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PRELIMINARY TEST FOR OBTAINING AN ONION PUREE SICILIAN

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ABSTRACT

The onion (Allium cepa) is highly valued for its flavor and becomes part of many culinary preparations in the world, plays an important role in the Mediterranean diet and in the preparation of foods that use this condiment. Onions grown in facility of Sicily have been transformed and evaluated the following parameters: the color, the content of total soluble solids, titratable acidity and organoleptic value.

Keywords: *Onion puree (Allium cepa); food "easy to use"; condiments, mediterranean aperitif.*

1. Introduction

The onion (*Allium cepa*) , bulbous plant of the family Liliaceae highly valued for its flavor, becomes part of many dishes. Apart from the taste and smell that is typical characterized by good nutritional properties due to the presence of trace elements (iron, potassium, magnesium, fluoride , calcium, manganese , phosphorus and sulfur) different vitamins (A, B , C, E) and flavonoids with antioxidant and diuretic. In recent years the market has been increasingly looking for ready-made products, the so-called "easy to use", also in the category of seasonings for culinary preparations. Since in the process of trimming and cutting the onion often causes tearing has evaluated the possibility of producing a puree of onion ready for use which, besides bringing advantages in terms of convenience, would eliminate this problem. The onion puree is especially suitable as a seasoning for meat dishes but especially for the preparation of food for the aperitif.

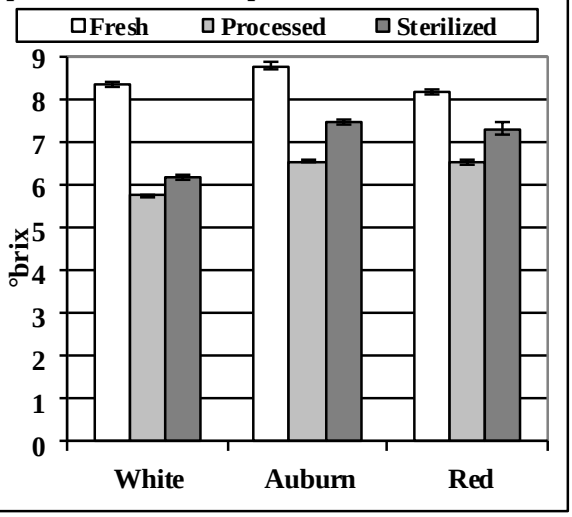
2. Materials and methods

The distribution were found three types of onion are characterized by different colors of the outer housing and the scales (White, Auburn, Red). Each type of onion has been processed separately. Before proceeding with the preparation of puree, onions, have been cleaned and washed under running water and air dried. Then we proceeded to cut into pieces of media magnitude that have been immersed in water at 80 ° C for 5 minutes. Once draining of excess water, the onions were chopped and pureed end with an immersion blender. The puree obtained was placed in glass jars sterilized volume of 100 mL, sealed with metal plugs, and subsequently subjected to the process of sterilization by autoclaving at 120 ° C for 20 '. To evaluate the quality of the raw material and the modifications of the same during the production process to the final product, were analyzed some qualitative parameters of the fresh product, the puree just obtained and sterilized. Were studied: the total soluble solids content (using digital refractometer), titratable acidity (using 0.1 N NaOH with bromothymol blue as an indicator color change of the solution to pH 7.8), the color (coordinates L *, a *, b *, measured with digital Minolta CR400 colorimeter from which were calculated the Croma and the angle of Tint). Product packaged and sterilized was also evaluated the visual quality and organoleptic characteristics by means of a group of ten tasters who evaluated the product with a score from 1 (unpleasant) to 5 (very pleasant). The data collected were subjected to analysis of variance. The averages of the

values tabulated were separated by means of the Duncan test. The remaining data are presented with the respective standard error.

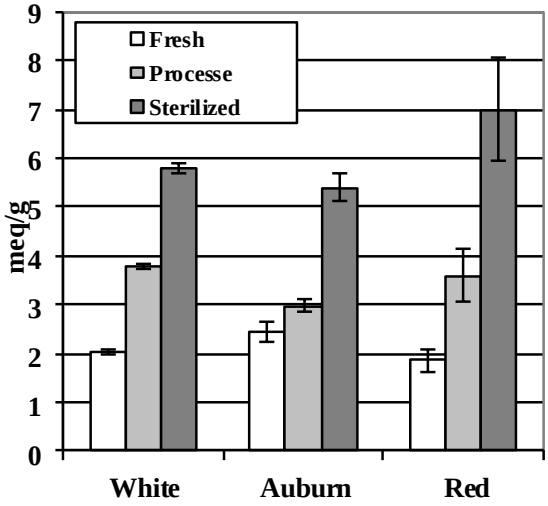
3. Results and disscussion

Fig. 1
Content of total soluble solids during the production of onion puree.



Except for the color variations types of onions used showed modest initial changes with regard to the content of total soluble solids and titratable acidity. Nevertheless by the type of onion used in various stages of preparation of the product and the production reduced in all types of used onions and especially in the case of white onions (<6 ° brix). The thermal treatment of stabilization The onions are appreciated and used in the different culinary preparations also for their coloration as well as for their aroma. onions auburn had a significantly higher level of SST of the other two types (Fig. 1). process has resulted in a significant increase in the sterilized product than the fresh puree and onions intact. the blanching and preparation of mashed SST levels were After at high temperature has probably resulted in the hydrolysis of complex sugars doing some slightly increase again the value of SST. The titratable acidity (Fig. 2) was not influenced.

Fig. 2 Changes in titratable acidity during the production of onion puree.



The three different choices are representative of the colors available on the market and is therefore interesting to analyze the changes that affected their color as a result of the preparation and sterilization of the puree. The white onion and auburn have a similar brightness and obviously much higher than the red onion (Table 1).

It remains almost constant parameter L * value around 30 while the white onion and auburn lose color brightness at different stages of the production process.

This is indicative of a tendency browning which is manifested in a more marked following the sterilization. The process of thermal stabilization also seen a very marked effect on the value and chroma of red onion. This parameter indicates the intensity and color saturation. The red onion is strongly conditioned, taking after heat treatment a little intense color and off, thus losing the typical coloring and attractive

Table 1 Changes in color during the preparation of mashed onion.

	L*			Quaver			Hue angle		
	white	auburn	red	white	auburm	red	white	auburm	red
Sleeves	73,05a	74,13a	29,32g	4,51d	14,64b	23,05a	102,6f	109,8e	360,1a
Mashed	67,61b	61,33c	33,88f	7,42c	8,42c	21,41a	106,1ef	114,3e	332,1b
Processed	52,60c	54,45c	28,40g	3,19d	7,22c	21,22a	174,9c	124,9d	323,7b
Sterilized	43,79d	40,33e	27,47g	6,45c	7,48c	4,95d	89,5g	86,8g	331,5b

A different letters correspond to different values at P <0.05 (LSD multiple range test)

The changes experienced by the color in part also have influenced the judgment on the organoleptic quality of the finished product. The tasters evaluated negatively the quality of mashed red onion with a score less than 2 (Fig. 3). The onion copper had a browning than the white onion, which, even from the organoleptic point of view reached the highest score 3.26.

4.Conclusions

The preparation of a puree of onion was a technological process simple enough that you get a product that could meet the favor of consumers for the convenience and versatility of use.

During the technological treatments and cooking, in the tissues of onion occurring chemical and biochemical reactions that can result in important changes in the bioavailability and activity of nutrient compounds and functional. It is therefore considered that further research is necessary to limit the negative effects of heat treatment in stabilization and help to improve the nutritional, aesthetic and organoleptic increasing appreciation by consumers.

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THE PRESERVATIVE ACTIVITY OF CITRIC ACID COATED ON THE SUMMER SALAMI DURING STORAGE UNDER REFRIGERATION

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ABSTRACT

The citric acid was coated on the summer salami and stored under refrigeration (at 4-5°C) with the aim of establish their preservative activity. The citric acid was used as powder as well as 1% and 10% solution. The following parameters were monitored: water and chloride content, pH, antioxidant activity. After 14 days of storage, the most reduced variation of the above mentioned physical-chemical parameters occurred in the package containing the summer salami modified with 10% solution of citric acid. Besides these parameters, the organoleptic characterization (flavor, color, aspect, consistence) of the stored summer salami was also performed. It was observed that the unpacked summer salami samples were more dried and more deep colored during storage as compared with those packed**.** During storage, differences between the unmodified summer salami and that coated with citric acid was observed. The color, aspect and consistence remain the same in all packages, but the flavour of condiments at unmodified summer salami is more weaker compared to the samples containing citric acid. Of all samples coated with citric acid, the sample containing citric acid solution 10% preserves at more than summer sausage quality.

Keywords*: citric acid, summer salami, package, refrigeration*

1. Introduction

Citric acid, salts of calcium, potassium and sodium are the most commonly used food preservatives, especially in beverages. These preservatives are used to inhibit bacteria, molds and having inhibitory effect against yeasts. Citric acid it is also used as a flavoring agent and preservative in food, especially in the fruit juice [1, 2].

Garcia et al. [3] studied the extension of the shelf life of chilled hake (*Merluccius merluccius*) by a novel icing medium containing natural organic acids and showed that the presence of organic acids in the novel icing led to an inhibitory effect on the *Enterobacteriaceae*, bacteria that occur during chilled storage of fish [3].

Jiang et al. [4] have studied the maintenance quality of fresh-cut Chinese chestnut using solution of citric acid, and they have demonstrated that the application of citric acid as a preservative was useful in extending shelf life and maintaining quality of fresh-cut Chinese water chestnut slices during storage [4].

Virto and Sanz [6] have investigated the inactivation of *Yersinia enterocolitica* bacteria using the citric acid and lactic acid at different temperatures (4, 20, 40 °C) and they have demonstrated that the inactivation of *Y. enterocolitica* by citric and lactic acids was dependent on tratment temperature and concentration of acids. If the acid concentration increases then *Yersinia enterocolitica* inactivation occurs faster [6].

Many researchers have demonstrated that the application of citric acid as a preservative reduce food alteration and so they extend their shelf life [2-4].

The purpose of this article is to test the activity of citric acid preservative coated on the summer salami unpacked and packed in polyethylene during storage under refrigeration (4-5°C).

2. Materials and methods

2.1. The coating of citric acid on the summer salami

For this experiment citric acid was deposited on the surface of the summer salami, using solid citric acid solution, 10% citric acid and 1% citric acid solution. Solid citric acid was deposited on the surface of the salami by dabbing, as follows: put a certain amount of citric acid on a watch glass and soak in the salami, covering the whole salami piece with citric acid. This dabbing was carried out on two pieces of salami, one being wrapped, one unwrapped. When depositing 10% citric acid solution on the surface of the summer salami was processed as follows: the piece of salami taken was sprayed with 10% citric acid solution.

In order to coat 1% citric acid solution on the surface of the summer salami was processed as above except that in place of 10% using 1% citric acid solution.

Both solutions of citric acid (1% and 10%) were deposited on two pieces salami, one being packaged, unpackaged one.

For the summer salami packaging was used polyethylene (supermarket Kaufland, Romania) and for each piece of salami was cut square with an area of 400 cm². The summer salami used was manufactured by Ferma Zootehnica and purchased from Ferma Zootehnica's store network, Baia Mare, Romania.

Each piece of coated salami, packed and unpacked (20 g) was placed in a refrigerator (at 4-5°C) for testing the activity of the preservative, citric acid.

2.2. Orgaloleptic analyses

Orgaloleptic analyses consisted in establishing the variations of aspect, color, flavour and consistence of the food samples deposited in studied packages, at different time ranges.

2.3. Humidity

A sample of 3 g (G) was mixed with calcined cuartz sand and the mixture is weight (G_1). The mixture was then treated with 5 ml of ethanol (S.C. Chemical Company S.A. Iaşi, România) and introduced in the Binder oven at 40...60°C, for 2 h and then at 105...110°C, until the weigh is constant [8]. After cooling the mixture is weigh again (G_2). The percentage of water is calculted with the formula:

$$\%water = \frac{G_1 - G_2}{G} \times 100 \qquad (1)$$

where: G_1 - mass of food sample and sand before drying (g),

G_2 - mass of food sample and sand after drying (g)

G - mass of food sample (g)

2.4. pH

A sample of 3 g was mixed together with 10 mL ultrapure water and then filtered. In the obtained filtrate the pH was measured using the Inolab pH 730 pH-meter.

2.5. Chlorine content

A sample of 3 g was mixed with 15 ml acetone and then 50 ml of ultrapure and a small quantity of sodium carbonate were added. The mixture was filtered and the entirely quantity of extract was added to 0.5 ml of potassium chromate 10% (S.C. Chemical Company S.A. Iaşi, România) were added. The mixture was titrated with 2.906% solution of silver nitrate (S.C. Chemical Company S.A. Iaşi, România) until the color changed in red-brown [8].

The chlorine content was calculated using the formula:

$$\%NaCl = \frac{V}{m} \qquad (2)$$

where: V – volume of $AgNO_3$ solution used to titration (ml).

m – sample mass (g)

2.6. Antioxidant activity

The antioxidant activity of the samples was performed according to the method of Brand-Williams [7]. The method is based on the reduction of DPPH in the presence of a hydrogen donor antioxidant. DPPH is a purple

colored solution and has an intense absorption band at 515 nm. Adsorption and the color is low when the DPPH is reduced by an antioxidant compound [7].

The preparation of the blanck consisted in mixing of 1 ml solution 0.1 mM DPPH and of 1 mL MeOH. This solution obtained was spectrophotometrically measured at 515nm (t=0 min). For the salami sample processing, an amount of 1 g sample (summer salami) was mixed with 10 mL methanol. The mixture was stirred using the Hettich Zentrifugen, EBA20 centrifuge, for 10 minutes at 5000 rot / min. A volume of 1 mL of the obtained extract was, then, mixed with 1 mL DPPH. This solution was kept in dark for 30 min and was spectrophotometrically measured at 515 nm.

The antioxidant activity was calculated using the formula:

$$\%Antioxidant\,activity = 100 - \frac{A_{DPPH}}{A_{sample}} \times 100 \quad (3)$$

where: A_{DPPH} – the absorbance of DPPH measurements at 515 nm (%)

A_{sample} – the absorbance of sample measurements at 515 nm, storage in dark place for 30 min (%).

3. Results and disscussion

3.1. Organoleptic analysis

The summer salami sample tested as reference (0 day) is fresh, the shells are dried, without microorganisms or slime, adherent to the composition. The colour of the sample was pink, uniformous, without stains, the consistence was dense and juicy, the flavour was nice.

After 14 days of storage, the colour of the packed salami samples does not change. The fresh sausage, salami and the unpacked samples becomes brown and the flavour is deeply altered. In undoped packaging, especially in the unpacked one, changes regarding the smell can be observed. It was observed a stale smell, flavor spices weaker compared to the other samples. the unpacked salami samples after 14 days are dry as compared with packaged salami (Figure 1).

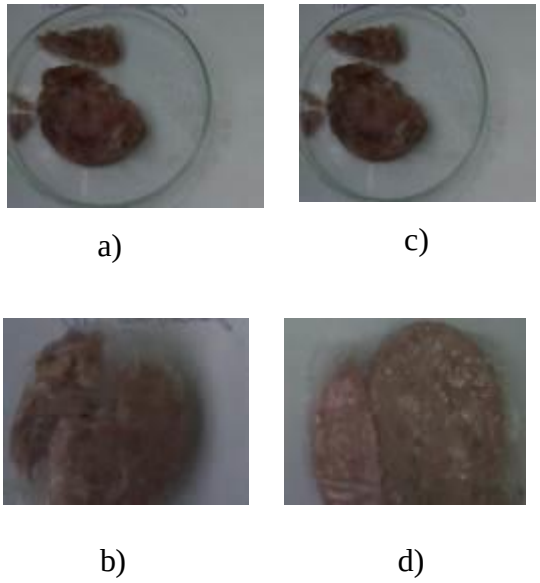
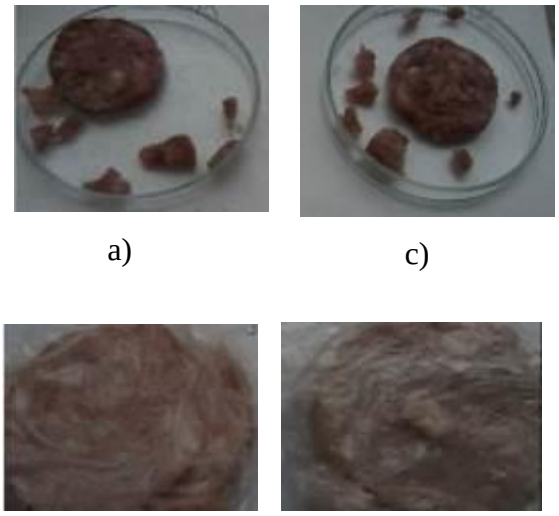


Figure 1. Images of summer salami samples storage during 14 days: a) unpacked summer salami buffered with citric acid, b) packed summer salami buffered with citric acid, c) unpacked summer modified with 1% solution of citric acid, d) packed summer modified with 1% solution of citric acid

After 14 days of storage, the smell of the sample packed in polyethylene modified with 1% citric acid, is a little stale, the spice aroma is slightly weaker as compared to the other samples stored in packages modified with citric acid. In comparison, the smell of the sample kept in package containing the summer salami modified with 1% solution of citric acid and the package containing the summer salami modified with powder of citric acid was almost the same as to that of the reference sample, and the flavor is not so altered as that of the sausage kept in package containing the summer salami modified with 1% solution of citric acid (Figure 2).



a) c)

b) d)

Figure 2. Images of summer salami samples storage during 14 days: a) unpacked summer modified with 10% solution of citric acid b) packed summer modified with 10% solution of citric acid, c) unpacked undoped summer salami, d) packed undoped summer salami

3.2. Physico-chemical parameters

The chloride content (Figure 3) of the summer sausage deposited in all the investigated packages decreased durring the storage. After 14 days of storage, the chloride content drastically decreases from 2.94 % (0 day) to 0.86 % in the sample stored in unmodified package, to 0.7 % in the sample stored in the unpacked summer salami, to 1.86% in the summer salami packed in polyethylene modified with 1% citric acid, to 1.00% in unpacked summer salami sprayed with 1% citric acid, to 1.95 % in packed summer salami sprayed with 10% citric acid, to 1.72 % in unpacked summer salami sprayed with 10% citric acid, to 2.02 % in unpacked summer salami buffered with citric acid, to 2.35 % in packed summer salami buffered with citric acid. The most accentuated decrease is observed in undoped packages both packed and unpacked. The most reduced decrease was observed for the summer sausage kept in samples that have a contains a high amount of citric acid. This can be explained by the already known [3,4] preservation activity of the citric acid.

The decrease in the chloride content due to the oxidation of chloride ion is released from the photolysis takes place according to the reaction:

$$Cl^{-} \rightarrow Cl\cdot \qquad \text{(eq.1.)}$$

$$Cl\cdot + Cl\cdot \rightarrow Cl_2 \qquad \text{(eq.2.)}$$

Photolysis process is more pronounced in undoped samples, especially those unpacked and in other samples this process is slower due to the presence of citric acid in samples that show their power conservation.

The pH (Figure 4) of the summer sausage kept in all packages decreases due to the fact that the degradation of the active principles in salami occurs during storage. The most reduced acidification of the sample was to observed during storage in samples packed in polytehylene modified with 10% citric acid, after 8 days of storage (from 7.74 to 6.95 in unpaked salami; from 7.74 to 7.10 in packed salami) and after 14 days, the process is the similar. The pH decreased from 7.74 to 6.71 in the unpacked salami and from 7.74 to 6,73 in the packed salami. The presence of citric acid in the samples makes it manifest their power conservation and protect the food against microorganisms growth and alteration processes.

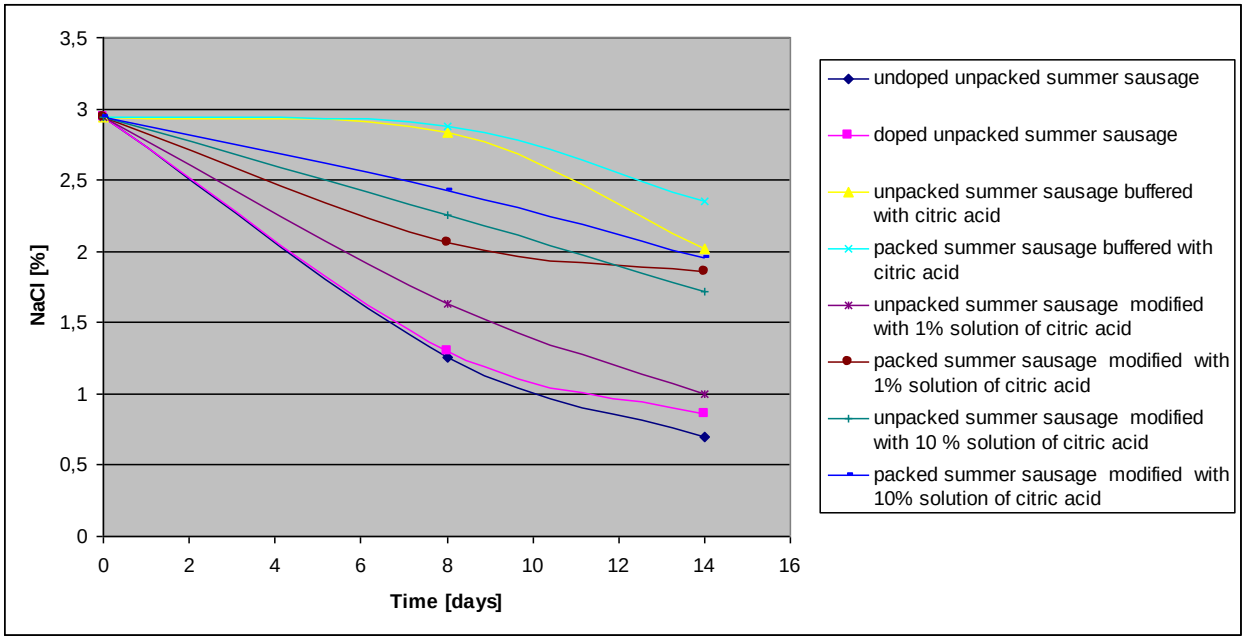


Figure 3. The variation of NaCl in summer sausage packed and unpacked.

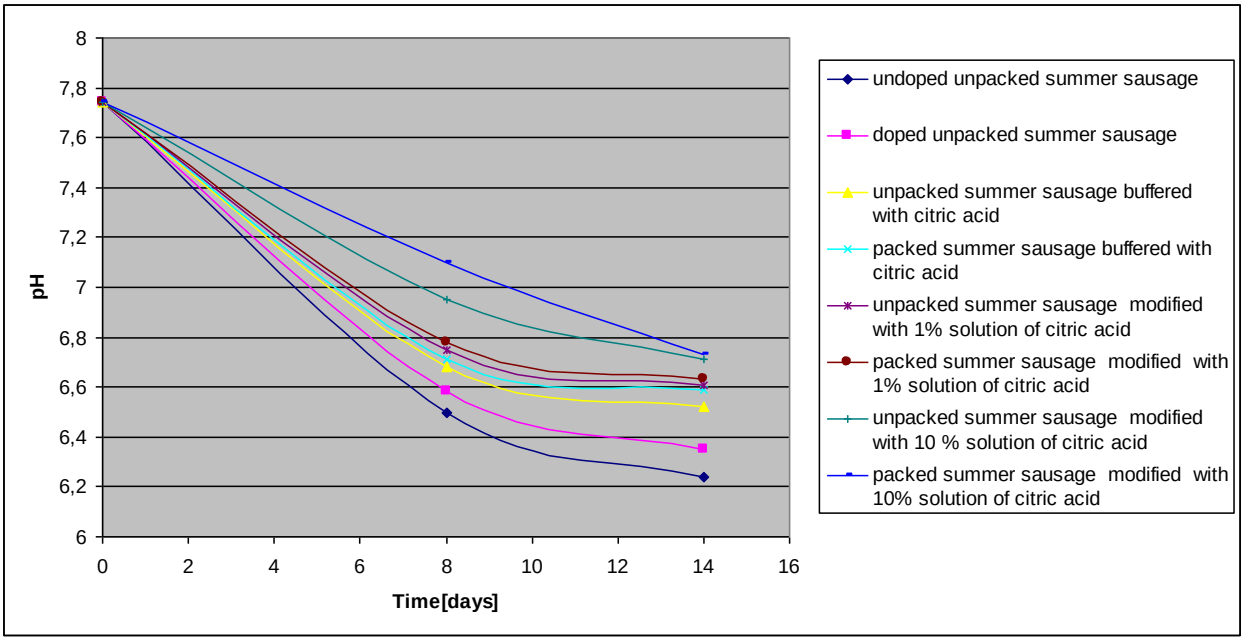


Figure 4. The variation of pH in summer sausage packed and unpacked.

In the samples in which the citric acid is not present, the oxidation processes occur more quickly as compared to the samples containing citric acid, which means that the deterioration is intensified. The water content (Figure 5) of the summer salami has been changed during deposition in the packages modified with citric acid. The water content of the summer salami kept in packages unmodified with citric acid decreases drastically in 14 days (from 67.60% to 47.69%) as compared with that of the sample modified with citric acid (from 67.60% to 56.84%). Samples that are not packaged salami lose more water, evaporating it and stay dry salami surface within 8 days and 14 days are dry salami inside.

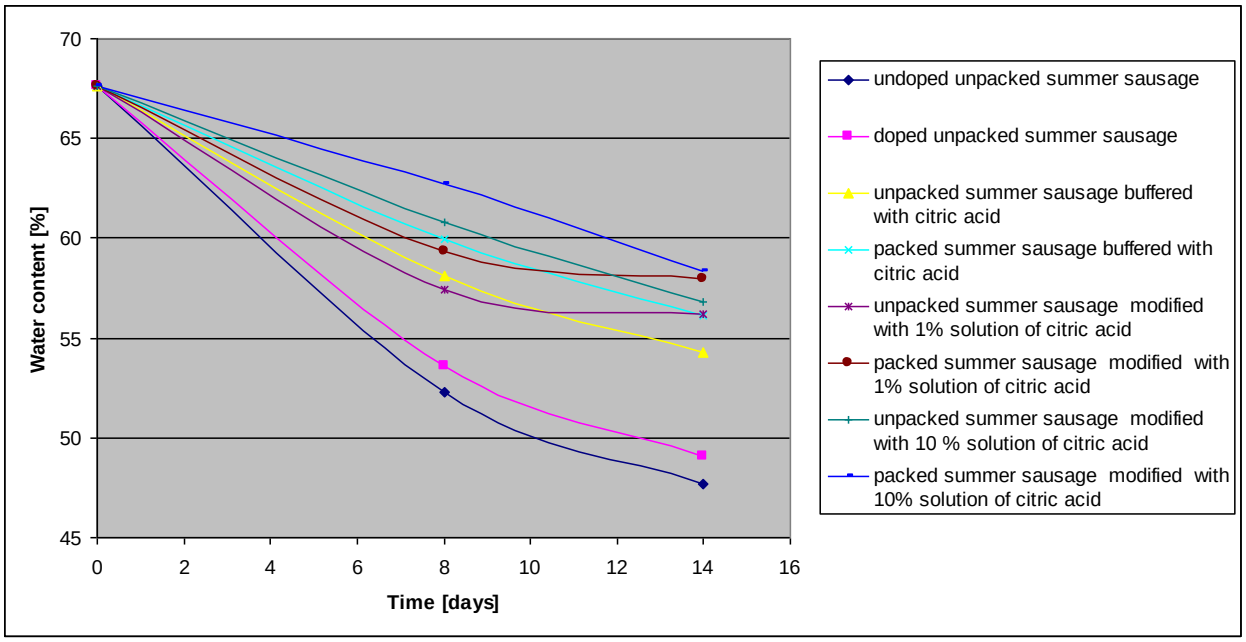
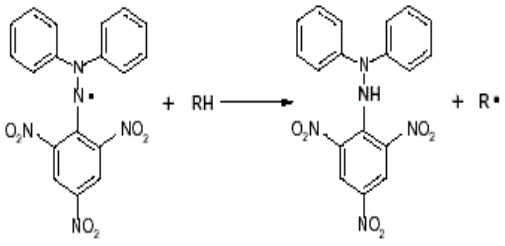


Figure 5. The variation of water content in summer sausage packed and unpacked.

Of all samples with citric acid, packed and unpacked, it's best to keep in storage, packed samples because the polyethylene foil prevents the reduction of water content being a barrier to oxygen and also are low oxidation processes in food, so the samples packed to its citric acid exhibits the best activity.

Antioxidant activity determines the number of compounds in the food structure able to oxidise and initiate degradation byproducts. The antioxidant activity was achieved with the DPPH (radical 1,1-diphenyl-2-picril hidrozil), which is reduced by the reducing agent (compound that oxidizes) and get in shape, and the reducing agent passes the oxidized (eq.3).



(eq.3)

The antioxidant activity (Figure 6) changes during storage in all the packages of sammer salami. In packaged samples of summer salami containing citric acid, the antioxidant activity increases, after 8 days of storage. In the samples packed in polyethylene modified with 10% solution of citric acid, the antioxidant activity increases from 56.61 % to 67.5 %.

After 14 days of storage, the antioxidant activity of the sample packed in polyethylene modified with 10% solution of citric acid decreased from 67.5 % to 63.76%. The antioxidant activity of the unpackaged sample decreased during storage. The antioxidant activity of the samples packed in polytehylene modified with 1% solution of citric acid decreased from 56.61 % to 51.17% after 8 days of storage and from 51.17% to 50.1 % after 14 days of storage. The antioxidant activity of the samples modified with citric acid decreased during storage in all the investigated package types, because the citric acid is oxidized in the presence of oxygen and antioxidant role is reduced.

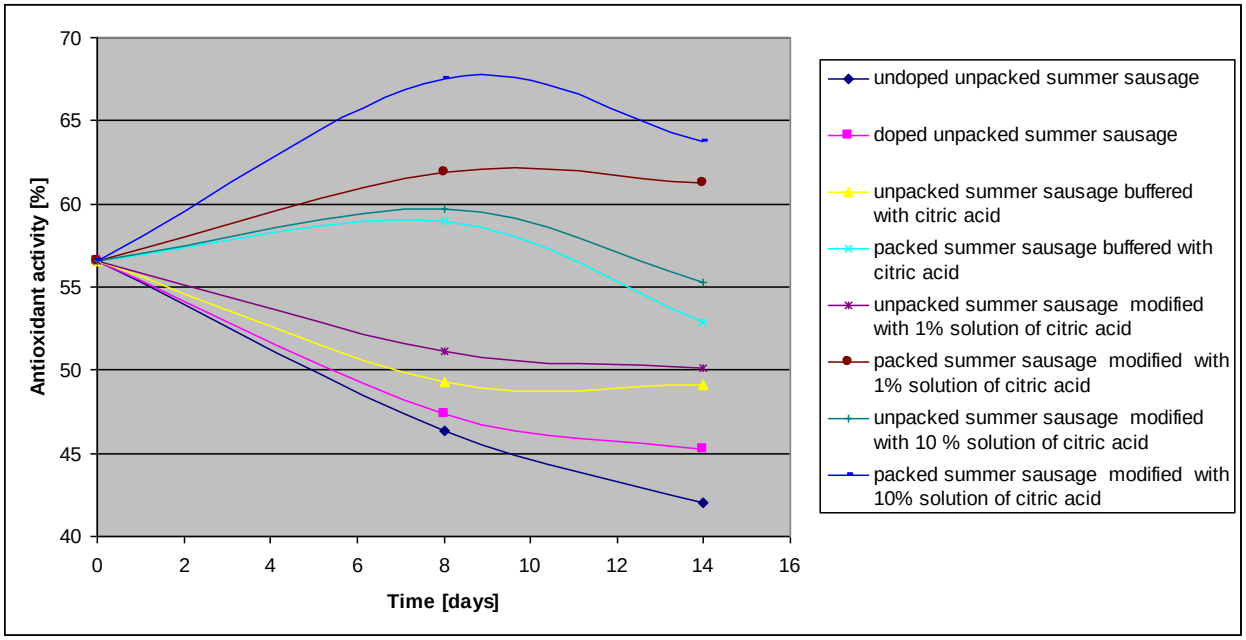


Figure 6. The antioxidant activity in summer sausage packed and unpacked.

4. Conclusions

This study have demonstrate that the citric acid can be successfully used as dopant that act as preservative during the summer salami storage at (4-5°C).

Of all the samples of summer salami modified with citric acid has been shown that the sample covered with citric acid solution 10% keeps the best summer sausage, due to the activity of citric acid preservative and antioxidant role it fulfills.

As a result, citric acid can be used with confidence to conserve summer sausage during storage.

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AGRIMONIA EUPATORIA L. EXTRACT INFLUENCE ON THE AGENT OF RHIZOCTONIA SOLANI KÜHN IN BEET

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ABSTRACT

We have analysed the influence of agrimony water-ethanol extract on the pathogenicity, the aggressiveness and growth of the pathogen the agent of the Rhizoctonia solani Kuhn in fodder beet, sort Pliska. In order to determine the influence of the extract on the pathogenicity and aggressiveness of the pathogen we conduct laboratory examination with root crops from the laboratory fields of the institute preliminary processed and non-processed with 10% water solution from extract, injured, not infected and infected with the pathogen. It is established that the preliminary processing decreases Rhizoctonia solani Kuhn with 0.5 grades, it delays the pathogen penetration with 0.5mm/25h and the relative quantity of putrefied mass with 3.5mg/100g. It suppresses the pathogen growth – for seven days the colonies diameter decreases with 2sm, and the dry mycelium mass with 1.5%.

