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CONTENT

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DETECTION AND CONTROL OF BIOGENIC AMINE CONTENT AND PHYSICAL/CHEMICAL PROPERTIES BASED ON HPLC METHOD IN FERMENTED FOOD

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ABSTRACT
In this paper, high performance liquid chromatography (HPLC) method for determination of the content of biogenic amines, methods and good stability, high precision, high recovery rate. Fermentation agent is the most important biogenic amines tyramine and histamine categories, because of its physiological toxicity, and cadaverine will strengthen due tyramine and histamine toxicity. Therefore, the reason why the event biogenic amines poisoning often occurs because we are biogenic amines thorough understanding is not enough, but also the lack of fast, efficient detection methods, the paper established HPLC detection of biogenic amines in food type and content of wine , soy sauce biogenic amines were measured and used to strengthen vaccination effectively reduce biogenic amines in fermented sausages content, which improve the quality of life and protect the public food safety has important practical significance.

1. Introduction

Food poisoning and certain toxicological properties of histamine and tyramine are closely linked, and therefore the study of biological amines can enhance and improve food quality and safety (Lu, 2014). China has a large number of hypertensive patients, they eat cheese, yogurt and other high tyramine containing foods, nausea, vomiting, and other symptoms touyunnaozhang This is because high blood pressure in patients with long-term use of reserpine, excellent drop Ning and other antihypertensive drugs, these drugs can inhibit the body monoamine oxidase activity, so food tyramine not decompose, accumulate in the body, more and more strongly stimulated the peripheral vascular system, causing indirect effects of sympathomimetic amines, vascular resistance increased, so more and more high blood pressure (Muthurs, 2014; Kim, 2014). In severe cases, intracranial hemorrhage and coma and even death. Clinical studies can inhibit monoamine oxidase in vivo as well as anti-TB drug isoniazid, anti-tumor methyl thousand navel, styrene solution antidepressant and anti-inflammatory bacteria hang furazolidone tablets (Zarifi, 2014).

Use fermenting agent in the production of fermented sausages vital leaven quality is directly related to the quality of fermented sausage products, if you choose an inappropriate leaven production of fermented sausage, the product quality is likely worse than manual irrigation system, natural fermentation sausage (Yu, 2014). Current domestic production of fermented sausages imported multi-use commercial fermentation agent, but imports of commercial starter cultures may be due to the traditional Chinese sausage production technology and raw meat

sausage incompatibility can not become the dominant bacteria causing sensory quality reduction (Leroy, 2014). Therefore, based on Western-style fermented sausages, combined with China's traditional fermentation technology, the use of traditional meat products selected from the strain out of production for Chinese-style fermented sausage is worthy of further study (Thakur, 2015; Sunano, 2015).

Biogenic amines are a class of nitrogen-containing aliphatic, aromatic or heterocyclic compounds of low molecular weight, it is essential for the biological activity of cellular components, but biogenic amines have potential toxic effects, when accumulated to a certain extent the body will produce toxicity, so far in food research has become a hot issue of biogenic amines. In fermented sausages, biogenic amines is an important factor affecting the safety of dry fermented sausages, at home and abroad in recent years for the study of biogenic amines in fermented foods are deeply concerned. In this study, the content of biogenic amines index, from this laboratory strain deposited preferred high-quality security, for fermented sausage production strains, and strains were identified; In the preferred mixed culture of bacteria and strains of a single production of fermented sausage products and with the commercial production of fermented sausage products business fermenting agent quality compared to a suitable fermentation of a preferred agent in the production fermented sausage, fermented sausage industrial production basis.

2. Materials and methods

2.1. Biogenic amines and their physiological significance

Biogenic amines are a class of nitrogen-containing aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, serotonin) low molecular weight compounds, mainly through amino acid decarboxylase or aldehyde and ketone amine and transamination formation (Barbosa, 2014). Animals, plants and micro-organisms living cells have an important physiological role, the amount of biogenic amines contribute to the body's normal physiological function, the excess will cause adverse physiological reactions that can lead to high blood pressure, headache, his face flushing, rash. Sometimes mainly gastrointestinal disorders, including sudden vomiting and diarrhea, accompanied by abdominal pain and other symptoms, biogenic amines are often produced in the food rotting or fermenting process (Kang, 2015).

Metabolic pathways generated by biogenic amines was shown in Figure 1, the most common monoamines (histamine, tyramine and serotonin), diamine (putrescine and cadaverine), histidine, tyrosine, tryptophan, ornithine, and lysine, polyamines (spermine and spermidine) is formed by putrescine. biogenic amines not only generate hormones, nucleic acids, proteins and other substances precursors, but also generate nitroso carcinogens and substances precursors. Monoamine compound has obvious blood vessels and muscle relaxation or contraction, can inhibit epileptic seizures, mental activity and cerebral cortex plays an important role in the regulation of the heart have different degrees of positive inotropic and positive-frequency effects. Polyamines organism during growth, can promote the synthesis of DNA, RNA and protein, to accelerate growth and development of the organism, it is essential for the biological activity of cellular components, they have an important in regulating protein synthesis and function of nucleic acids role, biofilms may also be related with the stability. Due to the diversity of their roles in cell metabolism and growth, so they need to have a great amount of rapid growth in the organization. All cells can be synthesized by the use of external resources polyamines.

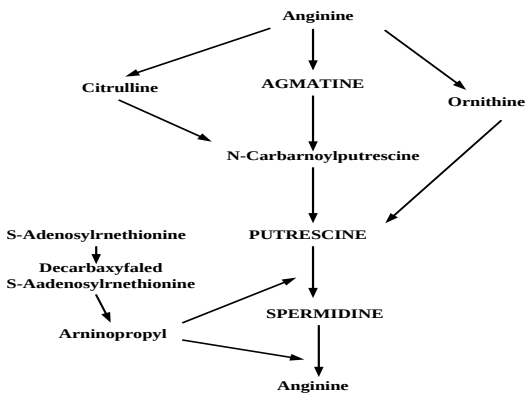

Figure 1. Metabolic pathways generated by biogenic amines

2.2. Preferred strains fermented sausages

In fermented sausage production process, the role of lactic acid bacteria play an important role, but not all are suitable for use as meat lactic acid fermentation agent. If the lack of meat in a competitive environment, some affect the sensory quality of meat, and some affect the safety of meat quality, in fermented foods and beverages, the use of leavening agents can affect biogenic amines produced by different interaction between microflora can be directly or indirectly affected. Therefore, strains used as fermenting agents screening criteria should include an analysis of the situation to produce biogenic amines.

Weigh accurately tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine each 50 mg, 0.4 mol than with perchlorate volume to 50 mL, made 1 mL reserve liquid reserve. Were taken over standard stock solution with 0.4mol than $HClO_4$ formulated into a final concentration of mixed standard solution 0.5, 1.0, 2.0, 5.0, 10, 20mL, the use of aluminum foil in the dark, refrigerator. The column was Asilent ZORBAXXDB-C18 (250 mm x 4.6 mm, 5 μm), the detection wavelength was 254 nm, injection volume 20 μL, column temperature 30°C, mobile phase A and water, using the gradient elution program displayed in the Table 1.

Table 1. Gradient elution program

Elution time/min	Mobile phase A/%	Mobile phase B/%
0.0	35.0	65.0
5.0	31.0	70.0
20.0	0.0	101.0
24.0	0.0	101.0
25.0	38.0	67.0
30.0	37.0	67.0

2.3. The main media

MRS medium: Peptone 10g, beef extract 10g, yeast extract 5g, hydrogen citrate diamine 2g, glucose 20g, Tween 801mL, sodium acetate, 5g, dipotassium hydrogen phosphate 2g, magnesium sulfate 0.5g, manganese sulfate 0.25g, distilled water 1000 mL, pH value 6.2-6.4, 121°C sterilization for 20min.

MS Medium: trypsin Chen 10g, beef extract 1 g, sodium chloride 75g, 1% phenol red reagent 2.5ml, D- mannitol 10g, distilled water 1000ml, PH value 7.2-7.4, 122°C sterilization for 20min.

Underlying medium: pancreatic peptone 0.5%, yeast extract 0.5%, beef extract 0.5%, 0.25% sodium chloride, glucose, 0.05%, 0.02% magnesium sulfate, 0.005% manganese sulfate, ferrous sulfate 0.04%, citric acid by 0.020 % vitamin B6 0.005%, 1.0% amino acids, 0.2% dipotassium hydrogen phosphate, calcium carbonate, 0.01%, agar 2.0%, pH 5.5.

Standards of biogenic amines: tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine Spermine, Dan sulfonephthalein chlorine (Sigma Aldrich Company).

HPLC grade acetonitrile and acetone; ammonia, perchloric acid, sodium hydroxide, sodium bicarbonate, analytical grade; L a tyrosine, histidine, lysine and biochemical reagents; homemade ultra pure water.

Acetonitrile: Shandong Yuwang Industrial Co., chromatography; Acetone: Nanjing Datang Chemical Co., chromatography; Ammonia: Nanjing Chemical Reagent Factory, AR; Perchlorate: Tianjin Xinyuan Chemical Plant, AR; Sodium hydroxide: Nanjing Chemical Reagent Factory, AR; Sodium bicarbonate: Nanjing Chemical Reagent Factory, AR; Wahaha purified water: purchased in the supermarket.

2.4. The main instruments and equipment

SW-CJ-l-type clean bench: Su net Group Suzhou Aetna; LDX a 50KBS vertical automatic electric pressure steam sterilizer: Shanghai Shen An Instrumentarija; HZO a high and low temperature oscillation incubator F160A: Shanghai Yuehua Instrument Co., Ltd.;

MDL9000 (B) -H-30-based desktop laboratory ultrapure water systems: Nanjing total Hing water equipment company; 2004MP analytical balance: STARIOUS; Desktop high-speed refrigerated centrifuge: Beckman Company; HPLC System: Waters Corporation USA.

3. Results and discussions

3.1. Qualitative analysis of the strains

In this experiment, B Lotus (double plate method) strains produce an amine preliminary analysis of the situation. As can be seen from Table 2, separated from the Dong acid meat production TD3 a 7 tyramine, ZJ3 a 4 tyramine and serotonin production. Only from qualitative analysis, staphylococcus AL> 3 can be used as an excellent safety fermenting agent. However, since some strains produce amines weak, reach BAP method detection limit, so the next step to make quantitative analysis.

Table 2. Strains were used and their activity of producing BA

Bacterial strain	Source	Tyramine	Histamine	Cadaverine
TD3-1	Dong Sour Meat	.	.	.
TD3-7	Dong Sour Meat	+	.	.
ZJ3-4	Dong Sour Meat	+	·	+
ZJ3-8	Dong Sour Meat	.	.	.
ALX3	Chinese mushroom	.	.	.

Figure 2 shows that the initial fermentation (0d), fermented group (A, B, C, D, E) of the total number of bacteria between 107-107.9, while the total number of bacteria in the control group F group was 10. By the end of the fermentation (2d), the total number of bacterial fermentation group in 8.0lg cfu / g between a 9.0 lgcfu/g, while the control group, the total number of bacteria increased 7.6 1gcfu/g and at 7d reaches a maximum and then decreased slowly. to the production end (28d), the total number of bacteria in the test group 7.619cfu/g - 8.319cfu/g, the difference between the experimental groups was not significant ($P<0.05$), while the control group, the total number of bacteria was 7.0 lgcfu/g. The results showed that in the control group throughout the production process the total number of aerobic mesophilic bacteria was significantly lower in the experimental group ($P <0.05$). Lactic acid bacteria and staphylococcus as the application leaven fermented sausages can be accelerated.

Sausage initial *E. coli* contamination from slaughter and segmentation as well as the use of appliances. Similar in all groups *Enterobacteriaceae* initial number of 4.0lg cfu/g a 5.0 lgcfu/g. But in the process of maturation of each group (A between B, C, D, E, F) change

significantly different (P <0.05). At the end of fermentation (2d), F group compared with the control group, mixed fermentation agent B, group C decreased significantly, respectively 3.5 lgcfu/g and 3.7 lgcfu/g. may be due to lactic acid bacteria produce lactic acid fermentation agent group classes to accelerate the rapid decline in the value of B, the demise of the *Enterobacteriaceae* group C, and the other may be lactic acid bacteria produce bacteriocins, thus inhibiting intestinal bacilli growth.

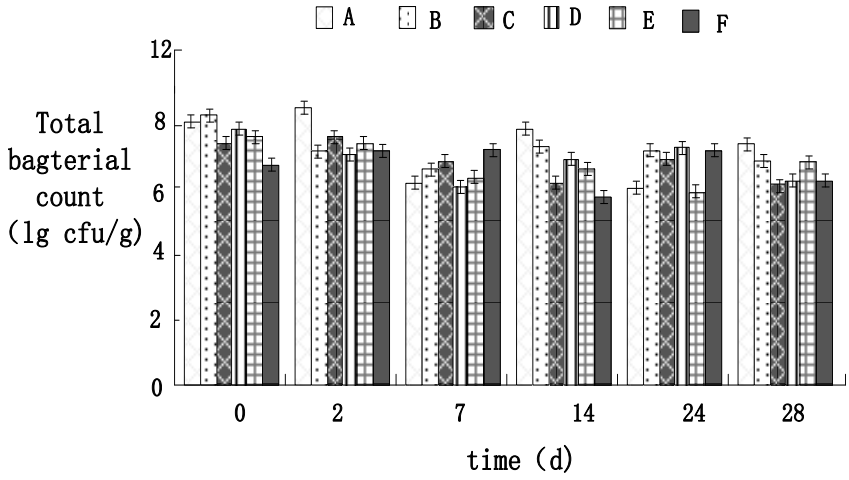

Figure 2. Different ferment fermented sausages during ripening total bacteria change

3.2. Different biogenic amines standard curve

In Figure 3, the standard curve biogenic amines can be seen, eight kinds of biogenic amines and their corresponding concentrations peak area was linear correlation coefficients were greater than 0.99. By this method can accurately measure the content of biogenic amines in the sample.

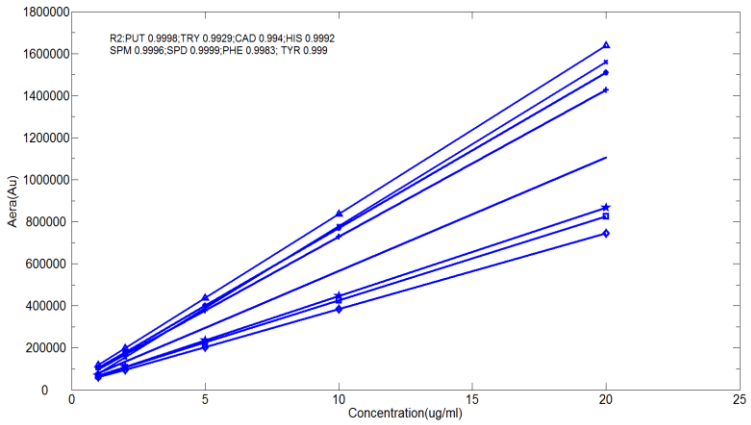

Figure 3. Standard curves of different levels of biogenic amines

Fermentation agent is the most important biogenic amines tyramine and histamine categories, because of its physiological toxicity, and cadaverine will strengthen due tyramine and histamine toxicity. Therefore, in this study, tyramine, histamine and cadaverine as standard strains qualitative analysis was an amine. In this experiment, all isolates are not producing histamine, may be due to these strains do not carry histidine decarboxylase, histamine may be too little, not enough to make flat-panel color

change, it needs to do next quantitative analysis of biogenic amines.

This part of the study was determined by high performance liquid chromatography five strains strains tryptamine, a phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine amount. ALX3 not produce an amine, was identified, respectively, sausage *Lactobacillus, Leuconostoc* bacteria and *Staphylococcus* xylose, these three bacteria are common bacteria can be used as a leavening agent, and from fermented meat products more suitable for use as ferment fermented sausages.

Biogenic amine content of the fermentation process changes the situation shown in Figure 4. The results can be seen: raw meat itself with phenylethylamine, cadaverine, spermidine, spermine, and increased in the fermentation process, and raw meat substantially free of putrescine, histamine, tyramine.

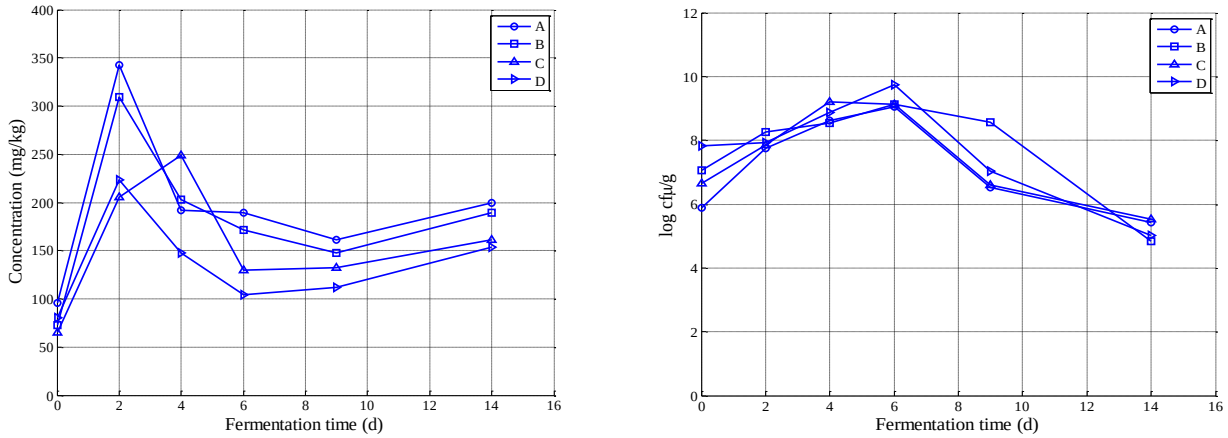

Figure 4. Biogenic amine content of the fermentation process changes the situation

Effect of putrescine and cadaverine and other secondary amines and other vascular amines (tyramine, histamine, tryptamine, phenylethylamine) is different, there is no adverse human health, but they are able to inhibit detoxification enzymes: diamine oxidase and light-methyl-converting enzyme activity and the potential impact of histamine content. Furthermore, the microbiological quality of their accumulation and products declined linked histamine toxicity in the presence of their increasing trend. From another point of view, it can also be considered biogenic amines are carcinogenic, as secondary amines can react with nitrite and are likely to form carcinogenic N-nitrosamines, because primary amine can be converted into a secondary amine, so not only heat, but also can be stored at room temperature the amine is further reacted with a nitrite.

3.3. Colony counts and pH change

Currently, sausage microbial fermentation agent commonly used are: bacteria (*Lactobacillus, Micrococcus, Staphylococcus*), yeasts and molds. The results (Figure 5 and Figure 6) can be seen, the number of lactic acid bacteria, total bacteria are showing a downward trend after the first rise. The first decline in six days a lot of lactic acid bacteria breeding trends and the corresponding pH value is, which is due to lactic bacteria break down carbohydrates produce a result of lactic acid. When the pH value of the product fell to its lowest value, along with the continuous loss of water, NaCI mass fraction relative increase, such an environment is no longer suitable for the rapid growth of lactic acid bacteria.

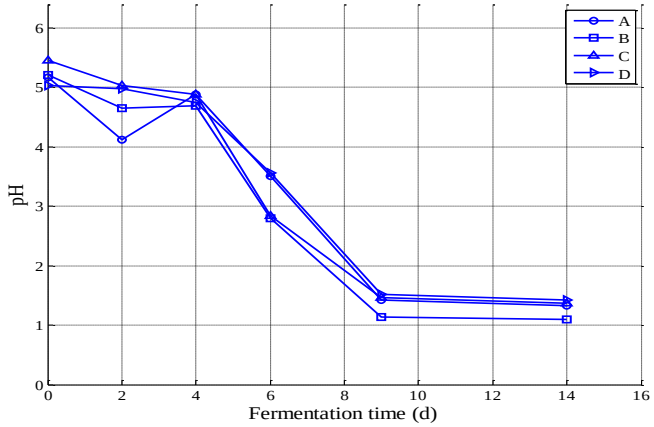

Figure 5. Different treatments in the fermentation process of change in the total number of bacteria

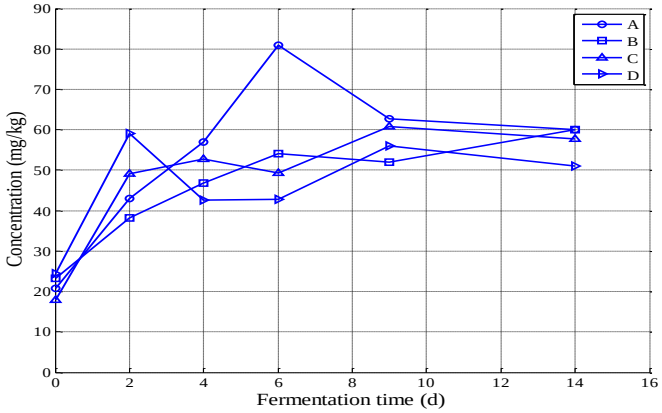

Figure 6. Sausage fermentation process pH changes

Therefore, the number of colonies of lactic acid bacteria is no longer a significant increase in pre-lactic acid fermentation bacteria quickly developed into advantages, the number of whole fermented sausage production process is much higher than bacteria, very close to the total number of bacteria, lactic acid is therefore the production of fermented sausages dominant bacteria and the final flavor of fermented sausages produced plays an important role. However, some species of lactic acid bacteria is to produce an amine, and as such, decarboxylase activity isolated from fermented sausages out of lactic acid bacteria have been studied extensively.

Between the same type of product, the content of biogenic amines are very different, leading to many reasons for this change. The process and differences can lead to other conditions there of biological fermented sausage different amine content. To prevent or reduce the formation of biogenic amines in fermented sausages need to proceed in terms of raw meat, leavening agents and process conditions, etc., that use raw meat, do not produce amines ferment and leaven strains were grown in favor of process conditions to control and reduce the amount of amine.

3.4 Physical indicators

Color and flavor of the meat important sensory indicators for evaluating color quality of

fermented sausages important sensory index, is the first impression that people on food quality evaluation and consumer appetite and desire to buy has a great influence from Table 3 can be A, B, C, D, E group luminance values, red values were significantly higher than other groups protected, yellowness value lower than the other groups, and the difference is significant. this may be because B, C, D, E group as a leavening agent added aureus, Staphylococcus nitrate reductase and catalase, the formation of sausage color plays an important role from the color analysis results, improved red Staphylococcus added value products and brightness values, improving the sausage color, improve the overall acceptability of the product.

Table 3. Color analysis of fermented sausages manufactured by different starter cultures

Process	L^*	a^*	b^*
A	33.71±0.94b	12.17±0.44b	6.05±0.34b
B	39.86±3.94a	15.23±13.68a	4.77±0.49c
C	36.82±3.57ab	15.75±1.15a	4.97±0.30bc
D	37.88±1.94ab	13.97±3.19ab	4.71±0.69c
E	33.56±2.54b	13.03±0.30b	5.60±0.54bc
F	34.08±1.21b	11.55±0.94b	8.20±0.94a

Means within column with different letters were significantly different ($P<0.05$).data were shown as mean SD.

4. Conclusions

In this study, tyramine, histamine and cadaverine as standard strains qualitative analysis was an amine. The number of colonies of lactic acid bacteria is no longer a significant increase in pre-lactic acid fermentation bacteria quickly developed into advantages, the number of whole fermented sausage production process is much higher than bacteria, very close to the total number of bacteria, lactic acid is therefore the production of fermented sausages dominant bacteria, and the final flavor of fermented sausages produced plays an important role. However, some species of lactic acid bacteria is to produce an amine, and as such, decarboxylase activity isolated from fermented sausages out of lactic acid bacteria have been studied extensively.

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VEGETABLE FARMERS' USE INTENTION TOWARDS BIOPESTICIDES AND ITS INFLUENCING FACTORS: BASED ON THE SURVEY IN JIANGSU PROVINCE, CHINA

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ABSTRACT

To understand the vegetable farmers' use intention towards biopesticides and its influencing factors, a survey was conducted in Nanjing, Yangzhou, and Xuzhou city, Jiangsu province, China. Penalized logistic regression model was used to analyze. The results showed that the vegetable farmers had low cognitive level of biopesticides, and 68.63% of them would like to use biopesticides if they vary little with chemical pesticides on controlling effect. The farmers with higher education level, higher cognitive level of chemical pesticide residues or biopesticides, smaller vegetable planting area, or vegetable planting years less than 10, as well as under 40 years old, were more willing to use biopesticides. Meanwhile, biopesticide price, government supervision of pesticides, and skill training on biopesticide use significantly influenced the use intention towards biopesticides. Therefore, some suggestions were made, such as developing those biopesticides that take effect promptly and are easy to use, training farmers on biopesticide use and subsidizing those farmers who use biopesticides.

1. Introduction

The planting area of vegetables in China have increased to more than 21 million hm^2, with annual outputs over 700 million tons, occupying the first place in the world (FAO, 2013). Meanwhile, vegetables, with per capita consumption over 500 kg a year (NBSC, 2014), play an important role in food consumption of Chinese. However, using pesticides repeatedly while planting vegetables, combined with short interval between pesticide use and vegetable harvest, may lead to excessive levels of pesticide residues in vegetables. These poisons can enter and accumulate in human body through diet, and pose great threats to human health (Khawaja, 2001).

Biopesticide, a kind of product using living organisms or their preparations to control crop diseases and pests, is characterized by low toxicity and good environmental compatibility. It may be an alternative for chemical pesticide to improve quality safety of agricultural products. To reduce pesticide residues at the source, it is essential to widely use biopesticides in vegetables. Owing to high price, slow efficacy, and high use skill, the application of biopesticides proves to be very difficult. Because the generalization of biopesticides depends on the use intention of vegetable farmers, it is very important to study the use intention and its influencing factors. This may be helpful to provide some policy suggestions on the acceleration of biopesticide application.

2. Materials and methods

2.1. Literature review

Biopesticides have been attracting extensive attention from governments and

academic communities. Currently, the focus is mainly on two aspects. One is the introduction of biopesticides from the macro perspective. Ma et al. (2011) introduced application status of various biopesticides in China and Zheng (2006) analyzed competitiveness of biopesticide industry from the perspective of economics. Uri (1998) explored the development and application of biopesticides in America, which remarkably affected consumers' preference for green agricultural products, as well as government's multiple agricultural decision-making in the fields of pesticide supervision, crop planting, and ecosystem protection, etc. However, Skovmand (2007) pointed out that biopesticides in Southeast Asia lacked of competitiveness because of high cost and low efficacy. The other is different types of vegetable farmers' biopesticide-use intention or behavior studied from the micro perspective. Adetonah et al. (2007) analyzed whether cotton farmers in western Africa were willing to apply biopesticides to effectively control cotton bollworm. Doss and Morris (1999) pointed out the striking effect of biopesticide use in corn planting in Ghana. Zhang et al. (2004) concluded that the characteristics of farmer population and cultivated land, and knowledge of farmers on pesticides were obvious factors affecting farmers' use intention towards biopesticides.

2.2. Research hypotheses

In summary, biopesticide use by farmers has been studied systematically, but there is little information on the use intention of Chinese vegetable farmers towards biopesticides. Based on previous findings on biopesticide use, along with actual situation of vegetable planting industry in China, this paper classifies the main factors influencing farmers' use intention towards biopesticides, and then makes some hypotheses as follows.

2.2.1. Individual characteristics of farmers

It mainly includes three factors: gender, age, and education level. When choosing the pesticides, females paid more attention to their own health than males, while males were more normative and rational than females (Wu et al., 2011). Biopesticide is a new type of pesticide. Because younger and higher-educated farmers are more easily to accept new concepts, they were more willing to use biopesticides (Zhu et al., 2012). Based on the above statements, it can be hypothesized that:

[H_1]: Individual characteristics of vegetable farmers will affect their use intention towards biopesticides.

2.2.2. Planting characteristics of farmers

It mainly includes four factors: planting area, planting years, the purpose of planting vegetables and the proportion of vegetable income to total. Vegetables from farmers with large planting area usually reach high degree of commercialization. If adopting biopesticides, those farmers will take higher risk from technology and market, thus they were more inclined to use chemical pesticides that they were familiar with (Ngowi et al., 2007). In addition, in the study on the use intention of the rice farmers in Sichuan province, China towards biopesticides, Fu and Song (2010) found that planting years and area of rice exerted significant influence on farmers' use intention. Based on the above statements, it can be hypothesized that:

[H_2]: Planting characteristics of vegetable farmers will affect their use intention towards biopesticides.

2.2.3. Farmers' cognition on pesticides

It mainly includes two factors: farmers' cognition on chemical pesticide residues and biopesticides. Wang et al. (2015) studied farmers' choice behavior on pesticide use, and found that the more they knew about the degree of the harm caused by chemical pesticide residues, the more likely they would choose non-polluted pesticides. Meanwhile, the more the farmers knew about biopesticides' friendly features such as safety, low toxicity and free pesticide residue, the higher the use intention towards biopesticides (Fu and Song, 2010). Based on the above statements, it can be hypothesized that:

[H_3]: The cognitive level of vegetable farmers on chemical pesticide residues and biopesticides will affect their use intention towards biopesticides.

2.2.4. Market factors

It mainly includes three factors: pesticide price, vegetable price and safety test. Amaza and Ogundari (2008) researched the intention of the soybean farmers in Nigeria to use new technology, and the result showed that the price of soybean remarkably improved farmers' use intention towards biopesticides. Ma and Yang (2011)

suggested that the price of pesticides and rice had great influence on the intention of the farmers in Jiangsu Province, China to use non-polluted pesticides. Ngowi et al. (2007) found that to pass the safety tests on pesticide residues before vegetable sale, the farmers in Tanzania were active in using biopesticides. Based on the above statements, it can be hypothesized that:

[H_4]: Market factors will affect vegetable farmers' use intention towards biopesticides.

2.2.5. Government factors

It mainly includes three factors: pesticide supervision, skill training on pesticide use, and agricultural subsidy. According to Ngowi et al. (2007), strict supervision of Tanzanian government on pesticides and skill training on pesticide use could dramatically enhance farmers' use intention towards biopesticides. Ma and Yang (2011) also suggested that national laws and regulations for agricultural products could restrict farmers' use behavior on pesticides, and improve their use intention towards non-polluted pesticides. Hruska and Corriols (2002) researched corn farmers' use intention towards pesticides in Nicaragua, and the result showed that skill training on pesticide use and agricultural subsidy to farmers who used biopesticides could dramatically stimulate their enthusiasm in using biopesticides. Based on the above statements, it can be hypothesized that:

[H_5]: Government supervision of pesticides, skill training on pesticide use and agricultural subsidy will affect vegetable farmers' use intention towards biopesticides.

2.3. Survey design

Based on the results of previous researches and the purpose of this study, the questionnaire was designed to investigate on the intention and behavior of vegetable farmers to use biopesticides. It mainly included three parts: farmers' basic characteristics, the knowledge of biopesticides as well as use intention and behavior. The questionnaire was modified according to the result of the pre-research, and then sent out.

Formal survey was conducted in April, 2015, and stratified and random sampling was used to select samples. Because the purpose of this study was to investigate vegetable farmers' use intention towards biopesticides, and whether pesticides have been used by farmers or not may directly influence the result of this study, the respondents who have used pesticides were selected.

Jiangsu is a large province of vegetable planting with the area of 1.287 million hm^2, ranking third in China (Fan, 2007). As the three regions, southern, middle, and northern Jiangsu province, have distinct economic development levels, three vegetable planting districts including Liuhe District of Nanjing, Feng County of Xuzhou and Hanjiang District of Yangzhou were chosen to represent the developed, relatively developed and under-developed region, respectively. Then two towns were selected randomly in each district, two villages in each town, and 20 farmers in each village.

In view of farmers' low education level, the face-to-face interview was conducted to guarantee sufficient comprehension of the interviewees on the questionnaire, so to ensure authenticity of' the intention and behavior of vegetable farmers to use biopesticides. A total of 240 questionnaires were distributed to the farmers, and finally 204 valid ones were obtained.

2.4. Questionnaire analysis

2.4.1. Sample characteristics

Table 1 shows the basic information of the respondents. The total of 138 males and 66 females participated in this investigation, accounting for 67.65% and 32.35%, respectively. Farmers' age concentrated in 40-49 and 50-59, which accounted for 30.39% and 38.24%, respectively. The education level of the respondents was relatively low. There were 76.96% of farmers with junior high school education level or below, and only 7.84% with junior college degree or above. The proportion of the respondents with vegetable planting area less than 5 mu was 72.55%, and that of the respondents with vegetable income occupied less than 50% of household income was 52.94%, which suggests that vegetable income is not their major income source.

2.4.2. Statistics on vegetable farmers' cognitive level of and use intention towards biopesticides

Vegetable farmers from Jiangsu province mainly used chemical pesticides such as herbicide, microbicide and caricide to control insects and diseases. There were 138 respondents having no knowledge of biopesticides, 26 having a little knowledge and only 8 having rich knowledge, which takes up 67.65%, 12.75% and 3.92%,

respectively. In addition, 64.22% (131) of the respondents doubted the efficacy of biopesticides, and only 28.43% (58) suggested that they have ever used or are using the biopesticides. Given that there were little difference in the efficacy on insect and disease controlling between biopesticides and chemical pesticides, 68.63% (140) of the responders showed willingness to use biopesticides.

2.5. Model choice and variable setting

2.5.1. Model choice

Probabilistic model is an ideal estimation approach to discrete choice analysis. When the discrete value of dependent variable belongs to two types, binary probabilistic model should be applied (Lin, 2000). Because the type of the dependent variable is 0-1, and most of the independent variables are also the same type in this paper, the binary logistic model is applied to the regression parameter estimation. $\boldsymbol{Y_i}$ as the dependent variable refers to farmers' use intention towards biopesticides, and if the ***i***th farmers shows willingness to use biopesticides, $\boldsymbol{Y_i} = 1$, otherwise, $\boldsymbol{Y_i} = 0$. Detailed expression is as below:

$$\Pr ob(Y_i = 1 | X_i, B_i) = \Lambda_i = \left\{1 + \exp(-\sum_{r=1}^{n} B_r X_r)\right\}^{-1} \quad (1)$$

Table 1. Basic characteristics of vegetable farmers

Test variable	Grouping variable	Sample size	Percentage
Gender	Male	138	67.65
	Female	66	32.35
Age	20-29	6	2.90
	30-39	18	8.82
	40-49	62	30.39
	50-59	78	38.24
	≥ 60	40	19.61
Education level	Primary school or below	57	27.94
	Junior high school	100	49.02
	Senior high school	31	15.20
	Junior college	14	6.86
	Bachelor or above	2	0.98
Vegetable planting area	< 1 mu	41	20.10
	≥ 1 and < 5 mu	107	52.45
	≥ 5 and < 9 mu	42	20.59
	≥ 9 mu	14	6.86
Vegetable planting years	≤ 5	55	26.96
	6-10	73	35.78
	11-20	45	22.06
	≥ 21	31	15.20
Proportion of vegetable income to total	< 30%	33	16.18
	≥ 30% and < 50%	75	36.76
	≥ 50% and < 70%	42	20.59
	≥ 70%	54	26.47
Purpose of planting vegetable	Self-consumption	62	30.39
	Selling	142	69.61

In formula (1), ***Prob*** refers to the possibility when the dependent variable equals to 1, $\boldsymbol{B_r}$ is the estimated parameter, and $\boldsymbol{X_r}$ is the independent variable. Corresponding log-likelihood function and score equation are as below:

$$lnL = \sum_{i=1}^{n} [Y_i \ln \Lambda_i - (1 - Y_i) \ln(1 - \Lambda_i)] \quad (2)$$

$$U(B_r) = \partial \ln L / \partial B_r = \sum_{i=1}^{n} \left[\left(Y_i - \Lambda_i \right) X_{ir} \right] \quad (r = 0,1,2,\cdots,15) \quad (3)$$

The basic sample size of binary logistic model is 100, and 50 samples should increase with every additional variable (Spicer, 2005); therefore at least 800 samples were needed for 15 variables in this study. If we use formula (3) to estimate parameter under the case of small sample volume, the model would be downward bias and separation could easily occur (Albert and Anderson, 1984). So under such condition, binary logistic regression can not be used alone. To avoid separation of the model and ensure the objectivity and validity of the result, according to Heinze and Schemper's advice (2002), $\boldsymbol{Y_i}$ and $\boldsymbol{(1 - Y_i)}$ were given the weight, and the penalty function was added to the log-likelihood function.

$$lnL^* = lnL + 1/2 ln | I(B) | \quad (4)$$

Wherein, $\boldsymbol{I(B)}$ represents information matrix, $\boldsymbol{|I(B)|}$ represents determinant of information matrix. The formula (3) can be modified as follows:

$$\begin{aligned} U(B_r)^* &= \sum_{i=1}^{n} \left[Y_i - \Lambda_i + h_i (1/2 - \Lambda_i) \right] X_{ir} \\ &= \sum_{i=1}^{n} \left[(Y_i - \Lambda_i)(1 + h_i / 2) + (1 - Y_i - \Lambda_i) h_i / 2 \right] X_{ir} \\ &(r = 0,1,2,\cdots,15) \end{aligned} \quad (5)$$

Wherein, h_i is the ith element on the principal diagonal of H ($H = W^{1/2}X(X'WX)X'W^{1/2}$, X is independent variable matrix, $W=diag[L_i(1- L_i)]$). The problem of model separation is solved by adding the weight of $(1 + h_i/2)$ and $h_i/2$ to Y_i and $(1 - Y_i)$, respectively.

2.5.2. Variable setting

The factors influencing vegetable farmers' use intention towards biopesticides were defined as 15 variables according to preceding hypotheses (see Table 2).

3. Results and discussions

3.1. Results

The formula (5) was dealt with Newton-Raphson iteration method by Matlab. The iterative equation was as follows:

$B^{(S+1)} = B^{(S)} + I^{-1}(B^{(S)})U(B^{(S)})^*$, wherein S means the Sth iteration. Table 3 shows relevant parameter estimation results of penalized logistic regression model.

Table 2. Variable table of hypothetical model

Variable	Symbol	Definition and assignment	Mean value	SD
Use intention	Y	Whether farmers are willing to use biopesticides or not, yes = 1, no = 0	0.6863	0.4491
Characteristics of farmer				
Gender	GEND	Dummy variable, male = 1, female = 0	0.6765	0.4690
Age	AGE	Dummy variable, 40 or older = 1, otherwise = 0	0.8824	0.3230
Education level	EDU	Dummy variable, junior high school or above = 1, otherwise = 0	0.7206	0.4498
Characteristics of planting				
Planting area	AREA	Dummy variable, 5 mu or larger = 1, otherwise = 0	0.2745	0.4474
Planting years	YEAR	Dummy variable, 10 or longer = 1, otherwise = 0	0.3725	0.4847
Purpose of planting	PURP	Dummy variable, for selling = 1, otherwise = 0	0.6961	0.4641
Proportion of vegetable income to total	INC	Dummy variable, 50% or higher = 1, otherwise = 0	0.4706	0.5004
Cognition of pesticides				
Pesticide residue	PESL	Dummy variable, know harm of pesticide residue = 1, otherwise = 0	0.6912	0.4631
Biopesticide	BIPE	Dummy variable, know biopesticide = 1, otherwise = 0	0.3529	0.4791
Market factors				
Biopesticide price	PEPR	Dummy variable, consider high = 1, otherwise = 0	06421	0.4805
Vegetable price	VEPR	Dummy variable, think that vegetable price is higher after using biopesticides = 1, otherwise = 0	0.5294	0.5004
Safety test	SEL	Dummy variable, think that the vegetable is easier to pass the safety test after using biopesticides = 1, otherwise = 0	0.5735	0.4958
Government regulation				
Pesticide supervision	SUP	Dummy variable, strict supervision on pesticides = 1, otherwise = 0	0.6225	0.4859
Training on pesticide use	TRA	Dummy variable, farmer has undergone skill training on pesticide use = 1, otherwise = 0	0.3627	0.4820
Agricultural subsidy	SUB	Dummy variable, government supplies subsidy to farmers for biopesticide use = 1, otherwise = 0	0.8137	0.3903

The education level of farmers, vegetable planting area, purpose of planting vegetables and biopesticide price had significant impact on the use intention towards biopesticides ($P < 0.05$). While, farmers' cognition on pesticide residues had more significant impact ($P < 0.01$) than the former, and other seven variables including farmer age, planting years, proportion of vegetable income to total, farmers' cognition on biopesticides, safety test, government supervision on pesticides and skill training on pesticide use had less significant impact ($P < 0.10$).

Among those variables, six of them, namely the education level of farmers, farmers' cognition on pesticide residues, farmers' cognition on biopesticides, safety test, government supervision on pesticides and skill training on pesticide use were positively correlated with farmers' use intention towards biopesticides. While planting area, planting years, the purpose of planting vegetables, proportion of vegetable income to total and pesticide price were negatively correlated with the use intention.

3.2. Discussions

Among the variables reflecting farmer's personal characteristics, age exerted a remarkable negative impact on the use intention towards biopesticides ($P < 0.10$). This demonstrates that farmers older than 40 are less likely to use biopesticides than those younger than 40. It is similar to the result of Zhu et al. (2012), but inconsistent with that of Ma and Yang (2011). It is probably because that the elder relies much on his own experience obtained from practice on insect and disease controlling, therefore, the degree of accepting new type of biopesticides is relatively low. However, the education level exerted a remarkable positive impact on the use intention ($P < 0.05$). This demonstrates that farmers with high education level may easily master the skills of biopesticide use, which can help lower the technical risks of biopesticide use and then enhance the farmer's use intention.

Table 3. Results of penalized logistic regression model

Variable	B	Penalized LR	P-value	O.R.
GEND	0.5060	3.3792	0.1435	0.4287
AGE*	-0.9641	4.6103	0.0622	0.6223
EDU**	1.0834	9.0256	0.0375	3.9204
AREA**	-0.5022	5.4453	0.0199	0.9981
YEAR*	-0.9823	2.1581	0.0842	0.3756
PURP**	-0.3297	6.1552	0.0469	1.2174
INC*	-0.5040	3.4912	0.0620	0.4302
PESL***	0.8077	5.0339	0.0003	2.0804
BIPE*	0.6930	7.7141	0.0792	1.5353
PEPR**	-0.8244	2.9783	0.0384	2.3257
VEPR	0.6105	1.0503	0.8239	0.3561
SEL*	0.3038	4.0098	0.0922	1.0392
SUP*	0.5199	3.1882	0.0664	0.8193
TRA*	0.3759	1.4814	0.0724	0.7136
SUB	0.4351	8.5778	0.3448	0.2704
CONT	-0.5290	2.7371	0.3905	-
Omnibus tests: Penalized LR = 208.9960, df = 15				
Goodness of fit: Pseudo $R^2 = 0.4725$				

***P < 0.01, **P < 0.05, * P < 0.10.

Among the variables reflecting farmer's plant features, planting area and years, the purpose of planting vegetables and proportion of vegetable income to total had negative impacts on farmers' use intention towards biopesticides. For farmers who possess large planting area and high income ratio were less likely to use biopesticides than those of opposite counterparts. It is possibly because that the larger area one possesses, the higher commercialization of vegetables, and the farmers will be more dependent on vegetable income. This will give rise to the higher economy and market risks faced by farmers. Because biopesticides itself lack of fast and efficient controlling on insect and disease, farmers with large planting area and high income ratio are more likely to use better chemical pesticide that they are familiar with, which is out of pursuing economic interest. Therefore, their use intention towards biopesticides decreased. It is consistent with the conclusion of Amaza and Ogundari (2008) and Ngowi et al. (2007). Meanwhile, farmers with less 10-year-planting experience were more likely to use biopesticides. It is probably because that farmers with less

experience are much younger and more willing to try new technology. This enhances their use intention towards biopesticides.

Vegetable farmers' cognition on pesticide residues significantly influenced farmers' use intention towards biopesticides ($P < 0.01$). The use of chemical pesticides not only damages farmers' own health but also jeopardizes badly vegetable eaters' health and ecological environment. Consequently, the more clearly vegetable farmers know about the hazard caused by chemical pesticides, the higher the use intention towards biopesticides they express. However, the cognition on biopesticides had no such significant influence on the use intention ($P < 0.10$). The reasons may lie in that vegetable farmers need to promptly and effectively resolve the problems of plant diseases and insect pests, but the biopesticides take effects slowly and their working range is relatively narrow, bringing great damage to the growth of vegetables, which leads to the economic loss for farmers. Compared with chemical pesticides, biopesticides are featured by higher safety and environmental compatibility. But, driven by economic interests, vegetable farmers are unwilling to use biopesticides, which is contrary to the results of Fu and Song (2010).

Biopesticide price ($P < 0.05$) and safety tests on biopesticides ($P < 0.10$) exerted significant influence on farmers' use intention towards biopesticides. Biopesticide price was significant negatively correlated to the use intention. This illustrates that the larger price gap between biopesticides and chemical pesticides may cause the higher cost farmers have to pay for pesticides, which finally leads to lower use intention towards biopesticides. In addition, safety test positively influenced farmers' use intention towards biopesticides ($P < 0.10$). The low residue property of biopesticides enables vegetables to pass the pesticide residues inspection more easily. Due to the unsound traceability system, vegetables produced by using biopesticides can not be sold at relatively high price. Therefore, the influence of vegetable price on farmers' use intention towards biopesticides is insignificant, which is contrary to the result of Ma and Yang (2011).

Both government supervision and skill training on pesticide use exerted influence on farmers' use intention towards biopesticides. Government supervision over pesticides positively influenced the use intention ($P < 0.10$), which indicates that more strictly the use of highly toxic and pestilent pesticides is supervised, more capable farmers become of regulating their behavior and enhancing their use intention towards biopesticides. Due to the complexity of biopesticide use, government training for farmers will reduce the risk from biopesticides and thus strengthen farmers' use intention towards biopesticides. Government policy of agricultural subsidy for farmers to use biopesticides exerted insignificant influence on the use intention, inconsistent with the findings of Hruska and Corriols (2002). One reason for it may lie in low or even entirely no subsidy, which can not motivate farmers to use biopesticides.

4. Conclusions

4.1. Main conclusions

This study conducted a survey on vegetable farmers from Nanjing, Yangzhou, and Xuzhou city in Jiangsu Province, China, to investigate the farmers' cognitive level of and use intention towards biopesticides. By employing penalized logistic regression model, the major factors affecting the use intention were analyzed. The main conclusions are as follows:

Vegetable farmers mainly use chemical pesticides to control plant diseases and insect pests. The farmers have low cognitive level of biopesticides. Up to 67.65% of the investigated farmers know nothing about biopesticides and have doubts about the controlling effects. Only 28.43% used to apply or are now applying biopesticides. Under the assumption that biopesticides vary little with chemical pesticides on the controlling effects, 68.63% show their willingness to use biopesticides.

Several factors, namely farmer age, vegetable planting area and years, the purpose of planting

vegetables, the proportion of vegetable income to total, and biopesticide price, negatively influence farmers' use intention towards biopesticides at different significant levels. Whereas, farmers' education level and cognitive level of chemical pesticide residues, government supervision over chemical pesticide use, safety test of vegetables, and skill training on biopesticide use positively influence the use intention at different significant levels.

4.2. Policy suggestions

First, related research institutions should develop those biopesticides that take effect promptly and are used easily. They should lower the difficulty of using biopesticides as well as the cost of biopesticides' production and marketing, thus enhancing market competitiveness of biopesticides.

Second, with support of vegetable production cooperative organization, the government should choose farmers under 40 years old, with high education level and small planting area as pilot objects to gradually promote biopesticides throughout the country.

Third, effective measures should be made to control pesticide residues in vegetables, to improve vegetable safety test system, and to strengthen supervision over pesticide use. Government should also establish a marketing network to sell high-quality agricultural products and ensure a relatively high price of vegetables used biopesticides, thus effectively encouraging farmers to use biopesticides.

Fourth, currently, biopesticide use by vegetable farmers in Jiangsu Province is motivated mainly by their internal willingness, while the external driving force from the government is not powerful enough. There is a necessity for the government to increase the propaganda of biopesticides, thus improving farmers' cognitive level of biopesticides. Meanwhile, it is essential for the government to reward for biopesticide use, and conduct skill training for farmers on biopesticide use, which may be helpful to promote the prompt application of biopesticides in vegetable-planting industry.

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STUDY OF FREEZE-THAWING ON THE PROCESS OF TILAPIA FILLETS HEAT PUMP DRYING

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ABSTRACT

In order to investigate the effects of freeze-thaw pretreatment on drying time of tilapia fillets by heat pump drying. The effects of freezing temperature (-10, -20, -30, -40 and -50°C), freezing time (0.5, 1, 1.5, 2 and 2.5 h), thawing temperature (5, 10, 15, 20 and 25°C) and melting time (1, 1.5, 2, 2.5 and 3h) on heat pump drying time of tilapia fillets and the drying speed of dried tilapia fillets were investigated in this paper. Furthermore, the heat pump drying speed curve of tilapia fillets had been gained under different freeze-thaw conditions, and results indicated that: drying time and drying speed were improved under moderate freeze-thaw condition. Along with the increase of freezing temperature, freezing time, melting temperature and melting time, drying time decreased firstly and then increased with a nadir. So, while under the same conditions, if the frozen temperature was between -20°C and -30°C, the melting temperature was 20°C and the freezing time was restricted by an hour, then the drying time was shortened and the drying speed was accelerated. Moderate freeze-thaw condition can reduce the drying time of tilapia fillets and accelerate the drying speed by heat pump drying and a reference for the low temperature heat pump drying pretreatment technology to update similar aquatic products.

1. Introduction

Tilapia is one of the aquatic products with high moisture and protein content, as a result of which, fresh fish is very susceptible to spoilage, so drying dehydration is a quite common way for storage (Sismotto et al., 2014; Cheng, 2015; Kituu, 2010). Undoubtedly, hot air drying (Xiao et al., 2010) is the most common tilapia drying process, but the quality of dried products is greatly affected by the drying time and drying temperature, therefore, it is difficult to control appropriate drying conditions to improve the quality of the drying tilapia fillets and shorten the drying time simultaneously (Zhen-hua et al., 2011). Additionally, the simply low temperature heat pump drying (Goh et al., 2011) can not avoid the action of microorganisms resulting in quality mutation of the products because of a longer drying time. However, adding pretreatment reagents is favorable to shortening the drying time, and improving the performance of dry goods (Zheng et al., 2013). But adding the pure chemical pretreatment reagent is limited by the volume index of national standard. Freeze-thawing as a method of drying process in the physical pretreatment have also been used in the drying process of other fields (Yucheng, 2009), and as a preliminary study has been pretreated before drying fruits and vegetables. And it is said that it is easy for food biological tissue or reactive porous media to receive dehydration after freeze-thawing treatment (Ting et al., 2013). For now, freeze-thawing

treatment research is mostly concentrated in the freeze-thawing damage to the organization on aquatic products and other meat products (Ramírez et al., 2011). Previous studies had shown that dried and cold swap could promote drying process. Overall, tilapia fillets would be used as a raw material in this paper, and this experiment tried to match freeze- thawing conditions better , in order to apply the freeze - thawing method to the tilapia fillets dry pretreatment process , which could strengthen the advantage of freeze-thawing that it benefits the dehydration and drying, while weakening its disadvantage of mutating the organizational structure. So the aim was to explore a new method of drying pretreatment which would provide the basis for the development of dehydration pretreatment process of aquatic products and other meat products.

2. Materials and methods

2.1. Raw materials and Sample preparation

Materials: fresh tilapia, purchased from Zhanjiang Gongnong market, weighing about 1.5 kg/bar.

Sample preparation: The average initial moisture content of the tilapia fillet samples was 5 kg/kg expressed in dry basis (d.b.) or 80% in wet basis. A commercially available fresh tilapia was killed quickly, the meats slices were taken after removing scales, head and tail, innards. And then it was required to control the standard of the fish fillet is 100 mm × 50 mm × 5 mm each (weight was about 30g). After that, external water of fillets was fully absorbed by absorbent paper after cleaning, before being weighed standby.

2.2. Main Instruments and Equipment

Self-built heat pump drying device (Guan et al., 2013): 3P power, temperature -20-80^{o}C, humidity 20% - 80%; HHS-type electric heated water bath (Shanghai Industrial Co., Ltd. Boxun medical equipment factory);DZF-6050 vacuum oven (Shanghai Jing Hong Laboratory Instrument Co., Ltd.); BD-730LT-86L-I ultra-low temperature freezer (Qingdao Haier Group); FYL-YS-50L incubator (Beijing Fuyi Electric Co.); T-18 homogenizer (Germany IKA group); GL-10LMD refrigerated centrifuge (Hunan Xingke Scientific Instrument Co., Ltd.); Sigma 1-14 high-speed desktop centrifuge (Germany Sigmal Company); AY120 analytical balance (Shimadzu Corporation); Drying temperature and air velocity data were collected by multi-channel digital instrument XSD (XSD/A-H3IIIS2, Automation Equipment Co., Ltd., Guangzhou Kunlun). The temperature was measured by Duwei ATH402 plastic pipe temperature and humidity transmitter (Hefei Dewey Instrument Co., Ltd. production). The hot air velocity was measured by Deweida EE65 air velocity transmitter (Shenzhen Deweida Instrument Co., Ltd. Production).

2.3. Methods

2.3.1. Process flow

Raw fish—section—weighing—freezing—thawing—weighing—heat pump drying—determining index

2.3.2. Points in operation

Fish fillets freezing and thawing: according to the experimental conditions, the cryostat's and the incubator's temperature were set to a predetermined value, and the fish fillets will be loaded into the polyethylene bags sent to the cryostat later for being frozen for a period of time. Afterwards, the fish fillets would be moved into the incubator for thawing for a period of time.

Heat pump drying: For tilapia fillets with 5 mm thickness, because there would be a better drying quality under 45^{o}C as the heat pump temperature, 2.5 m/s as wind speed and 30% humidity conditions around, heat pump drying tested the tilapia fillets under this circumstance (Li Min et al., 2011). Firstly, the parameters of heat pump drying device were required to be adjusted to predetermined value of the test requirements. Secondly, after removing the frozen-thawed fish fillets from polyethylene

plastic bags, absorbent paper gently and repeatedly suck the seeping water on the surface and fillet weight would be measured accordingly .Finally, move the fish fillets into the clean barbed wire tray of heat pump oven afterwards. Then, the fish fillets would receive drying treatment.

2.3.3. Experimental procedure

With the heat pump drying conditions and fish size unchanged, drying pretreatment experiments were performed at different freezing temperatures (-10, -20, -30, -40 and -50°C), at different freezing time (0.5, 1.0, 1.5, 2.0 and 2.5h), at different thawing temperatures (5, 10, 15, 20 and 25°C) and at different thawing time (1.0, 1.5, 2.0, 2.5 and 3.0h) pretreatment, weighting, then added in the heat pump dryer. The weight of the sample was measured at one hour intervals. For every batch of dried sample, the moisture content was determined, the drying procedure was not stopped until the moisture content of dry basis reached 0.30±0.02 g/g. Moreover, the experimental group without freeze-thawing pretreatment worked as the contrast group. Each run in the experiment was done in triplicate.

2.4. Indicators and Evaluation Methods

The moisture content of the test sample was determined according to the vacuum oven method (AOAC, 2005). At regular time intervals during the drying period, samples were taken out and dried in a dryer at 105°C for drying to constant weight and weighed (DZF-6050, Shanghai Experiment Instrument Co. Ltd., China).

Calculation of moisture ratio (W)

W represents the moisture ratio of dry basis and can be expressed as follows:

$$W = \frac{m_t - m_1}{m_1} \quad (1)$$

where m_t is the moisture content of the product at each moment, m1 is the samples dry weight.

Determination of drying rate curves

The drying rate represents the average changes of dry basis moisture content in per unit time. In order to reflect the rate of drying, the drying rate curves were plotted. The average drying rate was calculated by the formula as follows:

$$v = (w_2 - w_1)/(t_2 - t_1) \quad (2)$$

where, v is the rate of drying; w_1, w_2, respectively, represent the moisture content on a dry basis (g/g) in t_1 and t_2; t_1, t_2 are the drying time (h).

2.5. Statistical Analysis

All the tests were done in triplicate and data were averaged. Standard deviation was also calculated. Analysis of variance was used to evaluate significant differences ($P<0.05$) between the means for each sample.

3. Results and discussions

3.1. Drying curve and drying rate curve of different freezing time

Fixed melting time was 2.0 h, freezing temperature was at -40°C and melting temperature was at 25°C and under this condition, freezing-thawing pretreatments of fish fillets were using different freeze time: 0.5h, 1.0h, 1.5h, 2.0h and 2.5h respectively, to investigate the effects of different freezing time on the drying time and drying rate of fish fillets heat pump drying. The results are shown in Figure 1.

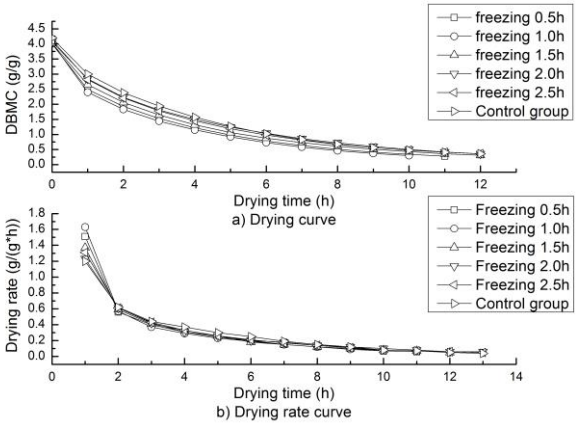

Figure 1. Effect of different freezing time of freeze-thaw pretreatment on drying time and drying rate (DBMC- Dry Basis Moisture Content, The same below)

As can be seen in Figure 1(a), different freezing time of freeze-thawing pre-processing can generate obvious effects on the drying time of fish fillets which dried with the same moisture content finally. Along with the increase in freezing time, results showed that the drying time of slices firstly decreased and then increased. When freezing time was less than 2.0h, the drying time of experimental group was less than that of contrast group without pretreatment, and surprisingly, there was a minimum drying time which was 10 hours experiencing the freezing time of 1.0h, which was significantly shorter than the drying time of 13h of the untreated control group. However, when the freezing time was more than 1.5h, with further increase in freezing time, the gap of drying time between the control group and the others was getting smaller and smaller. So, it showed the best freezing time was 1.0h by this test. Under a certain freezing temperature condition, along with the increase in freezing time, ice crystals would be formed layer by layer and get inward penetration constantly, which might cause a certain degree of damage to the muscle tissue, and the time period of 1 hours could just freeze the fillets thoroughly, and this freezing time might match the thawing conditions well. Furthermore, the fish fillets might not be frozen sufficiently when the freezing time was less than 1h, so that the permeability and detachment of internal moisture was not sufficient, which made the improvement of the drying time not obvious. If the freezing time was more than one hour, with prolonged freezing time, the condition would result in excessive frozen fillets, and increase the compacting properties of ice crystals, the competition pattern of the ice crystal interface had changed, and reduce the moisture from the proteins or other polymer compound, therefore, it could not reach to the maximum extent of tissue damage in the limited melting time, which may be the cause of the above phenomena.

