, R., Lo Scalzo, R., Nani, R. C., and Torreggiani, D. (2003). Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice. *Food research international*, 36 (9-10), 999–1005.

Sanchez-Patan, F., Barroso, E., van de Wiele, T., Jimenez-Giron, A., Martin-Alvarez, P.J., Moreno-Arribas, M.V., Martinez-Cuesta, M.C., Pelaez, C., Requena, T., and Bartolome, B. (2015) .Comparative in vitro fermentations of cranberry and grape seed polyphenols with colonic microbiota. *Food Chemistry*, 183, 273-282.

Sapers, G.M., Burgher, A.M., Phillips, J.G., Jones, S.B., and Stone, E.G. (1984). Color and composition of highbush blueberry cultivars. Journal of the American Society for Horticultural Science, 109 (1), 105–111.

Tsuda, T. (2012). Dietary anthocyanin-rich plants: Biochemical basis and recent progress in health benefits studies.

Molecular Nutrition and Food Research, 56 (1), 159-170.

Vanessa, R.D.S., Patrícia, A.P.P., Thais, L.T.D.S., Luiz, C.d.O.L., and Rafael, P.F.Q. (2014). Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chemistry*, 156, 362–368.

Wang, S.Y., Chen, C.T., Sciarappa, W., Wang, C.Y., and Camp, M.J. (2008). Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. *Journal of Agricultural and Food Chemistry*, 56 (14), 5788–5794.

Wang, S.Y., Chen, H.J., Camp, M.J., and Ehlenfeldt, M.K. (2012a). Genotype and growing season influence blueberry antioxidant capacity and other quality attributes. *International Journal of Food Science and Technology*, 47 (7), 1540-1549.

Wang, S.Y., Chen, H.J., Camp, M.J., and Ehlenfeldt, M.K. (2012b). Flavonoid constituents and their contribution to antioxidant activity in cultivars and hybrids of rabbiteye blueberry (*Vaccinium ashei Reade*). *Food Chemistry*, 132 (2), 855-864.

Xin, Y., Zhang, M., Xu, B.G., Adhikari, B., and Sun, J.C. (2015). Research trends in selected blanching pretreatments and quick freezing technologies as applied in fruits and vegetables: A review. *International Journal of Refrigeration*, 57, 11-25.

You, Q., Wang, B., Chen, F., Huang, Z., Wang, X., and Luo, P. G. (2011). Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars. *Food Chemistry*, 125 (1), 201–208.

Acknowledgements

This work was supported by the Anhui Province Natural Science Foundation (Grant No. 1508085SMC217) and Anhui Province District Key Project (15czz03101).

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

PHYSICAL AND MECHANICAL PROPERTIES AND QUALITY INDICATOR OF WHEAT

Kateryna Kostetska[1*], Yana Yevchuk[1]

[1]Uman National University of Horticulture, Uman city, Ukraine;
*Corresponding author:*katarin182@mail.ru*

Article history:
Received:
06 March 2016
Accepted in revised form:
31 May 2016

Keywords:
Grain;
Wheat;
Variety;
Physical and mechanical properties;
Quality

ABSTRACT

The study was conducted during 2011–2015 in the Department of technology of storage and grain processing of Uman National University of Horticulture and on the production complex farm "Prolisok +" in Graniv village, Haysyn ditrict, Vinnytsia region.The aim of the research is to study the physical and mechanical properties and quality of wheat grain depending on weather conditions and properties of the variety.Studies of eligibility of certain varieties of grain for use in the processing industry is new.Wheat grain of Podolyanka, Trizo, Lazurna and Midas varieties has marked peculiarities of type and variety, meets the requirements in terms of external geometric parameters, volume, area of the outer surface, sphericity, specific and volume weight, volume of surface layers of grains and mass fraction of endosperm starch, indicating its suitability for processing. There was a tendency of changes in the geometric characteristics of the grain of the varieties studied under the influence of weather conditions of the year of study. Significant difference in physical indicators of grains of different growing years was recorded in the wheat grain of Trizo variety in terms of length, width, volume, area of the outer surface, specific surface area and volume of surface layers of the grains; Midas – volume, external surface area, specific surface; Lazurna – sphericity. Technological properties of wheat grain are high enough

1. Introduction

1.1. Setting of the problem

Indicators of properties of grain can be divided into two groups: properties peculiar to grain of the crop, as well as properties that vary within the same crop. The technical process of grain processing should be improved towards obtaining maximum endosperm, increasing product yield of highest grades and improving their quality (Kazakov et al., 2005; Merko, 2001; Savchuk et al., 2005).

Studies of eligibility of certain varieties of grain for use in the processing industry is new. In addition, there are no recommendations for triticale grain production for the moment. Eligibility of grain for industry is characterized by its quality as a raw material for recycling.

1.2. Analysis of recent studies and publications

Wheat is the most important food crop. It contains all necessary elements of food: proteins, carbohydrates, fats, vitamins, enzymes and minerals. There is good reason that wheat is the staple food in 43 countries with a population of over 1 billion people (Likhochvor, 2004; Nettevich et al. 1990; Osokina et al., 2016).

For grain, as a raw material for processing, its biometric characteristics, size and

uniformity of grain mass have the main technological importance (Osokina et al., 2016).

The shape and linear grain size influence the choice of sieves or separators as well as the characteristics of shelling machines. In addition, the geometric characteristics of the grain determines its density when forming the layer and peculiarities of moving grain while transportation. Different from the average, values of grain shape affect the porosity, the angle of repose and the angle of friction. The larger geometric size of grain is, the greater the angle of slope is, which has a positive effect on gravity feed of grains during transportation by gravity pipes. Because of the complexity of the processes, many cereal and flour mills are characterized by a significant extent of processing grain products, which reaches a few kilometres of machines and different mechanisms for average powered plants (Gortinskyi et al., 1989; Osokina et al., 2016; Ostapchuk et al., 2005; Zverev, 2007).

That is why the study of physical and mechanical properties of grain has not only theoretical but also practical meaning. Given that these properties vary considerably depending on weather conditions, growing technologies and features of varieties, it requires thorough study. In addition, physical and mechanical characteristics of triticale grain have not been studied enough and thus it determines the relevance of the study.

2. Materials and methods

2.1. The aim of the research is to study the physical and mechanical properties and quality of wheat grain depending on weather conditions and properties of the variety.

2.2. Research Methodology

Wheat grain of Podolyanka, Trizo and Lazurna varieties were grown on the experimental field of educational research and production department of Uman National University of Horticulture, while wheat of Midas variety was grown in the experimental field of the farm "Prolisok +" in Graniv village, Haysyn district of Vinnitsa region.

The study was conducted during 2011–2015 in the Department of Technology of storage and grain processing of Uman National University of Horticulture and on the production complex farm "Prolisok +" in Graniv village, Haysyn ditrict, Vinnytsia region.

Linear dimensions were measured for the grain of wheat by the method described by G.A. Egorov (Egorov, 2000).

Grains volume (V) and an external surface area (F) were calculated by the formulas:

$$V = k \bullet a \bullet b \bullet \ell, \ mm^3 \qquad (1)$$

where – a, b, ℓ are width, thickness and length of grain;

k – research coefficient (for wheat grain k=0,52).

$$F = 1{,}12 \times a^2 + 3{,}76 \times b^2 + 0{,}88 \times \ell^2, \ mm^2 \qquad (2)$$

Peculiarity of grain form is evaluated by its sphericity, which is the ratio of external surface area equivalent grain bullet (F_{sh}) for up to actual grain area (F):

$$\Psi = \frac{F_{sh}}{F}, \qquad (3)$$

Thus: $F_{sh} = 4 \times \pi \times r^2$; $r = 0{,}62 \times \sqrt[3]{V}$

Specific surface of grain was set by the ratio of the area of the outer surface (F) to the volume of grains (V):

$$F/V \qquad (4)$$

The volume of surface layers ($V_{s.l.}$) of grain was determined by the formula:

$$V_{s.l.} = F \times G, \ mm^3 \qquad (5)$$

where G – the thickness of tissue (for wheat grain 0,065 mkm).

Mass fraction of starchy endosperm was calculated by the formula:

$$m_e = \frac{V-V_{s.l.}}{V} \times 100 - m_z, \% \quad (6)$$

where m_z is mass of a bud (for wheat grain $m_z = 2,5\%$).

Specific gravity (density) of grain was determined by the formula:

$$\rho = m/V, \quad (7)$$

where m – mass of grain, g/cm^3.

(Egorov, 2000; Gortinskyi et al., 1989; Kazakov et al., 2005; Osokina et al., 2016; Ostapchuk et al., 2005; Zverev, 2007).

To determine the quality of the grain standard methods were used: sampling [GOST 13586.3–83; GOST 24104–88]; determination of the color and smell [GOST 10967–75]; contamination [GOST 13586.6–93; GOST 13586.4–83]; debris [GOST 30483–97]; humidity [GOST 13586.5–93]; nature (bulk density) [GOST 10840–64]; 1000 grain weight [GOST 10842–89]; glasslike structure [GOST 10987–76].

3. Results and discussions

The geometric characteristics of the grain determine its density when forming layer (porosity) and features of the moving grain during transportation. Because of the complexity of the processes cereal and flour mills are characterized by a significant extent of processing grain products, which reaches a few kilometres of machines and various mechanisms (pneumatic pipes, elevators, conveyors, etc.) for average powered plants (Gortinskyi et al., 1989; Osokina et al., 2016; Ostapchuk et al., 2005; Zverev, 2007).

10 average-sized grains of wheat were selected and their size was measured. According to the conducted measurements, indicators of geometric characteristics of the grain vary rather greatly.

To characterize the geometric features of grain, it is not enough only to specify linear dimensions. The value of volume, area, sphericity, specific surface of grains, specific and bulk density that play an important role in moisturizing, heating and cooling of the grain were determined by the average value of linear dimensions of wheat of varieties studied, as well as the volume of surface layers of the grains and mass fraction of endosperm starch which characterize a possible yield of grain and flour from such grain (table 1).

The obtained values of physical and mechanical indicators of wheat and triticale (table 1) are within the limits given in the sources of literature (Likhochvor, 2004; Nettevich et al. 1990; Osokina et al., 2016; Savchuk et al., 2005). However, the grains of wheat of Podolyanka, Midas, Trizo and Lazurna varieties have the thickness up to 7 % larger, and the length and width, respectively, 4–11 and 2–10 % lower than average. Grains of soft winter variety of Lazurna have the largest linear dimensions, grains of spring soft wheat of Trizo variety grown in 2014 have the smallest dimensions.

These characteristics affected the volume and area of the outer surface of the grain, values of which are lower than the average values according to the sources of literature 4–8 mm^3 respectively and 4–12 mm^2 for wheat of Podolyanka, Trizo, Midas varieties, whereas for the grains of Lazurna varieties they are 0,8 and 0.3 % larger respectively. Value of sphericity of grains wheat – 0,58–0,63.

Specific surface of grains was determined by the ratio F/V. This indicator is extremely important in grain drying because it is responsible for the intensity of the heat exchange and moisture diffusion in the grain. The value of this indicator for wheat is 2,15–2,61 and exceed the average literature data for corresponding crops except wheat of Lazurna variety of 2014 (table 1).

Table 1. Physical and mechanical properties of wheat grain

Variety	Year	Size, mm			Grains volume, V, mm^3	Sphericity, φ	External surface area, F, mm^2	Specific surface of grain, F/V	Volume of surface layers, $V_{s.l.}$, mm^3	Mass fraction of starchy endosperm, m_e, %	Specific gravity (density), ρ, г/см^3	Bulk density, kg/dm^2
		length, ℓ	width, a	thickness, b								
Podolyanka	2011	6.6	3.7	3.1	38.8	0.62	89.4	2.30	5.81	82.5	1.33	0,78
	2012	6.8	3.8	3.1	40.8	0.62	93.0	2.28	6.05	82.7	1.34	0,78
	average	6.7	3.7	3.1	39.8	0.62	91.2	2.29	5.93	82.6	1.33	0,78
Trizo	2013	6.5	3.6	3.1	37.7	0.62	87.8	2.33	5.71	82.4	1.38	0,76
	2014	6.1	3.4	2.9	30.9	0.61	76.4	2.47	4.97	81.4	1.38	0,76
	2015	6.4	3.7	3.0	36.9	0.63	85.2	2.31	5.54	82.5	1.40	0,76
	average	6.3	3.6	3.0	35.2	0.62	83.1	2.36	5.41	82.1	1.39	0,76
Midas	2014	6.2	3.9	3.1	32.9	0.54	86.0	2.61	5.59	80.5	1.34	0,77
	2015	6.3	3.9	3.2	40.9	0.63	90.5	2.21	5.88	83.1	1.35	0,77
	average	6.2	3.9	3.1	36.9	0.58	88.2	2.39	5.74	81.8	1.34	0,77
Lazurna	2013	6.7	3.9	3.2	43.4	0.58	94.6	2.18	6.15	83.3	1.35	0,79
	2014	6.7	4.0	3.2	44.6	0.63	95.9	2.15	6.23	83.5	1.35	0,79
	average	6.7	3.9	3.2	44.0	0.61	95.2	2.16	6.19	83.4	1.35	0,79
According to literature sources*		4,8–8,0	1.6–4.0	1.5–3.3	12.0–54.9	0.36–0.68	58–115	-	3.77–7.48	77.0–85.0	1.33–153	0.73–0.84
		7,0	4.0	3.0	43.7	0.63	94.9	2.17	6.17	83.4	-	-
LSD 5%		*0,32*	*0.19*	*0.16*	*3.20*	*0.03*	*4.50*	*0.12*	*0.30*	*4.11*	*0.07*	*0.04*

Note. * – according to (Likhochvor, 2004; Nettevich et al. 1990; Osokina et al., 2016; Savchuk et al., 2005): above the line – the border; below the line – average.