Keywords: *extract, agrimony, pathogen, Rhizoctonia solani Kühn, fodder beet, putrefied mass, aggressiveness, mycelium.*

1. Introduction

The beet root decay is one of the most harmful blights for the crops. Regardless of the etiology this disease has heavy aftermaths for the beet production and it can cause preliminary dying of the crop when it appears in the crop as "cutting" in the beginning of the vegetation, or to highly reduced productivity as root decay and decay of the root crops at the end of the vegetation and during the storage period (Toporovskaya,1975; Seno et al.,1996; Ogoshi, 1987).

The agent of Rhizoctonia solani Kuhn is known in the science literature as a highly harmful pathogen mainly in the sugar beet. As an agent of the root decay and the root crops decay during the period of vegetation and storage, the soil pathogen Rhizoctonia solani Kühn is fixed for the conditions in Kyrgyzstan (Umralina et al.,1987), France (Comporota, 1989), Japan (Seno et al., 1996), USA (Allan et al.,2001). For Bulgaria, this is the most aggressive and dangerous agent of root decay and root crops decay during the vegetation of the sugar beet (Tanova, 2002). Isolated from the traditionally biggest regions of sugar beet production for the country – Veliko Turnovo, for Northern Bulgaria, Plovdiv and Stara Zagora – for Southern Bulgaria, this pathogen is with primary economic significance for producing raw materials for the sugar production. Practically an attack of the pathogen on a formed root crop with an index of 2 grades calculated by the 5 grade scale causes decrease of the sugar content with 3-6 points, considerably worsened quality and impossibility for the processing of the obtained raw material (Tanova, 2004).

The agent of Rhizoctonia solani Kühn for the conditions in Bulgaria is also isolated from fodder beet and it is found out that the isolates from the sugar beet also infect fodder beet from half-sugar sort. Our researches for the restriction of the Rhizoctonia solani Kühn show that applying chemical means is not sure and is expensive action, especially at the appearance and development after the formation of the root crops and their ripening (Tanova, 2008).

Similar results are obtained by Schnaider et al. (1986,1992**)** and Jacobsen et al. (1999). The problem leads the research to ecological ways and the usage of the power of the nature products in the field of the approaches for

restricting the pathogen. They are connected with plant protection (Durand, 2003).

According to Isman et al. (2006), the nature products used in plant protection, are biologically active substances as extracted or purified substances (ethereal oils, growth regulators, flavonoid compounds).The influence of extracts from Origanum marjoram, Lavandula vera, Hyssopus officinalis, Hypericum perforatum, Filipendula vulgaris, Cinnamomum verum, Pimpinella anisum, Nigella sativa, Syzygium aromaticum, Azadirachta indica, Aloe vera, Ocimum sanctum, Ocimum basilicum, Lantana camara, Artemisia vulgaris, Lantana camera, Michelia champaka, Passiflora foetida, Punica granatum, Strobilanthes flaccidifolius, Abrus precatorious, Acacia auriculiformis, Bougainvillea glabra, Convolvulus arvensis, Hibiscus rosa-sinensis, Morus alba, Thevatia peruviana and Withania somnifera as inhibitors of the mycelium growth of this pathogen fungus has been examined (Yakimov et al., 2008;Tanova et al., 2008; Abdulaziz, 2009;Gujar et al.,2012;Mangang et al.,2012; Sejapal, 2009). In vitro conditions the ethereal oils influence extracted from Origanum minutiflorum, Origanum onites, Thymbra spicata, Satureja cuneifolia, Zataria multiflora, Thymus vulgaris and Thymus kotschyanus on the pathogen has been tested (Dogmus Lehtijärvi, 2006;Amini et al., 2012).

In connection with the requirements of the modern agriculture for ecologically clean production and also because of the low effectiveness of the chemical means for restrictions in the Rhizoctonia solani Kühn in the beet with the current research we had a goal to test the influence of the Agrimonia eupatoria L. extract on the pathogenicity, aggressiveness, and the growth of the pathogen, agent of the Rhizoctonia solani Kühn in fodder beet.

2. Materials and methods

The research is executed in the laboratory at the phytopathology at the Agricultural Institute in Shumen in 2011. The experiment objects are root crops from half sugar sort of fodder beet sort Pliska, inoculated with agrimony extract.

The extract is obtained by modified fractional method by Yakimov from plants that inhabit the area of Shumen freely. As raw materials for the preparation of the extract are used blossoms and leaves from agrimony because the content of flavonoids in these parts is the highest (Correia et al., 2006).

For the purpose of this research we conduct a laboratory try with the following variants:

* Injured, not infected root crops, not processed with the extract.
* Injured, not infected root crops, processed with 10% water solution from the extract.
* Injured, infected root crops, not processed with the extract.
* Injured, infected root crops, processed with 10% water solution from the extract.

Every variant of the experiment consists of 20 root crops, in 4 repetitions. In laboratory conditions in vivo root crops from the half sugar sort fodder beet Pliska with weight 150-200g are injured and inoculated. For inoculum we use a ten day aggressive crop of the agent of Rhizoctonia solani Kühn isolated from root crops of the same sort of fodder beet. Before inoculation the root crops from variants 2 and 4 are processed with 10% water solution from the extract for 24 hours. The inoculation and the decay index reading are done by Shevchenko's approach by using 5 grade scale (1985). After the inoculation the root crops from all the variants are incubated in darkness in temperature 24-25 degrees Celsius in thermostat. When the first signs of decay appear, in a period of ten days, we check the diameters of the damages every day. In order to define the aggressiveness of the pathogen in the infected root crops we define the speed of its penetration by the damage diameter for a 3 day period.

To determine the influence of the extract on the pathogen growth we have conducted a laboratory test with seven days

pathogen crops cultivated in Chapec environment by adding 10% water solution from the extract in concentration of 20ml/l of nutritive environment. The cultures are cultivated at temperature of 25-26 degrees of Celsius in darkness. We periodically check the colonies diameter on the third, fifth and seventh day of their cultivation. By the weighing approach we figure out the quantity of dry mycelium mass produced for the relevant periods. In order to extract the produced mycelium mass from the nutritive environment we use the approach described by Nakova (1991). The obtained results after the statistic processing are presented in table and graphical forms.

3. Results and discussion

The research results are put in table 1. According to the data for the influence of Rhizoctonia solani Kühn attack index put in column 1 in the chart it turns out that the processing of the root crops with 10% water solution from the flavonoid extract decreases the grade of the decay with 0.5 for the variant with the infected root crops and 0.2 for the variant with the injured but not infected root crops. The pathogen penetration speed decreases with 0.5 mm/24h (column 2 and 3). The relative quantity of decayed mass for ten days period (column 4) decreases in the root crops processed with the extract. For the infected root crops the decrease of the relative quantity of decayed mass is with 3.5mg/100g mass and for the injured but not infected ones – 0.5mg/100g mass.

These results are significant for the stronger influence of the flavonoid extract from agrimony on the growth and aggressiveness of the agent of Rhizoctonia solani Kühn in fodder beet than the uninfected decay and its harmfulness. As a result of this influence the pathogen changes its aggressiveness by decreasing the relative quantity of decayed mass with 14%. In uninfected decay the decrease of this indicator is 5% (column 4).

The data from the laboratory test with the cultivated pathogen on nutritive environment containing flavonoid compounds are presented in table 1. The growth of the mycelium colonies in progress shows suppressing effect from extract addition in nutritive environment for cultivation. This effect is the most significant on the fifth day after the cultivation. For this period the difference in the colonies diameter length cultivated on clean nutritive environment and on environment with added extract is the biggest – 1.5cm, in favor of the cultivation on nutritive environment without the extract. It the beginning of the experiment (on the third day after the cultivation) and also in the end of the experiment we also see suppressing effect. The established differences for the both periods – the third and the seventh day after the cultivation are 1.0cm.

In the same chart we can also see the impact of the extract in nutritive environment for cultivation on the producing of dry mycelium mass for the same period as a supplementary factor for the growth. For production of dry mycelium mass given as %/100 dry mycelium mass we can also see a suppressing effect from the extract. This effect is the most significant at the end of the period – on the seventh day after the cultivation when the difference in gaining dry mycelium mass between the cultures is the biggest. As we can see from the figure the dry mycelium mass produced in cultivation by adding extract in nutritive environment has decreased with 1.0%/100g dry mass

The decrease after the third day of the cultivation is 0.5%/100g dry mycelium mass. The least is the influence of the extract on the production from dry mycelium mass after the fifth day of the cultivation - 0.3 %100g dry mycelium mass.

Table 1 The impact of flavonoid fractional extract from Agrimonia eupatoria L.on the aggressiveness of the agent of Rhizoctonia solani Kühn in beet

Variants	Attack	Penetration speed		Decayed mass
	scale	sm/24hours	%	mg/100g
Not infected injured root crops not processed with extract	0.5	1.5	15.0	10.0
Not infected injured root crops processed with extract	0.3	1.0	10.0	9.5
Infected injured root crops not processed with extract	2.0	10.0	100.0	25
Infected injured root crops processed with extract	1.5	9.5	95.0	21.5
GD 5%	0.23	0.36	2.30	3.60
GD 1%	0.34	0.53	3.40	5.30
GD 0.5%	0.49	0.80	4.90	7.90

Table 2 The impact of flavonoid fractional extract from Agrimonia eupatoria L.on the the mycelium growth of Rhizoctonia solani Kühn in beet.

Variants	Attack	Penetration speed		Decayed mass
	scale	sm/24hours	%	mg/100g
Not infected injured root crops not processed with extract	0.5	1.5	15.0	10.0
Not infected injured root crops processed with extract	0.3	1.0	10.0	9.5
Infected injured root crops not processed with extract	2.0	10.0	100.0	25
Infected injured root crops processed with extract	1.5	9.5	95.0	21.5
GD 5%	0.23	0.36	2.30	3.60
GD 1%	0.34	0.53	3.40	5.30
GD 0.5%	0.49	0.80	4.90	7.90

4.Conclusions

The preliminary processing of root crops from fodder beet, sort Pliska, with flavonoid extract from agrimony (Agrimonia eupatoria L.) in concentration 10%:

- Decreases the decay with 0.5 grades;
- Delays the pathogen penetration with 0.5mm/24h and the relative quantity of decayed mass for 100g with 3.5 grades.
- It suppresses the growth of the mycelium – for 7 days the diameter
- of the colonies decreases with 2 sm and the dry mycelium mass with 1.5%

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ANALYSIS OF NUTRITIVE VALUE AND BRAND MARKETING STRATEGY OF AMWAY NUTRILITE

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ABSTRACT

As an emerging health care product, protein powder enters into supermarket, drugstore and consumers' home with the development of social health industry. Protein powder is convenient to take. It is good protein with low fat and cholesterol. Long term medication will not lead to obesity, hyperlipidemia and cardiovascular and cerebrovascular disease. This paper plans to detect the ingredients of Amway Nutrilite and analysis its functions. It proceeds juvenile rats growing experiment to detect the quality of protein, analyzes the nutritive value and discusses the marketing strategy and product advantage in the perspective of nutriology.

Keywords: *Amway Nutrilite, protein powder, nutritive value, marketing strategy*

1. Introduction

There are various kinds of food. Every food is different in protein content and amino acid composition mode. Degree of digestion, absorb and utilization of human body are also different. The evaluation of nutrient value of protein is of great meaning to identification of food quality, research and development of new food resource, crowd dietary guidance, etc [1].

Food protein of high quality meets the demands of body for nitrogen source and amino acid and ensures healthy growth of body. Quality and quantity should be taken into account in evaluation of nutritive value of food protein. That is, the protein content of food, amino acid pattern of protein and digestion, absorption and utilization [2].

Traditionally, we study nitrogen balance or growth rate according to the nutrition of rats [3]. In means of growth rate detection, Experiment on protein efficiency ratio is widely applied this methods has widely used for predict the protein quality of protein required by human. It was adopted by American FDA for protein quality judgment in Nutritional Labeling and Education Act (NLEA). The improved methos based on PER is protein purification value. As to the evaluation of nitrogen balance, common indexes are digestibility, biological value (BV) and net protein utilization (NPU). This research carries out experiment according to AOAC standard. It works out PER, apparent digestibility (AD), true digestibility (TD), biological value and net protein utilization.

Digestibility of protein refers to the absorption degree of food protein after hydrolysis by digestive enzyme. It is expressed by ratio of absorption amount of nitrogen and total amount of nitrogen which can be used for detection of apparent digestibility and true digestibility. Biological value of protein is expressed as retention degree after protein absorption, which is the common method for measuring protein nutritive value [4]. Net protein utilization of protein reflects the degree of practical utilization of protein. It combines biological value and digestibility to evaluate

nutrient value of protein. Protein nutrition of protein powder is evaluated according to the growing of rats and above common indexes. And it is compared to other high quality protein. Then a relative comprehensive evaluation result is obtained.

2. Material and Method

2.1 Materials

Nutrilite protein powder; fat: edible peanut oil; starch: three brands of corn starch purchased in supermarket is detected protein content by kjeldah method; corn starch with low content of protein is used for fodder preparation (protein content < 1 %); cellulose: gold fruit and vegetable cellulose powder; inorganic salt: NaCl, KI, KH_2PO_4, $CaCO_3$, $MgSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 5H_2O$, $CoCl \cdot 6H_2O$; cellulose: vitamin AD drop, vitamin E soft capsule, vitamin K, folic acid, vitamin B12, compound vitamin B group.

Major reagent: sulfuric acid, copper sulfate, sodium hydroxide, hydrochloric aid, methyl red, bromocresol green, boric acid, absolute ethyl alcohol, distilled water. Instrument: electronic scales, kjeldahl apparatus and digestion furnace, digital thermostat water bath, micro burette. Other material: ruler, filter paper, grinding dish.

Laboratory animal: 36 SPF level male Wistar rats with early weaning, weight of 40 g; 12 SPF level male Wister rat, weight of 180 g.

2.2 Feed formula

According to AOAC method, groups of feed formula are shown in Table 1.

Table 1. Groups of feed formula (g/100g)

Ingredient	Nitrogen free group	Protein powder group	Casein group
Sample	0	12.5	10.1
Moisture	5.0	5.0	5.0
Fat	8.0	8.0	8.0
Starch	80.0	67.2	69.9
Cellulose	1.0	1.0	1.0
Mixed inorganic salt*	5.0	5.0	5.0
Mixed vitamin #	1.0	1.0	1.0
Protein (estimated value)	0.5	10.5	10.4
Heat energy (Keal)	394	382.4	393.2

Note: * each 1 kg mixed inorganic substance (g): NaCl 139.3 KI 0.79 KH_2PO_4 389.0 $CaCO_3$ 381.4 $MgSO_4$ 57.3 $FeSO_4 \cdot 7H_2O$ 27.0 $MnSO_4 \cdot H_2O$ 4.01 $CuSO_4 \cdot 5H_2O$ 0.44 $ZnSO_4 \cdot 5H_2O$ 0.54 $CoCl \cdot 6H_2O$ 0.023

each 1 kg mixed vitamin contain: VA2000IU (600ugRE) VD200IU (5ug)
VE 10IU (1mg α-tocopherol) VK 0.5mg choline 200mg
para aminobenzoic acid 10mg Inositol 10 mg niacin 4 mg
calcium pantothenate 4 mg VB2 0.5mg

2.3 Rats growth experiment

36 SPF level male Wister rats with early weaning and weight of 40 g were selected. The rates were divided into three groups and 12 for each group by randomized block method according to the weight. The average weight of animals between groups should not exceed 5 g and the weight within the group should not exceed 10 g. Rats would enter test period after 3 days of adaption period. The indoor temperature was (21±2) ℃ and the indoor relative humidity(RH) was (70±5)%. In the adoption period, rats were feed regular feed and free to eat and drink. In experiment period, rats were feed different formula of fodder for three days and conducted relative index test and observation.

Experimental animal feed was prepared refer to the feed formula requirements of PER determination method in AOAC method 982.30. All ingredients were mixed in Laboratory Animal Center in Southern Medical University. The protein content of protein powder group and casein feed group is 10.5% and 10.4%. Animals in nitrogen free group were feed nitrogen free feed to control the environment factor. All rats were feed in single cage and free for eating and drinking. Weight and height were detected and recorded every 3 days and at weekend. Food-intake was record every day. The experimental period is 28 days. The purpose of adding nitrogen free group is to observe accuracy of home-made fodder and eliminate the effect of environment [5].

2.4 Digestibility detection of Nutrilite powder

12 infancy male Wister rats of 180 g were conducted nitrogen metabolism experiment according to AOAC method 991.29. Every rat was moved into single stainless metabolic cage. After adopting the environment, the rats were divided into 2 groups and 6 for each group. Average value distinction of rats in every group was not exceed 5 g. When the experimental group was feed test fodder, the control group was also feed nitrogen-free fodder. The experiment was carried in SPF level animal room with （21±2）℃ of temperature range and 60%~70% of relative humidity. The rat fodder was feed fodder and water in united time every day. And the food should be limited for 15 g/day. Filter paper processed by 0.5 m sulfuric acid was used for avoiding excessive wetting of food by urine. Experiment was divided into 4 days of initial stage and 5 days of average stage (total of 9 days). In the 5 days of balance period, food consumption was weighed every day. And overflowed food and feces of every rat were collected every day. Rat urine was collected by sealed container. Sulfuric acid filter paper was put into the container and 0.5 m sulfuric acid was used for washing funnel in metabolism cage. And the washing fluid was also put into the container. Te overflowed food was air dried and weighed. Rat feces were dried to constant weight in the vacuum drying oven. Then it was weighed and grinded. Then kjeldah method was used for detecting nitrogen. Urine, filter paper and sulfuric acid were fully mixed, constant volumed and then detected the nitrogen content by kjeldah method [6].

Fecal and urine nitrogen of experiment animals are detected by kjeldah method. The specific method is as same as the former.

2.5 Experimental index and detection method

(1) Protein content in fodder: detect by kjeldah method

(2) Detection of food consumption: food consumption= food input- surplus (the food intake of rats should be recorded every day and food intake of every week should be counted)

(3) Protein intake in experimental period= experimental animals intake * protein intake in fodder

(4) Protein efficiency ratio= increased weight (g)/ protein intake (g) *100. PER calculation was corrected and compared to standard PER value of casein (2.5)

(5) Protein apparent digestibility= (food nitrogen-fecal nitrogen)/ food nitrogen

(6) Protein true digestibility= (food nitrogen - (fecal nitrogen-fecal nitrogen metabolism)/ food nitrogen

(7) Biological value (BV) = retention nitrogen/ absorption nitrogen * 100

Absorption nitrogen= food nitrogen-(fecal nitrogen- fecal nitrogen metabolism)

Retention nitrogen= absorption nitrogen- (urine nitrogen - urine endogenous nitrogen)

(8) Protein net utilization= retention nitrogen/ food nitrogen

(9) Calculation of protein digestibility corrected amino acid score (PDCAAS)

AAS = amino acid content in every gram in protein (or protein) (mg)/ amino acid content in every gram (or protein) in ideal pattern (mg)*100%

PDCAAS= protein digestibility (TD)*AAS. The maximum value is 1.0. The result above 1.0 was all recoded as 1.0. It expresses that protein can provide 100% amino acid to meet the demand of human body after digestion.

Experimental result is expressed as mean ± standard deviation. One-way analysis of variance was applied in SPSS 11.0 statistical software for statistical analysis.

3.Result and discussion

3.1 Growth experiment of rats

Growth change value of experimental animals is shown in Table 2 after four weeks of feeding. Statistical analysis shows that protein in protein powder group has no significant distinction (P> 0.05) in height and weight compared to casein group.

Table 2. Growth experiment of rats

Time	Height (cm)				Weight (g)			
(week)	Protein powder group	Casein group	F value	P value	Protein powder group	Casein group	F value	P value
0	11.1±0.42	11.3±0.40	0.000	>0.05	38.8±3.10	40.2±3.28	0.001	>0.05
1	13.8±0.25	13.6±0.49	2.252	>0.05	73.4±5.57	73.4±6.01	0.013	>0.05
2	16.1±0.54	16.1±0.49	0.285	>0.05	107.6±5.69	107.6±5.69	1.111	>0.05
3	18.3±0.49	18.1±0.43	2.129	>0.05	144.7±10.35	144.7±10.35	0.003	>0.05
4	20.0±0.34	19.6±0.40	2.703	>0.05	183.1±15.14	183.1±15.14	0.017	>0.05

3.2 Nutrient evaluation of Nutrilite protein

Research shows that food utilization of Nutrilite protein powder group have no significant distinction (P=0.365) compared to casein group. PER of Nutrilite protein Powder is 2.22±0.08. It is a kind of high quality protein [7], as shown in Table 3. Nitrogen metabolism of animal experiment is shown in Table 4. AD, TD, BV, and NPU result of Nutrilite protein is shown in Table 5.

Table 3. Nutritional evaluation index of animal experiment of Nutrilite protein powder ($\bar{x}\pm$s, n=12)

Index	Nutrilite protein powder group	Casein group	F value	P value
Weight increment (g)	152.08±14.78	146.63±17.61	0.562	0.463
Food intake (g)	461.19±32.59	434.88±31.99	3.328	0.085
Food utilization (%)	32.93±1.15	33.01±2.02	0.865	0.365
PER	3.12±0.11	3.50±0.21	26.972	0.000
Correction PER	2.22±0.08	2.5*	128.201	0.000

Note: * PER value of casein in standard situation

Table 4. Intake and discharged nitrogen in nitrogen metabolism experiment ($x\pm$s, n=6)

Index	Nutrilite protein powder	Nitrogen free group
Intake nitrogen (g)	0.920±0.124	0.129±0.012
Fecal nitrogen (mg)	4.954±1.337	3.642±1.511
Urine nitrogen (mg)	92.726±42.493	32.921±15.654
Fecal nitrogen metabolism(mg N/g)		0.545±0.139
Urine endogenous nitrogen (mg N/g)		0.818±0.356

Table 5. Apparent digestibility, true digestibility, biological value and net utilization of Nutrilite protein

Index	Nutrilite protein powder (%)
Apparent digestibility (AD)	99.45±0.16
True digestibility (TD)	99.46±0.16
Biological value (BV)	93.14±5.43
net protein utilization (NPU)	92.60±5.91

Research result on evaluation index of protein nutrient value shows that AD and TD reaches 99.45% and 99.46%. BV is 93.14% and NPU is 92.60% as shown (Table 5). PDCAAS's full score is 1.0 (Table 6), which was constant with casein, whey protein, soy protein and egg protein.

Table 6. PDCAAS of Nutrilite protein powder

EAA species	EAA content in protein powder (mg/g protein)	FAO/WHO/UNU Amino acid pattern of preschool child (mg/g protein)	AAS	PDCAAS
He	48.4	28	172.9	1.0
Leu	96.6	66	146.4	1.0
Lys	78.4	58	135.2	1.0
Met+Cys	30.2	25	120.8*	1.0*
Phe+Tyr	80.1	63	127.1	1.0
Thr	41.5	34	122.1	1.0
Trp	12.5	11	113.6	1.0
Val	48.5	35	138.6	1.0

Animal experimental result shows that rats have good digestibility on Nutrilite protein powder. It can obviously promote growth and development of animals and balance nitrogen metabolism. This experiment detected that PER of Nutrilite protein powder was close to casein and evaluation indexes such as apparent digestibility, true digestibility, biological value, net protein utilization, etc were all high [8]. It illustrates that Nutrilite protein powder have a high nutrient value of protein.

3.3 Discussion

This research studied the nutrient value of Nutrilite protein powder by biological method. PER method is the official method speculated by International AOAC. It is a biological analysis method used for detecting protein content from different food or food ingredients. Weight increasing of male rats in weaning period feed by Nutrilite protein was compared to another control group of rats feed on casein as only dietary source. The higher protein quality is, the better animals grow. In America, food industry regards PER as protein quality standard of food protein. Daily recommended amount of protein on food label speculated by FDA is also based on PER.

Protein quality of Nutrilite protein powder was compared to casein control group. Protein whose PER exceed 2.0 is regarded as high quality. 1.5~2.0 is medium quality. Less than 1.5 is regarded as low quality. This paper made an experimental research on the nitrogen metabolism of rats in growing period taking Nutrilite protein as nitrogen source [9]. The result shows that rats have a good digestion on Nutrilite protein powder and Nutrilite protein powder can obviously promote growth and development of animals and balance nitrogen metabolism. PER result of protein is 2.22, which hints that Nutrilite protein is of high quality. Protein digestibility and biological utilizability of amino acid were contained in experimental range to some extend because PER method is in vivo test.

Protein content, amino composition, digestibility and utilization are important factors in determining protein value. Protein digestibility is an index reflecting the degree of decomposition and absorption of food protein in digestive tract. It refers to the percentage of absorbed protein account for intake protein in digestive tract. It is a biological method for evaluating nutritive value of food protein.

Digestibility can be divided into apparent digestibility and true digestibility according to whether endogenous fecal nitrogen metabolic factor is taken into account. Apparent digestibility disregards the protein digestibility of endogenous feces. And true digestibility takes fecal metabolism digestion into account.

Biological value is a biological method for evaluating nutrient value of protein. It refers to the quality of human body protein transformed from every 100 g of food source protein. BV reflects the complexity of protein applying for protein tissue of human body protein after digestion in vivo. PDCAAS is evaluation index of protein quality based on the demand of amino acid and digestion ability [10].

Biological value of Nutrilite protein is close to eggs and higher than fish, beef and soybean. The apparent digestibility corrected amino acid score of protein is 1.0. It is constant with high quality protein such as whey protein [11], casein, egg protein, soybean, etc. Nutrilite protein is health food with high nutrient value. It is easy to digested and absorbed. It can promote growth and development and is the source of good protein.

4. Conclusion

Nutrilite protein powder has a high content of protein. And its amino acid composition pattern is very reasonable. BCAA content of Nutrilite protein powder is higher than meat, milk and eggs. Its amino and chemical score is higher than isolated soybean protein. It has a low content of fat and a certain amount of phospholipid. It also contains certain amount of micronutrient such as choline, folic acid, Fe, Zn, Se, etc and daidzein. Scientific ratio of whey protein and isolated soybean protein keeps the functional component as possible.

Rats experiment shows that Nutrilite protein powder is easy to digest and absorption and can promote the growth and development of animals and balance nitrogen metabolism. PER of protein is close to casein. Evaluation index such as apparent digestibility, true digestibility, biological value and net protein utilization are high.

And its protein nutritive value is also high. We gather the most nutritious part on the most suitable soil by method closet to nature in the period of most nutrient value. The high nutritive value and product quality ensure the competition of Amway in similar products.

Nutrilite is a nutrition food brand whose organic farming, nutrient extract, production and processing, sales and service are all shouldered by one corporation, which is rare in the world.

Creating food with high nutritive quality from the source is also marketing strategy and magic weapon of Amway.

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IMPACTS OF WATER PROPERTIES ON THE QUALITY OF STEAMED BREAD

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ABSTRACT

The main aim of this study was to provide steamed bread industry with theoretical knowledge about the properties of water used in steamed bread production. This paper analyzed the different water properties, including water content, water temperature, water hardness, and water pH value on the quality of steamed bread. The result showed that to produce steamed bread with high quality, the water content, water hardness and water pH value should be 46%, 1% and 6 separately and water temperature should be controlled between 25°C to 35°C. The mechanism for the phenomena discovered in this study showed that water properties can impact a lot of factors, such as the activity of yeast, physical properties of dough and activity of enzymes, which are critical to the quality of steamed bread.

Keywords: *Water; Steamed bread; Specific volume; Whiteness; Sensory evaluation; Mechanism*

1. Introduction

As a traditional refined flour food, Chinese steamed bread is favored by a lot of people in Eastern Asian countries (Hao & Beta, 2012). The property of Chinese steamed bread is a major concern in the food industry. A lot of factors, particularly the processing conditions and additives, which can influence the quality and sensory scores of Chinese steamed bread, have been analyzed (He, Liu, Javier Peña, & Rajaram, 2003; Ying, Jun, Yunzi, Zhuo, & Zaigui, 2009). For instance, compared with the conventional milling, the milling after debranning showed positive impacts on the quality score, volume and structure of Chinese steamed bread (Lijuan, Guiying, Guoan, & Zaigui, 2007). The addition of dietary fiber can improve the water holding ability of dough in the steamed bream production (Ming-Yin, Yung-Ho, Sy-Yu, & Cheng-Chang, 2012; Phimolsiripol, Mukprasirt, & Schoenlechner, 2012). These studies provided steamed bread processing industry with a lot of theoretical knowledge. However, the study on impacts of water properties on the quality and sensory score of steamed bread was rare.

Water, which can influence the rheological properties of dough, is an important content in refined flour food. How to improve the quality of refined flour food by adjusting the water properties is a hot topic in food industry. Some research on the quality of bread indicated that high water content, which caused an increase in the percentage volume of pores and irregular distribution of very large bubbles, is unfavorable to the quality of bread (Mastromatteo, Guida, Danza, Laverse, Frisullo, Lampignano, et al., 2013). Chemicals contained in water can also impact the properties of bread (Gómez, Jiménez, Ruiz, & Oliete, 2011). Previous study on bread indicated that some water properties can influence the quality of bread greatly. However, the impact of some water properties, such as hardness and pH value has not been analyzed.

In this study, to provide the steamed bread processing industry with theoretical

knowledge, the impact of water content, water temperature, water pH value, and water hardness on the quality and sensory evaluation of Chinese steamed bread were analyzed. Furthermore, the mechanism for these phenomena was discussed in detail.

2. Materials and methods

2.1. Chemicals

Flour for steamed bread, edible acetic acid and sodium bicarbonate were obtained from supermarket; dry yeast was obtained from Angel Yeast Co., Ltd.

2.2. Water properties

In this study, water content (%) referred to the ratio of water weight and total weight of water, flour and yeast. $CaCO_3$ was used to adjust the water hardness and the water hardness was expressed as the ratio (%) of weight of $CaCO_3$ and weight of water. Water temperature was measured by the thermometer. Edible acetic acid and sodium bicarbonate were used to adjust the water pH value to the expected level and the water pH value was evaluated by pH test paper.

2.3. Steamed bread making and evaluation

The main steps of making steamed bread were listed as follows. 48 mL distilled water and 1g dry yeast were added into 100g flour. The mixture of water, flour and yeast was stirred and then kneaded for 3 min. Then the dough was formed. The dough was fermented at 38ºC for 60 min, then removed and kneaded for 3 min again. The dough was formed into the shape of steamed bread and fermented at 38ºC and 85% humidity for 15 min. Then the dough was steamed for 20 min, then moved out and cooled to ambient temperature (Fan, Sun, Zhao, Ma, Li, & Li, 2009). In the analysis of impacts of water content on steamed bread quality, the amount of water added into flour would be changed. In the analysis of impacts of water hardness and water pH value on steamed bread, the hardness and pH value of distilled water were adjusted before being added.

The volume and weight of steamed bread were measured and the specific volume (mg/mL), which was the ratio of volume and weight of steamed bread, was calculated. The whiteness of steamed bread was evaluated by whiteness meter according to GB/T 12097-89, which was set by the Standardization Administration of the People's Republic of China. In this study, the sensory score of steamed bread was evaluated according to the Ministry of Commerce of People's Republic of China standard (SB/T10139-93) by an evaluation team with 10 members (Fan, Sun, Zhao, Ma, Li, & Li, 2009). The main content of this standard was listed in Table 1.

3. Results and disscussion

3.1. Impacts of water content on steamed bread

Impacts of water content on specific volume, whiteness and sensory scores of steamed bread were shown in Figure 1, Figure 2 and Table 2, separately.

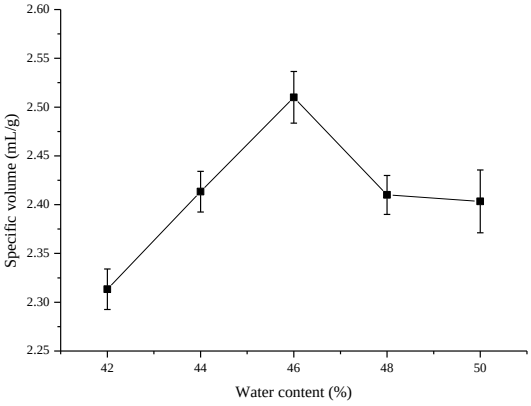

Figure 1. Impact of water content on specific volume (n=3)

When the water content was 46%, the specific volume and whiteness of steamed bread reached the highest points. When the water content was higher than 46%, the specific volume and whiteness of steamed bread decreased obviously. The sensory score of steamed bread was highest (94.7%) when the water content was 48%. However, there was no significant difference between sensory scores when the water contents were 46% and 48%. Therefore, the most appropriate water

content in steamed bread production should be 46%.