As can be seen from the drying rate curve in Figure 1(b), for the samples of the freeze-thawing pretreatment of different freezing time, there was a great difference among the drying rate of the first 1h, the drying rate after 1h freezing time had the maximum, while the drying rate after 2h freezing time was the minimum. But both of them were higher than the control group. Therefore, freeze-thawing pretreatment promoted the water escape of the drying section so that the water loss rate of initial drying period greatly increased. When to the drying time was 2h, the drying speed was substantially parallel.

3.2. Drying curve and drying rate curve of different melting time

Fixed freezing time was 1.5h, freezing temperature was -40^{o}C and melting temperature was 25^{o}C, and under this condition, freeze-thawing pretreatment of fish fillets were carried out in different melting time: 1.0h, 1.5h, 2.0h, 2.5h and 3.0h respectively, to investigate the effects of different melting time on the drying time of fish fillets, the results were shown in Figure 2.

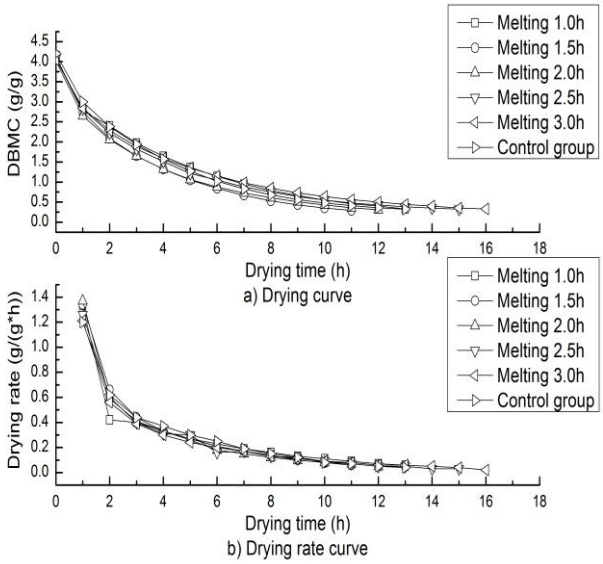

Figure 2. Effect of different melting time of freeze-thaw pretreatment on drying time and drying rate

As can be seen in Figure 2(a), along with the increase in melting time, results showed the

drying time of slices firstly decreased and then increased. When melting time was 1.5h, there was a minimum drying time that was 11h. It may be due to the short thawing time that was only 1 hour, so the sample was not completely thawed. What's more, ablation of ice would be more and more with the increase of thawing time. As a result, the ice crystals melting might reach the best point while the melting time was 1.5h, and no damage to voids left after ice melting, which led that 1.5h melting time played a role in promoting drying. Generally, due to the very fresh fish fillets were frozen, there will be a strongly rigor mortis phenomenon after thawing in a certain period of time , resulting in significant shrinkage deformation of fish tissue , which might cause the ice pore to be blocked again (Coleen et al., 2012; Xiufang et al., 2012). However, with the further increase of the melting time, the rigor mortis of backwardness may be more obvious, so excessively extend melting time would lead that the drying process was inhibited.

As can be seen from the drying rate curve in Figure 2(b), for the samples of the freeze-thawing pretreatment with different melting time, there was a great difference between the first 1 hour's drying rate, and the drying rate of 2h's melting time was the maximum, while the drying rate of 3h's melting time was the minimum, which were all higher than those of the control group. Freeze-thawing pretreatment promoted the water escape of the drying section, so that the water loss rate of initial drying period would greatly increase.

3.3. Drying curve and drying rate curve of different freezing temperature

Fixed freezing time was 1.5, thawing time was 2.0h and melting temperature was 25^{o}C. Under this condition, freeze-thawing pretreatment of fish fillets were carried out in different freezing temperature (-50^{o}C, -40^{o}C,-30^{o}C, -20^{o}C and -10^{o}C respectively), to investigate the effects of different freezing temperature on the drying time of fish fillets, the results were shown in Figure 3.

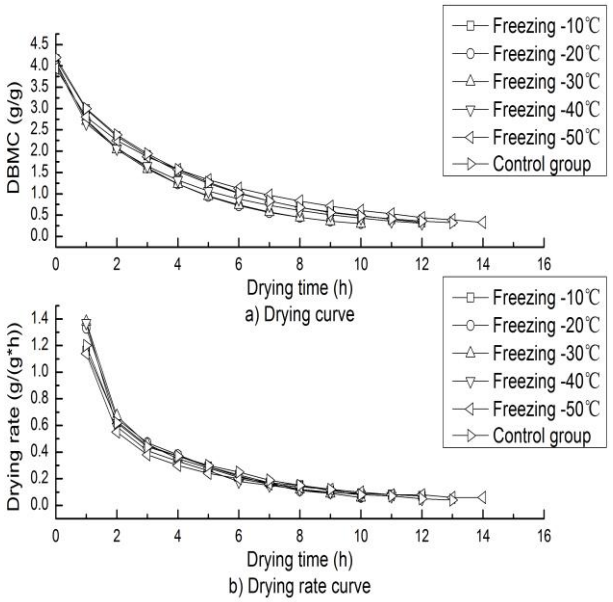

Figure 3. Effect of different freezing temperature of freeze-thaw pretreatment on drying time and drying rate

As can be seen in Figure 3a, compared with the control group, the fish fillets drying time did not have an improvement when the freezing temperature was too high or too low. On the contrary, under the condition of -50^{o}C freezing temperature, the drying time of samples was slightly longer than that of the control group. And when the freezing temperature varied from -20^{o}C to -30^{o}C appropriately, fish had the shortest drying time 10h.

During freezing initial period the water among cells formed ice crystals firstly when the fish fillets were at an increasingly low temperature. Because the freezing point of water inside the cells is lower so this water remained liquid. Moreover, under the condition of steam pressure difference, the moisture inside the cell would spread out of the cells and make cell shrink .At the same time, the ice growth in the freezing process would produce extrusion, drag and drop even amyxis of fish fillets thus, the structure was brittle and the water would be lost after being re-warmed which were conducive to dehydration after freeze-thawing tilapia fillets. But higher freezing temperature may not make the water of organization completely frozen. At this time, ice crystal formation was weak, which might

explain why the drying time of samples was longer at -10°C freezing temperature than a lower temperature after the freezing pretreatment, and why the drying time was shorter at the temperature of -20°C ~ -30°C. In unidirectional freezing process, for one, advancing ice crystals squeezed out the free water of the tissue, and for another, they would gradually stripped adsorbed water and bound water. Under the freezing conditions at -50°C, faster cooling rate that can make the low temperature quickly pass through the maximum frozen crystal creating belt would lead that the quantity of ice crystal formation is big and the volume is small, and that bound water and adsorbed water was frozen before being stripping from the proteins and other macromolecules (Delgado et al., 2005). Therefore, the over low freezing temperature is not conducive to drying process after melting. Their drying rate curve can be seen as Figure 3b. As can be seen from the drying rate curve in Figure 3b, for the samples of the freeze-thawing pretreatment with different freezing temperature, there was a great difference between the first 1 hours of drying rate, it was easy to find that the drying rate of -30°C freezing temperature was the maximum, while the drying rate of -50°C freezing temperature was the minimum. Compared with the control group, the drying rate of -10°C and -50°C freezing temperature was lower. Frozen impermeable or excessive freezing is the cause of this result.

3.4. Drying curve and drying rate curve of different melting temperature

Fixed freezing time was 1.5h, thawing time was 2.0h and freezing temperature was -40°C, under this condition, freeze-thawing pretreatment of fish fillets were carried out in different melting temperature of 5°C, 10°C, 15°C, 20°C and 25°C respectively, to investigate the effects of different melting temperature on the drying time of fish fillets, the results were shown in Figure 4.

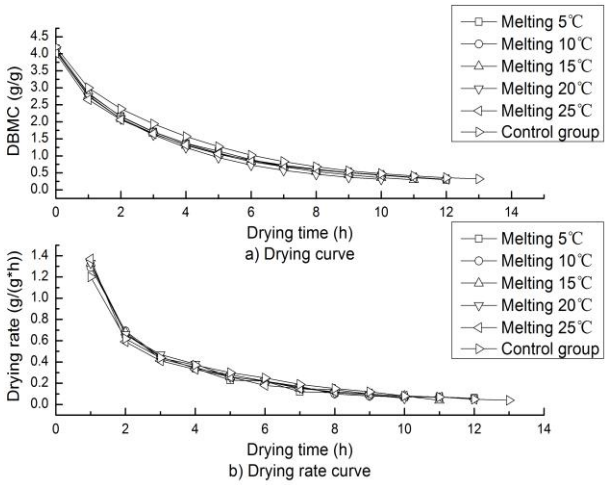

Figure 4. Effect of different melting temperature of freeze-thaw pretreatment on drying time and drying rate

The drying time of the tilapia fillets using different melting temperature treatments was shorter than that of the control group without freeze-thawing pretreatment which was shown in Figure 4. And when the melting temperature was lower than 25°C, with the increasing melting temperature, the drying time got shorter, which explained that in a certain range of temperature, a higher the melting temperature was more beneficial to be dried. But when the melting temperature reached 25°C, the drying time increased. In fact, the lower the melting temperature is the less quality loss will get after thawing meat. However, the higher melting temperatures will cause the muscle fibers to form larger pores, and result in greater and more extensive destruction of tissue morphology (Boonsumrej et al., 2007). At 0°C the thermal conductivity of water is only about a quarter of the thermal conductivity of ice, so if the melting temperature is too low, these may lead that outside heat can not be sufficiently passed through the thawed layer towards inside food in a shorter defrosting time (Morenoa et al., 2013) and therefore this thawing can not play a full role in the thawing process that led to changes in the organizational structure and removal action of moisture. In addition, the lower temperature after thawing was, the lower

internal temperature of the sample during initial drying time was, and water evaporation was difficult to be evaporated.

4. Conclusions

The factors in the freezing and thawing process including the frozen time, frozen temperature, melting temperature and melting time effect the drying time of the heat pump drying of Tilapia. And corresponding to the different drying stages, there exist different drying rates. When the fish fillets were operated by freeze-thawing pretreatment before drying with other conditions the same, the impact of 1.0 h freezing time for drying rate is the best, -20 ~ -30^{o}C freezing temperatures is the best, 1.5 h melting time is the best, and 20^{o}C melting temperature is the best in the range of this experiment. In a word, it can be seen that a suitable freeze-thawing condition pretreatment can quickly improve their dried speed, shorten the drying time.

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EFFECTS OF THE DEVELOPMENT OF TOURISM IN WUYI MOUNTAIN SCENIC SPOT ON LOCAL TEA INDUSTRY AND THEIR CORRELATION

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ABSTRACT

The rapidly developing Chinese tourism which has increasingly larger influence has significantly motivated the development of other industries. Tea industrial chain includes planting, processing, packaging, transportation, sales and brand marketing. The development of tourism can produce influence on tea industrial chain. There are a lot of successful cases of tea tourism in China; however, few researches concern about the influence of tourism on tea industry. The deep understanding of the effect of tourism on tea industry is of great significance to the tea industry in the areas which focus on tea tourism. On account of this, we analyzed the influence of tourism on tea industry in the perspective of industry chain and industry integration, taking the future direction of improvement of tea industry in Wuyi Mountain as an example. This work aims to further promote the development of tea industry and the optimization of industrial structure based on the influence of tourism on tea industry. Based on theoretical analysis model which is constructed with influence indexes, we discussed over the effect of tourism in Wuyi Mountain which is a typical case of tea tourism on tea industry in perspectives of planting, processing, sales and new operating mode.

1. Introduction

Currently, tourism has been one of the strongest and largest industries in global economy (Ksenija and Andreas, 2009). The rapid development of tourism is in demand of more tourism products. People begin to be unsatisfied with the single and rough travelling pattern, but hope to require more knowledge about foreign history, life, culture, production, folk custom and art (Tsang, 2011; Lucock et al., 2013). China, the country of origin of tea, has rich tea cultural accumulation. Tea tourism which is a case of the primary industry extending and penetrating to the third industry is a reformation and improvement on traditional tea industry and is a new form of modern tea industry. The exploitation and development of tourism can motivate the development of tea industry, tea cultural products, scenic spots, local economy and tea tourism consumption centered on cultural tourism, which is beneficial to stimulate tea consumption and tea market and promote the development of Chinese tea industry (Xiao and Jolliffe, 2007; Qian, 2008; Qiao, 2011).

Foreign researchers launch researches concerning the effect of tourism mainly in perspectives of the economic effect, social cultural effect and ecological environment effect of tourism. For instance, Minkyung Park et al. (Park and Stokowski, 2009) studied rural tourist destination based on the theory of social disruption. Takamitsu et al. (2011) studied from the perspective of the attitude of local people in tourist area on the changes of social culture caused by tourism. Researches on the

economic effect of tourism are few in China; and their content mainly concentrates on analysis of economic effect of tourism, the economic effect brought by tourism, evaluation of regional economic performance and the correlation between tourism and economy. Based on the above condition, this study systematically analyzed the tourism related factors influencing tea industry, applied tourism influence related theories into the practice, constructed a structural model of the effect of tourism on tea industry and finally proposed some suggestions for the development of tea industry and tourist products, the perfection of tourism infrastructure and the improvement of tourism service quality, which can guide the development of tea and tourism of Wuyi Mountain city.

2. Materials and methods

2.1. Promotion of core competitiveness of Wuyi rock tea industry

2.1.1 Promotion of tea cultural construction project

Wuyi rock tea possessing rich tea culture resources occupies a very important position in the history of Chinese tea and even tea in the world. However, tea culture of Wuyi rock tea has not been fully excavated and used for a long time due to the development concept of focusing on tea only, instead of emphasizing tea culture. Therefore, Wuyi rock tea industry has to promote the tea cultural construction and improve the core competitiveness by making full use of unique cultural charm of Wuyi rock tea, thereby promoting the sustainable development of tea industry. Propaganda is an essential means in the tea culture promotion. The rapid development of tourist industry in Wuyi Mountain scenic spot precisely provides an effective way for popularizing tea culture.

2.1.2. Enhancement of brand cultivation

Although numerous Wuyi rock tea brands have been created, there are few well-known brands, thus brand effect is unable to be formed. Consequently, in the face of an increasingly competitive tea market, joint efforts of government and enterprises are required so as to build Wuyi rock tea brands and improve core competitiveness of Wuyi rock tea industry. An increasing number of tourists coming to tourist area provide an opportunity to improve core competitiveness.

2.1.3. Perfection of learning system

The lack of a sound leaning system in enterprises in Wuyi rock tea industry and strong learning atmosphere results in low overall technical ability of Wuyi rock tea industry. Hence, it is very necessary to perfect leaning system of Wuyi rock tea industry and create a sound learning atmosphere, thereby improving core competitiveness of Wuyi rock tea industry. The most important thing in the promotion of learning system is to give hope to enterprises and employees and the development of local tourist industry just has such an effect.

2.1.4. Optimization of industrial structure

Some problems still exist in the industrial structure of Wuyi rock tea. For example, quite a number of tea gardens in the city are low-yielding and aged; most of tea processing production enterprises and factories in the whole city are equipped with old-fashioned and backward facilities and have small family workshops. Hence, to improve core competitiveness of Wuyi rock tea industry, its industrial structure must be optimized and adjusted. The development of tourist industry in Wuyishan City introduces a lot of money in the local and provides a financial support for the transformation and development of tea industry.

2.2. Construction of conceptual model on tourist and tea industries

2.2.1. Constitution of tourist and tea industrial chains

(1) Constitution of tea industrial chain

Modern tea industrial chain mainly consists of 4 basic links of tea planting, processing,

circulation and consumption (Dutta et al., 2010). Tea set, ware, packaging, culture, exhibition, creativity, show, food and derived new forms of tea industry are involved in the 4 links. They are related to each other and directly or indirectly correlated with other industries. Figure 1 below shows tea industrial chain.

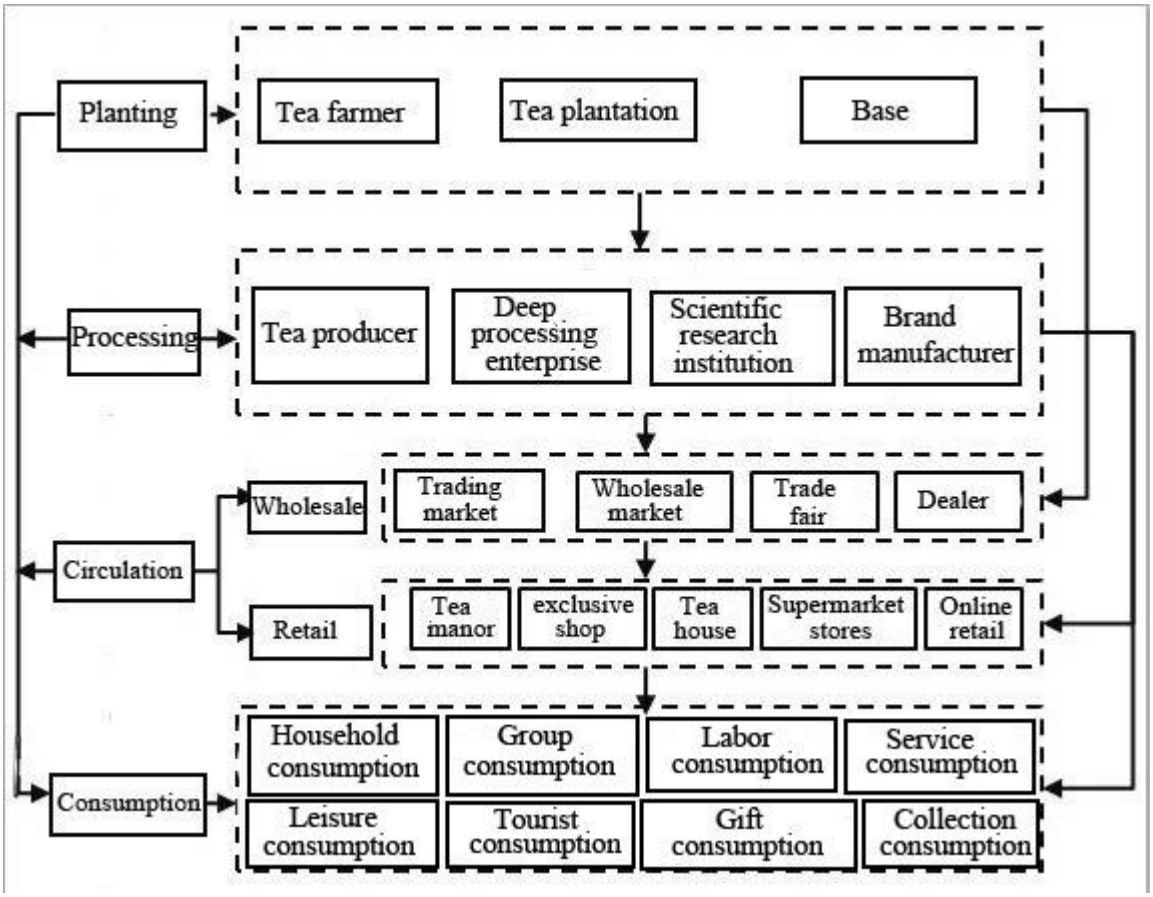

Figure 1. Tea industry chain

(2) Constitution of tourist industrial chain

This study focuses on optimization and updating of the influence of tourist industrial chain in Wuyishan city on tea industry and its drive effect. Figure 2 displays tourist industrial chain. Usually, factors constituting tourist industrial chain can be summarized as travel demand (Chen, 2010), travel agency, wholesalers and retailers of tourism products and some industrial sectors supplying service and products for tourists.

2.2.2.Analysis on relationships between tourist industry and tea industry

(1) Similarities and differences of tourist industry and tea industry

① Similarities of tourist industry and tea industry

Both of tourist industry and tea industry are comprehensive industries and meanwhile they provide products and services and keep the sustainable development of industry depending on persistent innovation (Hu and Zhang, 2008).

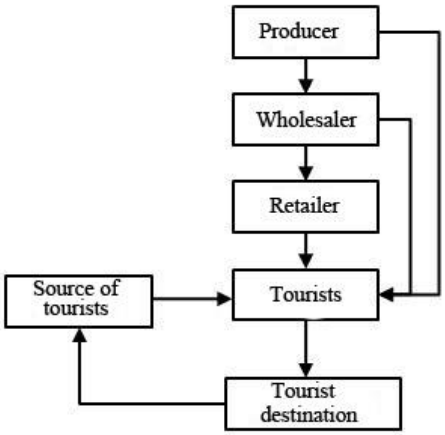

Figure 2. Schematic chains of the tourism industry

Constitution of tourist industrial chain is shown in Figure 3.

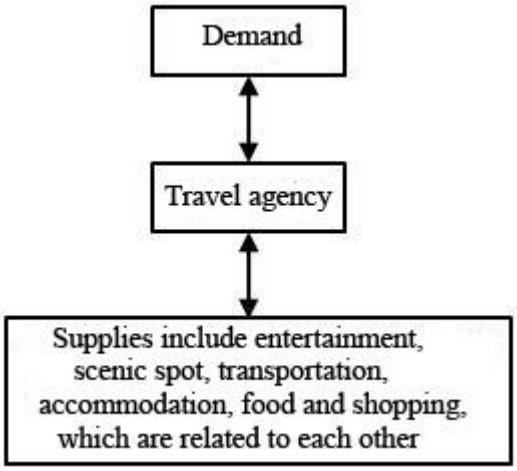

Figure 3. Basic composition element chains of the tourism industry

② Differences of tourist industry and tea industry

Product development in tourist industry lays emphasis on designing products according to different tourist groups, but tea products in tea industry are geared to the needs of the general public. The development of tourist industry needs several industries to coordinate with each other, which is more important than their competitions to some extent. However, tea industry is on the contrary.

(2) Intersection of tourist industry and tea industry

Tourist industry and tea industry are largely fused in addition to the above differences and similarities, performing as travel and leisure, tea theme scenic spot, tea cultural trip, tea theme restaurant, etc. Wuyishan City combines tea with travel, such as tea expo garden, impression Dahongpao, tea food, tea culture and so forth. Tourist industry and tea industry build a relationship based on mutual effect and benefit.

2.2.3.Theoretical model on analysis of influence of tourist industry on tea industry

(1) Model construction

The key of constructing a theoretical model on analysis of influence of tourist industry on tea industry is to establish a frame model on the relationship between tourist industry and tea industry. Generally speaking, the following links are involved: ① confirmation of the relationship between tourist industry and tea industry; ② selection of research objects; ③ investigation; ④ data analysis and construction of theoretical frame model.

(2) Theoretical model

Good tourism resource is the premise of the continuous development of tourism industry. It is required to make a plan on tourism resource in order to transform tourism resource into tourism products and the developed and planned tourism resource should be passed to the tourist market by various channels, which is tourism marketing. Finally, tourism products are delivered to customers so as to promote tourist purchase and consumption. This kind of tourist industry processes are corresponding to suppliers in tourism industrial chain, manufactures, retailers and consumers. The fusion of tourist industry and tea industry happens in the whole process of tea product production and consumption, i.e., from identification of tourism resources to consumption. Based on the above, it can be

seen that the development of tourist industry will bring certain influence on tea industry from the perspective of industrial chain and industry integration (Figure 4).

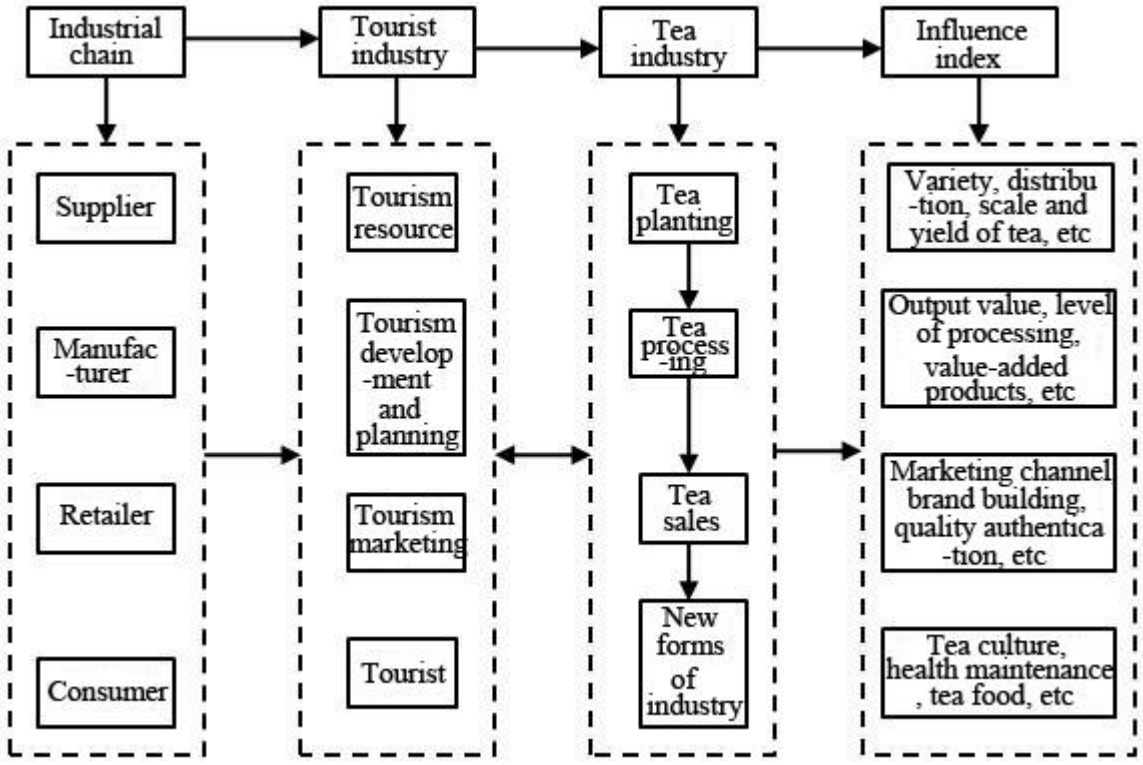

Figure 4. Tourism impact analysis model of tea industry

(3) Data acquisition and sorting

Data are collected from tea bureau, forestry bureau, tourist administration, industrial and commercial administration in Wuyishan City as well as official websites of tea expos holding in Mainland and Taiwan.

3. Results and discussions

3.1. Effect of tourism of Wuyi Mountain on local tea industry

3.1.1. Effect of tourism on tea planting

(1) Effect of tourism on planting area of tea

With the development of tourism in Wuyi Mountain, more and more tourists know Wuyi rock tea, which improves the sales and promotion of Wuyi rock tea. Moreover, the planting area of rock tea, for example, tea expo, can also be used as tourism resources to attract more tourists (Cao *et al.,* 2012). According to the statistics provided by the Ministry of Tea suggest that, the area of tea garden in Wuyi Mountain city had increasingly changes from 2007 to 2014 (Figure 5).

(2) Effect of tourism on spatial distribution of tea planting

In early stage, tea is planted in ravine area of Danxia landform whose spatial height is consistent with scenic spots; but afterwards, the spatial distribution of planting changes due to the diversity of demand (Gao, 2012).The changes reflect on the transfer of planting space and transformation of land use pattern. Some areas where is suitable for growing tea change land use pattern due to the development of tourism. For example, rest pavilion and steps are constructed besides seed tree of Da Hong Pao; some areas combines tourism and tea together spatially, for instance, the areas where scenic spots highly concentrates are also

planted with highly-concentrated tea, which can not only satisfy tourists but also promote the better development and utilization of tourism resource.

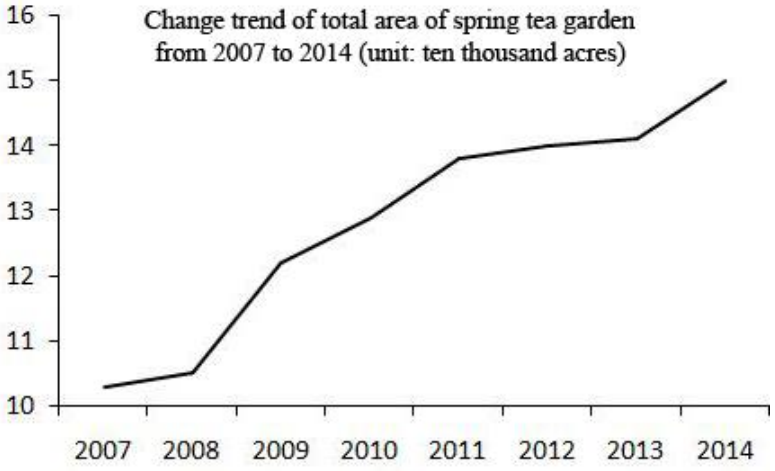

Figure 5. From 2007 to 2014, spring tea tea garden area of change trend chart (unit: ten thousand mu)

(3) Effect of tourism on tea output

The development of Wuyi Mountain city promotes the sales of tea, which affects the output of tea in Wuyi Mountain city. The variation of tea output from 2007 to 2014 is shown in Figure 6. The large number of tourists Increases the demand of tea. The diverse demand changes the category of tea needed as well as the output of tea. Tea of some brands exhibited in tea expo has been recognized and favored by the public, which can promote the increase of output.

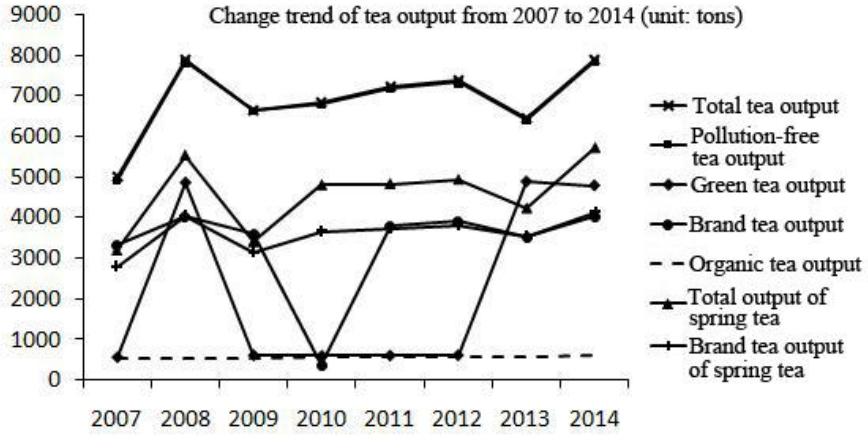

Figure 6. Tea yield trend chart in 2007-2014

3.1.2. Effect of tourism on the processing of tea

(1) Effect of tourism on tea processing levels

Tea production factory is the center of

production, processing and handling of tea which can directly affect the quality of tea products. The previous processing level has not able to satisfy the development of tea industry currently. Hence some tea production factories in Wuyi Mountain city took the leading in improving the processing level of tea. The detailed works done by those tea factories includes emphasis on the cleaning of internal and external environment of processing factory, introduction of advanced processing equipment and training of processing staffs.

(2) Effect of tourism on high added-value tea product

With the increase of tourist, highly processed tea products and development projects are introduced to Wuyi Mountain city. The government has tried to develop and produce products with high added-value using low and medium grade tea or the scraps of tea such as tea stem, single leaf and tea dust. Deeply processed tea products mainly include tea beverage, traditional Chinese medicine health care tea, tea wine, tea food, tea polyphenol and tea polysaccharide. Deep processing can increase the technological content and economic value added, expand consumption channels of tea products, promote the optimization of tea product structure and prompt the transformation and upgrade of tea industry.

3.1.3. Effect of tourism on tea consumption

(1) Effect of tourism on output value of tea

The development of tourism in Wuyi Mountain city, for example, the activities such as tea expo, tea cultural festival and live-action performance of Da Hong Pao, improves the popularity of Wuyi rock tea especially Da Hong Pao. As a result, the price of tea grows and thus the output value of tea increases. Figure 7 shows the output value of spring tea from 2007 ~ 2014. Advertisement implant, naming and sponsoring of tea enterprises also improves the output value of tea. A large-scale international seminar held along with Zhuyi Cultural Tourism Festival can attract many foreign tourists, improve the popularity of Wuyi Mountain and increase the output value of tea.

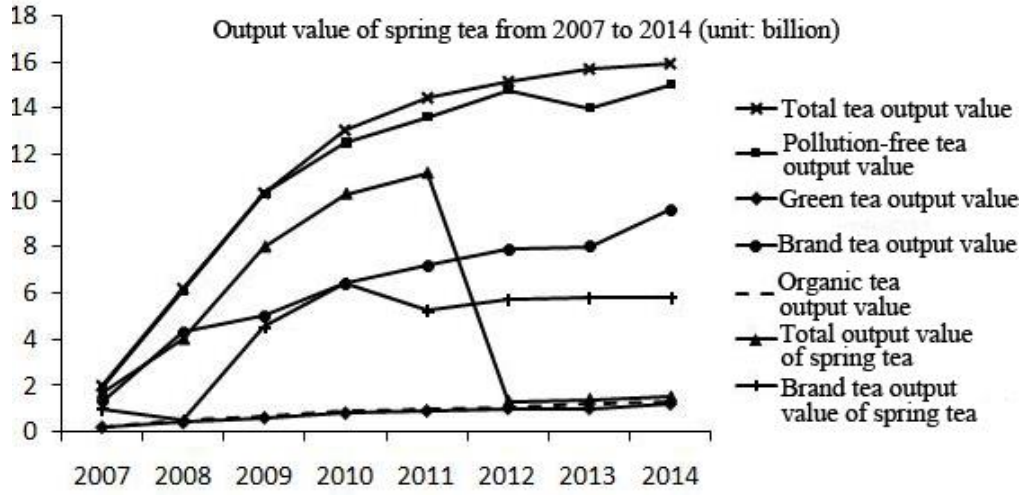

Figure 7. 2007-2014 season tea production

(2) Effect of tourism on tea marketing channels

Tea marketing channels in Wuyi Mountain includes store sales, online sales, sales along with activities, tea expo, etc. Tourists can

purchase tea directly from tea farmers. The development of tourism improves the informatization of tea industry, for example, the launching of intelligence tourism. Platforms such as Qunar, Tuniu and Taobao provide nonlocal tourists with the choice of tourism packages, hotels and restaurants. Such kind of online marketing pattern has been gradually accepted and recognized and applied into tea sales.

3.1.4. Effect of tourism on new operational pattern of tea

(1) Effect of tourism on tea industry

To promote the constant development of tourism in Wuyi Mountain city, Wuyi Mountain city combines the local culture with tourism, aiming to explore new approaches for the joint development of culture and tourism. The city is constantly enriching the local tea culture by collecting stories and paper relating to tea, exploring tea culture and constructing tea building. The current tea buildings include Wuyi Mountain expo garden and Da Hong Pao museum. Combing Wuyi rock tea and local tourism together, the city has released several travelling routes, for instance, "travelling Chinese tea country and exploring the source of tea ceremony" and "Travelling of rooting seeking of Taiwan Dongding oolong tea". Travelling routes such as "Leisure tourism of tea culture" and "tourism of health" which focus on tea motivate the promotion of tea culture.

(2) Effect of tourism on heath maintenance of tea

Since 2007, Wuyi Mountain scenic spot has began to build itself an international health maintenance and leisure tourist destination as well as the brand of leisure and health maintenance. Wuyi rock tea growing in special environment contains many mineral substances and microelement such as potassium, zinc and selenium (Zhao et al., 2014; Chun-Hua et al., 2013). Wuyi city is constantly exploring the content of health maintenance of tea. For example, Wuyi rock tea is found to be effective in improving eyesight, benefiting thinking, refreshing mind, promoting digestion, eliminating phlegm, treating asthma, resisting radiation, tumor and aging as well as lowering blood lipid, blood pressure and cholesterol.

(3) Effect of tourism on tea food

A variety of tea foods including tea pie, tea beverage and tea feast have been developed to satisfy the demand of more and more tourists. More and more tourism travelling in Wuyi Mountain choose to live tea garden and taste tea meals. Wuyi Tea feast which takes Wuyi rock tea as the major seasoning have favored by many tourists. To better satisfy tourist, tea used in the feast includes not only black tea and oolong but also green tea.

4. Conclusions

This study explored the effect of tourism in Wuyi Mountain on tea industry and found that the rapid development of tourist industry produced obvious effects on the planting, processing and sales of tea as well as new operational type of tea. We obtain four conclusions. First, tourism of Wuyi Mountain affects the planting area, spatial distribution of planting, output and price. Secondly, the growing consumption market promotes the adjustment and updating of tea processing, including processing levels and high-value-added product. Thirdly, effect of tourism of Wuyi Mountain on the sales of tea mainly reflects on the marketing channel and output value of tea. Fourthly, to satisfy different demands of tourists on tourist products, Wuyi Mountain city integrates the element of tea into eating, walking, accommodation, traveling, amusement and shopping, which extends the application of tea products. The new operational types of tea mainly include tea culture, tea health and tea food.

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INFLUENCE OF DEVELOPMENT OF CHENJI DIOSCOREA OPPOSITA THUNB. CV. TIEGUN SERIES PRODUCTS ON ECONOMIC DEVELOPMENT IN SHANDONG PROVINCE

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ABSTRACT
In recent years, Dioscorea opposita shares a bigger market as people pay more and more attention to health. Dioscorea opposita Thunb. cv. Tiegun is deeply favored by people as it has high edible value, medicinal value and nutritional value. Relatively concentrated harvest season of Dioscorea opposita Thunb. cv. Tiegun and its thin skin are not conducive to the preservation and long-distance transport. Meanwhile, local yam industry is in extensive form and few enterprises perform deep processing, which lead to limited utilization of yam resources. Therefore, Chenji Dioscorea opposita Thunb. cv. Tiegun should be further processed to improve its additional value, so as to bring certain economic benefits to the Chenji area and increase the economic income of local farmers. Taking Chenji Dioscorea opposita Thunb. cv. Tiegun as an example, this study explored its starch enzymolysis as well as the stability of yam series products, providing a scientific basis for better developing henji Dioscorea opposita Thunb. cv. Tiegun juice. In addition, this work also analyzed the influences of development of Chenji Dioscorea opposita Thunb. cv. Tiegun series products on economic development in Shandong province.

1. Introduction

Chinese yam is mainly used for tonifying spleen and kidney, nourishing stomach, profiting lung and arresting seminal emission (Sheng-sheng et al., 2013; Chui-Jie et al., 2013). Medical researches indicate that Chinese yam containing a variety of nutrients such as fat, carbohydrate, protein and vitamin also has multi-trace elements and more than ten kinds of amino acids which can not only strengthen spleen and benefit kidney (Liu et al., 2013), but also reduce blood fat, resist aging and regulate the immune. Hence, Chinese yam has been deeply favored by people in recent years. Nowadays, increasing importance has been attached to health. Scholars in China and overseas have done relevant researches on Chinese yam and yam series products and tried to improve the utilization rate of yam.

For example, Koo et al. *(*2014) attempted to transform the state from current rough machining to finish machining for medical use or consumption and truly reflected its nutritional value and regulating effect. Kondo and Fujita (2012) signified that Chinese yam is a functional food for health care and beauty. Regular consumption of Chinese yam can ward off disease and promoting longevity. Functional health food - yam fruit series mainly includes chocolate fruit yam and yam jelly (Kondo and Fujita, 2012). Qu et al. *(*2014), after briefly analyzing nutrition and health functions of Chinese yam and lactic acid bacteria fermented food, introduced processing process, operating points and matters of lactic

acid bacteria fermented yam beverage, yam beverage and yam yoghourt in detail, which provided a reference for the development of yam series beverage. Taking Chenji yam in Shandong province as an example, this study discussed effects of Chenji Dioscorea opposita Thunb. cv. Tiegun on starch enzymolysis under different volumes of addition, times and temperatures, providing a scientific foundation for the development of yam series products. Besides, this research also simply analyzed the influence of yam on economic development in Shandong province.

2. Materials and methods

2.1.Materials

Dioscorea opposita Thunb. cv. Tiegun, phenol, sodium sulfite, sodium hydroxide, citric acid, xylitol, sodium carboxymethylcellulose and medium-temperature α - amylase were used in this experiment.

2.2. Main instruments

Thermostatic water bath, food processer, electronic scales, refrigerated centrifuge and ultraviolet spectrophotometer were applied.

2.3.Experimental methods

2.3.1 Technological process

Chinese yam was made into final goods through a series of treatments, for instance, skin peeling, slicing, soaking, drying, grinding, precooking, beating, gelatinization, centrifugal separation, enzymolysis, allocation, homogenizing, degassing and sterilization.

2.3.2.Operational requirements

Chinese yam was skinned and sliced into cuboids with the length and thickness of 10 and 2.5 cm respectively, and the width could be set according to the yam itself (Pathak et al., 2015). After that, the yam was soaked into preservative (benzoic acid) for 3.5 h, in order to bleach it. It was baked for 12 h until dry and then ground into flavor powder. The skinned yam was precooked in boiling water (100±1°C) for 6 min. Then, the precooked yam was beaten with water (1: 6) and grounded into yam serous fluid. Considering the fined serous fluid containing organization fiber which would affect the taste, the serous fluid was centrifuged at 3500 r/min for 8 min and the obtained yam serous fluid was processed with enzymolysis. Medium-temperature a - amylase in 1.2 mg/mL was added into the yam serous fluid and stirred for 6 min and then heated at a constant temperature of 95 °C for 20 min after 23-enzymolysis at 65 °C. 0.18% of citric acid and 0.12% of carboxy methylated cellulose (CMC)-Na were added into the ground raw material and taken as a suspending agent, and an appropriate amount of white sugar and organic acid could also be added to make flavoring beverage (Mamede et al., 2015). The prepared raw material was homogenized once at 72 °C under over 18 mPa pressure. Additionally, the yam product was sterilized at 125 °C for 10 min under high pressure and then canned, which was a finished beverage.

2.4. Experiment content

Except the water, starch has the highest content (20%) in Chinese yam tuber. The insoluble starch restrains the application of Chinese yam. Especially in production and storage processes of beverage, the character of Chinese yam is not stable. But enzymolysis technology based on amylase can solve the problem; it can not only improve the taste, but also stabilize Chinese yam fluid (Mastrantonio et al., 2014). Medium-temperature enzymolysis technology based on a-amylase is characterized by mild condition, high efficacy and few side reactions. Hence we processed Chinese yam starch with enzymolysis using medium-temeprature enzymolysis technology based on a-amylase (enzyme activity: 6000 U/g). The enzymatic hydrolysate includes glucose, maltose, maltotriose and a-limit dexitrin. Reducing sugar obtained from enzymolysis of starch can change the color of 3, 5-dinitrosalicylic acid into brownish red. The optimal enzymolysis technical parameters for medium-temperature a-amylase can be

confirmed using response surface experiment and taking the content of reducing sugar obtained from enzymolysis.

2.4.1. Detection of the content of reducing sugar with dinitrosalicylic acid (DNS)

DNS method refers to the generation process of 3 - amino - 5 - nitro salicylic acid based on the redox reaction of DNS and reducing sugar. The product is brownish red in boiled water; the color depth is in a proportional relation with the content of reducing sugar; the content of reducing sugar is detected using colorimetric method (Gao et al., 2010; Montouto-Graña et al., 2012). The depth of color is correlated to the number of free reducing group instead of the category of reducing sugar. Hence the method is suitable to be used in multiple reducing sugar systems generated in polysaccharide enzymolysis.

2.4.2. Preparation of reagenst

Glucose standard solution: 100 mg of glucose was dried at 80 °C. After the dissolution in flask, the solution was moved into a 100mL volumetric flask. Then it was diluted into 100mL with distilled water.

3, 5 - dinitrosalicylic acid reagent: 3.15 g of DNS and 131mL of NaOH solution (2mol/L) was added into a 250mL hot solution containing 92.5 g of potassium sodium tartrate. Then 2.5 g of phenol and 2.5 g of sodium sulfite were added. After stirring and cooling, it was dissolved into 500mL with drilled water. Finally it was stored in a brown bottle.

2.4.3. Glucose standard curve

0mL, 0.2mL, 0.4mL, 0.6mL, 0.8mL, 1.0 mL and 1.2 mL of glucose standard solution (1 mg/ml) were added into 25mL test tubes respectively (Cheuk-Chun et al., 2007). Then the solutions were added with drilled water until the volume became 2.0mL. Then 2mL of DNS reagent was added. After 5-min boiling water bath and cooling with ice water, the solution was added with drilled water until the volume became 25 mL. Absorbance was detected at the wavelength of 540 nm. Standard curve was drawn, taking the content of glucose as the horizontal coordinate and the absorbance as the vertical coordinate (Figure 1). R is 0.99917, suggesting an obvious linear correlation.

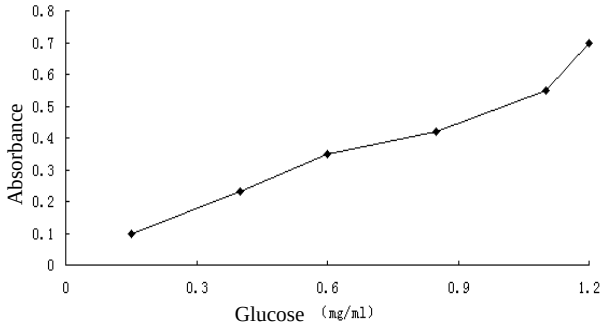

Figure 1. Glucose standard curve

2.4.4. Determination of samples

An amount of 0.2 mL of sample was put in a plugged test tube (25 mL) and distilled water was added to 2.0 mL. DNS reagent (2 mL) was added into boiling water bath for 5 min and the absorbance was then measured at the wavelength of 540 nm. The quantity of glucose (mg/mL) was figured out according to standard curve and reducing sugar content in the sample was calculated.

3. Results and discussions

3.1 Confirmation of temperature, time and concentration in alkali peeling

Yam was skinned with sodium hydroxide solution (7%, 8%, 9%) at 65 °C, 75 °C and 85 °C respectively. The optimal peeling condition was confirmed taking edible rate and peeling situation as indexes (Table 1). As shown in Table 1, higher alkali liquor concentration and temperature showed shorter peeling time. With the increase of alkali liquor concentration, edible rate was lower.

Table 1. Influences of temperature, time and concentration in skin peeling with sodium hydroxide solution

Number	Length of yam (cm)	Temperature (°C)	Time (min)	Concentration of sodium hydroxide solution (%)	Edible rate (%)	Peeling situation
1	6	65	13	7	85.74	With rhizome
2	6	75	11	7	84.12	With rhizome
3	6	85	5	7	87.96	Complete
4	6	65	11	8	85.12	With rhizome
5	6	75	7	8	79.23	Complete
6	6	85	5	8	90.12	Complete
7	6	65	7	9	86.12	Complete
8	6	75	5	9	85.23	Complete
9	6	85	3	9	83.65	Complete

Yam was completely skinned with the concentration of 8% at 75 °C taking 6.5 min, with the edible rate of 79.23%; at 85 °C, yam was completely skinned taking 4.5 min, with the edible rate of 90.12%. Peeling time was shorter and the edible rate decreased when the concentration was 9%. To reduce the loss of nutrients in yam and increase of corrosion to equipment when the temperature was too high, the optimal peeling condition was considered as concentration 8%, temperature 75 and 7 min.

3.2. Confirmation of the optimal proportion of Dioscorea opposita Thunb. cv. Tiegun and water

Material-water ratio of yam directly affected nutrition, flavor and taste of yam juice. Setting material-water ratio as 1:4, 1:6 and 1:8, enzymatic hydrolysate was determined and results are shown in Table 2.

Table 2. Influence of material-water ratio on yam quality

Material-water ratio	Structural state and taste
1:4	Dirty solution, uniform structural stat, mild taste
1:6	Dirty solution, uniform structural stat, moderate taste
1:8	Dirty solution, uniform structural stat, light taste

It could be seen from Table 2 that the comprehensive index was better when the solution ratio was 1:6; color and taste were better when the material-water ratio was 1:6.

3.3. Confirmation of enzymolysis parameters

3.3.1. Influence of enzyme volume of addition on starch hydrolysis effect

Accurately weighted yam and water were beaten (1:6) and 0.4, 0.6, 0.8, 1.0, 1.2 mg/mL of moderate-temperature a-amylase was added respectively (Figure 2).

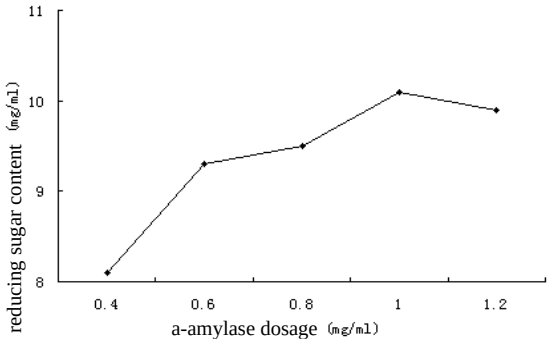

Figure 2. Influence of a-amylase dosage on starch hydrolysis

As shown in Figure 2, reducing sugar content increased with the increase of amylase dosage when volume of addition of moderate-temperature a-amylase was small. However, when amylase dosage reached to over 1.0 mg/mL, we found that reducing sugar content changed slowly as

amylase dosage increased. This explained that the reaction rate speeded up with the increase of amylase dosage and the effect of amylase was the best when amylase was completely combined with the substrate (Nithiyanantham et al., 2012; Engelen et al., 2003). Amylase failed to be completely combined with the substrate in the case of more molecular content and amylase effect was unable to be displayed. Therefore, the best dosage of amylase was 1.0 mg/mL.

In the experiment, we found that reducing sugar content increased obviously with the increase of amylase dosage and it changed slightly when amylase dosage increased to more than 1.0 mg/mL.

3.3.2.Influence of temperature on starch enzymolysis

Accurately weighted yam and water were beaten (1:6). Starch amylase was added for enzymolysis for 30 h at 45, 55, 65, 75 and 85 °C respectively, with the dosage of 1.0 mg/mL (Figure 3).

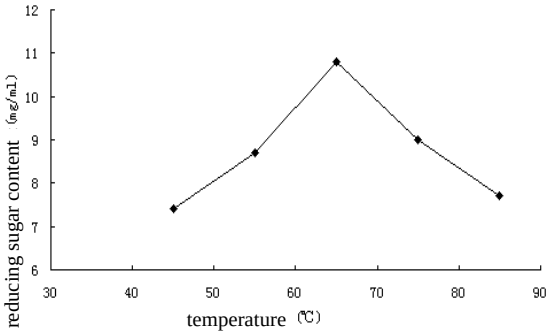

Figure 3. Influence of temperature on starch enzymolysis

It could be known from Figure 3 that reducing sugar content increased fast at 45 ~ 65 °C while it decreased gradually at 65 ~ 85 °C (Kimberly and Neuberger, 2011), which suggested that starch amylase had good activity at an appropriate temperature and higher or lower temperature was not beneficial to the enzyme activity. Therefore, the temperature was set as 65 °C in this study.

3.3.3. Influence of time on starch enzymolysis

Accurately weighted yam and water were beaten (1:6). Starch amylase was added for enzymolysis at 65 °C for 15, 20, 25, 30 and 35 min respectively, with the dosage of 1.0 mg/mL (Figure 4).

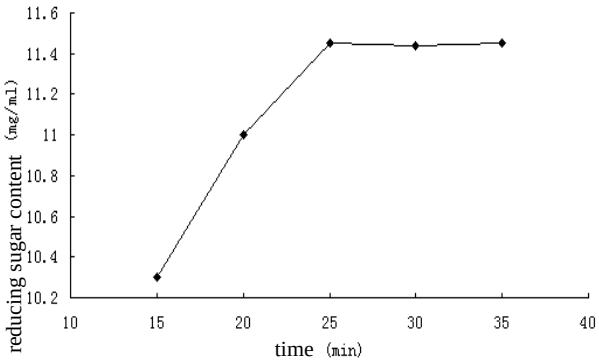

Figure 4. Influence of time on starch enzymolysis

As shown in Figure 4, reducing sugar content increased gradually within 25 min while it tended to be stable after 25 min, indicating that enzymolysis was incompletely performed on amylase in a short time while the degree of starch enzymolysis did not increase in a too long enzymolysis time. Hence, the enzymolysis time was set as 25 min.

3.4. Single factor experiment on stabilizer

Protein and other precipitates in Dioscorea opposita Thunb. cv. Tiegun juice would affect the quality, appearance and taste of products as well as destroy the stability of products, thus shortening the shelf life of products (Rodriguez-Aguilera et al., 2011; Coloma et al., 2014). To date, centrifugal observation, standing observation, viscosity density test, microscopic examination, electrophoresis detection and dynamic spectrum analysis are mainly used to confirm the stability of products. This study combining centrifugal observation with spectrophotometer detection detected and determined the stability taking the ratio of absorbance before and after centrifugation.

On the basis of preliminary experiment and data reference, the two factors mostly affecting the stability of yam juice were selected, i.e., CMC-Na and xanthan gum.

They were processed with single factor experiment according to the ratio of 0.02 %, 0.04 %, 0.06 %, 0.08 % and 0.10%.

3.4.1.Influence of differently concentrated CMC-Na on the stability of yam juice

The influence of differently concentrated CMC-Na on the stability of yam juice is shown in Table 3.

Table 3. Influence of differently concentrated CMC-Na on the stability of yam juice

Test number	Concentration (%)	Stability coefficient
1	0.02	86.49
2	0.04	88.23
3	0.06	87.16
4	0.08	87.12
5	0.10	86.89

As shown in Table 3, the ratio of absorbance increased gradually with the increase of CMC-Na dosage when other conditions were the same and the peak was 0.04%. After 0.04%, the ratio showed a downward trend. Hence, the appropriate dosage of CMC-Na was around 0.04% by comprehensive consideration.

3.4.2.Influence of differently concentrated xanthan gum on the stability of yam juice

Table 4. Influence of differently concentrated xanthan gum on the stability of yam juice

Test number	Concentration (%)	Stability coefficient
1	0.02	85.13
2	0.04	86.31
3	0.06	86.51
4	0.08	84.23
5	0.10	83.12

It could be known from Table 4 that the ratio of absorbance increased gradually with the increase of xanthan gum dosage when other conditions were the same and the peak was 0.06%. After 0.06%, the ratio showed a downward trend. Hence, the appropriate dosage of xanthan gum was around 0.06% by comprehensive consideration.

Chinese yam, as a kind of medicine as well as food, can not only be made into health food, but also has medicinal value on treatment of diseases (Bhatia et al., 2014). The Chenji yam studied in this paper is a product of geographical indication, with its protective range within the administrative region of Chenji town, Dingtao county, Shandong province, including 26 administrative villages which covers a total area of 83.9 square kilometers. It has been favored for thousands of years for its sweet and soft taste (Blackwell, 2003; Huhn, 2002) as well as medicine and health care practical value by local people.

Due to various advantages of yam, the sales volume of yam products is high nationwide, which to some extent has led to the economic development of Shandong province and has made some contribution for coordinated regional economic development in Shandong Province. Coordinated development of regional economy (Durbec and Disant, 2015; Xiong et al., 2008) refers to the state and process of realization of the interdependence, mutual adaptation and mutual promotion among regions under a fully open premise. It consists of coordination between economic growth rate, economic development quality and economic aggregate as well as coordination between industrial type and quantity inside and outside the region, which is beneficial for the coordination of economic layout of economic development in developed regions and development of the underdeveloped regions (Xia et al., 2011) as well as the coordination between interest relation, exchange relation, exchange relation and status relation among regions.

Through the experiment, we found that the polyphenol oxidase contained in Dioscorea opposita Thunb. cv. Tiegun during the production process could easily cause enzymatic browning and

affect color and luster of products. Thus, pretreatment are usually performed on yam so as to lower the enzymatic activity to inhibit enzymatic browning.

Usually, boiling water pre-cooking method is applied to realize enzyme inactivation in raw materials, cause starch dextrinization, increase juice yield and achieve a bactericidal effect. Since Dioscorea opposita Thunb. cv. Tiegun has hard texture, moisture cannot reach the inside part of yam with short precooking time and thus the enzyme deactivation effect will be unsatisfactory. However, with overlong precooking time, pulping effect can be influenced because the yam texture becomes too soft. Taking the above situation into consideration, the precooking time of yam is selected as 5 min.

4. Conclusions

The enzymolysis of yam starches was carried out with α-amylase and optimal enzymatic hydrolysis parameters were determined with response surface analysis as follows: dosage of enzyme: 1.0mg/mL; time: 25 min; temperature: 65 °C.

The compound stabilizer combination of yam juice was as follows: CMC-Na 0.04%; xanthan gum: 0.06%. Also, the development of Chenji Dioscorea opposita Thunb. cv. Tiegun series products have positive effects on economic development of Shandong province, especially on coordinated development of regional economy. However, due to limitations on conditions, there are some deficiencies on the results and analysis of this study, which will be improved in future studies.

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THE DEVELOPMENT OF OYSTER SPORTS BEVERAGE AND ITS ANTI-FATIGUE ACTIVITY ON ATHLETES AFTER TRAINING

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ABSTRACT

Sports beverage can effectively supplement the energy and nutrient substances consumed during sports and relieve fatigue for athletes. Taking oyster enzymatic hydrolysate as the raw material, we developed a good-taste and nutritional oyster sports beverage by adding some auxiliary materials according to the formulation requirements for sports beverage based on the international standard and figured out the best sterilization method. Finally, an animal experiment was carried out to evaluate the anti-fatigue activity of the beverage.

1. Introduction

Oyster is the largest cultivated shellfish in the world as well as one of the four largest cultivated shellfishes in China. Pacific oyster (Jouaux et al., 2013; Böhm and Gudehus, 2014) has drawn extensive attention for its high output and good benefit. Zymolytic oyster protein can be more easily absorbed by human body and has antihypertensive effect and oxidation resistance (Jiapei et al., 2008; Umayaparvathi et al., 2014). Therefore, more importance has been attached to preparing hydrolysate with zymolytic oyster in recent years. However, the fishy smell of oyster and the peculiar smell produced in hydrolysis process severely affect the application of oyster zymolyte.