It is obvious that with decreasing grain size decreases ratio value of volume and area of the outer surface; therefore, small grains should have a higher content of shells and smaller content of the endosperm.

Furthermore, cereals and flour are obtained by means of endosperm and coat, aleurone layer and embryo should be sent in by-products and waste. It is therefore important to have information about the content in the grain endosperm of the parties and the amount of surface layers of the grains to make a prediction about the possible yield of the product.

The largest mass fraction of starchy endosperm is defined in the grain of winter wheat of Lazurna variety – 83,4 %, and in the grain of other varieties studied it is 1,7–3,4 % less.

The volume of surface layers of grains of wheat during the years of study varied within 4,97–6,23 mm^3 (table 1). Among the varieties studied, grains of Lazurna variety had the highest figure and Trizo variety had the lowest figure (13 % less).

The highest value of bulk density was determined in the grain of wheat of Lazurna variety – 0,79 kg/dm^2.

Specific gravity (density) of the grain as a whole describes chemical composition, structure, fullness, hardness, strength, maturity of the grain and has a great impact on productive properties. Starch and minerals have the highest specific mass, therefore with the increase of their share density of grains increases, and, conversely, increased protein and lipid lower the density of grain. The value

of this index (table 1) for wheat is 1,33–1,40 g/cm^3, with the advantage of Trizo variety.

The quality of the finished product depends on the quality of raw materials. Study of grain quality showed that the samples have smell and taste typical for crops.

Technological properties of grain are a combination of features and indicators of its quality which characterize the state of grain in processing and production processes and affect the yield and quality of the product.

Table 2 present comparative characteristic of technological properties of wheat grain of the varieties studied.

Research results of studies of technological grain quality indicators (table 2) showed that wheat varieties studied meet the quality standards. Thus, moisture of wheat grains is 0,4–1,2 % subtolerance.

The total content of impurities is 0,8 % lower than minimum standards for the second-class grain of Trizo variety and Podolyanka, Lazurna is 0,7 % lower and the first-class grain of Midas variety is 0,4 % lower than minimum standards. Grain impurities in the wheat grain is 3,0 and 3,1 % on average which is 2,0 and 1,9 % less than minimum standards for the first-class wheat (table 2).

Table 2. Characteristics and quality standards of wheat

Indicator	Permissible limits (DSTU 3768:2010)*	The actual quality grade													*LSD 5%*
		Podolyanka			Trizo				Midas			Lazurna			
		year													
		2011	2012	average	2013	2014	2015	average	2014	2015	average	2013	2014	average	
Moisture. %	not more 14.0	12.9	12.5	12.7	12.9	12.6	13.0	12.8	12.8	13.2	13.0	13.7	13.5	13.6	*0.65*
Waste impurities. %:	not more 1.0/2.0	1.2	1.2	1.2	1.6	1.5	0.6	1.2	0.6	0.6	0.6	1.8	0.9	1.3	*0.05*
- mineral admixture	not more 0.3	-													-
Grain impurities. %	not more 5.0/8.0	3.7	2.2	3.0	3.1	2.8	3.0	3.0	3.0	3.0	3.0	3.1	3.1	3.1	*0.15*
Contamination by pests. units of live specimens	not allowed in addition to mite infestation level 1	not found													-
Nature. g/l	no less 760/740	780	780	780	765	760	764	763	770	775	773	790	790	790	*38.80*
Weight of 1000 grains. g	35–75**	51.6	54.3	52.9	52.0	42.6	51.2	48.6	44.1	55.2	49.6	58.6	60.2	59.4	*2.64*
Vitrescence. %	no less 50/40	32.0	37.0	34.5	42.0	42.0	44.0	42.7	44.0	50.0	47.0	45.0	45.0	45.0	*2.11*

Note. * – before the line – 1 class; after the line – 2 class; ** – according to literature sources (Likhochvor. 2004; Nettevich et al. 1990; Osokina et al.. 2016; Savchuk et al.. 2005).

Weight of 1000 grains of wheat of Lazurna variety was 59,4 g, which is more than in grains of Trizo, Midas and Podolyanka varieties by 18, 16 and 11 % respectively. The greatest value of nature is defined in the grain of wheat of Lazurna variety – 790 g/l, and in grain of other varieties studied – by 3–4 % less.

In the specimens studied no pests were found.With the increase of vitrescence of grain there is a higher protein content and better technological properties. Yield of cereals and flour from with high vitrescence is larger. Samples of the grain investigated had floury endosperm. Vitrescence of wheat grain – 32–50 %.

4. Conclusions

Thus, comparing the geometric parameters of wheat it was found that grain of Midas variety has the most rounded shape and grain of Lazurna variety has prevailing linear dimensions. It should be used while preparation of grain for processing as well as the selection of sieves, machines and speed of rotation of their working bodies.

There was a tendency of changes in the geometric characteristics of the grain of the varieties studied under the influence of weather conditions of the year of study. Significant difference in physical indicators of grains of different growing years was recorded in the wheat grain of Trizo variety in terms of length, width, volume, area of the outer surface, specific surface area and volume of surface layers of the grains; Midas – volume, external surface area, specific surface; Lazurna – sphericity.

Large linear dimensions are found in the wheat grain of Lazurna variety.

Wheat grain of Podolyanka, Trizo, Lazurna and Midas varieties has marked peculiarities of type and variety, meets the requirements in terms of external geometric parameters, volume, area of the outer surface, sphericity, specific and volume weight, volume of surface layers of grains and mass fraction of endosperm starch, indicating its suitability for processing.

Technological properties of wheat grain are high enough. Grain moisture, content of waste and grain impuritiess are within acceptable standards.

5. References

DSTU 3768:2010. (2010). Grain. Wheat. *Tts.*, 1–17.

Egorov, G. A. (2000). Managing technological properties of grain. *Voronezh: VSU*, 348.

Gortinskyi, V. V., Demskyi, A. B., Boryskin, M. A. (1989). Processes of separation on the grain processing enterprises. *M.: Kolos*, 304.

Kazakov, E. D., Gavrilenko, G. P. (2005). Biochemistry of grain and grain products (3 revised and expanded edition). *Grain economy. SPb.: GIORD*, 512.

Likhochvor, V. V. (2004). Plant-grower: train aid. *K.: Center of educational literature*, 816.

Merko, I. T., Morgun, V. A. (2001). Scientific bases of technology of storage and processing of grain. *Odesa*, 207.

Nettevich, E. D., et al. (1990). Breeding of spring wheat, barley, oats. *M.: Rossel'khozizdat*, 172.

Osokina, N. M., Kosteska, K. V. (2016). Technology assessment barley, wheat and triticale for production of groats. *Collection of scientific papers of Uman UNUH. Uman*, (88), 111–125.

Ostapchuk, M. V., Stankevich, G. M., Goncharuk, G. A. (2005). System methods for determining the characteristics of the grain mass. *Storage and processing of grain*, (11), 31–34.

Savchuk, N. T., Podpryatov, G. I., Skaletska, L. F. (2005). Chemical control crop production. *K.: Aristey*, 83.

Zverev, S. V. (2007). Physical properties of grain and products of its processing. *M.: DeLiprint.* 86–101

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

STUDY OF ANTIBACTERIAL EFFECTS OF *TEUCRIUM POLIUM* ESSENTIAL OIL ON *BACILLUS CEREUS* IN CULTURAL LABORATORY AND COMMERCIAL SOUP

Maryam Keykavousi[1], Babak Ghiassi Tarzi[2], Razzagh Mahmoudi*[3], Hossein Bakhoda[4] Ata Kabudari[5], Seyyede Faezeh Rahimi Pir Mahalleh[5]

[1] M.Sc, Food Science and Technology, Islamic Azad University, Varamin-Pishva Branch, Iran.
[2] College of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[3]Department of Food hygiene and safety, School of Public Health, Qazvin University of Medical Sciences, Qazvin, Iran.
[4]Islamic Azad University, Science and Research Branch, Iran
[5]Student of Veterinary medicine, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
**Corresponding authors: Razzagh Mahmoudi, Department of Food hygiene and safety, School of Public Health, Qazvin University of Medical Sciences, Qazvin, Iran.*
*Corresponding author:*r.mahmodi@yahoo.com*

Article history:
Received:
06 March 2016
Accepted in revised form:
31 May 2016

Keywords:
Teucrium polium;
Antibacterial effects;
Essential oil;
Bacillus cereus.

ABSTRACT
According to the increasing use of processed and semi-processed food products and the raise in the awareness about the disadvantages of food additives in the products, consumer preferences has increased in the matter of using natural food additives. Considering that the essential oils of medicinal plants had the attributes that increasing shelf life as the antibacterial effect, tending to use this essential oils in food products has increased. Teucrium polium is the one of this essential oils , which its antibacterial effectson Bacillus cereus in medium and industrial soupare examined in this study. The Obtained essential oil from Clevenger system is injected to GC-MS. In this study the commercial barley soup was used as a food model. Sensory evaluation caused by adding the essential oil from Teucrium polium to barley soup was evaluated using sensory acceptance test. The results of this study showed, the samples with higher concentrations of essential oils, had lower bacteria and samples related to farther days had higher bacteria growth. The effect of time on the regression was also significant ($p<0.05$). Acceptance of adding essential oil from Teucrium polium to barley soup decreased significantly ($p<0.01$). The results also confirmed significant and notable role of essential oil on reducing microbial load of food models and increase of the quality and the safety of the final products..

1. Introduction

With the increase of urban population, tourism, immigration, a variety of food with different components, improve technology in the food industry, changes in food consumption culture and approach to food consumption, food preparation, and finally international trade in food, overburdened the more food illness in the present age, so that about 30 percent of people in developed countries at least once a year to develop food-borne diseases (Burt, 2004).Food storage methods which maintain the quality and extend the shelf life of food because of improved production, supply and trading is important. Human familiar to kept foods by different methods such as the use of heating,

cooling, drying and salting long time ago, but in order to reduce or eliminate pathogenic microbial agents and also prevent food spoilage new method is more in need,therefore one of these methods is the use of essential oils as antimicrobial additives in food.Essential oils have antibacterial effect, which mainly is due to the phenolics (Burt, 2004).In latest reports ofvalid scientific sources, antimicrobial properties offered diverse mix of herbs, spices, fruits, vegetables, leaves, bark and found animal,However, still many have not been scientifically studied natural ingredients (Cowan, 1999). In recent years, due to the harmful effects of synthetic and chemical food preservatives, consumers and producers of food prefer to use of natural preservatives such as essential oils which increase the shelf life of food (due to its antimicrobial properties) of a multiplicity of holders harmful chemicals are safe (Cutter, 2000; Akhondzadeh *et al*., 2007).

Preservatives are used to limit the growth and microbial activity in pharmaceutical products, food and cosmetics and by interfering with cell membranes, enzymes or genetic structure of microorganisms have a preventive effect. To apply the essential oils as chemical preservatives in food, investigate their antibacterial activities alone and in combination with other factors affecting the growth of microorganisms in food and nutrition is essential in laboratory models (Darabpour *et al*., 2010). Because essential oils are often used for some food as taste modifiers, protective properties and some of their antimicrobial can also encourage their use for this purpose. Essential oils, commonly called volatile or ethereal oils, are obtained through various distillation (Akin *et al*., 2010).Soil, harvest, plant age and climatic conditions affect the type and amount of the compounds in the oil. The studies also have shown that coincides with the time of flowering, have a stronger antimicrobial activity of essential oils is extracted from plants (Darabpour et al., 2010). Mainly phenolic compounds are responsible for the antimicrobial activity of essential oils (Burt, 2004).The higher amount of composition of the oil causesthe more anti-microbial properties.These materialsinclude eugenol, carvacrol and thymol (Akin *et al*., 2010).