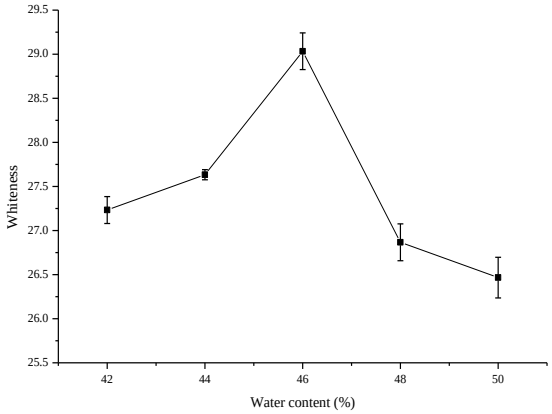

Figure 2. Impact of water content on whiteness (n=3)

When the water content was below 46%, the yeast was not active in fermentation process. So the foaming action was not obvious and the specific volume was low. With the increase of water content, the activity of yeast and the specific volume increased greatly. Benzoyl peroxide phthalocyanine which was an important brightening component added in flour absorbed water and produced oxidizing materials which determined the whiteness of steamed bread. When the water content was low, the process of producing oxidizing materials was thwarted. So the whiteness of steamed bread was negatively impacted. When the water content was higher than 46%, the density of flour mixed with water increased. The high density prevented the foaming action of yeast in fermentation process. So high water content was unfavorable to the specific volume of steamed bread. When the water content was low the fermentation process was negatively impacted. So the exterior appearance, elasticity and stickiness of steamed bread were low when the water content was below 46%. With the increase of water content, fermentation process was promoted and the sensory score increased.

3.2. Impacts of water temperature on steamed bread

Data in Figure 3 and Figure 4 indicated that specific volume and whiteness of steamed bread decreased with the increase of water temperature. Table 3 indicated that the total sensory score remained stable when the water temperature was between 25°C and 35°C and decreased obviously when the water temperature was above 35°C. Therefore, in order to improve the specific volume, whiteness and sensory score of steamed bread, the water temperature in steamed bread production should be controlled between 25°C and 35°C.

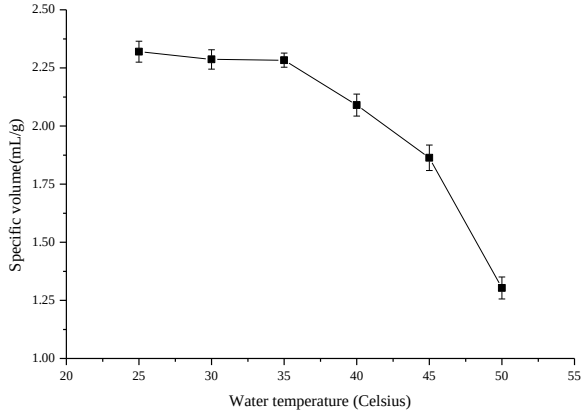

Figure 3. Impact of water temperature on specific volume (n=3)

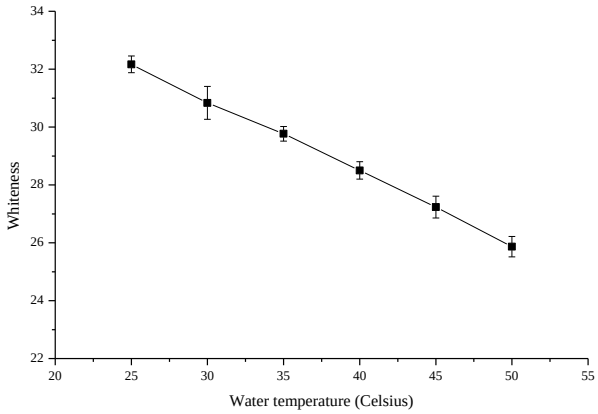

Figure 4. Impact of water temperature on the whiteness of steamed bread (n=3)

In the fermentation, the yeast can produce carbon dioxide which causes a foaming action in the production of steamed bread. High water temperature damaged the activity of yeast in fermentation. Then the foaming action of yeast was negatively

impacted. Figure 3 showed that 35°C is a critical point after which the activity of yeast in fermentation was seriously damaged. If the water temperature was controlled from 25°C to 35°C, specific volume of steamed bread can be 2.3mL/g or higher. Some enzymes, such as lipoxygenase, in flour can increase the whiteness of steamed bread (Zhang, Zhang, Lu, Bie, Zhao, Wang, et al., 2013). In this study, the whiteness of steamed bread decreased with the increase of water temperature. The main reason for this phenomenon is that in the high temperature water the enzyme became inactive and the catalytic activity was damaged. So the whiteness of steamed bread decreased seriously in hot water. Due to the damage of high temperature to enzymes in flour, the smell, exterior appearance and structure of steamed bread decreased when the water temperature was above 35°C.

3.3. Impacts of water hardness on steamed bread

Data in Table 4 indicated that the score of structure and elasticity increased with the increase of water hardness although the total sensory score remained stable when the water hardness was changing. So in order to improve the structure and elasticity of steamed bread, water hardness should be increased.

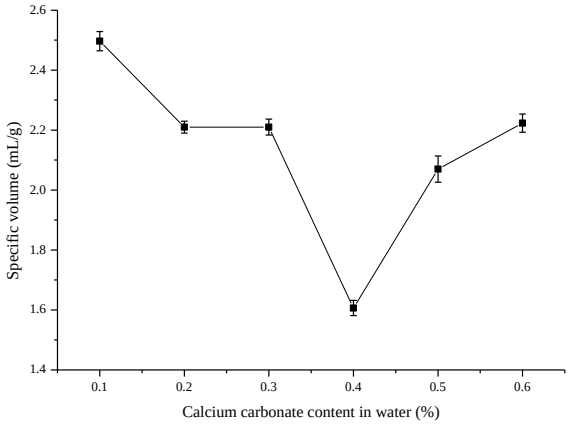

Figure 5. Impact of water hardness on specific volume (n=3)

The values of specific volume and whiteness were highest when the water hardness was 0.1%. So to increase the specific volume and whiteness of steamed bread, water hardness should be reduced. Therefore, in steamed bread production, high water hardness can improve structure and elasticity at the expense of whiteness and specific volume. In order to ensure the total quality of steamed bread, the water hardness should be adjusted according to the specific requirement on steamed bread quality.

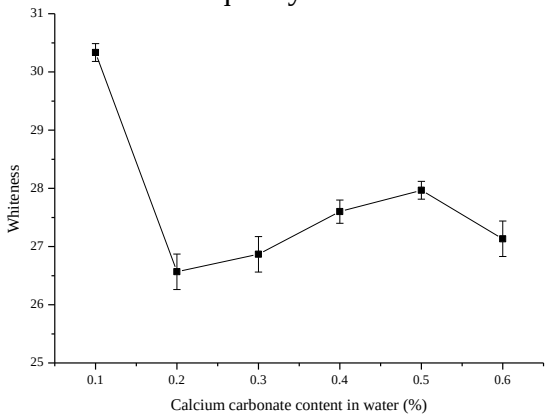

Figure 6. Impact of water hardness on whiteness (n=3)

Hard water contained more calcium ions which could modify gluten protein network structure. The gluten protein network structure can improve the scores of structure and elasticity. It was reported that citric acid could be used to modify gluten protein network structure (Chiou, Jafri, Cao, Robertson, Gregorski, Imam, et al., 2013). This study discovered that calcium ion could also be used in the modification of gluten protein network structure. Hard water could negatively impact the whiteness because the impurities in water could reduce the brightness of steamed bread surface. The impurities ($CaCO_3$) in water increased the density of steamed bread. As a result, the foaming reaction in fermentation was negatively influenced and the specific volume was reduced.

3.4. Impacts of water pH value on steamed bread

The result indicated that specific volume, whiteness, and sensory score of

steamed bread increased with the increase of water pH value (from 3 to 6) and then decreased with the increase of water pH value (from 6 to 9). The highest specific volume, whiteness and sensory score were 2.38mL/g, 30.08, and 93.2, separately, when the water pH value was 6. Therefore, the most appropriate water pH value in steamed bread production should be 6.

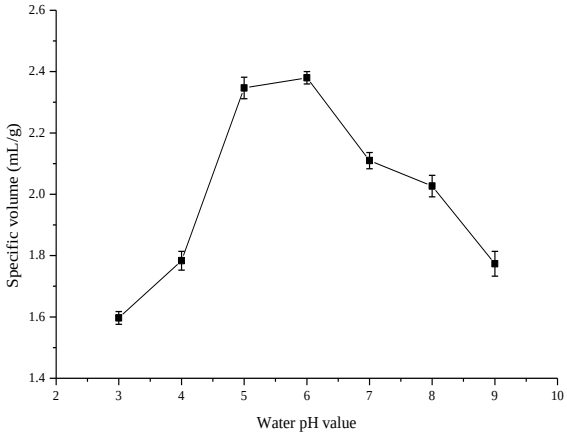

Figure 7. Impact of water pH value on specific volume (n=3)

High and low water pH values could prevent the growth of yeast in fermentation, even kill the yeast. Therefore, when the water pH value exceed the pH range preferred by yeast, the fermentation of steamed bread was thwarted.

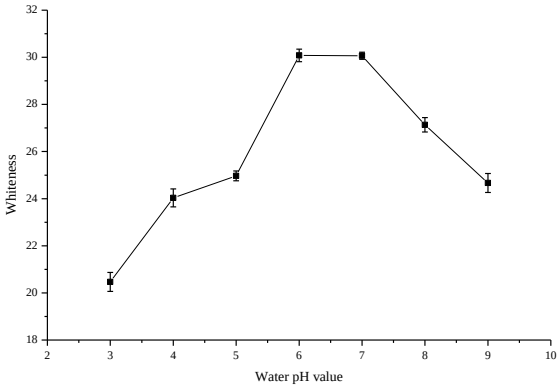

Figure 8. Impact of water pH value on the whiteness (n=3)

Then the specific volume and whiteness were negatively influenced. Furthermore, the scores of structure, stickiness, and elasticity were reduced by high and low water pH value because of the ineffective fermentation (Mustafa, Fink, Kamal-Eldin, Rosén, Andersson, & Åman, 2009). Therefore, in the production of steamed bread, water pH value should be adjusted according to the preferred pH range of yeast.

Table 1. Evaluation criteria of the sensory score of steamed bread (based on SB/T10139-93) (Fan, Sun, Zhao, Ma, Li, & Li, 2009)

Items	Score	Evaluation criteria
Specific volume	1-20	When the specific volume is 2.3 mg/mL, the score is 20; the score decreases by 1 point when the value of specific volume decreases by 0.1 mg/mL.
Exterior appearance	1-15	12.1–15: Crust is smooth; shape is symmetrical and stands upright; 9.1–12: middling; 1–9: crust is coarse; shape is asymmetrical and hard agglomerations occur.
Color	1-10	8.1–10: White or milk-white; 6.1–8: middling; 1–6: gray or dark.
Structure	1-15	12.1–15: Pores of longitudinal cut side are small and symmetrical; 9.1–12: middling; 1–9: pores of longitudinal cut side are large and asymmetrical.
Elasticity	1-20	16.1–20: Good rebound after pressing with a finger, strong to the bite; 12.1–16: middling; 1–12: poor rebound after pressing with a finger, soft to the bite.
Stickiness	1-15	12.1–15: Sample feels dainty and does not stick to teeth when steamed bread is chewed twice; 9.1–12: middling; 1–9: sample sticks to teeth when chewed.
Smell	1-5	4.1–5: Has fragrance of wheat and pleasant taste; 3.1–4: middling; 1–3: unpleasant taste.
Total score	100	

Table 2. Impact of water content on sensory scores of steamed bread (n=10)

Water content (%)	Specific volume	Exterior appearance	Color	Structure	Elasticity	Stickiness	Smell	Total score
42	20	13.4	8.2	13.5	17.5	12.4	4.2	89.2
44	20	14.1	8.2	14.2	18.4	13.1	4.3	92.3
46	20	14.8	8.5	14.3	18.9	13.2	4.4	94.1
48	20	14.6	8.4	14.9	18.8	13.3	4.5	94.5
50	20	14.2	7.9	14.4	18.8	11.7	4.2	91.2

Table 3. Impact of water temperature on sensory scores of steamed bread (n=10)

Water temperature (°C)	Specific volume	Exterior appearance	Color	Structure	Elasticity	Stickiness	Smell	Total score
25	20	15	9.6	13.2	16.8	14.7	4.8	94.1
30	20	14.5	9.8	12.5	15.6	14.9	4.9	92.2
35	20	14.6	8.9	12.3	15.1	15	4.1	90
40	17	10.8	7.7	10.2	13.4	12.2	3.5	74.8
45	14	9.4	6.8	9.5	10.5	10.4	2.9	63.5
50	10	8.9	5.9	7.1	9.2	8.3	2.9	52.3

Table 4. Impact of water hardness on sensory scores of steamed bread (n=10)

Water hardness (%)	Specific volume	Exterior appearance	Color	Structure	Elasticity	Stickiness	Smell	Total score
0.1	20	14.3	8.6	12.4	15.7	14.6	3.8	89.4
0.2	19	14.2	7.9	12.9	16.2	14.8	3.4	88.4
0.3	19	13.6	8.3	13.2	16.3	14.6	3.4	88.4
0.4	13	14.2	8.5	13.4	17.8	14.2	3.1	84.2
0.5	17	13.5	8.2	14.5	18.2	13.8	2.9	88.1
0.6	19	14.2	8.0	14.2	18.5	14.2	2.7	90.8

Table 5. Impact of water pH value on sensory scores of steamed bread (n=10)

Water pH value	Specific volume	Exterior appearance	Color	Structure	Elasticity	Stickiness	Smell	Total score
3	13	8.2	7.6	8.6	12.6	7.6	2.3	59.9
4	14	10.4	8.4	8.9	14.3	9.7	3.5	69.2
5	20	12.3	9.2	11.4	16.5	10.5	4.6	84.5
6	20	13.1	9.1	13.2	18.9	14.1	4.8	93.2
7	18	11.5	8.6	12.6	16.6	12.3	4.7	84.3
8	17	9.2	8.2	10.3	10.4	10.5	3.9	69.5
9	14	8.4	7.9	9.5	10.3	9.8	3.8	63.7

4.Conclusions

In order to produce steamed bread with high quality, the water content, water hardness and water pH value should be 46%, 1% and 6 separately and water temperature should be controlled between 25ºC to 35ºC. The mechanism for the phenomena discovered in this study showed that water properties can impact a lot of factors, such as the activity of yeast, physical properties of dough and activity of enzymes, which are critical to the quality of steamed bread.

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MONITORYNG OF NITRITE LEVELS IN FOOD LIQUID SAMPLES

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ABSTRACT

This study was performed to determine nitrite levels in food liquid samples (mineral water, wine, juice). A number of 17 brands of mineral waters, 6 brands of wine and 13 brands of juices were analysed. Nitrite levels were measured by spectrophotometrical method, by comparing the colour intensity at 540 nm with standard nitrite solution (red colour). The method provides a sensitivity of 0.005 mg NO_2/l. In the samples Juice 5 (3.64 mg NO_2^-/L) and Wine 4 (3.4 mg NO_2^-/L), the concentration of nitrite exceeds by far the maximum accepted contaminant level of 0.3 mg/L. Also, the following samples of food liquids: Wine 1 (0.49 mg NO_2^-/L), Wine 2 (0.31 mg NO_2^-/L), Wine 3 (0.64 mg NO_2^-/L), Juice 1 (0.44 mg NO_2^-/L), Juice 2 (0.67 mg NO_2^-/L), Juice 3 (0.26 mg NO_2^-/L), Juice 4 (0.68 mg NO_2^-/L), Juice 6 (0.48 mg NO_2^-/L), Juice 7 (2.12 mg NO_2^-/L), Juice 10 (2.46 mg NO_2^-/L), Juice 12 (0.57 mg NO_2^-/L) and Juice 13 (0.66 mg NO_2^-/L) can reach or exceed the maximum contaminant level (0.3 mg NO_2^-/L).

Keywords: *food liquids, mineral water, wine, juice, nitrite content.*

1. Introduction

Water is an important factor in the ecological balance and its pollution is a major global problem with more or less serious impact upon population. Water pollution means alteration of physical, chemical and biological characteristics of water. Water pollution can be caused directly or indirectly by human activities, which make the water unfit to use [1].

Nitrates and nitrites are natural compounds of ground, coming from mineralization of nitrogenous substances of vegetable and animal origin. Microorganisms in the soil, water and sewage change the nitrate to nitrite.

Plants use nitrites from the soil to satisfy nutrient requirements and may accumulate nitrites in their leaves and roots; due to its high mobility, nitrite also can leach into groundwater and surface water [2].

The major sources of nitrates in drinking water are runoff from fertilizer use, leaking from septic tanks, sewage, animal wastes and erosion of natural deposits.

Usually plants take up these substances hence through food and water, nitrates and nitrites reach in animals and human nutrition [3].

Nitrites value in water has to be 0 mg NO_2^-/L and maximum accepted contaminant level 0.3 mg NO_2^-/L [4]. Higher nitrite levels in water are toxic for public health being responsible for possible occurrence of blue baby syndrome and block the respiratory system.

The present work proposes to monitor the nitrites levels in liquid samples (mineral water, wine, juice) and to determine the analysed samples reaching or exceeding maximum contaminant level.

2. Materials and methods

The nitrites level is determined by the spectrophotometrical method by measuring the absorption of the azoic red compound, resulting from the acid nitration of aromatic amine with nitrite. The method provides a sensitivity of 0.005 mg NO_2/l [5].

The analyse was performed on 17 brands of mineral waters, 6 brands of wine and 13 brands of juices. In case of the pulp juice, the sample was filtered before analysis. To a volume of 10 ml of the free-solid suspensions sample, 2 ml of acetate solution of α- naphtylamine and 2 ml of sulfanilic acid solution were added. After 20 minutes, the absorbance at 520 nm was read by using a UV-VIS PG Instruments spectrophotometer.

The calibration curve (Figure 1) was developed by measuring the absorbance of several solutions of known concentration.

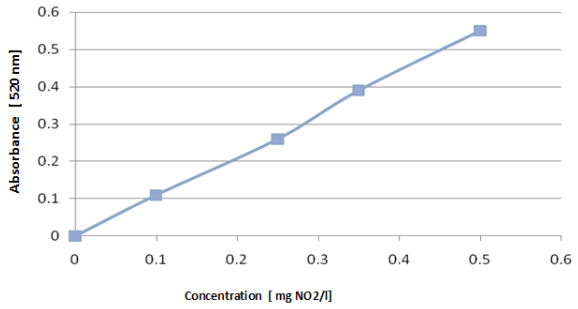

Figure 1. Calibration curve of the nitrite ions

3. Results and discussion

3.1. Nitrite levels in mineral water

A number of 17 brands of mineral waters were analysed to determinate the nitrite levels. Fig. 2 shows the nitrite concentrations in the investigated brands of mineral water.

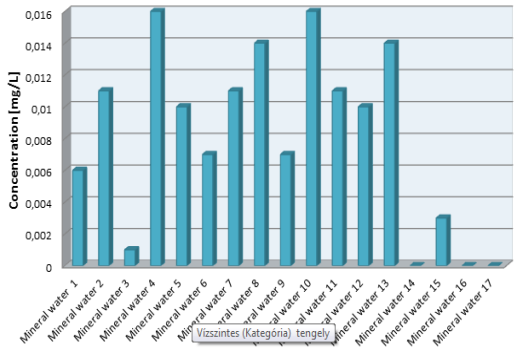

Figure 2 Nitrite levels in the investigated mineral waters

The results indicate that the samples of mineral water do not exceed the maximum contaminant level (MCL) except for samples 14, 16 and 17 which exceed the accepted nitrite level as per STAS (0 mg/L NO_2^-).

The topic have been followed up by other researchers too who have determinate the nitrite levels of packaged natural spring and mineral water consumed in Van city of Turkey. A total of 200 water samples, belonging to 10 different companies, each of 100 pet spring water and 100 bottled mineral water samples were used as material. Nitrite levels were measured by the spectrophotometric method. Mean nitrite concentrations were 0.045±0.003 mg/L in the spring water and 0.025±0.001 mg/L in the mineral water, respectively. Finally nitrite levels in water samples were in accordance with the related national and international regulations and considered safe for consuming [6].

3.2. Nitrite levels in wine

The nitrite levels in the investigated wine samples are shown in Figure 3.

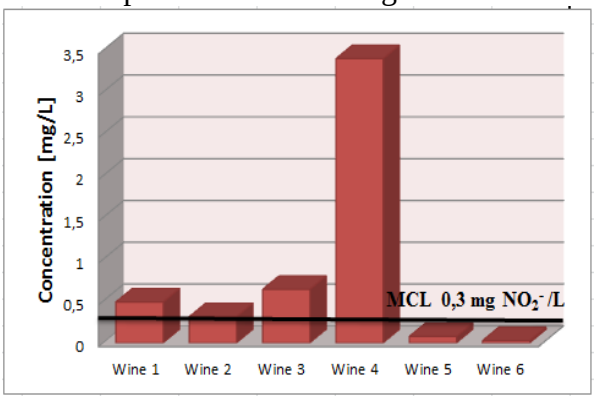

Figure 3. Nitrite levels in the wine samples

The main sources of nitrite in wine are water and raw materials. These raw materials could be contaminated through the soil.

Wine samples 1, 2, 3, and 4 indicated a high level of nitrite. The maximum contaminant was exceeded by 1.6 mg/L NO_2^- in Wine 1 and by 2.1 mg/L NO_2^- in Wine 2.

The highest exceeding was noticed in Wine 4 (3/4 mg NO_2^-/L). Low level of nitrite was found in 2 samples of investigated wine (Wine 5 and Wine 6).

3.3. Nitrite levels in juice

The nitrite levels in the investigated juice samples are shown in Figure 4.

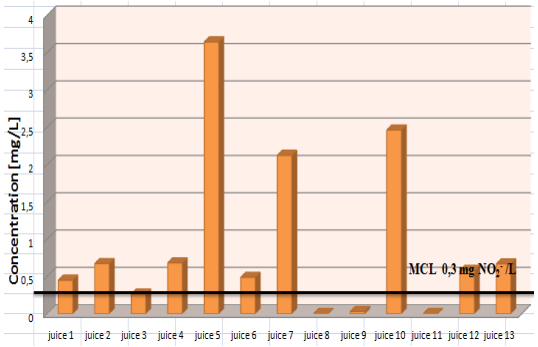

Figure 4. Nitrite levels in juice samples

In the above figure we can observe that excepting the samples Juice 3, Juice 8, Juice 9 and Juice 11, the level of nitrites was higher than 0.3 mg/L NO_2^-. Significant exceeding was observed in Juice 5, Juice 7 and Juice 10, while in Juice 12, MCL is exceeded by 12 times.

Norman et. al. [7] developed the same type of research by using HPLC method. Their outcome: vegetable and fruit juices had nitrate and nitrite concentrations (mg/L FW) of 27.6 and 0.04 (carrot juice), 26.1 and 0.09 (V8 juice), 12.9 and 0.07 (pomegranate juice), 9.1 and 0.14 (cranberry juice) and 0.6 and 0.01 (acai juice).

4. Conclusions

- Comparing test results with MCL the highest concentration was found in Wine 4 (3.4 mg NO_2^-/L), and Wine 6 (0.028 mg NO_2^-/L) while the lowest concentration of nitrite.
- A little higher level of nitrite in mineral water samples were found in Mineral water 10 and Mineral water 4 (0.016 mg NO_2^-/L); concentration of nitrite in Mineral water 14, Mineral water 16 and Mineral water 17 was below the limit of detection.
- In the juice samples, the highest concentration of nitrites was found in Juice 5, while the nitrite concentration in Juice 11 and Juice 8 was below the limit of detection.
- In some of the cases, the nitrite concentrations are even greater than the maximum contaminant level (0.3 mg/L). The highest exceeding was observed in Juice 5 (3.64 mg NO_2^-/L) and Wine 4 (3.4 mg NO_2^-/L), where the nitrite concentrations are higher that MCL. Also MLC is reached or even exceeded in beverages such as: Wine 1 (0.49 mg NO_2^-/L), Wine 2 (0.31 mg NO_2^-/L), Wine 3 (0.64 mg NO_2^-/L), Juice 1 (0,44 mg NO_2^-/L), Juice 2 (0.67 mg NO_2^-/L), Juice 3 (0.26 mg NO_2^-/L), Juice 4 (0.68 mg NO_2^-/L), Juice 6 (0.48 mg NO_2^-/L), Juice 7 (2.12 mg NO_2^-/L), Juice 10 (2.46 mg NO_2^-/L), Juice 12 (0.57 mg NO_2^-/L) and in Juice 13 (0.66 mg NO_2^-/L).

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DISCUSSION AND ANALYSIS FOR THE PRICE SENSITIVITY AND POST-PURCHASE BEHAVIOR OF TRAVELERS ON TOURISM FOOD-TAKING 'AGRITAINMENT' AS AN EXAMPLE

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ABSTRACT

Price is an important theme in marketing. This paper is to understand the food price perceptibility and post-purchase behavior of tourists through taking different food products (catering food is the main category) of 'agritainment' tourism as an example. The results indicate that population characteristics of tourists would influence its price sensitivity and post-purchase behavior. Life history does not influence price sensitivity and has influence on post-purchase behavior. Experience in purchasing would have tiny influence on price sensitivity，and it has no influence on post-purchase behavior. Research results have referential meaning for travel operators of 'agritainment' and appropriate authority in the process of establishing target market and formulating marketing strategy.

Keywords: *Agritainment, catering food, price sensitivity, post-purchase behavior*

1. Introduction

Agritainment tourism (hereinafter referred to as 'agritainment') is a kind of burgeoning travel and leisure modality, which could drive the development of holiday economy, and has obtained better social benefit and economic benefit. Consumer's purchasing behavior on a product would be first influenced by the price of this product. Price sensitivity measurement is a powerful tool, and could reveal the relation between price and value quality perceived by customers [1]. Many scholars at abroad could study the price problem of service industry. The products with high price indicates the purchasing power of the crowd who buy the products, symbolic role of price should be well understood before determining product price[2].

In the process of purchasing behavior, customers would make corresponding evaluation for the objective price of goods in their mind according to their own experience and standard [3]. In the marketing process of enterprise, demographic variable like age, sex, income etc are usually used to segment market and formulate corresponding strategy and tactics. These variables are closely linked with individual requirement and could not be ignored by enterprise operators [4]. Experience in purchasing of consumers would influence their consuming behavior. Many researchers measure the experience in purchasing with buying frequency. The purchasing behavior of consumers could be divided into the use and disposal of products, the communication of post-purchase experience, legal act, re-purchase behavior and business relationship behavior etc.

Food and beverage service is the important constituent part of 'agritainment', if the operation is appropriate, catering

would become the most profitable projects in 'agritainment' (Wei Zhao et al, 2007). Eating agriculturalism meal is the first purpose of citizens' suburb tourism(Bing Wang et al, 2006).As the Willow ditch village of Yanqing county in Beijing has developed bean curd ,it had received 42,0000 tourists in 2007, the earning of folk tourism is 15 million yuan, every household has more than 200000 yuan (Puquan Liu, 2008). The price is the important factor which affects customers' quality expectations; reasonable pricing is beneficial for the profiting of operator, as well as the promotion of market segment. In addition, consumers begin to know the solving degree of their consuming problems, and take a series of action. Studying consumer's post-purchase behavior has great significance to enterprise's knowing about whether consumers are satisfied or not and improving products and service .For the operators of 'agritainment', product up-gradation should be realized，and different agritainment products should be provided, tourists' knowledge about the price sensitivity and post-purchase behavior of catering product has great significance to product development and pricing[5].

2. Materials and method

On the basis of summary for the existing literature, researchers have put forward following hypothesis, as tourists' population characteristics, experience in purchasing and life history are different, their price sensibility of catering food and post-purchase behavior is different (see Table 1). Tourists' purchasing experience is measured by tourists' travel times of rural tourism and investigated local villages, tourists' life experience is mainly about whether they have rural life or not. With regard to the agritainment food conditions of different places and big characteristics gap, as well as the better suburban county of developing agritainment, researchers have selected three villages and towns in different suburban county for handing out questionnaire, the specialty catering of point A is more famous，the villages and towns have made the receiving price be uniform, and the evaluation of tourists on local service is of attractive and reasonable pric, as it is shown in Figure 1. Point B locates near the more famous beauty spot in suburban area, as it is shown in Figure 2. Point C is also the famous characteristic in suburban area and has attracted many tourists, as it is shown in Figure 3. The research has selected some better places of developed agritainment for issuing questionnaire. With regard to the different type s of catering food in agritainment, this paper has also handed out questionnaire at C, the total number of questionnaire is 600, 384 effective questionnaire are recycled, there are 314 questionnaire at point A, and there are 59 questionnaire at point B, 11 questionnaire are at point C. As the number of questionnaire about the type of food and beverage point is less than the minimum number required statistically, thus the type of studied food and beverage point only have other 3 classes. The situation of sample is shown as Table 2 and Table 3.

Table 1 6 hypothesis

Hypothesis 1	Tourists with different population characteristics, their price sensitivity on catering food is different.
Hypothesis 2	Tourists with different purchasing experience, their price sensitivity on catering food is different.
Hypothesis 3	Tourists with different life history, their price sensitivity on catering food is different.
Hypothesis 4	Tourists with different population characteristics, their post-purchase behavior is different.
Hypothesis 5	Tourists with different purchasing experience, their post-purchase behavior is different.
Hypothesis 6	Tourists with different life history, their post-purchase behavior is different.

Table 2 The situation of a sample

Project	Characteristic point of catering	Content	Number of people	Percent /%
Gender	A	Male(M)	172	54.8
		Female(F)	142	45.2
	B	M	32	54.2
		F	27	45.8
	C	M	45	50
		F	45	50
Years of age	A	≤25	113	36.0
		26-45	163	51.1
		46-60	32	10.2
		≥61	6	1.9
	B	≤25	21	35.6
		26-45	33	55.9
		46-60	5	8.5
		≥61	0	0
	C	≤25	18	20
		26-45	49	54.5
		46-60	21	23.3
		≥61	2	2.2
Education degree	A	High school or below	41	13.1
		Junior college and bachelor degree	138	75.8
		bachelor or above degree	35	11.1
	B	high school or below	8	13.6
		Junior college and bachelor degree	43	72.9
		bachelor or above degree	8	13.6

	C	high school or below	20	22.2
		Junior college and bachelor degree	64	71.1
		Bachelor or above degree	6	6.7
Month income/ yuan	A	＜2000	93	29.7
		2000-5000	164	52.3
		＞5000	57	28.7
	B	＜2000	11	18.7
		2000-5000	35	59.3
		＞5000	13	22.0
	C	＜2000	24	26.7
		2000-5000	56	62.2
		＞5000	10	11.1

Table 3 The situation of sample b

Project	Characteristic point of catering	Content	Value	Percent %
Experience in purchasing (1: average time of agritainment this year 2: average time of agritainment in investigated places)/time	A	1 / 2	3.22 / 1.516	
	B	1 / 2	4 / 1.80	
	C	1 / 2	4.4 / 2.17	
Life history (1: having rural life; 2: having no rural life) /people	A	1 / 2	123 / 191	39.2% / 60.8%
	B	1 / 2	18 / 41	30.5% / 69.5%
	C	1 / 2	47 / 43	52.2% / 47.8%

Figure 1 Point A

Figure 2 Point B

Figure 3 Point C

3. Survey result and analysis

3.1 Analysis for the food and beverage cost of tourists

Tourists' average cost of catering at point A is 22.0 yuan per person, point B is 36.2 yuan, point C is 43.6 yuan, p value of variance analysis is 0.00, it indicates that the average catering cost of tourists in three groups has significant difference .The gender, education degree and income of tourists at different points do not influence their catering cost. The factors influencing tourists' catering cost at point A includes age and life history, the catering cost of tourists in group of 26～45 years old and 46～60 years old is higher than other groups，the results of variance analysis is 0.014. For the tourists who have no rural life history, their catering cost is higher than control group; the result of t test is 0.024.Only the life history influences the point B. For the tourists who have no rural history life, their catering cost is higher than control group, the result of t test is 0.043.Only the age influences the point C, the catering cost of tourists in group of 26～45 years old and 46～60 years old is higher than other groups, the result of variance analysis is 0.032.

3.2 Analysis for the price sensitivity of tourists

(1)Tourists' sensitivity analysis for cheap price

For the catering cost this time, tourists are inquired about why they think the price is too cheap in the questionnaire, thus their suspicion about catering condition emerges. The result indicates that, if the catering price of point A is 12.8 yuan per person, the catering price at point B is 17.7 yuan per person, and it is 17.5 yuan at point C, tourists would be doubted about the catering condition. The gender, age, education degree, income, purchasing

experience and life history do not influence their sensibility of cheap price.

(2)Tourists' sensitivity analysis for quality assurance of price

With regard to how to fix a price, tourists would think that when the quality is guaranteed and the price is not expensive, the average price answered by tourists at point A is 24.4 yuan per person, it is 32.7 yuan at point B, it is 39.2 yuan at point C. The population characteristics of point A have no influence. The income and age of tourists at point B have influence, tourists think that the older the age is ,the higher the quality assurance of price will be, p value of variance analysis is 0.017, p value of income's variance analysis is 0.006.Generally speaking, the higher the income is, the higher the quality assurance of price will be, but the quality assurance of price in the group below 2000 yuan will be considered to be higher than group of 2000～5000 yuan. The income and age of tourists at point C has influence, the older the age is, the higher the quality assurance of price will be, p value of variance analysis is 0.013; the p value of income's variance analysis is 0.005, the higher the income is, the higher the quality assurance of price will be, but the group under 2000 yuan think that quality assurance of price is higher than group of 2000 ～ 5000 yuan .Through combining with the interview, researchers find that students' income are mainly below 2000 yuan, they care more about the safety and quality of food, and would rather pay a higher price for the assurance of food quality. Life experiences and purchase experience have no effect.