Sports beverage can effectively supplement the energy and nutritional substances consumed during sports and help people to rapidly recover from fatigue. Sports beverages sold in the current market are electrolyte beverages added merely with saccharides, vitamins and mineral substances which cannot rapidly relieve thirst and fatigue; besides, electrolyte is difficult to be absorbed by human body due to the interference and rejection between some factors (Tomlin et al., 2013; Byars et al., 2010; Jason et al., 2011). Oyster protein enzymatic hydrolyzate contains active substances such as taurine, bioactive peptide and glycogen. It has been found that, those substances with activities of antioxidation, immunity strengthening and anti-virus can be rapidly absorbed and utilized by human body to relieve fatigue (Yang et al., 2014; Jiang et al., 2013; Shi et al., 2013). Oyster as a kind of marine organism contains a large amount of mineral substances. But if the product of oyster is eaten without desalination, it may influence the taste, flavor as well as the health of people. But those adverse factors are natural favourable factors for sports beverages, because saline ions are needed by people after sports. A new generation of oyster sports beverage which can be rapidly absorbed and effectively relieve fatigue can be developed if we add some other auxiliary materials into oyster enzymatic

hydrolysate. By doing that, the sports beverage market can be diversified and the raw material of oyster can be better developed.

Based on the study of flavor changes during the enzymolysis of oyster protein, we explored a method to reduce nutritional loss and improve flavor and developed a kind of nutritional and good-taste oyster sports beverage with strong anti-fatigue activity taking oyster protein enzymatic hydrolyzate as the raw material. The development of the product provided a theoretical basis and a practical guidance for the development of oyster product, which has economic benefits and social benefits.

2. Materials and methods

2.1 Experimental materials

The materials used included self-made oyster protein enzymatic hydrolyzate, citric acid, orange juice powder, apple juice powder, mint powder, green tea powder, mango juice powder, blackcurrant juice powder, vitamin B6, vitamin C, vitamin B12, glucose, food-grade otassium chloride, sodium chloride, sucrose, maltodextrin and high methoxyl pectin. The experimental animal was specific pathogen male (SPF) Kunming mice weighed from 17 to 23 g. Test boxes used included glycogen test box, blood urea nitrogen (BUN) test box, malonaldehyde test box, lactic acid test box and protein quantification test box. Instruments used included precision electronic balance, constant temperature vibrator, vertical type automobile pressure steam sterilizer, high-speed tissue stamping machine, vertical type refrigerated centrifuge, high-pressure homogenizer, high performance liquid chromatography, ultraviolet spectrophotometer, rotary evaporator, mini-shaker and minitype freezing point osmometer.

2.2 Experimental methods

2.2.1 Detection of nutritional components

Water was detected using direct drying method; ash was detected using dry cineration method; protein was detected with Kjeldahl determination; crude fat was detected using method of chloroform-methanol, and glycogen was detected using anthrone-sulfuric acid method (Liu et al., 2015). Calcium, iron, zinc, sodium, potassium, magnesium, aluminum and copper were detected using inductive coupling plasma-atomic emission spectroscopy.

2.2.2. Debugging of the taste of oyster sports beverage

Orange juice powder, apple juice powder, mint powder, green tea power, mango juice powder and blackcurrant juice powder of proper quantity were added. Then the best powder and the corresponding amount were confirmed by sensory evaluation.

2.2.3. The effect of sterilization method on the quality of oyster sports beverage

Oyster sports beverages were subpackaged into glass bottles. After capping, they were sterilized by water bath at 70, 90 and 120 °C for 30 min, 20 min and 4 min respectively. The best seasoning powder and sterilization method was confirmed based on organoleptic score, loss rate of vitamin C and total count of bacterial colony.

2.3. Design of experiment

The mice purchased were put into a raising room to adapt to the environment for one week and they ate and drank freely in that period. The temperature of the raising room was set as 25 °C and the humidity was set as 60%; day (12 h) alternated with night (12 h). All mice were given two-day adaptive swimming (water depth: 30 cm; water temperature: 25 °C) after one week. Mice which were unable to swim were excluded. Then the remaining mice were grouped. Mice in the normal saline group were fed with normal saline; mice in the Gatorad group were fed with Gatorad sports beverage; mice in the oyster sports beverage group were given oyster sports beverage. The volume was 0.1 mL/10g in all groups. The gavage lasted for two weeks; mice could freely eat and drink in the process. Grouping of mice is shown in Table 1.

Table 1. Grouping of mice

Group	Group A: anti-fatigue group	Group B: fatigue relief group	Group C: weight carrying swimming group	Group D: control group
Category	Normal saline group/10 mice Gatorad group/10 mice Oyster sports beverage group/10 mice	Normal saline group/10 mice Gatorad group/10 mice Oyster sports beverage group/10 mice	Normal saline group/10 mice Gatorad group/10 mice Oyster sports beverage group/10 mice	Sedentary group/10 mice

2.3.1. Detection of duration of weight carrying swimming

Mice in group C were given test substances every day. After 30-min rest, they received 39 min of swimming training after every 30-min rest, lasting for 14 days. Thirty minutes after the last gavage, the mice carrying 4% iron wire were put into a 25 °C swimming box. The time from the beginning of swimming to the immersing of head in water for 8 s was recorded as the swimming duration.

2.3.2. Evaluation of anti-fatigue activity of oyster sports beverage

Mice in group A were given 0.1 mL/10 g test substances every day. After 30-min rest, they were trained to swim for 30 min, for 14 days. Thirty minutes after the last gavage, the mice carrying 4% iron wire were put into a 25 °C swimming box. Ninety minutes later, the mice were taken out of water. Eyeballs, blood, liver and muscle were collected. Serum and tissue homogenate were prepared. Then the content of blood lactic acid, glycogen, muscle glycogen, BUN and malonaldehyde in the test substances were detected according to the instruction of kit.

2.3.3. Evaluation of anti-fatigue activity of oyster sports beverage

Mice in group B were trained for 30 min every day. Then they were taken out of the water, dried and given 0.1 mL/10g test substances. After 14-day swimming, the mice swam for 1.5 h without carrying weight. Then they were dried and given oyster sports beverage, normal saline and commercially available sports beverage respectively. After 30-min rest, eyeballs, blood, liver and muscle were collected from the mice. Serum and tissue homogenate were prepared. Then the content of blood lactic acid, glycogen, muscle glycogen, BUN and malonaldehyde in the test substances were detected according to the instruction of kit.

3. Results and discussions

3.1 Nutritional components of oyster protein enzymatic hydrolysate

Through analyzing the nutritional components of oyster protein enzymatic hydrolysate, we found that it contained many nutritional components including 1.02 g/100 mL protein, 0.76 g/100 mL sugar and 0.2 g/100 mL taurine. Sugar and protein can provide human body with energy rapidly and effectively, and taurine can effectively relieve fatigue. In addition, oyster protein enzymatic hydrolysate contains a large number of microelements and saline ions including 2.74 μg/mL Al, 39.49 2.74 μg/mL Ca, 1.15 μg/mL Cu, 49.87 μg/mL Fe, 268.51 μg/mL K, 30.04 μg/mL Mg, 768.45 μg/ml Na and 19.32 μg/ml Zn. It can effectively supplement electrolyte missing during sports and maintain the balance of osmotic pressure of body fluid. Therefore, oyster sports beverage developed based on

oyster enzymatic hydrolysate has good nutritional value and physiological property.

3.2. Allocation of oyster sports beverage

3.2.1. Basis of the addition of electrolyte

Best sports beverage should provide people with electrolyte which are lost in sweat during exercise(Morgan et al., 2004). However, the taste of sports beverages needs to be considered during allocation; hence sports beverages cannot contain all the electrolytes in actual application. Too much intake of electrolytes would affect taste and result in strong feeling of thirst. Therefore, on the premise of not influencing taste, electrolytes need to be added as more as possible by referring to the composition and content of electrolytes in human body and sweat. It has been found that, water can be absorbed well when the ratio of glucose to sodium is close to 2 (Guo et al., 2004). Therefore, the content of Na could be confirmed as 40 mg and the content of k as 10 mg.

3.2.2. Basis of the addition of saccharides

The content of carbohydrate in sports beverage can be influenced by the absorption speed of the stomach and intestine to carbohydrate and water absorption. Saccharides in low content cannot supplement human body with energy timely; and saccharides in high content can increase the burden of the stomach and intestine. Research results demonstrate that, the absorption speed of the stomach and intestine to carbohydrate is 112 g/min; and athletes need to supplement 50 ~ 1042 J/h heat if doing exercise for more than one hour. Therefore, a supplement of 40 ~ 80 g/h saccharides can achieve a relatively good effect. In addition, applying compound glycogen can not only maintain blood glucose at a proper level, but can also regulate the osmotic pressure and taste of sports taste and promote the absorption of saccharides and water. Therefore, the content of glucose, sucrose and maltodextrin could be confirmed as 4 g, 1 g and 1 g.

3.2.3. Adjustment of osmotic pressure of sports beverage

Osmotic pressure represents the quantity of particles in solution. Osmotic pressure of normal blood (or body fluids) is 280 ~ 330 mOsm/kg. The intake of a large quantity of low infiltrated drink during sports, water, for example, can lower osmotic pressure of plasma and promote the precipitation of particles; as a result, the desire of drinking water is inhibited immediately. In contrast, the intake of too much beverage with high osmotic pressure can result in gastrointestinal discomfort such as satiety and abdominal distension, increasing burden. Osmotic pressure of isotonic beverages is 280 ~ 330 mOsm/kg, which is balanced with body fluid; therefore, it is more beneficial for the supplement of nutritional substances and energy after sports. Hence the osmotic pressure of sports beverage needs to be considered. In this experiment, osmotic pressure of the oyster enzymatic hydrolysate was detected as 154 mOsm/kg by a freezing point osmometer.

Oyster sports beverage was made referring to the international standard and the components of commercially available sports beverages such as Red Bull, Mizone and Gatorad. Assume that every athlete drinks 500 ~ 1000 mL of sports beverage every day, then per 100 mL of sports beverage should contain 2 g of oyster enzymatic hydrolysate, 40 mg of Na, 10 mg of K, 0.3 mg of VB6, 12 μg of VB and 40 mg of VC. To improve the flavor of oyster liquid, orange juice powder (0.2%) and mint powder (0.1%) were added. Finally the osmotic pressure of oyster sports beverage was measured to be 315 mOsm/kg.

3.3. Influence of sterilization method on the quality of oyster sports beverage

Table 2 shows the influence of different sterilization methods on the quality of oyster sports beverage. Though pasteurization has little influence on the color, smell and vitamin C of beverage, its effect of sterilization is not satisfactory and the microbiological indicator cannot meet the international standard (cfu < 100/mL). The flavor of beverage sterilized at

90 °C for 20 min is acceptable, though the quality is affected slightly; besides, the sterilization method can achieve good sterilization effect. Therefore, 90 °C and 20 min were selected as the optimal sterilization conditions for oyster sports beverage.

Table 2. Influence of difference sterilization methods on oyster sports beverage

	Color	Smell	Flavor	Loss rate of VC	The number of colony
70 °C, 30 min	No obvious change	The previous flavor was completely remained; free from extraneous odour	Good flavor and no peculiar smell	2.14±0.05%	1466
90°C, 20 min	Slightly dark	The previous flavor was fairly completely remained; but the delight had slight reduction.	Relatively good flavor with slight burning small, but acceptable	7.77±0.11%	58
120 °C, 4 min	Very dark	Relatively strong peculiar smell; no delight	Relatively strong burning small and fishy smell, unacceptable	10.18±0.25%	None

3.4. Quality index of oyster sports beverage

Oyster sports beverage was made according to the above requirements. Then indexes of the product were detected, and the results are shown Tables 3 and 4.

Table 3. Sensory index of oyster sports beverage

Item	Color	Fragrance	Taste	Texture status
Index	Gentle color, light yellow, even	Special fragrance of oyster and no peculiar smell	Special fragrance of oyster; sour and sweet; delicate taste; no peculiar smell	Transparent, stable and even; little sediment after long-time placement; no visible foreign matters

Table 4. Physicochemical and microbiological indexes of oyster sports beverage

Item	Actual content	International requirement
Soluble solid %	7.8	3.0-8.0
Protein (g/100mL)	1.15	—
Na (g/mL)	450	50-1200
K (g/mL)	150	50-250
Ascorbic acid (g/mL)	45	≤120
VB6 (g/mL)	3	—
VB12 (ug/100mL)	2	—
Taurine (g/100mL)	0.1	—
Total bacterial count	30	≤100
Coli group	0	≤3
Mycete and saccharomycetes (cfu/mL)	0	≤20
Other pathogenic bacteria	Not detected	None

3.5. Influence of oyster sports beverage of exhaustive swimming time of mice

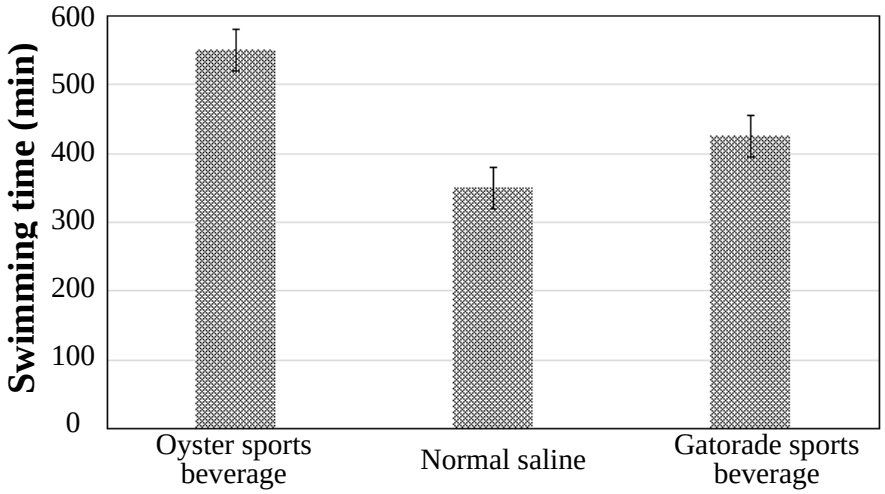

Figure 1. Influence of oyster sports beverage on exhaustive swimming time of mice

Loaded exhaustive swimming experiment is usually used for evaluating anti-fatigue performance of drugs. The swimming duration is correlated to exercise tolerance. Long swimming time indicates good anti-fatigue effect of drugs. Figure 1 shows that, the swimming time of three groups had significant difference. The swimming time of oyster group was 551 min, significantly longer than that of the other two groups ($p < 0.05$); the swimming time of Gatorade group was 422 min, which was longer than that of normal saline group. The above findings suggested that, oyster sports beverage could significantly prolong the exhaustive swimming time of mice and had certain anti-fatigue performance.

3.6. Evaluation of anti-fatigue performance of oyster sports beverage

Table 5. Influence of oyster sports beverage on biochemical indexes of mice

	Content of lactic acid (mmol/L)	BUN (mmol/L)	Hepatic glycogen (mg/g, liver tissue)	Muscle glycogen (mg/g, muscle)	Malonaldehyde (nmol/mgprot)
Oyster sports beverage	8.04±0.37	8.66±1.05	16.25±1.07	3.36±0.62	47.14±10.07
Normal saline	11.85±1.44	9.74±1.36	11.92±2.17	1.92±0.74	106.33±18.74
Gatorade sports beverage	8.97±1.47	9.51±0.85	12.78±1.25	2.56±0.44	118.25±21.07

A large amount of energy can be consumed in sports. Glycolysis reaction provides energy for muscle, but also generates a large amount of lactic acid. H+ isolated from lactic acid can result in the increase of pH in muscle, break the balance of internal environment and lead to fatigue. The content of lactic acid in blood increases when lactic acid in muscle penetrates into blood. After sports, the content of blood lactic acid gradually decreases and fatigue is relieved due to the termination of glycolysis and removal of lactic acid. Therefore, anti-fatigue activity of the test substance can be determined by detecting the content of lactic acid in serum.

Table 5 shows that, the content of lactic acid in the serum of mice in oyster sports beverage group was much lower than that in normal saline group ($p < 0.05$); but there was no significant difference between oyster sports beverage group and Gatorade sports beverage ($p > 0.05$). The above findings suggest that, oyster sports beverage with strong effect in removing lactic acid could effectively relieve fatigue.

The content of serum BUN represents the metabolic status of nitrogen substances in human body and can be used for evaluating the loaded tolerance capability of human body under special condition. When glycogen and blood glucose supply are insufficient, proteins decompose to provide partial energy.

Urea generated from the decomposition of protein in ornithine cycle can result in the increase of BUN. Lower content of BUN indicates less decomposition of nitrogen substance and stronger adaption capability of human body to load (Yu et al., 2006).

Table 5 suggests that, the content of BUN of oyster sports beverage significantly decreased (8.66 mmol/L) compared to the other two groups ($p < 0.05$), but the difference of normal saline group and commercially available beverage group was insignificant ($p > 0.05$).

As BUN is the metabolite of protein, protein rarely involves in energy supply when the sports time is too short, resulting in the insignificant change of BUN, which may be due to the failure of detection of regulatory effect of oyster sports beverage on nitrogen metabolism of serum blood urea.

Glycogen can maintain blood glucose at a normal level and it is also the source of energy in muscle fiber shrinkage. As glycogen with a highly branched structure can lead to the distribution of a large quantity of glucose at the non-reducing end of glycogen molecule, glycogen can be rapidly decomposed to supply energy. Higher storage quantity of glycogen can improve exercise tolerance.

Hepatic glycogen can supplement the blood glucose consumed in motor process, which is of great significance to maintain the balance of glucose. When the storage of glycogen is insufficient, fatigue can be induced (Jia and Wu, 2008). Though muscle glycogen cannot directly provide blood glucose for human body, it can provide the energy which is needed in muscle contraction. Table 5 suggests that, the content of hepatic glycogen of oyster sports beverage (16.25 mg/g) was much higher than that of the other two groups ($p < 0.05$); the content of hepatic glycogen of Gatorade group and normal saline group had no significant difference ($p > 0.05$); the content of muscle glycogen of oyster sports beverage group was 3.36 mg/g, 1.44 mg/g higher than normal saline group ($p < 0.05$) and 0.8 mg/g higher than Gatorade group respectively ($p > 0.05$).

Acute exercises and exhaustive exercises can increase the content of endogenous free radicals in human body and strengthen lipid peroxidation, thus damaging cellular membrane system. Free radicals and lipid peroxidization injury are in a relatively obvious correlation to exercise induced fatigue.

Malonaldehyde, the product of lipid peroxide, is an important index for evaluating the metabolism of free radicals. The content of malonaldehyde can be used for measuring the level of free radicals. In this experiment, the content of malonaldehyde of oyster sports beverage was 47.14 nmol/mgprot, which was much lower than that of the other two groups ($p < 0.05$). It indicated that, oyster sports beverage could reduce the generation of malonaldehyde during intensive exercises and improve antioxidant ability.

3.7 Evaluation of refection activity of oyster sports beverage

Table 6. Influence of oyster sports beverage on biochemical indexes

	Lactic acid (mmol/L)	BUN (mmol/L)	Hepatic glycogen (mg/g, liver tissue)	Muscle glycogen (mg/g, muscle)	Malonaldehyde (nmol/mgprot)
Oyster sports beverage	11.65±1.87	8.67±0.65	18.97±1.82	2.09±00.48	53.78±18.25
Normal saline	10.58±0.99	9.56±0.82	12.23±2.15	2.41±0.77	111.47±22.48
Gatorade	11.68±1.96	8.85±0.87	17.44±2.25	2.18±0.74	105.78±22.47
Control	16.77±1.84	9.97±1.45	18.95±2.48	2.95±0.38	66.47±19.36

Table 6 shows the content of different components including lactic acid, BUN, hepatic glycogen and muscle glycogen in different sports beverages. It can be known that, the content of lactic acid of sports groups was much less than that of the sedentary group, which suggested sports could remarkably improve the activity of lactic dehydrogenase and thus reduce the content of lactic acid. However, no significant difference of the content of lactic acid was observed between oyster sports beverage group, normal saline group and commercially available group ($p > 0.05$). That might be because the decline of the content of lactic acid was insignificant in such a short rest time.

The content of BUN of oyster sports beverage group was lower than that of normal saline group and Gatorade sports beverage group. However, we found the level of BUN of three groups had no significant difference ($p > 0.05$) and the level of BUN was normal in three groups in static state. That might be because few proteins are consumed in such a short swimming time. Therefore, the inhibition effect of oyster sports beverage on BUN was not observed.

The content of malonaldehyde in serum of mice in oyster sports beverage (53.78 nmol/mg) was much lower than that of normal saline group and Gatorade sports beverage group ($p < 0.05$); no significant difference was found between Gatorade sports beverage group and normal saline group. In addition, the content of malonaldehyde of mice in oyster sports beverage group recovered to the normal level after 30-min rest, and there was no significant difference with mice in sedentary group ($p > 0.05$). It indicated that, oyster sports beverage group could rapidly eliminate lipid oxidation product, relieve fatigue and restore physical power.

By detecting hepatic glycogen of mice in different group, we found oyster sports beverage group was significantly different with normal saline group ($p < 0.05$), but insignificant with sedentary group ($p > 0.05$), suggesting the storage quantity of hepatic glycogen could be rapidly recovered after 30-min exercises. The comparison of the content of muscle glycogen between different groups suggested that, there was no significant difference between three experimental groups and sedentary group. Hepatic glycogen not only can be synthesized with glucose, but also can be converted from nonsugar substances such as pyruvic acid, glycerin, lactic acid and amino acid through gluconeogenesis. However, muscle glycogen can only be synthesized with glucose; as a result, it cannot be synthesized in a short time.

We developed a kind of oyster sports beverage taking oyster enzymatic hydrolysate as the raw material according to the international standard as well as the formula of commercially available sports beverage. Every 100 mL of sports beverage contains 2 g of oyster protein enzymatic hydrolysate, 4 g of glucose, 1 g of sucrose, 1 g of maltodextrin, 4 mg of Na, 10 mg of K, 60.3 mg of VB6, 121 μg

of VB, 40 mg of VC, 0.2% orange juice powder and 0.1% mint powder. Different sterilization methods were compared taking sense, loss rate of VC and the total bacterial count as the evaluation indexes. Finally, water bath (90 °C and 20 min) were confirmed as the optimal sterilization conditions. The loaded swimming time of mice given oyster sports beverage 30 min before exercises was much longer than that of normal saline group and Gatorade group ($p < 0.05$); besides, oyster sports beverage could significantly reduce the generation of lactic acid and malonaldehyde and increase the content of hepatic glycogen and muscle glycogen. It indicated that, oyster sports beverage could effectively strengthen physical functions, regulate the balance of internal environment, relieve fatigue and improve athletic ability. The content of lactic acid, BUN and muscle glycogen of mice in oyster sports beverage group (oyster sports beverage was taken immediately after sports) had no significant difference with that of mice in normal saline group and Gatorade group within 30 min; besides, the content of malonaldehyde and hepatic glycogen recovered to the normal level rapidly. It indicated that, drinking oyster sports beverage could rapidly eliminate lipid oxidation product produced during exercises and effectively restore the storage quantity of hepatic glycogen, but was ineffective in rapidly eliminating lactic acid and increasing the storage quantity of muscle glycogen.

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APPLICATION OF FOOD RISK ASSESSMENT AND EARLY WARNING METHOD BASED ON DATA MINING

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ABSTRACT

In recent years, continuously emerging food safety issues have attracted wide attention and become more and more obvious as trade liberalization, economic globalization and food trade develop rapidly. This study first gives an introduction on comprehensive assessment mathematical model, multidimensional data model, association rule mining as well as Apriori algorithm, then analyzes core concept of each algorithm; secondly, discusses process and core concept of risk assessment through analyzing food risk assessment and early warning system concretely, establishes multidimensional data model based on food safety risk indicators and observes data from different angles to make better decisions; finally, performs instance analysis according to risk assessment method.

1. Introduction

With the advent of poison rice, substandard milk powder and poison bean sprout, food safety issues have impacted people over and over again, resulting in more concerns on food safety.

China has conducted extensive researches on food safety risk assessment as attentions on food safety heat up gradually. Scholars from various countries have put forward some methods for evaluating food safety risks in recent years, including probability exposure assessment model (Changhua et al., 2012; Liwei and Hong, 2008; Jingxian et al., 2009) and gray relative analysis method (GRA) (Huixi and Rentian, 2008; Zeyi and Yaobo, 2000), and China mainly develops index assessment method (Liling et al., 2003; Rentian et al., 2008) and comprehensive assessment method. Establishing food safety risk assessment index is the foundation. Chen Yinyu et al (Yinyu et al., 2007; Beibei et al., 2008) take category of risk factors in food safety into an overall consideration, which involve features of relevant food, establishment of enterprise safety and health system as well as running conditions, national laws and regulations on production and supervision, quality of law-executor, etc., but fail to analyze risk factors under the category. Pan Chunhua et al (Chunhua et al., 2010; Jianjun and Shengpu, 2011) set some rules combining national safety standard, and then early warning information for food safety is produced after simple statistics on food detection data according to some rules. Therefore, food safety risk assessment and pre-warning are the preconditions of guaranteeing food security.

2. Materials and methods

2.1. Mathematical model for comprehensive assessment

Comprehensive assessment is a mathematical method for evaluating multiple abstract systems based on several indexes, considering transforming several indexes into an index that can reflect the comprehensive situation as the core concept (Shoukang, 2003). The assessment with sole index and clear process is called as "individual assessment", however, place of comprehensive assessment different from individual assessment lies in assessment standard or index system varying complexity, but not the number of objects involving in the assessment (Shun and Shuxin, 2010). For example, evaluating a student's performance belongs to an individual assessment, while an assessment on the teaching quality of a school is considered as a comprehensive evaluation.

Mathematical model for comprehensive evaluation is shown below:

(1)Confirmation of evaluation index set

Evaluation index set is on behalf of evaluation index system, generally speaking, it is expressed with a vector, and every component can present the state of the system in many respects. To analyze and evaluate running or development conditions of objects being evaluated comprehensively, it is usually expressed as $x=\left\{x^{(1)},x^{(2)},...,x^{(n)}\right\}$, of which, n state vectors is $x^{(i)}=\{x_{i1},x_{i2},...,x_{im}\}^T(i=1,2,...,n)$, and m refers to m evaluation indexes.

(2)Confirmation of evaluation set

Evaluation set, showing the level that evaluation index is likely to belong to, is usually expressed with $v=(v_1,v_2,...,v_k)$, of which, $v_i(i=1,2,...,k)$ means different evaluation levels may exist in k.

(3)Confirmation of evaluation index weight

In the practical application analysis, evaluation factors have various influences on evaluation goal, and corresponding weight vector is confirmed as $w=(w_1,w_2,...,w_n)^T$ according to practical effect of m evaluation indexes, besides, $\sum_{i=1}^{m} w_i=1$ exists.

(4)Confirmation of comprehensive evaluation function

After confirming evaluation index set and weight, comprehensive evaluation function is presented as $y=f(w,x)$, thus, function value of comprehensive evaluation index is figured out: $y_i=f(w,x^{(i)})(i=1,2,...,n)$, and n systems are classified based on evaluation set according to $y_i(i=1,2,...,n)$, to confirm evaluation level.

2.2. Multidimensional data model

Data warehouse (DW), the core issue of multidimensional data model, is on the basis of multidimensional data model, together with online analytical processing (OLAP) tool. In other words, multidimensional data model shows a multi-dimensional space, "dimension" stands for the object that user observes, and points in the space are metrics (Shengen and Shan, 2005). In the multidimensional data model, data are organized into a multidimensional model structure, and each dimension contains several abstract layers with hierarchical definition, which provides flexibility for user observing data from multiple perspectives (Ming and Xiaofeng, 2007). For example, user may be interested in time, commodity, region and sales volume of the object when analyzing food selling (Figure 1), in the following figure, little blocks can be assumed as sales volume or other application instances for storing data according to actual situations.

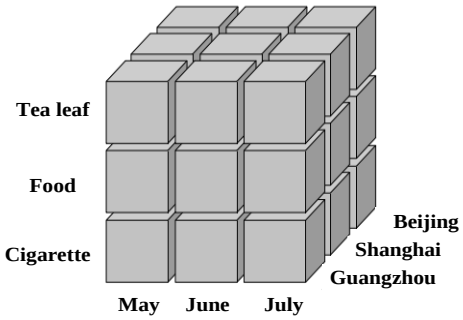

Figure 1. Data cube

2.3. Association rule mining

Association rule mining is a branch of data mining technology. Association rules, mainly applied in discovering the connection between different commodities in the transaction database, are able to reflect customer's purchasing behaviors, including input attribute and output attribute. Comparing traditional rules, difference of association rules is in that it has one or more output attributes, and output attribute of one rule can be taken as input attribute of another rule (Hongxia and Song, 2010; Jun et al., 2010; Haipeng, 2010).

2.4. Apriori algorithm

In 1994, Agrawal et al (Chunyuan, 2012) proposed that Apriori algorithm could be used in finding frequent item set in database, and the difference of which consisted in applying prior knowledge in data mining, compared with traditional algorithm. In addition, frequent item set was found to have 2 very important anti-monotone natures.

One is that sub-item set of frequent item set is surely frequent item set.

The other one is that superset of infrequent item set must be infrequent.

Based on above two natures, Apriori algorithm uses layer-by-layer iterative search algorithm together with K - item set to explore (k+1) - item set. L_k is the set of frequent K - item set and C_k refers to the set of candidate K - item set.

3. Results and discussions

3.1. Analysis on application of food risk evaluation and early warning system

3.1.1.Integrative construction of risk evaluation system

Food safety risk evaluation system is an important part of food safety risk assessment and early warning, and its reliability not only provides theoretical foundation for food risk warning, but also creates advantages for food safety supervision.

This study sets up a scientific risk evaluation system due to shortcomings in risk evaluation system and inconvenience for experts evaluating risk indexes. This system first analyzes and discusses food safety risk indexes that produce risks in detail and perfects risk evaluation index system; secondly, builds multidimensional data model on food safety risk evaluation indexes to formalize information knowledge so that experts can set level and weight; and finally, evaluates food risks using comprehensive evaluation method according to set level and weight. Process of food safety risk evaluation is shown in Figure 2.

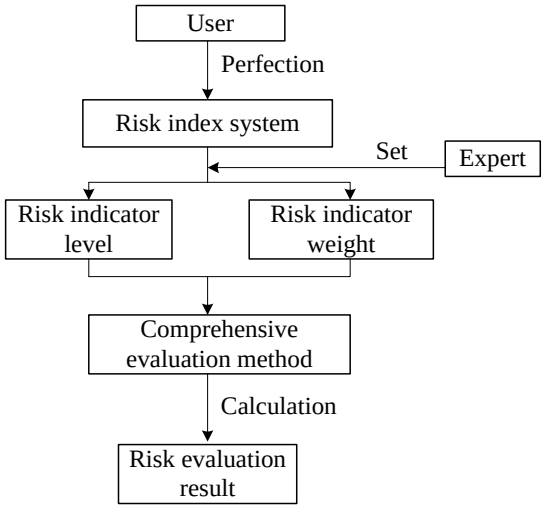

Figure 2. Process of food safety risk evaluation

3.1.2. Risk early warning

Though numerous scholars have done a large number of studies on food safety early warning, shortages exist in many aspects, for instance, early-warning object and source of the early warning information.

To date, food safety early warning is mainly targeted to test item or spot check, and early warning on food is relatively less. After food inspection, supervision department can obtain food risk level; if the risk level is high, early-earning information is sent and relevant departments make corresponding responses.

From another perspective, the food safety early warning is aimed at analyzing food safety monitoring data statistically, then, experts formulate corresponding rules for early warning, but the loss of original data and noise lead to deviation and error in the source of the early warning information.

3.2. Analysis on multidimensional data model of food safety risk index

3.2.1 Establishment of multidimensional data model

When experts evaluate food safety risks, level and weight should be set for risk evaluation index, and setting one-time cannot be suitable for all situations will happen in the future.

According to analysis above, plenty of elements are required to be considered when setting level for food safety risk evaluation indexes. Thus, traditional two-dimensional data analysis can no longer realize expected results, and multidimensional data modeling is demanded for risk evaluation index.

3.2.2. Display of multidimensional data cube

Multidimensional data cube with multidimensional data model is able to show the relationship between various dimensions more intuitively and make a convenience for setting risk index. On the foundation of diverse factors and dataset considered by experts in setting risk index, multidimensional data cube is analyzed from different angles.

Specialists focus on time, food, enterprise, nation and unqualified food when setting evaluation level for credit in enterprise. Here, because four-dimensional data cube is fairly complicated to be established, time dimension is confirmed first to analyze the influence of changes of other dimensions on setting risk evaluation index. Three-dimensional data cube made up of nation, enterprise plus food is displayed in Figure 3.

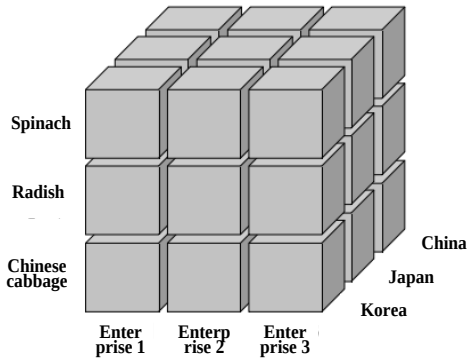

Figure 3. Credit in enterprise three-dimensional data cube

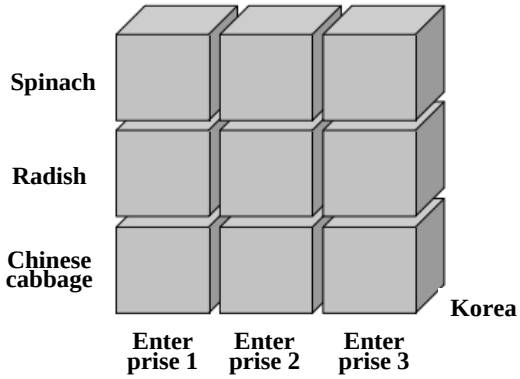

Figure 4. Volume analysis of credit in enterprise data cube

Data stored in small blocks in Figure 3 can be used for expressing unqualified food, credit in enterprise is related with time, food, enterprise and nation, and those correlation dimensions decide the unqualified food value. From a point of view of nation dimension, rolling up multidimensional data cube can acquire the tangent plane (Figure 4).

Specialists pay much attention to time, food, nation, test item and standard residual quantity when setting evaluation level for foreign technology. Time dimension is confirmed to analyze the influence of changes of other dimensions on setting risk evaluation index, and data cube consisting of nation, test item plus food dimensions is shown in Figure 5.

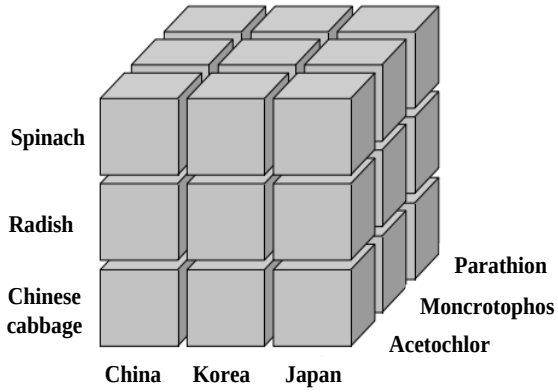

Figure 5. International technology multidimensional data cube

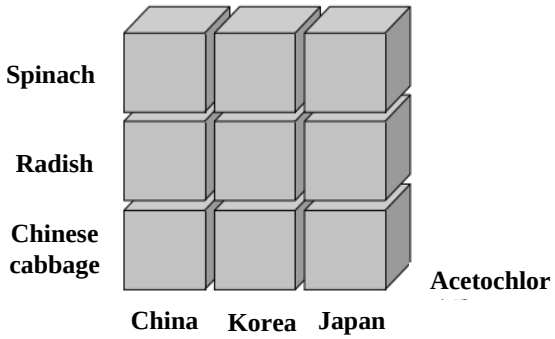

Figure 6. Volume analysis of international technology multidimensional data cube

Data stored in small blocks in Figure 5 are digital measured value, presenting standard residual quantity. Standard residual quantity is correlated with test item, food name, nation and set time in data model, and those correlation dimensions decide the standard residual quantity of test item. From nation dimension, rolling up multidimensional data cube can obtain the tangent plane (Figure 6). Relevant statistics data of standard acetochlor residues of each food in various countries in a specific period can be seen from it, and some food's standard acetochlor residues in each country are observed as well.

3.3. Mining process and result analysis

Factors that can affect food safety risk are analyzed using Apriori algorithm in association rules mining. Apriori algorithm is conducted for Boolean data while food category, risk evaluation result and time are multivalued data, so the obtained data should be converted. Food category mapping is S_{xy}, of which, x refers to category number and y expresses subclass number. Time information quarter mapping is $J_1 - J_4$, month mapping is $M_1 - M_{12}$, and enterprise risk level is set as Q_L, Q_M, Q_S, high risk, medium risk and low risk respectively. The official sets above risks as G_L, G_M, G_S, trade risk as T_L, T_M, T_S, and assumes risk evaluation result as P_L, P_M, P_S. Above data are encoded as shown in Table 1.

Table 1. Coded data

S_{01},	S_{015},	Q_S,	G_S,	T_S,	J_3,	M_9,	P_S
S_{02},	S_{021},	Q_M,	G_M,	T_L,	J_2,	M_4,	P_M
S_{02},	S_{022},	Q_M,	G_S,	T_L,	J_2,	M_4,	P_M
S_{01},	S_{015},	Q_L,	G_M,	T_S,	J_3,	M_8,	P_M
S_{02},	S_{021},	Q_M,	G_M,	T_M,	J_2,	M_5,	P_M
S_{02},	S_{021},	Q_M,	G_L,	T_M,	J_2,	M_6,	P_M
S_{02},	S_{022},	Q_S,	G_M,	T_M,	J_1,	M_1,	P_S
S_{01},	S_{015},	Q_L,	G_L,	T_M,	J_2,	M_5,	P_L

Encoded data in Table 1 are iterated applying Apriori algorithm, and minimum confidence coefficient and minimum support are set as 75% and 30% respectively. Data mining experiment is performed using Apriori algorithm, and data are the results of food inspection and reported record after food safety risk assessment in inspection and quarantine database in Qingdao from 2014 to 2015. Minimum confidence coefficient and minimum support are set as 80% and 20% respectively, to carry out association rules mining. After obtaining 1, 200 rules, association rules are restrained and screened from prior information because of too much data. As to risk evaluation level of predicted and inspected food, association rules are restrained as follows: consequent must have and only risk assessment results, and antecedent has to contain time

types, together with some other attributes, the more the better. In this way, the risk evaluation results of inspected food will be more accurate.

After deleting and constraining the association rules, the number of association rules decreases by 70%, and key association rules are found out:

(1) Food category = plant food, enterprise risk = high risk, official risk = high risk, quarter = the second quarter ——> risk assessment level = high risk;

(2) Food subclass = Chinese cabbage, enterprise risk = high risk, official risk = medium risk, trade risk = low risk, quarter = the third quarter ——> risk assessment level = medium risk;

(3) Food subclass = spinach, enterprise risk = medium risk, month = August ——> risk assessment level = medium risk.

It can be observed from above example that rules acquired from association rule mining reveal connotative correlations between risk evaluation indexes in risk evaluation data. The generation of the rule has a practical significance; on the one hand, these rules obtained from a large number of historical evaluation data mining are in line with the actual meaning; on the other hand, enterprise risk will impact food export to some extent.

4. Conclusions

Realizing food safety evaluation and early warning system construction with computer technology is of great help for food safety supervision, but it comes true with difficulty due to relatively complicated information of food safety issue. Thus, this study puts emphasis on presenting the application of evaluation method, in the meantime, proposes corresponding points to process and core concept of early warning system. However, accuracy of risk early warning remains to be further improved as a certain differences are bound to exist in the collected data and actual data.

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APPLICATION PROSPECT OF ISOMALTULOSE IN PHYSICAL ACTIVITY

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ABSTRACT

The purpose of this study is to evaluate the effects of different content of carbohydrate and volume (concentration) on glycemic index (GI) of isomaltulose beverage and to provide a reference for the application of solid sports beverage and the development of sports beverage which takes isomaltulose as the raw material. Twenty healthy adult females were selected as research objects. The test was carried out according to the international standard of food GI detection. The objects were given 250 ml (20 g/100 ml), 500 ml (10 g/100 ml), 250 ml (10 g/100 ml) and 250 ml (5 g/100 ml) of isomaltulose beverage which contained 50 g, 50 g, 25 g and 25 g of carbohydrate respectively. Finger blood collected was tested for GI with YSI-1500 glucometer. Totally 1342 blood samples were collected, including the samples used in pre-experiment. GI values of 250 ml (20 g/100 ml), 500 ml (10 g/100 ml), 250 ml (10 g/100 ml) and 250 ml (5 g/100 ml) isomaltulose beverage which contained 50 g, 50 g, 25 g and 25 g of carbohydrate were 33.24 ± 2.15, 32.14 ± 2.47, 38.21 ± 6.14 and 37.55 ± 5.84 respectively; there was no remarkable difference between them. But GI of isomaltulose beverage containing 25 g of carbohydrate was relatively high. It is concluded that, the concentration of carbohydrate has no significant influence on GI. Hence to improve the competitive ability, athletes can select beverage in different concentrations according to their own needs.

1.Introduction

Researches relating to saccharides have attached much attention in sport nutrition field (Bingham et al., 2012; Tomlin et al., 2013). The reasonability of saccharides intake is of great significance to both ordinary people and athletes, especially to those athletes who want to improve competitive levels and abilities (Richards, 2015; Edwards and Casto, 2013; Wang et al., 2013). Glycemic Index (GI) will rise after people eat food containing saccharides. As more and more people, especially patients and their family members, start to detect GI with glucometers, scientific researchers devote more to studying various glucometers (Perard et al., 2014; Ramtoola et al., 2014; Byounghoon et al., 2014). In 2011, Philis-Tsimikas et al. (Athena et al., 2011) explored the preciseness, accuracy and acceptability of OneTouch SelectSimple glucometer. They selected 100 diabetes patients and tested GI with YSI-2300 STAT glucometer and OneTouch SelectSimple glucometer. Health specialist agencies evaluated the results and the users evaluated the operation of OneTouch SelectSimple glucometer and moreover filled questionnaire survey. The statistical results suggested that, OneTouch glucometer was of high preciseness and accuracy and easy to be operated and accepted.

Glycemic index (GI) was proposed by Jenkins (Reyes-Pérez et al., 2013; Jenkins et al.,

2012; Vega-López et al., 2009) in 1981 at first. It refers to the response extent of blood glucose two hours after the intake of carbohydrate. GI can definitely reflect the physiological status of body after the intake of carbohydrate and it is also an effective index for measuring blood glucose response induced by food containing carbohydrate (Wolever et al., 2008; Wolever et al., 2013). GI, a relatively new concept in nutriology, is of great reference significance to the energy control and supplementation of athletes. Testing GI of athletes taking sports beverage and developing different sports beverages can provide more choice for athletes and body-building group.

Isomaltulose as a kind of functional disaccharide with special performance has become more and more popular in food industry (Park et al., 2014; Min-Wen et al., 2014). Especially in recent years when people concern more about health, the production and development of isomaltulose arouses much attention. Besides, the advancement of biocatalysis production technology provides a wide prospective prospect for industrial production of isomaltulose (Jördening et al., 2008; Park et al., 2014). Different sports beverages can be produced using different matching methods of saccharides. Due to the different sources of saccharides, GI of different beverages may differ. Hence, GI of isomaltulose which is regarded as the raw material of sports beverage needs to be detected. This study explored the influence of isomaltulose beverage with different carbohydrate content and volume on GI.

2. Materials and methods

2.1. Experimental objects

Twenty female postgraduate students from Beijing Sports University were selected. Written informed consent was obtained from all subjects. The participants conformed to the following conditions: nonsmokers; aged 20 ~ 28 years (average 24.1 ± 1.4 years); normal weight (body mass index BMI = 18±2.1 kg/m^2); no family history of metabolic disease and diabetes; no food allergy or intolerance; normal physiological indexes; completing test according to the requirements; no gastrointestinal disease and upper respiratory infection recently or during experiment; no intake of drugs or nutritional supplement influencing GI. Prior to the test, they were forbidden to eat and drink for more than 10 hours. Sports were not allowed in the morning of the test day.

2.2. Experimental method

2.2.1. Detection method of GI

GI detection was performed according to international standard ISO 26642:2010 (Food, 2010). Food products -- Determination of the glycaemic index (GI) and recommendation for food classification (ISO 26642:2010) is the current international standard for GI determination. As required by the standard, objectives included should not develop good allergy or intolerance and digestive system disease and are forbidden to eat more than 10 hours before test as well as drink and are not allowed to do exercise in the morning of test day, and peripheral blood such as finger blood is suitable to be the blood sample, as it is less likely to mutate compared to venous blood.

The calculation formula for GI was: GI = (increment area under the curve (IAUC) of blood glucose within 2 h after intake of tested food/ IAUC of blood glucose within 2 h after intake of glucose in same quantity) ×100.

The internationally recognized GI detection standard includes the following procedures.

(1)Glucose tolerance test

Participants should be forbidden to eat for at least ten hours prior to test. Fasting blood was collected twice in the morning of next day; there was an interval of 5 min. Blood glucose level was detected in statistic state, and the average value was taken as the baseline value. Then participants were given 50 g of glucose solution for oral administration (20 ml) and it is required to be completed in 12 ~ 15 min.

Timing starts from the time of oral administration. Blood was collected for detecting blood sugar concentration in the 15^{th}, 30^{th}, 45^{th}, 60^{th}, 90^{th} and 120^{th} min.

(2)Food blood glucose response test

Participants who were tested to be qualified in glucose tolerance test received food test after two days at least. Blood was collected twice in the morning of next day; there was an interval of 5 min. Besides, blood glucose level was detected in statistic stage; the average value was taken as the baseline value. Then participants were given food containing 50 g of available carbohydrate and 250 ml water; it is completed in 12 ~15 min. Timing starts from the moment of intake of food. Finger blood was collected for detecting blood glucose level in the 15^{th}, 30^{th}, 45^{th}, 60^{th}, 90^{th} and 120^{th} min.

Heparin anticoagulant capillary tube was used to collect not less than 25 µl of finger blood after the first drop of blood contacts with ACCU-CHEK Performa Tiras Reactivas. Then a 25 µl YSI-1500 glucometer sample injector was used to inject the blood samples into YSI-1500 glucometer.

2.2.2. IAUC of glucose response

Glucose response curve was drawn taking time as horizontal coordinate and blood glucose level at different time points as the vertical coordinate. The following formula was used to calculate IAUC of glucose response (Brouns et al., 2005; Nilsson et al., 2008; Di et al., 2011).

Suppose blood glucose concentration at time point t_0, t_1… t_n (0, 15… 120 min) as G_0, G_1… G_n.

$$\text{IAUC} = \sum_{n}^{x=1} A_x \qquad (1)$$

Ax refers to IAUC from time point t_{x-1} to t_x)

In the first period (x=1):

If $G_1 > G_0$, $A_1 = (G_1 - G_0) \times (t_1 - t_0)/2$ otherwise, $A_1=0$.

In other periods (x > 1):

If $G_x \geq G0$ and $G_{x-1} \geq G0$, then $A_x = ((G_x - G_0)/2 + (G_{x-1} - G_0)/2) \times (t_x - t_{x-1})$;

if $G_x \geq G_0$ and $G_{x-1} < G_0$, then $A_x = ((G_x - G_0)2/(G_x - Gx\text{-}1)) \times (t_x - t_{x-1})/2$;

if $G_x < G_0$ and $G_{x-1} \geq G_0$, then $A_x = ((G_{x-1} - G_0)2/(G_{x-1} - G_x)) \times (t_x - t_{x-1})/2$;

if $G_x < G_0$ and $G_{x-1} < G_0$, $A_x = 0$.

2.2.3. Calculation of GI

Food GI is usually calculated by taking glucose as reference (Edwards and Casto, 2013).

GI = (IAUC of blood glucose within 2 h after intake of tested food/ IAUC of blood glucose within 2 h after intake of glucose in same quantity) ×100.

2.3. Test design

YSI-1500 glucometer was used for testing blood glucose. GI of 250 ml (20 g/100 ml), 500 ml (10 g/100 ml), 250 ml (10 g/100 ml) and 250 ml (5 g/100 ml) isomaltulose beverage which contained 50 g, 50 g, 25 g and 25 g of carbohydrate was detected. Then the GI was compared.

2.4. Statistical processing

Data were expressed as mean ± standard error (SE). SPSS for Windows 16.0 was used for statistical analysis.

GI of beverage with different content of carbohydrate and volume was tested by double-factor variance analysis. When there was obvious difference, difference of data was tested with Post Hoc Tests using Tukey HSD test method by taking the content of carbohydrate and volume as processing factors. $\alpha = 0.05$ was considered as the significant level.

3. Results and discussions

3.1. Glucose response of glucose beverages with different content of carbohydrate and volume

Glucose response of females drinking beverages with different content of carbohydrate and volume was detected at different time points. The significant difference

of blood glucose level concentrated on time points of 60th, 90th and 120th min. Blood glucose concentration of females taking beverage containing 50 g and 20 g of carbohydrate had remarkable difference (table 1). Results suggested that, IAUC of glucose was in a correlation to the intake of glucose (50 g or 25g), *i.e.*, IAUC of blood glucose of beverage containing 50g of glucose (500 ml or 250 ml) was much larger than IAUC of blood glucose of beverage containing 25g of glucose (500 ml or 250 ml); IAUC of blood glucose of glucose had no relationship with the intake of liquid (500 ml or 250 ml), *i.e.*, IAUC of blood glucose of the intake of 50g or 25g of glucose had no significant difference, no matter the volume of beverage taken was 500 ml or 250 ml. Details are shown in Figures 1 ~ 6.

Table 1: Glucose response of females drinking glucose beverage detected with YSI-1500 glucometer (mmol/l)

Volume of beverage	**250ml**		**500ml**	
Content of glucose	25g	50g	25g	50g
0min	4.17±0.12	4.41±0.05	4.11±0.14	4.14±0.15
15min	6.44±0.32	7.19±0.41	7.01±0.29	7.29±0.46
30min	7.93±0.36	8.31±0.31	8.14±0.32	8.71±0.38
45min	7.02±0.31	8.24±0.54	7.28±0.19	7.33±0.59
60min	5.33±0.29^*	7.23±0.51	5.71±0.25^*	6.42±0.41
90min	3.99±0.24^*	5.98±0.36	4.02±0.15^*	6.35±0.21
120min	4.02±0.15^*	4.76±0.21	3.51±0.14^*	5.42±0.27^

^: $p < 0.05$ compared to 50 g (250 ml); *: $p < 0.05$, compared to 50 g (500ml)

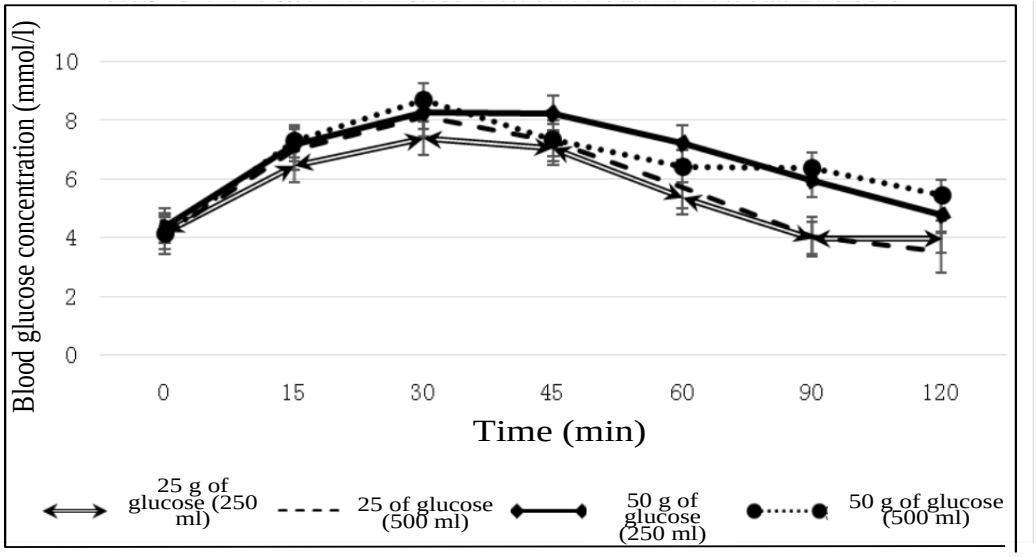

Figure 1. Glucose response of glucose beverage with different content of carbohydrate and volume

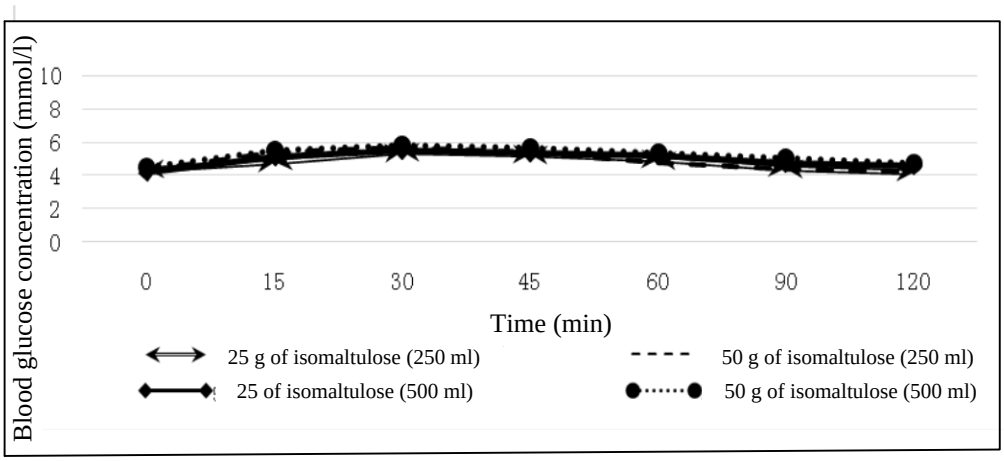

Figure 2. Glucose response of isomaltulose with different content of carbohydrate and volume

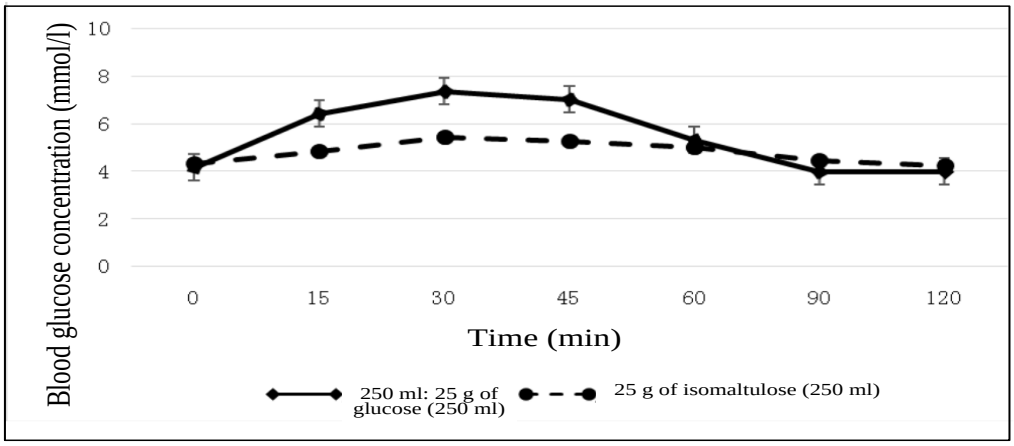

Figure 3. Glucose responses of 25 g of glucose and isomaltulose (250 ml)

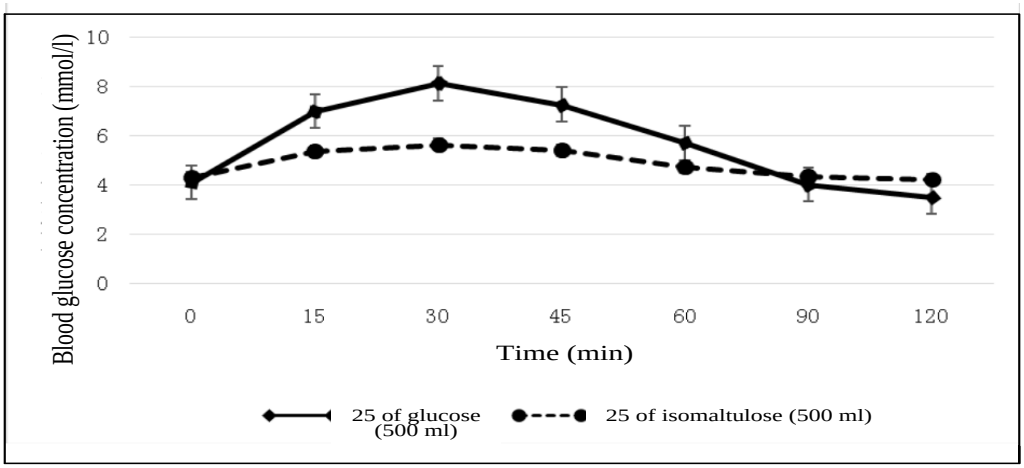

Figure 4. Glucose responses of 25 g of glucose and isomaltulose (500 ml)

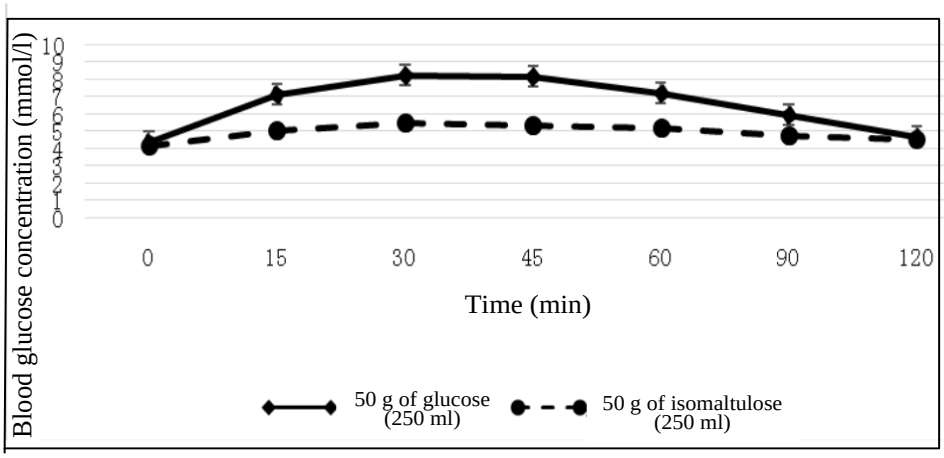

Figure 5. Glucose responses of 50 g of glucose and isomaltulose (250 ml)

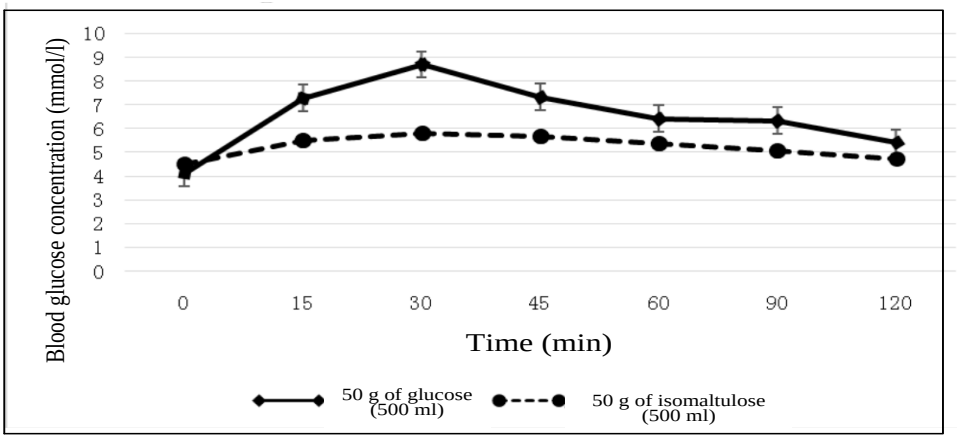

Figure 6. Glucose responses of 50 g of glucose and isomaltulose (500 ml)

3.2. Glucose response of isomaltulose beverage with different content of carbohydrate and volume

The difference of blood glucose level mainly reflected on time points of 60th min, 90th min and 120 min (table 2). IAUC of blood glucose of isomaltulose was much smaller than that of glucose in the same conditions. IAUC of blood glucose of 50 g of isomaltulose was slightly higher than IAUC of blood glucose of 25 g of isomaltulose, but there was no remarkable difference. Similar to glucose, the volume of beverage taken had no influence on IAUC of blood glucose of isomaltulose.