Mary peas is a herb belonging to the mint family, plateau, a height of 10 to 30 cm, with a white cottony appearance, usually in areas poor in nutrients and organic matter, rocky areas and sand dunes in Europe, the Mediterranean region, North Africa and south west Asia, including Iran, especially Khorasan province, for example, different areas of the north , west, south, and Central and Mountain arid Tehran and Alborz region have dispersion.Teucrium genus include about 340 species of annuals and perennial herbaceous species, of which 12 are endemic to Iran Scientific studies have shown that this herb has antioxidant effects, antipyretic and antimicrobial and antispasmodic effect(Zare *et al*., 2011). It has been reported that ethanol extract of this herb is also antibacterial activity against gram positive and gram negative microorganisms of the show itself (Darabpour *et al*., 2010). 10 combined terpenoids such as coderol, linalool Gaol and beta pinene have been found in the oil (Akin *et al*., 2010).

1 to 20 percent of all outbreaks of food poisoning in the world is due to Bacillus cereus (Gilbert and Kramer, 1998).At least from 1906 Bacillus cereus is known to cause food poisoning (Mulet-Powell *et al*., 1998).The bacteria produces extracellular material such as lesitinase C and beta hemolysin, which are important in the bactreria identification.The enterotoxin produced by the bacteria causes diarrhea and nausea, and can induce the syndrome of

diarrhea and vomiting syndrome.In 1950, Bacillus cereus known as causes food poisoning (Rahimifard *et al*., 2007).The bacteria in raw and processed meat, vegetables, rice, cereals and milk and milk-based soups has the ability of proliferation and toxin formation (Gilbert and Kramer, 1998).

2.Materials and methods

View the treatment and study duration is specified in Table 1.

In order to extract the essential oil of TP, the plant was completely milled and dried using a Clevenger apparatus for 3 hours, volatile oil was extracted by water distillation.After dehydrationby dry sodium sulfate, essential oils were stored in dark glass containers and in the refrigerator until the anibacterial properties and the components were identified.The prepared sample extract was injected into gas chromatography connected to mass spectrograph and mass spectra compounds were obtained (Rahimifard *et al*., 2007).

Table 1. Time and the evaluated treatments profile

Number	Sample's name	Added essential oil content (ppm(	Time (Day)
1	Soup 1(Witness)	0	1-5
2	Soup 2	625	1-5
3	Soup 3	1250	1-5
4	Soup 4	2500	1-5
5	Soup 5	5000	1-5

In order to determine the minimum inhibitory concentration of essential oils Micro Broth Dilution Method 2 was used in the study.In this experiment, 96 wells of microplates were used.To increase the solubility and uniform development of oil in the medium, dimethyl sulfoxide, 5% as emulsifiers and for stabilizers agar - agar was used as the 0.05 percent (Akhondzadeh *et al*., 2007).Concentration of essential oil samples were studied for serial dilution.In short, sterile BHI broth in each 80 ml well, 100 ml studied oil dilution was added and eventually 20 ml bacterial culture (containing approximately cfu / ml 106 × 5 of the studied bacteria) were added per well(Akhondzadeh *et al*., 2007).

In each experiment, positive control which contained no oil wells was containing sterile BHI broth along with the studied bacteria and negative control which contained the well containing sterile BHI broth without bacteria was used.After adding the desired concentration of essential oils and bacteria, microplate were incubated at a temperature of approximately 37 °C for 24 hours (Akhondzadeh *et al*., 2007).

After the incubation period for determining the minimum inhibitory concentration, microplate wells werestudied by determining the amount of cloudiness using the naked eye.Finally, theminimal concentrations in which create the inhibition zone or lack of certain opacity compared with the control group was designated as MIC (Akhondzadeh *et al*., 2007).

Barley soup was used according to the manufacturer after the purchase, preparation and meal as models.Autoclavable samples in flasks of 100 ml volume of distribution (50 ml per flask barley soup) and were sterilized.After adding oil at a concentration of 2500 ppm MIC, two lower concentrations of 625 and 1250 ppm and a higher concentration of 5000 ppm, the correct amount of bacteria under sterile conditions into flasks were inoculated. The final number of bacteria in each sample was Cfu / ml 103, which was confirmed by surface culture (Moosavy *et al*., 2008).

Table 2. Treatments table

Treatment	Day	Average essential oil concentrations PPM
Witness	1	0
1	1	625
2	1	1250
3	1	2500
4	1	5000
Witness	2	0
5	2	625
6	2	1250
7	2	2500
8	2	5000
Witness	3	0
9	3	625
10	3	1250
11	3	2500
12	3	5000
Witness	5	0
13	5	625
14	5	1250
15	5	2500
16	5	5000

In order to assess the sensory characteristics of samples generated from sensory acceptance test was used. In order to do so, the prepared barley porridge was devided into seven parts (including 1000 ml flask with a volume of 500 ml barley porridge), and oil concentrations were added to each flask.Sensory evaluation was conducted by the assessment team 7 seater. Panel members made the sensory evaluation of the essential oil-containing barley soup using the intuitive 9 point scale.

The score of 9 was very high, score 8 washigh, score 7 was good, score 6 was relatively good, score 5 was so so, score 4 was relatively bad, score 3 was bad, score 2 was very bad, and the score 1was super bad, for organoleptic characteristics were evaluated in terms (Meilgaard *et al*., 1991).

Data analysis was performed using response surface method optimal design.Statistical data analysis and charting software was design expert editing 8.To change the relationship between the dependent variables Pearson correlation coefficient and SPSS statistical software version 20 was used.The behavior was Cubic model or Grade 3. Due to the abnormal data analysis of variance in normal mode, it was normalizedusing logarithmic transfer function instead.

3. Results and discussions

According on the analysis made on Kalpureh, 23 volatile compounds were identified in the essential oil, which are shown in table 3 (analysis results using chromatography connected to mass spectrograph- the relevant mass spectrums identified in GC/MC is also available in the appendix).

Table 3. Oils agronomic criteria analysis using the chromatography connected to mass spectrograph

Combine name	Quatz index （K）	Percent
Alpha-Pinene	939	16.2
Beta-Pinene	979	7.1
Myrcene	991	4
Limonene	1029	5.2
Alpha-Campholenal	1126	1.1
Verbenol<trans>	1145	6.3
Camphor	1146	2.1

Menthone	1153	1.3
Mentha-1,5-dien-8-ol <para->	1170	3.2
Myrtenal	1196	1.5
Verbenone	1205	0.75
Pulegone	1237	2.9
Bornyl acetate	1289	2.8
Carvacrol	1299	8
Caryophyllene<(E)->	1419	3.7
Germacrene D	1485	3.5
Bicyclogermacrene	1500	1.9
Spathulenol	1578	10.6
Caryophyllene oxide	1583	5.7
Unknown	1680	3.1
Beta-Eudesmol	1654	5.7
Valerianol	1658	1.6
Cryptomerione	1725	1.7

In this study there were 16 treatments, which are shown in table 4, along with the results of microbial tests on the related samples.

Table 4. Microbial test results (count Bacillus cereus)

Treatment	**Day**	**Average essential oil concentrations) PPM (**	**Average number of Bacillus cereus (cfu / ml)**
Witness	1	0	21700
1	1	625	19000
2	1	1250	13000
3	1	2500	10000
4	1	5000	5000
Witness	2	0	7000
5	2	625	5000
6	2	1250	4500
7	2	2500	4000
8	2	5000	3000
Witness	3	0	3400
9	3	625	2000
10	3	1250	1200
11	3	2500	1100
12	3	5000	1000
Witness	5	0	2000
13	5	625	600
14	5	1250	500
15	5	2500	400
16	5	5000	100

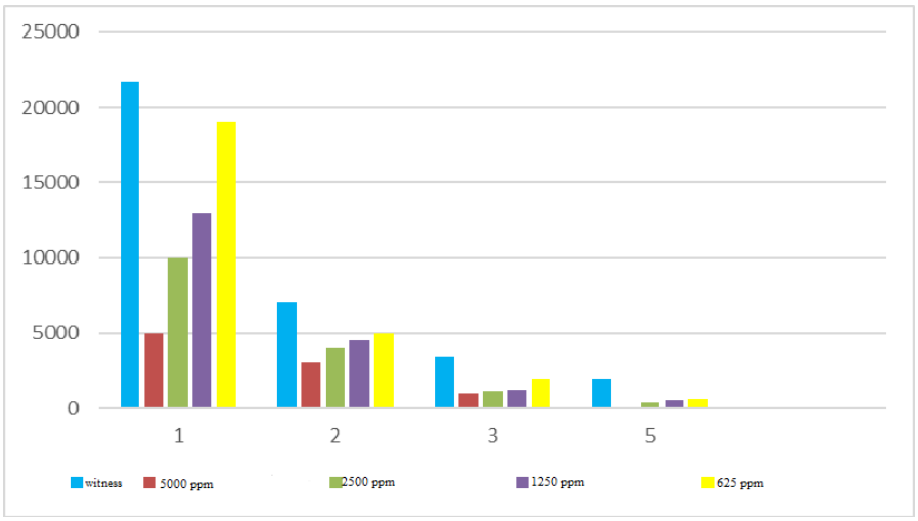

Figure 1. Bar chart of the day and the average number of B. cereus in different concentrations

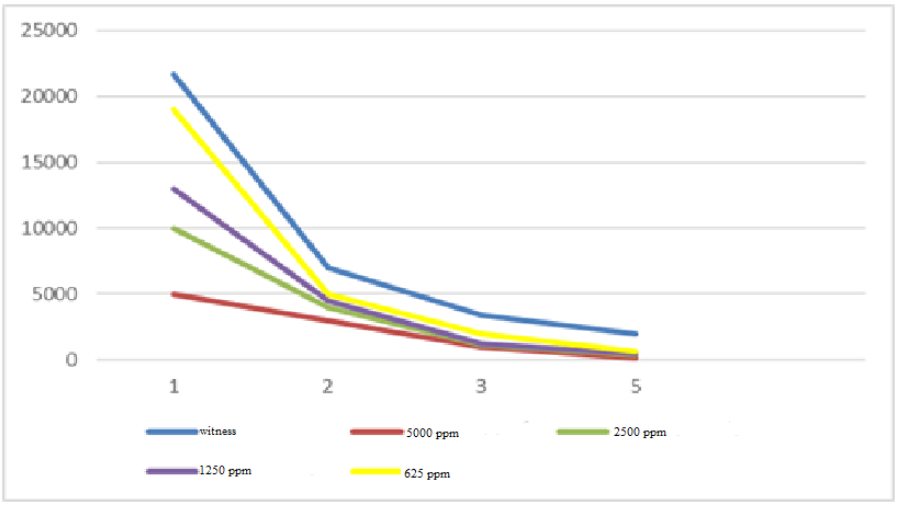

Figure 2. - Line graph B. cereus per day and the average number of different concentrations

The highest concentration was 5000 ppm causing the lowest bacterial count, which was 100 CFU/ml. The highest and lowest bacterial count belonged to the first and last day respectively, as the antimicrobial effect of the essential oil increased by time and in also amplified by increased concentration, as the 5-day-stored sample with the concentration of 5000 ppm caused the lowest bacterial count. Therefore, the two factors of the essential oil concentration and the storage period was effective on the survival rate of Bacillus cereus, leading to meaningful changes during the test.

In order to evaluate the sensory characteristics of adding the essential oil of Klapureh to barley soup, sensory acceptance test number 4 was used. Sensory evaluation was made by a 7-member group. The group members used a 9-numbered scale in order to make a sensory evaluation about the essential oil-containing soup. The analysis of the sensory test was done by Friedman test.

Meaningfulness in smell : P=0.002, therefore ranking in 0.01 is meaningful.

Meaningfulness in taste : P= 0.000, therefore ranking in 0.01 is meaningful.

Meaningfulness in color : P=0.4, therefore the ranking is not meaningful. Increase in concentration and time causes the correlation to increase by 24000 and then decrease, demonstrating the meaningfulness of the time effect.