(3) Tourists' sensitivity analysis of more expensive price.Upon responding to how to fix a price，tourists think although catering price is more expensive, when it is worthy of consumering, the average price answered by tourists at point A is 50.6 yuan per person, the price at point B is 54.8 yuan, the price at point C is 80.1 yuan. The population characteristics of tourists at point A and point B have no effect. For the tourists at point C, the higher the income is, the more expensive the price that they could accept will be, the one-way analysis of variance's significance among groups is 0.012, and it indicates that there exists significant difference about the price sensitivity of this problem among the tourists with different income. That whether the tourists have rural life history and purchasing experience has no effect.

(4) Tourists' sensitivity analysis for too expensive price

Upon responding to how to fix a price，when tourists think the catering price is too expensive to purchase, the average price answered by tourists at point A is 61.5 yuan per person, the average price at point B is 82.8 yuan, the average price at point C is 93.4 yuan per person. Tourists' population characteristics at point A have no effect. The gender of tourists at point B has influence, the average price that men think to be too expensive to accept is 91.9 yuan, for female, it is only 74.3 yuan, t inspection result is 0.032, the price recognized by male is significantly higher than female. The higher the tourists' income at point C is, the more expensive the price will be, and the significance of variance analysis among groups is 0.000. Life history and purchase experience have no effect.

(5) Tourists' sensitivity analysis for the right price.

When the tourists are inquired about what price they think is the most appropriate, the average price answered by tourists at point A is 27.7 yuan per person, which approaches to the price, is that they think it has quality assurance and is not expensive. Tourists' average price at point B is 37.3 yuan per person, tourists' average price at point is 59.0 yuan per person. Tourists' population characteristics at both point A and point B have no effect. Tourist' income at point C has influence, the higher the income is ,the higher the right price that tourists think would be, but the right price that the group below 2000 yuan consider is higher than the group of 2000～5000 yuan. Life history and purchase experience have no effect.

3.3 Analysis for post-purchase behavior of tourists.

This research is to measure the post-purchase behavior of tourists with two indicators including revisit intention and recommending intention to others.

(1) Analysis for tourists' revisit intention.

Tourists' population characteristics at point A do not influence their revisit intention. Point B has influence on the income of three tourists, significance of variance analysis is 0.000 and 0.003 respectively, it indicates that the higher the income is, the stronger the revisit intention will be, the revisit intention of the group below 2000 yuan is higher than that in group of 2000 ～ 5000 yuan. Through in-depth analysis, the group below 2000 yuan mainly contains students, whose interest to agritainment is relatively profound, thus they have stronger revisit intention. Tourists' age of two groups could also influence revisit intention, the revisit intention of group above 61 years old is significantly lower than other groups. Life history does not influence the revisit intention of three groups. The results of purchasing experience indicate that agritainment frequency of tourists at point A and point B is related with revisit intention, the correlation coefficient of two-tailed test at 0.01 level are 0.314 and 0.463 respectively, namely the bigger the frequency of agritainment is, the stronger the revisit intention will be. Tourists' agritainment frequency of investigated places at point B also influences the revisit intention, the bigger the frequency is, the stronger the revisit intention will be, the correlation coefficient of two-tailed test at 0.01 level is 0.426. The correlation coefficient of tourists' buying experience and revisit intention at point C is not up to standard.

(2) Analysis for the recommend intention of tourists. Tourists' population characteristics at point B do not influence their recommend intention.

The age and gender of tourists at point A have influenced the recommend intention. The recommend intention is arranged from high to low as following: the group of 26～45 years old, group of 46～60 years old, group above the age of 61 and group under the age of 25.The recommend intention of female is higher than male. The age and income of tourists at point C have influenced the recommend intention, recommend intention is divided to be group of 26~45 years old, group of 46~60 years old, group above the age of 6 years old and

group under the age of 25 from high to low. The higher the income is, the stronger the recommend intention will be. Life history of tourists has no influence on the tourists' recommend intention of three groups. The correlation coefficient of tourists' purchasing experience and recommend intention at point C is not up to significant standard. Tourists' agritainment frequency of point A and point B in that very year is related with recommend intention, the correlation coefficient of two-tailed test at 0.01 level are 0.313 and 0.311 respectively, namely the bigger the agritainment frequency is, the stronger the recommend intention will be.

4. Discussion and conclusion

4.1 Research results

Generally speaking, the proprietor of agritainment takes the local agricultural products for processing and satisfying the needs of guests, the cost is low，thus consumption will not be high. And beautiful natural or pastoral scenery and tourist attractions from place to place is around agritainment, it could relieve the tight thoughts of modern people and provide convenience for traveling outside. Thus it is warmly welcomed by many people. Reasonable consumption and affordable price are the two points which attract travelers most [7].

With the enhancement of people's living standard, agritainment tourism has already entered into people's life slowly and become people's main approach of feeling nature and experiencing pastoral life .But recently, various regions in China are developing agritainment, and there must be some problems among them, such as inaccurate orientation, unkown characteristic and 'similar face', as well as impowerful attraction ability.

Developing 'agritainment tourism' could not only enrich the content of tourism activity and expand the tourism capacity, but also drive the adjustment of agricultural production structure and increase farmers' income to become rich, promote the development of rural economy and society. Thus, in the process of comprehensively constructing well-off society, 'agritainment tourism' should be developed according to surrounding circumstances ,and helping farmers with tourism will make great contribution to solving 'agriculture problem', promoting the construction of new socialist countryside and playing a more active role.

Developing 'agritainment' should take 'agriculture' as the root. Farmers should demonstrate the rural landscape, agricultural production process and farmers' life scenes through their own fertile land, orchard and courtyard, fishpond, pasture and so on. Tourists would be attracted through displaying. Restaurant reception facilities could be accomplished through reconstruction and improvement by taking advantage of their own homestead and existing living facilities, the local flavor of village, agriculture, peasant and farmers should be fully expressed. As 'agritainment' has already become a kind of brand，many city dwellers also play the "rural" brand in their opened catering service facilities in the city，which is at opposite poles with authentic 'agritainment' in practice.

Developing 'agritainment' should take 'home' as shaping. 'Agritainment'

should take ‘household’ as the unit，do not pursue perfection and large-scale. Its form should represent the form of ‘household’. Since it is ‘home’, its scale should be moderate, craving for big and foreign things are wrong. The development should be characteristic, not in a popular style. The ‘agritainment’ in Pan Shi Zhou Cun of Pingjiang County is famous for being folk, unique and various. Thus ‘home’ is the isotopic carrier of ‘agritainment’, ‘agriculturalism’ could not be formed without home.

Developing ‘agritainment’ should take ‘enjoyment’ as the soul. What does ‘agritainment’ takes to seek pleasure? Nowadays, a lot of ‘agritainment’ are stereotyped, and provide projects like catering, playing cards, Kara OK and so on. The city dwellers make joke that it is equal to change places for playing cards. Actually, ‘enjoyment’ should also make an issue of ‘agriculture, farmer and rural area’, design the projects with strong property of participation，simple farming, and farm work, such as picking, grinding, seedling dish and so on. Taking ‘enjoyment’ as the soul is to carry forward the cultural connotation of ‘agriculturalism’, dig deeper, and prominent feature, implement projects such as farmers’ loved festive lantern (as displayed on the lantern festival), shadow play, folk song (sung in the fields during or after work), dragon and lion dance, flower drum, walking on stilts, taletelling and so on, which could make ‘agritainment tourism’ full of charm and realize sustainable development. [7]

This research takes the tourists of ‘agritainment’ as object, studies their price sensitivity of catering food and post-purchase behavior, some conclusion is obtained. Some of the prior assumptions have gotten verification, while some of them do not get validation.

(1)Population characteristics of tourists could influence their price sensitivity and post-purchase behavior. The income of tourists have the biggest influence on price sensitivity and post-purchase behavior, the second is the age of tourists, gender has the smallest influence.

(2)The rural life history has no influence on the price sensitivity of catering food and post-purchase behavior，while it has no influence on cost. The tourists who have no rural life history would pay higher catering food cost than the tourists in control group, but it has no influence on price sensitivity and post-purchase behavior.

(3)Buying experience had no effect on price sensitivity, but has effect on post-purchase behavior. The more the purchasing experience is, the stronger the recommend intention and revisit intention will be.

4.2 The significance of this study

(1)Food price of ‘agritainment tourism’ is studied from the perspective of price sensitivity.In the recent research about ‘agritainment tourism ’, there is no discussion directly performed from the perspective of price sensitivity. This research could be a beneficial supplement. Tourists’ demographic characteristics of three groups do not influence their sensibility of cheap price; it indicates that tourists’ opinion about the basic catering condition is consistent. In the interview, many tourists have also put forward

criticism about that some operators only pay attention to earn money, but regardless of catering hygiene. The operators of 'agritainment' only provide the catering food in accordance with popular demand, only by that could satisfy the basic requirement of tourists. As 'agritainment' is at different places, its catering characteristic and management system is different, which would have influence on the food price sensitivity of tourists [8].

(2) Marketing segment of rural tourism has reference value

The more intense the market is, the more important the marketing would be, while the behavior analysis of consumers is the basis of forming marketing strategies [9], one of the most important enterprise marketing strategy is to select market segment, while there is significant difference between the demand of market segment and other requirement of market, the finer the market segment is, the closer its provided products to the real demand of this market will be, thus the requirement of market segment consumers could be better satisfied, and the enterprise benefit would be realized. The results of the survey show that young tourists pay more attention to the quality of catering food, and would rather pay higher price to obtain the guarantee of food quality. With regard to that they are important future consumers, the operators of 'agritainment' should not only formulate reasonable price, but also pay more attention to the quality of catering food. As the income of tourists is different, the catering price that they could accept is also different. Operators of 'agritainment' could manage the catering products at various levels according to the real situation of their own, and then find out their position in the intense market competition. For local relevant management institution, as the basis of each village and town is different, the attractive resources are also different. Management institutions concerned could organize and lead 'agritainment leaders ' to make their own characteristics on purpose according to local resources, thus establish the suitable target market segment, formulate corresponding marketing strategy, implement dislocation competition, and further enhance the satisfaction degree and quality of 'agritainment', expand development scale[10].

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NANOMATERIALS USED IN ANTIMICROBIAL, ACTIVE AND INTELLIGENT FOOD PACKAGING

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ABSTRACT

Nanomaterials are the most studied materials of our days, due to their properties and their wide range of applications and can be considered materials of the future. Many studies focus on their capability to improve our life by making us healthier, protecting the environment or developing new nanostructured materials used in various industries andone of these industries is food industry. Food is for humans an essential factor for sustaining life and we are influenced very much by the eating habits that we have. But we must consider that what we eat define us and our health and therefore it is very important to maintain food characteristics during transportation, distribution and storage. These objectives can be completed by good control of storage environment factors or by improving packaging materials, resulting new functions that can sustain food shelf life. Food packaging materials improved with nanosized particles can have antimicrobial activity, can release components or can alert consumers about changes during time in food. These aspects and also regulations regarding nanomaterials used in food packaging can be found in this review.

Keywords: *nanomaterials, antimicrobial, active packaging, intelligent packaging*

1. Introduction

Nanomaterials are unique structures which have dimensions from 1 to 100 nm. In essence, the word nano is originating from ancient Greek, where the noun "nanos" was used with the meaning "dwarf". Nanomaterials are, in the most cases, powders consisting of nanoparticles. Because the size of particles is reduced compared to the macroscopic structures of the same compound, they have different chemical and physical properties [1]

Nanotechnology on the other hand is the science which is involved in the design, synthesis, characterization and application of small-size materials. In fact is the art of small particle manipulation. It has been also called "technology at nanoscale" because it creates nanostructured materials with new properties and functions. Applications of nanotechnology can be found in different areas: environment protection, pharmaceutical industry, new materials development, agriculture, food processing and packaging. [2] Concerning food nanotechnology, research is in progress for developing new foods, supplements and packages with unique properties, applications and functions due to the involvement of nanomaterials in this fields. It is well known that nanotechnology can be applied in all phases of food chain production, for example: in the agricultural sector, the use of nano-emulsions pesticides, in the food production sector, the use of nanoceramic devices for larger surface areas, in the conservation sector, the use of incorporated nanoparticles into packages or nanosensors for monitoring and detection. [3]The most common nanoparticles used in food industry are: metal nanoparticles for packaging materials and storage, different structures at nanoscale for nanosensor manufacturing, incorporated active nanoparticles for migration properties, nanopore filters for purification, nanoencapsulated additives, nanosized food and nutrients used as supplements. [1]

2. Definitions and regulations concerning nanomaterials used in packaging

Despite their occurrence in food nanotechnology and the rapid developments, very little is known regarding the toxicity of nanoparticles. Nanotechnology food contact materials will change the whole food packaging industry, so it is important to start from understanding the concept of nanomaterial and after to discuss the applications and benefits to the food industry, especially in food packaging. [1]

According to European Union Commission Recommendation 2011/696/EUof 18 October 2011, the definition of nanomaterial is the following:

"'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm.

By derogation […] fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

For the purposes […] 'particle', 'agglomerate' and 'aggregate' are defined as follows:

(a) 'particle' means a minute piece of matter with defined physical boundaries;

(b) 'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;

(c) 'aggregate' means a particle comprising of strongly bound or fused particles.

A material should be considered as falling under the definition […] where the specific surface area by volume of the material is greater than 60 m^2/cm^3." [4]

On the other side, the definition of engineered nanomaterials from the Regulation of the European Parliament and the Council No. 1169/2011 of 25 October 2011 is the following:

"'engineered nanomaterial' means any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale.

Properties that are characteristic of the nanoscale include:

(i) those related to the large specific surface area of the materials considered; and/or

(ii) specific physical-chemical properties that are different from those of the non-nano form of the same material" [5]

The revised form of this definition can be found in the European Union Commission Delegated Regulation No. 1363/2013 of 12 December 2013 and it is the following:

"Point (t) of Article 2(2) of Regulation (EU) No 1169/2011 is replaced by the following:

'(t)"engineered nanomaterial" means any intentionally manufactured material, containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm to 100 nm.

By way of derogation:

(a) food additives covered by the definition set out in the first paragraph shall not be considered as engineered nanomaterials […]

(iv) "intentionally manufactured" means that the material is manufactured to

perform/fulfil a specific function or purpose;."[6]

Concerning active and intelligent materials and articles intended to come into contact with food, the European Union Regulation No. 450/2009 of 29 May 2009 is the following:

"Many different types of active and intelligent materials and articles exist. The substances responsible for the active and/or intelligent function can be contained in a separate container, for example, inclusion in a small paper sachet or, the substances can be directly incorporated into the packaging material, for example, incorporation in the plastic of a plastic bottle.[…]

The active and intelligent materials and articles may be composed of one or more layers, or parts of different types of materials, such as plastics, paper and cardboard or coatings and varnishes.[…]

Active materials and articles may deliberately incorporate substances, which are intended to be released into food.[…]

Intelligent packaging systems provide the user with information on the conditions of the food and should not release their constituents into the food." [7]

The definitions of active and intelligent packaging from Regulation No. 450/2009 are the following:

"(a) 'active materials and articles' means materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food; [7]

(b) 'intelligent materials and articles' means materials and articles which monitor the condition of packaged food or the environment surrounding the food; [7]

(c) 'component' means an individual substance or a combination of individual substances which cause the active and/or intelligent function of a material or article, including the products of an in situ reaction of those substances; it does not include the passive parts, such as the material they are added to or incorporated into;

(d) 'functional barrier' means a barrier consisting of one or more layers of food contact materials which ensures that the finished material or article complies with Article 3 of Regulation (EC) No 1935/2004 and with this Regulation;

(e) 'releasing active materials and articles' are those active materials and articles designed to deliberately incorporate components that would release substances into or onto the packaged food or the environment surrounding the food;

(f) 'released active substances' are those substances intended to be released from releasing active materials and articles into or onto the packaged food or the environment surrounding the food and fulfilling a purpose in the food."[7]

3. Antimicrobial, active and intelligent food packaging

Active and intelligent packaging materials are food contact materials because they come into direct contact with food. The substances responsible for giving active or intelligent functions to those materials have to be safe and evaluated in accordance by the Regulation No. 450/2009. The substances or combinations which are authorized to be used as active and intelligent components are included in the Community list of substances that may be used in this purposes. The stationary parts, into which these substances are included, usually are normal packaging materials used along time to cover and preserve food into good conditions, maintaining their properties and avoiding chemical and microbiological contamination. This inert materials have to be characterized by the overall migration

limit, because the active component incorporated into the food contact material can exceed a certain limit due to the release of the active substance. [7]Active materials and articles which yet are not in direct contact with food, but can be found on the market and used as packaging materials, have to provide certain information regarding the allowed way of use and the products that fit to be packed, but also the maximum quantity of active substances released. When a new substance is supposed to be used in the future as an active or intelligent material, authorities give sufficient time to the applicant to provide certain safety information about the material, a period of 18 months, during which the applicant can obtain authorization.

The incorporation of nanoparticles into the package matrix has the purpose to improve antioxidant, antimicrobial and scavenger functions. Apart from the most common used packages that remove moisture, oxygen or carbon dioxide from the headspace of the food packaging systems, there are some other types that can absorb volatile compounds, ethylene or can emit antimicrobials, such as ethanol, silver ions or glucoseoxydase. There are some active packages which entered mass production and are used and commercialized, but most of them remained at the stage of research.

Nanomaterials used in food industry for packaging, apart from the mechanical improved properties, such as strength, reduced weight and heat resistance, can provide shelf-life extension and improve the safety of food, acting as barrier for humidity, UV radiation or chemical compounds. They can also act as sensors to alert consumers about food contamination (gas indicators, freshness indicators, microorganism activity biosensors) or as active scavengers for different compounds (oxygen, ethylene, carbon dioxide, water or undesirable flavors). Smart packaging reacts to the conditions of the environment or alerts the consumers when contamination or pathogens are present in food. Sensors give information about enzymes produced by degradation of food molecules, making products unsafe for consumption. [1]

There is the possibility for packages to eliminate internal compounds from the package atmosphere, such as air, water or enzymes which apart from increasing shelf-life, has the advantage that in the production process, a smaller quantity or preservatives is used. For example, the new improved polymer plastic combines polyamide with layers of silicate barriers, consisting of silicate nanoparticles, which reduce the entrance of oxygen or other gases from the surrounding atmosphere and the elimination of moisture. Another example is from the plastic beer bottles, which have nanocomposite particles included and have the function to stop the loss of carbon dioxide and the entry of oxygen, maintaining the freshness. [8]

Oxidation is the main factor which limits food shelf life and occurs often in products containing a big amount of fatty acids. The chemical reaction starts when these acids with unsaturated bonds are in presence of a catalyst, such as enzymes, iron and copper ions or in presence of other environment factors, such as light exposure and heat. It is a slow auto-oxidative process, in which during its stages, conjugated dienes, hydroperoxides, alkenes or carbonylic compounds are formed, giving rancid odors and other unpleasant flavors that damage food organoleptic properties. In order to protect the food from spoilage, oxygen can be removed from packages by oxygen scavengers or by delivery of antioxidants from the package material. Scavengers are used in packaging for food products to eliminate some compounds or to release active species on or into food. High oxygen concentration from headspace

packages accelerates growth of aerobic microorganism and organoleptic changes. Antioxidant agents and active packaging inhibit lipid oxidation and the deterioration of unsaturated fatty acids, giving the required shelf life. [9]Active packaging materials can contain metal-based nanocomposites, natural components, such as enzymes, extracts and essential oils, chemical synthesized organic acids or antimicrobial macromolecules. [23]

3.1. Metal and oxide-based nanomaterials in antimicrobial/active food packaging and their action mechanism

3.1.1 Metals

Silver has been used as agent against microorganisms for a long time in food and beverage packaging and its first applications were vessels for water, milk or wine storage since ancient times. Silver has a large spectra of antimicrobial activity, a long stability in time. It is the most efficient antimicrobial agent against bacteria, fungi, algae and even viruses, being also the least toxic to animal cells. [10] It is suitable for incorporating in food packaging materials, such as paper or polymer materials. Although silver has a history of antimicrobial activity, the action mechanism it is still a mystery. Metallic silver is inert, but moisture contact leads to silver ion formation. In order to have antimicrobial properties, silver must be in its ionized form. There are some possible explanations, such as the protein inactivation mechanism in which silver ions can bind to the thiol groups of enzymes causing deactivation by forming stabile bounds in cell membranes, blocking ion transportation and energy generation. [10] It is supposed that silver acts as an catalyst for oxidation reactions in cells, in which oxygen molecules and hydrogen atoms from two thiol groups react and release water and the thiol groups bind together resulting disulfide bonds. This new formed bonds change enzyme structures, especially for those used in cellular respiration and change cellular wall permeability. [11] Silver ions also bind to the ribosomal complex of cells and block protein translation and phosphorylation of adenosine diphosphate to obtain adenosine triphosphate by inactivating synthetase enzymes involved in tricarboxylic acids cycle. This important changes of cells proteins lead to cellular lysis.Another possible action mechanism is the DNA denaturation caused by silver intercalation between two base pairs of anti-parallel strings, such as purine and pyrimidine, disrupting hydrogen bonds. [25] Silver nanoparticles have been studied for their high antimicrobial effectiveness. It is demonstrated that smaller silver nanoparticles have an more inhibitory effect for microorganisms. Due to their size, they have an advantage for cell membrane penetration and also have a much larger specific surface area. This means that more silver atoms contained in the nanoparticle will be in direct contact with the cells components and will be able to participate to the cell destruction. [13] Antimicrobial activity is influenced by the nanoparticles shape. There are three possible shapes of silver nanoparticles: spherical, rod-shaped and triangular. The structural differences between these shapes are the number of active facets (electron dense facets) on the nanoparticles. Antimicrobial activity is increased for silver nanoparticles with more active facets. The triangular shape nanoparticles have more facets than the spherical ones, which are not perfect spherical and which have more active facets than the rod-shaped ones. [14]

Gold has been very few explored as an antimicrobial agent, although it has an good bactericidal effect and it is non-toxic. This properties are related to the

nanoparticles shape and size. At macroscale, gold is inert, but at nanoscale, gold nanoparticles express an antibacterial activity.It is possible to incorporate gold nanoparticles on different supporting materials, such as silica or zeolites and to use them against bacteria from food and water. This kind of bacteria can express an resistance to antimicrobials based on silver. [15] As action mechanism, it is similar to the silver, in which gold nanoparticles closely bind to the cell surface, causing visible damages and destruction of cellular organelles. They collapse membrane potential, disorganize membrane structures, bind to nucleic acids and inhibit ribosome activity. As difference, gold nanoparticles are not involved in chemical reactions with reactive oxygen species. [16]

Copper nanoparticles have similar properties to those of other noble metals, such as gold and silver andcan express antimicrobial properties when are incorporated in different coatings or plastics.[17]Although there are few studies about its antibacterial functions, copper nanoparticles show an important bactericidal effect. Their surface affect polysaccharides and peptides from the cell membrane of bacteria causing rupture and cell destruction. The limitations of these nanoparticles can be found in their rapid oxidation when in contact with air. [18] During copper nanoparticle preparation and storage, copper oxidizes resulting his oxides and after that the ionic form, so it is difficult to synthetize copper nanoparticles in the ambient environment. The alternative to this is synthesis in presence of polymers, such as polyethylene glycol and chitosan or surfactants used as stabilizers which form on the nanoparticles surface an protective coating against aggregation and oxidation. Recently, it has been developed a method for stabilizing nanoparticles with plant extracts. [17]

3.1.2. Metal oxides

Titanium dioxide is the most common and widely spread nanomaterial under metallic oxide form due to its non-toxicity, chemical structure stability, biocompatibility, high catalytic activity or self-cleaning characteristics. [19] Titanium dioxide can be found under three mineral forms: anatase, rutile and brookite. Anatase has an crystalline structure corresponding to the dipyramidal crystalline system and it is mainly used for photocatalysis under UV irradiation. Rutile has an prismatic crystal structure and it is used as white pigment in paint. Brookite has an orthorhombic crystalline structure and is not efficient on photocatalysis.[19] There are many applications of titania based photocatalytic systems used for decomposition of toxic organic compounds from water and removing bacteria and other microorganisms from food or beverages, which can be drug resistant strains. The term "photocatalysis" refers to any photoinduced reaction which is activated by photonic absorption with sufficient energy. The process of photocatalysis begins when under UV light with a wavelength <400 nm, titania generates electrons and holes. Because of light absorption with an energy equal or greater than the band gap, the excitation promotes electrons from the valence to the conduction band. These holes can react with molecular species nearby to form oxidants. For example, after the reaction with water molecules it results hydroxyl radical or in the case of the reaction with oxygen it results superoxide molecules. [20]The biocidal activity mechanism of titania consist of an oxidative attack of these radicals on the internal and external cell membrane of microorganisms, changing essential genes from the wall structure involved in lipopolysaccharide, peptidoglycan and protein metabolism,

destruction of enzymatic activity of coenzyme-A inhibiting acetyl-CoA synthetase, CoA-transferaseand also dehydrogenase, thiolase, reductase and an fatty acid oxidation complex, inhibition to assimilate and transport iron and inorganic phosphorus, a lower capacity for biosynthesis and degradation of heme or DNA damage induced by hydroxyl radicals. [21]

Zinc oxide is an efficient antimicrobial material, considered safe for human health. Because of these properties, zinc oxide nanoparticles can be introduced in different food packages, such as cans for preserving the natural color of products and for food spoilage. [20] Recently, studies have shown that these nanoparticles exhibit an toxic selectivity to different bacteria but express minimal effect on human cells. The antibacterial activity is resulting due to a reaction between zinc oxide surface and water. It is known that zinc oxide nanoparticles induce membrane damage caused by direct or electrostatic interaction between nanoparticles and the cell surface of microorganisms, cellular internalization and production of species such as hydrogen peroxide, which are oxygen active and cause considerable alteration of cellular morphology.[20]When bacteria are exposed to environment changes, they try to adapt to the new conditions and adopt different mechanisms in order to do these changes. One of these is the synthesis of proteins with defensive role in response to different stresses, mainly oxidative stress. In their attempt to eliminate reactive oxygen species, defensive enzymes such as superoxide dismutase, catalase and hydroperoxide reductase try to eliminate toxic intermediates and to repair the damages caused to molecules by oxidation. These is also the effect of zinc oxide nanoparticles when are in direct contact with microorganisms, increasing the level of oxidative stress and exhibiting antimicrobial activity. [22]

3.1.3. Other nano-based active packaging systems

Other advances in food packaging include the use of novel carbon nanotubes to improve mechanical features of the materials, but recent research developed the antibacterial properties of carbon nanotubes in which the immediate contact of bacteria with aggregates lead to their extinction. [26]

Nanocomposites based on polymer-clays are novel packaging materials, with improved mechanical, thermal or barrier properties for gas and water and with future prospects due to their benefits. [27]

Nanocoatings are improvements of packaging materials using an aqueous-based barrier coating consisting of nanocomposites, that provide oxygen barrier and carbon dioxide retaining, with a layer of only 1-2 microns for packaging use or the deposition of amorphous carbon using plasma into polyethylene bottles, acting as gas barrier. Nanocoatings have antimicrobial activity and are scratch-resistant and corrosion-resisant. There are also edible nanocoatings, which consist of 5 mm thin coating layers and can be used for a variety of food types, such as fruits, vegetables, meat or cheese. Edible coatings can provide a barrier for exchanging gases and moisture or even deliver different compounds to the food, such as antioxidants and antibrowning agents. [23]

Nanoemulsions are used in food packaging decontamination and also in food packaging equipment for the same reason. Nanoemulsions have the advantage of high clarity, which can allow the incorporation into beverages of flavours, without changing their aspect. They can remove pesticide residues from the surface of fruits and vegetables. Also, nanoemulsions have destructive effect on food pathogen

microorganisms, such as Gram-negative bacteria. There can be produced a large variety of nanoemulsions, such as single-layer, double-layer and triple-layer nanoemulsions, depending on the polyelectrolyte used. [26]

Nanofilters are small scale filters used for nanopackages with the ability of self-cleaning, for example the removal of microorganisms from milk or water without thermal treatment.

Biopolymer nanoparticles are very high bioactive solid particles, which are used to release controlled and targeted functional compounds and can also serve as carriers for antimicrobial compounds. Bio-nanocomposites are innovative materials with improved mechanical, thermal and barrier properties for gases and water vapours and are formed from a biopolymer matrix that contain different nanoparticles. [12] Another benefits of bio-nanocomposites are antimicrobial activity, great capability of immobilization for enzymes, use as biosensors, biodegradability, transparency, UV barrier or self-cleaning properties. This biopolymer matrix can be from natural sources, for example carbohydrates such as cellulose, chitosan, starch or proteins, such as casein, gelatin, from chemical synthesis, such as polylactic acid or compounds from microbial metabolism, such as polyesters. Nanoparticles used can be different metals and metal oxides, silica, silicate clays or carbon nanotubes. Biopolymer clay nanocomposites are often used in multilayer packaging systems. As an active packaging material, oxygen scavengers can be incorporated into biopolymer nanocomposites or antimicrobial agents and growth inhibitors for micoorganisms. [27]

3.2. Intelligent food packaging

Indicators have the function of alerting the consumer about changes that occur in the interior of the food package, usually by a change of his characteristics, such as color. [24]Temperature is one of the most important factor that can induce food spoilage. Together with time, this two factors contribute to sensorial and nutritional changes. Every food product has an expiration date marked on the package, but this does not guarantee the safety, because the conditions in which the product is held can damage its qualities. Time-temperature indicators are efficient for monitoring changes caused by this factors and have cumulative effect. This means that they express if a food product has been exposed to cold or high temperature and also the effects of the exposure, based on its duration. For example, microbial-based time-temperature indicators respond to food spoilage related to microorganism development. The indicator consists of a nutritive medium with a dye, in which lactic acid bacteria are inoculated and introduced to the package and in theory the growth of bacteria from the indicator mirrors the growth rate of microorganisms from food products. Another indicator of this type is a small, transparent freshness indicator used over the barcodes of food products. It consists of an adhesive label in which selected lactic bacteria are immobilized. When a product is not fit for consumption, bacteria develop different colors and the barcode is rejected. Enzymatic time-temperature indicators use the same shape of adhesive label and on their surface enzymes are immobilized. [24]

Polymer-based time-temperature indicators use the polymerization reaction of substituted diacetylene, resulting an intense colored compound. Photochromic time-temperature indicators are printed inks on the inner or outer side of the package that show the accumulated effect of time and temperature on chilled products and are usually applied as spots or rings. The

photochromic properties of dyes used allow the change of color in time, a change dependable of the intensity and duration of these factors. [24]

Another type of indicators are integrity indicators, which appear in the form of labels or printed on packages and change their color by an enzymatic or chemical reaction in presence of gases like oxygen or carbon dioxide in package headspace. This type of indicators must be in direct contact with food, because they monitor the gaseous composition of package atmosphere. The major chemical factor of food spoilage is oxygen and integrity indicators are based on a color change due to oxygen binding reactions, light-activated reactions or redox reactions. [24]

Freshness and spoilage indicators alert consumers about food spoilage caused by microorganisms, which is a main cause of food degradation. In order to determine contamination, microbial metabolites such as glucose, acetic or lactic acid, ethanol, amines, carbon dioxide and sulfuric compounds are usually estimated and their presence is signaled in a color change of the indicator. [24]

Biopolymer nanocomposites can be use as indicators that monitor package time and temperature conditions during transportation and storage and also package integrity or can be used as nanobarcodes. [27]

4. Conclusions

The quality of food is mostly influenced by packages and packaging materials evolved during time. Nowadays we can find active packages, which maintain food shelf life by releasing different compounds and intelligent packages, which can alert consumers if food is not safe to eat. Antimicrobial packages have now probiotics incorporated as a new method of biopreservation. Future trends show that active packages will become extra-active packages, because they will incorporate many functions, such as antimicrobial activity, atmosphere control, protective barrier, scavenging functions, edibility and biodegradability, all in one package. Another trend is to develop new packages with high barrier properties, which can reduce the quantity of material needed, because they are made from light weight materials.

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ULTRASOUND ASSISTED EXTRACTION OF ANTIOXIDANT FROM *Coleus tuberosus* PEELS

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ABSTRACT

Black potatoes (*Coleus tuberosus)* is one of the crops that contain tri terpenic acids such as ursolic acid and oleanolic acid as the main component for antioxidant compounds. The previous study showed that *Coleus tuberosus's* peel has higher antioxidant content than its flesh. Therefore, this research was aimed to extract the antioxidant compound from black potatoes 's peel by investigating the effect of solvent types and ratio of material to solvent on extraction yield. Moreover, this research also aims to compare the yield obtained by conventional and ultrasound assisted extraction methods, and to evaluate the effect of temperature and extraction time on the activity of antioxidants. The antioxidant activity of the extract was analyzed by using DPPH and FT-IR assay. The results showed that the extract obtained by ultrasound assisted extraction method at temperature of 60ºC for 60 minutes, solvent methanol and ratio material to solved ratio of 1:75 (w/v) provided the highest antioxidant activity (89.35%) as compared to any other variables.