3.3. GI of isomaltulose beverage with different content of carbohydrate and volume

No remarkable difference was observed in GI of isomaltulose beverage with different content of carbohydrate and volume (Table3).

Table 2: Glucose response of isomaltulose beverage detected by YSI-1500 glucometer

Volume of beverage	**250ml**		**500ml**	
Isomaltulose	**25g**	**50g**	**25g**	**50g**
0min	4.35±0.09	4.25±0.08	4.31±0.09*	4.51±0.09
15min	4.85±0.12	5.14±0.16	5.39±0.24	5.52±0.24
30min	5.45±0.15	5.53±0.16	5.66±0.18	5.81±0.18
45min	5.29±0.20	5.39±0.15	5.42±0.13	5.69±0.20
60min	5.02±0.15	5.26±0.14	4.74±0.12*	5.39±0.16
90min	4.45±0.15*	4.79±0.18	4.36±0.10*	5.10±0.18
120min	4.26±0.12*	4.58±0.16	4.24±0.16*	4.73±0.18

^: $p < 0.05$, compared to 50 g (250 ml); *: $p < 0.05$, compared to 50 g (500 ml).

Table 3: GI and IAUC of blood glucose of glucose and isomaltulose beverage with different content of carbohydrate and volume

Volume of beverage – carbohydrate content	**IAUC of blood glucose**		**GI**
	Glucose	**Isomaltulose**	
250ml-25g	161.11±13.87*ab	60.14±13.78^	38.21±6.14
250ml-50g	282.56±28.77*cd	91.36±11.68^	33.24±2.15
500ml-25g	190.32±12.87*ab	69.47±13.48^	37.55±5.84
500ml-50g	301.22±16.87*cd	93.74±9.56^	32.14±2.47

^: $p < 0.05$, compared to IAUC of blood glucose of glucose;
*: $p < 0.05$, compared to IAUC of blood glucose of isomaltulose;
a: $p < 0.05$, compared to 50g-250 ml;
b: $p < 0.05$, compared to 50g-500 ml;
c: $p < 0.05$, compared to 25g-250ml;
d: $p < 0.05$, compared to 25g-500ml.

4. Conclusions

We carried out the experiment according to the international standard ISO 26642:2010(E). In the experiment, we compared differences of glucose, IAUC of blood glucose and GI as well as the effects of glucose and isomaltulose with different content of carbohydrate and volume on them. Detection of glucose response IAUC of glucose and isomaltulose suggested that blood glucose IAUC of glucose was in a correlation to the intake of glucose (50 g or 25 g), i.e., IAUC of blood glucose of 500 ml or 250 ml isomaltulose beverage containing 50 g of glucose was much larger than IAUC of blood glucose of 500 ml or 250 ml isomaltulose beverage containing 25 g of glucose; IAUC of blood glucose of glucose had no relationship with the intake of liquid (500 ml or 250 ml), i.e., IAUC of blood glucose of beverage containing 50 g or 25 g of glucose had no significant difference, no matter the volume of beverage taken was 500 ml or 250 ml. Besides, GI of isomaltulose beverage with different content of carbohydrate and volume had no significant difference.

The internationally used GI detection method is to add 250 ml of water into 50 g of carbohydrate (20%) (Wong et al., 2009; Donaldson et al., 2010; Luscombe et al., 1999). In this study, we used the above method and finally obtained results consistent with the international GI table.

Usually, the concentration of carbohydrate of most liquid sports beverages ranges from 5% to 10%. GI of the beverage which was prepared by adding 50 g of carbohydrate into 500 ml of water (10%) and that of the beverage which was prepared by adding 50 g of carbohydrate into 250 ml of water (20%) had no remarkable

difference. If the concentration of beverage is set as 5% and moreover intake of 50 g of carbohydrate is required, then intake of 1000 ml of liquid is necessary. However, intake of such a large quantity of liquid seems to be difficult for some participants. In such a condition, intake of 25 g of carbohydrate is easy to be accepted. It was found that, detection result of GI of 25 g of carbohydrate was a little higher than GI, but there was no remarkable difference.

The purpose of the experiment is to observe effects of different content of carbohydrate and volume on GI of isomaltulose beverage. Experimental results suggested that, GI of isomaltulose beverage with four different contents of carbohydrate and volumes had no significant difference. Hence, it is considered that, concentration has no obvious impact on GI. Athletes can select sports beverage in different concentrations according to their needs, thus to improve competitive ability.

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DEVELOPMENT OF CONTROL SYSTEM FOR FOOD PROCESSING TESTING MACHINE

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ABSTRACT

Computer control system has been widely applied in food industry with the development of computer industry. This thesis introduces a kind of control system that uses the upper and lower computer structures taking tablet machine as the upper computer and single chip microcomputer (SCM) as the lower computer. This study mainly includes contents of overall system design, hardware and software design and design of upper computer application software. It first makes an introduction on the overall design of the system including working process of food processing testing machine, hardware and software design of testing machine control system as well as overall control scheme design of the testing machine; moreover, provides detailed information on hardware design of lower computer of testing machine control system, puts forward development direction conforming to practical application and discusses temperature control module and on-off input and output module; finally researches the software design of upper computer of testing machine, realizes real-time record and monitoring of field data and works out machine running, record query, alarm and other dynamic operation pictures. This system is able to manage food processing production in a modern way to improve the efficiency of production; and meanwhile, control the precision and achieve significant economic benefits. It also has excellent reference and guiding effects on other automatic food processing industries.

1. Introduction

Food has been always important strategic supplies in every historical stage of each country. An increasing number of new technologies and methods were used in food industry, and a variety of comprehensively used technologies drove the development of food industry as scientific and technological revolution in the 20th century had a profound impact on the food processing industry. For example, Voicu et al. (2008) conducted researches on new apricot hybridization. Jun et al. (2004) explored processing industries of apple and Goff (2013) discussed mechanical processing modes of yoghourt, cheese, ice cream and other dairy products.

Food processing industry combining agriculture and industry with the tertiary industry is inseparably interconnected with agriculture and helps each other forward (Rehber and Rehber, 2000). Food processing industry in developed countries as an important growth point of national economy has turned into a significant manufacturing sector and export

sector for earning foreign exchanges (Viaene and Gellynck, 1999). Developed countries develop food industry early and considerably due to early and high industrialization and urbanization plus fast science and technology development. Food processing industry with a great variety of goods and high degree of mechanization and automaticity guarantees the production efficiency of processing enterprises, at the same time, ensures the stability, integrity, reliability and standardization of product quality. Japan possesses advanced food processing technology, exquisite manufacturing and high degree of automation (Ijiri et al., 2007); food processing industry in France has a very strong development momentum for nearly 30 years and its industrial output grows rapidly (Gopinath and Munisamy, 2005); food production in Germany is featured by complete and diverse variety (Martínez, 2010). America as a great corn production, processing and consumption country has large corn yield, which brings huge economic benefits for the United States (Davis et al., 2000).

Food processing automation in China starts late and has relatively low technical level although it develops rapidly, and its technological content remains to be further improved. Besides, complete sets of food equipment matching food industry are also relatively lagging. Featured by small scale, backward equipment, weak foundation, low technical content, serious disconnection of automatic control system with process design and mechanical manufacturing, poor stability and kitting of products and low precision and degree of automation, most of the enterprises related to food industry still depend on importing a large number of foreign packaging machineries and components, which is not suitable for the rapid development requirements of China's food industry. Therefore, improving the automation level of food processing industry has been of a very considerable practical significance with the popularization and application of microcomputer. Based on current situation of China's food processing industry above, this study describes the technical indicators of food processing testing machine as well as functions of control system in detail.

2. Materials and methods

2.1.Working process of food processing testing machine

Working process is shown in Figure 1.

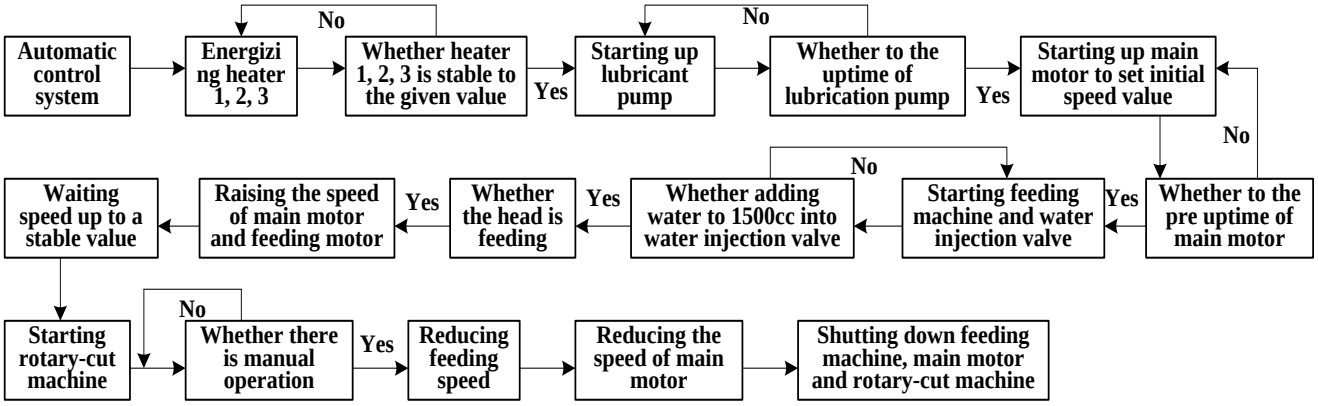

Figure 1. Working process

2.2. Overall design of hardware in testing machine control system

The upper computer uses PPC-1501 panel industrial personal computer (IPC) as man-machine interaction device.

Frequency control device uses a frequency converter series applied in controlling the speed of three-phase current motor, i.e., MICROMASTER420 variable voltage and variable frequency (VVVF) (Siemens).

Sensor adopts temperature sensor (PT100) and pressure sensor (30MPa and 0.2MPa).

2.3. Overall control scheme design of food processing testing machine system

(1) Distributed control system (DCS) and RS-485 computer bus are used.

(2) PPC-1501 panel IPC is taken as host computer.

(3) Based on the design of single chip microcomputer (SCM), input and output modules exchange information with host computer by RS-485 computer bus, so as to collect temperature, pressure and cooling parameters, receive orders from the host computer and output controlled variable.

(4) VVVF device from Siemens as frequency control device receives speed governing order and outputs a given rotational speed by communicating with RS-485 computer bus and host computer.

(5) Application software does a secondary development based on Kingview 6.51 (Wellin Tech) and realizes the given control requirements, development contents include control algorithm and field-bus communication.

3. Results and discussions

3.1. Hardware design of lower computer of testing machine control system

(1) Design of temperature control module

① Scheme constitution

Featured by high precision and excellent stability, thermal resistance is capable of transmitting, displaying and recording temperature output after appropriate data processing (Doyle and Mazzotta, 2000). PT100 thermal resistance sensor is taken as a temperature measurement device, and SCM system consisting of AT89C52 control center controls external heating device and realizes the accurate control of temperature by various control algorithms. Principle of the system is in Figure 2.

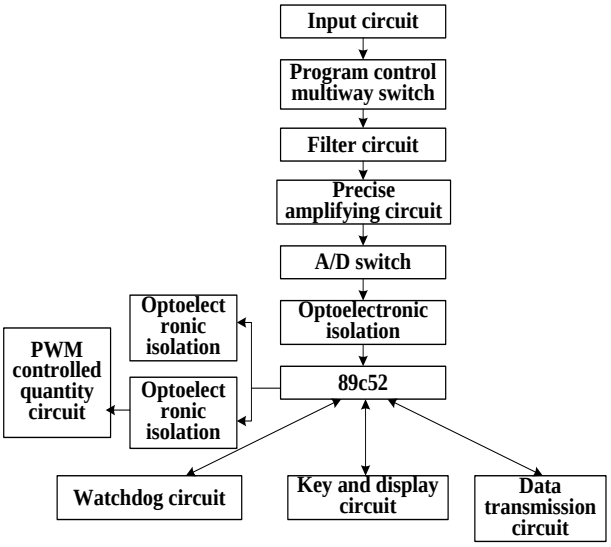

Figure 2. Principle of the system

② Design of input channel

A. PT100 wiring circuit

Resistance of thermal resistance is positively associated with temperature. The following formula describes the characteristics of PT100, and it can be obviously seen that temperature has a non-linear relationship with electrical resistance.

$$R_1 = R_0 \times (1 + A \times t + B \times t^2 + C \times (t - 100) \times t^3) \quad (1)$$

Of them, $A=3.9082\times10^{-3}1/°C$, $B=-5.802\times10^{-7}1/°C^2$, $C=-4.2735\times10^{-12}1/°C^4$, and R_0 is the resistance at 0°C.

B. Design of program control multiway switch

An 8 channel analog is used in measuring, and two differential motion four channel analog switch CD4O52 was applied in selecting each channel (Chen et al., 2004).

C. Filter circuit

To inhibit interference noise, a primary hardware filter circuit is added before the signal amplification and the structure is symmetrized to make sure line balance.

D. Precision differential amplifier circuit

Precision differential amplifier circuit has plenty of advantages, such as slow input, signal amplification, strong common-mode rejection ability as well as single-ended output (Prokopenko et al., 2008). Hence, this thesis uses three pieces of operational amplifier OP07 with low offset voltage and current plus low drift to constitute a two-level precision differential amplifier circuit.

E. A/D switching circuit

Differential amplifier, V/F switch and optoelectronic isolation are used in converting analog input into frequency signal for processing, and V/F switch is realized by applying LM331. In addition, LM331 adopting temperature-compensation circuit is able to improve the conversion accuracy in the whole operating temperature range.

③ Design of control circuit

AT89C52 SCM (ATMEL Corporation) is taken as the control center of analog quantity module (ShengXue, 2009; Hongli *et al.,* 2008), mainly functioned by collecting analog quantity, controlling the output according to different control algorithms; transmitting collected analog state to upper computer by serial port of network communication; receiving data and orders sent from upper computer and controlling the electronic luminescent display (ELD) status.

A. Upper computer

Eight T89C52 SCM (ATMEL Corporation) is taken as the control center of analog quantity module, mainly functioned by collecting analog quantity, controlling the output according to different control algorithms; transmitting collected analog state to upper computer by serial port of network communication; in the meanwhile, receiving data and orders sent from upper computer and controlling the display state of light emitting diode (LED).

B. Watchdog circuit

25045 watchdog circuit resets automatically when the system enters into an endless loop or system voltage decreases to below minimum operating voltage due to break down (Hirose et al., 2004). 40% serial bit in the 25045 is used in expanding PROM storage control parameters, alarm parameters and other control information and data, and exports those information automatically when backroll after power-on reset.

④ Design of output channel

There are mainly two kinds of output quantities in this module; one is to control working condition of external device, and the other one is an alerting signal. Output signal sends drive circuit to control controlled objects by optoelectronic isolation and Schmidt level switch. Seven Darlington circuits UNL2003 are used in driving. Thyristor outputs a string of complete sine wave by adjusting power and zero-crossing trigger technology in the control cycle, and then the breakover time of thyristor in the control cycle is changed by altering the number of sine wave to control the power.

⑤ Design of key and display circuit

Keyboard and display as components realizing man-machine interaction can not only interfere with the state of equipment and data input, also report operating condition and processing results to people (Horacek, 2008).

A. Keys

To save interface line, matrix keyboard interface made up of row line and column line is designed, and keys are located in the intersection of row and column. Five 1/0 ports can form a keyboard with 6 keys in this module.

B. Display circuit

Display adopts serial-in parallel-out shift registers 74HC164 consisted of 9 LED Nixie tube and its static drive circuit to control digital display. Display segment code is loaded serially by SCM and instructed by 74HC164 drive LED during working.

⑥ Design of communication interface

RS-485 serial interface bus is used for data acquisition and network control, sending end driver transmits transistor-transistor logic (TTL) level signal into differential signal and outputs it, and then reverts differential signal to TTL signal in the receiver. Communication distance can reach 1200m when transmitting speed is 100KBIT/S. Serial port of light 89C52 is connected to RS-485 bus via optocoupler 6N136 and RS-485 bus driver SN75176, and finally exchanges data with personal computer (PC) through 4852/232 module.

⑦ Design of power circuit

Multiple stabilized voltage supply is applied in supplying power for each part of the circuit to improve the ability of resisting disturbance of system, and meanwhile, realize the application of optoelectronic isolation technology in isolating input and output channel. 12V power supply is designed for operational amplifier, analogue switch and temperature sensor; 5VI for the connection part of central processing unit (CPU) and optocoupler; 5V2 for the connection part of optocoupler and input and output channel. Each power supply is made up of 7800 series three terminal regulator, and adjustable three-terminal voltage regulator LM317 can be used for forming 1.75V power supply, in order to power ELD Nixie tube.

(2) On-off input and output module

①Scheme constitution

Based on SCM and shifting register, this study designs an inexpensive and highly efficient intelligent multi-channel on-off input and output module (Tan *et al.*, 2003). Microprocessor uses 89C52 (Atmel Corporation) to expand on-off switch quantity condition input in the parallel port (Cristea and Danchiv, 2010), deserializer chip 74HC164 outputs all required control signal and controls 32-channel output. Hardware mainly includes SCM, input and output channel circuit, data communication circuit, and detailed process is shown in Figure 3.

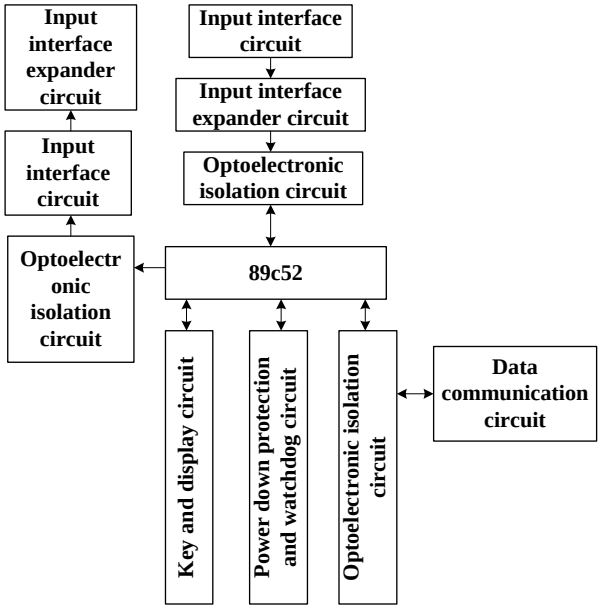

Figure 3. Process of on-off input and output

②Design of input channel

A. Design of input interface circuit

The selection of R1 needs to consider resistance and power consumption, and its volume should not be oversize (Huber and Jovanovic, 2000). The relationship between power consumption p, resistance R1 and alternating voltage effective value U that may be joined up wrongly is displayed in Figure 4.

$$P = \frac{1}{R}\left(\frac{V_{cc}}{2} + 0.45U\right)^2 \quad (2)$$

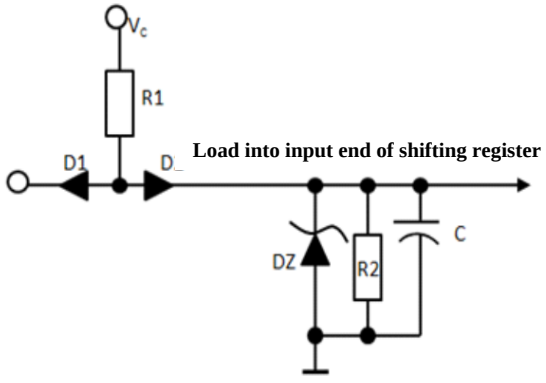

Figure 4: Signal conditioning of input channel and circuit protection

B. Design of input interface expander circuit

Four pieces of 8 bits parallel input serial output shift register 74HC165 is applied in expanding channel 32-channel switch input, and P0.0 port of SCM controls S/L of 74165 in a working way of parallel input or serial output.

③Design of control unit

AT89C52 SCM as the control center is able to collect switching value on time, process calculation, output control law, and transmit collected switch condition to upper computer by serial port of network communication, also receive data and orders from upper computer and display switch condition as well as confirm and report accident.

Watchdog circuit is similar to temperature control module, especially design of communication interface and power circuit. Here, it is not explained in detail.

3.2 Design of upper computer software of food processing testing machine

(1) Design of upper computer control software

① Input of control parameters and display of controlled variable

One advantage of this module lies in forming a visual man-machine interface combined with strong graphic function of computer. Some control parameters of lower computer are capable of making lower computer hardware produce corresponding actions by clicking and inputting screen of upper computer, and meanwhile, controlled variable can be displayed on the screen of upper computer in the graphic form.

② Realization of proportion integration differentiation (PID) control algorithm

As to the temperature control experiment, upper computer software adopts visual basic (VB) to realize time optimal PID control and transmits controlled quantity, i.e., breakover time of thyristor to lower computer by serial port, and then, lower computer transmits the obtained temperature value to upper computer as input of control system.

③ Communication with lower computer

Every communication of CP asks SCM 8 channel input instructions and 8 channel output control parameters and other command groups (Díaz et al., 2002). Figures 5 and 6 show the communication process of control host computer and SCM.

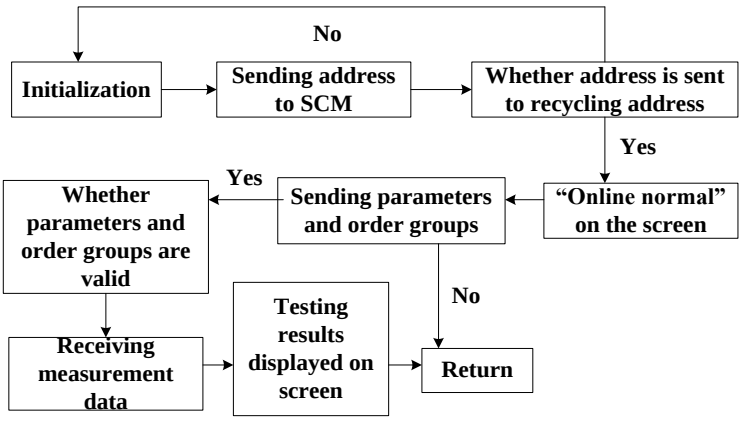

Figure 5. Process of PC communicating with SCM

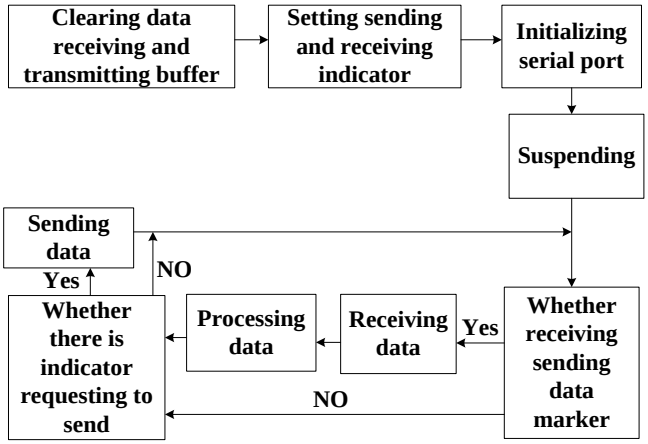

Figure 6. Process of SCM communicating with PC

(2) Design of upper computer monitoring pictures

Graphical interface is aimed to simulate actual process and corresponding device with abstract pictures. Database construction refers to establish a concrete database to reflect attribute of industrial control object, for instance, pressure and temperature inside charging barrel of food processing testing machine. Monitoring picture system of testing machine is composed of several monitoring pictures, including parameter setting and operating picture, running condition picture, temperature history curve and real-time curve picture, alarming window picture. Its system framework is in Figure 7.

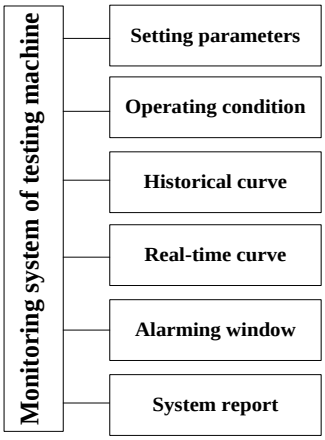

Figure 7. Framework of monitoring system

4.Conclusions

Based on current condition and development tendency of domestic food processing testing machine, this study proposes and designs a control system for food processing testing machine that meets the domestic market demand. This control system integrating data acquisition, real-time control and display in one not only reduces the cost of system, also makes full use of computer resources and speeds up the development. At the same time, this thesis carries out a thorough and extensive research on theoretical analysis, control strategy and industrial control software development according to the characteristics of food processing testing machine.

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EFFECT OF SOYBEAN POLYPEPTIDE FOOD ON PHYSICAL RECOVERY

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ABSTRACT

Objective: By analyzing the effect of soybean polypeptide food on physical recovery of basketball players, we explored the influence of soybean peptide on immune system and its antioxidant effect, thus to provide a theoretical basis for the development of soybean peptide.

Methods: By combining single factor experiment with response surface analysis, we did an optimization research on enzymolysis technology of soybean antioxidant peptides; gel filtration chromatography was adopted to separate bean pulp powder hydrolysate and 3 components of polypeptides were obtained; furthermore, in vitro and in vivo antioxidant experiments were performed.

Results: Soybean peptides could eliminate hydroxyl radicals (produced by Fenton reaction) and superoxide anion radical (produced by pyrogallol system); they could significantly inhibit H2O2-induced hemolysis of red blood cells; in addition, they had an inhibitory effect on lipid peroxidation to some degree.

Conclusion: Soybean peptides have health care effects such as enhancement of immunity, oxidation resistance and delaying senescence.

1. Introduction

In recent years, with the development of molecular biology, there have been new discoveries in function and nutrition of polypeptides which not only cover all kinds of amino acids, but also have regulatory effect on the physiological function of organisms (Kohsokabe and Kaneko, 2016; Qiang, et al., 2008). Soybean polypeptides are small peptides obtained by protease hydrolysis of soy proteins, featured with high absorptivity and fast absorption rate (Bamba, et al., 2015). Since soybean polypeptides are a kind of bioactive peptides, it is of great value to develop soybean polypeptides as food and health products, which has an important effect on the recovery of basketball players' physical fitness (Hoffman and John-Arne, 2012).

According to a recent investigation, the overall nutrition status of basketball players in China is not optimistic; consequently, basketball players' physical fitness is in need of targeted nutritional supplement (Altendorfer, et al., 2015). Nutrition intervention is effective in preventing basketball players from the decrease of blood sugar and serum iron during basketball training. In other words, nutritional intervention can effectively prevent fatigue, enhance immunity, increase energy, restore physical fitness and improve endocrine function (Rafiee, et al., 2014; Monnier, et al., 2012). The opinion that soybean polypeptide food has an effective effect on the recovery of basketball players'

physical fitness has been approved by a lot of experts and scholars. In 2014, Nikolaidis et al. found that increase of serum creatine kinase after exercise could be relieved by supplementing soybean polypeptides, which indicated that soybean polypeptides could promote the repair of tissue damage and reduce intracellular creatine kinase leakage effect (Nikolaidis, et al., 2014). In 2012, Cherif et al. analyzed basketball players' physical consumption characteristics and energy supply methods; furthermore, they analyzed the relationship between three major energy supply materials (sugar, fat, protein) and basketball training as well as competition, aiming to explore the importance of rational nutrition and balanced diet to the elimination of fatigue and physical recovery of basketball athletes (Cherif, et al., 2012).

Therefore, by increasing the specific immune function of organisms, soybean polypeptides can enhance body immune system so as to promote physical recovery.

2. Materials and methods

2.1. Function of soybean peptides

2.1.1. Good solubility and low viscosity at high concentration (Cwalina and Wagner, 2016)

After hydrolysis, reticular structure of soybean protein is destroyed, which leads to decrease of expansion and viscosity (Zhou, et al., 2015). When the concentration of soybean peptide in solution is 30%, its viscosity is only equivalent to the viscosity of 10% soybean protein solution. Soybean peptide is characteristic of low viscosity at high concentration, which makes it particularly applicable in high protein fluid food.

2.1.2. Good stability in resistance to acid and heat

Refined soybean peptide can remain in good dissolved state around isoelectric point pH 4.5 of soybean protein, which means the solution is clear and transparent, without being influenced by pH change or heating (Koji et al., 2011). With a high nitrogen solubility index (NSI) value (more than 90%) and good instant solubility, soybean peptide is applicable in the production of instant drinks, which provides favorable conditions for the development of acidic soybean beverages and protein-rich acidic food.

2.1.3. Absorption and retention of moisture (Liping, et al., 2006)

Moisture absorption and retention ability of soybean peptide is better than that of collagen polypeptide and silk protein peptide, which meets the psychological need of pure natural plant cosmetics and low cost.

2.1.4. Softening gel (Ueki, et al., 2016)

Soybean peptide can be applied in the production of ham, sausage, fish cake and other high protein food to soften the food, adjust the hardness and improve the taste of food.

2.1.5. Promoting fermentation (Palani, et al., 2016)

Soybean peptide can promote microbial growth and activate metabolism; moreover, it can promote the proliferation of lactic acid bacteria, bifidobacteria, yeast, mold and other fungi as well as secretion of useful metabolites. Therefore, soybean peptide can be applied in the production of fermented food, such as lactic acid beverage, cheese, vinegar, soy sauce and fermented ham (Quezada, et al., 2006).

2.2. Nutritional value of soybean peptide

Nutritional value of soybean peptide can be concluded as follows: firstly, advantageous in high absorbency, it can promote fat metabolism; secondly, it can enhance the physical fitness of athletes; thirdly, it can increase bone density and prevent osteoporosis (Black, et al., 2007); fourthly, it can lower blood pressure and blood sugar; fifthly, it can inhibit cholesterol; at last, it has functions such as oxidation resistance, low antigen and immune enhancement.

2.3. Experimental materials and instruments

Materials included defatted soy pulp, 2709 alkaline protease, neutral protease, efficient hydrolysis protease, flavor protease and papaya protease. Instruments included digital display thermostatic bath, PHS-25 precision pH meter, 722 spectrophotometer, centrifuge and micro Kjeldahl apparatus (Corral, et al., 2016).

2.4. Experimental methods

2.4.1. Enzymatic preparation

Table 1 shows the most suitable pH and reaction temperature for hydrolysis of defatted soybean pulp by 5 kinds of proteases. Under the condition of same substrate mass fraction and the same amount of enzyme addition, hydrolysis effect and hydroxyl radical clearance rates of the 5 kinds of proteases were compared.

Table 1. The most suitable pH and reaction temperature for protease hydrolysis

Protease types	Neutral protease	Alkaline protease	Efficient hydrolysis protease	Flavor protease	Papaya protease
pH	7	11	7	7	7.5
Temperature (°C)	50	55	50	50	50

2.4.2. Enzymolysis technology of defatted soybean pulp

Defatted soybean pulp was added into the hydrolysis reactor, and some distilled water was added; then, it was stirred mildly so that the soybean pulp was evenly dispersed in the water. It was heated at 90 °C for ten minutes; then, as it cooled off till its temperature was right for hydrolysis reaction, NaOH (1.0 mol/L) was used to adjust the potential of hydrogen (pH). And an accurate amount of protease was weighed and added into the hydrolysis reactor, and it was stirred slowly. During the reaction process, NaOH (1.0 mol/L) was added timely to adjust the pH value. When the scheduled reaction time was up, heating and stirring stopped; then, HCL (1.0 mol/L) was used to adjust the pH to 4.5, and the temperature rose up to 80 °C; it was heated for 10 minutes for passivation of proteases.

2.5. Determination method of antioxidant activity

Precisely, we mixed the following reagents in test tubes for sample group: 2.0 ml of PBS (150 mmol/L, pH 7.4), 0.2 ml of 1,10-Phenanthroline (7.5 mmol/L), 0.2 ml of FeSO4 (7.5 mmol/L), 0.4 ml of samples (of different concentrations), 0.8 ml of distilled water and 0.4 ml of H2O2 (1%). Each test tube was placed in 37 °C thermostatic water bath for an hour; absorbance value was measured at 536 nm (wavelength) and denoted as Asample. In the blank group, distilled water was used as sample, and the remaining steps were the same as in sample tubes; the measured absorbance was denoted as A0. In the blank control group, distilled water was used instead of sample and H2O2, and the remaining steps were the same as in the sample tubes; the measured absorbance was denoted as Ablank. Calculation formula for clearance rate is shown as follows:

$$E_{\cdot OH^-}(\%) = (A_{\text{sample}} - A_0)/(A_{\text{blankcontrol}} - A_0) \times 100\% \quad (1)$$

2.6. Protein supplement for basketball players

Due to the characteristics of basketball sports in strength, endurance and speed, protein synthesis and catabolism of basketball players have distinct features. On the one hand, synthetic metabolism needs to meet the need of physical exercise; on the other hand, endurance and speed training period determines muscle cell damage and intense protein decomposition which require renewal or reestablishment after training or during intermittence. Accordingly, basketball players' protein supplement should coordinate with the supplement of carbohydrate or saccharides (Rhodes, 2006) in recovery phase of exercise.

During high intensity exercise, timely supplement of high biological activity and high-quality protein and amino acids is not only beneficial to exercise training, but also has an important effect on the recovery of basketball players' physical fitness.

3. Results and discussions

3.1. Effect contrast of single enzyme enzymolysis

As can be seen from Figure 1, hydrolysis ability of neutral protease and efficient protease was similar; flavor protease was the least capable of hydrolyzing soybean protein, while hydrolysis ability of alkaline protease was the greatest with low cost (Alaskar, et al., 2015).

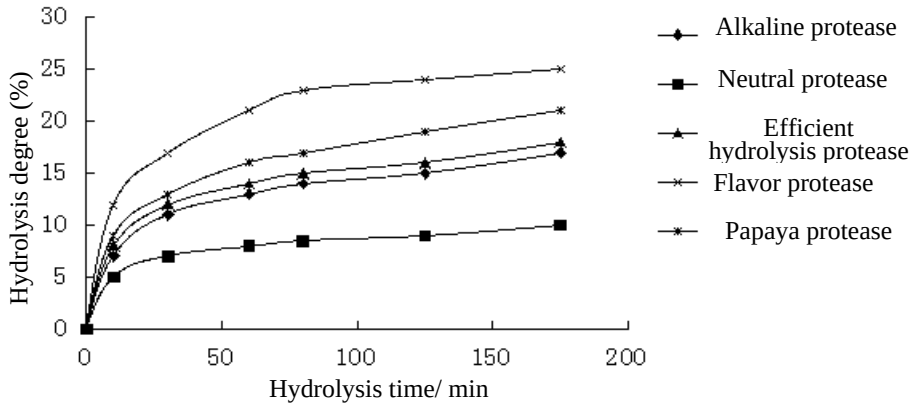

Figure 1. Graph of protease hydrolysis process

3.2. Clearance effect of different protease hydrolysates on hydroxyl radical ·OH⁻

As can be seen from Table 2, all the five kinds of proteases had antioxidant effect; however, their clearance effects were obviously different. Enzymatic hydrolysate of alkaline protease and papaya protease had better antioxidant effects.

Table 2. In vitro antioxidant activity of different protease hydrolysates

Protease hydrolysates	Clearance rate of hydroxyl radical·OH⁻ (%)
Flavor protease	17.3±0.8
Papaya protease	59.7±1.9
Efficient hydrolysis protease	51.4±1.6
Alkaline protease	66.7±1.0
Neutral protease	31.3±2.0

3.3. Effect of enzyme addition on antioxidant activity and hydrolysis degree

As can be seen from Figure 2 and Figure 3, with the increase of enzyme addition, hydrolysis degree improved continuously. Hydrolysis degree of substrate depended on protease concentration. Only when enzyme molecules tended to be saturated and part of them could not contact the substrate, increase of hydrolysis degree would slow down.

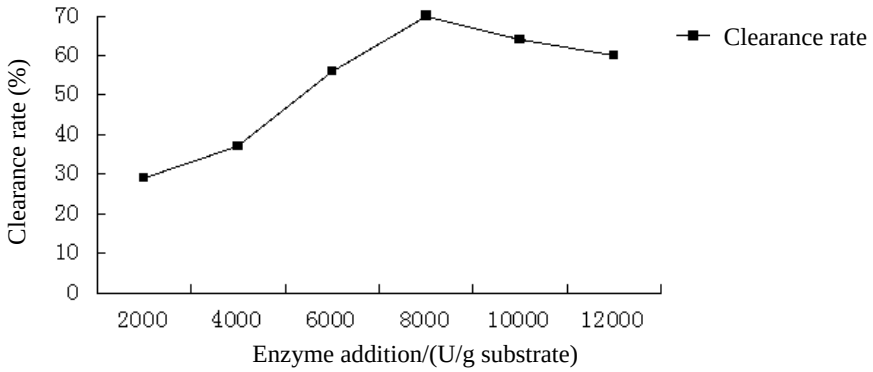

Figure 2. Effect of protease addition on clearance rate

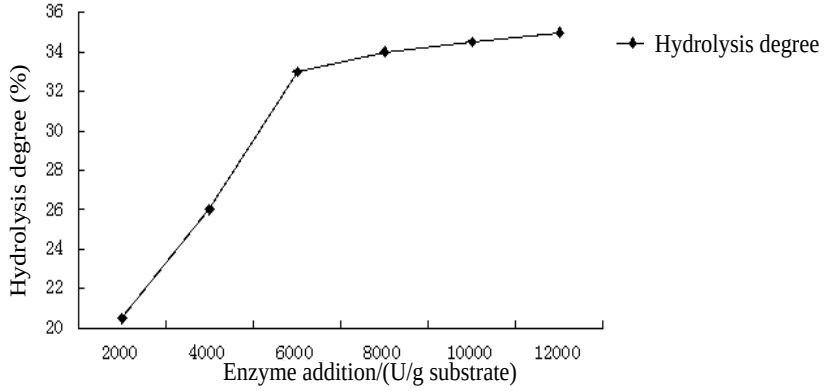

Figure 3. Effect of protease concentration on hydrolysis degree

Hydrolysis degree of soybean peptide increased with enzyme addition, thus distribution range of hydrolysate molecular weight decreased. Since hydrolysate only showed high antioxidant activity within certain molecular weight range, antioxidant activity of hydrolysate was relatively lower with insufficient or excessive hydrolysis (James, et al., 2009).

3.4. Effect of different substrate concentrations on hydrolysis degree and antioxidant activity

On condition that enzyme amount was 8000 U/g substrate, reaction time was 3 hours, reaction temperature was 55 °C, and the pH value was 11, defatted soybean pulp solutions of different concentrations (2%, 2.5%, 5%, 7.5%, 10%, 12.5%) were prepared for enzymolysis. The results are shown in Figures 4 and 5. According to Figure 4 and Figure 5, it can be concluded that when substrate concentration was low, substrates were not enough to combine with all the enzymes, in which case some dissociative enzymes did not play catalytic roles. Therefore, production speed increased with the concentration of substrates. Namely, under the condition of first-order reaction, more soybean peptides could be generated, thus the clearance rate of removing hydroxyl free radical was higher. However, when all the enzymes combined with substrates, reaction rate reached saturation condition (zero order reaction state) regardless of the increase in substrate concentration.

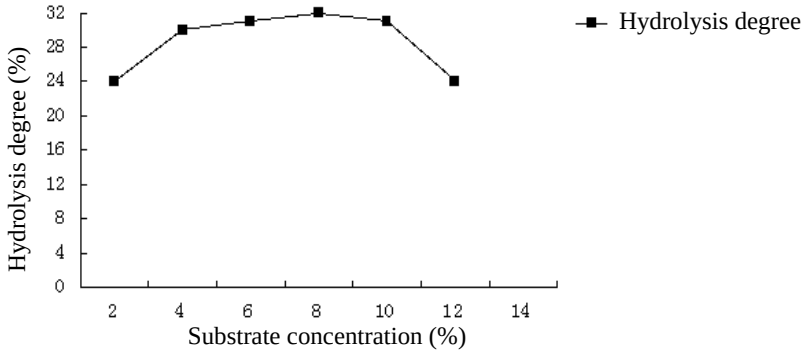

Figure 4. Effect of different substrate concentrations on hydrolysis degree

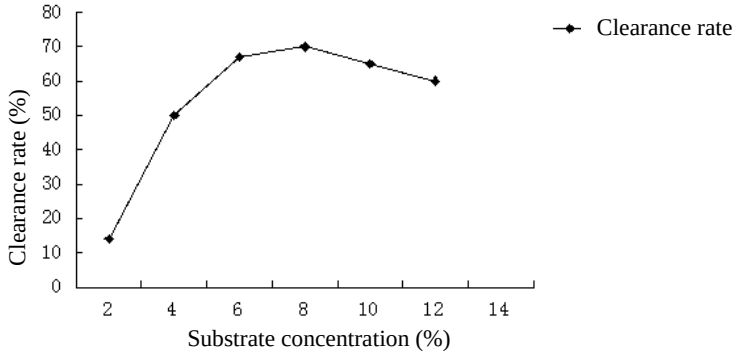

Figure 5. Effect of different substrate concentrations on clearance rate

3.5. Effect of different pH on hydrolysis degree and antioxidant activity

When the substrate concentration was 7.5%, enzyme amount was 8000 U/g substrate, the reaction temperature was 55 °C, and the reaction time was 3 hours, different pH values were selected for hydrolysis. The results are shown in Figures 6 and 7. It can be seen that when pH value was 11, the obtained soybean peptide had the highest hydrolysis degree; and hydroxyl radical clearance rate of the hydrolysate was the highest (Fréchette, et al., 2016). Therefore, the most suitable pH value was 11 for proteases to take effect.

3.6. Optimal process parameters of enzymatic hydrolysis

Response surface analysis method was applied. On the basis of single factor experiment, taking substrate concentration, enzyme addition and pH value as the factors, soybean meal hydrolysate of hydroxyl radical clearance rate as the response values, we designed a three-factor and three-level quadratic regression equation for fitting of the functional relationship between factors and indexes. Experimental factors and levels are shown in Table 3 while experimental results are shown in Table 4.

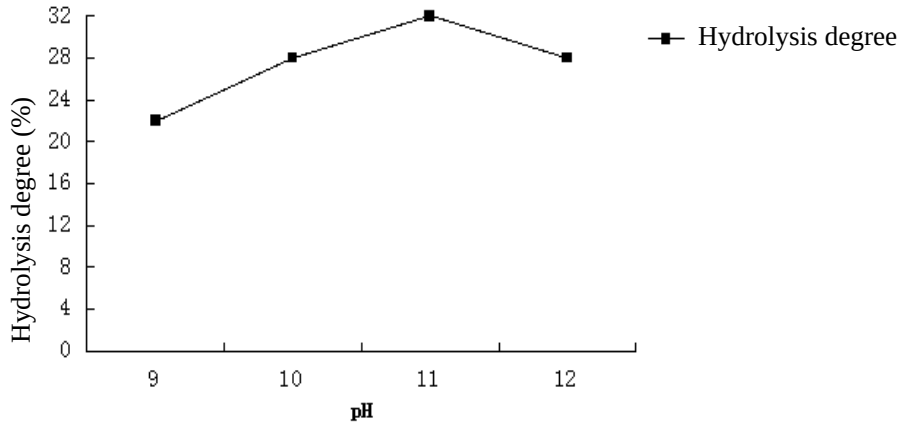

Figure 6. Effect of different pH values on hydrolysis degree

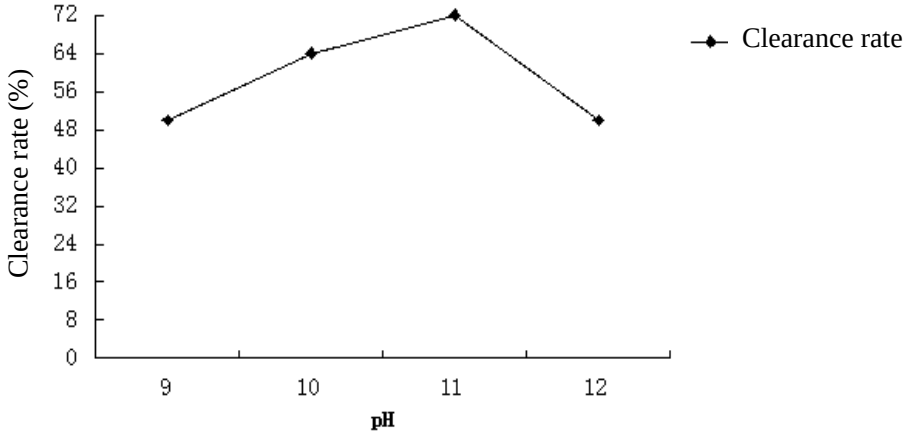

Figure 7. Effect of different pH values on clearance rate

Each experiment was carried out for three times; the mean value of three experiment results was selected as the corresponding response value. The experimental data were given quadratic regression analysis with Design-Expert software based on the equation:

$$Y_{DH} = +63.3 + 7.2 * X1 + 8.6 * X2 + 1.3 * X3 + 0.9 * X1 * X2 - 5.2 * X1 * X3 - 4.9 * X2 * X3 - 10.3 * X1^2 - 9.3 * X2^2 - 11.0 * X3^2 \quad (2)$$

Table 3. Experimental factors and levels

Factors	**Levels**		
	-1	**0**	**+1**
Enzyme addition/(U/g substrate)	6000	8000	10000
Substrate concentration (%)	5	7.5	10
pH	10	11	12

Table 4. Experimental design and results

Number	Enzyme addition	Substrate concentration (%)	pH	Clearance rate (%)
1	-1	-1	0	29.6
2	1	-1	0	40.6
3	-1	1	0	45.2
4	1	1	0	59.6
5	-1	0	-1	28.1
6	1	0	-1	54.5
7	-1	0	1	39.9
8	1	0	1	45.6
9	0	-1	-1	27.8
10	0	1	-1	54.6
11	0	-1	1	41.2
12	0	1	1	48.5
13	0	0	0	63.2
14	0	0	0	62.9
15	0	0	0	63.8

where: X1, X2 and X3 respectively represent enzyme addition, substrate concentration and pH value.

Variance analysis was adopted to verify the significance of models and parameters, as shown in Table 5.

Table 5. Variance analysis of response surface

Source of variation	Quadratic sum	Degree of freedom	Mean square	F value	Prob>F
Models	2221	9	247	146	<0.0001
X1	414	1	414	245	<0.0001
X2	589	1	589	349	<0.0001
X3	13	1	13	8	0.0386
X1X2	3	1	3	2	0.2361
X1X3	106	1	106	63	0.0005
X2X3	95	1	95	56	0.0007
$X1^2$	391	1	391	231	<0.0001
$X2^2$	317	1	317	187	<0.0001
$X3^2$	445	1	445	263	<0.0001
Lack of fit	8	3	3	12	0.0781

As can be seen from Table 5, if Prob > F value of model was less than 0.0001, it means the model was extremely significant. Lack of fit represented the probability of model's predictive value not fitting with the actual value (Gragg and Yang, 2016). Coefficient of variation (CV) reflected the confidence coefficient of models: the lower the CV value, the higher the model confidence coefficient. In the experiment, CV value was 2.77%, indicating that the model equation could reflect the actual experimental value efficiently (Wan-Kai, et al., 2005; Bucher, et al., 2016). Therefore, the model was used to analyze changes of response values.

4. Conclusions

Through single factor experiment combined with response surface analysis (Qingxiang, et al.,

2013), this study carried out an optimization research on enzymolysis technology of antioxidative soybean peptides. Soybean pulp was used as substrate, and different proteases were selected for hydrolysis (Virunanon, et al., 2013). We determined the optimal enzymolysis conditions for preparing soybean peptide of high antioxidant activity with alkaline protease: substrate concentration was 8.50%; enzyme addition was 8750 U/g substrate; pH was 11; temperature was 55 °C; reaction time was 3 hours. Under such conditions, we obtained the soybean peptide with a hydroxyl radical clearance rate of 65.37%.

In summary, as a kind of bioactive peptide, soybean peptide plays a role in regulating physiological function of organisms, of which protein is not capable. Therefore, it is of great value to develop soybean peptide into food and health products which can benefit the recovery of basketball players' physical fitness.

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FOOD COLD CHAIN ROUGH SET MODEL BASED ON GRAY SIMILAR CORRELATION RELATION AND ITS APPLICATION

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ABSTRACT
This study makes a deep systematic analysis on food cold chain logistics operative system. Firstly, gray correlation analysis is used to determine key input index that has important influence on system output. Then based on gray correlation cluster analysis, absolute relational degree between indexes was calculated using gray modeling software and index correlation matrix is established. Cluster results are obtained according to determination of correlation degree and critical value. Key sequential parameters of system in operating state are confirmed. Comprehensive operating state of system is evaluated using constructive method based gray rough set model and combining with gray system theory and rough set methodology. At last, uncertain problem such as difficulty in processing gray based on one methodology is solved in an innovative way.

1.Introduction

In recent years, food safety accidents such as bad edible oil, Sudan red and melamine-tainted formula milk powder severely threatens health of people (Powell et al., 2014). A series of food safety issues make people think about the reason for such a situation. It is urgent to figure out how to solve or improve food safety problem. As one of China's traditional industry, the development of food industry is very rapid (Barbara et al., 2010). Safety, freshness and variety are the foundation that ensure value of food and create added value, especially for perishable food. In trans-regional or global supply chain which circulates food with high value, how to normalize cooperative operation and monitor management in a scientific way is concerned by both basic staff and managers in the whole network chain. Safety and freshness are also the bottleneck for ensuring quality of fresh and perishable food. Rough set theory and gray system theory are proposed aiming at processing different types of uncertain problems. Though they have different basis and analyze and process uncertain problems in different perspectives, there is intersection between their research fields, i.e., theory, research method and means of them has something in common. If they can learn from each other to make up their own weakness, uncertain information can acquire more effective processing. Research on food cold chain logistics cooperative system has great theoretical and practical meaning. Based on a deep analysis on food cold chain logistics cooperative system, this study first constructs a parameter system for food cold chain logistics system, calculates index which dominates the operation of food cold chain logistics system

and finally, evaluates cooperative operation state of food cold chain logistics system.

2. Materials and methods

2.1. Food cold chain logistics cooperative system and theoretical basis

Cold chain is used for describing a series of correlated operation such as production, delivery, storage and resale of refrigerated and frozen food (Zou et al., 2013). Logistics optimizes goods or service production network through predicting need and demand of customers and acquiring necessary capital, material capital, staff, technology and information, thus to create space utility, time utility and quality utility of logistics activities (Soysal, 2014). Rough set theory, proposed in 1980s, is a soft calculation method used for processing uncertain problems. Its largest advantage is that, apriori information except data set is not needed. It is suitable for discovering potential knowledge in data and can provide a complete set of method for simplification of information system and extraction of decision table rule (Subrat et al., 2013). Gray system theory believes that, whether system would appear problem of incomplete information depends on levels of recognition, information and decision. Uncertain quantity of low-level system is the certain quantity of high-level system. Rule of system can be revealed making full use of known information (Wang et al., 2014). The main body of food cold chain logistics system includes member enterprises on refrigeration network chain, raw material suppliers, food producer and processer, food dealer and wholesaler and food cold chain logistics service supplier (Wang et al., 2015). Structure of food cold chain logistics system differs greatly in practical economical operation, and there is no absolutely specified excellent structure. An adaptive structure is quite important for supply chain logistics system with different characteristics. Based on the above analysis on components of food cold chain logistics system, we make a division on category of structure in terms of constituent type of main body and scale of main body. Main body of food cold chain logistics system can be divided as follows according to different type of main body (Table 1):

Table 1. Structure category division based on type of main body

Structure category	Main body of logistics operation
Direct selling type	Supplier or producer-retailer
Complex type	Supplier-producer-retailer
Logistics enterprise in service type	The third party logistics enterprise provide logistics service
Logistics business in self support type	Enterprise engaging in production and processing or retailing and wholesale

Food cold chain logistics system has different scale. Scale of system is represented by number and scale of main body and equipment. Based on that, structure of food cold chain logistics system can be roughly divided as shown in Table 2.

Table 2. Structure division based on number and sale of main body and equipment

Structure category	Number and scale of main body	Object	Equipment needed
Convergence (T type)	Small amount but large scale of supplier and clients	Processing/selling foods	Large scale
Symmetry (H type)	Large amount but small scale of supplier of supplier; clients on the opposite	Food whose production place and consumption place is far away	Large-scale vehicle and small-scale refrigeration house

Divergence (A type)	Small amount of suppliers and large scale of supplier; clients on the opposite	Food with concentrated production place and dispersed selling place	Small-scale vehicle and large-scale refrigerator house

Customer is the end of food cold chain logistics system. It aims to provide satisfactory foods for customer realize maximum profit of member enterprises and reflect efficacy of food value chain. Food cold chain logistics system which is different from ordinary systems is characterized by high safety, irreversible quality, high sensitivity to environment, high cost, complex technology and highly uncertainty of information.

3. Results and discussions

3.1. Analysis of food cold chain logistics system cooperative model

3.1.1. Construction of state parameter of food cold chain logistics system

Based on dynamic systematic theory, state variables is a group of systematic parameters determining systematic behavioral characteristics (Christopher et al., 2013). Construction of state parameter system differs due to different selection of method and evaluator, but the basic principle following in construction of state parameter system is consistent (Francis et al., 2014). First, state parameter selected should be key variable which can describe system state; second, state parameter system constructed should be completed. Thirdly, state parameter selected should be distinguished according to role of member enterprises. Fourth, availability and accuracy of state parameter should be ensured.

State parameter system constructed based on qualitative analysis differs. This study constructs food cold chain logistics system state parameter based on pattern of input, conversion and output and dynamic systematic theory. According to the analysis on connotation of food cold chain logistics system in this study, systematic input state parameter system can be divided into main body, object, equipment and information. System switching is food cold chain logistics system cooperative process. System output is the objective which should be achieved by food cold chain logistics system coordination. System output state parameter system can be divided based on food safety, logistics operating efficacy, logistics cost and satisfaction of client.

3.1.2. Analysis of key index of food cold chain logistics cooperative system

In the process of system coordination, there would be few state parameters which completely dominate macroscopic behavior and ordering degree of system. This kind of state parameter is order parameter. Order parameter occupies a leading position in the cooperative behavioral process of system and also dominates other state parameter as well as order condition and changes of degree of order of subsystem (Forrest et al., 2008). When order parameter and slaving principle are used in food cold chain logistics system coordination system, the core idea lies on find out the index that plays a leading domination effect in system coordination among a large amount of parameters influencing food cold chain logistics state.

Gray correlation analysis is used to analyze the important degree of all input elements output by system. Procedures for calculation of gray correlation degree are as follows (Wang et al., 2011).

Suppose $R_0 = (r_0(1), r_0(2), r_0(3), \ldots, r_0(n))$ as sequence group of systematic characteristic behavior and

$R_1 = (r_1(1), r_1(2), \ldots, r_1(n)) \ldots R_i = (r_i(1), r_i(2), \ldots, r_i(n))$ as relevant behavioral sequence. In the system, there are two any irrelevant behavioral sequences.

(1) Solution of initial value image

$$R_i = \frac{r_i}{r_i(1)} = (r_i(1), r_i(2), \ldots, r_i(n)), i = 0,1,2,..m$$

(2) Difference sequence

$$\Delta_i(k) = |r_0(k) - r_i(k)|, \Delta_i = (\Delta_i(1), \Delta_i(2), \ldots, \Delta_i(n)), i = 1,2,\ldots,m$$

(3) Solution of maximum difference and minimum difference

(4) Solution of correlation coefficient

$$\gamma(r_0(k), r_i(k)) = \frac{\min\limits_i \min\limits_k |r_0(k) - ri(k)| + \zeta \max\limits_i \max\limits_k |r_0(r_0(k) - r_i(k))|}{|r_0(k) - r_i(k)| + \zeta \max\limits_i \max\limits_k |r_0(k) - r_i(k)|}$$

(5) Calculation of correlation degree

$$\gamma(r_0, r_i) = \frac{1}{n} \sum_{k=1}^{n} \gamma(r_0(k), r_i(k))$$

Thus according to comprehensive correlation degree between system input element index and output element index, we can determine which system input element plays important domination effect in objective of food cold chain logistics system coordination.

Evaluation and observation of food cold chain logistics cooperative system can be represented by a large number of state parameters. But in practice, some indexes are correlated and mixed. We consider dividing observation index into different categories, screening and deleting to simplify evaluation process or assessment standard on the condition that ensure basic correct evaluation and decision. Gray cluster is a method that divides observation indexes or evaluation objects which are mixed through gray correlation matrix or white function of gray number into several definable categories. Through gray correlation cluster analysis, we can determine cluster of index, thus to further confirm operation state of representation element representation system and avoid loss of information (Liu et al., 2012). The following procedures for gray correlation cluster analysis are shown in Figure 1.