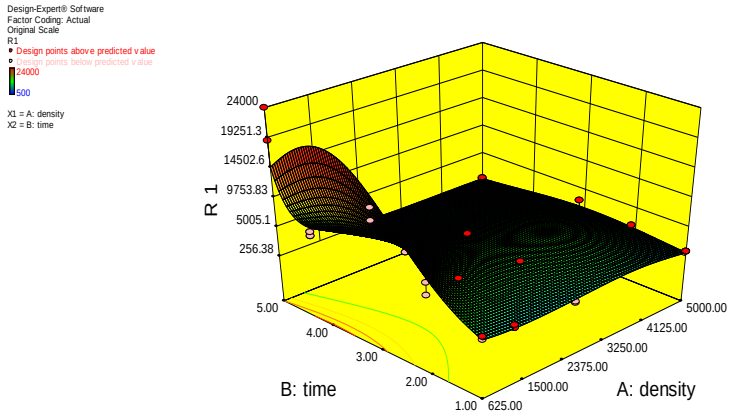

Figure 3. The combined effect of time and concentration on solidarity

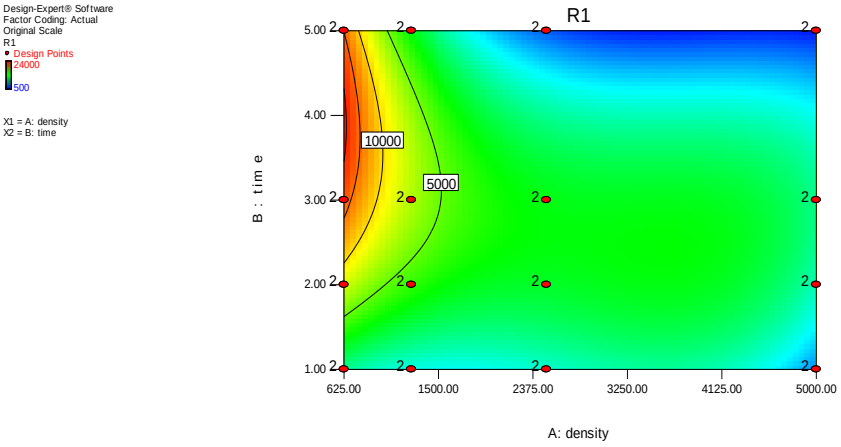

Figure 4. The combined effect of time and concentration on solidarity

According to the data of the analysis of One-way ANOVA, the effect of concentration wasn't meaningful. The applied modelling approach was Stepwise Regression.

Based on the results of Mahmoudi *et al* (2012), *L. casei* populations were not inhibited by low concentrations of the T. polium EO. However, increases in the EO concentrations lead to decreases in bacterial counts ($P < 0.05$) (Mahmoudi *et al*., 2010).

Zare et al (2011) results indicate that this essential oil has a high potential of antibacterial effect and resazurin can be used as a good growth indicator for different bacterial pathogens. Therefore, it can be suggested to purify and evaluate the active substances of this essential oil for future application as antibacterial agent and food preservative to combat pathogenic and toxigenic microorganisms (Zare *et al*., 2011).

4.Conclusions

The results of the present study showed that the commercial barley soup can cause increase in the food safety in refrigerator temperature. Therefore, the joint effect of Kalpureh essential oil and other bacteristat agents (such as natural preservatives) can cause enhancement in the usage of various essential oils in the food industry. Due to the results of this study, the samples containing higher concentration of the essential oil had less bacteria, and the samples of the nest days had more bacteria.

The effect of time on the regression was also meaningful. This fact demonstrates the meaningful and significant effect of the essential oil in decreasing the bacterial count of the food samples and the increase in quality and safety of the final product. However, the bitter taste of the essential oil caused low sensory acceptance.

Therefore, in case of using this essential oil in the food industry as a natural preservative, methods must be taken into consideration in order to eliminate the bitter taste such as microencapsulation of the essential oil.

5. References

Akhondzadeh, Basti, A.A. Misaghi, and Khaschabi, D. (2007). Growth response and modeling of the effects of Zataria multiflora Boiss. Essential oil, pH and temperature on Salmonella typhimurium and Staphylococcus aureus. *LWT Food Science and Technology*, 40,973 - 81.

Akin, M., Oguz, D,. Saracoglu, H.T. (2010), Antibacterial activity of essential oil from Thymbra spicata var. spicata L. and Teucrium polium (Stapf Brig.). *International Journal of Pharmaceutical and Applied Sciences*, 1, 55-58.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, 94(3), 223-253.

Cowan, M.M. (1999). Plant products as antimicrobial agents, *Clinical Microbiology Review*, 12: 564- 582.

Cutter, C.N. (2000). Antimicrobial effect of herb extracts against E. coli O157: H7, L. monocytogenes and S. typhimurium associated with beef. *Journal of Food Protection*.63(5), 601 - 7.

Darabpour, E., Motamedi, H., Seyyed, Nejad, S.M. (2010) Antimicrobial properties of Teucrium polium some clinical pathogens. *Asian Pacific Journal of Tropical Medicine*, 124-127

Gilbert RJ, Kramer JM.. *Biochemical Society Transactions.* (1984) Apr, 12(2), 198-200. Bacillus cereus enterotoxins: present status.

Mahmoudi, R., Zare, Z., Hassanzadeh, P., Nosratpour, S. (2012). Effect of Teucrium Polium essential oil on the physicochemical and sensory properties of pribiotic yoghurt. *Journal of Food Processing and Preservation*, 38 (3): 880-888.

Meilgaard, M, Civille, GV, Carr, B.T. (1991). *Sensory Evaluation Techniques*, 2nd Ed., 135-235. CRC Press Inc, Boca Raton, Florida.

Moosavy, M., Akhondzadeh, Basti, A.,Misaghi, A., Zahraei, salehi,T.,Abbasifar, R., Mousavi, H.,Alipour,M., et al., (2008). Effect of Zataria multiflora Boiss essential oil and nisinon *Staphylococcus aureus* and *Salmonella typhimurium* in a food model system and on the bacterial cell membranes. *Food Research International*, 41, 1050-1057.

Mulet-Powell, N., Lacoste-Armynot, A.M., Viñas, M. *and* de Bouchberg, M.S. *(*1998*)* Interactions between pairs of bacteriocins from lactic bacteria. J Food Prot 61, 1210–1212.

Rahimifard, N, Fatholahzadeh, B, Pirali Hamedani, M., Noory, Z, Saadati S.H.,Zavar, M., Pirouz, B., Asghari S.H.,Khezripour, M., Saberi, S. (2007) Bacillus cereus contamination in infant formula: a study in food and drug control laboratory. *Tehran University Medical Journal*; Vol. 65, No. 8, 64-68.

Zare, P., Mahmoudi, R., Ehsani, A. Biochemical and antibacterial properties of essential oil from Teucrium polium using resazurin as the indicator of bacterial cell growth *Pharmaceutical Sciences*, (2011), Vol. 17, No 3, Pp. 183 -188. [Persian].

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

REDUCTION OF ANTINUTRIENTS IN PEARL MILLET (*Pennisetum glaucum)* USING HURDLE TECHNOLOGY

Logasaranya S. M.[1*], Mahesh Kumar S.[1], Periyar Selvam[1]

[1]*Department of Food Process Engineering, SRM University, Tamil Nadu, India.*
*Corresponding author:*logasaranya@yahoo.co.in*

Article history:
Received:
4 March 2016
Accepted in revised form:
26 May 2016

Keywords:
Antinutrients,
Bioavailability,
Hurdle Technology,
In-Vitro Protein Digestibility,
Synergistic effect

ABSTRACT

Pearl millet (*Pennisetum glaucum*) is a staple food that supplies a major proportion of nutrition to large segments of the population living in Africa and Asia. The grain is nutritious but has some limitations due to the presence of antinutritional factors. Processing methods such as drying, washing, soaking, germination and autoclaving were used to reduce the antinutrients. The antinutrients analyzed were tannins, oxalates, polyphenols and phytic acid, which were found to reduce post processing. Germination, soaking and autoclaving reduced the level of antinutrients significantly. Hurdle technology was employed to study the combined effect of processing techniques and analyze the synergistic effect on the degradation of antinutrients. The individual treatments were assessed and the most suitable techniques for combination were found to be germination, soaking and autoclaving which were coupled with drying as the end technique to degrade the antinutrients and increase the *In Vitro* Protein Digestibility. Soaking + Germination + Drying combination degraded the antinutrients to the maximum level and increased IVPD from 51.032% to 66.571%.

1. Introduction

Pearl millet (*Pennisetum glaucum*) is a staple food in many parts of India because of its high protein content. The crop is adaptive and can survive in regions that are arid and production is likely to increase due to global warming. The grain is nutritious and is known to have higher protein and energy levels than maize and sorghum. However, the presence of antinutrients limits the absorption into the system. Antinutrients are known to interfere with carbohydrate, mineral bioavailability and protein digestibility (Pushparaj et al., 2001). Maximum utilization of the nutrient potential of the millet is limited by the presence of phytates, phenols, tannins, and enzyme inhibitors (Ramachandra et al., 1977). Domestic treatments had proved to improve the nutritional content of beans (Kataria et al., 1989). Protein digestibility is a measure of susceptibility of protein for the process of proteolysis. Protein with high digestibility is potentially of better nutritional value, as amino acids for absorption on proteolysis is high. Studies have shown that tannins contribute to lower nutritional value of dietary proteins by reducing the bioavailability of proteins to the body. Minerals and proteins bind with the antinutrients thereby retarding the digestibility (Reddy et al., 1994). Polyphenol content of pearl millet is considerably high which affects the mineral bioavailability and protein digestibility of grains.

Studies have proved that processing techniques reduce the level of antinutrients in pearl millet. Various physical and chemical treatments are used to reduce the antinutritional factors such soaking, germination, irradiation, roasting, drying, washing and fermentation. Fermentation had proved to increase the primary nutrients in finger millet (Antony et al., 1996). The antinutritional factors are generally found on the outer portion of the grain (Chavan et al., 1989). Decortication significantly decreases the amount of tannins with a corresponding increase in protein digestibility (Irén Léder, 2004).

Soaking and germination had been found to reduce the antinutrients in pearl millet (Obizoba et al., 1994). Pearl millet does not contain gluten hence it can be advised for patients suffering from Celiac disease. The low Glycemic Index (GI) of the grain is known to help in dealing with diabetes. The application of a single processing technique is frequently insufficient for the effective treatment hence combination of treatments is preferred.

Hurdle technology is used to reduce the level of antinutrients in the millet by employing multiple processing techniques that can reduce the antinutrients to the maximum level. After the primary processing, the results were analysed and the most suitable combinations were used to reduce the level of antinutrients. Soaking, germination and autoclaving were the most effective treatments against antinutrients hence they were applied in sequence. Drying was used as the end technique in all the combinations. In Vitro Protein Digestibility (IVPD) was used to assess the enhanced level of protein in the grain after various treatments and to analyze the amount of protein that can be absorbed by the body.

2. Materials and methods

Pearl millet samples were purchased in bulk from Chennai market. The raw material was then cleaned and extraneous materials were removed.

DRYING

The raw pearl millet samples were subjected to heating in a tray drier under constant temperature. The temperatures include 40°C, 50°C, 60°C, 70°C and 80°C. The dried samples were then cooled in a dessicator, ground and packed in an aluminium pouch. The samples were stored under dry conditions to avoid moisture absorption.

SOAKING

The raw pearl millet samples were subjected to soaking in distilled water in the ratio 1:3. The soaking times were 6, 12,18,20,22 and 24 hours. The soaked samples were then dried at 50°C for 3 hours and ground. The powdered samples were stored in air- tight aluminium pouches.

GERMINATION

The millet samples were initially soaked in water for 12 hours. After soaking, the samples were lined on sterile petri dishes lined with filter paper and sprouted for various time durations. The germination times are 20, 24, 28, 32, 36 and 40 hours. The sprouted samples were then dried at 50°C for 3 hours and ground. The powdered sample were sieved and stored in air- tight aluminium packets.

AUTOCLAVING

The raw samples were weighed and autoclaved at a pressure of 15psi for 15 minutes. The dry samples were allowed to cool in a dessicator, ground and stored for further analysis. The powdered samples were stored in air- tight aluminium pouches.

WASHING

The raw sample was soaked in water for 2 minutes and washed with distilled water. The washing times are 2, 4, 6, 8 and 10 minutes. After the washing procedure the samples were dried in tray-drier at a temperature of 50°C for 3 hours and ground. The powdered samples were stored in air- tight aluminium pouches.

EXPERIMENTAL TREATMENTS

The combination of individual processing techniques tested were,

Soaking + Autoclaving + Drying
Soaking + Germination + Drying
Autoclaving + Germination + Drying
Autoclaving + Soaking + Drying
Germination + Autoclaving + Drying
Germination + Soaking + Drying

Experiments were performed in triplicates and samples were

taken immediately after processing and stored in air tight pouches.

2.1.Analytical methods

2.1.1.Estimation of tannins

Determination of tannins based on the method of A.O.A.C (1975). Accurately weighed 0.5 g of the powdered material was transferred to a 250ml conical flask. Add 75ml water. Heat the flask gently and boil for 30 minutes. Centrifuged at 2,000 rpm for 20 minutes and collect the supernatant in 100 ml volumetric flask and make up the volume. Transfer 1ml of the sample extract to a 100ml volumetric flask containing 75 ml water. Add 5 ml of Folin-Denis reagent, 10ml of sodium carbonate solution and dilute to 100 ml with water and shaken well. Read the absorbance at 700 nm after 30 minutes. The

tannin concentration was determined by the standard graph of tannic acid solution.