Keywords: *ultrasound, extraction, antioxidant, Coleus tuberosus, peel, ursolic acid, oleanolic acid*

1. Introduction

Plant-derived antioxidants, such as ursolic and oleanolic acids have shown high values due to their potential health benefits. Consumption of plant foods containing antioxidants is advantageous to health because it down-regulates many degenerative processes and can effectively lower the incidence of cancer and cardio-vascular diseases(Sultana et al, 2009). One of the plants that could be potential as antioxidant is *Coleus tuberosus,* which is in Indonesia also known as black potatoes. Black poetatoes will not be consumed due to their higher cyanide contents. The presence of triterpenic acids compounds in black potatoes has given considerable potential as source of antioxidant and immune enhance system (Nugraheni, 2012). There are two types of triterpenic acids compounds contained in *Coleus tuberosus* namely ursolic acid and oleanolic acid (Nugraheni et al., 2010). As the triterpenic acids are the most dominant antioxidant compounds, the extraction of these acid compounds is still interest of study. Black potato's peel has not been explored so far for their potential of antioxidant. Nugraheni et al (2011) found that ursolic acid content of ethanolic extract of flesh and peel of Coleus tuberosus are 3.41 ± 0.04 μg/ml and 13.78 ± 0.15 μg/ml, respectively and Oleanolic acid content of ethanolic extract of flesh and peel of 3.71 ± 0.07 μg/ml and 1.98 ± 0.30 μg/ml, respectively.

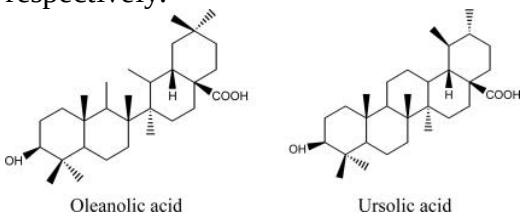

Figure 1. The chemical structure of oleanolic and ursolic acids as the main antioxidant in black potatoes (Xia et al,2011, 2012)

Extraction is a common technique to recover antioxidant compounds from plant material, while solvent based extraction is the most frequently used method for isolation of plant antioxidant compounds. The yield of extraction is strongly dependent on the nature of solvent due to the varied characteristic of

chemistry in the antioxidant compounds. In addition, the polarities of antioxidant compounds become another consideration in the choosing the suitable solvents. For most polyphenols from a plant matrix, they are usually extracted by using polar aqueous solvents such as ethanol, methanol, acetone, propanol or ethyl acetate (Peschel et al, 2006). Among these solvents, ethanol and methanol have been extensively used to extract antioxidant compounds from various plants and plant based foods such as rice bran, wheat grain, rosemary, while ethyl acetate was also used for extracting the compounds from citrus peel (Abdille et al, 2005; Rehman et al, 2006). Therefore, the study of effectiveness of various solvents in antioxidant extraction of black potatoes is still interesting for being studied. Several conventional methods such as maceration, soxhlet or reflux methods have been intensively used to extract the antioxidant compounds. However, these methods are sometimes solvent- and time-consuming and obtained low yield of extract (Xia et al, 2012; Lin et al, 2007). Process intensification by applying ultrasonic – assisted extraction (UAE) has shown significant improvement of extraction time of plant material (Lin et al, 2007).

The ultrasound-assisted extraction may enhance the extraction efficiency due to disruption of cell walls, particle-size reduction, and enhancing mass transfer of the cell contents as a result of cavitation bubble collapse(Wu et al, 2001; Melecchi, 2006). Therefore, UAE is a highly efficient and reduced solvent- and time-consuming method. However, it is unknown whether the extraction efficiencies of oleanolic acid and ursolic acid from black potatoes peel could be improved by the ultrasonic-assisted extraction. Effects of several experimental parameters, such as type and concentration of extracting solvent, extraction temperature and extraction time, on the extraction efficiencies of oleanolic acid and ursolic acid from black potatoes also need to be evaluated.

Therefore, this research was aimed to evaluate the effect of solvent type, ratio of sample and solvent on batch extraction, effect of ultrasound assisted ultrasound extraction compared to conventional batch extraction, and the effect time and temperature used on ultrasound assisted extraction toward antioxidant activity from the extract obtained from *Coleus tuberosus* peel.

2. Materials and methods

The materials used were *Coleus tuberosus* peel, methanol, ethanol, 2-propanol, and ethyl acetate as a solvent, DPPH (diphenylpicrylhydrazil) as a reagent to test the antioxidant activity. The conventional extraction was carried out in an extraction vessel heated and stirred by magnetic stirrer plus heater. The ultrasound assisted extraction was carried out in an ultrasonic cleaning bath (Bransonic 2510E-DTH with ultrasonic power of 100W and frequency of 42kHz).

Preparation of Raw Material

Coleus tuberosus was washed to remove any dirt from its skin. *Coleus tuberosus*' skin was peeled to $\pm$ 1mm thick using a potato peeler. The peel then dried in an oven for 24 hours at 40°C. After the drying process done, the peel was grinded using a blender.

Conventional Extraction

The conventional extraction was carried out in an extraction vessel heated and stirred by magnetic stirrer plus heater. The bath temperature was 40°C and the extraction time was 45 minutes. The solvents used in the extraction were methanol, ethanol, 2-propanol, and ethyl acetate. The solid to solvent ratio was varied from 1:25 to 1:75 (w/v). The extract was then filtered and concentrated into 25 mL. The extract was stored in the refrigerator for further analysis.

Ultrasound Assisted Extraction

The extraction was carried out in an erlenmeyer flask. Sample solid to solvent ratio and type of solvent used in the ultrasound

assisted extraction was based on the optimum condition obtained from the conventional

extraction method. The bath temperature was varied from 30 to 60°C and the extraction time was varied from 30 to 60 minutes. The extract was then filtered and concentrated into 25 mL. The extract was stored in the refrigerator for further analysis.

Fourier Transform Infra Red (FT-IR) Assay

FT-IR assay was done to identify the functional groups contained in the extract. Sample preparation was done using Diffuse Reflectance Attachment method (DRS-8000), where the dried extract was grinded and mixed with Potassium Bromide (KBr). The FT-IR assay was done using Fourier Transform Infrared Spectrophotometer Shimadzu IR Prestige-21.

Determination of Antioxidant Activity

The antioxidant activity assay was conducted based on the DPPH method of Celli et al. (2011). About 100µL of crude extract were added to 3.9 mL of 60µM DPPH solution. After 30 minutes of incubation in the dark at room temperature. The absorbance then was measured at 515 nm. The antioxidant activity can be calculated according to the following formula :

$$Antioxidant\ activity(\%)=\left[1-\left(\frac{Absorbance\,sample_{t=30}}{Absorbance\,control_{t=0}}\right)\right]x100\%$$

3. Results and disscussion

Functional Groups Identification using FT-IR Assay

FT-IR (*Fourier Transform Infra Red*) assay was done to identify functional groups contained in the *Coleus tuberosus* peel extract. The functional groups were identified to analyze whether the desired antioxidant substances (ursolic acid and oleanolic acid) is present in the extract.

The FT-IR spectrum of *Coleus tuberosus* peel extract is shown on the Fig. 2. There are 9 peaks detected on the spectrum. The peaks represent the functional groups detected in the *Coleus tuberosus* peel extract. The functional groups detected in the extract are alcohols (O-H stretch, C-O stretch), carboxylic acids (O-H stretch, C=O stretch, C-O stretch), alkanes (CH_3 bend, CH_2 bend, C-H stretch), and aromatics (C=C stretch). The functional groups detected in the extract show the functional groups of ursolic acid and oleanolic acid.

Effect of Solid to Solvent Ratio

The effect of solid to solvent ratio on the antioxidant activity of *Coleus tuberosus* peel extract was investigated at various solid to solvent ratio (1:25, 1:50, 1:75 (w/v)) and solvent (methanol, ethanol, ethyl acetate, 2-propanol). The extraction was done at 45°C for 45 minutes using conventional method. The result is shown on Fig. 3. It is found that the higher solid to solvent ratio, the higher antioxidant activity of *Coleus tuberosus* peel extract regardless of the solvents used in the extraction.

The higher solid to solvent ratio gives larger concentration gradient of antioxidant (ursolic acid and oleanolic acid) between the solid and the solvent. Concentration gradient is one of driving forces in the extraction process. This causes the higher mass transfer from solid to solvent as the concentration gradient increase, therefore the antioxidant content in the solvent will increase (Vetal *et al*, 2013). The higher antioxidant content corresponds to the higher antioxidant activity of the extract (Nugraheni *et al*, 2011).

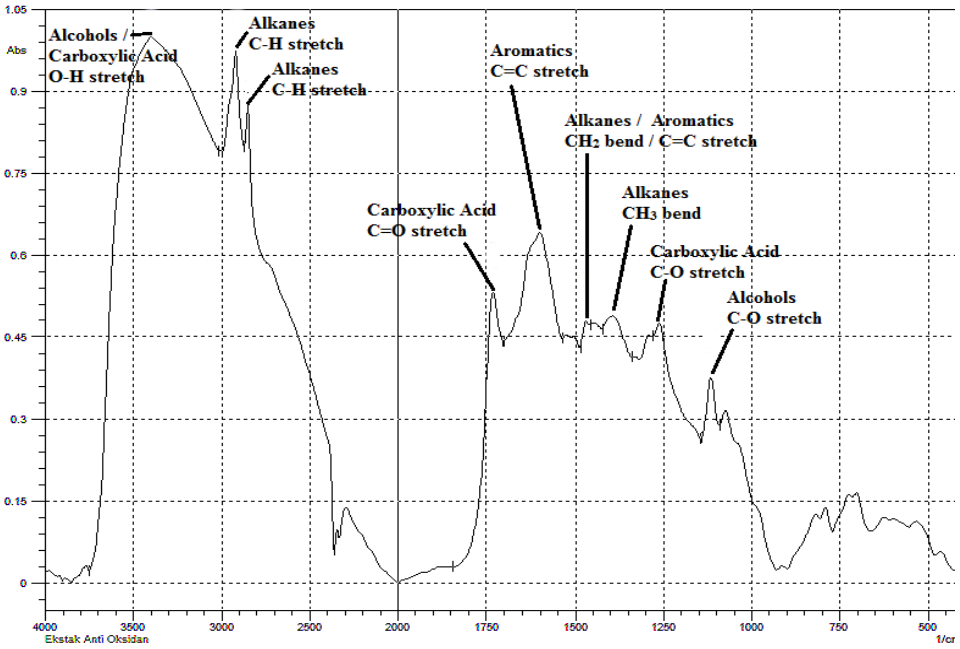

Figure 2. FT-IR Spectrum of Coleus tuberosus Peel Extract

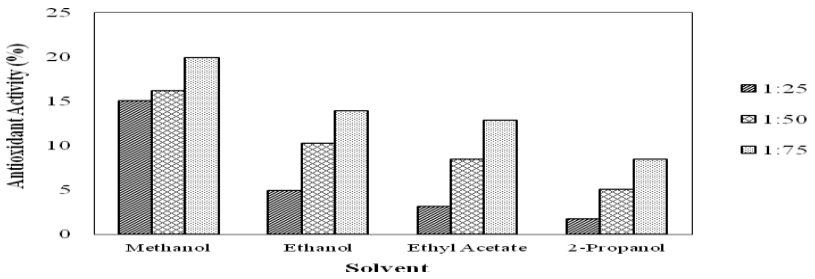

Figure 3 Antioxidant Activity of Coleus tuberosus Peel Extract on Various Solvent and Solid to Solvent Ratio

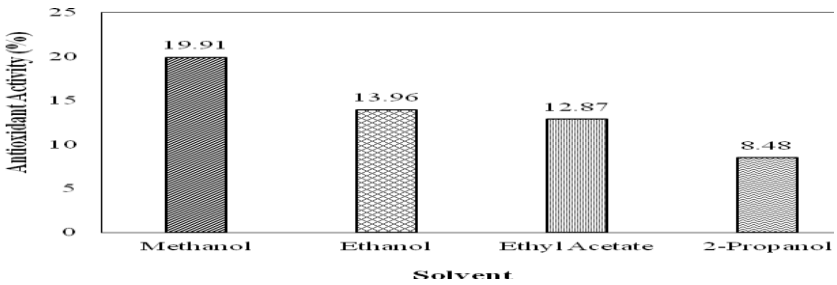

Figure 4 Antioxidant Activity of Coleus tuberosus Peel Extract on Various Solvents (Temperature : 45°C, Time : 45 Minutes, Solid to Solvent Ratio 1:75 (w/v))

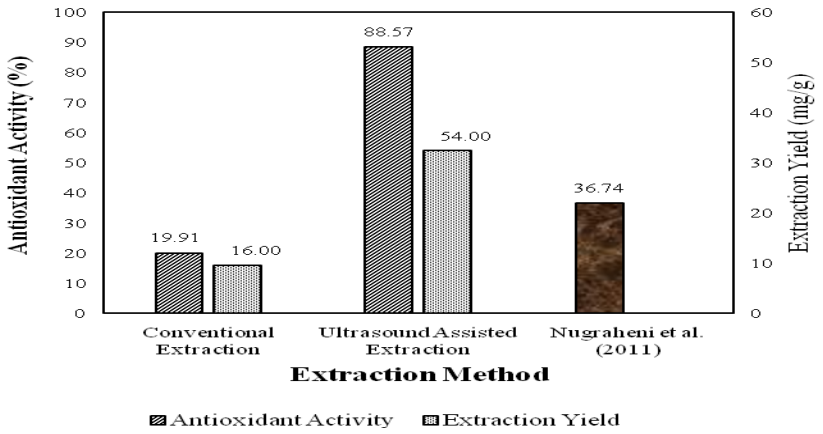

Figure 5 Antioxidant Activity of Coleus tuberosus Peel Extract on Various Extraction Methods (Temperature : 45°C, Time : 45 Minutes, Solvent : Methanol, Solid to Solvent Ratio 1:75 (w/v))

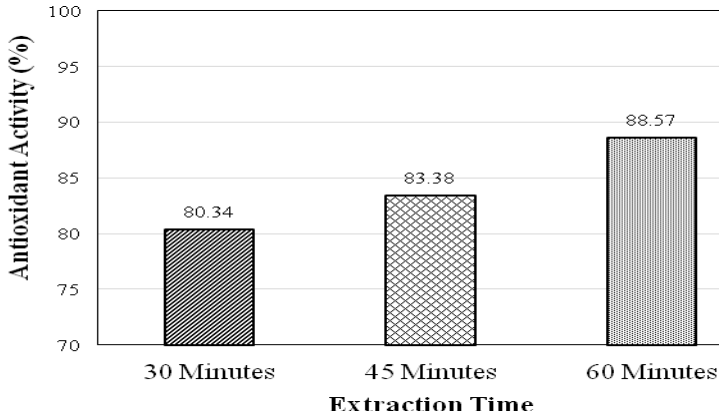

Figure 6 Antioxidant Activity of Coleus tuberosus Peel Extract on Various Extraction Time (Temperature : 45°C, Solvent : Methanol, Solid to Solvent Ratio 1:75 (w/v))

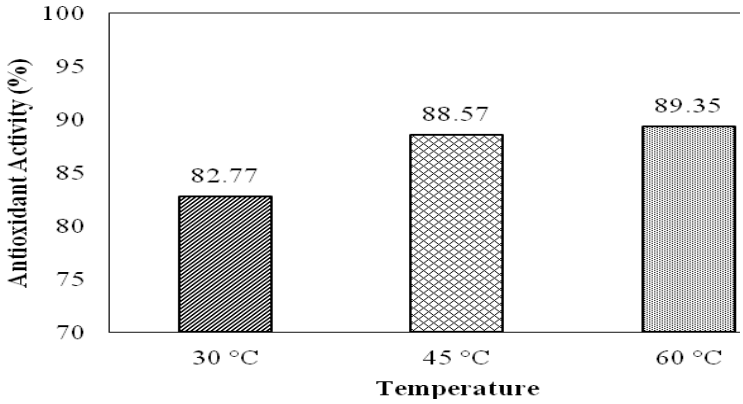

Figure 7 Antioxidant Activity of Coleus tuberosus Peel Extract on Various Extraction Temperature (Time : 60 minutes, Solvent : Methanol, Solid to Solvent Ratio 1:75 (w/v))

Effect of Solvents

The effect of solvents on the antioxidant activity of *Coleus tuberosus* peel extract was investigated using four different solvents (methanol, ethanol, ethyl acetate, 2-propanol). The extraction was done at 45°C for 45 minutes using conventional method and various solvents. Solid to solvent ratio 1:75 (w/v) was used to investigate the effect of solvents because it yields the highest antioxidant activity compared to the other solid to solvent ratio used in this study.

The result is shown on Fig. 4. Methanol yields the highest antioxidant activity among the other solvents. This is because methanol has the highest polarity compared to the other solvents. Polarity of a solvent has an important role in the extraction process because ursolic acid and oleanic acid are polar compund in nature and the solubility of ursolic acid and oleanic acid increases as the solvent polarity increases (Vetal et al., 2013). Furthermore, the higher polarity of a solvent increases the permeability of the cell wall and helps in increasing extraction yield (Vetal et al., 2013).

Effect of Extraction Method

The effect of extraction methods on the antioxidant activity of *Coleus tuberosus* peel extract was investigated using two different extraction methods (conventional extraction and ultrasound assisted extraction). The extraction was done at 45°C for 45 minutes using methanol as a solvent and solid to solvent ratio used was 1:75 (w/v). The result is shown on Fig. 5. It is found that the antioxidant activity increases significantly using ultrasound assisted extraction. Ultrasound assisted extraction yields antioxidant activity 4.4 times higher than conventional extraction. The antioxidant activity obtained using ultrasound assisted extraction in this research is higher than the antioxidant activity obtained in a research done by Nugraheni et al. (2011). The extraction of *Coleus tuberosus* peel done by Nugraheni et al. (2011) was conducted by maceration method at room temperature for 7 days using ethanol with solid to solvent ratio 1:5. The extraction yields obtained in this research are 16 mg dry extract per gram *Coleus tuberosus* peel for conventional extraction and 54 mg dry extract per gram *Coleus tuberosus* peel for ultrasound assisted extraction.

In the ultrasound assisted extraction, the cavitation effect caused by ultrasonic radiation helps penetration of solvent into the cells of *Coleus tuberosus* peel, so that the ursolic acid and oleanolic acid can be extracted easily (Kosior et al. 2013). Furthermore, the cavitation effect produces physical effects such as liquid circulation currents and turbulence which results in significant increase in the mass transfer rate and extraction yield (Vetal et al., 2013). In addition to cavitation effect, ultrasound assisted extraction also causes disruption of cell walls and increase mass transfer rate across cells membrane (Vetal et al., 2013).

Effect of Time

The effect of extraction time on the antioxidant activity of *Coleus tuberosus* peel extract was investigated at various extraction time (30, 45, 60 minutes). The extraction was done at 45°C using methanol as a solvent and solid to solvent ratio used was 1:75 (w/v). The result is shown on Fig. 6. It is found that the longer extraction time, the higher antioxidant activity of *Coleus tuberosus* peel extract. The longer extraction time gives the longer contacting time between solvent and solute, therefore the higher antioxidant (ursolic acid and oleanolic acid) extracted from *Coleus tuberosus* peel. The yield of an extraction increases as the extraction time increases (Petigny et al., 2013). The higher amount of antioxidant can be extracted from *Coleus tuberosus* peel extract corresponds to the higher antioxidant activity of the extract (Nugraheni et al., 2011).

Effect of Temperature

The temperature effect on the antioxidant activity of *Coleus tuberosus* peel extract was investigated over temperature range 30-60°C. The extraction was done during 60 minutes using methanol as a solvent and solid to solvent ratio used was 1:75 (w/v). The result is shown on Fig. 7. It is found that the temperature rise causes the antioxidant activity of *Coleus tuberosus* peel extract increases. The solubility of ursolic acid and oleanolic acid increases as the temperature increases, therefore the antioxidant content in the solvent will increase as the temperature increases (Fan et al., 2011). In addition to the solubility, the diffusion of ursolic acid and oleanolic acid increases due to reduction of solvent viscosity as the temperature increases (Vetal et al., 2013). The higher diffusion of ursolic acid and oleanolic acid, the higher mass transfer occurs from *Coleus tuberosus* peel's cells to the solvent, therefore the antioxidant content in the solvent will increase as the temperature increases (Xia et al., 2012). The higher antioxidant content corresponds to the higher antioxidant activity of the extract (Nugraheni et al., 2011).In spite of that, there is temperature limitation in the extraction of ursolic acid and oleanic acid from *Coleus tuberosus* peel, because the ursolic acid and oleanic acid is thermally stable up to 70°C (Xia et al., 2012). The ursolic acid and oleanic acid starts degraded if the extraction temperature is more than 70°C.

4.Conclusion

The results from this study showed the effect of solvent, solid to solvent ratio, extraction method, time, and temperature of extraction on the antioxidant activity of the extract obtained. The higher polarity of a solvent increases the permeability of the cell wall and helps in increasing extraction yield. Ultrasound assisted extraction yields antioxidant activity 4.4 times higher than conventional extraction. The higher solid to solvent ratio gives larger concentration gradient of antioxidant (ursolic acid and oleanolic acid) between the solid and the solvent. The longer extraction time gives the longer contacting time between solvent and solute, therefore the higher antioxidant (ursolic acid and oleanolic acid) extracted from *Coleus tuberosus* peel. The higher extraction temperature, causes the higher solubility of ursolic acid and oleanolic acid and reduces the solvent visosity. Extract obtained by ultrasound assisted extraction method at 60°C for 60 minutes of extraction time using methanol as a solvent with solid to solvent ratio 1:75 showed the highest antioxidant activity compared to any other variables with 89.35% of antioxidant activity.

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RESEARCH CONCERNING THE ROASTED COFFEE

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ABSTRACT

This paper aims to study the influence of the roasting process on caffeine content in SIDAMO coffee sort cultivated in Ethiopia and the influence of solubilization time on caffeine released in water. It was concluded that during the roasting process, the content of caffeine decreases by 0.715% as compared with the untreated coffee. A period of solubilization of 30 minutes is sufficient for a significant extraction of caffeine.

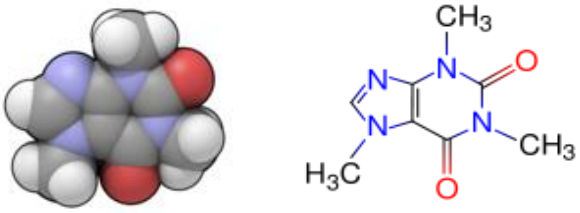
Keywords: *coffee, caffeine content, solubilization, UV-VIS spectrophotometry*

1.Introduction

Coffee is a beverage enjoyed by millions of people around the world for at least a thousand years. In addition, coffee is now one of the most studied products in the world, the subject of thousands of scientific published studies. Coffee beverages are increasingly popular worldwide due to their organoleptic characteristics and stimulating effects [1]. Its consumption continues to increase due to its physiological effects as well as its pleasant taste and aroma. We know about 80 varieties of coffee, of which is cultivated for industrial purposes following four types of coffee: Arabica coffee, Robusta coffee, Liberia and Maragogype coffee. [2]

Recent research suggests that drinking moderate amounts of coffee (two to four cups per day) provides a wide range of health benefits such as decrease of the risk of colon cancer, gallstones, cirrhosis of the liver and Parkinson's disease. [3]

One of the most relevant properties of coffee beverages is their antioxidant activity [4]. In fact, roasted coffee and brews are replete with antioxidant compounds, namely melanoidins [5] phenolic acids [6], caffeine [7, 8] and tocopherols [9], among several others. Caffeine is a substance which stimulates the central nervous system, reduces sleepiness and increases vigilance. It is one of the components that explain the popularity of that drink [10]. Caffeine structured in Figure 1 is the main alkaloid present in coffee and thus the determination of caffeine is required in food laboratories in order to inform the consumers about the characteristics of coffee samples [11].



Spatial formula *Structural formula*

Figure 1. Spatial and structural structure of caffeine [12]

This paper aims is to study the influence of the roasting conditions and of the solubilization time on the content of caffeine from the coffee sort SIDAMO cultivated in Ethiopia.

It has been observed that were many interfering matrices extracting with chloroform than dichloromethane, literature report indicated that even if both solvents were useful for decaffeinating caffeine from coffee beans the current most widely used solvent for decaffeinating in coffee beans was dichloromethane[13, 14]. The efficiency

of dichloromethane to extract caffeine from coffee beans is 98–99%. [11]

2. Materials and methods

2.1.Coffee sample preparation

Caffeine analysis was made according to the method indicated by A Belay et al., 2008 and includes the following steps: a) The roasted coffee beans are ground, then a precise amount of powder of 0.05 g is solubilized in 25 mL distilled water for 10, 20, 30, 40 and 50 minutes, meanwhile the solution is stirred. The solution was filtered by a glass filter to get rid of particles. A volume of 5 mL filtrate was mixed with 5 mL of dichloromethane for the extraction of caffeine from coffee. The time of extraction was 10 minutes. The procedure is repeated two times with 5 mL dichloromethane, collecting the extract in volumetric flasks. Finally, the absorbance of the solution was measured by UV/VIS spectrophotometer at 274 nm. b) The calibration curve was constructed using pure caffeine samples dissolved in dichloromethane and the absorbance was read at 274 nm. By plotting the values of absorbance against the concentrations of caffeine samples the calibration curve was designed. The caffeine concentrations in coffee samples were red from calibration curve and expressed as mg caffeine/ml of sample.

3. Results and discussion

3.1. Caffeine content in green and roasted coffee

As shown in Figure 3, the caffeine content in green coffee was 50.19 mg/g, but during the coffee roasting at 200^0C, the caffeine content decreases by 0.71%, reaching a value of 35.92 mg caffeine/g coffee.

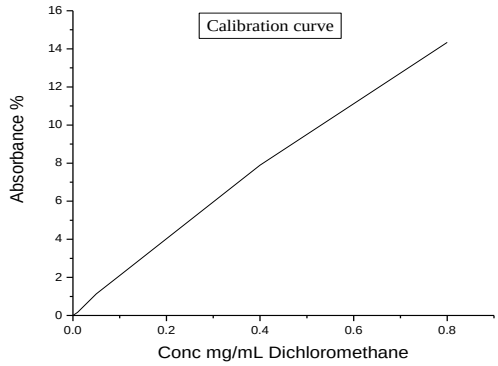


Figure 2. Calibration curve of the caffeine in dichloromethane

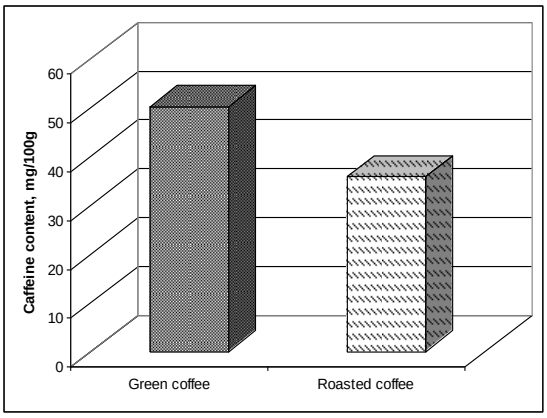


Figure 3. The influence of roasting process on the content of caffeine

3.2. Evolution of caffeine level during solubilization

The influence of the solubilization time (10, 20, 30 and 40 min) of the coffee powder in the solvent on the caffeine content is represented in Figure 4. It can be noticed the increase of caffeine concentration, but after 30 minutes of solubilization it is notice a flattening, a sign that the extraction of caffeine is no longer carried.

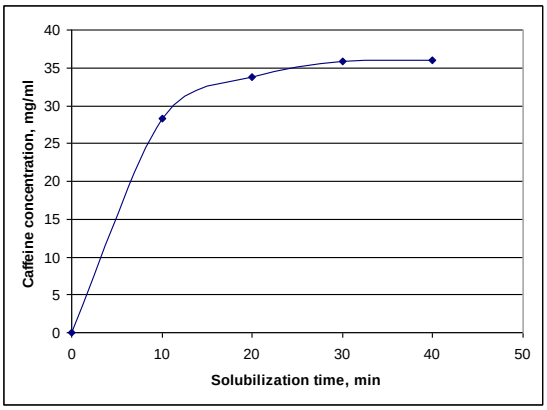


Figure 4. The influence of solubilizing time on the caffeine concentration

The extraction yield (represented by the maximum caffeine concentration in water) based on the time of solubilization is represented in Figure 5. In the first 10 minutes of solubilization, caffeine it's extracted in a percentage of 78.85%. After 20 minutes, the extraction yield was 1.12%, and after 30 minutes, the maximum concentration of caffeine was extracted (100%). After 40 min of extraction, the extraction yield decreased due to the fact that the caffeine extraction does no longer take place.

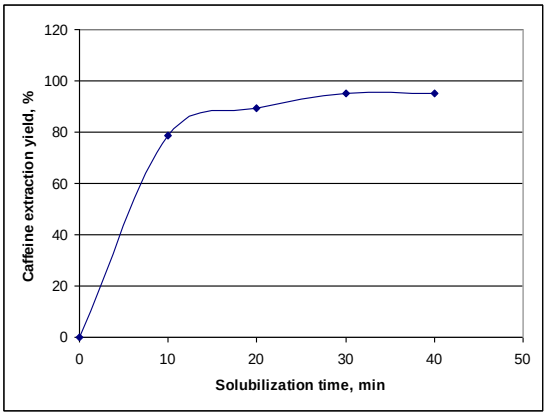


Figure 5. Caffeine extraction yield % versus solubilization time

4. Conclusions

The concentration of caffeine in roasted coffee decreases compared to green coffee. After 30 minutes to solubilization the caffeine extraction does not longer occur. Caffeine content in roasted coffee decreases due to sublimation of this substance during the process of green coffee roasting.

In term of caffeine solubilization the maximul yield is obtained after 30 minutes.

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COSTS CONTROL MODEL OF FOOD CROP LOGISTICS DISTRIBUTION

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ABSTRACT

Food crop is a kind of special commodities. Logistics distribution process from field to table and from acquisition of raw material to final customers all have special requirements. Due to various reasons, development of food crop logistics distribution in China are still in a relatively backward level, which lead to great loss in logistics distribution process and serious food crop safety incidents. At this stage, food crop logistics distribution problems not only show on storage technology but also show more on management of whole process of food crop logistics distribution. Therefore, launch of food crop logistics distribution research and exploration of new approach for solving food crop logistics distribution fundamentally is of great practical significance. And research result can make scientific contribution for enriching theory system of food crop logistics distribution. Applying theory and method of management of food crops, logistics distribution management and system theory, this paper establishes distribution costs model by literature analysis and site survey adopting basic mode of data algorithm. It is beneficial to redefine food crop logistics distribution system and clearly analyze the elements, structure, function and characteristics of food crop logistics distribution system.

Keywords: *food crop, logistics distribution, costs control model*

1. Introduction

Logistics distribution of food crop refers to entity flow of whole process from production place to consumption place of food crop. It includes steps such as production, purchasing, transportation, storage, handling, carry, package, distribution, distribution processing, distribution, information process and achieving value added and organization goal of food crops in this process. We often lack of preservation equipment in the process of food crop logistics distribution. Cold storage is the best preservation technology. Food crop abroad mostly applies cold chain system. However, amount of refrigeration house and refrigerated transport vehicle is seriously inadequate in China and terminal refrigeration equipments are also short of. Deficiency of refrigeration equipments greatly restricts fresh-keeping work of food crop logistics distribution of China. In the process of food crop logistics distribution, we often encounter problem of low degree of informatization. Most farmers can not search and release information, which lead to ineffective of market information and restriction of information flow. And many farmers can not find market while wholesaler is badly in need of food crop. Inefficiency and slow pass of market supply and demand information is caused by scatter of food crop information, untimely information publish and update, low quality of information and inconvenience of information search and acquisition. Third problem is particularity

of food crop can not make it achieve full standardization as industry products due to the defective standardized system. However, it can realize standardization on transportation equipment, technology, storage and trade process in order to accelerate logistics operation distribution and reduce the waste in this step. Fourth problem is imperfection of logistics distribution policy. If the overall situation of logistics distribution in China can not develop normally, then logistics distribution of food crops will be affected. Although rural reform promotes the development of countries to a great extend, it is falling far behind cities. China does not have good policy to guide the logistics distribution operation of agricultural food crops [1]. Problems which exist in logistics distribution of food crop trouble farmers. Then how many are the farmers and how serious is the problem? Following is the data which is found in State Statistics Bureau—percentage of food crop farming area and the price index of food crop in recent four years. We can have a general understanding of importance of logistics distribution of China by these data [2].

2.Material and method

Establishment of logistics distribution costs control model of food crop

2.1 Material

60, 000, 00 kg of food crop from some corporation is selected. Average price is 10110 yuan. Ordering cost is 150 yuan every time. Lead time of ordering is 3 days. Work day is 300 days a year. And storage cost is 8 yuan of single carton. Economic order quantity, total inventory cost and order point is going to be confirmed. Suppose that the distribution center P_0 of that corporation need to delivery food crop to wholesaler in key area P_i and P_j of that city. Amount of distribution food crop of every area is showed in the following table. Transportation cars are all of one model and loading capacity is 12 cases. The distribution is achieved by two cars and shipping lines are going to be confirmed [3].

Table1. Inventory statement of description of food crop

Food crops	Inventory	Proportion%	Costs (thousand yuan)	Proportion
Wheat	6328.5	4.1	750	19.4
Corn	15649.6	10	900.45	23.3
Soybean	20781.5	13.3	617.53	16
Barley	33799.1	21.7	689.52	17.8
Rice	64214.3	41.2	777.5	20.1
Highland barley	15240.5	9.8	128.23	3.3
Total	156013.5		3863.23	

2.2 Method

As shown in the figure, P_0 is distribution center which delivery goods to P_i and P_j. Suppose distance between P_0 and P_i as D_{0i} and between P_0 and P_j as D_{0j}. And suppose distance between two users as D_{ij}. There are only two kinds of delivery plans.