(6) Establish index correlation matrix and calculate absolute correlation degree

	r_1	r_2	.....	r_n
r_1	ε_{11}	ε_{12}	.....	ε_{1n}
r_2	ε_{21}	ε_{22}	.....	ε_{2n}
.....			.....	
r_n	ε_{n1}	ε_{n2}	.....	ε_{nn}

Figure 1. Procedures of gray correlation cluster analysis

(7) Cluster results can be obtained by making critical value r determination on ε_{ij} ($0 \leq r^{\varepsilon_{ij}} \leq 1$). The division is more detailed if r is closer to 1.

Through the above procedure, key index system which can represent food cold chain logistics cooperative system operating state can be obtained.

3.1.3. Food cold chain logistics cooperative system state evaluation

Typical rough set theory and method had been successfully applied for processing imprecise, inconsistent and uncertain data and knowledge and calculating order parameter with attribute reduction. But it cannot be applied for evaluating overall operating state of system (Gong et al., 2006). The most important thing is that, typical rough set theory has an assumed premise, i.e., all available individual objects can all be given complete description by attribute set. That means, when $C = \{r_1, r_2, \ldots, r_n\}$ stands for definite set of individual objects and $Y = \{y_1, y_2, \ldots, y_m\}$ stands

for attribute set, then for any y from Y, r from C, attribute value y(r) exists and is confirmed.

Though the food cold chain logistics coordination system researched in this study focuses on coordination of different elements, it is still a complex and highly uncertain system which is imperfect in acquiring information. Therefore, introducing gray system theory and rough set theory into innovative research of food cold chain logistics cooperative system is quite necessary. Table 3 shows the difference and correlation between rough set theory and gray system theory.

Table 3. Difference and correlation between rough set theory and gray system theory

	Rough set theory	**Gray system theory**
Similarity	Any apriori information except data requiring processing is needed.	
Difference	A large amount of data can be simplified.	Few data, poor information and little information much information
Correlation	Gray system theory makes up the limitation of rough set theory, i.e., only capable of processing strict equivalence relation and discover uncertain data or knowledge and rules implied in information.	

3.2. Empirical analysis of food cold chain logistics system cooperative model

The reason to make empirical analysis on food cold chain logistics system cooperative model is to prove the scientificity and practical meaning of state parameter system construction, key index calculation analysis and overall operation state evaluation model.

3.2.1. Empirical analysis for state parameter system of food cold chain logistics system

The empirical object is directing selling-type food cold chain logistics system, i.e., the third party cold chain logistics enterprise X provides logistics service. Main body involved includes food producer and processer, food wholesaler and retailer and cold chain logistics enterprise X, as shown in Figure 2.

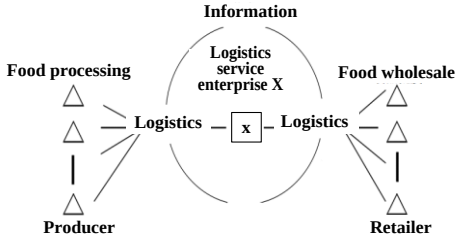

Figure 2. Structure of the food cold chain logistics system for empirical analysis

In this study, the purpose of confirming food cold chain logistics cooperative system operation is to ensure safety, improve logistics efficiency; decrease logistics cost and enhance client satisfaction.

3.2.2. Key index and state evaluation and calculation of empirical food cold chain logistics system

Input index and output index in food cold chain logistics system state parameter system is distinguished and gray correlation analysis is used to identify key elements that plays key role in system output. Degree of comprehensive correlation between input index and output index is shown in Table 4. According to gray correlation calculation result and research results obtained by other people, we can draw the conclusion that, input index that plays key influence on safety of logistics include quality of logistics staff, number of refrigeration vehicle, investment amount of information system and utilization rate of refrigeration house area. Input index that has key influence on cost of logistics include total value of circulated food, number of refrigeration vehicle, utilization rate of refrigeration house and utilization rate of refrigeration house volume.

Table 4. Analysis of gray correlation between input index and output inde

Degree of correlation	Number of logistics staff	Quality of logistics staff	Training of logistics staff	Mobility of logistics staff	Logistics quantity of food	Total value of circulating food
Timeliness ratio of goods collection	0.56	0.72	0.54	0.52	0.52	0.71
Timeliness of goods delivery	0.52	0.78	0.59	0.53	0.52	0.56
Logistics income	0.72	0.62	0.51	0.72	0.63	0.51
Total logistics cost	0.51	0.52	0.58	0.74	0.84	0.51
Satisfaction degree of producer	0.50	0.52	0.74	0.78	0.61	0.51
Satisfaction degree of retailer	0.50	0.52	0.91	0.55	0.53	0.51
Number of producer	0.67	0.60	0.50	0.50	0.50	0.75
Number of retailer	0.71	0.65	0.51	0.51	0.51	0.90
Rate of sales return	0.50	0.51	0.57	0.78	0.78	0.51

Input element which has the closest correlation with satisfaction degree of client include training rate of logistics staff, number of refrigerator, utilization rate of refrigeration house area and utilization rate of refrigeration house. Details are shown in Table 5.

Table 5. Input index that plays key effect on operation of system

Logistics safety	Quality of logistics staff, number of refrigeration vehicle, investment amount of information system and utilization rate of refrigeration house area
Logistics efficacy	Quality of logistics staff, food logistics quantity, number of refrigeration vehicles, utilization rate of refrigeration house area and investment amount of information system
Total logistics cost	Total amount of circulating food value, number of refrigeration vehicle, utilization rate of refrigeration house area and volume
Client satisfaction degree	Training rate of logistics staff, number of refrigerator, utilization rate of refrigeration house area and utilization rate of refrigeration house

Gray information table for empirical food cold chain logistics system is generated based on index system representing operation state of system (Table 6).

Table 6. Gray information table of empirical food cold chain logistics system

Time	Quality of logistics staff	Number of refrigeration vehicle	Investment amount of information system	Satisfaction degree of retailer	Total logistics cost
1	[7.5,11.8]	[6,6]	[1.0,1.5]	[95,96]	[38,40]
2	[10.6,12]	[10,10]	[1.5,1.5]	[94,95]	[37,39]
3	[7,10]	[13,13]	[2.0,2.0]	[93,95]	[33,37]
4	[8,10]	[20,20]	[2.0,2.0]	[94,96]	[30,35]
5	[9,11]	[20,20]	[3.0,3.0]	[94,98]	[30,32]
6	[11,12]	[20,20]	[3.0,3.0]	[94,95]	[28.30]

Data in Table 6 is first given standardization processing with gray range transformation formula; then ideal state vector of empirical food cold chain logistics system is calculated. Then gray interval correlation coefficient matrix corresponding to ideal state at different evaluation stage is constructed, as shown in Table 7 ($\lambda = 0.5$).

Table 7. Gray interval correlation coefficient matrix of evaluation object

$$(r_{ij}^{+})_{6\times 8} = \begin{bmatrix} 0.606 & 0.332 & 0.332 & 0.700 & 0.358 & 0.645 & 0.332 & 0.386 \\ 0.891 & 0.412 & 0.400 & 0.532 & 0.453 & 0.564 & 0.332 & 0.445 \\ 0.467 & 0.500 & 0.500 & 0.532 & 0.562 & 0.564 & 0.332 & 0.551 \\ 0.552 & 1.000 & 0.500 & 0.668 & 0.635 & 0.708 & 0.332 & 0.680 \\ 0.612 & 1.000 & 1.000 & 0.832 & 0.875 & 1.000 & 1.000 & 0.825 \\ 1.000 & 1.000 & 1.000 & 0.532 & 1.000 & 0.502 & 1.000 & 1.000 \end{bmatrix}$$

Though calculation, we get index weight $\eta = (0.158, 0.125, 0.096, 0.145, 0.123, 0.152, 0.072, 0.125)$

Average value of system state at different quarter is (0.208, 0.335, 0.355, 0.542, 0.774, 0.717). Estimated value of empirical food logistics cold chain system in 18 months can also be obtained, as shown in Figure 3. Though empirical cold chain logistics cooperative system shows fluctuation and unordered state at some time point, overall state shows a rising tendency.

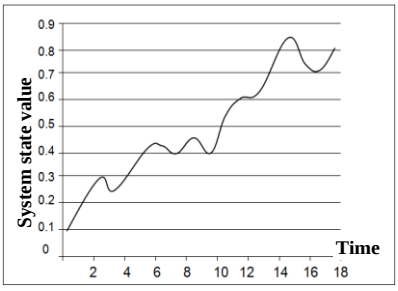

Figure 3. Evaluation value of empirical food cold chain logistics system state

System state can be divided into good, general and not ideal. White function of three gray categories is constructed as follows.

$$f_j^1(r) = \begin{cases} 0 & r < 0.6 \\ \dfrac{r-6}{0.2} & 0.6 \le r \le 0.8 \\ 1 & r > 0.8 \end{cases}$$

$$f_j^2(r) = \begin{cases} \dfrac{r-0.4}{0.2} & 0.4\, r \le 0.6 \\ \dfrac{0.8-r}{0.2} & 0.6 \le r \le 0.8 \\ 0 & r \notin [0.4, 0.8] \end{cases}$$

$$f_j^3(r) = \begin{cases} 1 & r < 0.6 \\ \dfrac{0.6-r}{0.2} & 0.6 \le r \le 0.8 \\ 0 & r > 0.8 \end{cases}$$

Through calculation, it is found that, cooperative operating state of empirical food cold chain logistics system in the first and second quarter is not ideal; cooperative operating state in the third and fourth quarter is general; operating state in the first and second quarter in the next year is good.

4. Conclusions

To sum up, food cold chain logistics system cooperation refers to member enterprises realize seamless joint of various logistics links through mutual cooperation, information resource share and complementary advantage when food circulates from supply source to reception source, thus to ensure food safety, improve operation efficacy of cold chain logistics operation, lower logistics cost, optimize food economical efficiency and enhance satisfaction degree of clients. We make an overall analysis and study on food

cold chain logistics rough set model based on gray similar correlation relationship and its application and prove the innovation and practical meaning of this study by evaluating system cooperative state and analyzing key index with gray system theory and rough set methodology.

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GAME ANALYSIS OF APPLICATION OF HAZARD ANALYSIS AND CRITICAL CONTROL POINT SYSTEM IN STRAWBERRY RAW MATERIAL PROCESSING INDUSTRY

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ABSTRACT
Food safety has become an outstanding global problem. International food safety hygiene organizations and governments from different countries are taking efforts to establishing a new food safety system to cope with food safety hazards. Hazard analysis and critical control point (HACCP) system is widely applied across the world for its characteristics of prevention, scientificity and systematicness. In terms of economics, this study analyzed the impact of HACCP system on cost-benefit of strawberry raw material processing industry as well as the benefits of implementing HACCP system in strawberry raw material processing industry, in order to provide a theoretical basis for future study. As an effective measure, HACCP system is now the most authoritative safety prevention and monitoring system aiming at food production process. However, its application in food processing enterprises in China is in a slow pace; moreover, the application scope and depth remains to be improved. Thus the application of HACCP in China requires efforts from government, producer and consumer.

1. Introduction

Hazard analysis and critical control point (HACCP) system, the most authoritative safety prevention and monitoring system aiming at food production process, has become the important precondition and security of food consumption and international food commerce (Khatri and Ray, 2007). In recent years, export of Chinese food has not been able to satisfy the international food safety standard. In addition, cost-benefit of HACCP system has not been widely extensively in China. But we can see that, HACCP system developed in the past thirty years has attracted much attention from Chinese scholars for its economical efficiency (Jin et al., 2008). Foreign researches suggested that, developed countries has released regulations and rules concerning HACCP system one after another; forcing implementation of HACCP system aims at ensuring food safety and improving hazard prevention level of food enterprise (Laurian, 2007; Unnevehr et al., 2001; Zaibet, 2000). Meanwhile, most researches has also suggested that, effective implementation and application of HACCP system can bring different levels of profits for food enterprise, which is one of the reasons that middle and large scale food

enterprise positively implementing this system. Furthermore, more and more scholars begin to discuss over and assess the value of HACCP system to enterprise and the whole society with wider research methods (Zhigang et al., 2007; Hooker et al., 2002).

Food is the basis for people's survival and development. Food safety directly concerns the health and safety of thousands of households as well as the development and stability of the society. Foodborne disease, a severe threat for human's health, develops in a serious condition, especially in developing countries and about 21 million people die of foodborne (waterborne) diarrhea (Tim and Sabine, 2006; Thomas and Juan, 2002). This study explored the benefits of HACCP system in terms of economics, in order to analyze the significance of HACCP system more deeply and provide a theoretical support for effective application of HACCP system for strawberry raw material processing industry under the current food production condition.

2. Materials and methods

2.1. Game theory

Game theory, a branch of applied mathematics, has become one of standard analysis tools in economics (Juan and Yadong, 2003). It means two people change their strategies accordingly in an equal game to win.

At first, game theory is mainly used to study and analyze success or failure issue in chess, bridge and gamble. At that time, scholars have only focused on experiential level and have not involved theoretical research. Verification of basic principle of game theory by Von Neumann in year 1928 signified the official birth of game theory. Afterwards, scholars made extension researches on game theory. Based on the information which two participants know about the rival, game theory can be divided into complete information game and incomplete information game. Complete information game means that, two participants exactly know characteristics, strategic space and revenue function of the opposite side, while incomplete information game means two participants do not exactly know characteristics strategic space and revenue function of the opposite side or they do not know accurate information about all characteristics, strategic space and revenue function of the opposite side.

2.2. Related theory about HACCP system

HACCP, a food safety security system based on preventing food safety issues and food safety-related disease, aims at preventing food safety issues and removing or reducing hazards to an acceptable level (Carol et al., 2013). HACCP system is not an independent system; plans about operation specifications and sanitation standard operation procedure are the basis of formulating and implementing HACCP system. HACCP system characterized by rationalization, strong systematicness, strong binding force and high applicability has become the internationally recognized monitoring and controlling system for effectively ensuring food safety and has produced large impact on government monitoring department, consumers and food processing enterprises. This study focuses on its application in strawberry raw material processing industry in economic perspective.

HACCP system is considered as the most effective and economic food safety controlling and monitoring system by the world. HACCP system can reduce and lower biological, chemical and physical pollution in the process from raw materials to consumption (William, 2014). Implementation of HACCP system further perfects food quality monitoring system in China. To be specific, it supplements the traditional food quality monitoring system, which makes food safety monitoring process more scientific and reasonable. Practical operation in enterprises suggested that, it is too late to remedy if we test the product at the last step of production, as the unqualified products have been formed. Such kind of responsive monitoring mode has not been able to satisfy the constantly improved production needs and cannot ensure food safety as well.

2.3. Theory of cost-benefit analysis

Cost is a category of value in commodity economics and also a component of commodity value. Production is bound to consume some resources and the monetary expression and objectification of the consumed resources is termed as cost (Els and Luca, 2014). Benefit, a concept corresponding to cost, refers to the contributions on system, including direct benefits and direct benefits. Benefit differs in meaning if we recognize benefits in different views. Cost-benefit analysis is one of the commonly used economical analysis methods. Managers from enterprises tend to use cost benefit to analyze and confirm operating target and scheme, i.e., calculating and assessing the cost and benefit of every possible scheme as well as the limitation conditions and probability of scheme implementation and then choosing the most beneficial one.

Generally speaking, cost and benefit is opposite and unified. In the case of unchanged index, reducing cost would increase benefits; however, when all economical indexes change, the ratio of benefit and cost must be increased, in order to improve benefits. Indeed, absolute volume of some necessary cost is possible to increase accordingly. But such increasing is aiming at obtaining greater benefit increase and cannot ignored compared to benefit increase.

3. Results and Discussions

3.1. Game analysis of food processing enterprise and local government

Strawberry raw material processing industry, one party of game, would compare the cost and benefit in the condition of implementing or not implementing HACCP system, while government, the other party, would also consider whether it should monitor safety production of strawberry raw material processing industry as well as how much monitoring strength can maximum the benefit. Government would also balance the cost and benefits if the monitoring is carried out in strawberry raw material processing industry and then choose the optimal decision based on that.

Table 1. Impact on cost and benefits if HACCP system is applied in strawberry raw material processing industry

Index	Perception of enterprise (%)					Tot（%）	Sample size
	1	2	3	4	5		
Production cost	1	50	40	5	0	100	20
Sales revenue	1	20	45	30	0	100	20
Trading profit	10	50	30	5	0	100	20
Ratio of profits to cost	10	45	30	10	0	100	20
Return on total assets	10	35	30	20	0	100	20
Return on net assets	1	45	25	25	0	100	20
Security surplus cash multiples	5	35	35	20	0	100	20
Growth rate of sales revenue in three years	1	50	25	20	0	100	20
Growth rate of assets in three years	5	35	25	30	0	100	20
Technical input ratio	20	35	30	10	0	100	20

Every index ranges from 1 to 5. The value stands for the increase of cost or benefit perceived by strawberry raw material processing industry after HACCP system implementation. 1 refers to no impact and 5 refers to maximum impact. 1 = no increase, 2= little increase, 3= a little increase, 4= much increase, 5=numerous increase.

Enterprises' perception on cost and benefits after HACCP implementation has been shown

in Table 1. Willing strength of HACCP system implementation of strawberry raw material processing industry is not only correlated to the punishment strength on strawberry raw material processing industry due to irregular production, but also correlates to the monitoring strength of local government to strawberry raw material processing industry.

Moreover, monitoring strength is correlated to the implementation degree of HACCP system in strawberry raw material processing industry as well as the loss. As to the game relationship between local government and strawberry raw material processing industry, strawberry raw material processing industry balances in the view of the impact of HACCP implementation on net benefits of enterprise and the impact of monitoring of local government on cost. Strawberry raw material processing industry would balance the extra benefits obtained from producing unsafe food and benefits brought by implementing food safety systems such as HACCP system. In the eye of most enterprise, the former is larger than the latter. They believe that, implementing HACCP system means continuous cost input which may increase the burden of enterprise and decrease profits and benefits. Therefore, strawberry raw material processing industry tends to acquire maximum benefits without implementing HACCP system and even producing foods illegally.

3.2. Game analysis of strawberry raw material processing industry and consumer

This study attempts to analyze the game relationship between product quality and price based on the previous studies. Product quality is defined as quality of safety.

3.2.1 Analysis of market demand of food in different quality

Here, we assume the demand of customers on a food in market as:

$$C = \begin{cases} \theta q - r \\ 0 \end{cases} \tag{1}$$

Where q refers to quality characteristics parameter of food, θ (θ>0) refers to the effectiveness obtained when q=1 (θ is the quality preference coefficient of customers), $\theta q - r$ stands for customer surplus. Assume θ as a random variable. The effectiveness obtained by customers is greater when θ is larger. Meanwhile F(θ) is used to express distribution function of θ and $f(\theta)$ is used for express its density function.

There are two foods in same category but in different quality q1 and q_2 ($q_1 > q_2$). q_1 is the quality of product A produced by strawberry raw material processing enterprise which has applied HACCP system, while q_2 is the quality of product B produced by strawberry raw material processing enterprise B which has not applied HACCP system. We assume $0 < q_1 < \bar{q}$ and $r_1 > r_2$. When $\theta q_2 > r_2$, customers prefer to purchase product A. That is because

$$\begin{aligned} (\theta q_1 - r_1) - (\theta q_2 - r_2) &= p_1(\theta \frac{q_1}{r_1} - 1) - r_2(\theta \frac{q_2}{r_2} - 1) \\ &\geq r_1(\theta \frac{q_2}{r_2} - 1) - p_2(\theta \frac{q_2}{r_2} - 1) \\ &= (r_1 - r_2) \quad (\theta \frac{q_2}{r_2} - 1) > 0 \end{aligned} \tag{2}$$

It can be seen that $C_1 > C_2$. Customers prefer to purchase product A produced by enterprise A which has implemented HACCP system. Then we come to consider the condition when $\frac{q_1}{r_1} \leq \frac{q_2}{r_2}$. We assume demand on two products has no difference, i.e., $C_1 = C_2$, when the quality selection of customers is $\bar{\theta}$. Thus we get:

$$\bar{\theta} q_1 - r_1 = \bar{\theta} q_2 - r_2, \bar{\theta} \square q = \square r, \quad (\square q = q_1 - q_2, \square r = r_1 - r_2) \tag{3}$$

$$\bar{\theta} = \frac{\Delta q}{\Delta r} \tag{4}$$

Necessary and sufficient condition for customer purchasing product A is $C_1 > C_2$ and $\theta \geq \frac{r_1}{q_1}$ or $\theta \geq \bar{\theta} = \frac{\Delta r}{\Delta q}$ and $\theta \geq \frac{r_1}{q_1}$. When $\frac{q_1}{r_1} \leq \frac{q_2}{r_2}$, then we

get $\frac{\square r}{\square q} \geq \frac{r_1}{q_1} \geq \frac{r_2}{q_2}$. This is because $\frac{\square r}{\square q} \geq \frac{r_1}{q_1} \geq \frac{r_2}{q_2}$ is equal to $q_1 \square r \geq r_1 \square q$ and meanwhile $q_1 r_2 \leq r_1 q_2$, .

Thus demand of customer on product A is:

$$r\left\{\theta \geq \frac{r_1}{q_1}, \theta \geq \frac{r_1 - r_2}{q_1 - q_2}\right\} = 1 - F\left(\frac{\square r}{\square q}\right) \square D_1(r_1, r_2, r_1, r_2) \quad (5)$$

Similarly, customers would purchase product B only when $U_1 \leqq U_2$, $\theta \geq \frac{r_2}{q_2}$。 or $\theta \leq \frac{\square r}{\square q}$, and $\theta \geq \frac{r_2}{q_2}$, thus demand on product B is:

$$r\left\{\frac{r_2}{q_2} \leq \theta \leq \frac{\square r}{\square q}\right\} = F\left(\frac{\square r}{\square q}\right) - F\left(\frac{r_2}{q_2}\right) \square D_2(r_1, r_2, r_3, r_4) \quad (6)$$

Then we can get demand functions of two products:

$$\begin{cases} D1(q_1, q_2, r_1, r_2) = 1 - F(\frac{\square r}{\square q}) \\ D_2(q_1, q_2, r_1, r_2) = F\left(\frac{\square r}{\square q}\right) - F(\frac{\square r_2}{\square q_2}) \end{cases} \quad (7)$$

When θ even distributes on [0, 1], we have

$$\begin{cases} D1(q_1, q_2, r_1, r_2) = 1 - \frac{r_1 - r_2}{q_1 - q_2} \\ D2(q_1, q_2, r_1, r_2) = \frac{r_1 - r_2}{q_1 - q_2} - \frac{r_2}{q_2} \end{cases} \quad (8)$$

$\frac{1}{q_1 - q_2}$ is the competitiveness strength of enterprise A.

From formula (8), we know that, demand of customers decreases when price of product rises; moreover, demand of customer rises when the price of product of the other enterprise becomes higher. If price of two products is the same, then demand of product A would rise with the improvement of product quality and declines with the quality improvement of product B.

3.2.2. Quality and price competition

Quality-price competition game model is three-stage and dynamic (Langfeng and Hanhui, 2008). In stage 1, enterprise A chooses quality q_1; enterprise B chooses quality q_2 in stage 2; and in stage 3, both enterprises choose price. Game structure is shown in Figure 1.

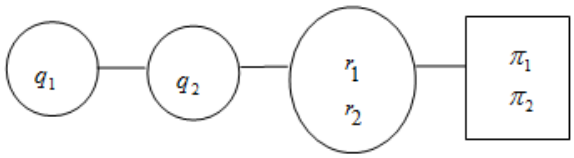

Figure 1. Three-stage dynamic game structure

4. Conclusions

There are two game relationships involving in the application of HACCP system in strawberry raw material processing enterprise. First is the game relationship between local government and food enterprise and the second is the game relationship between food enterprise and customers. In the second game relationship, quality and price are the key points. Customers prefer high quality and low price food. They would give priority to high quality food, *i.e.,* safe food. Application of HACCP system is bound to produce impact on cost and benefit of enterprises. Moreover, we find through game analysis that, application of HACCP system is beneficial to government, enterprise and customers, especially market development of enterprise.

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MATHEMATIC MODEL BASED ON ROTATING ELECTROMAGNETIC THEORY USED IN BUILDING A THERMAL CIRCUIT USED IN FOOD HEATER

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ABSTRACT
Rapid development of China's economy, changing population structure and reformed economic structure drives rapid growth of energy consumption. As a result, energy safety gradually becomes a hot spot in energy development. Electricity heating technology has been concerned for a long time as a safe and effective mean; as a result, it has developed into a subject integrating electric engineering, pyrology and materials science. A fundamental research is carried out on food heater in this study. First, static electromagnetic induction heating and dynamic electromagnetic induction heating were introduced. To solve reverse problems of temperature rise, thermal power mathematical theoretical model and thermal circuit model of food heater were established based on electromagnetic field and empirical formula. Finally, under certain rotating speed, thermal power in short-circuited winding as well as eddy current thermal power and magnetic hysteresis thermal power in solid iron core was analyzed.

1.Introduction

In recent years, energy consumption is booming in China. Since 1990s, demand of energy has exceeded supply and the problem becomes even more intensive. Moreover, imbalanced energy distribution and low power generation-transmission efficacy aggravate intense energy supply. When high speed economy in China is facing with dilemma of insufficient power, energy safety problem emerges (Tong, 2006). Electrothermics developed from electric engineering, pyrology and materials science has become an emerging interdisciplinary science. Its basic principle is transforming electric energy into thermal energy. In a broad sense, electrothermics can be researched as a reverse problem of electrical machine and appliance theory (Guoqun et al., 2011). Starting from reverse problem of electrical machine and appliance theory, energy can be completely transformed into thermal energy based on the concept and cause of electrical rotating machine loss using proper material, structure and method. Cheng (Shukang et al., 2008) once explored the above method. Nikrityuk et al. (Nikrityuk et al., 2003) attempted constructing model for thermal transmission in external magnet.

In daily life, food is usually heated by fire, microwave and chemical agent. Heating with fire wastes time and energy and pollutes environment; moreover, the temperature should be controlled by people. Open fire is forbidden in many occasions such as forest or chemical

plant. As to heating with microwave oven, microwave produced brings radiation to operator; problems of damaged nutritional ingredients and disturbed power grid also exist (Fukuoka et al., 2005; Halla et al., 2000). Heating food with chemical agents is widely applied in field, mainly for military used food. It will lose efficacy after being affected with damp, though this method causes no fire and smoke. In addition, water is required and improper disposal of chemical agents can pollute environment. To cope with defects of heating with fire, microwave and chemical agents, this study proposed a reusable food heater based on rotating rotating electromagnetic theory and constructed thermal power mathematical theoretical model and thermal circuit model.

1. Materials and methods

2.1. Electromagnetic induction heating

Static electromagnetic induction heating: Alternating electromagnetic field can produce Lorentz force or induced electric field force on free electron inside metal which locates in the electromagnetic field. Two forces produced can induce induced current, *i.e.,* eddy current. As eddy current loop metal has small resistance and current strength of eddy current is relatively large, joule heat produced is large. Induction heating has been applied in medium and high frequency melting technology and microwave cooker (Wang, 2011). As alternating magnetic field produced by coil called inductor is not correlated with movement or relative movement, traditional electromagnetic induction heating is termed as static electromagnetic induction heating (Souley et a.,, 2012). Electro-magnetic induction themogenesis is found in operation process of electromotor at the earliest. But it is considered to be harmful as it reduces efficacy of energy conversion. In industrial field, static electromagnetic induction heating technology is mainly applied in smelting of ferrous and nonferrous metals (Satoshi et al., 1993). Scholars from China and other countries have made a large quantity of theoretical analysis. Yang XG et al. (Xiaoguang, 2004) once comprehensively analyzed the solution of coupled fields, numerical simulation of induction heating and boundary condition of temperature field. Zhang HL (Hongliang et al, 2007) et al. analyzed heat treatment process of steel ball in columnar induction through heating equipment, established a mathematical model for calculating temperature field and made coupling calculation on dynamic eddy current field and temperature field.

2.2. Dynamic electromagnetic induction heating

Dynamic electromagnetic induction heating means transforming electric energy fully into thermal energy with proper materials, structure and method, i.e., transforming loss in conventional sense into effective thermal energy (Linhuang et al., 2014). Dynamic electromagnetic induction heating is also based on electromagnetic induction principle, but it has two points of difference with static electromagnetic induction heating. The first point is that alternating magnetic field is produced from multiphase rotating magnetic field or rotating permanent magnet rather than non-motor magnet exciting coil. The second point is that, dynamic electromagnetic induction heating based on eddy-current and magnetic hysteresis effect of iron core should also sense electric current effect of rotational voltage in closed coil using cutting magnetic line and meanwhile make use of eddy-current effect and magnetic hysteresis effect of block iron core in rotating magnetic field.

Multiphase rotating magnetic field or rotating permanent magnet which can produce thermal power when dragged by electromotor, water turbine and draught fan can be used to construct a novel environmental friendly dynamic electromagnetic induction heating equipment (Carrillo, 2008; Kaneda et al., 2000). As thermal energy produced by loss of rotating electromagnet is high efficient, safe

and environmental friendly, it is applied in power drying in some countries. Figure 1 demonstrates the drying equipment operating based on a low speed rotating cylinder driven by rotating magnetic field (Takashi et al., 2005).

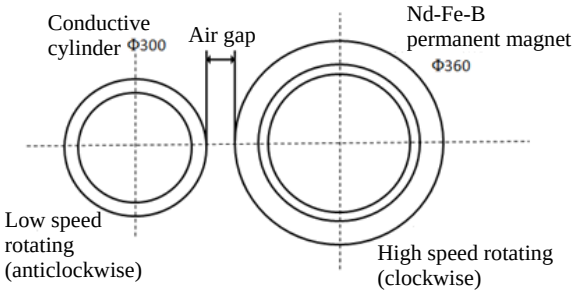

Figure 1. Principle of drying equipment

2.3. Mathematical model for heat generation of food heater

2.3.1. Magnetic hysteretic thermal power in iron core

Magnetic hysteretic thermal power on teeth and yokes should be calculated separately due to different distribution of magnetic density. They can be used to express function of maximum of magnetic density. On teeth, magnetic hysteretic thermal power in unit volume is:

$$P_{ht} = K_h f B_{tm}^{\beta} \ (\mathrm{W/m^3}) \quad (1)$$

$$P_{hy} = K_h f B_{ym}^{\beta} \ (\mathrm{W/m^3}) \quad (2)$$

where: k h is constant of material performance; β is a value between 1.6 and 2.2; f is frequency of magnetic field change; B tm is maximum of magnetic density on teeth; B ym is maximum of magnetic density on yoke.

Magnetic hysteretic thermal power of iron core in heater can be obtained from formula (1) and (2).

$$P_h = P_{ht}V_t + P_{hy}V_y = K_h f(B_{tm}^{\beta}V_t + B_{ym}^{\beta}V_y) \quad (\mathrm{W}) \quad (3)$$

2.3.2. Eddy-current thermal power in iron core

Insulating treatment between laminations leads to decrease of conductivity as well as low eddy-current parameter (Park et al., 2012). Based on that, it is assumed that, solid iron core is made of superposed laminations in same height; eddy current is radial and axial eddy current is ignored; moreover, lamination is not insulative, which means conductivity between laminations is conductivity of solid iron core. Eddy-current thermal power on teeth and yokes should be calculated separately as well. On teeth, eddy-current thermal power in unit volume is:

$$P_{et} = K_e (fB_{tm})^2 \quad (\mathrm{W/m^3}) \quad (4)$$

On yokes, eddy-current thermal power in unit volume is:

$$P_{ey} = K_e (fB_{ym})^2 \quad (\mathrm{W/m^3}) \quad (5)$$

In the formula, k_e is constant of material performance.

Thus eddy-current thermal power of iron core of heater can be obtained:

$$P_e = P_{et}V_t + P_{ey}V_y = K_e f^2(B_{tm}^2 V_t + B_{ym}^2 V_y) \quad (\mathrm{W}) \quad (6)$$

2.3.3. Thermal power of short circuit winding

Current in short circuit winding in static state is I (A). Based on Joule-Lenz's law, thermal power in winding is:

$$P_{Cu} = I^2 R \quad (W) \quad (7)$$

R is resistance of copper wire. Total length of winding I (m), wire cross section S (m^2) and electrical resistivity of wire ρ(Ω ·m) are substituted into formula (7), and then thermal power of short circuit winding of heater can be obtained.

$$P_{Cu} = I^2 \rho \frac{l}{S} \quad (\mathrm{W}) \quad (8)$$

2.3.4. Mathematical model for heat generation of food heater

Mathematical model for heat generation of food heater can be obtained based on formulas (3), (6) and (8).

$$P = P_h + P_c + P_{Cu} = K_h f\left(B_{tm}^{\beta} V_t + B_{ym}^{\beta} V_y\right) + k_e f^2\left(B_{tm}^2 V_t + B_{ym}^2 V_y\right) + I^2 \rho \frac{l}{S} \text{ (W)} \quad (9)$$

2.3.5. Thermal circuit model of food heater

Specific heat capacity refers to thermal absorbed or released by unit mass of substance when temperature rises or falls one degree (Wollti et al., 2001). Usually, expression for correlation between thermal variation and temperature variation is:

$$Q = C \cdot m \cdot \Delta T \quad (10)$$

where Q stands for heat, m stands for quality, ΔT stands for temperature variation and C stands for specific heat capacity.

Copper heat loss thermal power in short circuit winding and magnetic hysteretic and eddy-current thermal power in solid iron core are taken as effective heat source for heater in modeling of temperature rise of heater. It is assumed that, heat is isolated between heater and external environment; thermal source distributes evenly in stator core and thermal conductivity of stator is good; influence of heat-transfer patterns such as convection and radiation is ignored; thermal performance parameter of materials is not affected by temperature in transient heat transfer process.

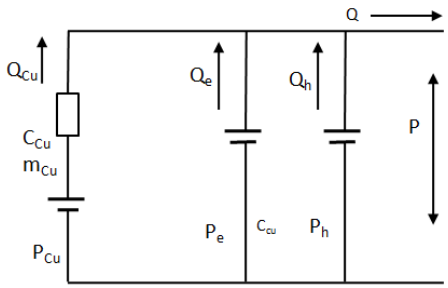

Figure 2. Equivalent thermal source

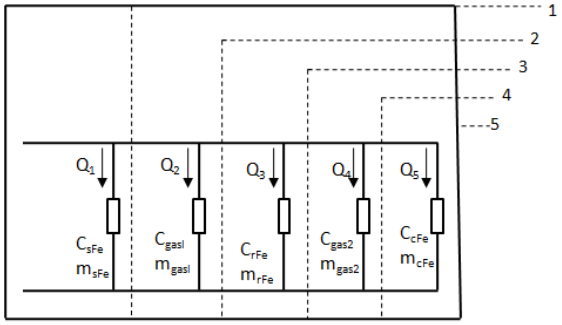

Figure 3. Equivalent thermal load of heater (1-level 1 load, 2-level 2 load, 3-level 3 load, 4-level 4 load, 5-level 5 load)

Mathematical model for temperature rise of heater

$$\begin{cases} Q = Cm\Delta T \\ Q = Pt \end{cases} \quad (11)$$

Where P stands for effective thermal power of heater and t stands for heating time. Establishing multiple-grade thermal circuit model is to heat different thermal load with the same thermal source. P_{Cu} is thermal power of short circuit winding of heater, Pe is eddy-current thermal power, Ph is magnetic hysteretic thermal power, Q_{Cu} is heat produced by short circuit winding in a period, Qe is heat produced by eddy current in a period, Q_h is heat produced by magnetic hysteresis in a period, C_{Cu}, C_{sFe}, C_{gas1}, C_{rFe}, C_{gas2} and C_{cFe} is specific heat capacity corresponding to different materials of heater (unit: J/ (Kg·°C)).

3. Results and discussions

3.1. Analysis of thermal power of food heater

Thermal power of short circuit winding: Induced electromotive force produced in copper on stator side by rotating magnetic field is bound to produce induced current; the condition is similar to operation of permanent magnet synchronous generator in short circuit condition (Ran et al., 2011). Analysis of heater model with finite element method suggests that, there are 9 complete short circuit current waveforms within one cycle, which conform to operation principle of 9-antipode permanent magnet synchronous generator.

Based on formula $f_c = \frac{F_c}{F_c} = 1 = h_c$, thermal power of short circuit current, P_{Cu} can be calculated as follows.

$$P_{Cu} = n \cdot R \cdot I^2 = n \frac{1}{\sigma} \frac{1}{S} \frac{1}{T} \int I^2(t) dt \quad (12)$$

In the formula, n stands for number of short circuit winding, σ stands for conductivity of copper (S/m); S stands for cross section area of copper bar (m^2) and $\frac{1}{T}\int I^2(t)dt$ stands for quadratic mean of current in one cycle.

3.2. Eddy-current thermal power of solid iron heater

To enhance eddy-current loss, iron core of food heater is made to be solid (Chy et al., 2010). We assume that, solid iron core is made of laminations in same height; there is no insulating treatment between laminations; conductivity of laminations is the same as solid iron core; eddy current in iron core is radial and axial eddy current is ignored; eddy current in iron core is two dimensional (Zhizhen et al., 2003).

It can be known from formula $P_e = K_e (Bf)^2$ (Pe stands for eddy-current loss and k_e stands for eddy-current loss coefficient), when rotating speed is constant, then frequency of alternating magnetization in stator core is also constant. Thus eddy-current thermal power is correlated to magnetic density amplitude. Figure 4 demonstrates magnetic density distributing along radial cross section at one time point. It can be seen from the figure that, the part of iron core embedded with copper, i.e., slotted section, has relatively large magnetic density. As distribution and amplitude of magnetic density on teeth and yokes are different, eddy-current thermal power on teeth and yokes should be considered separately. Cross section of magnetic density on teeth and yokes is shown in Figures 5 and 6 respectively. Magnetic density shown in figure 5 and 6 is at the same time point. An oscillograph involving maximum of magnetic density on teeth and yokes can be obtained by connecting maximum of magnetic density on teeth and yokes at every time point in one cycle according to the time order. Maximum of magnetic density on teeth and yokes changes intensively, and meanwhile maximum of magnetic density on teeth is higher than yokes, which conforms to distribution rule of motor field (Lahiri et al., 2014; Katoh et al., 2004).

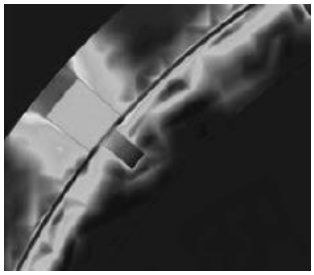

Figure 4. Distribution of magnetic density along radial cross section under a magnetic pole at a time point when rotating speed is 1, 400 r/min

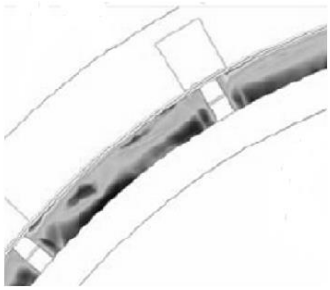

Figure 5. Distribution of magnetic density on teeth along radial cross section at a time point when rotating speed is 1, 400 r/min

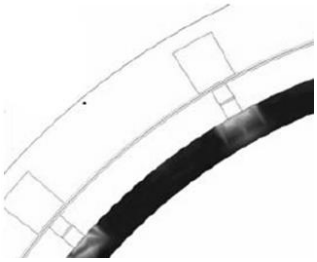

Figure 6. Distribution of magnetic density on yokes along radial cross section at a time point when rotating speed is 1, 400 r/min

Based on formula $P_e = K_e(Bf)^2$, eddy-current thermal power can be calculated as follows:

$$p_{et} = K_e \cdot \left(\frac{1}{T}\int B_{tm}(t)dt \cdot \frac{pn}{60}\right)^{\beta} \cdot (\pi r_1^2 - \pi r_2^2 - ah_t b) \cdot l \cdot \rho \quad (13)$$

where k_e stands for eddy-current thermal power coefficient, 0.00022W/kg·Hz2·T2; P stands for number of pole-pairs of rotor; n stands for rotating speed of rotor (r/min), $\frac{pn}{60}$ stands for frequency of alternating magnetization in iron core, $\frac{1}{T}\int B_{tm}(t)dt$ stands for average value of maximum of magnetic density on teeth in one cycle; β is 2; r_1 stands for external diameter of stator core, r_2 stands for distance from stator slot base to center, ht stands for height of iron core of teeth, α stands for number of short-circuit copper embedded with stator on one side, b is slot width of stator core, I stands for length of stator core, $(\pi r_1^2 - \pi r_2^2 - ah_t b)\cdot l$ stands for volume of iron core on teeth, ρ stands for density of iron core material, *i.e.,* type 10 steel. Eddy-current thermal power on yokes can be obtained using the following formula:

$$p_{ey} = K_e \cdot \left(\frac{1}{T}\int B_{ym}(t)dt \cdot \frac{pn}{60}\right)^{\beta} \cdot (\pi r_2^2 - \pi r_3^2) \cdot l \cdot \rho \quad (14)$$

where $\frac{1}{T}\int B_{tm}(t)dt$ stands for average value of magnetic density amplitude on yokes in one cycle and r_3 stands for inner diameter of stator core.

3.3. Magnetic hysteretic thermal power of solid iron core

In alternating magnetic field, magnetic domain orientation of ferromagnetic material tends to change under periodic repeated magnetization, leading to magnetic hysteretic loss (Tang et al., 2012). Thus magnetic hysteretic loss is considered to be closely correlated to frequency of alternating magnetization and magnetic density amplitude (Sakai *et al,* 2001). As to food heater, magnetic hysteretic thermal power in stator core can be ignored during analysis as frequency of alternating magnetization in stator core is low. In analysis of magnetic hysteretic thermal power of stator core, teeth and yokes should still be considered separately.

Based on formula (1), magnetic hysteretic thermal power on teeth can be calculated with the following formula:

$$p_{ht} = K_h \cdot \frac{pn}{60} \cdot \left[\frac{1}{T}\int B_{tm}(t)dt\right]^{\beta} \cdot (\pi r_1^2 - \pi r_2^2 - ah_t b) \cdot l \cdot \rho \quad (15)$$

where k_h stands for coefficient of magnetic hysteretic thermal power, 0.045 W/kg·Hz·T2; P is number of pole-pairs of permanent magnet in rotor, n stands for rotating speed of rotor (r/min), $\frac{pn}{60}$ stands for frequency of alternating magnetization in iron core, $\frac{1}{T}\int B_{tm}(t)dt$ stands for average value of maximum of magnetic density on teeth in one cycle, β is 2, r_1 stands for external diameter of stator core, r_2 stands for distance from slot base of stator to center, ht stands for height of iron core on teeth, α stands for number of short circuit winding embedded with stator on one side, b stands for slot width

of iron core of stator, I stands for length of iron core of stator, $(\pi r_1^2 - \pi r_2^2 - a h_t b)$ stands for volume of iron core on teeth and ρ stands for density of iron core material, *i.e.,* type 10 steel.

4. Conclusions

Food heater is based on electromagnet. Thermal power in food heater can be divided into three categories, *i.e.*, magnetic hysteretic thermal power and eddy-current thermal power in solid iron core and short-circuit current thermal current; and thermal power of short-circuit current is the main source of thermal power of heater. Taking food heater as research objects, this study constructed thermal power mathematical theoretical model and thermal circuit model and made a numerical analysis of various thermal powers of heater. Simulation results suggest that, thermal power of short-circuit winding accounts for 90% among total thermal power and eddy-current and magnetic hysteretic thermal power both account for more than half.

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PURIFICATION OF YEAST GLUCOSE TOLERANCE FACTOR AND ITS EFFECT ON INTRACORPORAL GLUCOSE CONVERSION IN PHYSICAL ACTIVITY

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ABSTRACT

Objective: this paper was aimed to study the separation and purification of yeast glucose tolerance factor (GTF) and its effect on intracorporal glucose conversion of body builders.

Methods: Yeast high-yield GTF was adopted in order to improve the detection sensitivity of chromium; in separation and purification process, the method high performance liquid chromatography- inductively coupled plasma- mass spectrometry/ atomic emission spectrometry (HPLC-ICP-MS/AES) was applied in microanalysis of the distribution of GTF; according to the analysis results, appropriate separation technique was selected for batch separation and purification; by comparing the blood indexes of fed mice, intracorporal glucose conversion of body builders could be deduced.

Results: Chromium/ protein ratio of Peak 1 was much lower than that of Peak 2; Peak1 was micromolecular chromium-binding protein, while Peak 2 was micromolecular chromium-binding protein. N-terminal amino acid sequence analysis results of peak PP1 showed that amino acids in peak PP1 were probably connected with chromium by coordination bonds instead of peptide bonds. In addition, triglyceride and cholesterol level in blood decreased.

Conclusion: Chromium-enriched yeast has an improvement effect on diabetes to some degree.

1. Introduction

Glucose tolerance factor (GTF) is a kind of micromolecular chromium-binding protein (Yuann et al., 2014), also known as a water-soluble complex of chrome with nicotinic acid, glutamate, glycine and cysteine. Due to the physiological functions of GTF, such as adjusting glycometabolism, improving fat metabolism and livestock production performance, GTF has become a research hotspot in the field of drugs, functional food and fodder (Mertz, 1975). As the metabolite of yeast, GTF can be absorbed by human body at high absorption rate and consumption security (Anderson et al., 1977). Researchers of various countries have made efforts to study the mechanism and functional properties of GTF. In 2012, Sarah et al. (Sarah et al., 2012) applied molecular docking method and speculated that GTF played a role by combining with hxt2p (member of hexose transporter family). In 2014, Qin et al. (Lili et al., 2014) found that homemade high-yield chromium-enriched yeast could prevent the occurrence of type-2 diabetes

in rats and reduce blood glucose level in rats with type 2 diabetes. In 2013, Yang et al. (Yang et al., 2013) analyzed the effect of GTF puerarin functional milk on the metabolism of glucose in the resting cells of beer. In this study, we carried out an experiment on the purification of yeast GTF as well as its effect on intracorporal glucose conversion by taking mice as example, which provided basis for the speculation of the effect on intracorporal glucose conversion of body builders.

2. Materials and methods

2.1. Major instruments and reagents

This study adopted the following instruments and reagents: nucleic acid and protein analyzer, high speed refrigerated centrifuge, N4S ultraviolet visible spectrophotometer, portable glucometer, ammonia water, nitric acid, perchloric acid guarantee reagent (GR), chromium trichloride, glucose, glycosylated hemoglobin determination kit.

2.2. Experimental methods

2.2.1. Cultivation and collection of chromium-enriched thalli cells

Ammonia extracting solution of chromium-enriched yeast was added into a 100 kD ultrafiltration centrifugal tube for 90-min centrifugation at the rate of 2250 r/min, and interception liquid was obtained; then, the centrifugated (100 kD) and filtrated liquid was added into a 50 kD ultrafiltration centrifugal tube for another 90-min centrifugation at the rate of 2250 r/min, and interception liquid was obtained; after that, the centrifugated (50 kD) and filtrated liquid was added into a 10 kD ultrafiltration centrifugal tube for 90-min centrifugation at the rate of 2250 r/min, and interception liquid was obtained; at last, the centrifugated (10 kD) and filtrated liquid was kept at 4 °C as standby.

2.2.2. Sephadex G-25 gel filtration chromatography

A certain amount of Sephadex G-25 dry glue was added to the ammonia acetate buffer (50 mM, pH 6.0) at 90 °C for 90-min intumescence (Neudachina et al., 2014); when the intumescence was over, the mixture was stirred mildly so as to remove floating broken particles; then, a certain amount of buffer was added to adjust the concentration to 73%. Before packing, ultrasonic wave was applied to remove bubbles for 30 min (Skilbeck et al., 2014). Then, ammonia acetate buffer (50 mm, pH 6.0) was used as the eluent; the flow rate was 0.5 mL/min, and the temperature was set at 0 ~ 4 °C; 2.6mL of eluent was collected for each tube. The eluent was traced and checked at 250nm and 270 nm; flame atomic absorption method was applied to detect metal content. At last, liquid in target tube was collected and merged for standby application after freeze drying processing.

2.2.3. Ultraviolet full wavelength scanning

N4S ultraviolet (UV) visible spectrophotometer was used to scan the absorbance of samples within wavelength coverage of 200 ~ 600 nm; blank control liquid and target samples were put into the quartz cuvette which was placed in a UV spectrophotometer for baseline correction, with deionized water as the blank control.

2.2.4. Determination of protein content

Referring to Bradford method, zymoprotein content was determined, with bovine serum albumin as a standard protein.

2.2.5. Analysis of amino acid composition

A certain amount of sample was weighed and placed at the bottom of a hydrolysis test tube, and 3 ml of muriatic acid (5.7 mol / L) was added. The sample was placed in an ultrasonic tank for oscillation and degassing; then, it was put in a blast burner and the pipe orifice was sealed; then, it was placed in an oven for 22-hour hydrolysis at 110 ± 1 °C, and proteins or peptides were hydrolyzed into free

amino acids (Lan et al., 2010). After cooling, the test tube was cut open, and the hydrolysate was filtrated into a 5 ml volumetric flask; test tube and filter paper were flushed with deionized water, and the hydrolysate was diluted to the constant volume. Then, 5 ml of filtrate was placed in an evaporation pan and dried by water bath. Residue was dissolved with 4.5 ml of deionized water and evaporated to dryness, which was repeated for 3 times. Precisely, 5 ml of citrate buffer solution (pH 2.2) was added to dissolve the extractive; 1.5 ml of sample was centrifuged at a high speed refrigerated centrifuge; with an injector, 50μl of supernatant was drawn and stored in the spiral pipe for computer analysis. According to the concentration and retention time of amino acid criterion liquid, concentrations of amino acid in sample solution were determined.

2.2.6. Sequence analysis of protein/ polypeptide-N terminal with Edman degradation method

Edman degradation method was used for sequence analysis of protein/ polypeptide-N terminal, aiming to analyze N terminal amino acid sequence of protein / polypeptide with free N terminus. It was applied to sequencing amino acids in a peptide. The amino-terminal residue was labeled and cleaved from the peptide without disrupting the peptide bonds between other amino acid residues (Edman *et al.*, 1950).

2.2.7. Determination of blood biochemical indicators

After being fed, the mice were on fasting; then, orbital blood was drawn and then centrifugated at the rate of 2250 r/min for 25 min, thus serum was separated; then, blood glucose level, total cholesterol and triglyceride were determined.

3. Results

3.1. Microanalysis on distribution of metal chromium and protein in Peak 1 and Peak 2 (from Sephadex G-75 gel chromatography) by HPLC-ICP- MS/AES

Sephadex G-75 was used to separate chromium-enriched yeast ammonia organic chromium extract, and two peaks (Peak 1 and Peak 2) that contained organic chromium were obtained. Peak 1 was a protein with large molecular weight (greater than 10 kD), while Peak 2 was a protein with smaller molecular weight (approximately less than 3000 D). This study adopted the method HPLC-ICP-MS/AES to analyze distribution of protein and chromium in the two groups of peaks, which indicated research direction for the subsequent experiment.

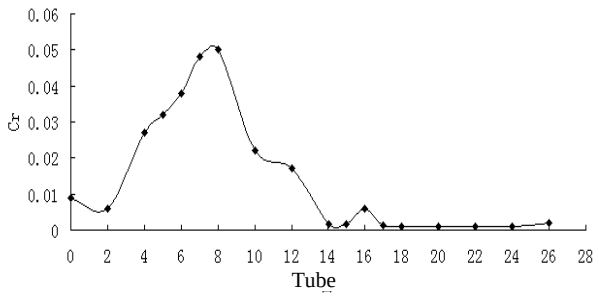


Figure 1. Distribution of organic chromium in high performance liquid chromatography (HPLC) of Peak 1

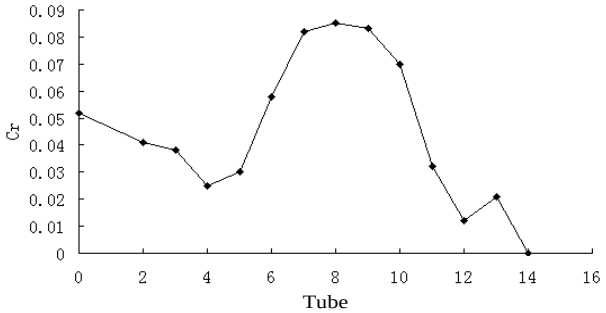


Figure 2. Distribution of organic chromium in HPLC of Peak 2

Figure 1 shows the distribution of organic chromium in HPLC of Peak 1. In Peak 1, protein was spread effectively, and the whole process of elution and separation lasted about

45 min. According to the distribution map, there was a peak value of chromium content in Peak 1, which was 0.05 μg; content of chromium was the highest in tube 6, 7, 8. Figure 2 shows the distribution of organic chromium in HPLC of Peak 2. It can be observed that there was a peak value of chromium content, which was 0.087 μg; the content of chromium was the highest in tube 7, 8, 9.

Table 1. Distribution of chromium and protein in ultra-filtration (100kD, 50kD, 10KD)

	Chromium (μg)	**Protein content (mg)**	**Chromium/protein (μg/mg·pro)**
Ammonia extracting solution	975.12	19.45	51.23
≥100kD	523.19	14.23	38.22
100~50kD	67.16	2.51	4.79
50~10KD	121.22	2.29	55.79
≤10KD	141.62	0.50	339.13

3.2. Ultrafiltration centrifugation

Membranes of different cut-off molecular weights were used for protein fractionation in organic chromium ammonia extracting solution. The distribution of protein and chromium is shown in table 1. In view of the absolute chromium content in cut-off products, chromium content and protein content (accounting for almost three quarters of the total protein) were the highest in the membrane cut-off components when membrane molecular weight was greater than 100 kD, while chromium content was the lowest when membrane molecular weight was 100 ~ 50 kD, and protein content was the lowest when membrane molecular weight was less than 10 kD.

3.3.Sephadex G-25 gel filtration chromatography

After ultra-filtration through the membrane (with the molecular weight of 100 kD, 50 kD and 10 kD) for 3 times, the ammonia extraction liquid was given Sephadex G-25 filtration chromatography. Ammonium acetate buffer (pH 6.0, 55 mM) was used as the eluent (Jitka et al., 2006), the flow rate was 0.25 mL/min; 2.6 mL was collected in each tube. During elution, the detection wavelengths were A280 and A260. From the experiment, it can be observed that the detection wavelength showed there were 6 protein peaks in the whole separation process, and the peaks overlapped. According to the distribution of protein peaks, the collected tubes were selected for metal content detection, and the results are shown in Figure 3. Through Sephadex G-25 gel chromatography, large quantities of impure proteins were eliminated.

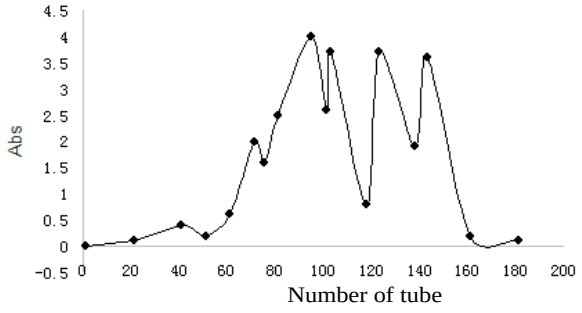


Figure 3. Distribution of metal chromium in Sephadex G-25 gel filtration chromatography

3.4. Sephadex G-25 gel desalination chromatography

Figure 4 shows the distribution map of organic chromium. According to the experimental process and figure 4, it can be

seen that there was only one protein peak in the elution process, while distribution of chromium also formed a peak, and protein peak coincided with chromium peak, with symmetrical distribution of peak curves. Through ultrafiltration with three cutoff membranes (100 kD, 50 kD, 10 kD) and combination of two gel chromatographic separation, we obtained the only protein peak (PP1) that contained chromium and had symmetrical distribution of peak curves.

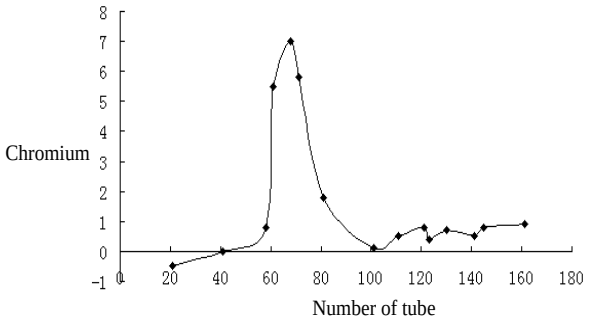


Figure 4. Distribution map of chromium in the process of separation and purification with Sephadex G-25

3.5. Analysis on separation purity of PP1 with combined method HPLC-ICP-MS/AES

Based on protein molecules of different sizes, gel filtration chromatography was applied to achieve the purpose of purification by fractionation; however, it was not suitable for the fractionation of protein components that had similar molecular weights. In the separation and purification of YS-3 yeast ammonia extract, fractionation was performed according to molecular size of protein (Liu et al., 2011). Although we obtained the only protein peak that contained chromium and had symmetrical distribution of peak curves, its purity could not be determined accurately. Therefore, we made further analysis on peak PP1 with the aid of HPLC-ICP-AES/MS method (Tabb et al., 2004); thus the specific purity could be analyzed; on the other hand, by HPLC, PP1 was given high efficiency separation so as to test the separation prospect (Dębski et al., 2004).

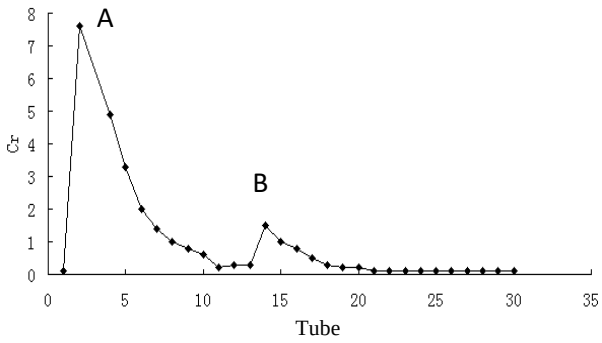
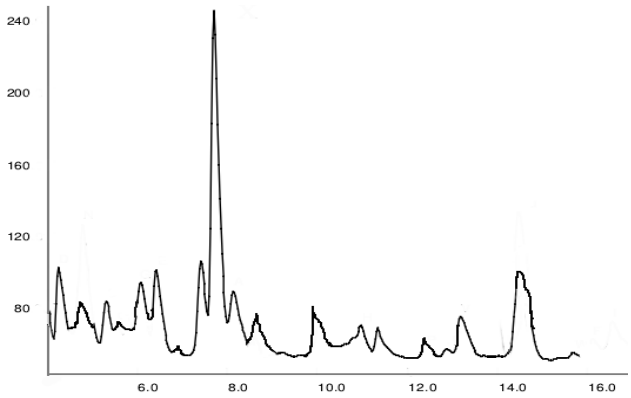


Figure 5. Distribution map of organic chromium in high performance liquid phase of PP1

Figure 5 shows the distribution of organic chromium of PP1. In tube 3 and tube 14, there were two chromium peaks (peak A and peak B). As shown in the figure, chromium content of peak A was significantly higher than that of peak B, while UV detection signal of peak A was weaker than that of peak B. Peak B showed symmetrical peak shape, while chromium distribution of peak A was asymmetrical.