2.1.2.Estimation of oxalates

Oxalate was determined by using the method of Sanchez-Alonso and Lachica (1987). Exactly one gram of the sample was placed in 250 ml volumetric flask, 190 ml of distilled water and 10 ml of 6M HCl were added. The mixture was then warmed in a water bath at 90°C for 4 hours and the digested sample centrifuged at 2,000 rpm for 5 min. The supernatant was then diluted to 250 ml. Three 50 ml aliquots of the supernatant was evaporated to 25 ml, the brown precipitate was filtered and washed. The combined solution and washings were then titrated with concentrated ammonia solution in drops until the pink colour of methyl orange changed to yellow. The solution was then heated in a water bath to 90°C and the oxalate was precipitated with 5% CaCl2 solution was allowed to stand overnight and then centrifuged, precipitate was washed with hot 25% H_2SO_4, diluted to 125 ml with distilled water and titrated against 0.05 M KMnO4.

Calculation:

1 ml 0.05 M KMnO4 = 2.2 mg Oxalate

Total amount of protein = (concentration) x (total volume of extract)

(1)

2.1.3.In vitro protein digestibility

In vitro protein digestibility was determined by calculating the difference between the amount of nitrogen in the sample before and after hydrolysis with pepsin (AOAC, 1965). Two hundred milligrams of whole seed or dehulled finger millet flour was incubated with 50 ml of 0.2% pepsin in 0.075 N HC1 for 24 h at 37°C. Digestion was performed in duplicate. The digests were filtered through Whatman No. 2 filter paper, and the residue was washed with warm water on the filter. Nitrogen in the residue was estimated by the micro- Kjeldahl method. IVPD was obtained by calculating the difference between the amount of total nitrogen in the sample before and after in vitro digestion with pepsin. Kjeldahl nitrogen was multiplied by the factor 6.25 to obtain crude protein.

2.1.4.Determination of protein content

The protein content was determined by the Lowry's method. BSA was used as the standard. Take 0.2 ml of BSA working standard in 5 test tubes and make up to 1 ml using distilled water. The test tube with 1 ml distilled water serves as blank. Add 4.5 ml of Reagent I and incubate for 10 minutes. After incubation, add 0.5 ml of reagent II and incubate for 30 minutes. Measure the absorbance at 660 nm and plot the standard graph. Estimate the amount of protein present in the given sample from the standard graph (Wilson and Walker 2000).

2.1.5.Determination of phytic acid

Phytic acid was determined by the procedure of Lucas and Markakas (1975). Two gram of sample was weighed into a 250 ml conical flask. One hundred ml 2% concentrated hydrochloric acid was used to soak sample for 3 hours and then filtered with a Whatmann No. 1 filter paper. Fifty ml of the filtrate and 10 ml of distilled water were added in each case to give proper acidity. Ten ml 0.3% ammonium thiocyanate solution was added into the solution as indicated and titrated with standard Iron II Chloride solution containing 0.00195 g Iron/ml, end point observed to be yellow which persisted for 5 min. The percentage phytic acid was calculated thus:

$$\% \text{ Phytic acid} = y \times 1.19 \times 100 \quad \text{where,}$$

$$y = \text{titre value} \times 0.00195 \text{ g}$$

(2)

2.1.6.Determination of polyphenols

The concentration of phenolic in sample extracts was determined using Singleton et al., 1999 method. Ethanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of ethanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was prepared**,** containing 0.5 ml methanol, 2.5 ml 10% Folin Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45˚C for 45 min. The absorbance was determined using spectrophotometer at 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance

was obtained. The same procedure was repeated for the standard solution of tannic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of Gallic acid equivalent (mg of GAE/g of extract). (Milan and Tanković, 2011).

2.1.7.Statistical analysis

Each determination was carried out on three separate samples and analyzed in triplicates. Regression and statistical analysis were performed using SPSS. Significance was accepted at $P \leq 0$.

3. Results and discussions

3.1. Effect of processing on the Protein and Tannin content of pearl millet

The processing techniques had shown to reduce the level of antinutrients in pearl millet. Germination and soaking have been the most effective methods. The reduction of tannins was observed the most in germination followed by soaking. The level of tannins is inversely proportional to the protein content. Germination the seeds for 36 hours had the highest quantity of protein. Soaking the grains for 24 hours reduced the tannin content effectively while drying had very less effect on the increase in protein content. Drying at 70°C showed low tannin content but increase in temperature can lead to the denaturation of protein hence temperature beyond 80°C was not preferred. High pressure cooking had also shown reduction of tannins and increase in protein but the levels were lower than that of germination. Washing the pearl millet had very little changes with respect to the tannin content.

Table 1. Effect of Drying on the Antinutrients and *In Vitro* Protein Digestibility (%) of Pearl millet

TEMPERATURE (°C)	PROTEIN (g/100g)	TANNINS (%)	OXALATES (mg/100g)	PHYTIC ACID (mg/100g)	POLY-PHENOLS (mg/100g)	IVPD (%)
UNPROCESSED	**7.213 ± 0.189**	**21.896 ± 0.014**	**2.42 ± 0.103**	***686 ± 0.014**	**304 ± 0.156**	**51.032 ± 0.024**
40°C	7.397 ± 0.119	21.311 ± 0.026	1.69 ± 0.00	679 ± 0.145	297 ± 0.293	52.417 ± 0.149
50°C	7.471 ± 0.519	20.940 ± 0.008	1.54 ± 0.103	650 ± 0.261	284 ± 0.137	53.963 ± 0.317
60°C	7.619 ± 0.361	20.647 ± 0.035	1.54 ± 0.00	627 ± 0.797	277 ± 0.419	54.414 ± 0.213
70°C	7.728 ± 0.174	20.392 ± 0.035	1.32 ± 0.00	604 ± 0.148	270 ± 0.145	54.890 ± 0.119
80°C	7.998 ± 0.213	19.978 ± 0.004	1.1 ± 0.00	582 ± 0.452	262 ± 0.352	55.287 ± 0.172

Values are means ± SD of three independent determinations Significant difference ($p \leq 0.05$) exist

3.2.Effect of processing on the Phytic acid content of pearl millet

The unprocessed pearl millet had considerable quantity of phytic acid. Phytic acid plays a significant role in deciding the nutritive value of pearl millet. The sprouted samples had higher levels of protein when compared to the control. Sprouts had low concentration of phytic acid and the lowest level was observed at 36 hours at 30°C.

A significant reduction in phytic acid level was observed by soaking the sample for 6 hours and the maximum at 24 hours.

Reduction in phytic acid concentration during soaking is due to the hydrophilic nature of phytates (Duhan et al., 1989).

Treating the millet sample at high pressure (15psi), were found to lower the level of phytic acid to a significant level while washing had negligible changes in the phytic acid content. The reduction of phytic acid after autoclaving could be due to the breakdown of phytic acid at high temperature. Drying at 80°C had effect on the levels of phytic acid but the preferred temperature was 70°C to avoid browning of pearl millet.

Table 2. Effect of Soaking on the Antinutrients and *In Vitro* Protein Digestibility (%) of Pearl millet

SOAKING TIME (hours)	PROTEIN (g/100g)	TANNINS (%)	OXALATES (mg/100g)	PHYTIC ACID (mg/100g)	POLY-PHENOLS (mg/100g)	IVPD (%)
UNPROCESSED	**7.213 ± 0.189**	**21.896 ± 0.014**	**2.42 ± 0.103**	**686 ±0.0149**	**304 ± 0.156**	**51.032 ± 0.024**
6	7.668 ± 0.213	21.224 ± 0.011	1.11 ± 0.103	653 ± 0.341	290 ± 0.268	52.129 ± 0.251
12	7.821 ± 0.124	20.909 ± 0.019	0.88 ± 0.00	617 ± 0.145	268 ± 0.134	52.972 ± 0.0139
18	8.119 ± 0.279	20.789 ± 0.016	0.88 ± 0.103	597 ± 0.296	251 ± 0.335	53.867 ± 0.233
20	8.327 ± 0.147	20.409 ± 0.005	0.66 ± 0.103	585 ± 0.167	242 ± 0.148	55.259 ± 0.161
22	8.551 ± 0.824	19.769 ± 0.010	0.44 ± 0.00	569 ± 0.172	221 ± 0.162	56.924 ± 0.217
24	8.671 ± 0.159	19.486 ± 0.014	0.44 ± 0.00	542 ± 0.214	208 ± 0.110	58.526 ± 0.156

Values are means ± SD of three independent determinations. Significant difference ($p \leq 0.05$) exist

3.3.Effect of processing on the Polyphenol content of pearl millet

The polyphenolic content in the sample reduced significantly with germination, drying, soaking and autoclaving. Autoclaving the sample decreased the level of polyphenols from 404 mg/100g to 286 mg/100g. Soaked and sprouted samples also had reduced polyphenolic values.

Table 3. Effect of Germination on the Antinutrients and *In Vitro* Protein Digestibility (%) of Pearl millet

GERMINATION TIME (hours)	PROTEIN (g/100g)	TANNINS (%)	OXALATES (mg/100g)	PHYTIC ACID (mg/100g)	POLYPHENOLS (mg/100g)	IVPD (%)
UNPROCESSED	**7.213** ± 0.189	**21.896 ± 0.0143**	**2.42 ± 0.103**	**686 ± 0.0149**	**304 ± 0.156**	**51.032 ± 0.024**
20	8.471 ± 0.176	21.221 ± 0.098	0.88 ± 0.00	651 ± 0.269	286 ± 0.132	56.251 ± 0.072
24	8.508 ± 0.974	20.776 ± 0.026	0.88 ± 0.0	627 ± 0.317	261 ± 0.219	56.682 ± 0.112
28	8.673 ± 0.175	20.201 ± 0.057	0.88 ± 0.103	586 ± 0.524	243 ± 0.135	57.853 ± 0.143
32	8.747 ± 0.110	19.967 ± 0.079	0.66 ± 0.103	559 ± 0.411	239 ± 0.211	58.274 ± 0.092
36	8.964 ± 0.845	19.317 ± 0.053	0.66 ± 0.00	521 ± 0.128	212 ± 0.259	60.129 ± 0.187
40	9.012 ± 0.114	18.993 ± 0.004	0.44 ± 0.103	501 ± 0.169	197 ± 0.249	62.728 ± 0.212

Values are means ± SD of three independent determinations, Significant difference ($p \leq 0.05$) exist

3.4.Effect of processing on the Oxalate content of pearl millet

The oxalate content in pearl millet is less when compared to other antinutrients. Germination and soaking the samples showed the lowest levels of oxalates while washing did not have any significant changes. Autoclaving resulted in reduction from 2.42 mg/100g to 1.10 mg/100g. Increase in temperature caused mild reduction in the level of oxalates in pearl millet sample.

3.5.Effect of processing on the *in vitro* protein digestibility (IVPD) of pearl millet

In Vitro Protein Digestibility of pearl millet increased after germination, the increase in digestibility was observed with increase in germination time. Germination time greater than 36 hours cannot be employed due to the formation of cyanide higher than the permissible limit (Panasiuk *et al.*,1984). Germination has been found to reduce antinutrients like phytic acid in millets (Khetarpaul et al.,1990). Soaking the samples for 24 hours significantly increased the digestibility of protein. High pressure cooking had pronounced improvement in the digestibility.

3.6.Effect of combined processing on the *in vitro* protein digestibility (IVPD) and antinutrients

The samples that were subjected to combination of techniques showed better degradation of antinutrients. Soaking + Germination + Drying, had the highest value of IVPD and low values of antinutrients, followed by Germination + Autoclaving + Drying.