Plan A is that distribution center delivery goods to wholesaler P_i and P_j respectively. Distribution lines are(as shown in figure 1):

$P_0 \_\_\_ P_i \_\_\_ P_0 \_\_\_ P_j \_\_\_ P_0$

Total distribution distance is:

$D_a = 2D_{0i} + 2D_{0j}$

Plan B is that distribution center P_0 delivery goods to wholesaler P_i and P_j in the meantime. Distribution lines are (as shown in the figure 2):

$P_0 \_\_\_ P_i \_\_\_ P_j \_\_\_ P_0$ or $P_0 \_\_\_ P_j \_\_ \_\_ P_i \_\_\_ P_0$

Total distribution distance is:

$D_b = D_{0i} + D_{0j} + D_{ij}$

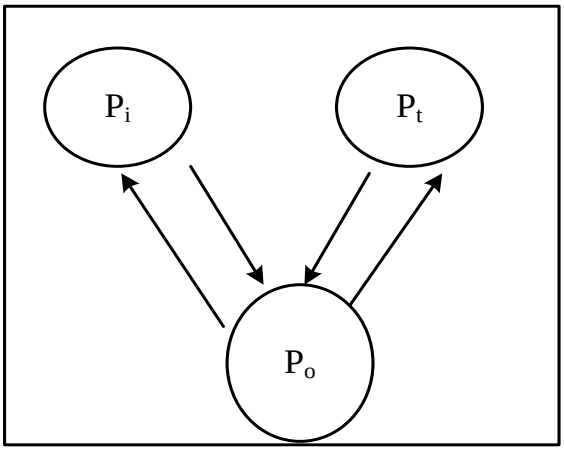

Figure 1. Distribution center (a)

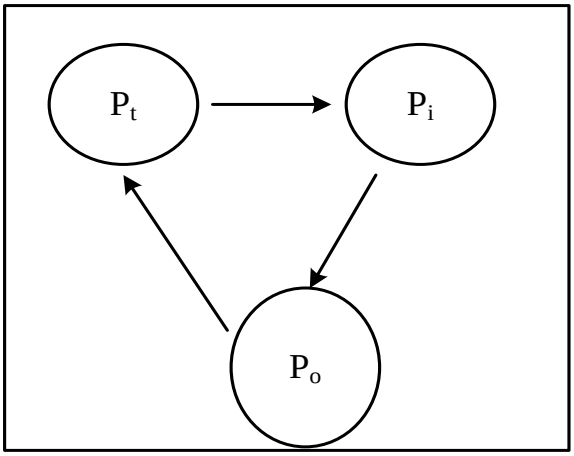

Figure 2. Distribution lines (b)

Compared with two plans, the smaller one in D_a and D_b is more reasonable. The smaller distribution distance is, the more reasonable the plan is.

$$D_a - D_b = D_{0i} + D_{0j} - D_{ij}$$

As shown in figure, P_0 , P_i and P_j can be regarded as three vertex of a triangle, then D_{0i} , D_{0j} and D_{ij} are length of three sides of this triangle. It can be known from the geometric property of triangle that sum of any two sides length is bigger than the third one. Therefore, $D_a - D_b > 0$ that is, $D_a > D_b$. Thus, distribution (b) is better than (a). It is the basic idea of saving method. We define a saving amount and express it by S_{ij} for convenient analysis, then

$$S_{ij} = D_a - D_b = D_{0i} + D_{0j} - D_{ij}$$

Therefore, according to the idea of saving method, if one distribution center P_0 delivery goods to n wholesalers, the more the wholesaler are in the distribution lines, the more reasonable distribution line is and the smaller distribution distance is. As time go on, the saved distance is considerable [4].

① According to the background description, we suppose that demand points are in P_i area. Highway transportation is considered only because P_0 area and P_i area is close. The whole emergency food distribution transportation can be divided into two parts including goods collection and distribution. First, emergency food of corporation is sent to specified goods collection site in P_0 area. Then emergency command center transports emergency food on the goods collection sites to demand sites of P_i area where emergency occur [5].

②Cooperative corporation can

make a fast reaction and quick processing after receiving order in P_0 area. Time of receiving order and arranging order processing can be condensed. At that moment, replenishment lead time t can suppose to neglect that period of time and only include transportation time of emergency food. However, lead time t refers to time of distribution from demand sites of P_0 area to those of P_i area. Time of the whole process can be controlled to within 24 hours and $t \le 1$ according to the background description.

③Demand of emergency food of P_i area is not fixed and will change according to the daily demand. Suppose daily demand as $X(x \in [a,b])$. Daily demand which meet certain state distribution is expressed as $F(x) = P(X \le x) = \int_{-\infty}^{x} f(u)du$.

Suppose μ_x as average daily demand and σ_x as standard deviation of daily demand [6].

④There are a lot of cooperative corporations that choose in P_0 area. Thus, corporations that can meet the requirement need to be selected among these corporations. Besides management order and business condition, we should also make a comparison of delivery situation when the emergency occur [7]. We can make a comparison of quantification. That is, when the command center issues an emergency order of Q to corporation in P_0 area, goods collection distance of corporation and distribution center and delivery speed is the main considering factors. Corporations whose goods collection distance is close should be given priority to and then delivery speed ca being considered. On this basis, emergency food cooperative corporation in P_0 area can be selected. Suppose shipment of cooperative cooperations as Q_i and $\sum_{i=1}^{j} Q_i = Q$.

Suppose number of packing lot as 0 and number of goods collection sites and distribution site as $1,2,\ldots\ldots,m$. Variables are defined as following for constructing mathematic model:

$$x_{ijk} = \begin{cases} 1, \text{Express car k drive from site i to site j;} \\ 0, \text{ otherwise} \end{cases}$$

$$y_{ik} = \begin{cases} 1, \text{express task of site i is fulfilled by car k;} \\ 0, \text{ Otherwise} \end{cases}$$

Symbol description:

C_T : Total costs of distribution;

K: amount of cars needed

q: maximum load capacity of cars

i,j: clients and parking lot, of which i=0 express parking lot;

d_{ij} : Direct distance between site i to site j;

d_i : demand of emergency food of site i;

a_{ai}、a_{bi}: Earliest and latest time of time window of site i;

f_1、f_2: Punishment coefficient when arrive site i earlier or later than service time;

t_i: arrive time of site i;

C_p、C_J: Distribution costs and goods collection costs;

S_i: Service time of site i

If site i and j are on the same line and j will be served after site i., then $t_j = t_i + s_i + t_{ij}$ and distribution cost is $C_p = \sum_{i=0}^{n}\sum_{j=1}^{n}\sum_{k=1}^{k} d_{ij}x_{ijk} + f_1\sum_{i=1}^{n}\max(a_{ai} - t_i, 0) + f_2\sum_{i=1}^{n}\max(t_i - a_{bi}, 0)$ If cars can not fulfill goods collection and delivery and arrive before the service time of site i, then it will be given punishment of coefficient f_1. Otherwise, it will be given punishment of f_2. Value of f_1、f_2 are confirmed according to the actual situation and range from 0 to 1000 generally, which means that every deviation unit of time correspond to increase of distance of f_1、f_2. If value of f_1、f_2 are too big, then it means that deviation of time window of service is not allowed. However, arriving between $[a_{ai}, a_{bi}]$ will not be punished [8].

When the model expresses goods collection costs, then f_1、f_2 are all 0. That is, goods collection is $C_J = \sum_{i=0}^{n}\sum_{j=1}^{n}\sum_{k=1}^{k} d_{ij}x_{ijk}$.

It can be calculated from assumption that total distribution costs of every order cycle time is: $C_T = C_P + C_J$

According to assumption, there are constraint conditions. The constraint expresses that total distribution time from P_0 area and P_i area is within one day (within 24*60 min) [9]. Replenishment lead time have $\frac{\mu_x}{Q}$ of picking period per unit time. Total distribution costs of unit time can be calculated:

$$\frac{\mu_x}{Q}C_T = \frac{\mu_x}{Q}(C_P + C_J)$$

Meet the constraint: $[a_{ai}, a_{bi}] \in [0,1440]$

3. Results and discussion

1.This paper gives priority to consider the timeliness of transportation of food crop and establish costs control model of logistics distribution of food crop and effective solution algorithm is given[10].

2.Costs control model of logistics distribution of food crop that put forward in this paper is reliable and application of genetic algorithm can better solve the transportation problem with complex constraint condition. Model that study in this paper is for the transportation and delivery step of food crop. In the further research later, we can comprehensively consider the step of warehouse management of logistics distribution of food crop to search for optimizing of total

costs of food crop logistics distribution [11].

4. Conclusions

In conclusion, we can apply systematic thought and activity-based costing to reexamine the existing business process, improve the operation, promote effectiveness and lower the distribution costs.

(1)Establishment of food crop logistics distribution system

Establishment of food crop logistics distribution system includes independence of logistics, set of distribution center of food crop logistics distribution and change of existing pattern of administrative division. Distribution center do not need to build in every city or county. Two levels of distribution pattern of regional logistics center and distribution center should be built. Establishment of distribution center by administrative division is broken. It can increase scale merit, reduce repeated construction and prevent idle of assets.

(2)Handle the relationship between market occupation and service cost.

Ideal market occupation rate need more service objects. With the increase of service objects, service cost increase significantly. Therefore, service objects should be screened especially the wholesalers with small sale, far distance and small market impact. First, it can increase the profitability of key and general wholesalers to solve the phenomenon that food crop wholesalers sell out cigarettes but do not make money; second is lower service costs; third is distinguishing key and general wholesalers and carry out differentiated service [12].

(3) Improvement of business process.

Code process should be optimized and relatively concentrated. With the standard and unification of provincial agricultural market, coding of food crop in the province should be transformed from business to concentration code of Industrial Corporation. Business corporations which take City Corporation as unit only code on food crop outside the province (or transform to concentration code of regional distribution center). The practice can reduce code equipment and code stuff.

(4)Support by informatization to improve the automatic level of logistics distribution

Rapid development of modern logistics is supported by modern information technology. Without informatization, high efficient logistics will not be mentioned. Thus, automatic level of logistics distribution should be constantly improved. For example, replacing labor promotion sale by phone promotion sale can improve 75% of efficiency; automatic sorting system is applied to ensure goods be sorting.

(5)Extend distribution period

Existing distribution period should be recalculated. And no matter it is city or rural wholesaler, sales volume should be reverified. Different distribution periods should be confirmed by different kinds of clients. The adjustment of distribution period should prevent not only the effect of service quality but also the market impact of increase of single delivery quantity.

(6)Distribution line optimization and improvement of loading rate

Current distribution is mainly one-way distribution, that is, car is loaded full before departure and empty when return.

Loop line distribution is carried out by line optimization to improve loading rate.

(7)Positive development of diversified distribution

So far, distribution of food crop is single product, whose sale only account for 10%. In addition, price difference is small and attraction to wholesalers is not strong. Thus, current cigarette sales network is applied to develop distribution of non-smoking products, enlarge distribution of diversified products, improve the reliability of wholesalers on tobacco distribution and lower the costs of cigarette distribution.

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ANTIFUNGAL EFFECTS OF SAGE ESSENTIAL OIL AGAINST *PENICILLIUM* INFECTION ON ORANGES STORAGED IN MODIFIED PACKAGING

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ABSTRACT

The objective of this study is to observe the influence of essential oil obtained from sage (*Salvia Officinalis*) on the mold *Penicillium* and create a package of a protective film for the preservation of oranges. The aim of this study was to access the in vitro and in vivo activity of essential oil obtained from sage against *Penicillium* and based on the results to create a package.

The results indicate that sage essential oil could be used for the control of diseases produced by *Penicillium* but can not be applied directly to the fruits because destroys their tissues. The basic compounds from the sage oil (*Salvia Officinalis)* influence the physico-chemical parameters of oranges but onlye in time.

Keywords: *essential oils, oranges, Penicillium, antifungal activity, package.*

1.Introduction

Generally, consumers prefer to have healthy foods and organic agricultural products. Many countries have special regulations for pesticide residue for import of agricultural crops, especially the fruits.

Fruits and vegetables are very essential for the human body diet because they contain important nutritional value such as vitamins and nutrients.

Quality of fruits and vegetables contains a number of parameters including flavor, texture, nutrition and safety, and the importance of each parameter depends on the product. The most important aspect of quality products is freshness, typicality quality fresh fruits and vegetables when they are harvested. [1]

Packaging plays an important role both in handling and in food preservation that can even increase shelf life. It is a factor of great importance effectively protecting the fruits from light, oxygen and microbial recontamination, thus directly preventing the alteration of fruits.

Since 1960, attempts have been made to create and maintain in modified atmosphere plastic films polymer, ethylene and water provides an additional tool to use packaging technology and maintain a desired atmosphere in the package. Conditions created and maintained in a pack are the result of the interaction of many factors, including those to fruit and vegetables and those related to the environment.[2]

The orange (specifically the sweet orange) is the fruit of the citrus species *Citrus sinensis* in the family *Rutaceae*. The orange is a hybrid, possibly between pomelo (*Citrus maxima*) and mandarin (*Citrus reticulata*). Which has been cultivated since ancient times. As of 1987, orange trees were found to be the most cultivated fruit tree in the world. Orange trees are widely grown in tropical and subtropical climates for their sweet fruit. The fruit of the orange tree can be eaten fresh, or

processed for its juice or fragrant peel. Oranges, like most citrus fruits, are a good source of vitamin C. The most destructive fungal rot of oranges is a mold which is caused by *Penicillium* spp.[3]

Salvia officinalis (sage, also called garden sage, or common sage) is a perennial, evergreen subshrub, with woody stems, grayish leaves, and blue to purplish flowers. It is a member of the family Lamiaceae and is native to the Mediterranean region, though it has naturalized in many places throughout the world. The strongest active constituents of sage are within its essential oil which contains cineole, borneol and thujone. Sagea leaf contains tannic acid, oleic acid, ursonic acid, ursolic acid, cornsole acid, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavonoid, glycosides and estrogenic substances.[4]

2. Materials and method

a) In vitro, on agar

The mold *Penicillium* was isolated from infected oranges and cultivated on PDA. In Petri-dishes. In the PDA, sages essential oil and pesticide Comet was tested on daily growth of *Penicillium.* Medium PDA was made from 500 ml of distilled water and 21 g PDA and sterilized in an autoclave at 121°C at 1-1.2 atm pressure for 20 minutes .The gas light comes on and it creates the right environment for the incubator contamination, then it is poured into the Petri-dishes are placed in the incubator for 20 minutes to solidify the medium. 10µl of sage essential oil, was placed over medium in Petri dishes and dispersed . The same will be done with the pesticide Comet. The control treatment did not have any essential oil. All the Petri-dishes were inoculated with young mycelium of the mold *Penicillium* from the margin of a fungal colony which was cut with a cork-borer (3mm).

Petri-dishes were sealed with parafilm and then were kept in incubator at 25°C for five days. Mycelium growth was measured daily with a ruler in order to watch how it is developing.

b) In vivo, on fruit

Fresh oranges are inoculated with *Penicillium* and cultivated on PDA disks then they are treated with sage essential oil, the control treatment did not have any essential oil .This process is done every half-hour, every hour and every hour and a half. After 5 days, the fruit infection was determinate.

c) Protective film for preservation the oranges

In each plastic cup was placed one fresh oranges samples. Whit the micropipette was taken 2 ml of 95% ethanol and 1 ml of sage essential oil and then dispersed on the surface of the plastic film by means of the Petri-dish. Perform 5 samples. The first sample contains only plastic sheet with protective film. A second sample containing plastic film protective film made of ethanol. The third sample contains plastic film protective film made of ethanol and sage essential oil.. A fourth sample contains the mold *Penicillium* and the plastic film protective made from ethanol. A fifth sample contains the mold *Penicillium* and the plastic film protective made of ethanol and sage essential oil.

3. Results and discussion

a) Results on PDA

As shown in fig.1, fig.2 and fig. 3, maximum mycelia growth of *Penicillium* was in the controlled group and in the treatment with sage. In the controlled group the growth of the mycelium was Complete, with the sage essential oil treatment in the first day had inhibitory effect but in the following days gad no effect on the mold *Penicillium* and the growth was Complete on the following four days. The treatment with sage was also able to

have an inhibitory effect on the mycelia growth but only within the first hours and on the first day. However, the treatment the sage essential oil was having an inhibitory effect but only within the first days after has no effect against the mold *Penicillium digitatum*.

Table 1. The effect of sage essential oil on the *Penicillium* mycelia

Treatments	Reply	1 Day	2 Day	3 Day	4 Day	5 Day
Control	1	Complete	Complete	Complete	Complete	Complete
Control	1	Complete	Complete	Complete	Complete	Complete
Control	2	Complete	Complete	Complete	Complete	Complete
Control	2	Complete	Complete	Complete	Complete	Complete
Control	3	Complete	Complete	Complete	Complete	Complete
Control	3	Complete	Complete	Complete	Complete	Complete
Control	4	Complete	Complete	Complete	Complete	Complete
Control	4	Complete	Complete	Complete	Complete	Complete
Comet	1	a 2,4	Complete	Complete	Complete	Complete
Sage	1	a 1,2	Complete	Complete	Complete	Complete
Sage	1	b 1,0	Complete	Complete	Complete	Complete
Sage	2	a 1,5	Complete	Complete	Complete	Complete
Sage	2	b 1,0	Complete	Complete	Complete	Complete
Sage	3	a 0,8	Complete	Complete	Complete	Complete
Sage	3	b 0,8	Complete	Complete	Complete	Complete
Sage	4	a 1,0	Complete	Complete	Complete	Complete
Sage	4	b 0,9	Complete	Complete	Complete	Complete

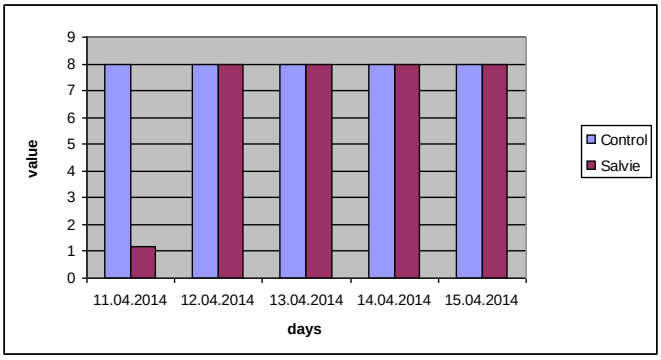

Figure 2 Influence of the sage oil on the Penicillium

Day	Day 1	Day 2	Day 3	Day 4
Treatment sage-control				

Figure 3 The growth of Penicillium on in vitro agar

b) Oranges experiment results

Percentage of infected fruits was determined in a few days of the experiment. Measuring method was observational based on percentage of fruit surface which contamination and the incidence with fungi. Amount of infection was scored between 0 for uninfected fruit and 100 for fully covered fruit with fungi. Based on figure 2. The effect of sage essential oil and pesticide Comet on *Pencillium* spp, haven't been favorable.

Figure 1a. Oranges fruits day 0

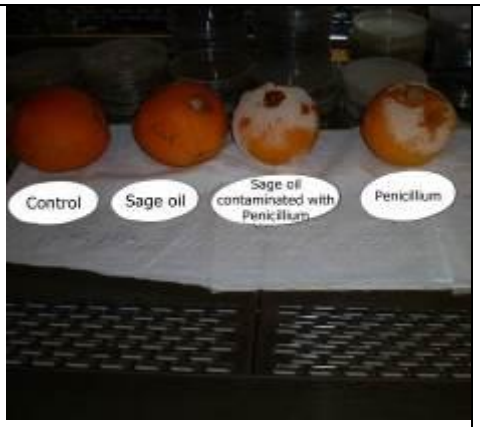

Figure 1b. Oranges fruits after 5 days,

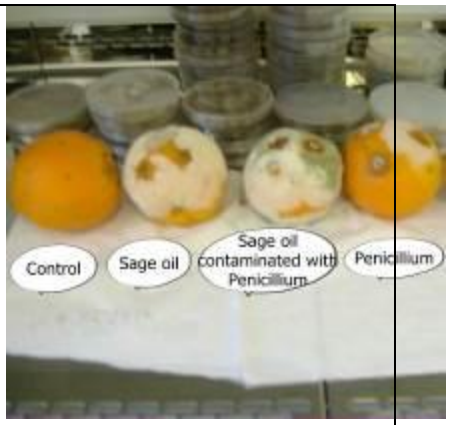

Figure 1c. Oranges fruits after 6 days

Figure 4. Mean comparison effect of different treatments with sage essential oil

c) Protective film for preservation of oranges fruits

The film was made from plastic glass covered with a protective film made of plastic foil and 2 ml of ethanol 95% and 1 ml of sage essential oil. 5 samples are done, the first is made only of plastic foil without the protective film, the second only the plastic foil with film made of ethanol 95%, the third is made of plastic foil and the protective film made of ethanol 95% and sage oil, and the fourth is made also of plastic foil and the protective film of sage essential oil, the fifth sample is made of plastic foil with the protective film made from protective film that contain ethanol 95% and sage essential oil and contaminated with *Penicillium* spp.

Figure 5 Package effect over attack of Penicillium spp.

4. Conclusion

The results show that, the sage essential oil has an antifungal activity and an inhibitory effect on the growth of the mold *Penicillium* spp in the first days, in time it has no effect, this results are for the in vitro on agar. The results for the in vivo, on fruit show that the sage essential oil has no inhibitory effect on the mould *Penicillium* and even destroyed the oranges tissue. Moldy oranges and excluded from human nutrition.

On the other hand the protective film had an inhibitory effect on mould *Penicillium* even on the sample contaminated with the mold *Penicillium* spp.

The sample contaminated with *Penicillium* spp, was only a bit damaged on the surface with a whitish and greenish color from the colonies of *Penicillium* spp this happened only after 10 days.

The conclusion of this study is that the sage essential oil has and antifungal and inhibitory effect on the mold *Penicillium* spp only if the sage oil it is not in a direct contact with the orange fruit, the protective film made of ethanol 95% and sage essential oil has a protective effect, this way we could extend the shelf life of fruits and vegetables and other edible products.

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HEAT TREATMENT OF *LISTERIA MONOCYTOGENES* IN LIQUID EGG PRODUCTS WITH LOW TEMPERATURE

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ABSTRACT

This study was done in order to express a method for thermal destruction of *Listeria monocytogenes* as a psychrotrophic bacterium with ability to grow at refrigerator temperatures in liquid egg products including egg white, egg yolk and homogenized liquid whole egg at lower temperature and longer than normal pasteurization to decrease the damages upon nutrient compounds of egg. Thermal destruction was done in the temperatures of 53°C, 55°C and 57.5°C. Application of thermal process was done by glass tube method. In present study, D-value and Z-value of *L. monocytogenes* in egg white, egg yolk and homogenized liquid whole egg at 53°C, 55°C and 57.5°C was determined. Thermal resistance was the highest in egg yolk. The lowest thermal resistance was observed in egg white, and in homogenized liquid whole egg, had a medium thermal resistance. For *L. monocytogenes*, D-values ranged from 6.69 min in egg white at 57° C to 24.99 min in egg yolk at 53°C. This study indicated that the temperatures of 53°C, 55°C and 57.5°C can be used to remove *L. monocytogenes* with maintain the egg quality.

Keywords: *Thermal resistance, Listeria monocytogenes, egg.*

1. Introduction

Listeria monocytogenes is an important foodborne pathogen that causes listeriosis in humans and animals. Foodborne listeriosis often associated with severe illness mainly in unborn children, infants and the elderly, as well as in immunocompromised persons. In the recent 25 years, food has been an important factor in the transmission of human listeriosis in developed countries (Tompkin 2002). *L. monocytogenes* leads to meningitis or meningo-encephalitis, bacterimia and septicaemia and serious bacterial infection in human (De Valk *et al.* 2005). Milk and milk products, meat and meat products, plant products and fish and fish products can considered as a source of *Listeria* spp. In epidemiological studies egg and egg products have never been caused listeriosis, but is most frequently isolated from egg shells and in the environment of laying hens (Rivoal *et al*. 2010)**.** Chemaly *et al.*, (2008) sampled from dust and faeces of 200 laying hen farms and showed that 15% of samples were contaminated with Listeria and reported which bacterium resistant under storage and handling conditions of shell eggs (Chemaly *et al*, 2008). *L. monocytogenes* is a psychrotrophic bacterium with the ability to grow at low temperatures. Slow growth of some *L. monocytogenes* strains was observed at a temperature of -1.5°C to -0.1°C and may tolerate high salt concentrations (23.8% NaCl)(Larson *et al*. 1999)**.** This organism can survive 90 and >14 days on egg stored at 5°C and 10°C, respectively, persist on inoculated eggs treated with sodium hypochlorite containing 100 ppm available chlorine(Gandhi *et al*. 2007, Bartlett 1993). It can grow in infected egg which stored at refrigeration and ambient temperatures (Gandhi *et al*. 2007). Eggs contain nutrients that form a suitable substrate for the growth and multiplication of microbes (St. Louis *et al*. 1988). The best method for controlling these pathogens in egg products (liquid whole egg, liquid egg white

and liquid egg yolk) is use of heat pasteurization (Tompkin 2002). For the standard pasteurization of egg products suggested by USDA, it is necessary that liquid egg white heated at 56.6ºC and liquid whole egg heated at 60ºC for minimum 3.5 min(USDA 1969). The heat resistance of *L. monocytogenes* depends on the age, pH, growth culture, salt, acid content, water activity and the presence of potential inhibitors (Palumbo *et al*. 1995, Palumbo *et al*. 1996). Usually the D-value (decimal reduction time, or time required to inactivate 90% of the population, characteristic to the heat sensitivity of the bacterium. Another factor is Z-value (negative reciprocal of the slope of the regression line between decimal logarithms of D-values) that is important in heat treatment (Murphy *et al*. 2002). Our purpose is to develop a model for a deletion *L. monocytogenes* in egg products at 53 °C, 55 °C and 57.5 °C.

2. Materials and methods

Collection of Samples: Samples (fresh eggs) were collected from a Shahrekord egg processing plant 24 hours before the experiments and were stored in a refrigerator until testing. The eggshells washed with 70% ethanol and allowed to air dry. Then eggs were broken in the aseptic laboratory condition and egg products were separated in three samples, containing homogenized liquid whole egg, egg yolk and egg white and transferred to a sterile glass bag. These samples were cultured on tryptic soy agar as enrichment media for 24 hours at 37°C and then on palcam agar as selective media to examine listeria contamination. As heat resistance of *L. monocytogenes* can be variable with pH of food matrix(Jayamanne *et al*. 2010), pH of samples were detected.

***Microbial strain*:** We prepared inoculum with concentration of approximately 5×10^8 CFU/ml of 24 h fresh culture of the *L. monocytogenes* ATCC 19114 grown on tryptic soy broth media at 37°C for 24 hours. one ml of this media (TSB containing bacteria) or 5×10^8 CFU of *L. monocytogenes* was inoculated in to 100 ml of each liquid egg samples (5×10^6 CFU/ml of egg samples).

***Heat process*:** Infected samples were incubated in water bath (model: LWB30T) at 53°C and sampling was done at different times (every 5 minutes). For reisolation of bacteria, serial dilution and culture on Tryptic Soy Agar with 0.6% yeast extract (TSAYE) media with surface plating method, was done (20). After 48 h incubation at 37°C, counting of bacterial colonies on TSA was done with a colony counter. These works also were done in 55°C and 57.5°C. All the experiments repeated three times.

***Parameters to evaluate lethality of treatments*:** To evaluate lethality of treatments the glass TDT tubes system (15) were used. At each temperature (53°C, 55°C and 57.5°C), time and temperature heating data were recorded. D-value and Z-value of *L. monocytogenes* for each sample were determined. D-values derived from negative inverse slope of the linear portion of survivor curves. The z-values were determined as the negative inverse slope of the $\log_{10}$D vs. temperature plot (Murphy *et al*. 2002). Using the Destruction time of *L. monocytogenes* in homogenized liquid whole egg, egg yolk and egg white were compared.

3. Results and disscussion

In the present study inactivation kinetics of *L. monocytogenes* was plotted by log-linear decline in surviving cells with time. Results are shown in the figure 1. Based on the linear portion of these survivor curves, D-values were calculated and z-values were obtained. Time required to thermal destruction of all bacteria (5×10^8 CFU/ml) at 53 ° C. in egg yolk, egg white and whole egg were 125, 65 and 95 minutes respectively.

At 55°C scale down rate of bacteria was observed that like to 53°C, but thermal destruction of *L. monocytogenes* was happened faster than 53°C. At the temperature of 53°C for 40 minutes thermal destruction in

egg yolk, egg white and homogenized liquid whole egg were 4.59, 1.45 and 2.97 logarithmic cycle respectively (Figure 2).

Thermal destruction of all liquid egg products were occurred very fast in this temperature. In fact the time required for deletion total of bacteria (5×10^6 CFU/ml) for egg yolk, egg white and homogenized liquid whole egg were 40, 50 and 70 minutes, respectively (Figure 3).

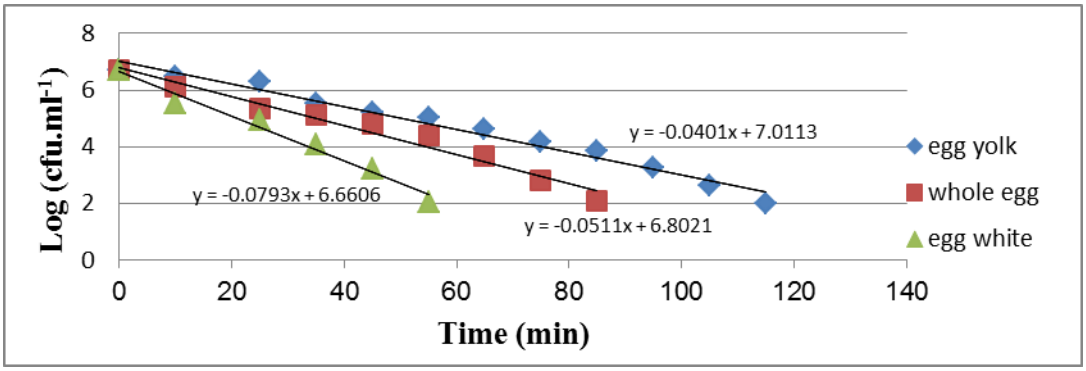

Figure 1. Heat destruction of L. monocytogenes in egg yolk, egg white and whole egg at 53°C.

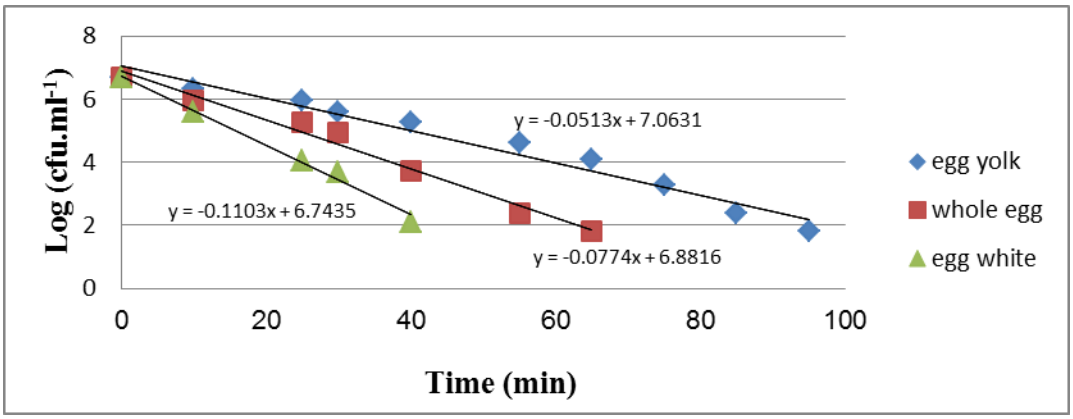

Figure 2. Heat destruction of L. monocytogenes in egg yolk, egg white and whole egg at 55°C.

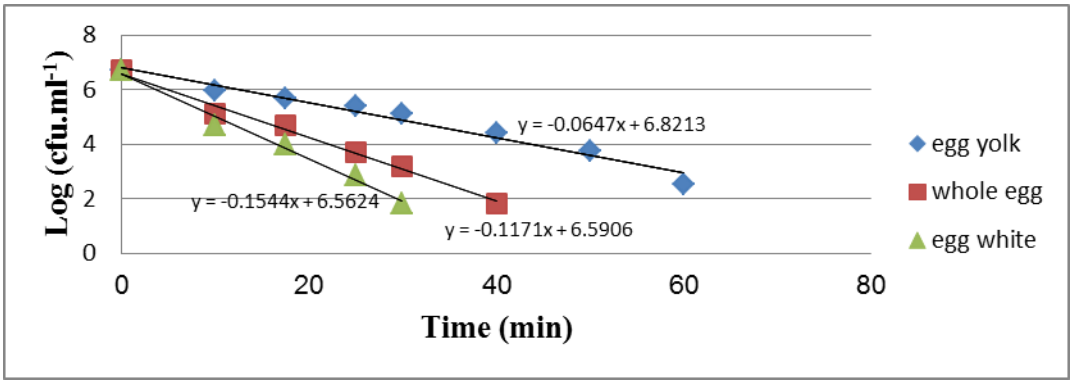

Figure 3. Heat destruction of L. monocytogenes in egg yolk, egg white and whole egg at 57.5°C.

In this study D-value for *L. monocytogenes* at 53°C was acquired highest amount (24.99 min) in the egg yolk, and lowest amount (6.69 min) was acquired for egg white at 57.5°C (Table 1).