3.6.Sequence analysis of protein / polypeptide-N terminal

The four graphs of Figure 6 show the liquid phase diagram of residue 1, residue 2, residue 3 and residue 4 (of amino acids). It can be seen that each peak shape and height did not change with hydrolysis, which indicated that this kind of substance seemed to remain unchanged without being cleaved.



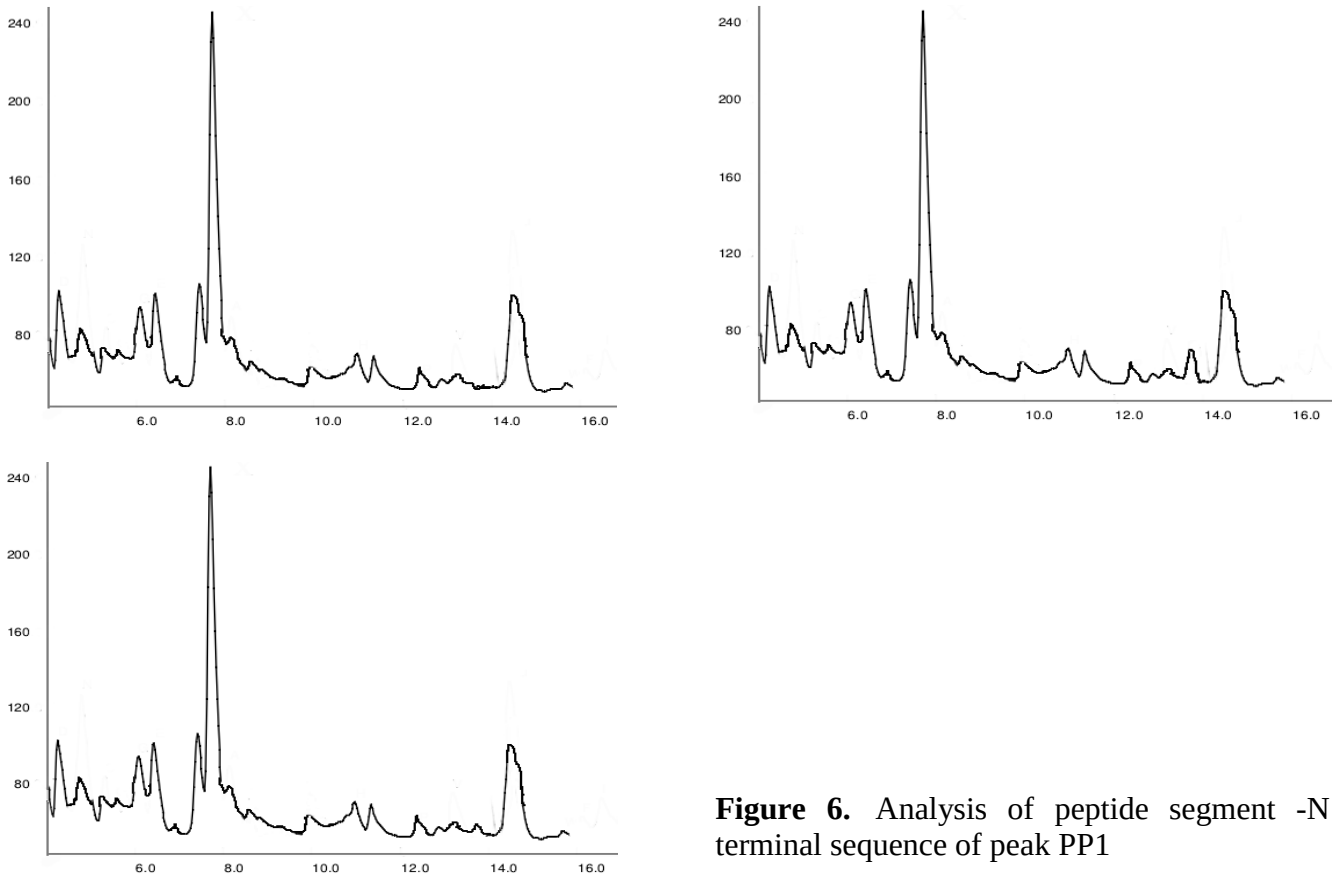


Figure 6. Analysis of peptide segment -N terminal sequence of peak PP1

3.7. Blood lipid level

Table 2. Blood lipid level in each treatment group after 15 weeks

	Normal	Model	Control	Low	Middle	High
Triglyceride	471.8±95.7	301.6±55.9	550.9±71.9	413.9±34.9	246.1±31.9	401.2±33.1
Total cholesterol	204.1±14.9	231.5±13.1	184.9±11.6	161.5±16.2	167.6±12.1	225.9±10.9

Table 2 shows blood lipid level in each group (normal group, model group, positive control group and low/ middle/ high dose chromium-enriched yeast groups) after 15-week medication. In comparison with control group, triglyceride in middle dose chromium-enriched yeast group decreased significantly ($p < 0.05$) and was lower than that in normal group; triglycerides in low dose group, high dose group and positive control group were higher than that of control group.

In comparison with the model group, total cholesterol level decreased significantly in low and middle dose groups ($p < 0.01$) as well as positive drug control group ($p < 0.05$), while no change of total cholesterol level was found in high dose group. The decrease of triglyceride and cholesterol level in blood indicated that chromium-enriched yeast had improvement effect on blood lipid level of diabetes to some degree.

3.8. Overall discussion

According to previous researches, GTF was reported to be low-molecular-weight chromium-enriched protein (peptide) (Koch et al., 2011). However, it has never been reported that macromolecular chromium-binding protein could be separated from chromium-enriched yeast. It was because previous studies adopted

cytoactive test to detect GTF in separation liquid, while this study detected the distribution of organic chromium in separation liquid; on the other hand, it was also related to the adoption of different separation methods were applied.

As an emerging method of metal proteomics, HPLC-ICP-MS/AES combines chromatographic separation ability with qualitative function of inductively coupled plasma mass spectrometry so as to realize the quantitative and qualitative analysis of complex mixtures, with fast separation speed, high efficiency and low metal detection threshold (Troccoli et al., 2004). In addition, the pretreatment process of samples can be simplified and sample analysis is more convenient.

According to chromium content determination results, it was found that chromium content was much higher in Peak 2 than in Peak 1, and Peak 2 was small molecular weight component, which was basically consistent with the previous report that GTF was micromolecular peptide. Based on the previous research reports and the analysis results of Peak 1 and Peak 2, subsequent purification work will focus on the separation of components; in addition, the tube with the highest chromium content in Peak 1 will be analyzed to explore the physicochemical properties of chromium-binding molecular protein.

GTF is a kind of compound of chromium and protein; therefore, single evaluation based on chromium content or protein content is one-sided. This study adopted the ratio of chromium and protein as a reference index. In reference to previous studies, separation range of Sephadex G-75 was 80 ~ 3 kD, in this study, the filtrate that was fractionated through 100 kD, 50 kd and 10 kD membrane was used for subsequent purification.

The binding of metal and protein is different from that of amino acids in proteins. Amino acids in proteins are bonded by peptide bonds (Beili et al., 2010), while metals bond with proteins by coordination. Each metal ion has certain coordination configuration; as long as the spatial arrangement of configuration atoms meets the basic requirements of the configuration, stable complexes can be formed. Metal ions bind to protein molecules through coordination with endogenous ligands and exogenous ligands (such as water molecules, porphyrin ring and small organic molecules), thus metal active sites are formed, which affects protein structure. Based on the experimental results, it was confirmed that peak PP1 was chromium-binding small molecular peptide; through sequence analysis of protein/ polypeptide-N terminal, it was found that some substance could not be dissociated to produce amino acids, suggesting that the amino acid in the substance is linked with chromium by coordination bonds instead of peptide bonds; therefore, amino acid analysis based on N-terminal dissociation could not be achieved.

In general, fasting glucose level reflects the amount of hepatic glucose output, without reflecting the sensitivity of peripheral tissues to insulin, which is due to the decrease in fasting insulin level and increase in glucagon level. Therefore, we speculated that the absence of Fyn in liver could influence gluconeogenesis under fasting conditions, since hepatic glucose output failed to balance the high clearance rate of glucose. Insulin resistance leads to the increase of intracellular hydrolysis in adipose cells, thus more fatty acids are released into blood vessels, which promotes liver to form more low-density cholesterol and triglyceride. If insulin sensitivity is increased, the opposite reaction will happen in blood lipid.

4. Conclusions

Through the experimental study, we found that chromium/ protein ratio of Peak 1 was far less than that of Peak 2; Peak 1 was chromium-binding macromolecular protein, while Peak 2 was chromium-binding micromolecular protein. According to the UV wavelength scanning on peak PP1 and amino acid composition analysis, it was found that peak PP1 was a chromium-binding small peptide consisting of aspartic acid, glutamic acid, glycine and ysteine. We

assumed that chromium-enriched yeast thalli had an improvement effect on diabetes to some degree. However, due to the limitations of various conditions in the experiment, there are still some defects in this study, based on which we will make some improvement in the subsequent researches.

5. Acknowledgement

Participated project: name of project (strategy research on mass sports development in construction of new-type urbanization) project number (14008) title (not concluded)

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SIMPLE ANALYSIS OF POTENTIAL IMMUNE REGULATION EFFECT OF CUCURBITACIN E ON PROFESSIONAL ATHLETES ENGAGED IN HIGH INTENSITY TRAINING

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ABSTRACT

This study was designed to analyze the immune regulation effect and functional mechanism of cucurbitacin E (CucE) taking professional athletes engaged in high intensity training as the main analysis objects, and then to further discuss the potential possibility of CucE to be a kind of immune regulation drug for professional athletes. Phorbol esters and ionomycin were used in this study to activate human peripheral blood mononuclear cells (PBMC) and Jurkat T cells; immunofluorescent staining combined flow cytometry method was adopted for analysis of CD3+ T cell activation antigen expression in human PBMC; quantitative polymerase chain reaction (PCR) method was used to detect the effect of CucE on expression of interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) mRNA; moreover, immunofluorescence assay was used to observe the nuclear translocation of NF-κB/p65, thus to further discuss whether CucE had regulating effect on the adaptive immunologic function. Results showed that high intensity training could lead to immunosuppression of professional athletes as well as the increase of various kinds of inflammatory cytokines, and CucE could significantly inhibit the expression of cytokines from the mRNA level and protein level through lowering NF-κB signal path, and CucE also had significant regulating effect on adaptive immunity.

1. Introduction

Professional athletes belong to a special group who are of strong physique, and athletes engaged in different sport events have different body shapes. Physiologically, energy consumption of athletes in unit time is large, thus athletes are required to be physically healthy and strong as well as have strong cardio-pulmonary function and anaerobic metabolism ability (Miyagi et al., 2012; Tylee and Walters, 2010). Immunologic function reflects the ability of body to resist diseases, which is an important part of evaluation of human body functions. As a kind of stress stimulation (Jung et al., 2009), long-term high intensity training can have short-term or long-term effect on the overall immunologic function of athletes, such as leading to immunosuppression, decrease of immunologic function of athletes and especially the increase of infection rate of the upper respiratory tract (Dever et al., 2016;

Petrelli, et al., 2016). Therefore, how to relieve sports inflammatory state of athletes is an urgent problem to be solved. In recent years, the anti-inflammatory effect of cucurbitacin E (CucE) has been verified by a large number of experiments. Gursoy et al. (2012) carried out an in vitro cell culture experiment in 2012, which verified that CucE could inhibit the production of nitric oxide (NO) from LPS/INF-γ activated mice macrophage RAW 264.7 and WRL-68 cell; meanwhile, CucE could play an anti-inflammation role through inhibiting the activity of cyclo-oxygenase and active nitrogen. In 2013, Mielgo-Ayuso et al. (2013) carried out an in vivo test and verified that intraperitoneal injection of CucE could effectively relieve the edema of feet of mice induced by carrageenin. In 2014, Ramos et al. (2014) discovered that CucE could inhibit T cells activation and expression of cytokines and had certain regulating effect on adaptive immunologic function.

While strengthening the competitive ability training of athletes and improving their sport performance, it is of great significance to monitor changes of immunologic function of athletes timely to avoid decrease of immunologic function as well as injury and diseases (Bennell et al., 2011; Silva et al., 2013). Through the discussion on regulating effect of CucE on adaptive immunologic function and its possible mechanism, this study mainly aims to explore the potential possibility of CucE to be an anti-inflammatory drug which can inhibit the increase of pro-inflammatory cytokines of athletes (Chew and Ong, 2016), so as to relieve the increase of inflammatory cytokines of athlete to some extent through dietary instruction, which has certain practical significance.

2. Materials and methods

2.1. Reagents and equipment

Reagents used in this study include paraformaldehyde, absolute ethyl alcohol, phorbol esters (PDB), ionomycin (Ion), 2-mercaptoethanol, L-glutamine, et al.; equipment used in this study include a clean bench, a refrigerated centrifuge, a microplate reader, an inverted microscope, an electronic scale, a pipette, et al.

2.2. Detection of activation surface molecules of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were inoculated in a 24-well plate, 1×10^6 cells for each well and the volume was 0.5 ml. Four groups which were blank group, CucE group, PDB+Ion model group and CucE+PDB+Ion drug group were set and cultured for one day in an incubator (37 °C, 5% CO_2). After that, supernatant was collected for cytometric bead array (CBA) (Malpass, et al., 2013).

2.3. Extraction and inverse transcription of RNA

(1) Jurkat cells were collected and added with 1 ml of Trizol; cells were separated and dissolved and then transferred to an eppendorf (EP) tube for 5-6 min of incubation (at about 25 °C), thus to completely separate the ribonucleoprotein complex.

(2) Then 0.2 ml of chloroform/Trizol mixed liquor was added and the obtained solution was vibrated quickly for 10-20 s and had about 3 min of reaction.

(3) The tube was put in a 5 °C environment and centrifuged in 8000 g for 15 min; then the supernatant was transferred to a new EP tube; then 0.5 ml of isopropanol was added and the reaction lasted for 10 min at room temperature.

(4) After the reaction, the tube was

centrifuged in 12000 g for 10 min at 2-8 °C; the supernatant was removed and 1 ml of 75% diethyl pyrocarbonate-ethyl alcohol was added; sediments were washed once and the solution was shaken up.

(5) After that, the tube was centrifuged in 13000 g for 2-3 min at 2-8 °C and the supernatant was removed; sediments were dried for about 8 min; 20 μL of D.D•H_2O was added and vibrated to fully dissolve RNA (Burns et al., 2015).

2.4. Real-time quantitative polymerase chain reaction (Table 1)

Table 1. Real-time quantitative polymerase chain reaction

	Sense	Antisense
IL-2	5'-TGAAGGACGAGGAGTACGAGC-3'	5'-TGCAGGAACGAGTCTCCGT-3'
TNF-α	5'-CGTGGAACTGGCAGAAGAG-3'	5'-TGAGAAGAGGCTGAGACATAGG-3'
IFN-γ	5'-AGATCTGGCACCACACCTTCT-3'	5'-CTTTGATGTCACGCACGATTT-3'
β-Actin	5'-TGGTACCATGTACCCAG-3'	5'-AAGGGTGTAAAACGCAGCTC-3'

2.5. Extraction of nuclear protein

(1) Blank control group, PDB+Ion model group and drug pretreatment group were set in the experiment. Jurkat T cells in logarithmic phase were selected and cell concentration was adjusted. Cells were inoculated in 75 ml cell culture flasks, 5×10^7 cells for each flask.

(2) In the drug pretreatment group, flasks were added with CucE in 0.1 mol/L, 0.3 mol/L and 1 mol/L respectively for 0.5 h of pretreatment; then PDB+Ion was added in PDB model group and CucE+PBD+Ion drug group; flasks in the two groups were cultured in 5% CO_2 at 37 °C for 0 min, 0.5 h, 1 h and 2 h respectively, then the reaction was terminated.

(3) Lysis buffer was added into cells to remove cytoplasm protein and cells were washed by lysis buffer once.

(4) 2×SDS-PAGE loading buffer was used to extract nuclear protein and nuclear protein was split using boiling water bath; after the centrifugation in 16000 g for 20 min, the supernatant was removed and nuclear protein was obtained (Lentz and Shideler, 2016).

2.6. Immunofluorescence analysis

(1) Blank control group, PDB+Ion model group and CucE+PBD+Ion drug pretreatment group were set in the experiment. Jurkat T cells in logarithmic phase were selected and cell concentration was adjusted. Cells were inoculated in culture dishes in 5% CO_2 at 37 °C overnight, 1.5×10^4 cells for each.

(2) CucE+PBD+Ion drug pretreatment group was pretreated using CucE for 60 min and then added with PDB and Ion and cultured in 5% CO_2 at 37 °C for 120 min, then the reaction was terminated; PDB+Ion model group was stimulated using dulbecco's modified eagle medium (DMEM) complete culture containing PDB and Ion for 120 min (Yamamoto, et al., 2013).

(3) Then culture medium was removed and 4% paraformaldehyde was added to cover cells; cells were fixed for 15 min at room temperature, then stationary liquid was removed and cells were washed twice by cold phosphate buffer saline (PBS); then iced methyl alcohol was added for 10 min of transparence at -20 °C.

(4) After that, cells were washed three

times by cold PBS and blocking buffer was added for 60 min of treatment; then the blocking buffer was removed and 500 μmol/L of Hoechst 33342 was added for about 10 min of staining; a fluorescence microscope was used for observation and recording (Masuda et al., 2015).

2.7. Statistical analysis method

Graphpad Prism 5.0 software was used for one-way analysis of variance; Tukey test was adopted for comparison between groups; $p < 0.05$ was considered to have statistical significance and $p < 0.01$ represented that the difference was highly significant.

3. Results and discussions

3.1. Inhibition effect of CucE on expression of PBMC cytokines (Figure 1)

Activated T cells can secrete IL-2, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and other cytokines which play an important role in congenital immunity and adaptive immunity. Figures 1a-c show that in PDB+Ion model group, the specific value of $CD3^+CD69^+/CD3^+$ increased significantly compared with that of $CD3^+CD25^+/CD3^+$ ($p < 0.01$); in the meantime, in the CucE+PDB+Ion drug treatment group, the expression of CD69 and CD25 of T cells activation surface molecules was inhibited significantly in a dose-dependent way. Compared with the blank group, expression of TNF-α in CucE treatment group was improved, and expression level of IL-2, TNF-α and IFN-γ in PDB+Ion group was increased significantly ($p < 0.01$); compared with the activation model group, expression level of IL-2, TNF-α and IFN-γ in PDB+Ion+CucE drug treatment group was significantly inhibited in a dose-dependent way (Amezquita-Garcia et al., 2015; Yousef et al., 2015).

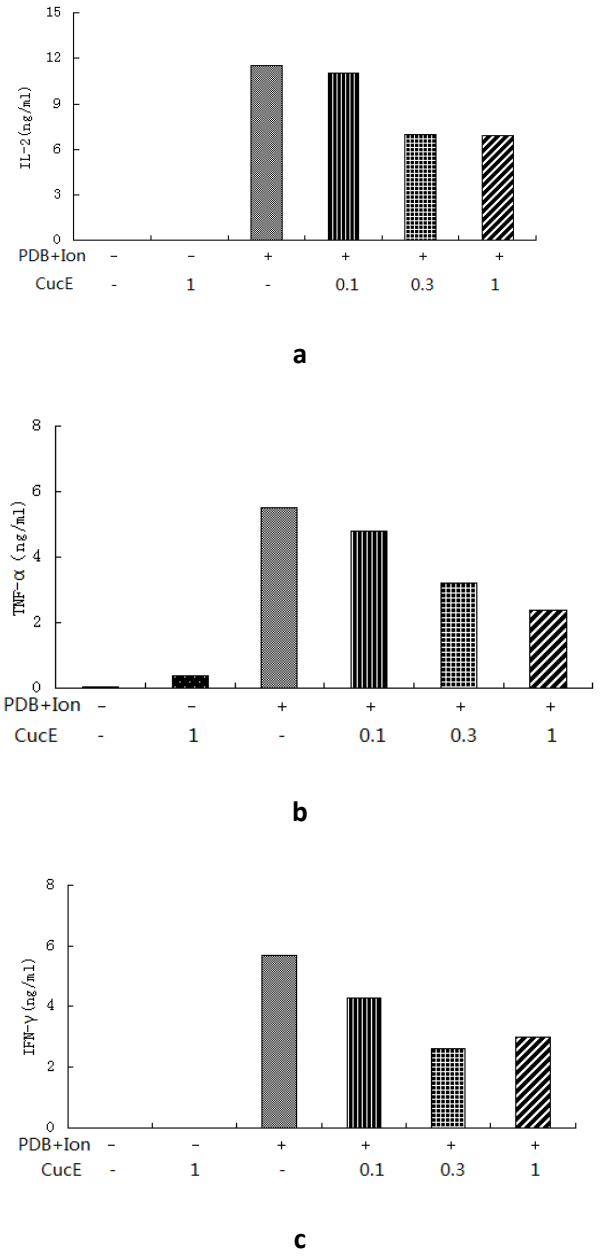


Figure 1. Inhibition effect of CucE on expression of PBMC cytokines (a-c)

3.2. The inhibition effect of CucE on expression of Jurkat T cytokines (Figure 2)

As shown in Figure 2, compared with the blank group, the expression level of IL-2 and IFN-γ after 6 h and 24 h in PDB+Ion model group both increased significantly ($p < 0.01$).

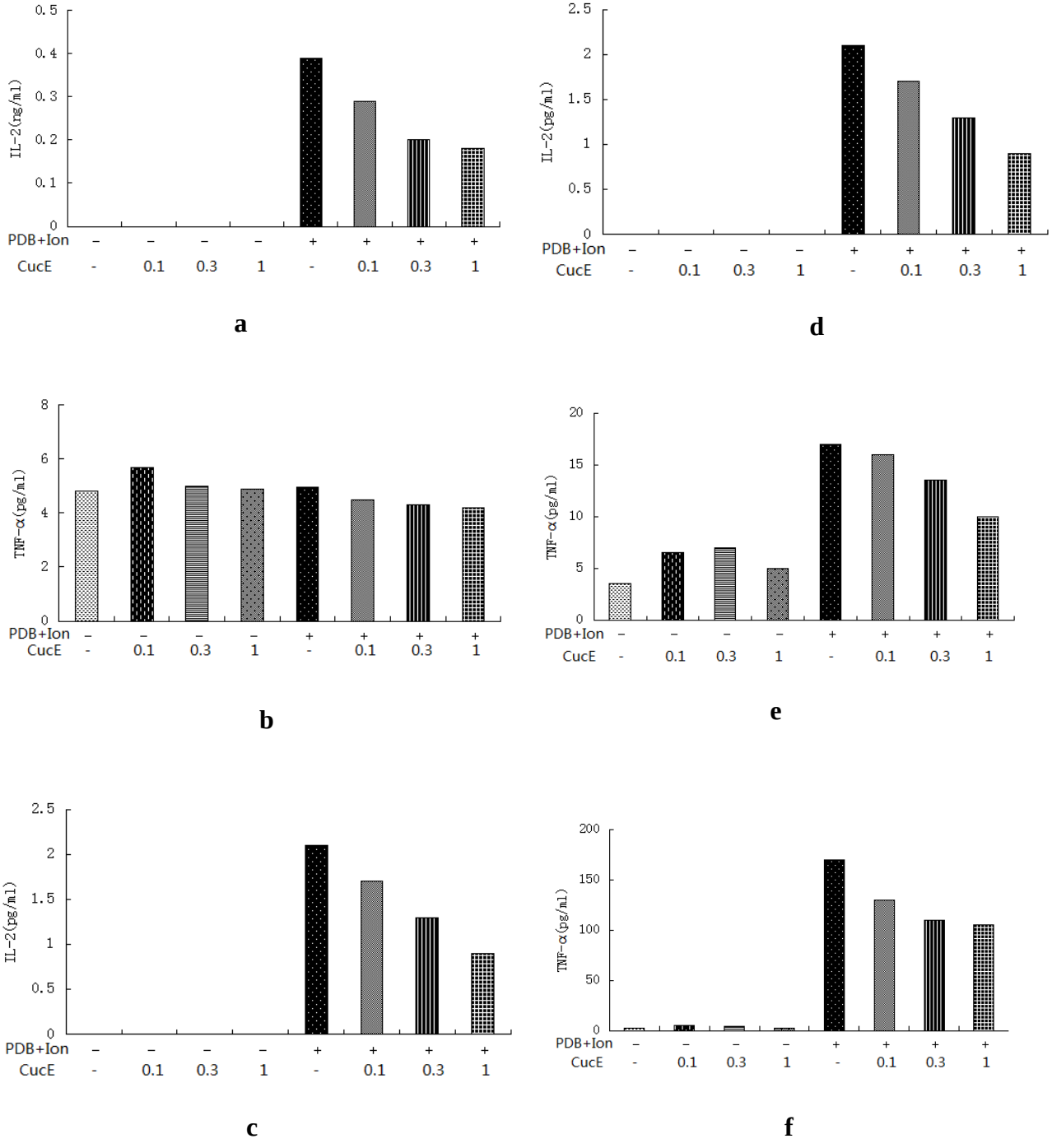
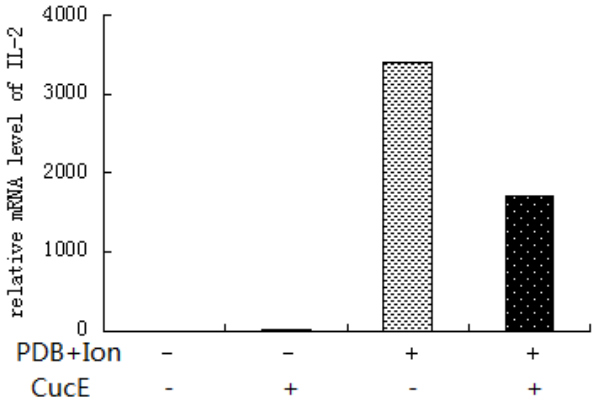


Figure 2. The inhibition effect of CucE on expression of Jurkat T cytokines **(**a-f)

After 24 h, compared with the PDB+Ion model group, the expression level of IL-2, TNF-α and IFN-γ in PDB+Ion+CucE drug treatment group was inhibited significantly in a dose-dependent way (Wei et al., 2015).

3.3. The inhibition effect of CucE on mRNA expression level of IL-2, TNF-α and IFN-γ in Jurkat T cells

In the experiment, blank group, CucE treatment group, PDB+Ion model group and CucE+PDB+Ion drug group were all cultured in a 5% CO_2 incubator at 37 °C for 3 h and 6 h; then the supernatant was collected to detect the mRNA expression level of IL-2, TNF-α and IFN-γ (Figure 3). Figure 3 shows that after the addition of PDB+Ion and 3 h of culture, the mRNA expression level of IL-2, TNF-α and IFN-γ increased significantly, while after the addition of CucE, the mRNA expression level of IL-2 and IFN-γ decreased significantly; after the addition of PDB+Ion and 6 h of culture, the mRNA expression level of IL-2, TNF-α and IFN-γ increased significantly, while after the addition of CucE, the mRNA expression level of IL-2, TNF-α and IFN-γ decreased significantly. Therefore, a conclusion could be drawn that CucE could effectively inhibit the mRNA expression level of IL-2, TNF-α and IFN-γ in Jurkat T cells (Suttorp et al., 2013; Chih-Kang et al., 2009).



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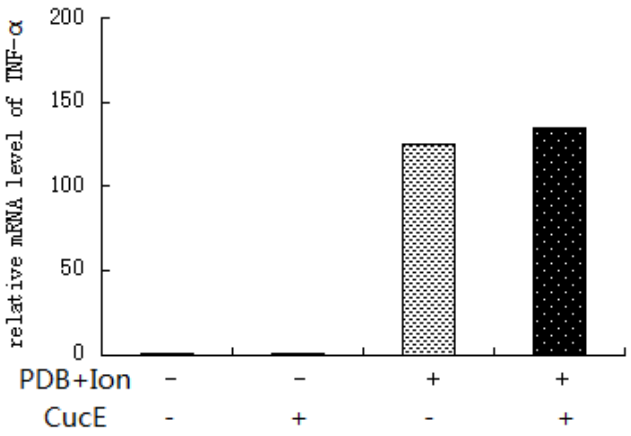

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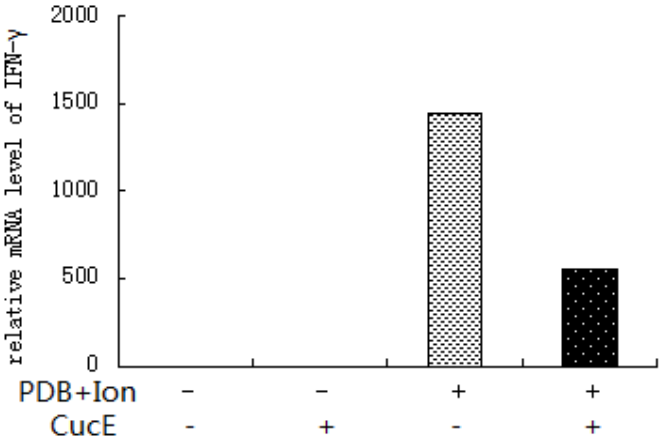

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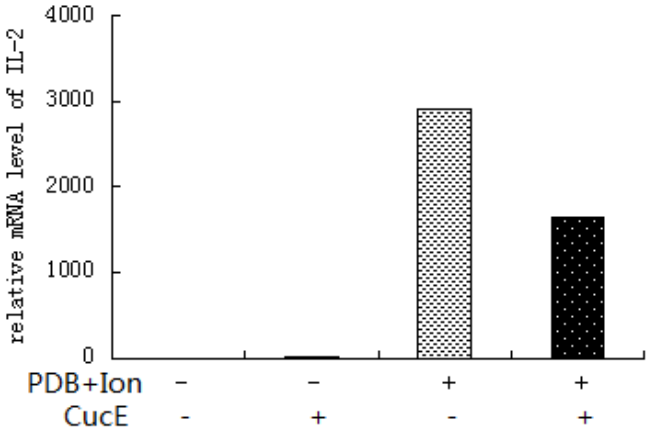

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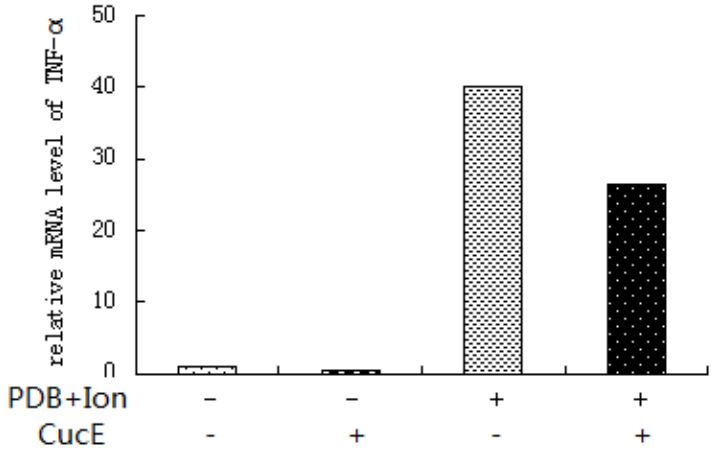

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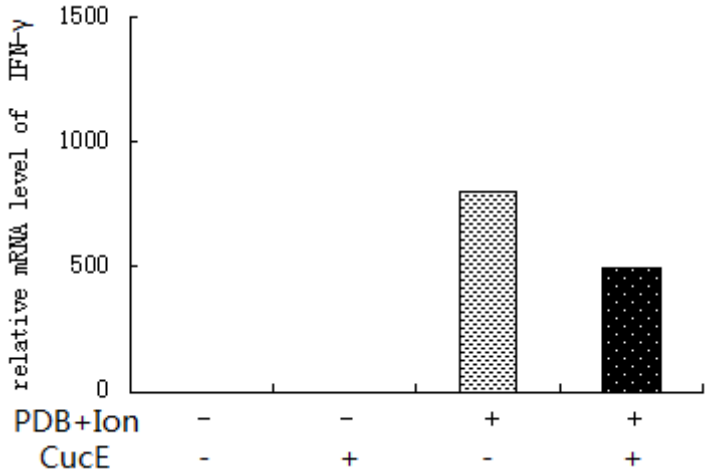

f

Figure 3. The inhibition effect of CucE on decreasing mRNA expression level of Jurkat cytokines

3.4. The influence of CucE on NF-κB signal path and MAPKs signal path

After the 1 h of pretreatment using CucE in 1 μmol/L final concentration, PBD and Ion was added; Protein expression of NF-κB signal path and MAPKs signal path was analyzed using Western Blot method. Results are shown in Figure 4 and Figure 5.

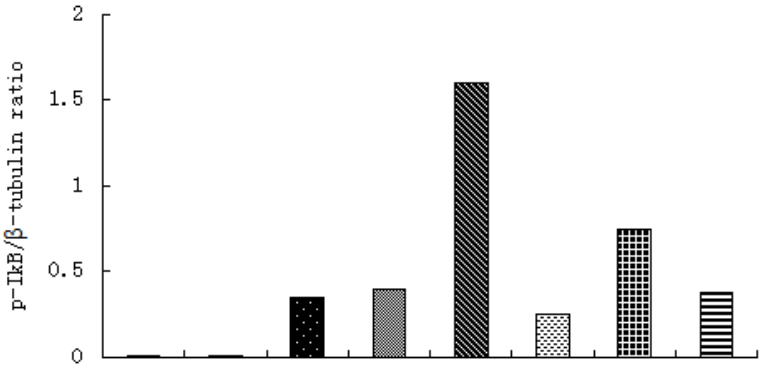

Figure 4. Analysis of protein expression of NF-κB signal path using Western Blot method

Figure 4 indicates that in CucE drug group, phosphorylation level of IκB and NF-κB/p65 decreased significantly at 30 min and 60 min, suggesting that CucE could significantly inhibit the phosphorylation level of IκB and NF-κB/p65.

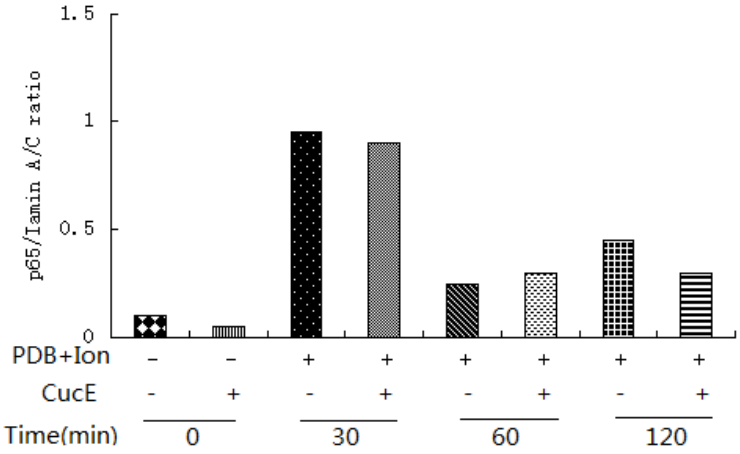

Figure 5. Analysis of intra-nuclear expression level of NF-κB/p65 using Western Blot method

Figure 5 shows that in CucE drug group, the intra-nuclear phosphorylation level of NF-κB/p65 at 2 h decreased significantly, thus CucE could significantly inhibit the intra-nuclear expression level of NF-κB/p65.

In addition, results of using Western Blot method to detect the effect of CucE on protein expression of MAPKs signal path indicated that, after the activation of Jurkat cells through PDB+Ion stimulation, phosphorylation level of JNK, Erk1/2 and p38MAPK increased significantly (Xiao et al., 2011).

4. Conclusions

(1) Researches showed that PBMC in vitro could be activated by PDB+Ion, expression of Jurkat T cells surface molecules CD69 and CD25 increased significantly, and the expression of cytokines IL-2, TNF-α and IFN-γ also increased significantly; however, CucE could significantly inhibit the activation of T cells as well as the expression of IL-2, TNF-α and IFN-γ (Petrelli et al., 2016). Results of Western Blot method showed that such kind of

anti-inflammatory effect might be realized through inhibiting NF-κB signal path (Carvalho et al., 2015), suggesting that CucE might inhibit the expression of IL-2 and its co-stimulatory effect through inhibiting the expression of surface molecules like CD69, thus to inhibit the activation of T cells; CucE has cetatin regulating effect on adaptive immunologic function (Zhao et al., 2016; Molander et al., 2015).

(2) IFN-γ is mainly produced by activated T cells and natural killer (NK) cells, which can regulate immunologic functions, activate NK cells, accelerate differentiation of T cells, induce cells to produce antiviral protein and induce expression of MHC-I and II-type molecules in antigens, etc. (Omokoko et al., 2016).

(3) Cucurbitacin can regulate NF-κB signal path and affect nuclear translocation of NF-kB/p65; CucE can not only inhibit the nuclear translocation of NF-kB/p65, but also can significantly inhibit the phosphorylation level of IkB and p65 (Maroni et al., 2015).

(4) CucE has certain potential medicinal value in regulating adaptive immunologic function and inhibiting inflammatory reaction (Fujita et al., 2015). In conclusion, CucE can significantly inhibit the activation of PBMC in vitro as well as inhibit the protein expression level and mRNA expression level of IL-2, TNF-α and IFN-γ, and it has good immunomodulatory effect on professional athletes engaged in high intensity training in particular (Ye et al., 2014; Miloski et al., 2014). The inhibition effect of CucE on expression of cytokines is realized by decreasing NF-κB path, suggesting that CucE can regulate adaptive immune response, prevent cell fatty degeneration and inhibit fibroplasias, etc.; in addition, it can also eliminate jaundice, lower serum alanine transaminase, zinc sulfate turbidity, eliminate ascitic fluid and improve protein metabolism, which can be used as a new-type drug to relieve sports inflammation of professional athletes.

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SYMMETRY AND ASYMMETRY MECHANISM OF DIFFERENT TRUST DIMENSIONS IN FOOD SAFETY MANAGEMENT AND THE CAUSES

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ABSTRACT
In recent years, the frequent occurrence of food safety issues has not only harmed the health of consumers, but also severely influenced the development of food industry; moreover, people begin to distrust food safety and government's relevant management. At present, food biotechnology (such as food additives, pesticides and genetically modified food) is the focus of people's attention as well as researches in terms of food safety. Therefore, we researched on the symmetry and asymmetry mechanism of different trust dimensions in food safety management. Through variance analysis, we concluded that in food safety management, relational trust is symmetrical, while calculative trust is asymmetrical; specifically, in the field of food additives and pesticides, relational trust is symmetrical, awareness of government's behavior and familiarity are asymmetrical; in the field of genetically modified food, relational trust and government's behavior and quality are symmetrical, awareness of government's behavior is asymmetrical.

1. Introduction

It is said that hunger breeds discontentment, while food safety comes first. As a global problem, food safety not only relates to our health and lives, but also concerns the operation of economy, government's credibility as well as our national image (Qijun, 2010). The management of food safety issues is closely connected with the construction of a harmonious society (Fischer Arnout et al., 2006).

According to Ge Wu's survey, it could be concluded that 86% of the interviewees considered inadequate government supervision as the cause of food safety problems. Patil's research indicated that government's regulatory measures and the implementation of relevant laws and policies could effectively enhance consumers' trust in food safety (Patil et al., 2005). In the view of Kathariou, trust of government had positive influence on food safety trust (Kathariou, 2002). Franz pointed out that market failure in trading and government's regulation failure led to the lack of trust in dairy (Franz et al., 2004). In addition, other researches also proved that government's regulation and information exchange affected the trust in quality standard (QS) certification of food enterprises (Wilcock et al., 2004); the trust in food safety system or management was an important part of food safety trust, corresponding to government behavior (Piggott and Marsh, 2004); government behavior significantly affected consumers' trust in food safety (Shan et al., 2015). At present, the public is relatively trustful to the government; the people with higher education level have less faith in scientists; and the people with higher

social status show more trust to government (Miraglia et al., 2009). The research conclusions above reveal that the improvement of trust in food safety management is significant to improving consumers' trust in food safety which can promote the development of food industry and the prosperity and stability of society. Therefore, we did a research on the trust in food safety management in order to improve people's trust in food safety and promote the economic development in food industry.

2. Materials and methods

2.1. Materials

Aiming at food additives, pesticide and genetically modified food, the measurement of trust in food safety includes eight aspects: moral, care, impartiality, openness, perceived ability, reliability, familiar and controllability. Considering that education level, professions, location and income level could affect people's perception of risk and trust in government, we conducted extra measurement based on these aspects.

2.2. Research Methods

In this study, we analyzed the symmetry and asymmetry of different trust dimensions in food safety management with empirical methods. Before the interviewees received the information, we measured their general trust in seven levels (from complete distrust to fully trust); afterwards, we measured the acceptance levels (also seven levels, from fully unacceptable to fully acceptable) of food additives, pesticide and genetically modified food; after the interviewees received relevant information, we measured their general trust again in seven levels (from complete distrust to fully trust). In addition, aiming at food additives, pesticides and genetically modified food, we measured the trust from the aspects of moral, care, impartiality, openness, perceived ability, reliability, familiar and controllability in seven levels (from completely disagree to completely agree).

In this study, SPSS 17.0 was applied in descriptive statistics analysis, t test, correlation analysis and variance analysis.

3. Results and Discussions

3.1. Effects on Overall Trust Evaluation

Table 1 lists the effects of information potency and specificity on some overall trust evaluation— general trust after receiving information; overall trust; trust of food additives, pesticides and genetically modified food. Information potency has significant impact on overall trust ($F(1, 168) = 4.408$, $p < 0.05$, $\eta^2 = 0.026$) and the trust in food additives ($F(1, 168) = 3.956$, $p < 0.05$, $\eta^2 = 0.023$), pesticide trust ($F(1, 168) = 4.730$, $p < 0.05$, $\eta^2 = 0.027$) and genetically modified food ($F(1, 168) = 3.614$, $p = 0.059$, $\eta^2 = 0.021$) except on the general trust ($F = 0.985$, $p > 0.05$) after receiving information. Specifically, the degree of negative information to reduce overall trust is higher than the degree of positive information to improve overall trust, which means people are more likely to believe negative information ($M = 4.15$) rather than positive information ($M = 3.77$). Similarly, in terms of the trust in food additives, pesticides and genetically modified food, the average values of trust in negative information are respectively 4.19, 4.23 and 4.03; and the average values of trust in positive information are respectively 3.82, 3.82 and 3.67. Thus we can conclude that the trust in food additives, pesticides and genetically modified food and the overall trust are asymmetrical.

Table 1. The effects of information potency and specificity on overall trust

		SS	df	MS	F	p	η^2
General trust after receiving information	Information potency	0.032	1	3.032	0.986	0.321	0.07
	Information specificity	1.257	1	1.257	0.407	0.523	0.03
	Potency×specificity	1.761	1	1.761	0.573	0.452	0.04

	Error	517.198	167	3.078			
Overall trust	Information potency	6.022	1	6.022	4.407*	0.038	0.027
	Information specificity	2.901	1	2.901	2.123	0.148	0.013
	Potency×specificity	0.777	1	0.777	0.568	0.453	0.004
	Error	229.477	167	1.367			
The trust of food additives	Information potency	5.713	1	5.713	3.957	0.047	0.024
	Information specificity	3.244	1	3.244	2.247	0.137	0.012
	Potency×specificity	0.762	1	0.762	0.528	0.468	0.004
	Error	242.556	167	1.445			
The trust of pesticides	Information potency	7.003	1	7.003	4.731*	0.032	0.028
	Information specificity	3.235	1	3.235	2.186	0.142	0.014
	Potency×specificity	0.157	1	0.157	0.104	0.748	0.002
	Error	248.683	167	1.481			
The trust of genetically modified food	Information potency	5.407	1	5.407	3.615	0.058	0.022
	Information specificity	2.284	1	2.284	1.528	0.217	0.008
	Potency×specificity	1.902	1	1.902	1.272	0.262	0.007
	Error	251.278	167	1.495			

* Refers to $p < 0.05$.

In addition, after the information is received, information specificity has no significant effect on the general trust ($F = 0.408$, $p > 0.05$), overall trust ($F = 2.124$, $p > 0.05$) and the trust in food additives ($F = 2.246$, $p > 0.05$), pesticides ($F = 2.185$, $p > 0.05$) and genetically modified food ($F = 1.527$, $p > 0.05$); interaction effects of information potency and specificity are not significant on general trust ($F = 0.572$, $p > 0.05$), overall trust ($F = 0.569$, $p > 0.05$), trust of food additives ($F = 0.527$, $p > 0.05$), trust of pesticides ($F= 0.105$, $p > 0.05$), trust of genetically modified food ($F = 0.127$, $p > 0.05$) .

In terms of overall trust (general trust, overall trust and trust of food additives, pesticides and genetically modified food) evaluation in food safety management, the results of variance analysis indicate that information potency has no significant effect on general trust after information is received, this might result from people's impression of food safety risks. Different from the risks of earthquake, flood and nuclear industry, food safety risks are not highly lethal (Law, 2012). At the same time, it can be concluded from this study that people trust food safety management to some degree (M = 4.55), which indicates that food safety risks are acceptable to some extent. As was mentioned, for the minor risks, trust is symmetrical (Li and Liu, 2007).

3.2. Effects on the Trust of Food Safety Management

Table 2 shows the effects of information potency and specificity on relational trust and calculational trust in food safety management. The results reveal that information potency has no significant effect on relational trust ($F = 0.421$, $p > 0.05$), and there is no evident difference between negative information to reduce relational trust and positive information to improve relational trust, which proves relational trust is symmetrical. However, information potency has significant effect on calculative trust ($F (1, 168) = 8.714$, $p < 0.01$, $\eta^2 = 0.049$), and the degree of negative information to reduce calculational trust is higher than the degree of positive trust to improve calculational trust. That is, in terms of the calculative trust, people are more likely to believe negative information (M = 3.98) rather than positive information (M = 3.42), which reveals that calculative trust is asymmetrical.

In addition, information specificity has no significant effect on relational trust ($F = 1.842$,

p > 0.05) or calculational trust (F = 1.741, p > 0.05); interaction effects of information potency and specificity with relational trust (F = 0.495, p > 0.05) and calculational trust (F = 0.466, p > 0.05) are not significant.

As for the trust in food safety management, the results of variance analysis show that information potency has no significant effect on relational trust, and there is no significant difference between positive trust improving relational trust and negative information reducing relational trust. Therefore, relational trust is symmetrical. However, information potency affects calculational trust significantly, and negative information reduces calculational trust more greatly than positive information improves calculational trust, which proves that calculational trust is asymmetrical. Here, relational trust reflects people's evaluation of government's quality. Without objective standards, the evaluation is mainly based on the mutual relationship (of consumers and government) and their shared values. As long as they have similar values (even if there is some deviation), the relational trust will not be reduced significantly. Calculational trust reflects people's evaluation of government's behavior and ability in food safety management with objective behavioral standards. Once government's management behavior is not in accordance with people's cognitive standards, calculational trust will decline sharply (Lin et al., 2010).

Table 2. The effects of information potency and specificity on the trust of food safety management

		SS	df	MS	F	p	η^2
Relational trust	Information potency	0.782	1	0.782	0.422	0.518	0.003
	Information specificity	3.428	1	3.428	1.843	0.178	0.012
	Potency × specificity	0.921	1	0.921	0.496	0.482	0.002
	Error	312.476	167	1.861			
Calculational trust	Information potency	12.778	1	12.778	8.715**	0.005	0.048
	Information specificity	2.552	1	2.552	1742	0.187	0.011
	Potency × specificity	0.683	1	0.683	0.467	0.495	0.004
	Error	246.334	167	1.467			

* Refers to $p < 0.05$; ** refers to $p < 0.01$.

3.3. Effects on Trust Dimensions of Food Additives

The effects of information potency and specificity on trust dimensions (relational trust, awareness of government's behavior and familiarity) in food additives are listed in table 3. According to the results, in terms of food additives, information potency has no significant effect on relational trust (F = 0.422, p > 0.05), and there is no evident difference between positive information improving relational trust and negative information reducing relational trust; therefore, relational trust is symmetrical. However, information potency affects the awareness of government's behavior (F (1, 168) = 3.882, p = 0.05, η^2 = 0.023) and familiarity (F (1, 168) = 11.009, p < 0.01, η^2 = 0.061) significantly, and the degree of negative information reducing awareness of government's behavior and familiarity is higher than the degree of positive information improving them, which means people are more likely to believe negative information (averages are M = 4.11 and M = 3.96) rather than positive information (averages are respectively M = 3.72 and M = 3.23). Therefore, it can be concluded that the awareness of government's behavior and familiarity are asymmetrical. Since calculational trust consists of the awareness of government's behavior and familiarity, it can be deduced that calculational trust is asymmetrical.

Table 3. Effects of information potency and specificity on trust dimensions of food additives

		SS	df	MS	F	p	η^2
Relational trust of genetically modified food	Information potency	0.957	1	0.957	0.423	0.518	0.002
	Information specificity	3.355	1	3.355	1.478	0.225	0.008
	Potency×specificity	0.446	1	0.446	0.198	0.656	0.002
	Error	380.898	167	2.268			
Awareness of government's behavior	Information potency	6.545	1	6.545	3.881*	0.04	0.022
	Information specificity	2.358	1	2.358	1.397	0.238	0.007
	Potency×specificity	1.596	1	1.596	0.948	0.333	0.005
	Error	283.248	167	1.685			
Government's behavior and quality	Information potency	22.155	1	22.155	11.008**	0.002	0.062
	Information specificity	6.432	1	6.432	3.195	0.075	0.018
	Potency×specificity	0.012	1	0.012	0.007	0.942	0.000
	Error	338.108	167	2.012	0.423	0.518	

* Refers to $p < 0.05$; ** refers to $p < 0.01$.

In addition, information specificity has no significant effect on relational trust (F = 1.479, p > 0.05), awareness of government's behavior (F = 1.398, p > 0.05) and familiarity (F = 3.196, p > 0.05), and there is no significant interaction effect of information potency and specificity with relational trust (F = 0.197, p > 0.05), awareness of government's behavior (F = 0.947, p > 0.05) and familiarity (F = 0.006, p > 0.05).

As for the three specific food safety issues, the results of variance analysis indicate that information potency has no significant effect on relational trust, and there is no significant difference between negative information reducing relational trust and positive information increasing relational trust, namely, relational trust is symmetrical. In addition, in the field of food additives and pesticides, information potency affects awareness of government's behavior and familiarity significantly, and negative information reduces them more greatly than positive information improves them, namely, awareness of government's behavior and familiarity are asymmetrical. Since calculational trust is composed of awareness of government's behavior and familiarity, we can deduce that the calculational trust is asymmetrical.

3.4. Effects on Trust Dimensions of Genetically Modified Food

Table 4 shows the effects of information potency and specificity on trust dimensions (relational trust, awareness of government's behavior, government's behavior and quality) in genetically modified food. The results reveal that in the field of genetically modified food, information potency has no significant effect on relational trust (F = 0.285, p > 0.05), and there is no significant difference between negative information reducing relational trust and positive information improving relational trust, which indicates relational trust is symmetrical. At the same time, information potency has no significant effect on government's behavior and quality (F= 0.044, p > 0.05) which prove to be symmetrical as well. However, the effects of information potency on awareness of government's behavior (F (1, 168) =9.678, p<0.01, η^2=0.054) are significant, and negative information reduces awareness of government's behavior more greatly than positive information improves the awareness. In brief, people are more likely to believe negative information (M= 3.86) rather than positive information (M= 3.24), this suggests that awareness of government's behavior is asymmetrical. Since awareness of government's behavior is part of calculational trust, to some degree, we can deduce that calculational trust is asymmetrical.

Table 4. Effects of information potency and specificity on trust dimensions of genetically modified food

		SS	df	MS	F	p	η^2
Relational trust of genetically modified food	Information potency	0.684	1	0.684	0.284	0.595	0.003
	Information specificity	5.508	1	5.508	2.287	0.131	0.012
	Potency×specificity	1.492	1	1.492	0.61	0.431	0.003
	Error	404.265	167	2.405			
Awareness of government's behavior	Information potency	15.93	1	15.93	9.679**	0.003	0.055
	Information specificity	1.08	1	1.08	0.663	0.416	0.003
	Potency×specificity	2.003	1	2.003	1.216	0.271	0.008
	Error	276.704	167	1.648			
Government's behavior and quality	Information potency	0.102	1	0.102	0.045	0.832	0
	Information specificity	1.537	1	1.537	0.677	0.413	0.003
	Potency×specificity	2.507	1	2.507	1.101	0.294	0.008
	Error	382.412	167	2.275			

* Refers to $p< 0.05$; ** refers to $p < 0.01$.

In addition, information specificity has no significant effects on relational trust ($F = 2.289$, $p > 0.05$), awareness of government's behavior ($F = 0.662$, $p > 0.05$) and government's behavior and quality ($F = 0.676$, $p > 0.05$); and there are no significant interaction effects of information potency and specificity with relational trust ($F = 0.620$, $p > 0.05$), awareness of government's behavior ($F = 1.217$, $p > 0.05$) and government's behavior and quality ($F = 1.102$, $p > 0.05$).

In the field of genetically modified food, information potency has significant influence on awareness of government's behavior, and negative information reduces it more greatly than positive information improves it, accordingly, awareness of government's behavior is asymmetrical. Since awareness of government's behavior is calculational trust, to some extent, we can deduce that calculational trust is asymmetrical.

3.5. Effects of Previous Attitudes on Symmetry and Asymmetry Mechanism of Trust

Table 5 lists the effects of previous attitudes (general trust and acceptance level before receiving information) on the asymmetry principle of food safety management. It can be observed that after the introduction of previous attitudes, information potency has greater impact on trust measurement. Before receiving the information, people have a low degree of general trust in food safety management and not ready to accept food biotechnology. Due to negative previous attitude, information potency has greater effect on the asymmetry of trust.

Table 5. Effects of previous attitudes on the asymmetry principle of food safety management

	SS	df	MS	F	p	η^2	Original η^2
Overall trust	7.141	1	7.145	6.115	0.015	0.034	0.025
Trust of food additives	7.031	1	7.031	5.677	0.019	0.032	0.024
Trust of pesticides	7.962	1	7.962	6.077	0.014	0.034	0.026
Trust of genetically modified food	6.475	1	6.475	5.078	0.025	0.028	0.022
Calculational trust	14.112	1	14.112	11.127	0.002	0.061	0.048
Food additive 1,	8.375	1	8.373	5.595	0.018	0.031	0.024
Food additive 2	21.821	1	21.821	11.612	0.002	0.064	0.062

Pesticide 1,	8.647	1	8.647	5.358	0.021	0.031	0.027
Pesticide 2,	26.582	1	26.582	16.636	0.000	0.08	0.07
Genetically modified food	17.348	1	17.348	12.584	0.002	0.04	0.053

Note: food additive 1 and 2 respectively refer to the awareness of government's behavior and familiarity in terms of food additives; pesticide 1 and 2 respectively refer to the awareness of government's behavior and familiarity in terms of pesticides; genetically modified food refers to the awareness of government's behavior in terms of genetically modified food.

To sum up, information specificity has no significant effect on all kinds of trust measurements in food safety management. As for overall trust evaluation, information potency has no significant influence on general trust after the information is received, while overall trust and the trust of food additives, genetically modified food and pesticides are asymmetrical and significantly affected. The fact that information potency has no significant effect on relational trust indicates relational trust is elastic and symmetrical; calculational trust is affected significantly and the degree of negative information reducing calculational trust is higher than positive information improving calculational trust, accordingly, calculational trust is asymmetrical. In terms of the three food safety issues, calculational trust is symmetrical. In the field of food additives and pesticides, awareness of government's behavior and familiarity are asymmetrical; in the field of genetically modified food, awareness of government's behavior is asymmetrical, while government's behavior and quality are symmetrical.

3.6.Suggestions for Food Safety Management

Government and managers are expected to formulate and implement corresponding policies so as to maintain and enhance the trust of food safety management as well as the acceptance of food biotechnology. It is also suggested that government should include public expectations in food safety management with righteous attitude to consider multiple opinions, which will improve people's evaluation of government's morality, care and justice (Tompkin, 2001). In addition, more attention should be paid to high-income people's attitudes and suggestions in food safety management.

Government and managers can improve the trust of food safety management by enhancing people's acceptance of food safety issues. In order to improve people's trust in food safety management, the government should constantly strengthen their ability and food safety control; furthermore, they are supposed to improve people's familiarity and reliability evaluation of government's decision content and decision process; at the same time, they need to avoid the generation or deterioration of food safety issues (Liu et al., 2010). What's more, it's also important for government to improve their quality (such as sense of responsibility and integrity) and make a good impression on the public so as to earn better evaluation of relational trust.

4. Conclusions

In this study, we researched on the trust in food safety management, specifically in food additives, pesticides and genetically modified food. The results reveal that the trust in food safety management includes two dimensions: relational trust and calculational trust. The trust of food additives and pesticides both include relational trust, awareness of government's behavior and familiarity; the trust of genetically modified food include relational trust, awareness and quality of government's behavior. In subsequent study, we should expand the types of food safety risks: on the one hand, we can seek the general trust structure in food safety management; on the other hand, we can explore different trust structure of food safety.