Table 4. Effect of Washing on the Antinutrients and *In Vitro* Protein Digestibility (%) of Pearl millet

WASHING TIME (minutes)	PROTEIN (g/100g)	TANNINS (%)	OXALATES (mg/100g)	PHYTIC ACID (mg/100g)	POLYPHENOLS (mg/100g)	IVPD (%)
UNPROCESSED	**7.213** ± 0.189	**21.896 ± 0.0143**	**2.42 ± 0.103**	**686 ± 0.0149**	**304 ± 0.156**	**51.032 ± 0.024**
2	7.213 ±0.189	21.896 ± 0.0143	2.42 ± 0.103	686 ± 0.014	304 ± 0.156	51.032 ± 0.024
4	7.225 ± 0.121	21.861 ± 0.0167	2.42 ± 0.00	681 ± 0.165	302 ± 0.153	51.347 ± 0.125
6	7.250 ± 0.173	21.837 ± 0.0241	2.42 ± 0.00	673 ± 0.213	296 ± 0.314	51.621 ± 0.219
8	7.311 ± 0.146	21.796 ± 0.0173	2.20 ± 0.00	669 ± 0.137	291 ± 0.239	51.842 ± 0.168
10	7.338 ± 0.129	21.753 ± 0.0142	2.20 ± 0.103	658 ± 0.184	288 ± 0.157	51.998 ± 0.153

Values are means ± SD of three independent determination.No Significant difference exist ($p \geq 0.05$)

Table 5. Effect of Autoclaving on the Antinutrients and *In Vitro* Protein Digestibility (%) of Pearl millet

SAMPLE (PRESSURE)	PROTEIN (g/100g)	TANNINS (%)	OXALATES (mg/100g)	PHYTIC ACID (mg/100g)	POLYPHENOLS (mg/100g)	IVPD (%)
UNPROCESSED	**7.213** ± 0.189	**21.896 ± 0.0143**	**2.42 ± 0.103**	**686 ± 0.0149**	**304 ± 0.156**	**51.032 ± 0.024**
15 psi	7.964 ± 0.345	19.521 ± 0.124	1.10 ± 0.103	602 ± 0.462	286 ± 0.132	54.128 ± 0.192

Values are means ± SD of three independent determinations. Significant difference ($p \leq 0.05$) exist

Table 6. Effect of combined treatment on antinutrients and *In Vitro* Protein Digestibility (%) of Pearl millet

SAMPLE	PROTEIN (g/100g)	TANNINS (%)	OXALATES (mg/100g)	PHYTIC ACID (mg/100g)	POLYPHENOLS (mg/100g)	IVPD (%)
UNPROCESSED	**7.213** ± 0.189	**21.896 ± 0.0143**	**2.42 ± 0.103**	**686 ± 0.0149**	**304 ± 0.156**	**51.032 ±0.024**
Soaking + Autoclaving + Drying	11.267 ± 0.168	18.368 ± 0.015	0.44 ± 0.103	523.102 ± 0.167	207 ± 0.214	63.129 ±0.067
Soaking + Germination + Drying	12.314 ± 0.212	18.779 ± 0.024	0.44 ± 0.103	489.171 ± 0.021	197 ± 0.251	66.571 ±0.182
Autoclaving + Germination + Drying	10.652 ± 0.180	19.327 ± 0.214	0.44 ± 0.00	506.954 ± 0.269	278 ± 0.134	64.375 ±0.015
Autoclaving + Soaking + Drying	9.851 ± 0.014	19.659 ± 0.287	0.66 ± 0.00	541.264 ± 0.154	243 ± 0.049	59.126 ±0.017
Germination + Autoclaving + Drying	11.627 ± 0.216	18.225 ± 0.203	0.44 ± 0.103	492.524 ± 0.583	221 ± 0.127	65.121 ±0.219
Germination + Soaking + Drying	11.983 ± 0.312	19.263 ± 0.381	0.44 ± 0.103	502.352 ± 0.552	219 ± 0.082	64.876 ±0.084

Values are means ± SD of three independent determinations. Significant difference ($p \leq 0.05$) exist

The synergistic effect of treatments had greater influence on the digestibility of protein. Autoclaving + Soaking + Drying, had relatively low value of protein digestibility. Germination + Soaking + Drying also showed significant reduction in antinutrients, but the millet is more prone to microbial contamination in this technique as soaking the seeds after germination may produce unfavorable odour and slimy texture. Treatments like soaking and autoclaving were effective in the reduction of phytic acid and polyphenols in pearl millet (Sharma *et al.*,1996). Soaking + Autoclaving + Drying is one the

traditional methods of processing pearl millet, the antinutrient reduction was significant and the IVPD was high.

4. Conclusions

The samples subjected to individual processing treatments had shown reduced levels of antinutrients and increased protein digestibility. It was observed that combination of techniques showed relatively better results than individual techniques. Combinations containing germination as one of the treatments had maximum desirability. Analyzing the combinations, it can be concluded that Soaking + Germination + Drying, gave the most preferred result of low antinutrients and high *in vitro* protein digestibility.

5. References

Antony, U., Sripriya, G., & Chandra, T. S. (1996). Effect of fermentation on the primary nutrients in finger millet (Eleusine coracana). *Journal of Agricultural and Food Chemistry*, *44*(9), 2616-2618.

AOAC (1975). Official methods of analysis (12th ed.). Washington, DC: Association of Official Analytical Chemists (AOAC).

Chavan, J. K., Kadam, S. S., & Beuchat, L. R. (1989). Nutritional improvement of cereals by germination. *Critical Reviews in Food Science & Nutrition*, *28*(5), 401-437.

Duhan, A., Chauhan, B. M., Punia, D., & Kapoor, A. C. (1989). Phytic acid content of chickpea (Cicer arietinum) and black gram (Vigna mungo): varietal differences and effect of domestic processing and cooking methods. *Journal of the Science of Food and Agriculture*, *49*(4), 449-455.

Kataria, A., Chauhan, B. M., & Punia, D. (1989). Antinutrients and protein digestibility (in vitro) of mungbean as affected by domestic processing and cooking. *Food chemistry*, *32*(1), 9-17.

Khetarpaul, N., & Chauhan, B. M. (1990). Effects of germination and pure culture fermentation by yeasts and lactobacilli on phytic acid and polyphenol content of pearl millet. *Journal of Food Science*, *55*(4), 1180-1180.

Léder, I. (2004). Sorghum and millets. *Cultivated Plants, Primarily as Food Sources*, 66-84.

Lucas, G. M., & Markakas, P. (1975). Phytic acid and other phosphorus compounds of bean (Phaseolus vulgaris). *J. Agric. Educ. Chem*, *23*, 13-15.

Panasiuk, O., and Bills, D. D. (1984). Cyanide content of sorghum sprouts. *Journal of Food Science*, *49*(3), 791-793.

Pushparaj, F. S., & Urooj, A. (2011). Influence of processing on dietary fiber, tannin and in vitro protein digestibility of pearl millet. *Food and Nutrition Sciences*, *2*(8), 895.

Ramachandra, G., Virupaksha, T. K., & Shadaksharaswamy, M. (1977). Relation between tannin levels and in vitro protein digestibility in finger millet (Eleusine coracana Gaertn.). *Journal of Agricultural and Food Chemistry*,*25*(5), 1101-1104.

Reddy, N. R., & Pierson, M. D. (1994). Reduction in antinutritional and toxic components in plant foods by fermentation. *Food Research International*,*27*(3), 281-290.

Sanchez-Alonso, F., & Lachica, M. (1988). Oxalate salts in the leaves of plum (Prunus salicina L.) and cherry (P. avium L.). *New phytologist*, *108*(4), 505-508.

Sharma, A., & Kapoor, A. C. (1996). Levels of antinutritional factors in pearl millet as affected by processing treatments and various types of fermentation. *Plant Foods for Human Nutrition*, *49*(3), 241-252.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152-178.

Wilson, K., & Walker, J. (2000). *Principles and techniques of practical biochemistry*. Cambridge University Press.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

THE DRIED MYCELIUM OF *GANODERMA LUCIDUM* EXHIBITING IMPROVING INTRACELLULAR POLYSACCHARIDE CONTENT BY SUBMERGED FERMENTATION OPTIMIZATION IN LARGE-SCALE FERMENTATION PROCESSES AND ITS FOOD SAFETY

Hua-Wei Zeng[1]*, Hui Chen[2]

[1]*Department of Bioengineering, College of Life Sciences, Huaibei Normal University, Huaibei 235000, Anhui Province, PR China*
[2]*Jiangsu Alphay Biotechnology Co. Ltd, Nantong, Jiangsu 226009, PR China*
*Corresponding author: * huaweizeng@163.com; 170246405@qq.com*

Article history:
Received:
23 April 2016
Accepted in revised form:
29 May 2016

Keywords:
Ganoderma lucidum;
Large-Scale Fermentation;
Processes;
Intracellular Polysaccharide;
Content ;
Food Safety

ABSTRACT

This study mainly focused on increasing intracellular polysaccharide (IPS) content in the medicinal fungus *Ganoderma lucidum* by optimized cultured conditions (containing carbon sources, initial pH values, temperatures and carrier-to-noise ratios) in large-scale fermentor. The maximum IPS content of 6.68% in a 1500-L fermentor was achieved in a medium containing 30 g/L glucose, 5 g/L soybean meal, and 10 g/L corn flour at an initial pH 6.0 and temperature 28°C and was found to be 2.27 times higher than that of unoptimized conditons (2.93%). When expanding culture in a 10000-L fermentor was performed under optimum conditions, the highest IPS content (6.30%) and dry mycelium weight (about 17.85 g/L) were obtained at only 52h. According to the analysis of AS contents and Pb contents from fermentation raw materials by the determination of graphite furnace atomic absorption spectrophotometer, As content and Pb content of the dried mycelium were decreased to food safe range by adjusting fermentation raw materials. The results can promote its industrial-level production.

1.Introduction

Ganoderma lucidum (Leyss.:Fr.) Karst, a basidiomycete belonging to the polyporaceae, is one of very famous medicinal herbs in worldwide, especially in China, Korea and Japan. Because of its high medicinal value, *G. lucidum* has received wide popularity as a health food and medicine. Nowadays, the yield of fruiting bodies has not satisfied its consumer demand, so submerged culture for producing mycelium of *G. lucidum*, which has many advantages for industrial-level production, such as short production cycle, stable food safe (such as low heavy metal content and not pesticide contamination) and so on (Chen and Gu, 2008), has a good prospects for application. The polysaccharides isolated from fruiting bodies and cultured mycelium of *G. lucidum* have antitumor activity, immunomodulation and antioxidation (Hsiao et al., 2004; Mojadadi et al., 2006; Sudheesh et al., 2009), and its content is one of the key indexes for evaluating medicinal value of *G. lucidum* products. Improving intracellular polysaccharide (IPS) contents of mycelium by submerged cultured optimization have been studied in several reports (Babitskay et al., 2005; Simonić et al., 2008; Tang et al., 2011; Yang et al., 2013). However, their processes in all studies don't suit industrial-level production, such as long fermentation time, mall fermentation scale and unstable food safety. In the present study, submerged fermentation optimization for producing mycelium of *G. lucidum* exhibiting

high IPS content was performed in large-scale fermentor during short cultured time.

Furthermore, those mycelium with safe heavy metal contents were obtained by the adjusting of fermentation raw materials.

2. Materials and methods

2.1. Maintenance of *G lucidum*

The strain of *G.lucidum* used in this study was maintained on potato dextrose agar (PDA) slants. The slant with mycelium was incubated at 28°C for 6 days, and then stored at 4°C for the following experiment.

2.2. Cultured medium of *G lucidum*

Preculture medium included the following components (g/L): glucose 20, soybean meal (AS content of 0.24ppm, Pb content of 0.43ppm) 15, corn flour 10, corn steep powder 15, inorganic salt 2.0, vitamin 0.004, bean oil 1, antifoam 0.5. The initial pH value was adjusted to 6.0 by adding industrial grade solid caustic sodaor analytical grade solid caustic soda.

Cultured medium consisted of the following components (g/L): sucrose or glucose 30, soybean meal 5, corn flour 5, corn steep powder 10, inorganic salt 2.0, vitamin 0.004, bean oil 1, antifoam 0.5. The initial pH value was adjusted to 6.0 by adding industrial grade solid caustic soda or analytical grade solid caustic soda.

2.3. Preculture of *G lucidum* in flasks

For the first preculture in flasks, 500-mL flasks containing 200 mL of preculture medium were sterilized at 120°C for 30 min, and then cooled to room temperature. The strain from PDA slant was inoculated into 500-mL flasks and followed by 4-day incubation at 28°C on a rotary shaker (150 rpm).

For the second preculture in flasks, 1-L flasks containing 400 mL of preculture media were sterilized at 120°C for 30 min, and then cooled to room temperature. Preculture medium with *G lucidum* prepared in a 500-mL flask was inoculated into 1-L flasks with volume ratio of 10%, and then followed by 3-day incubation at 28°C on a rotary shaker (150 rpm).

For preculture in fermentor, 200-L fermentor with a working volume of 120 L of preculture medium was sterilized by steam at 120°C for 60 min, and then cooled to room temperature. 800 mL of preculture medium with *G lucidum* prepared in a 1-L flask was inoculated into 200-L fermentor, and then culture was performed with aeration rate (1.11 $V \cdot V^{-1} \cdot min^{-1}$) at 28°C for 3 d.