Table 1. D-value of *L. monocytogenes* in egg yolk, egg white and whple egg

Sample	D_{53}(min)	D_{55}(min)	$D_{57:30}$ (min)
Egg yolk	24.99 (R=0.997)	19.6 (R=0.997)	15.04 (R=0.998)
Egg white	12.65 (R=0.998)	9.09 (R=0.989)	6.69 (R=0.996)
Whole egg	19.60 (R=0.991)	12.98(R=0.989)	8.54 (R=0.99)

The z-value was obtained from the slope of log10 (D)vs temperature plot and was 20.83, 16.94 and 12.65°C for listeria in egg yolk, egg white and whole egg respectively.

Table 2. Z-value of *L. monocytogenes* in egg yolk, egg white and whple egg.

Sample	Z-value (°C)
Egg yolk	20.83
Egg white	16.94
Whole egg	12.65

Our result showed that in all temperature reduction of live germ count, most rapidly in the liquid egg white. Heat destruction in egg white was fastest and *listeria* in homogenized liquid whole egg had medium thermal resistance. Significant ($p > 0.05$) differences were observed among D-values in all temperature (53 °C, 55 °C and 57.5 °C) between egg white and egg yolk. This is maybe due to the difference between pH, water activity, and nature of constituents in tow samples(Yang *et al*. 2000). In addition to the alkaline pH of egg white (8.9±0.3), it can be due to the proliferation inhibiting and cell destroying effects of lysozyme, conalbumin and avidin(Castellano *et al*. 2001, Park *et al*. 2006, Board *et al*. 2008). On the other hand the absence of antimicrobial substances and the presence of lecithin in yolk egg causes the bacterial egg yolk was resistant to test temperature(Muriana *et al*. 1996). Egg yolk contains 7.87% fat but egg white does not have any fat(Sumner *et al*. 1991). This fat can be protection *L. monocytogenes* from heat at pasteurization process.

4. Conclusions

This study suggests that the lower thermal process is applied to liquid egg white than the liquid egg yolk. Hank *et al*., (2001) used low temperatures for shell pasteurization process and showed that pasteurization of egg at 55°C does not cause significant damage in total or soluble egg protein, and reported that 55°C had no effect on the protein quality of albumen(Hank *et al*. 2001). Hence the data from this work will be useful for pasteurization of eggs at temperatures of 53, 55 and 57.5 ° C. This temperature can eliminate *L. monocytogenes*, with maintain nutrients fragment in egg products. This work could be done for other bacteria, especially food-borne pathogens.

5. Acknowledgments

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PRODUCTION OF LOW-ALCOHOLIC BEVERAGES FROM *CITRUS RETICULATA* AND *ANANAS COMOSUS*

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ABSTRACT

India stands second in the world production of vegetables and fruits owing to the remarkable diversity of its geographical conditions. In the present study, baker's yeast was used for the production of low-alcoholic naturally carbonated beverage from Citrus reticulata (Kinnow) and Anasas comosus (pineapple) and their blends with other fruits and condiments. The prepared beverages were analyzed for their physicochemical properties and sensory qualities. The physicochemical characteristics of Citrus reticulata and Anasas comosus beverages determined after 72 hours were pH 3.3 and 3.4, TSS 80B and 3.60B, CO2 content of 120 mg/l and 110 mg/l, % acidity 0.9 and 0.5, brix-acid ratio of 8.89 and 7.2, respectively. Alcohol content was 0.5 per cent. The physicochemical characteristics of blended beverage of both the ratios were also determined. In the sensory analysis 2:1 kinnow-pineapple blended beverage was the most accepted with an average score of 8.5 out of 10. The results showed that the production of these blended beverages can be further explored on a larger scale for commercialization of the product.

Keywords: *Ananas comosus, Beverage, Citrus reticulata, Low-alcoholic, physicochemical properties, Saccharomyces cerevisae.*

1. Introduction

India is the second largest producer of fruits with 74.877 million metric tonnes production of fruits for the year 2010-11. The cumulative wastage in fruits and vegetables is estimated to be 5.8% to 18%. As per study by the Central Institute for Post Harvest Engineering & Technology, Ludhiana (published in 2010) post-harvest losses of major agricultural products including fruits and vegetables at National level were estimated to the tune of about Rs. 44,000 crore per annum (Press Information Bureau, Government of India, 2012). This can be attributed to the lack of proper handling and storage that results in losses in qualitative and quantitative features like flavour, texture, nutritional value and safety [1]. The alarming wastage of fruits and vegetables associated with its low level of industrial utilization in the developing countries calls for a great concern. To make even the seasonal fruits available throughout the year as well as to minimize their wastage, two different fruits viz. *Citrus reticulata* (vern. Kinnow) and *Ananas comosus* (Pineapple) were used to produce beverages which were low alcoholic and naturally carbonated without the use of additional preservatives using baker's yeast (*Saccharomyces cerevisiae*).

Citrus fruits are considered to be the rich source of ascorbic acid, pectin, carotenes, citric acid, and minerals like calcium and phosphorous. Consumption of high sugar drinks leads to various diseases such as diabetes, obesity and dental caries [2] where as these fruit-based beverages provider a healthier alternative. Citrus fruits can thus be used in the production of value added products such as fruit beverages. These types of citrus drinks are probably the most recognized and globally accepted fruit drinks[3,4]. *Citrus reticulata* is one of the most popular citrus fruit having attractive bright colour, appealing taste and flavor, which is cultivated in north India and can be exploited for production of ready to drink beverages. *Ananas comosus* has a unique attractive sweet flavour and therefore consumed widely as fresh fruit, canned fruit,

processed juice, and as an important ingredient in exotic foods. This fruit is a good source of manganese and contains significant amounts of vitamin C and B1 [5].

Fermentation of juices of these fruits was carried out in order to come up with ready to drink beverages with enhanced taste. The fermented beverage retains nutrients, and the additional CO_2 so produced is antimicrobial and adds tangy taste, fizz, and sparkle to the beverage[6]. Blending of fruit juices with other fruit juices and condiments improves taste as well as nutritional value of the beverages which can add to their commercial value.

2. Materials and methods

Extraction of juices

Healthy fruits and vegetables were washed with chlorinated water and peeled. Fruit juices were extracted aseptically using Electronic juicer. Extracted juices were filtered through mesh and later through muslin cloth.

Preparation of sugar solution

The granulated sucrose procured from local market, was boiled in equal water (500g/100litre) for 5 minutes and then cooled to room temperature to prepare sugar solution.

Preparation of starter culture

Baker's yeast was procured from local market. The yeast granules were inoculated aseptically into sterile YPD medium and incubated at 300 C for 48 hours. The inoculum was diluted to 1 x 108 yeast cells to be used as starter culture.

Fermentation of Kinnow and Pineapple juices

Fermentation of kinnow and pineapple juices was performed so as to check the fermentation potential of the cultured yeast. 750 ml of each fresh juice was taken and TSS (total suspended solids) in form of brix was calculated and adjusted to 16 0B. This was followed by pasteurization of juice at 82 0C for 15 sec. The juices were inoculated with 0.5 % (v/v) culture and incubated at 30 ± 2 0C for 48 hrs. After incubation, physicochemical tests were carried out to determine the pH, TSS (0Brix), carbon-dioxide, total acidity, brix:acid ratio and alcoholic content of the fermented juice.

Fermentation of juice blends

Three different blends of Kinnow juice with carrot, pineapple and mint juices and Pineapple juice with carrot, mint and lemon in the ratio of (3:1) and (2:1) were prepared. Blending of different juices was carried out so as to produce the beverages with enhancing nutritional value, flavor, and aroma.

TSS of blended juices was measured in form of brix and adjusted to 16 0B by using sugar solution [7]. The juices were pasteurized at 82 0C for 15 sec. The 3:1 and 2:1 blended juices were inoculated with the 0.5 % (v/v) culture and incubated at 30 ± 2 0C for 48 hrs. Physicochemical tests were carried out to determine the pH, TSS (0Brix), carbon-dioxide, total acidity, sugar acid ratio and alcoholic content of the fermented juice.

Physicochemical Analysis

The pH of the juice was determined using a digital pH meter (Euiptronics, Model EQ- 610). The total suspended solid (0brix) was determined by calculating the Brix value of the beverage. 0Brix was determined by calculating the Apparent Specific gravity of the beverage using the following conversion:

$^0Brix = [(182.4601 * SG - 775.6821) * SG + 1262.7794] * SG - 669.5622$

Total acidity expressed as % anhydrous citric acid was calculated by titration against standardized 0.1N NaOH [8]. The CO_2 content of the beverage was calculated by the Buret Titration Method 8223[9] by using 0.1 N Sodium Hydroxide as standard solution and

Phenolphthalein as an indication. The Brix acid ratio of the beverages was calculated from the values of ^{0}brix and the % acidity. The alcoholic content of the distilled beverages were determined after the 72 hrs of the fermentation using standard potassium dichromate titration. Distillation of the beverages was carried out with the help of distillation unit and then the distilled beverage was taken for the calculation of the total alcoholic content v/v by the alcohol meter. Statistical analysis was done by using CPCS1 software developed by Department of Maths, Statistics and Physics, Punjab Agricultural University, Ludhiana.

Sensory evaluation of blended beverages

Blended beverages were served to a group of ten people for the sensory evaluation. The beverage was evaluated on the basis of eight different parameters which included taste, color, aroma, appearance, mouth feel, astringency, flavor and overall acceptability [10] on nine-point hedonic scale in order to determine the most acceptable beverage blend.

Table1: Physicochemical Properties of *Citrus reticulata* and *Ananas comosus* beverages.

Incubation Period (in hrs) / **Physiochemical Properties***	***Citrus reticulata* (Kinnow)**		***Ananas comosus* (Pineapple)**		**F-Ratio**	**CD (@5%)**
	48	72	48	72		
TSS (^{0}B)	11.7	8	3.9	3.6	1468.94	0.33
pH	3.8	3.4	3.4	3.3	58.99	0.09
Total Acidity (% Citric Acid)	0.8	0.9	0.5	0.5	40.80	0.11
Brix-Acid Ratio	14.63	8.89	7.8	7.2	12131.63	0.10
CO_2 (mg/l)	100	120	95	110	11.80	10.53
Alcohol (%)	0.5	0.5	0.5	0.5	NS	NS

NS= Non-significant *Average of five replicates

Table2: Physiochemical Properties of 2:1 *Citrus reticulate (Kinnow)* and *Ananas comosus* (Pineapple) blended beverages.

Blends	K:C		K:M		K:P		P:C		P:M		P:L		F -Ratio	CD (5%)
Incubation Period (in hrs)	48	72	48	72	48	72	48	72	48	72	48	72		
TSS (^{0}B)	2.8	1.4	1.8	1.0	2.3	1.8	5.2	3.4	6.8	3.6	5.4	3.9	857.75	0.18
pH	4.2	3.9	4.4	4.1	4.1	3.9	3.8	3.7	4.3	4.3	3.3	3.2	40.96	0.17
Total Acidity (% Citric Acid)	0.57	0.57	0.64	0.64	0.53	0.54	0.43	0.48	0.35	0.39	0.65	0.65	178.98	0.23
Brix-Acid Ratio	4.91	2.46	2.81	1.56	4.34	3.33	12.09	7.08	19.43	9.23	8.31	6.00	15786.59	0.12
CO_2 (mg/l)	90	120	100	135	100	130	100	120	100	135	90	115	76.94	5.55
Alcohol (%)	0.5	0.5	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.7	0.7	NS	0.11

K:C= Kinnow: Carrot; K:M= Kinnow:Mint; K:P= Kinnow: Pineapple; P:C= Pineapple: Carrot;P:M=Pineapple:Mint;
P:L= Pineapple:Lemon ; NS: Non-significant

Table3: Physiochemical Properties of 3:1 *Citrus reticulate (Kinnow)* and *Ananas comosus* (Pineapple) blended beverages.

Blends	**K:C**		**K:M**		**K:P**		**P:C**		**P:M**		**P:L**		**F-Ratio**	**CD (5%)**
Incubation Period (in hrs)	48	72	48	72	48	72	48	72	48	72	48	72		
TSS (^{0}B)	7.3	5.2	7.1	5.0	8.0	5.5	6.5	3.9	7.9	3.9	6.2	3.5	898.37	0.15
pH	3.8	3.6	4.0	3.7	3.9	3.6	3.8	3.7	4.3	4.1	3.5	3.2	43.32	0.13
Total Acidity (% Citric Acid)	0.60	0.76	0.63	0.85	0.64	0.86	0.42	0.49	0.48	0.50	0.45	0.50	101.14	0.04
Brix-Acid Ratio	12.17	6.84	11.27	5.88	12.5	6.39	13.27	9.29	15.8	8.13	12.4	7.78	4585.31	0.14
CO_2 (mg/l)	95	125	95	125	100	135	100	125	90	110	95	120	34.99	7.69
Alcohol (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	NS	NS

K:C= Kinnow: Carrot; K:M= Kinnow:Mint; K:P= Kinnow: Pineapple;P:C= Pineapple: Carrot;P:M=Pineapple:Mint;
P:L= Pineapple:Lemon; NS: Non-significant

3. Results and disscussion

Fermentation of Kinnow and Pineapple juices

Fresh Kinnow and Pineapple juices inoculated with Baker's yeast were allowed to ferment and various physicochemical properties were studied after 48 and 72 hours of incubation. There was a general trend of decrease in TSS, pH and Brix acid ratio. However, total acidity increased in kinnow beverage while it remained same in case of pineapple beverage (Table 1). CO_2 content increased from 100mg/l to120mg/l in kinnow beverage while in case of pineapple beverage it increased from 95mg/l to 110mg/l. Alcohol content remained stable after 3 days at 0.5 per cent. Since baker's yeast is a low alcohol producer, this ability of the yeast strain was responsible for low alcohol production in the beverages. High carbonation is also an important attribute of baker's yeast which was important for the production of self carbonated beverage

Fermentation of Blended Kinnow and Pineapple juices

On blending the pure fruit juices with other fruit/vegetable juices/condiments, a similar trend was observed. Kinnow juice was blended with carrot, pineapple and mint juices in ratios of 3:1 and 2:1. It was found that there was a significant decrease in TSS as the incubation time increased for both the ratios. A very slight, though significant decrease in pH was also observed with increase in incubation period. Brix acid ratio of 2:1 kinnow: carrot beverage decreased from 4.91 to 2.46 while in 3:1 ratio, it decreased from 12.17 to 6.84. Similar trend was observed in other blends. The total acidity in different blendings remained almost constant with increase in incubation period though they differed significantly when compared to each other. CO_2 content generally increased slightly on incubation from two to three days (Table 2, 3).

Alcohol content stayed constant for all the blends after 48 and 72 hours of incubation for both the blends except in 2:1 Kinnow: Mint beverage (0.6 per cent) and 2:1 Pineapple: Lemon beverage (0.7 per cent).

Table 4: Effect of blending on sensory scores* of *Citrus reticulate (Kinnow)* and *Ananas comosus* (Pineapple)

Blends	**K:P**		**K:C**		**K:M**	
Ratios	2:1	3:1	2:1	3:1	2:1	3:1
Taste	6.1±0.184	5.3±0.050	7.4±0.10	6.3±0.080	6.9±0.150	5.0±0.078
Color	8.1±0.027	7.2±0.052	8.0±0.026	6.6±0.078	7.3±0.103	7.0±0.026
Aroma	8.0±0.026	6.2±0.052	7.1±0.039	5.8±0.103	7.5±0.052	6.1±0.052
Appearance	7.6±0.051	5.3±0.077	8.0±0.023	5.2±0.052	7.3±0.052	6.2±0.026
Mouth feel	7.2±0.052	6.0±0.058	7.3±0.077	6.2±0.052	7.5±0.051	5.6±0.052
Astringency	7.8±0.103	5.7±0.078	7.2±0.0516	5.6±0.052	7.3±0.052	6.0±0.056
Flavor	7.5±0.052	5.3±0.077	6.7±0.103	6.1±0.026	6.8±0.103	6.0±0.052
Overall Acceptability	8.5±0.052	6.0±0.052	7.5±0.052	6.3±0.053	7.8±0.052	5.5±0.077

Table 5: Effect of blending on sensory scores* of *Citrus reticulate (Kinnow)* and *Ananas comosus* (Pineapple)

Blends	**P:C**		**P:M**		**P:L**		**F-Ratio**	**CD (@5%)**
Ratios	2:1	3:1	2:1	3:1	2:1	3:1		
Taste	7.8±0.070	8.2±0.050	5.6±0.070	4.2±0.050	7.2±0.070	7.5±0.130	217.76	0.24
Color	7.0±0.123	7.2±0.077	6.6±0.026	4.6±0.103	7.0±0.077	6.8±0.103	125.06	0.22
Aroma	5.8±0.103	5.6±0.052	5.4±0.077	3.2±0.052	5.8±0.103	5.7±0.077	293.28	0.20
Appearance	6.8±0.103	6.7±0.091	6.6±0.078	4.7±0.078	6.8±0.103	7.1±0.026	211.01	0.194
Mouth feel	6.6±0.051	7.2±0.052	6.2±0.052	4.0±0.052	6.2±0.052	7.0±0.026	320.45	0.152
Astringency	6.0±0.052	6.0±0.051	5.2±0.052	5.0±0.077	6.0±0.051	7.0±0.026	187.82	0.181
Flavor	7.2±0.052	6.0±0.052	6.0±0.052	5.0±0.078	6.5±0.052	8.0±0.051	185.83	0.179
Overall Acceptability	7.8±0.103	8.3±0.077	6.8±0.103	6.0±0.051	7.5±0.052	7.8±0.103	214.21	0.191

K:C= Kinnow: Carrot; K:M= Kinnow:Mint; K:P= Kinnow: Pineapple;P:C= Pineapple: Carrot; P:M=Pineapple: Mint; P:L= Pineapple:Lemon

* mean value of ten replicates

4.Conclusions

Sensory evaluation was done by constituting a panel of 10 members. The different beverages scored between 5.5 to 8.5 points. 2: 1Kinnow: pineapple beverage has been adjudged the best beverage with the highest sensory quality characteristics. The beverage was rated as liked very much due to its taste, color, texture, aroma, effervescence and appearance. This was followed by

3:1Pineapple: carrot beverage with a score of 8.3. The overall score for all the beverages is given in table 4. It was observed that pineapple blended beverages had a greater overall acceptability as compared to kinnow beverages. This could be attributed to the bitter after taste of kinnow and its seeds during extraction.

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ANTIFUNGAL EFFECTS OF BASIL ESSENTIAL OIL AGAINST *BOTRYTIS CINEREA* INFECTION ON STRAWBERRIES STORAGE IN MODIFIED PACKAGING

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ABSTRACT

Objective of this study is to observe the influence of essential oil obtained from basil on mold *Botrytis cinerea* and create a package of a protective film for preservation strawberries. Two types of treatments were studied. The aim of this study was to access the in vitro and in vivo activity of essential oil obtained from basil against *Botrytis cinerea* and based on the results to create a package. The results indicate that basil essential oil could be used for the control of diseases caused by *Botrytis cinerea* but cannot be applied directly to the fruits because destroys their tissues.

Keywords: *essential oils, strawberries, Botrytis cinerea, antifungal activity, package*.

1. Introduction

Generally, consumers prefer to have healthy foods and organic agricultural products. Many countries have special regulations for pesticide residue for import of agricultural crops, especially the fruits.

Fruits and vegetables are very essential for the human body diet because they contain important nutritional value such as vitamins and pigments,

Quality of fruits and vegetables contains a number of parameters including flavor, texture, nutrition and safety, and the importance of each parameter depends on the product. The most important aspect of quality products is freshness, typicality quality fresh fruits and vegetables when they are harvested. (Beceanu D. et all, 2008)

Packaging plays an important role both in handling and in food preservation can even increase shelf life. Is a factor of great importance effectively protecting the fruits from light, oxygen and microbial recontamination, thus directly preventing the alteration of fruits.

Since 1960, attempts have been made to create and maintain in modified atmosphere plastic films polymer, ethylene and water provides an additional tool to use packaging technology and maintain a desired atmosphere in the package. Conditions created and maintained in a pack are the result of the interaction of many factors, including those to fruit and vegetables and those related to the environment. (Baldwin E.A., 2007)

Strawberries are among the earliest fruits that mature during the year (May-June). They are required for consumption fresh, frozen or processed. Fresh strawberries containing 5-12% sugar, 0.6-1.6% acid. These fruits are one of the best sources of vitamin C. In terms of vitamin C, strawberries are surpassed only blackcurrant. Strawberries belong to the family Rosaceae, in Rosoideae family, genus Fragaria. (Vitoratos Andrew, 2013)

Strawberry constituted a large portion of the fresh fruits on the market during the spring. Because of the high rate of metabolic activity and respiration as well as the need for high water control, strawberries are very susceptible to microbial rots and mechanical damages during transportation. The most

destructive fungal rot of strawberry is gray mold which is caused by *Botrytis cinerea*.

The strawberries are good sources of antioxidants, so keep the body young longer and dodge him degenerative diseases. In addition, low-calorie sugar and help maintain blood glucose levels within normal limits. (Andrei G., 1980)

Basil (Ocimum basilicum) is a plant of the genus Ocimum, family Lamiaceae. It is sensitive to cold herbs that loves heat and humidity. Its name comes from the Greek basileus means king.Throughout history, basil was considered a sacred plant and all civilizations revered the world. Current research revealed therapeutic determinate by chemical composition.Volatile oil containing eugenol (which gives specific flavor, which is Sustiva and other flat aromatic), camphene and camphor, anethole (anise the same substance), citronellol, pinene, ocinem, terpineol, linalool (specific flavor of coriander), methyl clavicol (tarragon flavor), geraniol (same in gernium), myrcene (found in bay leaves). (A.I.Husain, 2008)

Compounds from the aerial parts have intestinal antiseptic, carminative, stimulant of digestion and expectorant. It was found that basil essential oil has antibacterial and antifungal. It is appreciated more in folk medicine, with various traditional uses. The oil has uses in food, perfumes and cosmetics. (Ashok Kumar, 2011)

The aim of the present study is to analyze the changes induced by, modified packaging with basil essential oil tested on strawberries.

2. Materials and methods

a) In vitro, on agar

The Botrytis cinerea was isolated from infected strawberries and cultivated on PDA. In Petri-dishes. In the PDA, basil essential oil and was tested on daily mycelium growth of Botrytis cinerea. Medium PDA was made from 500 ml of distilled water and 21 g PDA and sterilized in an autoclave at 121 ° C and 1-1.2 atm pressure for 20 minutes .The gas light comes on and it creates the right environment for Avitar incubator contamination is then poured into the Petri dishes are placed in the incubator for 20 minutes to solidify environment. 10µl micropipette basil essential oil, was placed over medium in Petri dishes and dispersed . The control treatment did not have any essentials oil. All the Petri-dishes were inoculated with young mycelium from the margin of a fungal colony which was cut with a cork-borer (3mm).Petri dishes were sealed with parafilm and then were kept in incubator at 250C for five days. Mycelium growth was measured daily as diameter of fungal colony.

b) In vivo, on fruit

Fresh strawberries are inoculated with Botrytis cinerea and cultivated on PDA disks then are being treated with basil essential oil, the control treatment did not have any essential oil. This process is done every half-hour. After three days, the fruit infect situation was determined.

c)Protective film for the preservation of strawberries

In each plastic cup were placed 2-3 fresh strawberry samples. The micropipette and 2 ml of 95% ethanol and 1 ml of basil essential oil and then dispersed on the surface of the plastic film by means of the Petri dishes. Perform 5 samples. The first sample contains only plastic sheet with protective film. A second sample containing plastic film protective film made of ethanol. A third sample contains plastic film protective film made of ethanol and basil essential oil.. A fourth sample contains mold Botrytis cinerea plastic film protective film made from ethanol. A fifth sample contains mold Botrytis cinerea plastic film protective film made of ethanol and basil essential oil.

3. Results and discussion

Results on PDA

As shown in Fig.1, Fig.2, Fig.3, Fig4 maximum mycelia growth of Botrytis cinerea

was in the control group. In the first day, in the control group, the mycelium has developed less but in the second, third and fourth day the mycelium development was completed. The treatment with basil was able to have a strong inhibitory effect on the mycelia growth in all days of the experiment.

The basil essential oil was completed effective against Botrytis cinerea.

Table 1 The effect of the basil essential oil treatments on the mycelia growth of *Botrytis cinerea*

Treatment	Nr	Day 1		Day 2		Day 3		Day 4	
Control	1	a	7	a	8	8,5		8,5	
Control	1	b	7,3	b	7,5	8,5		8,5	
Control	2	a	7,4	a	7,5	8,5		8,5	
Control	2	b	7,4	b	7,5	8,5		8,5	
Control	3	a	7,4	a	7,5	8,5		8,5	
Control	3	b	7,4	b	7,5	8,5		8,5	
Control	4	a	7,4	a	7,5	8,5		8,5	
Control	4	b	7,4	b	7,5	8,5		8,5	
Ocimum basilicum	1	a	0	a	0	a	0	a	0
Ocimum basilicum	1	b	0	b	0	b	0	b	0
Ocimum basilicum	2	a	0	a	0	a	0	a	0
Ocimum basilicum	2	b	0	b	0	b	0	b	0
Ocimum basilicum	3	a	0	a	0	a	0	a	0
Ocimum basilicum	3	b	0	b	0	b	0	b	0
Ocimum basilicum	4	a	0	a	0	a	0	a	0
Ocimum basilicum	4	b	0	b	0	b	0	b	0

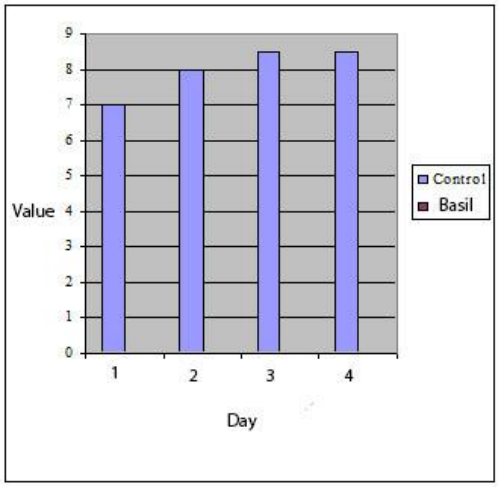

Figure 2 Variation of the influence of basil essential oil treatments against Botrytis cinerea

Day	Day 1	Day 2	Day 3	Day 4
Treatment Basil-Control				

Figure 3 Evolution of Botrytis cinerea, in vitro, on agar following treatment with basil essential oil

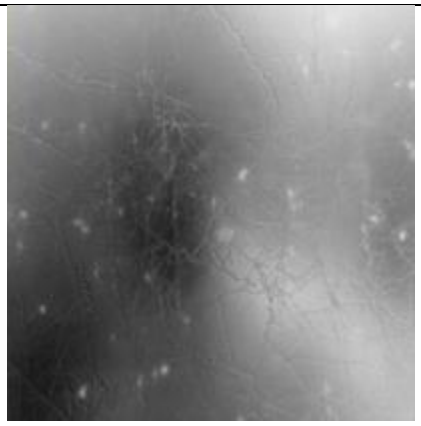	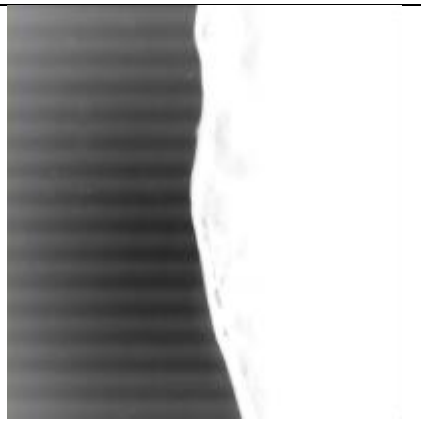
Figura 1. Control, *Botrytis mycelium* and formation of resting bodies (Sclerotia) magnification x40)	**Figura 3. *Botrytis* agar plug treated with Basil (magnification x40)**

Figure 4 The effect of the basil essential oil treatments on the mycelia growth of Botrytis Cinerea seen on the microscope

a) Strawberry experiment results

Percentage of infected fruits was determined in 3rd day of the experiment. Measuring method was observational based on percentage of fruit surface which contamination and the incidence with fungi. Amount of infection was scored between 0 for uninfected fruit and 100 for fully covered fruit with fungi. Based on figure 2., the effect of basil essential oil on Botrytis cinerea, haven't been favorable because after three days in those two situations all the strawberry were contaminated with Botrytis cinerea. In the first half hours, the contamination developed less but in the other two half-hour the mold developed almost completely.

		
Figure 5.1a. Strawberry infected and treated with Botrytis cinerea mycelium (Control, , Basil) after 96h (30min)	Figure 5.1b Strawberry infected and treated with Botrytis cinerea mycelium (Control, Basil) after 96h (60min)	Figure 5.1c. Strawberry infected and treated with Botrytis cinerea mycelium (Control, ,Basil) after 96h (180min)

Figure 5. The results of basil essential oil treatments against Botrytis cinerea on strawberry fruit in every half-hour

The most effective treatment against the development of *Botrytis cinerea* fungus hyphae complete is the volatile oil of basil. Comet pesticide treatment also has a rather effective against mould. Seen under a microscope, the development of micelles is much lower when treated with pesticide Comet, to control the development of the micelles is almost complete in the case of basil essential oil therapy mycelia development was null.

	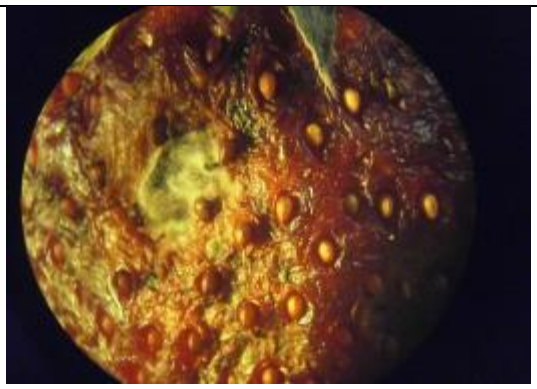
Figure 1a. Strawberry fruit infected with *Botrytis cinerea* mycelium (Control) after 72 h	Figure 1b. Strawberry fruit treated with basil and infected with *Botrytis cinerea* mycelium, after 72 h

Figure 6. Strawberry fruit infected with Botrytis cinerea control and surface treated with basil oil after 72 hours

c) Protective film for preservation strawberries

In the first day didn't happened nothing with the strawberry but in the second day all the strawberry in different situation with the treatment were contaminated with the mold *Botrytis cinerea*. The protective film for preservation strawberries didn't worked.

4. Conclusion

The results show that, the basil essential oil have antifungal activity, are effective on the fungus on PDA. In addition, inhibits the mycelium growth of *Botrytis cinerea* but the protective film made of basil essential oil and ethanol didn't worked because strawberries are highly perishable and the essential oil destroys their tissues. Ocimum bassilicum can be used as an antioxidant in food preservation to replace the chemical additives. The essential oil of Oc. Basillicum register limitation, in function of microorganism level of attack on strawberry substrate.

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FUNCTIONAL CLUSTER ANALYSIS OF RAW PORK TRACEABILITY TIME-SERIES DATA

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ABSTRACT

This paper discussed cluster problem of time-series data applying functional cluster analysis of raw pork traceability time-series data under general framework. We resolve problems and put forward research approach. We can also regard time-series data as function object rather than simple arrangement of individual observations which transform discrete data into functional data; replacing distance between primitive function distances of primary functional expansion coefficient vector can simplify arithmetic operation. Discrete cluster data is divided into continuous classification information by making functional cluster analysis on traceability time- series data, which can improve availability of traceability time-series data and then inspire decision maker to make a correct choice.

Keywords: *raw pork traceability, time-series data, cluster analysis*

1. Introduction

Nowadays, labor division becomes more and more detailed and production means of raw pork becomes more and more professional. Complex process in supply chain and more and more intermediate link from material production to final consumption involve safety of raw pork into many supply chains, such as production, processing, storage, transportation and sales. Increase of steps in production process will increase safety problems of raw pork along with storage time efficiency of raw material and complexity of raw pork chain [1].

This paper obtains related data by supply chain with the help of basic method and thought of FDA based on RFID technology. Analyzing cluster problem of time-series data under specified standard will show primary contradiction of problem clearly. Quality safety on effective statistic,

the following related introduction of scheme supervision and trace of cultivation, slaughter, circulate and consumption was made by research of data; it is a good test by record and supervision platform of data center [2].

2. Trace process of raw pork traceability

According to the introduction of meat institute, China is a great power pf producing raw pork and meanwhile it is also the biggest country of raw pork consumption in the world. Consumers in many areas are feed on pork as daily meat, especially in Spring Festival. And how to ensure pork on the table is safe? A transparent full-course raw pork safety traceability of cultivation, slaughter, processing, warehouse, logistic and customer can be realized by RFID technology in pork processing and feeding farm. Then we can have a look at process of raw pork [3]:

that can ensure safety traceability

full-course

Raw pork traceability is a step including making pig ear mark by RFED technology, marking it on gig ear, recording information of birth, slaughter, cold storage and sales of pigs by RFID read-write equipment and providing inquire service in public information platform for public to supervise the quality safety of raw pork. Then how do the slaughtering enterprises realizing pork traceability? Feeder and feeding farm can record the growing process by REID read-write equipment. And feeder registers every pig after purchasing pigs from feeding farm. Before entering into slaughtering workshop, pigs will be examined if they match conditions. All the qualified pigs will be covered number stamp and enter next step. At last, they will be sending to stores. And customers can inquire detailed information of pigs through relative platform by relative marks on pigs [4].