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6. Acknowledgements

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THE ANTIOXIDANT EFFECT OF COMPOUND E JIAO JIANG ON BALL GAME PLAYERS ENGAGING IN ENDURANCE TRAINING

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ABSTRACT

This study was designed to explore the antioxidant effect of compound E Jiao Jiang on ball game players engaging in endurance training, aiming to provide a theoretical basis for the effectiveness and security of compound E Jiao Jiang in the application of fatigue resistance of ball game players. Totally 50 ball game players, including 25 males and 25 females, were selected and divided into control group (placebo 3 times/day, one dose/time), experimental group (compound E Jiao Jiang 3 times/day, one dose/time) and high-dose group (compound E Jiao Jiang 3 times/day, two doses/time) using a double-blind design. Under the same training program, each group took compound E Jiao Jiang for 4 weeks according to prescription. Subjects' antioxidant indexes were measured before test, after 2 and 4 weeks oral administration respectively. Results revealed that, after 2 and 4 weeks of oral administration, methane dicarboxylic aldehyde (MDA) content decreased ($P<0.01$); superoxide dismutase (SOD) and catalase (CAT) content in experimental group and high-dose group increased as dosing time prolonged ($P<0.01$, $P<0.05$); MDA and carbonyl content in experimental group and high-dose group decreased more and more apparently after taking medication for 2 and 4 weeks ($P<0.01$, $P<0.01$). Besides, the antioxidant ability showed a time-response relation. After 2 weeks of administration, all subjects were found with reduced MDA content in experimental group and high-dose group ($P<0.01$); SOD and haemoglobin (Hb) in male subjects in experimental group rose ($P<0.05$); MDA content in male subjects in high-dose group dropped ($P<0.01$); and CAT ($P<0.05$), Hb ($P<0.01$) and total antioxidant capacity (T-AOC) ($P<0.05$) in female subjects in experimental group increased. After 4 weeks of administration, the antioxidant ability was observed without dose-response relationship. Thus, it can be known that compound E Jiao Jiang is capable of enhancing Hb content and maximum oxygen uptake of athletes, improving athletes' oxygen carrying capacity as well as aerobic endurance and also resisting fatigue.

1. Introduction

An adequate and regular exercise, belonging to a special kind of stressor, is able to change shape and function of the heart positively and actively, increase the contractility of cardiac muscle and reduce the risks of the occurrence of cardiovascular disease. However, excessive exercise will

damage morphological structure and function of the myocardial tissues (Veneroso et al., 2009; Berzosa et al., 2010; Comstock et al., 2013; Dolezal et al., 2000). Severe myocardial damage is likely to cause sudden death. Antioxidant substance existing at a low concentration can inhibit the oxidation reaction of free radicals effectively, which can directly act on free radicals, and also indirectly consume material easy to produce free radicals away, to prevent further reaction (Esmaeili and Sonboli, 2009). Human body produces free radicals, and meanwhile, generates antioxidants, so as to offset the oxidation attacks of free radicals to human cells. The stronger antioxidant ability of human body is likely to have better effect in eliminating free radicals. There are a variety of methods for enhancing antioxidant ability, involving improving the activity of antioxidant enzyme, increasing the amount, reducing the intake of materials easy to produce free radicals and increasing the intake of materials with antioxidant ability. A study (Hongzhong et al., 2007) reports that E Jiao Jiang supplements have significant effects in promoting red blood cells and haemoglobin (Hb) of ischemic animals and accelerating the proliferation and differentiation of hematopoietic stem cell (HSC). Compound E Jiao Jiang as a compound traditional Chinese medicine well promotes the hematopoietic function of marrow. Oxygen of each cell in the whole body increases and fatigue resistance strengthens with the improvement of hemachrome, which make athletes tolerance of high intensity exercise training (Maoxuan et al., 2014). Based on the current situation of traditional Chinese medicine tonic and sports fatigue, this study measures the antioxidant indexes of athletes after taking compound E Jiao Jiang, to confirm whether compound E Jiao Jiang can strengthen the antioxidant ability of body, thereby providing a theoretical foundation for the application of oral administration of compound E Jiao Jiang in athlete's physical exercise.

2. Materials and methods

2.1. Research objects

Fifty ball game players were selected as research objects, including 25 males and 25 females, with the age ranging from 18 to 25 years. They were randomly divided into three groups applying double blind method, i.e., control group, experimental group and high-dose group. All subjects ate and trained normally, athletes in control group were treated with oral administration of placebo that had the similar appearance and taste with compound E Jiao Jiang three times a day, one dose per time; experimental group took compound E Jiao Jiang three times a day, one dose per time; and athletes in high-dose group received the oral administration of compound E Jiao Jiang three times a day, two doses per time. All three groups took medication half an hour before meals. The qualified athletes were included as study subjects after they were screened based on grouping criteria, and research objects signed the informed consent.

2.2. Experimental methods

This experiment collected and detected samples three times, i.e., before oral administration, after 2 and 4 weeks of oral administration respectively, lasting 4 weeks.

(1)Collection of blood samples

Blood samples were collected from fingertip and vein of subjects with an empty belly in the early morning, finger-tip blood was used for Hb measurement, and venous blood was preserved in the refrigerator at - 80 °C for future application after serum was separated through stewing and centrifuging and serum samples were marked.

(2)Measurement of maximum oxygen uptake and blood antioxidant

Maximum oxygen uptake was measured through Astrand-Ryhnuiy method to predict

the maximum oxygen uptake of subjects, and the data was recorded. Blood antioxidant indexes were detected using relevant antioxidant index kit as per the instructions. Biochemical test was performed on corresponding indexes, and data was measured and recorded.

2.3. Documentation methods

To acquire the current situation of the field and the latest studies, relevant literatures on colla corii asini, ginseng, radix rehmanniae praeparata, compound E Jiao Jiang, antioxidant, fatigue resistance, index of athletes' body function were collected, sorted and analyzed by searching PUBMED, China National Knowledge Infrastructure (CNKI) and other professional websites, so as to provide a theoretical foundation for writing this thesis.

2.4. Statistics

Data was statistically processed using SPSS 17.0 software and expressed as mean ± standard deviation (SD). Test indexes measured before oral administration, after 2 and 4 weeks of oral administration received normal distribution test, and all of them were observed to be normally distributed (P>0.05). Bonferroni multiple comparison method from analysis of variance methods was considered as the statistical method for measuring the design repeatedly. The difference was considered to be statistically significant if P<0.05 and extremely significant in statistics if P<0.01.

3. Results and discussions

3.1. Analysis of general materials

Stature, weight, systolic pressure, diastolic pressure and average training year of athletes are displayed in Table 1. Before the test, natural conditions of subjects were collected to make the following comparison between some variables more comparable.

Table 1. Basic physical information of subjects

	Gender	Stature (cm)	Weight (kg)	Systolic pressure (mmHg)	Diastolic pressure (mmHg)	Heart rate (times/min)	Average training year
Control group	Male	168.35±5.53	76.78±14.46	118.24±7.54	74.76±5.76	60.53±7.71	6.3
	Female	164.63±2.43	62.23±11.66	107.18±3.35	71.50±2.68	66.42±11.20	6.5
Experimental group	Male	169.53±5.96	71.92±12.01	117.23±10.83	74.75±5.76	60.64±7.72	6.7
	Female	163.42±2.39	58.73±6.75	112.01±8.05	72.64±6.95	72.49±10.62	6.6
High-dose group	Male	169.43±5.85	67.34±12.03	117.28±11.34	71.26±4.96	63.85±11.73	6.7
	Female	162.65±2.53	59.46±9.86	107.50±6.82	70.53±2.54	78.01±6.72	6.9

3.2. Test results of antioxidant indexes in serum

Before the experiment, antioxidant indexes measured in this experiment, such as catalase (CAT), superoxide dismutase (SOD), methane dicarboxylic aldehyde (MDA), carbonyl and Total antioxidant capacity (T-AOC) received mean T test. Results showed that CAT, SOD, MDA and T-AOC had no difference in gender, while carbonyl had, and the content of carbonyl in male athletes was significantly higher than in female athletes (P<0.05) (Table 2). Only when plasma concentration achieved an effective level as drug entered into blood,

could drug take effect by acting on the target point. Test results of male and female players were set as follows because plasma concentration was affected by gender.

Table 2. Difference of antioxidant indexes in gender before test (mean ± SD)

	Females	Males
SOD (U/ml)	52.815±6.382	52.934±4.418
CAT (U/ml)	2.621±0.265	2.564±0.217
MDA (nmol/ml)	4.985±0.62	4.971±0.812
T-AOC (U/ml)	16.654±1.852	16.925±1.031
Carbonyl (mg/ml)	0.349±0.067*	0.428±0.104

Note: *: $P<0.05$ while comparing female athletes with male athletes, suggesting a statistical significance.

3.2.1. Test results of CAT content

(1) Dose-response relationship.

The content of CAT in all athletes after taking compound E Jiao Jiang increased to some extent in the same test cycle, but had no statistical significant difference. Details are shown in Figure1.

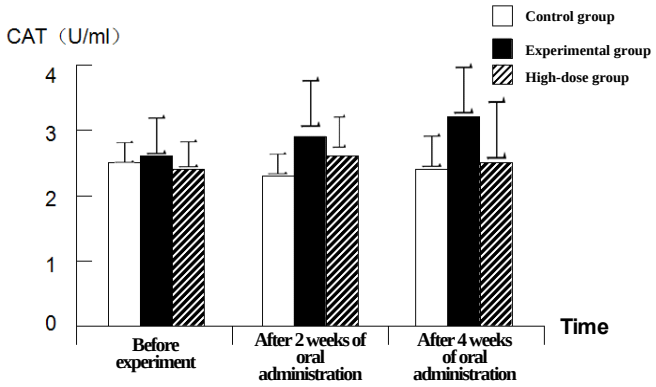

Figure 1. CAT content in all athletes

After male athletes took compound E Jiao Jiang, the pairwise comparison between control group, experimental group and high-dose group in the same test cycle was not statistically significant Figure 2).

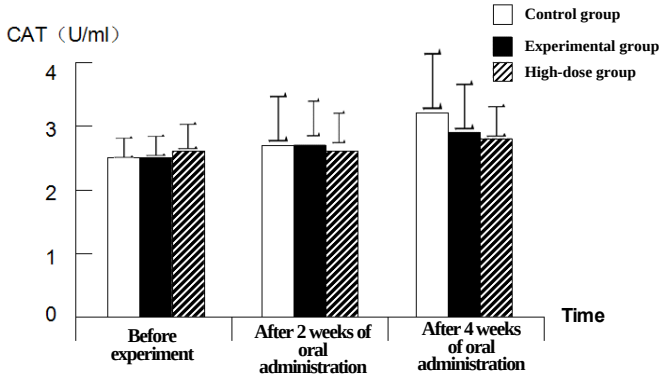

Figure 2. CAT content in male athletes

Comparing female subjects in experimental group with control group after 2 weeks of oral administration, CAT content increased, and the difference had a statistical significance (P<0.05), as shown in Figure 3.

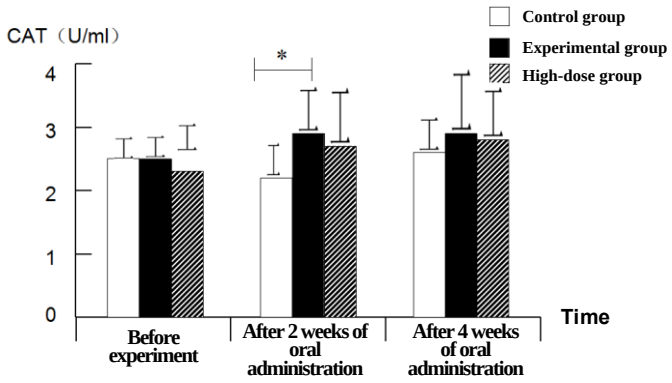

Figure 3. CAT content in female athletes

(2)Time-response relationship

After all athlete subjects took compound E Jiao Jiang, the content of CAT in experimental group after 2 and 4 weeks of oral administration increased significantly as time went on in comparison with before experiment (P<0.01). However, the content in high-dose group was in an increasing trend, without statistical significance. CAT content of male subjects in high-dose group rose gradually with time, and increased after 4 weeks of oral administration, the difference was statistically significant (P<0.05); while the content in control group and experimental group had a tendency to increase slightly, but without statistical significance. Compared with before experiment, CAT content of female subjects in experimental group increased notably after 2 weeks of administration with time, and there was a statistical significance in the differences (P<0.05), and it increased more markedly after 4 weeks of administration (P<0.05). However, as time went on, the content only showed a certain rising trend in high-dose group, and without statistical significance.

(3)Influence of gender on CAT content

Data indicated that the content of CAT in male and female players taking the same dosage in the same time had no difference (P>0.05) (Table 3).

Table 3. Comparison of CAT content in male and female (mean ± SD)

	Before experiment		**After 2 weeks of oral administration**		**After 4 weeks of oral administration**	
	Male	Female	Male	Female	Male	Female
Control group	2.557±0.125	2.625±0.244	2.674±0.605	2.451±0.301	2.958±0.513	2.497±0.365
Experimental group	2.553±0.215	2.670±0.185	2.756±0.419	3.272±0.574	3.065±0.474	3.274±0.585
High-dose group	2.584±0.302	2.546±0.360	2.678±0.365	2.933±0.664	2.945±0.505	3.012±0.831

3.2.2. Test results of SOD content

Compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, all subjects in

experimental group and high-dose group were observed with very significantly increased SOD content after 4 weeks of oral administration, and there was a very significant difference (P<0.01). Comparing male in experimental group with control group after 2 weeks of oral administration, SOD content increased obviously, with a significant difference (P<0.05); and the content in male in experimental group and high-dose group after 4 weeks of oral administration increased very notably, with a statistical significance (P<0.01). In comparison with subjects of the same sex taking compound E Jiao Jiang at the same dose before experiment, SOD content in all subjects in high-dose group increased very markedly after 4 weeks of oral administration (P<0.01), the content in male subjects in experimental group increased significantly after 4 weeks of oral administration (P<0.01), and the content in female subjects in experimental group increased very significantly after 2 and 4 weeks of oral administration (P<0.01). After female subjects in high-dose group took compound E Jiao Jiang for 4 weeks, SOD content increased obviously (P<0.05). Details are displayed in Table 4.

Table 4. Test results of SOD content (mean ± SD)

		Before experiment	After 2 weeks of oral administration	After 4 weeks of oral administration
Control group	Male (n=8)	50.225±3.554	45.812±5.714	44.664±4.253
	Female (n=8)	53.688±4.611	56.295±7.564	54.172±5.418
	All of them (n=16)	51.954±4.362	51.042±8.447	49.415±6.801
Experimental group	Male (n=8)	54.514±2.625	62.131±9.248*	63.994±5.427** ##
	Female (n=8)	53.861±4.902	56.815±5.818##	56.331±6.048##
	All of them (n=16)	54.184±3.828	59.454±7.979	60.162±6.826**
High-dose group	Male (n=8)	53.835±5.826	54.445±6.852	57.904±7.265**
	Female (n=8)	50.775±6.612	54.002±3.486	57.813±4.898#
	All of them (n=16)	52.301±6.225	54.204±5.245	57.854±5.987** ## ○○

Note: compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, *: P<0.05, suggesting a significant difference; **: P<0.01, suggesting a very significant difference; compared with subjects of the same sex taking compound E Jiao Jiang at the same dose before experiment, #: P<0.05, suggesting a significant difference; ##: P<0.01, suggesting a very significant difference; compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same dose for 2 weeks, ○: P<0.05, suggesting a significant difference; ○○: P<0.01, suggesting a very significant difference.

3.3. Free radical injury markers

3.3.1. MDA test results

Compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, all subjects in experimental group and high-dose group were observed with very significantly decreased MDA content after 2 and 4 weeks of oral administration (P<0.01). Comparing male in high-dose group with control group after 2 weeks of oral administration, we found MDA content was reduced obviously (P<0.05); and the content after 4 weeks of oral administration dropped very notably (P<0.01). Female subjects in high-dose group after 4 weeks of oral administration were found with obviously decreased MDA content (P<0.05). In comparison with subjects of the same sex taking compound E Jiao Jiang at the same dose before experiment, MDA content in all subjects declined after 2 and 4 weeks of oral administration, and the difference was very statistically significant (P<0.01), the content in male subjects in experimental group decreased very significantly after 4 weeks of oral administration (P<0.01); the content

decreased significantly after 2 and 4 weeks of oral administration at high dose, and the difference had a very statistically significance (P<0.01); female subjects in experimental group and high-dose group were observed with a very notably declined MDA content after 2 and 4 weeks of oral administration (P<0.01). The MDA content in high-dose group decreased very significantly after subjects took compound E Jiao Jiang for 4 weeks compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same dose for 2 weeks (P<0.01), and female subjects in high-dose group had a markedly dropped MDA content after 4 weeks of oral administration (P<0.05).

3.3.2. Carbonyl test results

Compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, all subjects in experimental group and high-dose group were observed with decreased carbonyl content after 4 weeks of oral administration, and the difference was very statistically significant (P<0.01). Female subjects in experimental group had a markedly dropped carbonyl content after 2 weeks of oral administration, and the difference was significant (P<0.05). In comparison with subjects of the same sex taking compound E Jiao Jiang at the same dose before experiment, carbonyl content in experimental group and high-dose group declined significantly after 4 weeks of oral administration (P<0.01); female subjects in high-dose group were observed with a notably dropped carbonyl content after 4 weeks of oral administration, with a statistical significance (P<0.05). The carbonyl content in female subjects in experimental group decreased markedly after 4 weeks of oral administration compared with subjects sharing the same gender who took compound E Jiao Jiang at the same dose for 2 weeks (P<0.05). No statistical significance was found in the remaining pairwise comparison.

3.4. T-AOC

Table 5 shows the T-AOC results detected three times from subjects sharing different genders and taking compound E Jiao Jiang at various doses. Compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, all athletes' T-AOC in experimental group were observed to be significantly increased after taking compound E Jiao Jiang for 4 weeks, and the difference was statistically significant (P<0.05). After taking compound E Jiao Jiang for 2 weeks, females subjects in experimental group had notably increased T-AOC in comparison with control group (P<0.05). The rest of pairwise comparison had no statistical significance.

Table 5. Test results of T-AOC content (mean ± SD)

		Before experiment	After 2 weeks of oral administration	After 4 weeks of oral administration
Control group	Male (n=8)	17.221±0.574	17.905±0.784	17.277±1.612
	Female (n=8)	15.795±1.818	14.752±2.785	14.032±1.922
	All of them (n=16)	16.504±1.522	16.324±2.566	15.644±2.402
Experimental group	Male (n=8)	16.685±1.552	17.424±1.435	18.085±1.398
	Female (n=8)	17.671±1.533	16.722±1.948*	17.642±1.616
	All of them (n=16)	17.174±1.569	17.063±1.695	17.861±1.485*
High-dose group	Male (n=8)	16.867±0.663	17.127±1.042	17.172±1.185
	Female (n=8)	16.392±1.983	15.681±2.093	16.345±1.872
	All of them (n=16)	16.631±1.428	16.415±1.762	16.761±1.574

Note: compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, *: P<0.05 suggesting a significant difference; **: P<0.01, suggesting a very significant difference.

3.5. Indexes of oxygen carrying capacity and aerobic endurance

Physical signs measured in this study included Hb and maximum oxygen uptake, both of which were influenced by gender differences. Compared with subjects sharing the same gender and taking compound E Jiao Jiang at the same time in control group, male athletes in experimental group had obviously increased Hb content after taking compound E Jiao Jiang for 2 weeks ($P<0.05$), and female athletes in experimental group had very obviously increased Hb content after taking compound E Jiao Jiang for 4 weeks ($P<0.01$). In comparison with subjects sharing the same gender and taking compound E Jiao Jiang at the same dose before experiment, female athletes in high-dose group were observed with significantly increased Hb content after 2 weeks of oral administration ($P<0.05$) and very markedly increased Hb content after 4 weeks of oral administration ($P<0.01$). Comparing female athletes in high-dose group taking compound E Jiao Jiang for 4 weeks with 2 weeks, Hb content increased notably ($P<0.05$). No statistical significance was found in the rest of pairwise comparison.

4. Conclusions

4.1. Influence of compound E Jiao Jiang on antioxidant enzyme

CAT, a marker enzyme of peroxysome (Seyhan et al., 2013), existing in the peroxide body of red blood cell and other tissues, mainly helps H2O2 decomposed into oxygen and water, eliminates H2O2 inside the body, avoids the damage of H2O2 to cells and restricts the generation of hydroxyl radicals resulted from oxygen and hydrogen peroxide under the action of iron chelates. Hydroxyl radical is a kind of lively active oxygen, which has strong destructive effect as it is capable of reacting with most of organic materials in the cell rapidly (Liang and Ramesh, 2010; László et al., 2014). It could be seen by analyzing results that the CAT content in athletes of different genders increased to some extent after taking compound E Jiao Jiang, and the increasing degree showed no gender differences. A study (Yee et al., 2014) proves that rehmannia glutinosa polysaccharide is able to increase the content of CAT and compound E Jiao Jiang can enhance the quantity of red blood cell as well as hematokrit, which directly increase the carrier of CAT. This is in accordance with the test results acquired in this study. SOD as an important antioxidant enzyme inside the biological body plays a crucial role in balancing oxidation and antioxidant of the body. It is effective in eliminating superoxide anion free radicals and protecting cells from damage (Alcely et al., 2003; Daizoh et al., 2001). By observing dose-response relationship of compound E Jiao Jiang, results indicated that SOD content in experimental group was significantly higher than in high-dose group after taking compound E Jiao Jiang. Thus, it could be seen that compound E Jiao Jiang had no dose-response relationship with SOD content. Through observing time-response relationship of compound E Jiao Jiang, SOD content was always in a rising trend as time went on from an overall perspective. Therefore, there was a time-response relationship between compound E Jiao Jiang and SOD content.

4.2. Influence of compound E Jiao Jiang on maximum oxygen uptake

Maximum oxygen uptake refers to body's oxygen intake per unit time when cardiopulmonary function and muscle using oxygen reach a limitation after human body does long-time strenuous exercise involving a lot of muscles. It reflecting the ability of body to inhale, transport and use oxygen is

one of the important indexes evaluating the aerobic working capacity of human body (Shephard, 2008). A variety of factors affect maximum oxygen uptake, including Hb content in the red blood cells, the ability to carry and transport oxygen; heart pump function, that is to say, the influence of cardiac output; the influence of pulmonary ventilation and gas exchange function, as well as the ability of muscle to use oxygen; genetic factors; age and gender; training level, etc (Shephard, 2008). Study results obtained by observing the time-response relationship between compound E Jiao Jiang and maximum oxygen uptake demonstrated that compound E Jiao Jiang could enhance maximum oxygen uptake. Besides, female athletes had obviously higher maximum oxygen uptake than male athletes as maximum oxygen uptake was affected by gender which was the key factor impacting drug sensitivity. However, no dose-response relationship was found between compound E Jiao Jiang and maximum oxygen uptake.

All in all, the study results indicate that compound E Jiao Jiang is able to improve the antioxidant ability of athletes, inhibit the production of free radicals and enhance the ability of body carrying and transporting oxygen, thereby improving the aerobic working ability as well as aerobic endurance. Eventually, it takes effect in resisting fatigue.

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Effects of *Lactobacillus salivarius* on oral cancer cell proliferation and apoptosis in vitro

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ABSTRACT
Oral cancer is a serious and growing problem in many parts of the world. Probiotic was suggested as a prophylactic measure in many cancers including oral cancer. The aim of this study was to investigate the possible inhibitory mechanism of *Lactobacillus Salivarius Ren* on oral cancer cells (TCA-8113). The effect of *L. Salivarius* Ren on cell proliferation, apoptosis, Expression of COX-2 were also assessed. The results showed that L. Salivarius Ren suppressed cellular proliferation accompanied by enhanced apoptosis, down regulated the COX-2 mRNA levels and protein expression significantly. The results from this study suggest that L. Salivarius Ren exhibits a marked antitumor effect. One of the antitumor mechanisms of *L. Salivarius* Ren may be that its inhibition of COX-2 led to reduced proliferation and induction of apoptosis.

1. Introduction

Oral cancer is a serious and growing problem in many parts of the world. It is estimated that each year there are over 484,000 people diagnosed with oral cancer in the world and approximately 261,000 people die of this disease (Jemal et al., 2011). In China, over 11,900 cases of oral cancers are diagnosed each year and approximately 5,000 patients die of the disease (Han et al., 2010). It is generally believed that oral mucosal carcinomas are predominantly caused by chemical carcinogens such as tobacco, excess consumption of alcohol and betel quid usage (Negri et al., 1993). Despite recent advances in surgery, chemotherapy and radiotherapy, the survival of patients with oral carcinoma remains poor. Furthermore, second primary oral tumors occur rather frequently, which cannot be predicted reliably in the individual patent (Zini et al., 2010). Therefore, the promising approach to reduce the occurrence and development of this malignancy is prevention. Dietary factors play an important role in human health and in the development of certain chronic diseases including cancer.

The suppression of proliferation and inhibition the over-expression of Cyclooxygenase-2 (COX-2) (Harris et al., 2007; Fong et al., 2008) are two most important mechanisms involved in the prevention. In addition, selective COX-2 inhibitors have been reported to suppress COX-2 activity, proliferation activity, and PGE2 production in cancer cell lines (Pandey et al., 2008). However, some of selective COX-2 inhibitors

were reported to be associated with a significant increase in the risk of myocardial infarction and with an increase in the risk of death from cardiovascular causes (Sporn et al., 2005). Therefore, a new strategy of prevention may be essential in the future.

The term probiotic refers to live microorganisms that survive passage through the gastrointestinal tract and have beneficial effects on the host (Parvez et al., 2006). The list of healthful effects attributed to probiotic bacteria is extensive. Epidemiologic and experimental studies suggest that the consumption of the probiotics or fermented milk products could decrease the incidence of certain types of cancer. The vast majority of studies on the anticancer effects deal with colorectal cancer (CRC) (Yamazaki et al., 2000; Caderni et al., 2003), although there are some studies on bladder and oral cancer (Biffi et al., 1997; Zhang et al., 2013). The precise mechanisms by which probiotics exert their anti-CRC are uncertain but might involve preventing the DNA damage induced by carcinogens, modulating the key biomarkers which can inhibit or promote the development of tumor, inhibiting the proliferation or inducing the apootosis of carcinoma cells via its metabolites (Lan et al., 2008; Goldin et al., 1984). However, it has not any reports about inhibition of oral cancer cells of the probiotics.

Lactobacillus Salivarius Ren (*L. Salivarius* Ren) was isolated from fecal samples from healthy centenarians living in villages located in Bama in the Guangxi Zhuang Autonomous Region in China, where has the world's highest longevity ratio. In the previous study, we reported that the *L. Salivarius* Ren could act as potential agents for oral cancer prevention. This is the first report demonstrating the inhibitory effect of the probiotics on oral carcinogenesis (Zhang et al., 2013). In current study, the possible inhibitory mechanism of *L. Salivarius* Ren on oral cancer cell was investigated in TCA-8113 cell line. The effect of *L. Salivarius* Ren on cell proliferation, apoptosis, Expression of COX-2 was also assessed.

2. Materials and methods

2.1. Cell culture

TCA-8113 cell line (derived from human tongue squamous carcinoma) was grown in Dulbecco's Modification of Eagle's Medium (Gibco Co., USA) supplemented with 10% fetal calf serum. The cells were cultured at 37℃ in a humidified atmosphere of 5% CO_2.

Preparation of *L. salivarius* REN cells and its metabolites. After 12h cultivation in Man-Rogosa-Sharpe (MRS) liquid medium (Oxoid), *L. salivarius REN* was harvested by centrifugation (4000g, 10min), washed twice and adjusted to appropriate concentrations in sterile saline (0.9% w/v) for oral administration. The metabolites were collected from the resuspending solution of the strain. This solution was incubated for 1h at room temperature and the water soluble secretion was harvested by centrifugation (4000 g, 10 min) and filtered through a 0.22 μm sterile filter. The concentration of the metabolites was defined as the concentration of the strain in sterile saline.

2.2. TCA-8113 Cell-based assays in general

In cell culture-based assays, TCA-8113 cells were exposed to three different dosages of *L. salivarius* REN metabolites (1, 2, 3×10^9 CFU/ml), *L.casei* metabolites (2×10^9 CFU/ml, negative control) and NS-398 (10mM, Sigma-Aldrich, positive control) for different time, after which COX-2 mRNA expression, protein expression and apoptosis cells were measured.

2.3. Cell Proliferation Assay

Cell proliferation was measured by the (4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay. Cells seeded on 24-well microplates at 5×10^4cells/well were treated for 24 h and exposed to 10 μL of MTT solution (5 mg/mL MTT in phosphate-buffered saline (PBS)) for 3 h. Live cells appeared purple in color in response to MTT. After discarding the medium, cells were dissolved in dimethyl sulfoxide. One hundred microliters of cell suspension was transferred to a 96-well plate and cell viability was determined using

VersaMax™ microplate ELISA reader (Molecular Device Co., Sunnyvale, CA, USA).

2.4. Flow cytometric detection of apoptotic cells

TCA-8113 cells were cultured in 6-well cluster plates, and stimulated with different treatment as described above for 24h. Subsequently, the assay was performed with the commercially Annexin V FITC/PI Apoptosis Detection kit (Roche Diagnostics GmbH) according to the manufacturer's instructions. Finally, the mixtures were analyzed with a FACSCalibur flow cytometer (BecktonDickson).

2.5. Quantitative real-time PCR

TCA-8113 cells were grown in monolayer and stimulated for 12h with different treatments as described above. After harvest, total RNA was extracted from cells with NucleoSpin RNA II kit (QIAGEN). RT-PCR was done as described24 using COX-2 and β-actin primers obtained from Invitrogen (Beijing). The sequences of the β-actin and COX-2 primers were as follows: β-actin, forward 5'-GGATCCGACTT CGAGCAAGAGATGGCCAC -3' and reverse 5'- CAATGCCAGGGTACATGGTGGTG -3'; COX-2, forward 5'-GCGAGGGCCAGCTTTCACCA -3' and reverse 5'- TTCCCTCAGCCAGATTGTGG CA -3'. Up regulation and down regulation of β-actin and COX-2 were determined by the $2^{-\Delta\Delta Ct}$ method (Pfaffl et al., 2002).

2.6. Immunohistochemistry

TCA-8113 cells were grown in coverslips and stimulated for 12h with different treatments as described above. The coverslips were rehydrated and subsequently incubated with a polyclonal primary antibody (COX-2, 1:50 solution, Cell Signaling Technology, Inc) at room temperature for 1h. The secondary antibody (anti-rabbit-mouse-goat-antibody) was incubated for 15 min at room temperature, followed by incubation with strepavidin-POD (Dako) for 15 min. Antibody binding was visualized using AEC-solution (Dako). The samples were then counterstained by haemalaun solution (Dako). Slides were subsequently reviewed in a blinded fashion. The immunoreactivity of the samples was graded on the basis of the number of positively stained cells. COX-2 positive cells were counted in eight randomly selected fields at a magnification of 400. The results of the cell counts were given as means of percentages of positive cells of all the cells counted in a defined field.

2.7. Western Blotting

TCA-8113 cells were grown in monolayer, and stimulate for 18h with different treatment as described above. The cells were harvested, washed with cold PBS, and lysed with ice-cold lysis buffer supplemented with protease inhibitors. Cell lysates were analyzed for Western blot analysis using COX-2 antibody (Cell Signaling). Blots were reprobed with actin to compare protein load in each lane.

2.8. Statistical analysis. Statistical analysis on the incidence of lesions and immunoreactivity COX-2 were performed using Fisher's exact probability test. The data of positive cells ratio in immunohistochemistry was analyzed using the Student's t test. The data of COX-2 mRNA levels and flow cytometry analysis were analyzed using the Duncan test. The results were considered statistically significant if the P value was less than 0.05.

3. Results and discussions

3.1. Anti-proliferative effect of *L. Salivarius* Ren on TCA-8113 cells

Cell proliferation is suggested to play an important role in multistage carcinogenesis, including oral tumorigenesis. Many of possible cancer preventive agents could suppress cell proliferation activity (Versalovic et al., 2008). In the present study, TCA-8113 cell line was treated with three doses of metabolites for 24 hours (Figure 1). After treatment, the proliferation of the cell lines was significantly

inhibited, especially in high dose (3×10^9 cfu/ml). Furthermore, the growth rate of cell lines was greatly decreased by incubation with NS-398 ($P<0.05$), and the viability of TCA-8113 cell lines treated with artesunate decreased in a dose-dependent manner, whereas the metabolites of *L. casei* had no significant effect on cell proliferation activity ($P>0.05$).

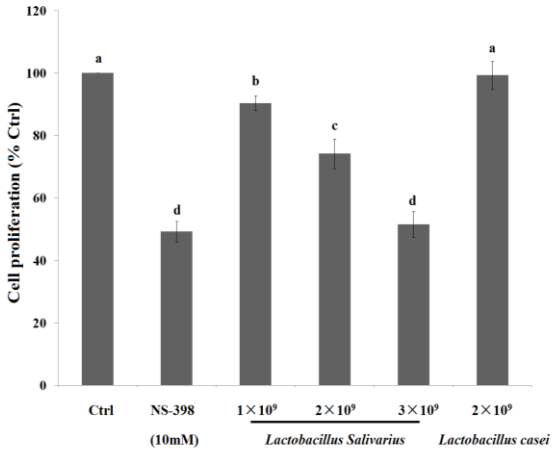

Figure 1. Anti-proliferative effect of *L. Salivarius* Ren on TCA-8113 cells

3.2. *L. Salivarius* Ren induced apoptosis in TCA-8113 cells

The neoplasia inhibition effect of anti-cancer agents is always associated with their ability in inducing apoptosis (Granado-Serrano et al., 2006). To determine whether the inhibitory effects of *L. Salivarius* Ren on TCA-8113 cellular proliferation are accompanied by enhanced apoptosis, we utilized Annexin V FITC/PI Apoptosis Detection kit to detect the different stage apoptosis. The results of flow cytometry analysis of apoptosis were shown in Fig 2. After 24h exposure, the metabolites of *L. Salivarius* Ren could dose-dependently increase the early and late apoptosis cells ratio. Under the treatment of high dose metabolites (3×10^9cfu/ml), 10.32% of treated cells entered the early apoptosis, and 28.15% entered the late apoptosis. In addition, under the treatment of NS-398, 7.44% of treated cells entered the early apoptosis, and 32.36% entered the late apoptosis, whereas the metabolites of *L. casei* had no significant effect on apoptosis cells ratio ($P>0.05$).

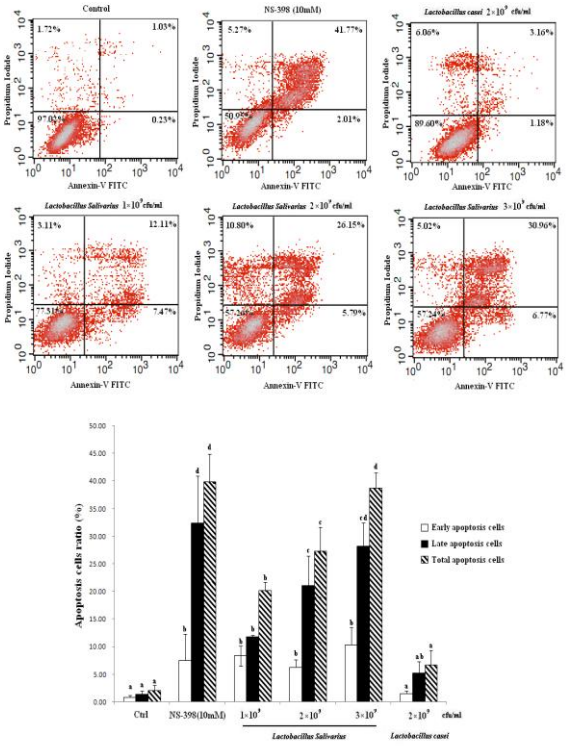

Figure 2. Flow cytometry analysis of apoptosis induced by the metabolites of *L. Salivarius* Ren

3.3 Effect of the metabolites of *L. Salivarius* Ren on COX-2 mRNA levels and protein expression

COX-2 is constitutively overexpressed in a variety of malignancies, including gastric cancer, breast cancer, bladder cancer, non-small-cell lung cancer, and colorectal cancer (Harris et al., 2007; Fong et al., 2008), and COX-2 over-expression is associated with carcinogenesis, progression, invasion, metastasis, and a poor prognosis (Hanahan & Weinberg, 2000; Greenhough, 2000). Therefore, inhibition of COX-2 expression may prevent or reverse gastric carcinogenesis. There is increasing evidence demonstrating that inhibition of expression of COX-2 has antitumor activity against gastrointestinal carcinoma. Given the apoptotic response displayed following *L. Salivarius* Ren treatment, we investigated whether L.

Salivarius Ren treatment reduces the overexpression of COX-2 of TCA-8113 cells in mRNA and protein levels.

The results of RT-PCR (Figure 3) revealed that the metabolites of *L. Salivarius* Ren could down regulate the COX-2 mRNA levels of TCA-8113 cell line significantly ($P<0.05$). After a 12h exposure, an approximately 9-fold decrease in COX-2 transcription was recorded under the treatment of high dose metabolites (3×10^9cfu/ml). NS-398, a selective COX-2 inhibitor, caused an approximately 10-fold decrease, whereas the metabolites of *L. casei* had no significant effect on COX-2 transcription ($P > 0.05$). These results suggested that the modulation of COX-2 by *L. Salivarius* Ren is positively correlated with its mRNA expression, which indicated the regulation of COX-2 by *L. Salivarius* Ren could be at the transcription level.

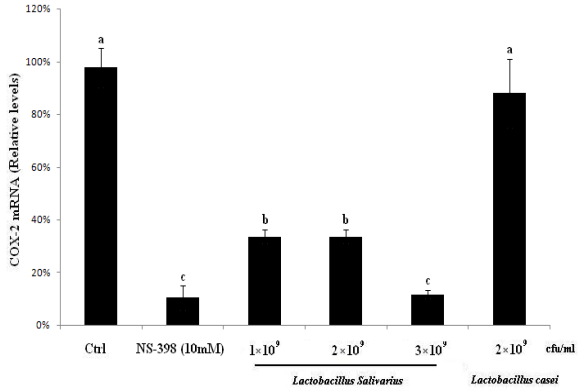

Figure 3. Effect of the metabolites of *L. Salivarius* Ren on COX-2 mRNA levels

The result of immunocytochemistry (Figure 4A, Table 1) revealed that metabolites of L. Salivarius Ren could downregulate the COX-2 protein expression significantly ($P<0.05$).The TCA-8113 cell lines were strong positive immunoreactivity of COX-2. In addition, NS-398 could decrease the immunoreactivity of COX-2, whereas the metabolites of *L. casei* had no significant effect on COX-2 protein expression ($P > 0.05$). We also used the Western Blotting to reconfirm the observation in immunocytochemistry assays. As shown in Figure 4B, the secretion of *L. Salivarius* Ren down regulated the COX-2 protein expression significantly in a dose dependant manner, whereas those of *L. casei* (positive control) had no significant effect.

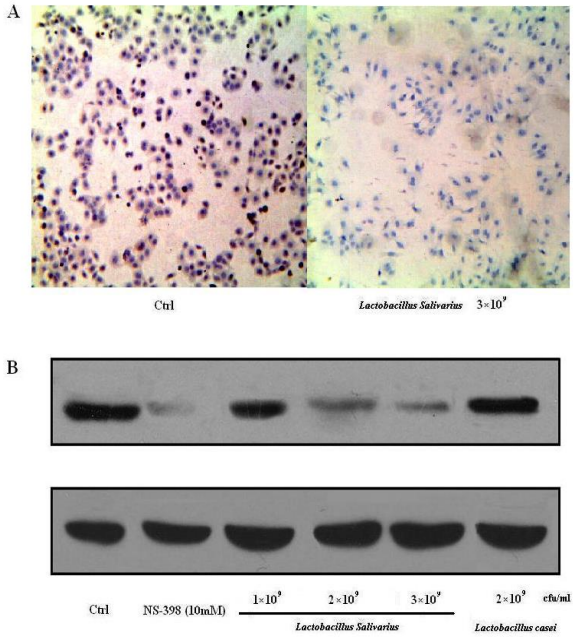

Figure 4. Effect of the metabolites of *L. Salivarius* Ren on COX-2 protein expression

Table 1. COX-2 immunocytochemistry staining

Treatment	COX-2 stainning		
	-	+	+ +
No treatment	0(0%)	1(11%)	8(89%)
NS-398(10mM)	7(78%)[a]	2(22%)	0(0%)[a]
1×10^9cfu/ml *L. Salivarius* Ren	1(11%)	4(44%)	4(44%)
2×10^9cfu/ml *L. Salivarius* Ren	3(33%)	3(33%)	3(33%)[b]
3×10^9cfu/ml *L. Salivarius* Ren	5(56%)[b]	3(33%)	1(11%)[a]
2×10^9cfu/ml *L. casei*	0(0%)	3(33%)	6(67)

4. Conclusions

In summary, the results from this study suggest that *L. Salivarius* Ren exhibits a marked antitumor effect. One of the antitumor mechanisms of *L. Salivarius* Ren may be that its inhibition of COX-2 led to reduced proliferation and induction of apoptosis. This study suggests the possible effectiveness of a

novel preventive approach for oral malignancy by using the probiotics, although further studies will be necessary.

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ANALYSIS ON EXTRACTION AND PURIFICATION OF CORN BRAN AND THE EFFECT OF ITS OXIDATION RESISTANCE ON INDUSTRIAL VALUE CREATION FROM AN INDUSTRIAL CHAIN PERSPECTIVE

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ABSTRACT

This paper studied the extraction and purification of corn bran as well as its oxidation resistance from an industrial chain perspective in order to improve the utility value of corn and increase the economic benefits of corn processing enterprises, which had great and far-reaching significance for the value creation of the corn industry. The microwave-assisted water-extraction and alcohol-precipitation method was applied for extraction of polysaccharide from corn bran. Then, the extracted polysaccharide was processed with Sevag method for deproteinization, dialysis method for removal of small molecular impurities and finally Sephadex G-75 chromatography for separation and purification. Thus, the oxidation resistance of the purified polysaccharide extracted from corn bran was studied. The results showed that corn bran polysaccharide CSP Ⅰ contained galactose, glucose, arabinose, xylose, wherein the content of glucose was the highest. Besides, no nucleic acid and small amount of protein was found in corn bran polysaccharide CSP Ⅰ. Corn bran polysaccharide CSP Ⅰ had varying degrees of scavenging effect on hydroxyl radical, DPPH • radical and superoxide anion free radical, of which the scavenging effect on hydroxyl radical was not obvious. If results of this study can be applied to the actual corn industrial chain, not only can the corn bran be turned into useful resource but also the industrial value creation can be improved through the development of high value-added products, which can bring a very broad market prospect and remarkable economic benefit.

1. Introduction

As one of the major crops in China, the production of corn reached 211 million tons in 2013. However, corn is not appropriate to be taken as staple food and about 90% of the domestic corn is used for industrial processing and consumed as animal feedstuff (Cappelli et al., 2015; England and Möller, 2015). Corn bran, containing hemicellulose, cellulose, etc., is a by-product of corn starch production process. Yet it is mainly used as feedstuff, with low economic value. With the increase of output quantity of corn non-starch components year by year, studies on the utility value of various components of corn have drawn much attention of the corn processing enterprises, which can lead the development of corn economy by extension of

corn processing chain (Weiss et al., 2010; Li et al., 2016). The corn bran contains corn bran polysaccharide, whose water-soluble and processing performance is better compared with corn bran fiber. Studies on the extraction of polysaccharide from corn bran can not only improve the value of corn, bring economic benefits to processing enterprises, but also is of great significance to the development of functional medicine and food (Nordberg, 2011; Panjkovich and Daura, 2010).

Polysaccharide, also known as N-glycans, is a kind of macromolecule substance synthesized by a great number of monosaccharide residues. Polysaccharide is the energy substance of living organisms as well as the indispensable structural material for metabolism which participates in various life activities of cells (Urai et al., 2015). In recent years, there are many experts and scholars in China and abroad who have studied the extraction and purification of corn bran and its oxidation resistance. Sadeghi et al. (2015) found in 2015 that polysaccharide was a biologically active substance which had obvious anti-tumor and anti-virus effect, without toxic and side effects. Guo et al. (2015) studied in 2015 the extraction of cottonseed meal polysaccharide by acid pickling method and obtained the optimal extraction process condition. Safa et al. (2014) in 2014 found that corn bran had lipid-lowering effect, laxative effect and anti-obesity effect, etc. Therefore, studying the extraction and purification as well as the oxidation resistance of corn bran has an important significance for industrial value creation.

2. Materials and methods

2.1. Extraction of corn bran polysaccharide

2.1.1. Materials and equipment

Materials used in this study are as follows: corn bran, phenol, concentrated sulphuric acid, ethanol, petroleum ether, glucose, thermostable α amylase.

Equipment applied in this study include: electro- thermostatic blast oven, centrifuge, rotatory evaporator, LAB-DANCER, ultraviolet and visible spectrophotometer, electric-heated thermostatic water bath, precise timing electric mixer, etc.

2.1.2. Extraction process of corn bran polysaccharide

An amount of 10 g of corn bran was weighed after it was cleaned and purified, crushed and sieved, with degreasing. Under certain microwave time, microwave power, material liquid ratio, extraction temperature and extraction time, the first extraction was carried out through centrifugation of the corn bran for 10 min with thermostable α amylase. Then, the filter liquor was collected and subsided for second extraction (Calín-Sánchez et al., 2011). Afterwards, the combined filtrate was concentrated under reduced pressure using a rotary evaporator to 10% of the original volume. Next, the concentrate was added with 3 volumes of 95% ethanol and placed for one day. Then, it was centrifuged for 10 min at 4000r/min and sediment was collected. Finally, the sediment was washed with anhydrous ethanol and acetone in turn and corn bran crude polysaccharide was obtained after processing of freeze drying (Madhurambal et al., 2015).

2.1.3. Determination of polysaccharide content

The formula for the extraction efficiency of polysaccharide content is as follow:

$$\text{Extraction efficiency},\% = \frac{\text{Extractive polysaccharide content (g)}}{\text{Gross of rawmaterial (g)}} 100 \quad (1)$$

2.1.4. Orthogonal experimental design for the extraction of corn bran polysaccharide

The orthogonal experiment was carried out based on the five factors of microwave power, microwave time, solid-liquid ratio, extraction temperature and extraction time, in which microwave power and microwave time were two main factors considered. Based on the single factor experiment, four levels were taken for each factor, taking the extraction efficiency of corn bran polysaccharide solution as the indicator, as shown in Table 1.

Table 1. Factors and levels of orthogonal experiment of corn bran polysaccharide extraction

level	A	B	C	D	E
	Microwave power (W)	**Microwave time (min)**	**solid-liquid ratio**	**Extraction temperature (°C)**	**Extraction time (h)**
1	280	2	1: 10	70	2
2	460	3	1: 20	80	3
3	600	4	1: 30	90	4
4	700	5	1: 40	100	5

2.2. Separation and purification of corn bran polysaccharide

2.2.1. Materials and equipment

Materials used in this section include: corn bran crude polysaccharide solution, phenol, concentrated sulfuric acid, phosphoric acid, chloroform, ethanol, n-butanol, sephadex G50, sephadex G75, sephadex G100 and bovine serum albumin.

Equipment applied include: electric constant temperature drying oven, centrifuge, ultraviolet and visible spectrophotometer, automatic sampling instrument, dialysis tube, etc.

2.2.2. Deproteinization with Sevag method

Firstly, the Sevag reagent was added into the corn bran water-soluble polysaccharide concentrated solution and mixed sufficiently. Then, the solution was centrifuged for 10 min at 4000 r/min. After the solution presented stratification state, the liquid supernatant was obtained. The above operation was repeated for several times. Then, V sample, V n-butyl alcohol: V chloroform, deproteinization frequency and deproteinization time were taken as four factors, with three levels in each factor. The orthogonal experiment was carried out, taking protein removal rate and polysaccharide loss rate of corn bran polysaccharide solution as indicators, as shown in Table 2.

2.2.3. Dialysis

The corn bran polysaccharide solution after deproteinization was put into a dialysis bag for 3 days of dialysis with distilled water.

2.2.4. Purification of Sephadex G-75 chromatographic column

The corn bran polysaccharide was firstly prepared into 2 mg / ml solution and then separated and purified with the Sephadex G-75 chromatographic column. Afterwards, elution was carried out on the solution with deionized water at a flow velocity of 0.26 ml/min. Then, a collector was used to collect the solution at a speed of 15 min/tube. Finally, a graph was drawn, taking number of tubes as x-coordinate and absorbance value of eluent at a wavelength of 490 nm as y-coordinate. After the eluent at peak part was combined, freeze drying process was carried out, thus corn bran polysaccharide was obtained (Akamatsu et al., 2016).

Table 2. Factor level of deproteinization with Sevag method

Level	V reagent: V sample	V n-butyl alcohol: V chloroform	Deproteinization frequency	Deproteinization time
1	1:1	1:2	2	15
2	1:2	1:3	3	20
3	1:3	1:4	4	25

2.3. Oxidation resistance of corn bran polysaccharide

2.3.1. Materials and equipment

Materials used in this section include: corn bran polysaccharide CSP I, ethanol, ferrous sulfate, salicylic acid, hydrogen peroxide, pyrogallol and DPPH.

Equipment applied include: LAB-DANCER, ultraviolet and visible spectrophotometer, thermostatic water bath pot, etc.

2.3.2. Determination of hydroxyl radical-scavenging ability

Two ml of 9 mmol/L FeS04 solution, 2 ml of mixed polysaccharide solution with polysaccharide of different concentrations, 2 ml of 9 mmol/L H2O2 solution were added into a tube successively and mixed sufficiently and then placed for 10 min. Next, 2 ml of 9 mmol/L salicylic acid solution was added into the tube and mixed sufficiently and then placed for 1 hour. The absorbancy A_i of the mixed polysaccharide solution at 510 nm was measured; distilled water was used to replace salicylic acid and then the background absorbance A_j of a certain concentration of polysaccharide was measured; the absorbancy A_0 of blank control was measured with polysaccharide solution replaced by distilled water. Thus, the calculation formula of clearance rate S is as follow:

$$S = \frac{A_0 - (A_i - Aj)}{A_0} \times 100\% \quad (2)$$

2.3.3. Determination of DPPH • radical-scavenging ability

0.2 mmol/L DPPH solution was prepared by dissolving DPPH • free radical into 95% ethanol (Noipa et al., 2011). 2 ml of mixed polysaccharide solution with polysaccharide of different concentrations and 2 ml of DPPH solution were added successively into a tube and mixed sufficiently and then placed for half an hour at dark place. Then, the absorbance A_i of the mixed polysaccharide solution at 517 nm was measured. The background absorbance A_j of a certain concentration of polysaccharide was measured by 2 ml of polysaccharide solution and 2 ml of 95% ethanol and the absorbance A_0 of blank control was measured by 2 ml of distilled water and 2 ml of DPPH solution. Thus, the calculation formula of clearance rate S is as follow:

$$S = \frac{A_0 - (A_i - Aj)}{A_0} \times 100\% \quad (3)$$

3. Results and discussions

3.1. Impact of microwave power on corn bran polysaccharide extraction rate

As can be seen from Figure 1, polysaccharide extraction rate increased with the increase of microwave power. When the microwave power reached 460 w, the polysaccharide extraction rate reached its maximum.

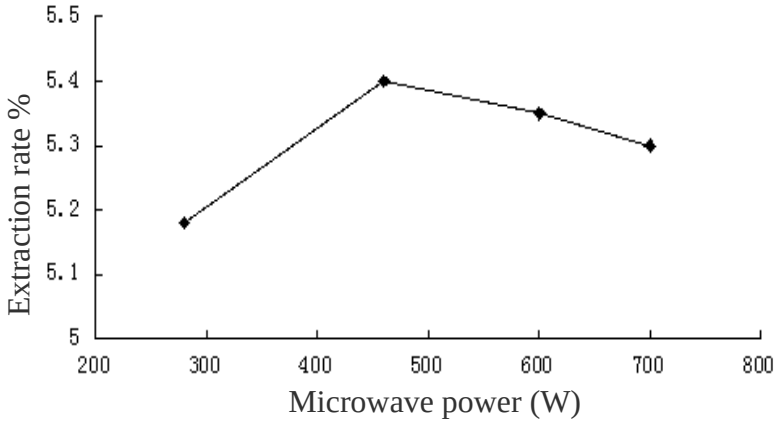

Figure 1. Impact of microwave power on corn bran polysaccharide extraction rate

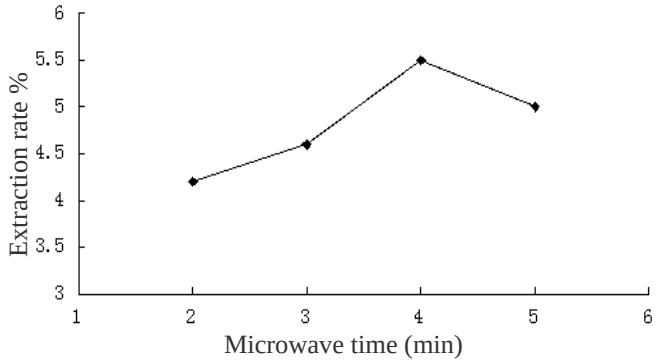

Figure 2. Impact of microwave time on corn bran polysaccharide extraction rate

However, with the continuing increase of microwave power, polysaccharide extraction rate decreased. When the microwave power was 460 w, it was in a relatively mild condition which could realize the dissolution of polysaccharide without destroying its structure. Therefore, it is considered as suitable condition when microwave power is 460 w (Cheng et al., 2015; Juanni et al., 2008).

3.2. Impact of microwave time on corn bran polysaccharide extraction rate

As can be seen from Figure 2, polysaccharide extraction rate increased with the increase of microwave time. When the microwave time reached 4 min, the polysaccharide extraction rate reached its maximum. However, with the continuing increase of microwave time, polysaccharide extraction rate decreased. When the microwave time exceeded 4 min, polysaccharide extraction rate decreased because of degradation of polysaccharide caused by saturation of solubility. Therefore, it is considered as suitable condition when microwave time is 4 min (Wlodarski et al., 2015; Bharate and Vishwakarma, 2015).

Table 3. Orthogonal experimental results of extraction of corn bran polysaccharide

Test number	Microwave power	Microwave time	liquid-to-solid ratio	Extraction temperature	Extraction time	Extraction rate %
10	3	2	4	3	1	5.1
11	3	3	1	2	4	4.7
12	3	4	2	1	3	5.2
13	4	1	4	2	3	5.4
14	4	2	3	1	4	4.9
15	4	3	2	4	1	6.0
16	4	4	1	3	2	4.5
A1	20.2	19.7	17.5	18.6	19.1	-
A2	21.6	20.2	21.1	20.1	21.3	-
A3	21.4	22.7	22.7	21.0	22.2	-
A4	20.8	21.5	22.8	24.4	21.4	-
B1	5.1	4.9	4.4	4.6	4.8	-
B2	5.4	5.0	5.3	5.0	5.3	-
B3	5.4	5.7	5.7	5.2	5.5	-
B4	5.2	5.4	5.7	6.1	5.3	-
Range (R)	0.3	0.7	1.3	1.5	0.8	-

3.3. Orthogonal experimental results of extraction of corn bran polysaccharide

As can be seen from table 3, the importance order of each factor which affects corn bran polysaccharide extraction rate is as follows: extraction temperature> liquid-to-solid ratio > extraction time > microwave time > microwave power. The best condition for corn bran polysaccharide extraction is as follows: extraction temperature: 100 °C; liquid-to-solid ratio: 1:40; extraction time: 4 hour; microwave time: 4 min; microwave power: 460w. According to the best extraction condition, corn bran polysaccharide was extracted twice and the extraction rate was 6.7% calculated by polysaccharide extraction rate formula (Müssigbrodt et al., 2015; Rahman et al., 2015).

3.4. Orthogonal experimental results of deproteinization with Sevag method

As can be seen from Table 4, the importance order of each factor which affects corn bran polysaccharide deproteinization is as follows: deproteinization frequency > V reagent: V sample > V n-butyl alcohol: V chloroform > deproteinization time. The best condition for polysaccharide deproteinization is as follows: deproteinization frequency: 4 times; V reagent: V sample: 1:2; V n-butyl alcohol: V chloroform: 1:3; deproteinization time: 25 min.

3.5. Purification results of Sephadex G-75 chromatographic column

As can be seen from figure 3, CSP Ⅰwas separated from corn bran polysaccharide through Sephadex G-75 chromatographic column. The peak pattern was close to normal distribution, suggesting its uniformity (Jie et al., 2015).

3.6. Determination of hydroxyl radical-scavenging ability

As can be seen from Figure 4, corn bran polysaccharide CSP Ⅰ had certain clearance effect on ·OH.

Table 4. Orthogonal experimental results of deproteinization with Sevag method

Test number	V reagent: V sample	V n-butyl alcohol: V chloroform	Deproteinization frequency	Deproteinization time	Protein removal rate %
1	1	1	1	1	36.4
2	1	2	2	2	41.6
3	1	3	3	3	42.7
4	2	1	2	3	44.0
5	2	2	3	1	46.7
6	2	3	1	2	38.3
7	3	1	3	2	41.5
8	3	2	1	3	35.2
9	3	3	2	1	33.9
A1	120.7	121.9	110.0	117.0	-
A2	128.9	123.5	119.5	121.5	-
A3	110.6	114.9	130.9	121.9	-
B1	40.2	40.6	36.7	39.0	-
B2	43.0	41.2	39.8	40.5	-
B3	36.9	38.3	43.6	40.6	-
Range (R)	6.1	2.9	7.0	1.6	-

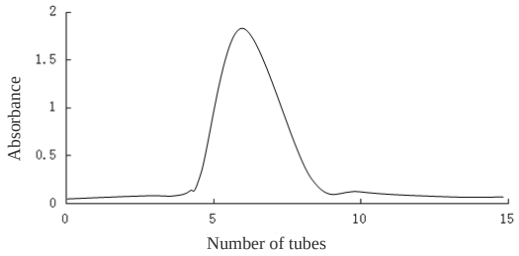

Figure 3. Sephadex G-75 elution curve of corn bran polysaccharide

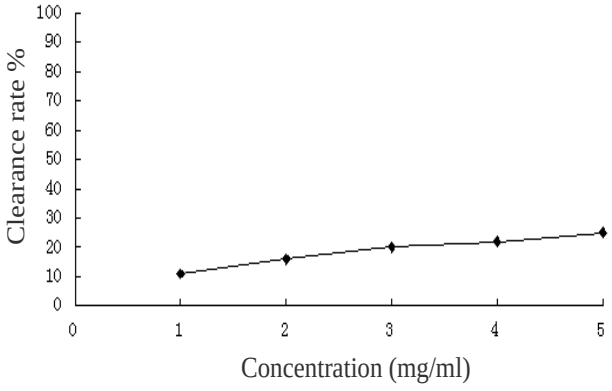

Figure 4. Hydroxyl radical-scavenging ability

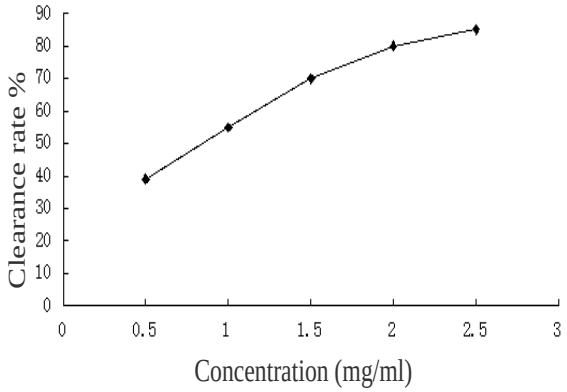

Figure 5. DPPH • free radical -scavenging ability

The clearance rate increased gradually with the increase of polysaccharide CSP concentration. However, the changes were not obvious, with the clearance rate below 50% within the range of determination (Kerry et al., 2012).