2.4. Fermentation culture of *G lucidum* in large-scale fermentors

For fermentation culture, 1500-L fermentor with a working volume of 800L of cultivation medium were sterilized by steam at 120°C for 60 min, and then cooled to room temperature. 120 L of preculture medium with *G lucidum* prepared in 200-L fermentor was translated into 1500-L fermentor with a working volume of 800 L of cultured medium, and then fermentation was performed with agitation speed (0 rpm) and aeration rate (0.93 $V \cdot V^{-1} \cdot min^{-1}$) at 28°C for 72h. For amplication culture, 10000-L fermentor with a working volume of 7000 L of cultured medium was sterilized by steam at 120°C for 60 min, and then cooled to room temperature. 120 L of medium with *G lucidum* prepared in 200-L fermentor was translated into 10000-L fermentor with a working volume of 800L of cultured medium, and then fermentation was performed with aeration rate (0.93 $V \cdot V^{-1} \cdot min^{-1}$) at 28°C for 60h.

2.5. Experimental design

Two different carbon sources at the concentrations of 30g/L (glucose or sucrose) were tested in order to determine the effect on IPS content, pH value and mycelium concentration. In optimum carbon source condition, the influences of two different initial pH value (6.0 and 4.5) and two different temperatures (28°C and 32 °C) were evaluated, respectively. In the optimum conditions, the variation of IPS content, pH value and mycelium concentration was tested by adjusting corn powder content and soybean meal content. Subsequently, the culture in 10000-L fermentor was performed in optimal

conditions. Finally, the experiment for reducing AS content and Pb content in mycelium of *G. lucidum* was carried out.

2.6 Analytical methods

For the analysis of IPS content, wet mycelium was dried at 85°C for 24h, then the dry mycelium (1.0 g) grinded in mortar was sieved by using 2μm sieve. Mycelium powder was extracted by boiling water for 2h, and extracted liquid followed a precipitation with absolute ethanol at 4°C for 12h.

The precipitated polysaccharide was collected by centrifugation at 3000 rpm for 10 min and was dissolved by distilled water. Polysaccharide content of the final solution was determined by anthrone-sulfuric acid method according to Chinese pharmacopoeia (Anonymous, 2005). IPS content was present as g/g (dry mycelium weight) 100%.

For the analysis of growth state of *G. lucidum*, mycelium concentration was determined in real-time. Fermentation broth (100 mL) was centrifuged at 3000 rpm for 30min, and then wet mycelium weight was determined by electronic balance. Mycelium concentration was describe as g (wet mycelium weight in 100 mL broth) /100mL·100%.

The pH value of cultured liquid was measured with a digital pH meter.

AS content and Pb content in dry mycelium were determined by graphite furnace atomic absorption spectrophotometer according to Xing et al., method (Xing et al., 2002).

3. Results and discussion

3.1. The effect of different carbon sources, temperatures and initial pH values on IPS content, pH value and mycelium concentration from *G. lucidum*

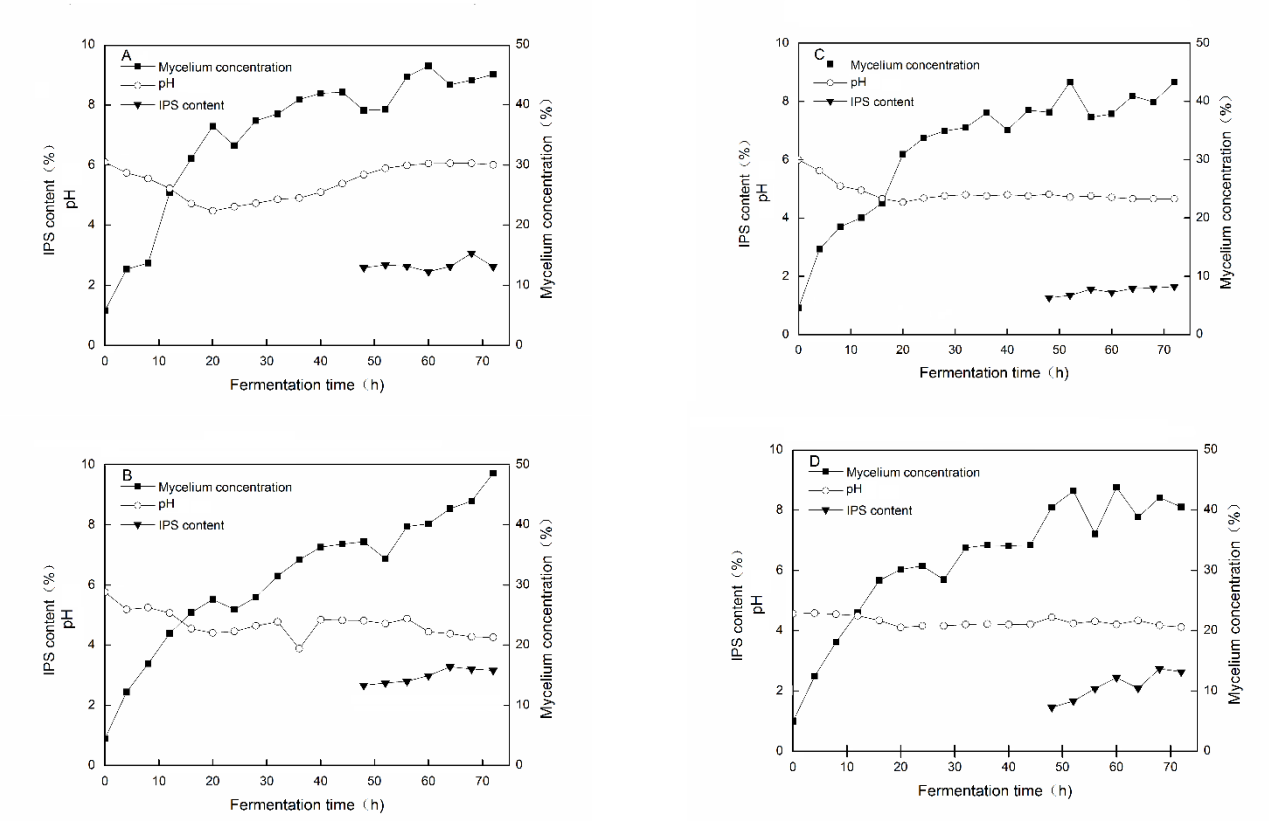

Figure 1. The time course of IPS content, pH and mycelium concentration by using *G. lucidum* under different cultured conditions in 1500-L fermentor. A, Sucrose as main carbon source; B, Glucose as main carbon source; C, Cultured temperature of 32°C; D, Initial pH value of 4.5

Fig. 1A and Fig.1B revealed that the maximum IPS content of 2.93% reached in the presence of sucrose was lower than the highest IPS content of 3.41% obtained in the presence of glucose.

The results weren't inconsistent to the previous report, which sucrose was the better substrate for IPS content compared to glucose (Tang and Zhong, 2002). The variations of pH value between Fig. 1A and Fig. 1B were different: pH value in the presence of sucrose sharply increased from 4.47 to 6.01 between 20 h to 72h, whereas pH value in the presence of glucose slightly decreased from 4.4 to 4.26 between 20 h to 72h.

Possible explain is that the pathways of utilization of *G lucidum* were different between glucose and sucrose, and lead its different secretion level of acid material and alkaline material. Mycelium concentration in the presence of sucrose rapidly increased during exponential phase (4h-20h), and then slowly rose to 46.45% at 60h (Fig.1A). Mycelium concentration in the presence of glucose gradually increased all the time, and the highest mycelium concentration of 48.6% was achieved at 72h (Fig.1B). The result indicated that glucose was a more suitable growth substrate than sucrose. Temperature has significant effect on synthesis of polysaccharides of *G. lucidum* (Babitskaya et al., 2005; Kim et al., 2006; Lee etal., 2007; Yang and Liau, 1998). When cultured temperature was increased from 28 to 32°C, the maximum IPS content was decreased from 3.41% to 1.71% (Fig. 1B and Fig. 1-C). The result is similar to the report of Babitskaya et al, which maximum end polysaccharides was obtained at 25-30°C (Babitskaya et al., 2005). Variation trend of pH value was similar between Fig. 1B and Fig. 1-C, pH values rapidly decreased from 0 h to 24h, and then almost no reduction of pH value was showed after 24h. The highest mycelium concentration of 43.4% at 32°C was lower than 48.6% at 28°C (Fig. 1B and Fig. 1C). The result is agreement to a previous report, which the optimum temperature for the culture mycelium growth was obtained at 28°C (Kim et al., 2006) .

The initial medium pH can greatly affect cell membrane function, cell growth, morphology and structure, salt solubility, the ionic state of substrates, the uptake of various nutrients, and product biosynthesis (Fang and Zhong, 2002a). When initial pH value was adjusted from 6.0 to 4.5, the highest IPS content was reduced from 3.41% to 2.74% (Fig. 1B and Fig. 1D). However, Fang et al found that lowering the initial pH from 6.5 to 3.5 gradually led to a higher IPS content (7.75%) (Lee et. al., 2007). Likewise, Simonić et al showed that IPS production in medium with initial pH 4.5 was the highest value among that of initial pH values (4.0, 5.0, 5.5 and 6.0) (Simonić et. al., 2008). Although the initial pH value was set at 4.5 or 6.0, their final pH value was about 4.0 at incubation of 72h and slightly higher than the pH value (3.54) in the literature (Yang and Liau, 1998). Fang and Zhong reported that at an initial pH of 6.5, a maximum in biomass of 17.3 g/L by dry weight was achieved (Fang and Zhong , 2002a).In *Ganoderma resinaceum* DG-6556, the maximum mycelium growth was obtained at an initial pH of 7.0(Kim et. al., 2006). Simonić et al found that biomass production increased gradually by increasing the initial pH and reached the peak at an initial pH of 5.5 (Simonić et. al., 2008). These authors confirmed the range of initial pH value (5.5-7.0) was the most suitable for growth. In the study, the highest mycelium concentration of 43.82% at initial pH value of 4.5 was lower than that at initial pH value of 6.0 (48.6%). This result was in the range mentioned above.

3.2. The effect of the regulation of carrier-to-noise ratios (C/Ns) on IPS content, pH value and mycelium concentration from *G. lucidum*

Previous studies indicated that C/N is thought as the important factor for the polysaccharide biosynthesis of *G.lucidum*, and suitable C/N can obviously enhance polysaccharide production (Babitskaya et. al., 2005; Lee et. al., 2007; Yuan et. al.,2012). In the study, corn flour and soybean meal was generally regarded as carbon source (a

general C/N ratio of 97.3) and nitrogen source (a general C/N ratio of 16.76). The highest IPS content of 3.47%, 2.77%, 5.45%, 6.68%, and 3.41% were shown in Fig. 2 A, B, C, D, and Fig. 1B, respectively. The maximum IPS content of 6.68% was revealed in the medium containing soybean meal 5 g/L and corn flour 10 g/L (Fig. 2D). We found that increasing nitrogen source content decreased IPS content (Fig. 1B, Fig. 2 A, and Fig. 2B), whereas increasing carbon source content enhanced IPS content (Fig. 1B and Fig. 2D).