3.7. Determination of DPPH • free radical - scavenging ability

As can be seen from Figure 5, corn bran polysaccharide CSP had certain clearance effect on DPPH·. The clearance rate increased gradually with the increase of polysaccharide CSP concentration and reached 50% (Feuchtenberger et al., 2008).

4.Conclusions

Corn bran polysaccharide CSP Ⅰ had varying degrees of scavenging effect on hydroxyl radical, DPPH • radical and superoxide anion free radical, of which the scavenging effect on hydroxyl radical was not obvious. If results of this study can be applied to the actual corn industrial chain, not only can the corn bran be turned into useful resource but also the industrial value creation can be improved through the development of high value-added products, which can bring a very broad market prospect and remarkable economic benefit.

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ANTIBACTERIAL AND ANTIOXIDATIVE ACTIVITIES OF MULBERRY RED PIGMENT

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ABSTRACT
In this article, the antibacterial and antioxidative effects of mulberry red pigment were studied in order to contribute to edible and medicinal values of mulberry. Antibacterial actions of mulberry red pigment against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas stutzeri*, *Asparagillus niger* and *Saccharomyces cerevisiae* were studied by inhibitory zone with filter paper and analysis of minimum inhibitory concentration, and the antioxidant effects of mulberry red pigment were investigated by scavenging DPPH free radical and scavenging superoxide radical. As a result, antibacterial and antioxidative abilities increased gradually with increase of concentration of mulberry red pigment. The mulberry red pigment showed antibacterial effects against six bacterial stains: *E. coli* > *S. aureus* > *B. subtilis* > *P. stutzeri* > *A. niger* > *S. cerevisiae*, and had the most strongly antibacterial activity on *E. coli* with IC_{50} value of 16.27 mg/mL and the most weakly antibacterial action on *S. cerevisiae* with IC_{50} value of 195.7 mg/mL. And mulberry red pigment could scavenge DPPH free radical and superoxide radical, and EC_{50} were 3.91 g/L and 1.93 g/L, respectively, but the antioxidant properties were lower than that of Vc. In general, mulberry red pigment had antibacterial and antioxidative effects, antibacterial ability against bacteria was higher than agaist fungi, and could scavenge DPPH free radical and superoxide radical.

1. Introduction

The mulberry belongs to the *Morus* genus of the Moraceae family which is widely distributed around the world. There are 24 species in the world, and there are 15 species and 4 variations in China (Muhammad et. al., 2012). Mulberry leaves are used for sericulture. Mulberry fruit is a kind of a purple fruit, and has a long history in China as one of the traditional fruit. And the mulberry fruit has been widely used in Chinese health foods and folk medicines for several thousands years. The mulberry fruit containes rich protein, polysaccharides, flavonoids, anthocyanins, amino acids and vitamins, etc, including antimicrobial, antioxidation and antitumor properties (Muhammad et. al., 2012; Bae and Suh, 2007; Chang et al. 2007). Muhammad Arfan reported the sugar-free extracts of *Morus* nigra had much oxidation resistance though

determining ABTS and DPPH (Muhammad et. al., 2012). Mulberry red pigment contained anthocyanins and carotene as a natural pigment, including antimicrobial and antioxidation properties. Duan Honglian reported the mulberry red pigment could strongly inhibit on *E. coli*, weakly inhibit on *S. aureus* and *B. subtilis*, and had no effect on fungi and yeast (Duan et al., 2007). Lu Yinghua reported mulberry pigment had excellent natural antioxidant and free radical scavenger (Lu et al., 2007). Niu Tianyu reported that mulberry had the highest anthocyanin content and had the strongest free radical scavenging capacity (Niu et al., 2016). Some studies had been done on antimicrobial and antioxidative activities of mulberry fruits. However, few studies had been done on researching mulberry red pigment both to inhibit food microorganism and to have antioxidant activity. As a good natural pigment, mulberry red pigment can be widely used in beverages, cold drinks, baked products, chewing gum, jelly and wine, etc. Therefore, mulberry red pigment has extensive effect on the food industry. In this paper, antibacterial and antioxidant activities of mulberry red pigment were studied in order to study food preservatives effect of mulberry fruits and mulberry red pigment. In this study, the alcohol abstraction method was used to gain mulberry red pigment, and inhibitory zone with filter paper and analysis of minimum inhibitory concentration were used to investigate antimicrobial effects of mulberry red pigment against *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* which were isolated from food. Antioxidant activities were studied by scavenging superoxide anion and DPPH free radical, and compared with Vc. The objective of this study was to evaluate antibacterial and antioxidant activities of mulberry red pigment in order to promote the comprehensive utilization of mulberry.

2. Materials and methods

2.1. The Mulberry samples collection

The mulberry fruits samples were collected from Yanbian county of Panzhihua city in Sichuan province of China. And the collected samples were freezed and preserved.

2.2. The mulberry red pigment extracts

The mulberry red pigment was extracted by the alcohol extraction. Fifty gram mulberry fruits were weighed, and were broken by mechanical method and soaked in 50% ethanol for 48 hours. The samples were filtered and centrifuged, and the supernatant was mulberry red pigment.

2.3. Antimicrobial sensitivity assay

Bacillus subtilis, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were separated and purified from food. The antimicrobial effects of mulberry red pigment against *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were tested by inhibitory zone with filter paper. The diameter of inhibition zones was measured and the average was calculated. And the inhibition rate was assayed by the inhibition zone diameters (Diao and Yang, 2014).

The inhibition rate (%) = (the inhibition zone diameters - filter diameter)/ the inhibition zone diameters × 100% (1)

Toxicity regression equations and 50% inhibiting concentration(IC_{50}) were got in order to determine antibacterial property of mulberry red pigment. The minimal inhibitory concentration (MIC) was scaled by agar dilution method (Wiegand et al., 2008; Eloff, 1999; Andrews, 2001)

2.4. Antioxidant effect analysis

Scavenging DPPH free radical and scavenging superoxide radical were determined and compared with Vc (Muhammad et. al., 2012; Bae and Suh, 2007; Chang et al. 2007).

3. Results and discussions

3.1. Antibacterial assay of mulberry red pigment

3.1.1. The antibacterial action

Figure 1 showed the inhibition rate of mulberry red pigment against *B. subtilis*, *S. aureus*, *E. coli*, *P. stutzeri*, *A. niger* and *S. cerevisiae* increased with the increase of concentration of mulberry red pigment. When concentration of mulberry red pigment increased from 0.2 mg/mL to 125 mg/mL, the suppression ratio against *E. coli* was the highest, with 92.89% ± 3.88%; it was followed by *S. aureus*, *B. subtilis*, *P. stutzeri*, and *A. niger*; and it showed the lowest inhibiting rates against *S. cerevisiae*, with 34.36% ± 2.96%. Wholly, mulberry red pigment could inhibit *E. coli*, *B. subtilis*, *S. aureus*, *P. stutzer*i, *A. niger* and *S. cerevisiae*, it had the strongest antimicrobial effect on *E. coli* and the weakest antimicrobial effects on *S. cerevisiae*, and antibacterial ability against bacteria was higher than against fungi.

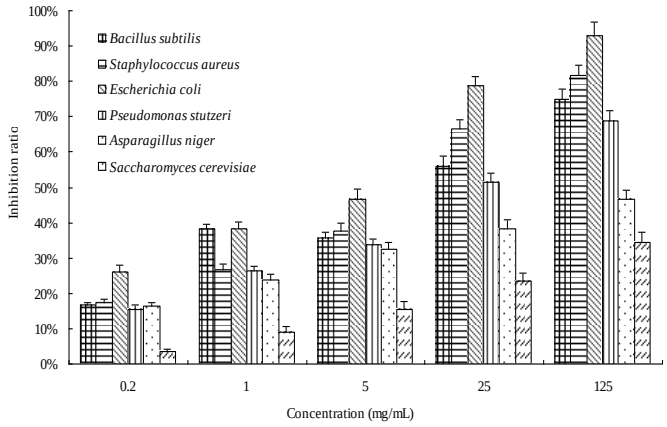

Figure 1. The antibacterial action of mulberry red pigment

3.1.2. Regression analysis of antimicrobial activity

As shown in Table 1, that regression equations and IC_{50} were obtained from the inhibition rates when *E. coli*, *B. subtilis*, *S. aureus*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were inhibited by mulberry red pigment. IC_{50} against *E. coli* was the lowest, with 16.27 mg/mL; it was followed by *S. aureus*, *B. subtilis*, *P. stutzeri* and *A. niger*; and it showed the highest IC_{50} against *S. cerevisiae*, with 195.70 mg/mL. There was significant difference between IC_{50}. Wholly, IC_{50} value descended orderly: *S. cerevisiae* > *A. niger* > *P. stutzeri* > *B. subtilis* > *S. aureus* > *E. coli*. Therefore, mulberry red pigment had the strongest antibacterial activity on *E. coli* and the weakest antibacterial action on *S. cerevisia*.

3.1.3. Analysis of minimum inhibitory concentration (MIC)

Table 2 showed that *E. coli*, *B. subtilis*, *S. aureus*, *P. stutzeri*, *A. niger* and *S. cerevisiae* were inhibited by mulberry red pigment, and minimum inhibitory concentration (MIC) were attained. MIC against *E. coli* was the lowest, with 45 mg/mL, it was followed by *S. aureus*, *B. subtilis*, *P. stutzeri* and *A. niger*; and it showed the highest MIC against *S. cerevisiae*, with 540 mg/mL. And there were significant difference between MIC. The MIC descended orderly: *S. cerevisiae* > *A. niger* > *P. stutzeri* > *B. subtilis* > *S. aureus* > *E. coli*. Therefore, mulberry red pigment had the most strongly antibacterial activity on *E. coli* and the most weakly antibacterial action on *S. cerevisiae*.

3.2. Antioxidative assay of mulberry red pigment

3.2.1. Scavenging effects of mulberry red pigment on DPPH free radical

It could be seen from Figure 2 that the scavenging effects on DPPH free radical increased with the increase of concentration of mulberry red pigment. When the concentration of Vc was in the 1 g/L, scavenging rate of Vc reached its maximum. When the concentration of mulberry red pigment was in the 8 g/L, scavenging effects on DPPH free radical reached its maximum, And the scavenging rate of mulberry red pigment was lower than that of Vc, and scavenging rates were 89.95% ± 2.86% and 97.38% ± 2.1%, respectively.

Regression equations of scavenging DPPH free radical was $y = 0.1128x + 0.059$ ($R^2 = 0.9815$), and EC_{50} of scavenging DPPH free

radical was 3.91 g/L. So mulberry red pigment had antioxidant abilities against DPPH free radical.

Table 1. Regression equations and IC_{50}

Strains	Regression Equation	R^2	IC_{50} (mg/mL)	T0.01
Bacillus subtilis	y = 0.0036x + 0.3312	0.9568	46.89	D
Staphylococcus aureus	y = 0.0043x + 0.3251	0.985	40.67	D
Escherichia coli	y = 0.0044x + 0.4284	0.9583	16.27	E
Pseudomonas stutzeri	y = 0.0035x + 0.283	0.9282	62.00	C
Asparagillus niger	y = 0.0018x + 0.2586	0.966	134.11	B
Saccharomyces cerevisiae	y = 0.002x + 0.1086	0.9429	195.70	A

Table 2. Minimum inhibitory concentration (MIC)

Strains	MIC (mg/mL)	T0.01
Bacillus subtilis	88	D
Staphylococcus aureus	80	D
Escherichia coli	45	E
Pseudomonas stutzeri	125	C
Asparagillus niger	450	B
Saccharomyces cerevisiae	540	A

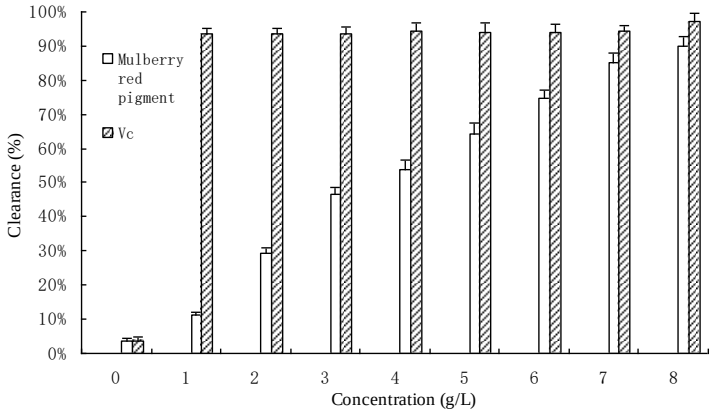

Figure 2. Scavenging effects of mulberry red pigment on DPPH free radical

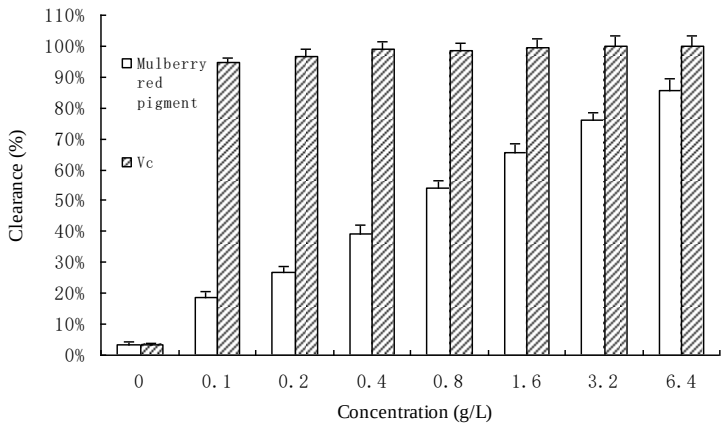

Figure 3. Scavenging effects of mulberry red pigment on superoxide radical

3.2.2. Scavenging effects of mulberry red pigment on superoxide radical

Figure 3 showed that the scavenging effect on superoxide radical increased with the increase of concentration of mulberry red pigment. When the concentration of Vc was in the 0.1 g/L, scavenging ability of Vc reached its maximum. When the concentration of mulberry red pigment was in the 6.4 g/L, scavenging effects on superoxide radical reached its maximum. But the scavenging rate of mulberry red pigment was lower than that of Vc, and scavenging rates were 85.78% ± 3.66% and 100% ± 3.46%, respectively. Regression equations of scavenging superoxide radical was $y = 0.1092x + 0.2889$ ($R^2 = 0.9594$), and EC_{50} of scavenging superoxide radical was 1.93 g/L. So mulberry red pigment had antioxidant abilities against superoxide radical.

4. Conclusions

Mulberry is widely distributed in China. Mulberry leaves are used as forage plant for silkworms, and they are also used as a herbal medicine. Mulberry fruit with rich nutrient is a natural fruit, but its storage period is short in natural condition, which can affect sales and prices of mulberry fruits. So extraction, antibacterial and antioxidative activities of mulberry red pigment were studied in order to promote further processing of mulberry fruits and to improve the comprehensive utilization of mulberry red pigment.

In this study, antibacterial and antioxidative effects of mulberry red pigment were studied. Mulberry red pigment could inhibit *E. coli*, *B. subtilis*, *S. aureus*, *P. stutzeri*, *A. niger* and *S. cerevisiae*, antibacterial ability against bacteria was higher than against fungi, and mulberry red pigment had the strongly antibacterial activity on *E. coli* and the weakly antibacterial action on *S. Cerevisiae*, the research result was are different from Duan Jianglian's reports (Duan and Xu, 2007). Mulberry red pigment could scavenge DPPH free radical and superoxide radical, and EC_{50} were 3.91 g/L and 1.93 g/L, respectively. The antioxidant properties were lower than that of Vc, but EC_{50} was lower than that of Lu Yinghua's reports (Lu et al., 2007)

The present study suggested mulberry red pigment had certain bacteriostatic and antioxidative action, and had nutrient hygienical function. So mulberry red pigment could be used in food additives, which contributed to food safety.

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OPTIMIZATION OF ULTRASONIC-ASSISTED EXTRACTION TOTAL FLAVONOIDS FROM *CORN COB* USING RESPONSE SURFACE METHOD

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ABSTRACT

The aim of the study was to extract total flavonoids from Corn Cob through optimizing process assisted by ultrasonic. [Method] Based on single-factor experiments, ethanol concentration, extraction temperature, extraction time and ultrasonic power were set as independent variables and the yield of flavonoids was response value. Effects of each variable and their interactions on flavonoids were determined using Box-Behnken method. The quadratic polynomial regression equations between flavonoids and each variable were built using Design-Expert software. [Results] The results showed that the optimal conditions of total flavonoids from Corn Cob were set as follows: ethanol concentration of 70%, extraction temperature of 59℃, extraction time of 44 min and ultrasonic power of 55Hz, respectively. The yield of flavonoids was 3.719 ± 0.030mg/g (N=6) under the modified conditions. The relative error was 0.216% compared to predictive value, indicating the model fitted well with experimental data. In addition, the extraction yield was increased by 28.7% as compared to the traditional extraction method. [Conclusion] The results could provide a theoretical foundation and scientific basis for the development of natural antioxidant products of Corn Cob.

1. Introduction

Corn is one of the important traditional crops. The *corn* planting area is large and the output is high. After threshing *corn cob* accounted for 20%-30% of corn quality. Currently, *corn cob* is mainly used for the animal feed, making pulp and sugar. In addition to cellulose and starch, *Corn cob* is abundant in flavonoids (Jiaming et al., 2012). Nowadays more and more attention is cast on flavonoids by biochemical and nutritional researchers due to their various biological activities used in health-care food or medicine, especially antibacterial, antiviral and antioxidant effects (Zunlai et al., 2013). *Corn cob* is a type of underutilized natural resource**.** For the sake of making better use of the resource, more research is immediately required for efficient procedures of *Corn cob* extracts.

Response surface methodology (RSM), has been widely used to determine the optimal operating conditions. In this methodology, mathematical and statistical techniques are collected for designing experiments, building models, evaluating the effects of factors and searching optimum condition of factors for

desirable responses (Box and Wilson, 1951). Box-Behnken (BBD) and central composite design (CCD) of the principal RSM have been widely used in various experiments. BBD, a spherical and revolving design, has been applied in optimization of chemical and physical processes because of its reasoning design and excellent outcomes (Ferreira et al., 2007; John and Borkowski, 1995; Yaqiang 2015). Ultrasonic-assisted extraction is widely applied in the extraction of food functional components because of its high efficient, time saving and simple operation (Lifen et al., 2011). In the present study, the total flavonoids content was considered as response value while ethanol concentration, extraction temperature, extraction time and ultrasonic power were considered for optimization parameters. Box-Behnken design, followed by canonical and ridge analyses, was employed to optimize the process parameters of total flavonoids extraction from the *corn cob*.

2. Materials and methods

2.1. Materials and instruments

Core cob was collected from Henan province (China). *Rutin* was purchased from the Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Ethanol, Sodium nitrite**,** Aluminum nitrate and Sodium hydroxide were of analytical grade and purchased from Tianjin chemical reagent manufacturing co., LTD (Tianjin, China). Pure water was purchased from Hangzhou wahaha group co., LTD (Hangzhou, China). TU-1810 spectrophotometer (Persee Corporation, China) was used for total flavonoids analysis of samples. KX-1740QT Ultrasonic cleaner (Kexi Corporation, China) was used for ultrasonic-assisted extraction of total flavonoids from *Corn Cob*. TGL-158 centrifuge (Shanghai Anting Scientific Instrument Factory, China) was used for centrifugal separation.

2.2. Extraction of flavonoids

Dry corn cob was ground in a blender to obtain a fine powder (particle diameter: 0.2-0.3mm). Assisted with ultrasonic, Samples of 1 g were extracted by ethanol solvent in a designed ethanol concentration, temperature, extraction time, ultrasonic power and ratio of liquid to material. The extraction solution was separated from insoluble residue by centrifugation (2000rpm for 5 min), and then the supernatant fluid of 5 ml was sucked out for determination of flavonoids. The formula to calculate the yield of total flavonoids was given as:

$$\omega = \frac{m}{m_0} \qquad (1)$$

where ω-the yield of total flavonoids, mg/g; *m*-the quality of the total flavonoids in the sample, mg; m_0 - the quality of sample, g.

2.3. Determination of total flavonoids content

The content of total flavonoids was determined by the colorimetric method with some modification (Jianfu et al., 2014). The supernatant fluid of 5 ml was moved into the volumetric flask of 10 ml, mixed with 0.3mL of 5% (w/w) $NaNO_2$ for 5 min, and then 0.3mL of 10%的 $Al(NO_3)_3$ (w/w) was added and mixed. 6 min later, 2mL of 1mol/mL NaOH was added and diluted to 10mL. With 15 min standing, the absorbance of the solution was measured at 510 nm with TU-1810 spectrophotometer against the same mixture, without the sample as a blank. The calibration curve ($y = -0.0117+10.884x$, where y is absorbance value of sample, x is sample concentration) ranged 0.005-0.06 mg/mL (R^2=0.9998).

2.4. Experimental design and statistical analysis

The yield of total flavonoids was affected by numerous parameters. Because it was impossible to identify the effects of all parameters, it was necessary to select the parameters that had major effects. The total flavonoids content in *corn cob* was influenced main by ethanol concentration, ratio of liquid to material, extraction temperature, extraction time and ultrasonic power, so the five

parameters were screened by single-factor experiment. Based on the preliminary results, the proper range for each factor was preliminarily determined, and a response surface methodology was conducted to design experimental project. As shown in Table 1, the four factors chosen for this study were designated as X_1, X_2, X_3, and X_4, and prescribed into three levels, coded +1, 0, –1 for high, intermediate and low value, respectively (Suresh et al., 2013).

Statistical analysis of the single-factor experimental data was performed with Microsoft Excel software. Design-Expert 8.0.6 was used for the experimental design and regression analysis of the experimental data. Student's t-test permitted the checking of the statistical significance of the regression coefficient, and Fischer's F-test determined the second-order model equation at a probability (P) of 0.001, 0.01 or 0.05. The adequacy of the model was determination by evaluating the lack of fit, the coefficient of determination (R^2) and the F-test value obtained from the analysis of variance (ANOVA) that was generated.

Table 1. Factors and levels of response surface methodology

Factors	Coded symbols	Levels		
		-1	0	1
Ethanol concentration(%,v/v)	X_1	50	60	70
Extraction temperature(℃)	X_2	50	55	60
Extraction time(min)	X_3	40	45	50
Ultrasonic power(Hz)	X_4	50	60	70

3. Results and discussions

3.1. The effect of ethanol concentration on the total flavonoids yield

Ethanol concentration was an important parameter of the total flavonoids extraction (Guowen et al., 2010). Different concentrations of ethanol (40, 50, 60, 70, 80 %, V/V) were prepared when other experiments were set as follows: particle size 100 mesh, ratio of liquid to raw material 20:1(mL/g), extraction temperature 60℃, extraction time 1 h, and ultrasonic power 70 Hz. It could be seen from Figure 1a that different ethanol concentrations had important effects on yield of flavonoids. The extraction yield was the highest when 60% ethanol was used as extraction solvent, which was (3.63±0.16) mg/g. when the concentration of ethanol was less than 60%, the yields increased with the increased concentration of methanol. Then, the yields declined from 60 to 80% of ethanol. So 60% ethanol was selected as the center point for further response surface methodology (RSM) experiment.

3.2. The effect of the ratio of liquid to material on the total flavonoids yield

In order to study the effect of the ratio of liquid to material on the extraction performance, different ratio of liquid to material (10:1, 15:1, 20:1, 25:1, 30:1) were prepared when other experiments were set as follows: Particle size 100 mesh, ethanol concentration 60%, extraction temperature 60℃, extraction time 1 h, and ultrasonic power 70 Hz. The results were displayed in Figure1b. The yield increased greatly when the ratio increased from 10:1 to 20:1, and then it maintained a mild slope when the ratio of liquid to material increasing. If the ratio of liquid to material is too small, total flavonoids in raw material could not be completely extracted up. If the ratio of liquid to material is too big, this would cause high process cost. Therefore, suitable ratio of liquid to raw material should be selected for extraction of targeted flavonoids (Lei et al., 2013). Taking into account of cost, the ratio of liquid to material 20:1 was adopted in this work.

3.3. The effect of extraction temperature on the total flavonoids yield

The choice of the extraction temperature was another important step (Yu, 2014). Extraction temperature was not constant during the extraction stages. Here, it was, respectively, set at 30, 35, 40, 45, 50, 55, 60 and 65℃ to

examine the influence of different temperature on the yield of the flavonoids extraction when other reaction conditions were as follows: particle size 100 mesh, ethanol concentration 60%, ratio of liquid to raw material 20:1(mL/g), extraction time 1 h, and ultrasonic power 70 Hz. Figure 1c indicated that the yield of total flavonoids rose gradually with the increase of temperature, and then reached the peak at 55℃, and finally dropped from 55 to 65℃. This phenomenon could be explained that solvent viscidity declined and movement of molecular accelerated with the increase of temperature, it was benefit for bioactive compounds to release from plant cells. However, much higher temperature promoted the degradation of some thermo-sensitive compounds. Therefore, the center point of extraction temperature was considered to be 55℃ in this experiment.

3.4. The effect of extraction time on the total flavonoids yield

Under the above optimal conditions of particle size 100 mesh, ethanol concentration 60%, ratio of liquid to raw material 20:1(mL/g), and ultrasonic power 70 Hz, effects of extraction time (20, 25, 30, 35, 40, 45, 50, 55, 60min) on the extraction yield of the flavonoids were tested. The results were displayed in Figure 1d. The yield of flavonoids was increased markedly with the increase of extraction time from 25min to 45min. Over 45min the yield lightly decreased. This might be due to the decomposition of active compounds during the prolonged extraction time. Therefore, the center point of extraction time chosen for RSM was 45min.

3.5. The effect of ultrasonic power on the total flavonoids yield

Ultrasonic power was also an important factor for extraction of active components from plant materials. It was associated with the final extraction efficiency, the energy cost and yield of total flavonoids (Qian et al., 2013). In this study, effect of different ultrasonic power (60, 70, 80, 90, 100, 120Hz) on the extracting yield was investigated. Six groups of samples were extracted with the optimal parameters obtained above. The results were displayed in Figure 1e. When the ultrasonic power was less than 60Hz, the yield of total flavonoids increased with the increased ultrasonic power. Then, the yields declined from 60Hz to 80Hz of ultrasonic power. This might be due to the much higher ultrasonic power promoted the decomposition of some structure- unstable compounds. Therefore, the 60Hz of ultrasonic power was adopted in this work.

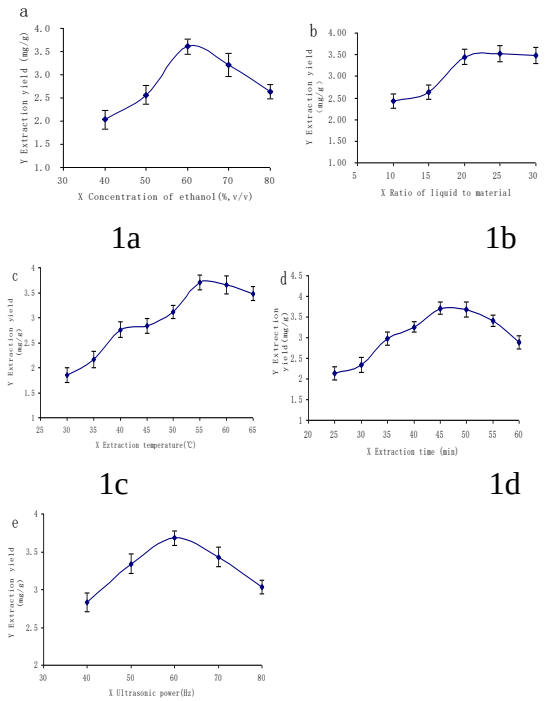

Figure 1. Effect of different extraction parameters (ethanol concentration, %, v/v; ratio of liquid to material; extraction temperature, ℃; extraction time, min; ultrasonic power, Hz) on yield of total flavonoids.

3.6. Optimization of the procedure

3.6.1. The model fitting and statistical analysis

The extraction of total flavonoids from *Corn Cob* was optimized through RSM approach. All 29 of the designed experiments were conducted for optimizing the four individual parameters in the current Box-Behnken design. Table 2 showed the experimental conditions and the results of total flavonoids yield according to the factorial design. By applying multiple

regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation: $Y = 3.64 + 0.13X_1 - 0.059X_2 - 0.007833X_3 - 0.11X_4 + 0.24 X_1 X_2 + 0.052X_1 X_3 - 0.008250X_1 X_4 - 0.13X_2 X_3 - 0.12X_2 X_4 + 0.016X_3 X_4 - 0.15X_1^2 - 0.17X_2^2 - 0.15X_3^2 - 0.22X_4^2$**,** where X_1, X_2, X_3 and X_4 were the coded values of ethanol concentration, extraction temperature, extraction time and ultrasonic power, respectively.

To determine whether or not the quadratic model was significant, the statistical significance of regression equation was checked by F-test, and ANOVE for response surface quadratic polynomial model were summarized in Table 3. The P-value was used as a tool to check the significance of each coefficient, which also indicated the interaction strength of each parameter. The smaller the P-values were, the bigger the significance of the corresponding coefficient. Here, the P-value of the model was smaller than 0.0001, which indicated the model was suitable for use in this experiment. The determination coefficient (R^2=0.9302) was close to 1, which indicated the satisfactory correlation between actual values and predicated ones. The Adj.R^2 value was 0.8605, which meat most variation (> 86.05%) of the total flavonoids content could be predicted by the models, which only 14% variation could not be explained by the model.

The lack-of-fit measured the failure of the model to represent the data in the experimental domain at points which were not included in the regression. The *F*-value of 1.37 and *P*-value of 0.4096 represented that the lack of fit was insignificant relative to the pure error. Insignificant lack of fit made the mode fit. Adequate precision compares the range of the predicted values at the design points to the average prediction error. A ratio greater than 4 indicated adequate model discrimination. In the present study, the value of 12.461 indicated an adequate signal. This model could be used to navigate the design space.

Table 2. Experiment design and result of response surface method analysis

NO.	X_1	X_2	X_3	X_4	Extraction yield(mg/g)
1	60	55	50	50	3.414
2	50	55	45	50	3.297
3	70	50	45	70	3.327
4	60	60	40	60	3.504
5	70	55	45	50	3.534
6	60	55	40	50	3.306
7	70	55	40	60	3.432
8	60	55	45	60	3.523
9	60	50	45	70	3.336
10	60	55	40	70	3.072
11	50	50	45	60	3.549
12	70	55	45	70	3.219
13	70	60	45	60	3.555
14	60	50	45	50	3.267
15	60	60	45	70	2.985
16	60	50	50	60	3.393
17	50	60	45	60	2.834
18	60	50	40	60	3.258
19	60	55	45	60	3.705
20	60	55	45	60	3.611
21	70	55	50	60	3.562
22	60	55	45	60	3.674
23	60	55	45	60	3.685
24	60	60	50	60	3.132
25	60	60	45	50	3.408
26	60	55	50	70	3.243
27	50	55	45	70	3.015
28	50	55	40	60	3.219
29	50	55	50	60	3.141

The regression coefficients and the corresponding *P*-values were also presented in Table 3. From the *P*-values of each model term, it could be concluded that the independent variables studied (X_1, X_2, X_4) and four quadratic term (X_1^2, X_2^2, X_3^2, X_4^2) significantly affected the total flavonoids yield. However, the analysis showed the interactions between two parameters (X_1X_2, X_2X_3, X_2X_4,) were in significant. The results of the study also

represented that the ethanol concentration and the ultrasonic power were the most significant parameters which influenced total flavonoids yield followed by extraction temperature and time.

Table 3. Regression analysis results of extraction parameter of total flavonoids

Source	Sum of Squares	Degree freedom	Mean Square	F-Value	p-value (Prob>F)
Model	1.29	14	0.092	13.34	<0.0001
X_1	0.21	1	0.21	29.85	<0.0001
X_2	0.042	1	0.042	6.11	0.0269
X_3	0.0007363	1	0.0007363	0.11	0.7490
X_4	0.15	1	0.15	22.15	0.0003
$X_1 X_2$	0.22	1	0.22	32.14	<0.0001
$X_1 X_3$	0.011	1	0.011	1.56	0.2316
$X_1 X_4$	0.0002722	1	0.0002722	0.039	0.8456
$X_2 X_3$	0.064	1	0.064	9.29	0.0087
$X_2 X_4$	0.061	1	0.061	8.75	0.0104
$X_3 X_4$	0.0009922	1	0.0009922	0.14	0.7106
X_1^2	0.15	1	0.15	21.40	0.0004
X_2^2	0.18	1	0.18	26.48	0.0001
X_3^2	0.15	1	0.15	21.68	0.0004
X_4^2	0.33	1	0.33	47.29	<0.0001
Residual	0.097	14	0.006917		
Lack of fit	0.075	10	0.007490	1.37	0.4096
Pure error	0.022	4	0.005484		
Cor total	1.39	28			
R^2	0.9302				
Adj.R^2	0.8605				
Pred. R^2	0.6645				
Adequate precision	12.461				

Note: $P < 0.05$, difference significant;
$P < 0.01$ difference was extremely significant.

3.6.2. Analysis of response surface

Three-dimensional response surface plots and two-dimensional contour plots, as presented in Figure 2 and Figure 3, were very useful to see interaction effects of the factors on the responses. These types of plots showed effects of two factors on the response at a time. In all the presented figures, the other two factors were kept at level zero.

It could be seen from Figure 2a and Figure 3a that the effects of ethanol concentration and extraction temperature on the yield of flavonoids. The interaction effects between ethanol concentration and extraction temperature were very significant. The yield increased with the increased of ethanol concentration and extraction temperature when the two factors were kept at high level.

Figure 2b and Figure 3b represented the effects of ethanol concentration and extraction time on the yield of flavonoids. With an increase of extraction time, the yield increased when the extraction time was less than 44 min, but it decreased when the extraction time was more than 44 min. In addition, it could be seen that the yield changed earlier and greater in a higher level of ethanol concentration. As ethanol concentration was increased in the range from 50% to 55%, extraction time had little effect on the yield of flavonoids. However, when the ethanol concentration was increased in the range from 55% to 70%, the yield increased greatly with the increased extraction time.

According to Figure 2c and Figure 3c, as the ethanol concentration increased in the range from 50% to 65%, flavonoids yield increased. At low ethanol concentration levels, the curve did not level off, indicating that this concentration was well below optimum for flavonoids yield. There was a linear increase in the yield with increase in ultrasonic power from 50 Hz to 59 Hz, but beyond 59 Hz, the yield decreased with increasing ultrasonic power. In addition, the result was consistent with the preliminary experimental result and could determine the accurate value of the parameter.

The effects of extraction time and extraction temperature on the yield of flavonoids were shown in Figure 2d and Figure 3d. The yield mainly depended upon extraction temperature and resulted in a curvilinear increase until zero level 58℃, and then decreased in flavonoids yield. The effect of extraction time was less significant than the extraction temperature.

The effects of ultrasonic power and extraction temperature on the yield of flavonoids could be seen in Figure 2e and Figure 3e. It was obvious that the high yield of

flavonoids could be achieved at a wide range of extraction temperature. However, the ultrasonic power should be strictly controlled. Increased ultrasonic power up to a threshold level led to increasing the yield. Beyond this level, the yield slightly decreased. The result was similar to other research findings (Chenxi et al., 2014; Javed et al., 2014).

Figure 2f and Figure 3f showed that extraction time exhibited a weaker effect whereas ultrasonic power represented a significant effect on the flavonoids yield. An increase in the yield of flavonoids could be significantly achieved with the increases of ultrasonic power, only at low levels of ultrasonic power. The yield of flavonoids decreased significantly at high levels of ultrasonic power.

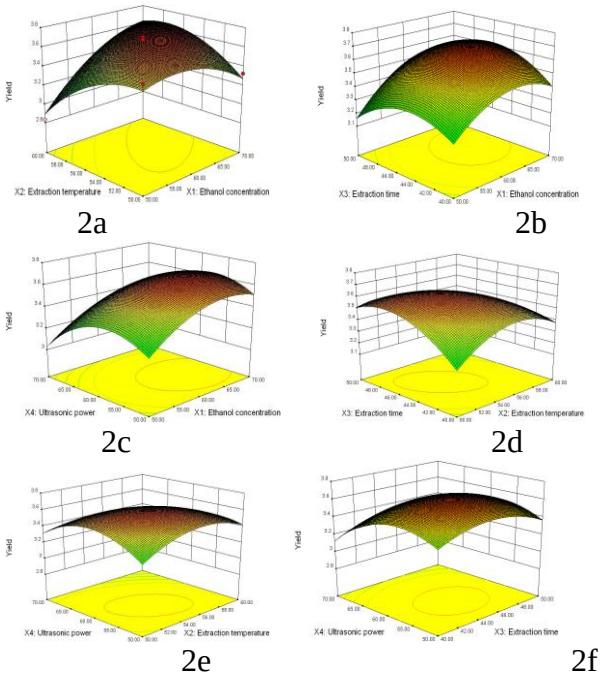

Figure 2. Response surface (3D) for the effect of different extraction parameters (X_1: ethanol concentration, %, V/V; X_2: extraction temperature,℃; X_3: extraction time, min; and X_4 ultrasonic power, Hz) added on the response Y.

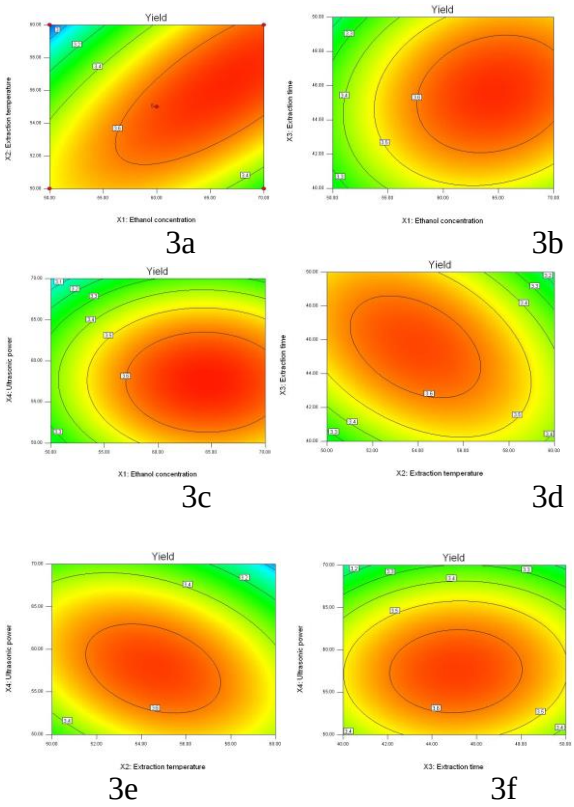

Figure 3. Contours (2D) for the effect of different extraction parameters (X_1: ethanol concentration, %, V/V; X_2: extraction temperature, ℃; X_3: extraction time, min; and X_4ultrasonic power, Hz) added on the response Y.

From what has been discussed above, the degree of influence on the extraction yield from big to small order is: ethanol concentration, ultrasonic power, extraction temperature and time.

3.6.3. Optimization of extracting parameters and validation of the model

In this study, the aim of optimization was to find the conditions which gave the maximum yield of flavonoids. The optimal values of the selected variables were obtained by solving the regression equation using Design-Expert 8.0.6 software. The optimum conditions for independent variables and the predicted values of the responses were also presented as follows: ethanol concentration of 70%, extraction temperature of 58.78℃, extraction time of 44.29 min and ultrasonic power of 55.19Hz,

respectively. The predicted extraction yield was give by the Design-Expert 8.0.6 software under the above conditions was 3.711 mg/g.

Considering the operability in actual production, the optimal conditions could be modified as follows: ethanol concentration of 70%, extraction temperature of 59℃, extraction time of 45 min and ultrasonic power of 55Hz, respectively. Under the modified conditions, the experimental yield of flavonoids was 3.719 ± 0.030mg/g (N=6), the relative error was 0.216% compared to predictive value, indicating that the model was adequate for the extraction process (Table 4). In addition, the extraction yield was increased by 28.7% as compared to the traditional extraction method (Lixin et al., 2014).

4. Conclusions

The use of multivariate optimization was of paramount importance in order to select the optimal operating conditions of interrelated variables, avoid or minimize degradation and achieve the best yields in the extraction process. RSM proved to be fairly accurate in predictive modeling and optimization of conditions for the yield of flavonoids, and that the yield of flavonoids to be reasonably approximated by quadratic non-linearity. In addition, the yield of flavonoids under the optimized extraction conditions was great higher than that of the non-optimized condition. This process could be considered as a sustainable alternative for the industry since it allowed simplified handing and the quantity of targeted extracts to be improved.

Table 4. Predicted and experimental values of the responses at optimum conditions

Optimum conditions	Ethanol concentration(%)	70
	Extraction temperature(℃)	58.78
	Extraction time(min)	45
	Ultrasonic power(Hz)	55.19
Extraction yield (mg/g)	Experimental	3.719± 0.030
	Predicted	3.711

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COMPARISON OF TOTAL BACTERIAL COUNT (TBC) IN BULK TANK RAW COW'S MILK AND VENDING MACHINE MILK

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ABSTRACT
Many of raw cow's milk suppliers in Slovakia decided to increase their profit through direct sale of milk from dairy farm or by using milk vending machines. Slovakia has currently about two hundred milk vending machines. Since the machines sold raw milk it is necessary to maintain good milk quality. The objective of this study was to determine bacteriological quality of raw cow's milk sold in vending machines. In this work we also compared total bacterial count in bulk tank raw cow's milk and vending machines milk respectively. From the observation we found that 64 out of 70 samples (91%) of raw cow's milk from the milk vending machines coincided with the criterion of maximum value of TBC 100 000 CFU.ml^{-1} (5.00 log$_{10}$ CFU.ml^{-1}) according the European Commission Regulation No. 1662/2006. We have found the average value of total bacterial count in bulk tank raw cow's milk samples 4.61 log$_{10}$ CFU.ml^{-1} and average value of total bacterial count in vending machine raw cow's milk samples 4.76 log$_{10}$ CFU.ml^{-1}.

1. Introduction

Milk and milk products provide a wealth of nutrition benefits. But raw milk can harbour dangerous microorganisms that can pose serious health risks. Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, microbial contamination can generally occur from three main sources: from within the udder, from the exterior of the udder, and from the surface of milk handling and storage equipment (Murphy and Boor, 2010). The health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning and sanitizing the milking and storage equipment are important in influencing the level of microbial contamination of raw milk. Equally important are the temperature and length of time of storage, which allow microbial contaminants to multiply and increase in numbers. All these factors will influence the total bacteria count (Murphy and Boor, 2010). Increased total bacterial count can be caused by growth of bacteria on unsanitary milking equipment, contamination from soiled cow udders, occasionally by milking of mastitic cows. The total bacterial count increases mainly in inadequately cooled milk. The poor hygiene increases the risk of presence of pathogenic bacteria in raw milk (Murphy and Boor, 2000; Hayes et al., 2001; Chambers, 2002; Costello et al., 2003, Elečko et al., 2004,

Zajác et al., 2008, Pantoja et al., 2009, Dudriková, 2010). The presence of pathogenic bacteria in raw milk was studied with several authors (Vasiľ et al., 2004, 2008, 2010, 2012, Pyorälä and Taponen, 2009). The resistance of bacteria to sanitising agents and the effectiveness of sanitation process were studied with (Kalmus et al., 2011, Lavová et al., 2010, Čanigová et al., 2004, Fabianová et al., 2010, 2011). Other sources of bacteria contaminating raw milk may be: equipment, hoose, transport containers, equipment of vending machines, personnel and environment (Čapla et al., 2008, Zajác et al., 2011). With the number of bacterial count increase the risk of drug residues in raw milk (Zajác et al., 2004). Soiled udders and teats are common sources of faecal contamination and often indicate inadequate premilking cow preparation. Increased numbers of coliforms in bulk milk can also occur when coliforms grow on residual milk left on milk contact surfaces or in poorly sanitized milking equipment (Guterbock and Blackmer, 1984; McKinnon et al., 1990; Chambers, 2002). Our microbiological survey suggested appropriate preventive measures that should be applied in terms of risk management (Valík et al., 2011).

Microbial contamination of raw milk can occur from a variety of microorganisms from a variety of sources. Because of this, determining the cause of bacterial defects is not always straightforward. Although there is often one source of bacteria that cause high bulk tank counts, high bacteria counts can also result from a combination of factors i.e., dirty equipment and marginal cooling (Murphy and Boor, 2010). The problematic of contamination of raw milk was studied Vasiľ et al., (2010). Raw milk must be immediately after milking placed in a clean place which is designed and equipped so as to avoid contamination, and cooled to a temperature of not more than 8°C and less than 4°C. In the case of raw milk intended for sale not cooled must be sold within two hours to the final consumer. If this milk is not soled within two hours, it must be immediately cooled to a temperature of up to +8 ° C and +4°C at least and sold within 24 hours of milking.

Date of consumption of not cooled raw milk intended for direct sale is not more than 24 hours of milking. Date of cooled raw milk is not more than 48 hours of milking. Direct sale of raw milk to the final consumer at the holding milk production takes place in a separate room from the premises where animals are housed and equipped with a cooling device. When producer of milk delivers milk to approved establishments for the collection or processing of milk, then the room for the direct sale of raw milk must be separated from milk rooms. Primary producer in an appropriate manner in a prominent place at the place of sale of raw milk must place these information and data: a) Notice for the end consumer, "Before eating raw milk should be boiled. It is not suitable for direct consumption of children, sick and old people or people with weakened immune" to be stated the type of raw milk; b) The date the consumption of raw milk with the words "use by" and the date and storage conditions of raw milk; c) Information about: name, surname and address of the primary producer or a trade name and place of business, if it is a primary producer who is a natural person - entrepreneur, or a business name and seat of the food business operator, if it is a primary producer, which is a legal entity, breeding for milk production.

Leading primary producer in addition to records relating to traceability and hygiene must have special written records, which shows the total amount of produced raw milk, which has been sold: a) Each day during each buyer; b) During the calendar year.

Primary producer keep records for at least one year after the calendar year in which they were made, and, on request, make available to the competent veterinary authority (Decree of Government of the Slovak Republic no. 360/2012).

Analysis of consumers' opinion on organic food and their safety and availability in the Slovak food market studied Kozelová et al., (2006, 2010, 2011a, 2011b).

From above mentioned, it is necessary to eliminate contamination of raw cow's milk and prevent bacterial growth with storage of milk in proper temperatures. In this work we were compared total bacterial count in bulk tank raw cow's milk and vending machines raw cow's milk. We were focused on the risk of secondary contamination of milk and we have quantified this risk. Milk vending operators should be aware of what may be at risk for the consumer. They should perform a regular sanitation of all equipment that comes into direct contact with the milk. The same attention should be given to the vessels used for the transport of raw milk. The bottles, which are filled in raw milk, must be clean and materials suitable for contact with food. Also, consumers should be aware that raw milk consumption increases the risk of disease caused by the presence of pathogenic bacteria. The all milk vending machines in the Slovak Republic must be in accordance with applicable legislation states that raw milk can be consumed only after cooking.

2. Materials and methods

2.1. Milk samples

We used samples of raw cow's milk taken from bulk tanks in dairy farms and dairy vending machines located in western area of Slovakia. Bulk tank milk samples were taken directly from dairy farms after the morning milking 6 to 8 a.m. Subsequently, the farmers fill this milk into the vending machines, from which we were sampling second sample. Vending machines milk samples were taken in the afternoon at 12 to 14 p.m. and analysed within the same day at 16 p.m. Milk samples were collected in sterile sample bottles in a volume of 500 ml. Samples were taken in accordance with standard ISO 707:2010. Samples were transported in portable dry ice box at 1 to 5 °C to the National Reference Laboratory for milk and milk products in Nitra, which is accredited according to the international standard ISO 17025 where the samples were subjected to microbial testing. We have analysed total number of 70 bulk tank raw cow's milk samples and 70 dairy vending machines raw cow's milk samples. Each bulk tank raw cow's milk sample corresponded with vending machines raw cow's milk sample.

2.2. Testing period

Samples were collected and analysed during the years 2010, 2011 and 2012.

2.3. Total bacterial count analysis

Total bacterial counts in raw cow's milk samples were determined by the standard plate count method, which is recognized as the standard method for enumerating total bacteria count in raw milk. Laboratory testing was performed according to the requirements of these standards: a) STN EN ISO 4833 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 degrees C; b) STN EN ISO 7218 Microbiology of food and animal feeding stuffs. General requirements and guidance for microbiological examinations; c) STN EN ISO 6887-1 Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilutions; d) STN EN ISO 6887-5 Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 5: Specific rules for the preparation of milk and milk products.

To determine total bacterial count in raw cow's milk samples OXOID Plate Count Agar (Tryptone Glucose Yeast Agar) code CM0325 was used.

2.4. Statistical analysis

We have performed basic statistical analysis in the R language (www.r-project.org).

3. Results and discussions

The total bacterial count reflects microbial quality of raw cow's milk. In the Slovak

Republic, the evaluation of the microbiological quality of milk is based on requirements of: Slovak Technical Standard STN 57 0529 : 1999 (Raw cow milk for dairy treatment and processing and European Commission Regulation no. 1662/2006 of 6 November 2006 amending Regulation (EC) No. 853/2004).

The European Union currently imposes a regulatory limit of 100.103 CFU.ml-1 (5.00 log10 CFU.ml-1) of raw cow's milk (EC reg. no. 1662/2006). Slovak Technical Standard STN 570529 determines limit max 50.103 CFU.ml-1 (4.70 log10 CFU.ml-1) and 100 x103 CFU.ml-1 (5.00 log10 CFU.ml-1) for Q (quality) and 1st class respectively.

The results of total bacterial count of bulk tank raw cow's milk samples collected from dairy farms in Slovakia show that 60% of samples met Q class limit and 34% met 1st class limit presented in STN 570529. We have found that, 6% of samples were not conforming to legislation limit presented in Regulation (EC) No. 1662/2006 and STN 570529 respectively. These results are presented in the Figure 1.

The results of total bacterial count of raw cow's milk samples collected from vending machines in Slovakia show that 37% of samples met Q class limit, 54% met 1st class limit presented in STN 570529. We have found that, 9% of samples were not conforming to legislation limit presented in Regulation (EC) No. 1662/2006 and STN 570529 respectively. These results are presented in the Figure 2. We have found the average value of total bacterial count in bulk tank raw cow's milk samples was 4.61 log10 CFU.ml^{-1}. The percentage of unsatisfactory results did not mean that farmers had to be immediately penalised, because in Slovakia a rolling geometric mean is used, according to the Commission Regulation EC No. 1662/2006.

However, there is a large potential to improve the quality and safety of raw cow's milk, as well as economic losses in dairy farms in Slovakia (Zajác et al., 2011). We have found the average value of total bacterial count in vending machine raw cow's milk samples was 4.76 log10 CFU.ml^{-1}.

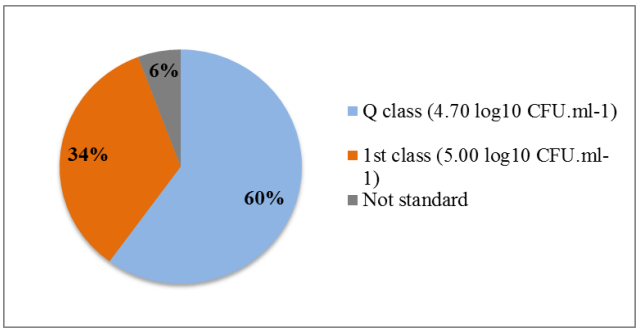

Figure 1. Evaluation of total bacterial count in bulk tank raw cow's milk samples

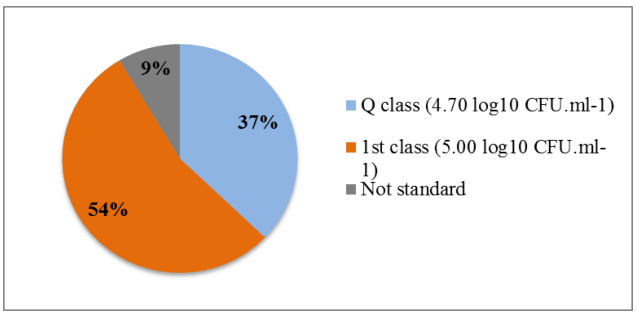

Figure 2. Evaluation of total bacterial count in vending machines raw cow's milk samples

In our previous study we were analysed 19,830 results of total bacterial count of bulk tank raw cow's milk samples taken from dairy farm directly with dairy companies in Slovakia in 2011. These results shown that 84.54% of samples tested for total bacterial count, met the European Union legislation limits and 15.47 % of samples did not met the legislation limit the European Union legislation. The average value of total bacterial count in bulk tanks raw cow's milk samples was 4.84 log10 CFU.ml-1 (Zajác et al., 2011)

Valík et al., (2011) were analysed microbiological quality or raw cow's milk from vending machines. The analyses and evaluations were focused to bacterial indicators only. Refer to total bacterial counts, the criterion ≤ 100,000 CFU.ml^{-1} met 12 from 15 samples (80%) and all samples complied with

the supplementary criterion of 5×104 CFU.ml-1 for psychrotrophs. The average value of total bacterial count in vending machine raw cow's milk was 4.75 log CFU.ml^{-1}. This value is in agreement with our average result of vending machine raw cow's milk total bacterial count 4.76 log10 CFU.ml^{-1}. Assuming a constant rate of bacterial growth we may expecting the increase in the numbers of different groups of microorganisms after 24 hours and we can also assess the density quite well after 48 hours. The average total bacterial count value after 24 h increased by one logarithmic order of 5.8 log10 CFU.ml^{-1}. Based on this assumption, the number of bacteria after 48 h should achieve an average of 6.8 log10 CFU.ml^{-1} (6.3.106 CFU.ml^{-1}) (Valík et al., 2011). Histogram of total bacterial count in bulk tank raw cow's milk samples is presented in Figure 3. Histogram of total bacterial count in vending machines raw cow's milk samples is presented in Figure 4. Histogram of paired t-test of total bacterial count in bulk tank raw cow's milk samples and vending machines raw cow's milk samples. We were confirmed our hypothesis (p-value <0.001), that we can expect increase the number of bacteria in the raw cow's milk in vending machine.

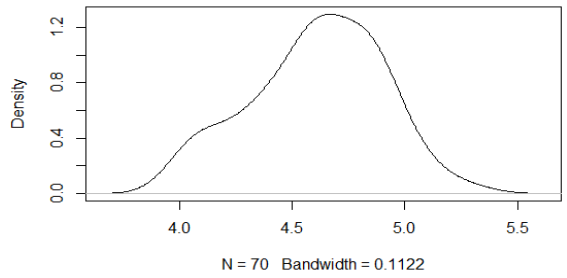

Figure 3. Histogram of total bacterial count in bulk tank raw cow's milk samples

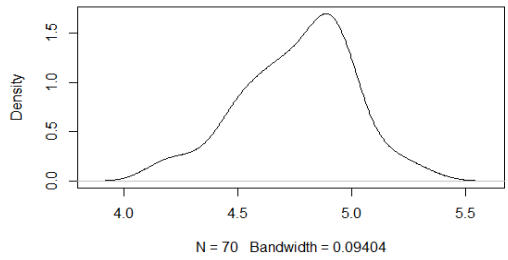

Figure 4. Histogram of total bacterial count in vending machines raw cow's milk samples

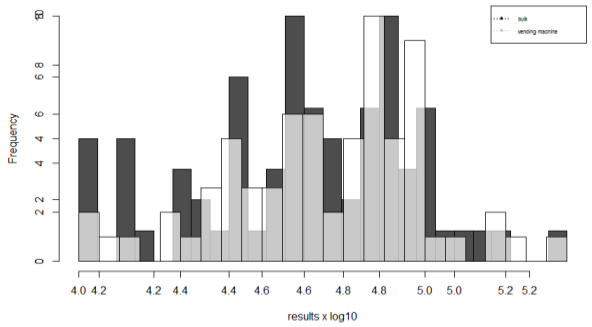

Figure 5. Histogram of total bacterial count in bulk tank raw cow's milk samples and vending machines raw cow's milk samples

4. Conclusions

In this research, we focused on the comparison of microbial quality of raw cow's milk from bulk tank and vending machines.

The results of total bacterial count of bulk tank raw cow's milk samples collected from dairy farms in Slovakia show that 60% of samples met Q class limit and 34% met 1st class limit presented in Slovak technical standard STN 570529. We have found that, 6% of samples were not conforming to legislation limit presented in Regulation (EC) No. 1662/2006 and STN 570529 respectively. The results of total bacterial count of raw cow's milk samples collected from vending machines in Slovakia show that 37% of samples met Q class limit, 54% met 1st class limit presented in Slovak technical standard STN 570529. We have found that, 9% of samples were not conforming to legislation limit presented in Regulation (EC) No. 1662/2006 and Slovak technical standard STN 570529 respectively. We have found the average value of total bacterial count in bulk tank raw cow's milk samples 4.61 log10 CFU.ml-1 and average value of total bacterial count in vending machine raw cow's milk samples 4.76 log10 CFU.ml-^{1}. Comparing the results of total bacterial count of bulk tank milk samples and milk samples obtained from vending machines we verified that presence of secondary

contamination of milk during the transport and storage. Also, we can expect intensively bacterial growth. Despite the fact that most of raw cow's milk (94% bulk tank and 91% vending machines) samples meet hygiene limits for total bacterial count, it is necessary to perform heat treatment of this milk before the consumption, to reduce the risk of foodborne illness.

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