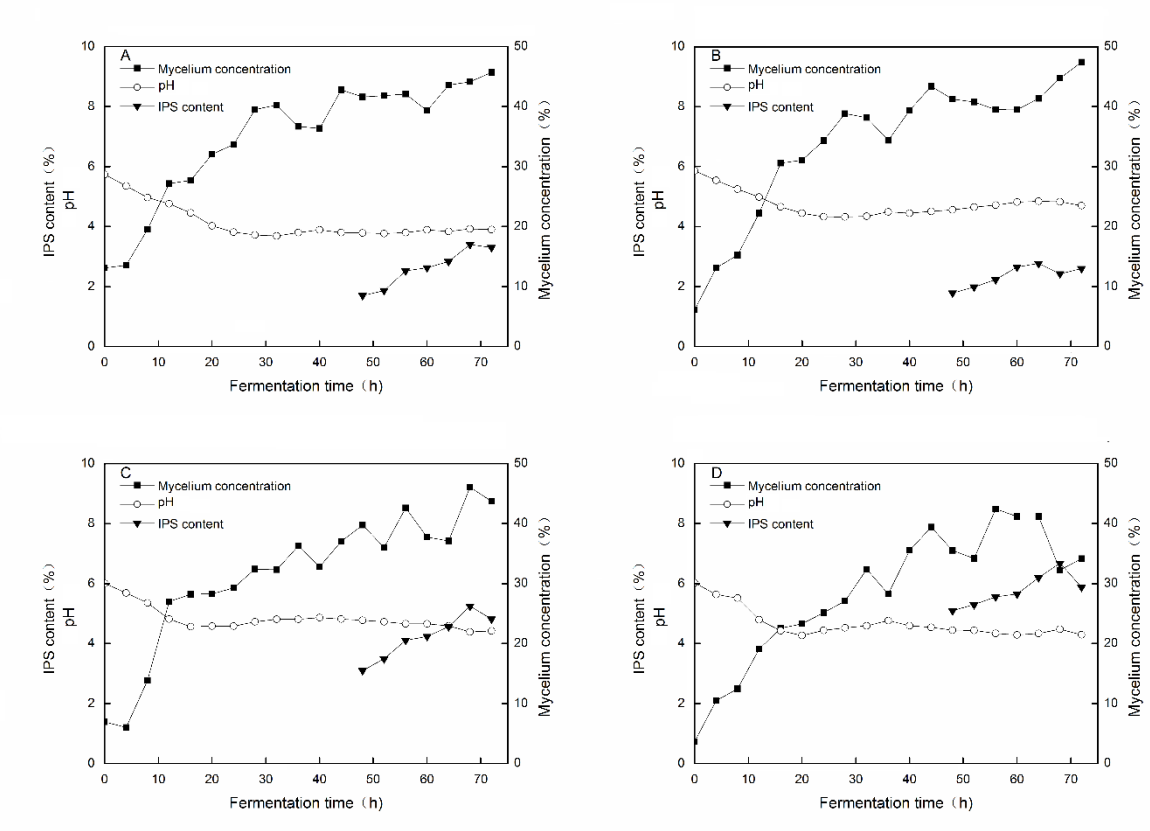

Figure 2. The time course of IPS content, pH and mycelium concentration by using G. lucidum under differnent C/Ns in 1500-L fermentor. A, soybean meal 10g/L and corn flour 5 g/L; B, soybean meal 15 g/L and corn flour 5 g/L; C, soybean meal 10 g/L and corn flour 10 g/L; D, soybean meal 5 g/L and corn flour 10 g/L

However, the result was converse to previous report which the endo polysaccharides reduced with increasing C/Ns in *G. applanatum* (Babitskaya et. al., 2005). This converse variance of IPS content from the same kind of fungus may differ due to strains and their culture conditions. Variety trend of pH value by regulation of C/Ns among Fig. 2A, B, C, D, and Fig. 1B showed similarly that pH value quickly decreased before 20h and then almost not changes was observed after 20 h.

Mycelium concentration sharply increased at exponential phase, and then slightly changes at stationary phase (Fig. 2A, B, C, D, and Fig. 1B), the maximum mycelium concentration was 45.9%, 47.45%, 46.1%, 42.45%, and 48.6% in Fig. 2A, B, C, D, and Fig. 1B, respectively.

3.3. Fermentation amplification in 10000-L fermentor under optimum conditions and reducing Pb content and As content of mycelium from *G. lucidum*

The profiles of IPS content, mycelium concentration and pH value in fermentation process was shown in Fig. 3A. IPS content firstly increased from 48h to 52h, and then decreased. The maximum IPS content of 6.3% was obtained at 52h. Although previous

reports showed the maximal IPS content of 23%(Tang et. al., 2011), their measure method of phenol-sulfuric acid were different from the measure method in present study.

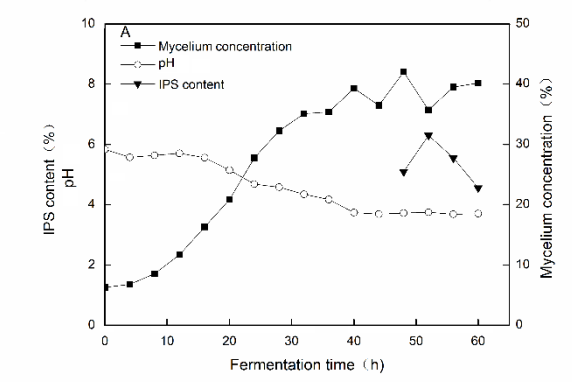

Figure 3 A. The time course of IPS content, pH and mycelium concentration by using *G. lucidum* under optimum conditions in 10000-L fermentor.

In our pervious research, we noticed that IPS content with phenol-sulfuric acid method was obviously higher than that with anthrone-sulfuric acid method, so the comparison did not make among those results. The authors found that the highest IPS content was only 2.74% among those fruiting bodies from 18 varieties of *Ganoderma Lucidum* Karst (Xu and Xu, 2004).

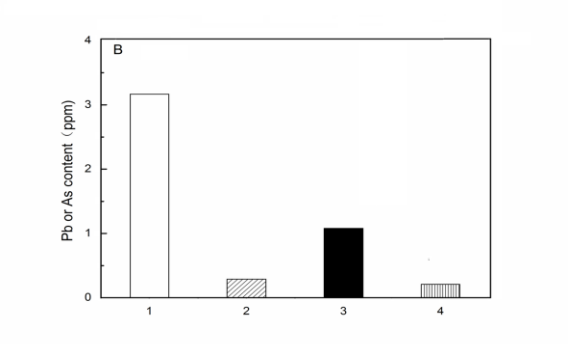

Figure 3B. The comparison of AS content or Pb content in *G. lucidum* of before and after treatment. 1, Pb content using industrial grade solid caustic soda ; 2, Pb content using analytical grade solid caustic soda ; 3, As content using industrial grade solid caustic soda ; 4, As content using analytical grade solid caustic soda

Mycelium concentration sharply increased from 0 h to 32h and then slightly increased after 32h, the concentration of 35.7% was achieved at 52h (Fig. 3A). According to the calculation of water content of 95%, dry mycelium weight was about 17.85 g/L at 52h and was moderate level among that of other study (14.7 g/L, 29.2 g/L, and 12.4 g/L) (Fang and Zhong , 2002a; Habijanic et. al.,2013; Simonić et. al., 2008). The pH value sharply decreased from 0 h to 40 h, and the final pH value was 3.71 (Fig. 3A). Short cultivation time is a very important element for industrial application. The cultivation time of 52h in present study was the shortest compared to those values in the above reports (168h and 336h) (Simonić et. al., 2008; Tang et. al., 2011).

Excessive heavy met. al., content in mycelium from *G. lucidum* do harm to personal health, so it must be controlled in safe range. In previous study, the contents of Pb (3.17 ppm) and As (1.08 ppm) in dried fermented mycelium was obtained using industrial grade solid caustic soda and far exceed food safety standard. The Pb and As contents in those fermentation raw materials were determined to solving the solution, and the results showed in table 1.

Table 1. The contents of Pb and As in different fermentation raw materials(ppm)

Fermentation raw materials	As	Pb
Glucose	0.02	0.16
Sucrose	0.03	0.13
Soybean meal	0.24	0.43
Corn flour	0.04	0.56
Corn steep powder	0.11	0.60
Bean oil	0.03	0.12
Antifoam	0.04	0.31
Industrial grade solid caustic soda	5.12	8.11
Analytical grade solid caustic soda	0.11	0.08

All materials, except for industrial grade solid caustic soda, contained low contents of Pb and As (below 1ppm). Industrial grade solid caustic soda has high contents of Pb (5.12ppm) and As (8.11ppm). The Fig. 3B showed Pb content (3.17 ppm) and As content

(1.08 ppm) in the dried fermented mycelium using industrial grade solid caustic soda was 10.93 and 5.14 folds higher than Pb content (0.29 ppm) and As content (0.21 ppm) using analytical grade solid caustic soda which did not exceed the limits of Chinese national standards (Pb content below 1ppm, As content below 2 ppm).

4. Conclusions

In the work, the different effects of carbon sources, initial pH values and regulation of C/Ns on IPS content, pH value and mycelium concentration were investigated under submerged fermentation of *G. lucidum* in 1500-L fermentor, and then the optimal cultured conditions for IPS content were identified. Subsequently, the highest IPS content of 6.3%, dry weight of 17.85g/L and safe heavy met. al., contents was achieved during incubation of only 52h by amplification fermentation in a 10000-L fermentor under the optimum conditions. The result can promote industrial-level production for mycelium of *G. lucidum*.

5.References

Anonymous. (2005). Pharmacopoeia of People's Republic of China. Committee of Chinese Pharmacopoeia, Chemical Industry Press, Beijing.

Babitskaya, V. G., Shcherba, V. V., Puchkova, T. A., et al. (2005). Polysaccharides of *Ganoderma lucidum*: factors affecting their production. *Applied Biochemistry and Microbiology*, 41(2): 169-173

Chen, Z.J., Gu, Z.X. (2008). Progress of studies on the main active substances and submerged fermentation technology of *Ganoderma lucidum*. *Food Researh and Development*,29(3):186-190

Fang, Q. H., Zhong, J. J. (2002) Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. *Process Biochemistry* , 37(7): 769-774.

Habijanic, J., Berovic, M., Boh, B., et al. (2013). Production of biomass and polysaccharides of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst.(higher Basidiomycetes), by submerged cultivation. *International journal of medicinal mushrooms*, 15(1):81-89

Hsiao,W. L.W., Li, Y. Q., Lee, T. L., et al. (2004). Medicinal mushroom extracts inhibit ras-induced cell transformation and the inhibitory effect requires the presence of normal cells. *Carcinogenesis*, 25(7): 1177-1183.

Kim, H. M., Paik, S., Ra, K. S., et al.(2006). Enhanced production of exopolysaccharides by fed-batch culture of *Ganoderma resinaceum* DG-6556. *Journal of microbiology*, 44(2): 233-242.

Lee, W. Y., Park, Y., Ahn, J. K., et al. (2007) Factors influencing the production of endopolysaccharide and exopolysaccharide from *Ganoderma applanatum*. *Enzyme and Microbial Technology*, 40(2): 249-254.

Mojadadi, S., Ebtekar, M., Hassan, Z. M. (2006). Immunomodulatory effects of Ganoderma lucidum (W. Curt.: Fr.) P. Karst.(aphyllophoromycetideae) on CD4+/CD8+ tumor infiltrating lymphocytes in breast-cancer-bearing mice. *International Journal of Medicinal Mushrooms*,8 (4): 315-320

Simonić, J., Stajić, M., Glamočlija, J.,et. al.,. (2008). Optimization of submerged cultivation conditions for extra- and intracellular polysaccharide production by medicinal Ling Zhi or Reishi mushroom *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllophoromycetideae). *International Journal of Medicinal Mushrooms*, 10 (4): 351-360

Sudheesh, N.P., Ajith, T.A., Janardhanan, K.K. (2009). *Ganoderma lucidum* (Fr.) P. Karst enhances activities of heart mitochondrial enzymes and respiratory chain complexes in the aged rat. *Biogerontology*.10(5):627-636

Tang, Y. J., Zhong, J. J. (2002) Fed-batch fermentation of *Ganoderma lucidum* for hyperproduction of polysaccharide and ganoderic acid. *Enzyme and Microbial Technology* , 31(1): 20-28.

Tang, Y. J., Zhang, W., Liu, R. S., et al. (2011). Scale-up study on the fed-batch fermentation of *Ganoderma lucidum* for the hyperproduction of ganoderic acid and *Ganoderma* polysaccharides. *Process Biochemistry*, 46(1): 404-408.

Xing, Z., Tang, Q., Zhou, C., et al. (2001) Analysis of trace elements and their safety in different *ganoderma* fruit bodies and coarse polysaccharide. *Mycosystema*, 21(1): 107-111.

Xu, L.C., Xu, S.C.(2004) Determination of content of polysaccharides of 18 varieties of *Ganoderma lucidum* karst with different origins. *World Science Technology-Modernization of Traditional Chinese Medicine*,6(2):57-60.

Yang, F. C., Liau,C.B.(1998). The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged cultures. *Process Biochemistry*, 33(5): 547-553.

Yang, H., Min, W., Bi, P., et al. (2013). Stimulatory effects of Coix lacryma-jobi oil on the myceliuml growth and metabolites biosynthesis by the submerged culture of *Ganoderma lucidum*. *Biochemical Engineering Journal*, 76: 77-82.

Yuan, B., Chi, X., Zhang, R (2012). Optimization of exopolysaccharides production from a novel strain of Ganoderma lucidum CAU5501 in submerged culture. *Brazilian Journal of Microbiology*, 43(2): 490-497.

Acknowledgements

This work was financially supported by Ph.D. Accumulation Program in Company of Jiangsu Province in China and Ph.D. Scientific Research Foundation of Huaibei Normal University

ERRATA

In the paper: ANALYSIS AND COMPARISON OF CHINESE AND FOREIGN FOOD SAFETY LEGAL SYSTEM AND THE ESTABLISHMENT OF SCIENTIFIC LEGAL IDEA, by Fanyou Zhou and Shiyan Bai publish in the Carpathian Journal of Food Science and Technology, Volume 7, issue 1, 2015, page 26-31

At the page 26 the line under the author names:

*[1]Northeast Normal University, Changchun, Jilin, 130117, China; *bsyshi@163.com*

Will be replace with:

[1]College of Humanities and Sciences of Northeast Normal University (for both authors) Changchun, Jilin, 130117, China; *bsyshi@163.com