Pork processing enterprises can make informationalized supervisor mode of quality traceability by RFID electronic mark technology. It can not only ensure the safety of pork production, but also can let customers enjoy health, safe and reliable pork. With the strict implementation of security control on pork, technology will be more and more applied in more and more industries [5].

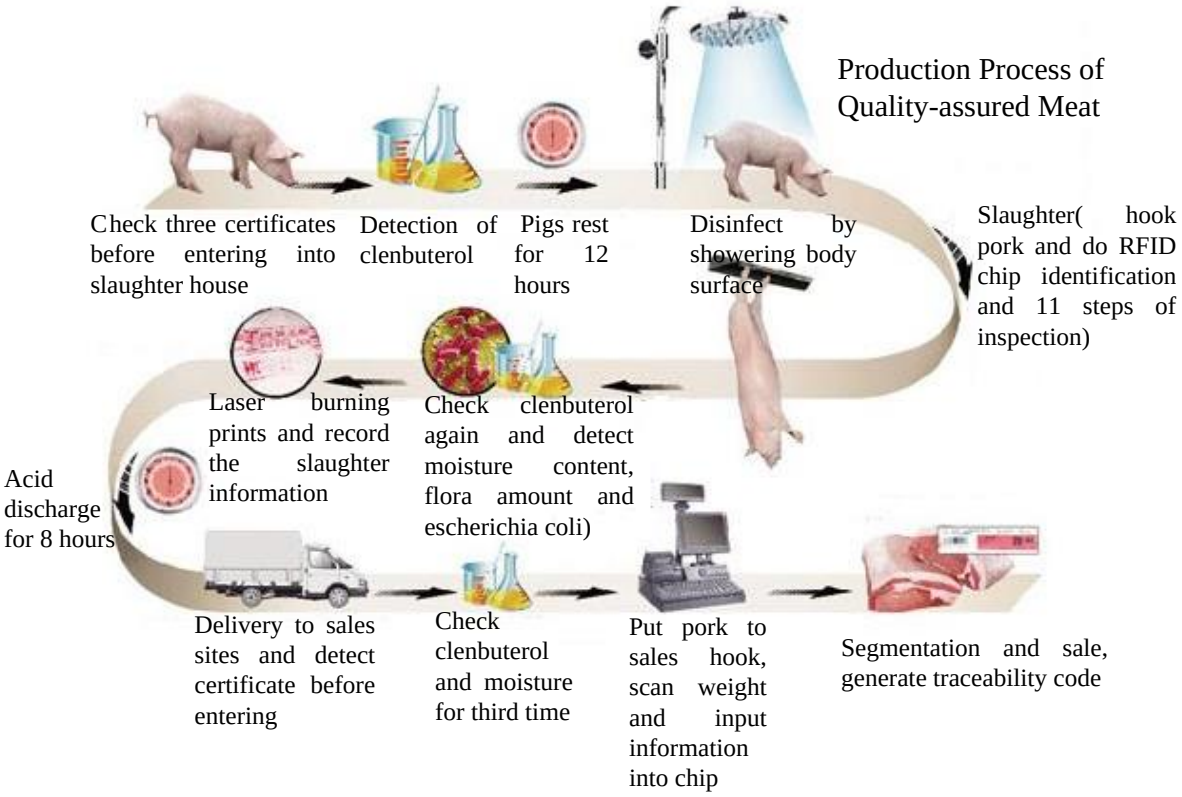

Figure 1. Overall process of traceability tracking of raw pork

3. Functional cluster analysis of functional cluster analysis of raw pork traceability time-series data

3.1 Material

Data of pork supply chain contact surface and microbial pollution of carcass surface from May 1, 2014 and May 10, 2014 is selected. Pollution factor is analyzed by supervising microbial pollution situation in process of slaughter, transportation and sales. Total number of bacteria of unit area is used to measure microbial pollution index. As time going, effect of moisture and temperature on pork surface and contact surface will lead to the change of bacterial amount. If sample size is small, hierarchical clustering method can also be used. In this method, clustering result can be observed by clustering tree diagram [6].

Specific steps is: 1. expand first order derived function by same basis function; 2. calculate distance matrix between individuals according to formula (4); 3. Make hierarchical cluster based on that distance matrix. In this case, Ward is used to measure between class distances. Figure 3 is clustering tree diagram obtained last. Flora amount of refrigerator car and tray can be judged abnormal by this diagram [7].

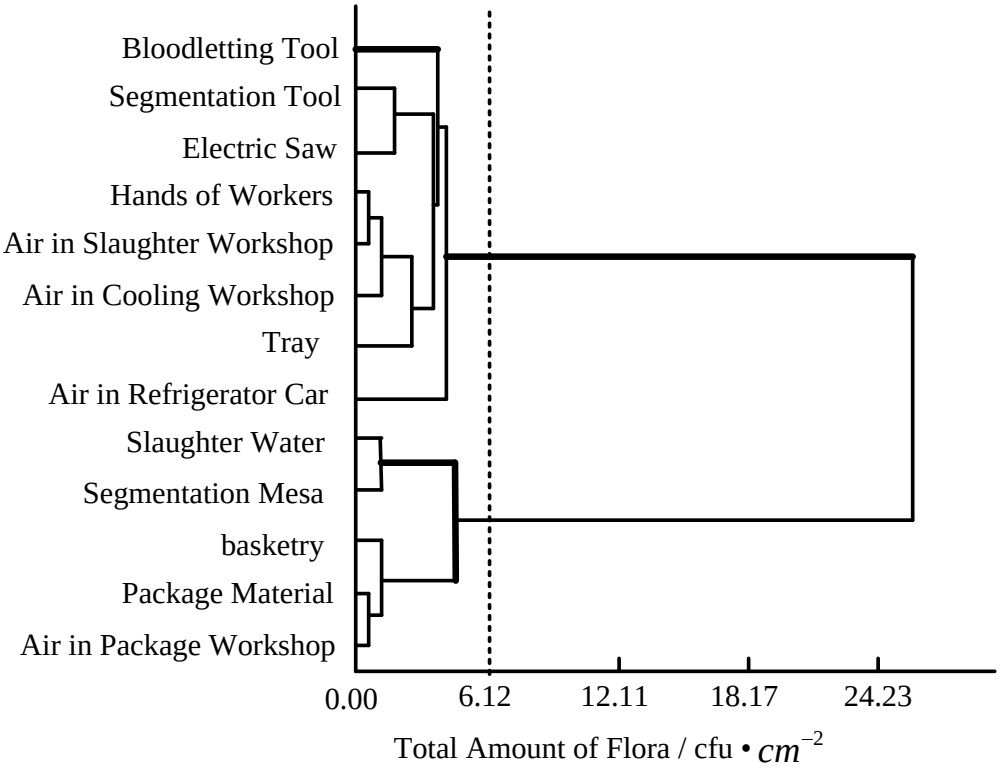

Figure 2. Hierarchical clustering tree diagram

3.2 Method — functional clustering analysis

We all know that from processing to consumption of pork is a dynamic process. Thus, most of data in data center is a kind of time-series data,

such as bacterial index of cold fresh pork, environment index of slaughter workshop, temperature of transportation cars, location data, flora amount of contact surface of market and sales data, which reflect characteristic of attribute value on time sequence. This paper discussed clustering problem of time-series data of pork chain under general framework with the help of FDA basic method and idea by extracting first hand data of pork supply chain traceability system based on RFID technology. Effective statistic, analysis, supervision and trace is made on quality safety of cultivation, slaughter, circulating and consumption by data excavation. Relative data result is published on public network platform based on WEB technology by a unified data center and supervision platform [8].

FDA always takes a serious function (curve) as research object. However, in fact, value on limited time of function is the only one that can get, that is, original time-series data. Therefore, primary task of FDA is to transform discrete time-series data into continuous and smooth function form [9].

Suppose $a^q(t)(q=1,2,...,n)$ is function object of No. n selected pork chain and obtain T_q of observed value $b^q=(b_1^q,b_2^q,...,b_{T_q}^q)'$. Existence of data error leads to the following model:

$$b_p^q=a^q(t_p^q)+\varepsilon^q(t_p^q)$$

$$q=1,2,..n,p=1,2,..T_q \quad (1)$$

First, $a^q(t)$ should be expanded on a group of primary function $\Phi(t)=\{\phi 1(t),\phi 2(t),...,\phi k(t)\}'$ in order to calculate $a^q(t)$. That is, $a^q(t)$ is expressed into linear combination of primary function:

$$a^q(t)=\sum_{k-1}^{k}c_k^q\phi_k(t)$$

$$q=1,2,...,n \quad (2)$$

Matrix is:

$$a^q(t)=c^q\Phi(t),c^q=(c_1^q,c_2^q,...,c_k^q)'$$

$$q=1,2,...,n \quad (3)$$

Substitute formula (2) into formula (1) at point-in-time t_p^q and then apply the least squares principle. Then primary expansion coefficient vector of function $x^q(t)$ can be obtained:

$$c^q=\arg\min_{c^q}\sum_{p-1}^{T_q}a_q^2\left[b_p^q-\sum_{k-1}^{k}c_k^q\phi_k(t_p^q)\right]^2=(B^{q'}B^q)^{-1}B^qb^q \quad (4)$$

Where matrix element of matrix $B^q=(\phi_k(t_p^q))_{T\times K}$ is value of No. k primary function on point-in-time t_p^q. Every function object $a^q(t)$ is independently evaluated by observation

vector. Thus, it is not required that data should be collected on the same point-in-time. Meanwhile, once primary function is set, and then information of function set $\{a^1(t), a^2(t), ..., a^n(t)\}$ will be uniquely reflected by coefficient vector set $\{c^1, c^2, ..., c^n\}$[10].

Measurement index of distance between clustering objects is the primary problem of clustering analysis. There many kinds of measurement index of distance, among which European index possess the best mathematical properties. European index can take as primary similarity measurement index of function clustering. As t o set Functions

$$D_{az} = \int (a(t) - z(t))^2 dt = \int (a'\Phi(t) - z'\Phi(t))^2 dt$$
$$= \int ((a-z)'\Phi(t))^2 dt = \int ((a-z)'\Phi(t)\Phi'(t)(a-z))dt$$
$$= (a-z)' \int (\Phi(t)\Phi'(t)dt(a-z) \quad (6)$$

Set K order matrix $W = \int (\Phi(t)\Phi'(t)dt$

then $D_{az} = (a-z)'W(a-z)$. If primary function is standard orthogonal basis, matrix W will degenerate into unit matrix. And distance between functions become European distance between coefficient vectors. If primary function is not orthogonal, D_{az} can be understood as weighting European distance which take coordinated differential matrix of primary function as weight between coefficient vectors [11].

3.3 Result

Then a general method and solution which can solve clustering problem of time-series data in every step a (t) and z (t), their European distance can be expressed as:

$$D_{az} = \int_0^T (a(t) - z(t))^2 dt \quad (5)$$

However, if functional process of formula (3) is directly used, data of the whole pork supply chain need large amount of numerical integration, which will lead to increase of complexity of algorithm time. Expand a (t) and z (t) by same K dimensional primary function $\Phi(t)$ in order to simplify calculation. Primary function expansion coefficient vector a (t) and z (t) are expressed as a and z, then:

of pork product chain can be-obtained. Its universality is showed on the following two points: 1. no matter the primary function is orthogonality or not orthogonality, original time-series data can be expanded by any primary function. 2. Any clustering method based on European distance can be applied in time-series clustering analysis. Every relative function objects if multivariable time-series is composed by several functions because a large amount of data exceed two variables in real operation. For example, environment function in slaughter workshop is composed of bacteria on surface of carcass, supervision of split face, temperature of workshop; heavy metal index function of pork is composed of content change of fodder, water change of farm and heavy metal content of different part of pork.

If function $a_l(t)$ and $z_l(t)(l = 1,2,..., p)$ respectively express the

function of first variable of p dimensional multivariable function a (t) and z (t), then European distance of function a (t) and z (t) can be defined as:

$$D_{az} = \int_0^T \sum_{l=1}^{p} (a_l(t) - z_l(t))^2 dt \quad (7)$$

Use x_l and z_l to respectively express coefficient vector which will be expanded by same primary function, then:

$$D_{az} = \sum_{l=1}^{p} ((a_l - z_l)' W (a_l - z_l)) \quad (8)$$

We can find that there are lots of problems in pork processing and sales supply chain. And Pork will have several changes of physical condition in the whole supply chain. Thus, pigs, pig carcass and segmentation body should be marked on any point of supply chain process. And unique authentication code must be applied. Information connection of every process of pork processing should be ensured. We found that it is of great importance to establish safety traceability system by research of this paper. Traceability system can collect, settle and statistic pork product by mark, identification, storage and read-write on individual identity in trade project. When health and safety problem appear, we can according to these identifications to track and trace in traceability system, inquire location identity in supply chain, find out the problem step and recycle to narrow range of safety problem. Customers can find the detailed processing information on quality information public shared platform according to the information on the paper label and feedback to supply chain when they find the problem pork. Supervision department and members of pork production chain can confirm the responsibility of safety problems, stop supply of good of this lot into market and govern it in time and effectively.

4. Research conclusion

Research of this paper went on according to the original time-series data in process of traceability process of pork and a series of coefficient vectors are obtained; coefficient vectors are made clustering analysis according to the weighting and non-weighting European distance applied relative clustering method. Traditional pork traceability can only solve simple trace. Describing types of time-series data by functional data clustering analysis can wide the channel of traceability application of pork. A large amount of first hand data is used to dissect in order to explore real phenomenon, which is good to clear understanding of pork processing for customer by convenient approach and mastering of production and processing situation for government. Ultimate goal is to adopt correct action on pork safety issue in first time.

Existing information traceability system on agriculture is mainly for area that have complete data chain and whose location of production and processing is relatively focused. However, pork production and processing current situation of China is that steps in supply chain disperse production and supply and belong to different independent nodes. Especially in cultivation, slaughter houses purchase pigs from small peasant

household or livestock farm. However, the large-scale livestock farm with existing production information management system have not been shaped, which lead to the irregular and disunity of data source of pork production and processing supply chain. In addition, the united of production quality early warning and traceability system still have room for improvement. Take rapid alert system of food and fodder as example, it emphasizes the parameter analysis of production quality safety. However, data of production process of supply chain have not been managed completely and natively, which lead to lack of early warning before accident. There are the innovation and development direction for traceability system.

Traceability system of pork production and processing information will establish eight friendly and practical open software system platform and database of traceability canter for pork quality safety respectively for full-course information management of supply chain, information management of cultivation step, information system of processing step, real time industrial control of processing step, information management of logistic step, information management of sales step, public share of quality information and supervision warning of safety production quality. The system defines information traceability data project according to world unified identity. It standardize data of traceability project of production processing supply chain to achieve trace and track of production and processing information, quality supervision and intelligent warning in process of production of processing and information exchange of quality safety between customers and supply chain member. The system can help to confirm the source of safety problem for supply chain member, customer and supervision department which can improve the quality of pork, reduce food safety accidents and provide a platform for customers to understand and supervise production of pork. Establishment of system can build traceability system of production and processing information which conform to practical situation of production and processing industry in China. Based on the current situation of livestock production, the system can ensure the systemic, integrity and consistency of traceability data and avoid repeated construction under the guidance of industry standard combining with advanced automated and intelligentized technology. The system is fully integrated with similar general system in the world and also set good examples for establishment of system and traceability system on sanitary safety of other agricultural products.

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PREPARATION TECHNOLOGY OF FRESH BEEF FRANKFURTERS: PHYSICAL-CHEMICAL PROPERTIES

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ABSTRACT

Meat and meat products are concentrated sources of valuable quality protein whose amino acid composition compensates for the shortcomings of the other raw materials. This study aims to establish the technology for preparation and some physical-chemical properties of beef frankfurters. The results showed that from 1603,245 kilograms of beef meat, 687.105 kilograms of fat bacon used as raw materials and 724.3491 kilograms of auxiliary materials, 2800 kilograms of beef frankfurters can be prepared. Physical-chemical properties have consisted in the determination of water and sodium chloride contents, readily hydrolysable nitrogen, nitrite and ammonia with Nessler reagent contents. The average value of water content was 63.58%, of sodium chloride content was 2.54%, of readily hydrolysable nitrogen was 26.58%, and of nitrite content was 4.748 mg %. The results showed that the physical-chemical parameters fall in the quality system.

Keywords: *beef frankfurters, technology of preparation, physical-chemical analyses*

1. Introduction.

The content of proteins, lipids, vitamins and minerals in meat gives it a high nutritional value, essential in a healthy diet. In many developing countries, meat production is managed efficiently, animal slaughter and meat processing is based on the results of scientific discovery.[1]

The manufacturer intends to ensure a greater degree of product quality and is directly responsible for product safety, because he is committed to the consumer to achieve a high level of quality overall. [1] In the meat products manufacture it can be used also bovine meat with the nutritional value reduced to 25% and 50%. [1]

The processing, thermal state, organoleptical, chemical and bacteriological properties, shall comply with STAS 2713-74 [4].

This study aims to establish the procedure to prepare the beef frankfurters and to determinate it's physical-chemical properties. [1]

2. The technological scheme of beef frankfurters

In Scheme 1 is presented the technological steps for frankfurters preparation. For beef frankfurters manufacturing the following raw materials were used: beef meat, fat bacon, edible organs**.** (1) beef (cattle and buffalo) - meat provided from cattles with different ages by normal slaughter cuts approved by the veterinary service for industrialization and corresponding provisions ref. [4]; (2) beef meat obtained from normal cuts - processed to obtain all the meat types used in the processing technologies.[1]

Bacon is subcutaneous fat from pigs. For industrialization bacon is collected from the skinned or scalded pork. In the manufacture of different meat products is used only bacon without rind.[1]

It can be also used the soft bacon which is the bacon surrounding the anatomical parts of the pork. This type of bacon it appears in small chunks without the rind and is used in the manufacture of meat

assortments without structure, especially in the frankfurters manufacture.

At the reception, raw or salted bacon used in the frankfurters manufacture should require conditions of STAS 2273-85 and 1117-85, according to the Romanian Rules for Consumer Safety.[5]

The edible organs that are used in the manufacture are: tongue, heart, liver, kidneys, spleen, lungs, and udder. The processing, thermal state, organoleptic, physical-chemical and bacteriological characteristics of the edible organs must require also the conditions of STAS according to the Romanian Rules for Consumer Safety.[1]

The auxiliary materials are: water, ice, salt, sugar, dextrose, protein derivatives, spices, sodium, nitrite and nitrate, ascorbic acid, sodium ascorbate and sodium polyphosphate.[1]

The raw materials are stored until they entry into production under appropriate conditions of temperature and humidity. In the refrigerating rooms the temperature must be $2\text{-}4^0$ C and the maximum storage period is 72 hours. Trenching is the operation by which the housing is sectioned. Deboning is the operation by which the meat is detached from the bones. The selection of meat is the operation by which the meat is sort by quality.

The temperature in the sections used for these operations must be to a maximum of 12° C and the relative atmospheric humidity of about 80%. These sections should be well ventilated and lit, also they must meet all sanitary conditions.

Preparing the composition is carried out at cutter where are added crushed ice and spices, which are sprinkled over the entire paste.

Filling the composition in membranes is a process of plastic deformation. The paste is introduced into the intestines of sheep, pigs or artificial membranes prepared in advance for this purpose.

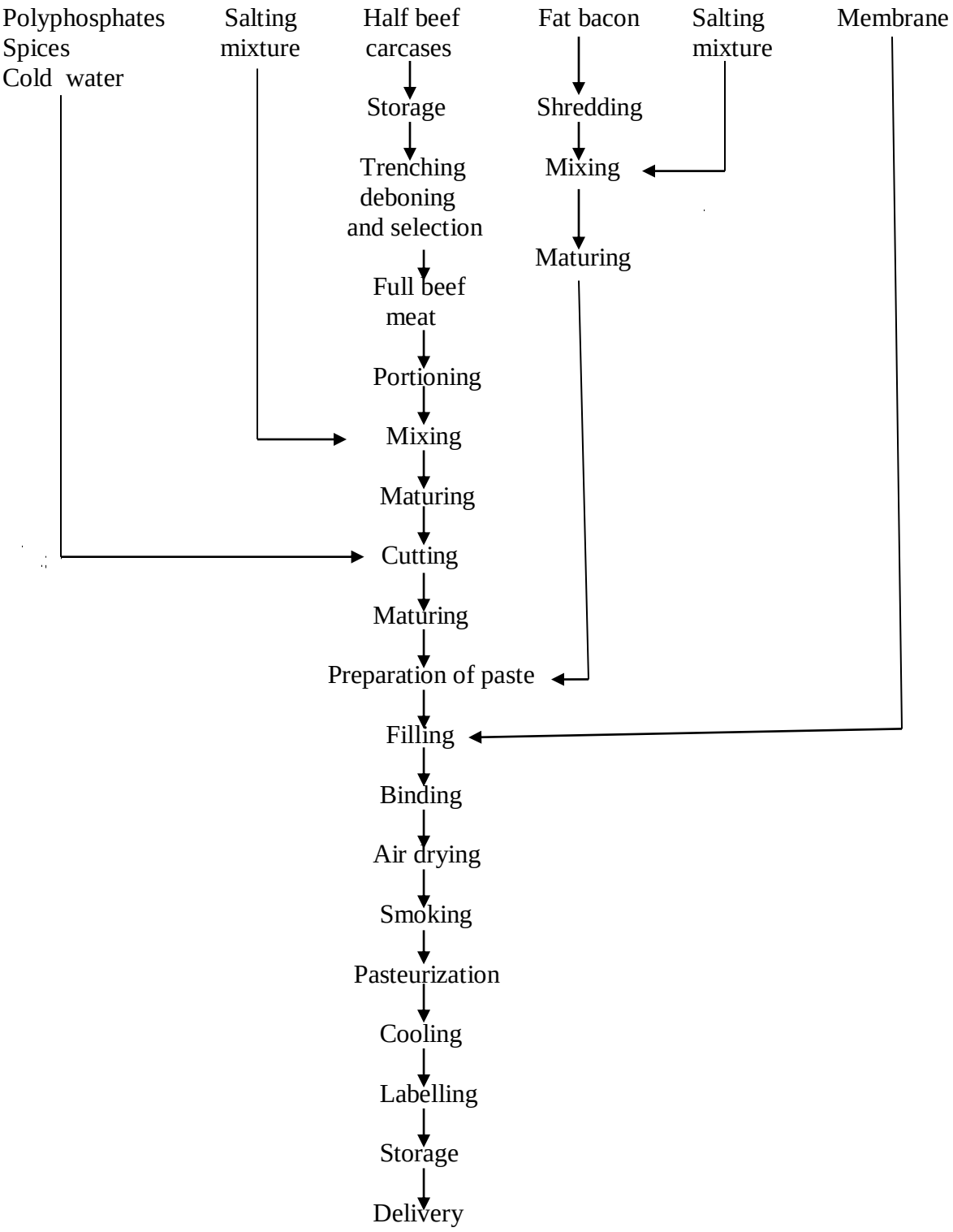
The binding of the bars is carried out by manual or mechanical twisting in short equally spaced pieces at about 12 cm long. After about 10 minutes, the products are subjected to heat treatment.

By hot smoking is obtained a sterilization an increase in the membrane resistance and browning of the membrane. At the same time, in the bulk of frankfurther takes place flavoring and pasteurization processes.

Boiling is carried out at a temperature of 72-75°C for 10-20 minutes, and is made in the water boilers or steam cells. After boiling the product is cooled in pools with running water or under shower with cold water.

The products are positioned on sticks, store in refrigerator at a temperature of 2...4ºC until delivery.

The delivery of finished products is made under hygienic conditions avoiding the causes which might contaminate them or to produce damage. To this end, will be taken into account the means of transport, distance and weather conditions during transport. [2]



Scheme 1. Technological scheme of beef frankfurters preparation [2]

3. Experimental for the physical-chemical analyses

3.1. Determination of moisture content by drying in the oven at 103^{0}C

The analysis was performed according to ref. [6]. In a weighing ampoule with cap and glass wand is inserted 10 to 15 g of calcined sea sand and dried in an oven for 30 minutes at a temperature of 103 ± 2°C. After cooling to room temperature in a desiccator, the ampoule with cap and glass wand are weighed together with a precision of 0,001 g. In the ampoule are inserted about 5 g of the sample ready for analysis and is reweigh with a precision of 0,001 g. After weighing, in the ampoule is poured about 5 mL of ethyl alcohol and by using the wand, the sample is homogenized by crushing the meat particles.

The ampoule (without cover) is placed in a water bath set at a temperature of 60 - 80°C where they keep agitating the wand from time to time until the alcohol is evaporated. The oven temperature is set to 103 ± 2 ° C and the heating of the ampoule is continued at that temperature for two hours. The ampoule is covered with the lid and placed it in the desiccator. After cooling to ambient temperature, the ampoule is weighed with 0,001 g precision. Repeat the heating in the oven (one hour), the cooling and weighing, until two successive weighing results do not differ by more than 0.1% of the mass of the sample. Two parallel determinations of the same sample prepared for analysis are performed.

The water content of the product tested (moisture) is expressed as percentage (grams/ 100 grams of fresh product) and is calculated in Eq.1 :

$$A = \frac{m_1 - m_2}{m_1 - m} \cdot 100\ [\%] \qquad \text{(Eq. 1)}$$

where: m - is the mass of the ampoule, wand and sand, in grams; m_1- is the mass of the ampoule, wand, sand and the sample before drying, in grams; m_2- is the mass of the ampoule, wand, sand and the sample after drying, in grams

The final result will be the arithmetic mean of two parallel determinations, which do not differ by more than 0,005 grams of water at 100 grams of sample. [3]

3.2. Determination of sodium chloride by Mohr method

The analysis was performed according to ref. [3]. An amount of 10 g sample are placed together with 100 ml of distilled water in a 250 ml flask. The mixture is kept at room temperature for 30 min and occasionally homogenized with a glass wand. Then, is filtered to obtain the aqueous extract. A volume of 10 ml filtrate is measured and placed in an Erlenmeyer flask of 250 ml and is neutralized with 0.1 N NaOH solution, in the presence of a drop of phenolphthalein used as indicator. Then, a volume of 1 ml of 10 % $KCrO_4$ solution was added as indicator and the mixture was titrated with 0.1 N $AgNO_3$ solution until the color of the solution changes from yellow to orange.

The content of NaCl was calculated using the formula (Eq. 2):

$$\%NaCl = \frac{0{,}005844 \cdot V}{m} \cdot \frac{100}{10} \cdot 100 \qquad \text{(Eq. 2)}$$

where: 0,005844 - the amount of NaCl, g, corresponding to 1 ml $AgNO_3$ 0,1 N; V – volume of 0.1 N $AgNO_3$, used for titration,

ml; m - mass of the sample taken for determination, g

3.3.Quantitative determination of easily hydrolysable nitrogen by titration with hydrochloric acid

An amount of 10 g of the meat sample previously prepared using 250 mL of water is placed in the flask. Then, 1-2 g of MgO and 5-10 cm^3 paraffin oil (antifoaming), 25 ml H_3BO_3 solution (40 g H_3BO_3 are dissolved in water up to 1000 ml) and 4 drops of Tashiro indicator (0.2 g of methyl red and 0.1 g of methylene blue was dissolved in 100 ml ethyl alcohol of 95% volume)were added. Then, the distillation unit is assembled. In a burette is placed HCl solution 0,1 N and fixed above the holding vessel and start distillation. As ammonia uptake in the holding vessel indicator turns pink tint acid to alkaline yellow tint. Now HCl 0,1 N is added dropwise until the indicator returns to acid tint. The distillation and the titration are continued until the pink tint acid is maintained at least 5 minutes.

Easily hydrolysable nitrogen content, expressed as mg of ammonia in 100 g sample is given by (Eq. 3):

Easily hydrolysable nitrogen [mg/100 g]

$$(NH_3) = \frac{0{,}0017 \cdot V \cdot F \cdot 1000}{m} \cdot 100 \qquad \text{(Eq. 3)}$$

where: 0,0017 - the amount of ammonia corresponding to 1 ml HCl 0,1 N, g; V – volume of HCl 0,1 N used for titration of the distillate, ml; F – HCl 0,1 N correction factor; m – mass of the sample taken for determination, g.

As a result is taken the arithmetic mean of the two determinations made in parallel if the difference between their results does not exceed 2 mg easily hydrolysable nitrogen to 100 g product.[3]

3.4. Determination of nitrite

From well chopped and homogenized sample, 10 g are weigh and mixed with about 80 mL distilled water into a 100 ml volumetric flask. The flask is put into a water bath for one hour at 60°C, vigorous stirring occasionally. Then is added 5mL of a saturated solution of mercuric chloride, is mix well, is cooled, is filled with water to the mark and filtered through a fluted filter. In a clean test tube is inserted 1ml of Griess reagent, 1ml aqueous extract of the sample and 8 mL water. After stirring is left at room temperature for at least 20 minutes, (for color development) then is compared to the standard scale or read at the photometer Perkin Elmer Lambda 35, at 520 nm.

The content of nitrite was calculated using the formula (Eq.4):

$$Nitrite(NaNO2) = \frac{C * V}{m * V1} \text{x } 100[\text{mg}/100\text{g}] \qquad \text{(Eq. 4)}$$

where:
C = the quantity of nitrites in the standard test tube that matches the color intensity of the sample (0.001 ... 0.009 mg), in mg, or the quantity read from the standard curve;
V = the volume of volumetric flask, in ml ;
m = mass of the sample taken into work in g (10g);
V_1 = the volume of solution used for the determination ;
100 = percentage factor.

4. Results and discussion

4.1. Moisture

This parameter is one of the most important physical-chemical analysis of the food industry. If the water content of the product is higher, the nutritional value and the power of conservation are lower.

In Figure 1 is presented the mean value of the moisture obtained from the replicates presented in Table 1. and the value accepted according to the standard method.

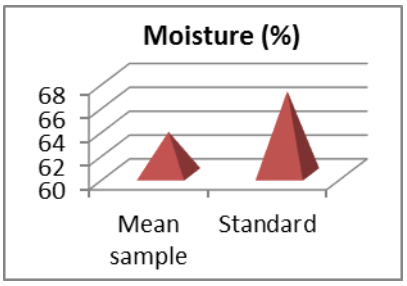


Figure 1. Determination of the moisture

The moisture of the sample was below the legal limit. The minimum value was 61.84% and the maximum 65.34% resulting an average of 63.58%. The low value of this indicator favors product conservation.

4.2. Sodium chloride

The role of sodium chloride in the meat products is to increase the water retention capacity and the hydration capacity of the meat as well as that of improving the taste.

In Figure 2 is presented the mean value of the sodium chloride obtained from the replicates presented in Table 1 and the value accepted according to the standard method.

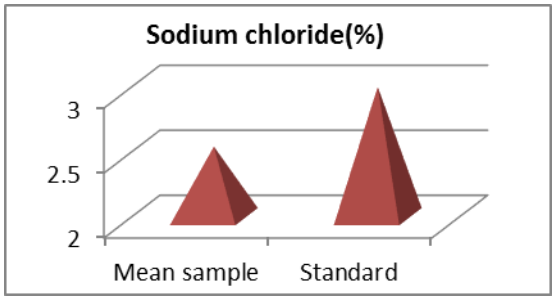


Figure 2. Content of the sodium chloride in the prepared frankfurters

The content of the sodium chloride was in the range 2.35% to 2.74%. The average value was 2.54%. The proportion of sodium chloride for the samples examined was below the legal limit.

4.3. Easily hydrolysable nitrogen

Easily hydrolysable nitrogen is the main indicator of meat freshness. In Figure 3. is presented the average value of the easily hydrolysable nitrogen obtained from the replicates presented in Table 1. and the value accepted according to the standard method.

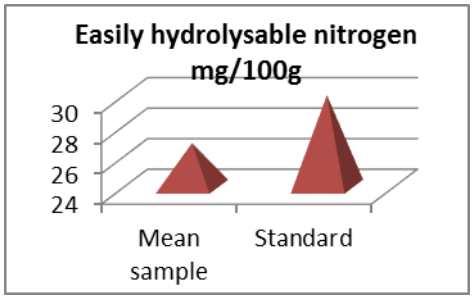


Figure 3. Determination of easily hydrolyzable nitrogen

The amount of easily hydrolysable nitrogen is between 25.29% and 28.71%, with an average of 26.85%.

The average value of easily hydrolysable nitrogen obtained for test samples is below the maximum legal limit.

4.4. Nitrites

In Figure 4. is presented the average value of the content of nitrites obtained from the replicates presented in Table 1 and the value accepted according to the standard method.

The content of nitrites was between 4.35 mg% and 5.10 mg%, resulting in an average of 4.748 mg%. The results indicate that it was used a small amount of nitrites in the salting mixture.

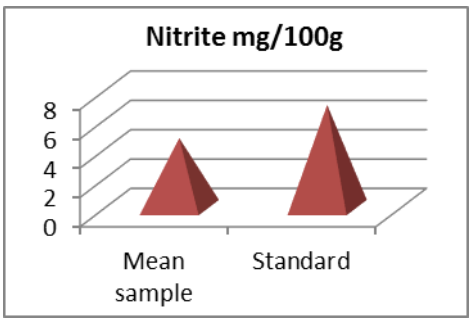


Figure 4. Determination of nitrites from frankfurters samples

The roles of nitrites in meat products are to give a reddish color as well to help their conservation.

5. Conclusions

Frankfurters manufacturing technology is a complex process being required to go through a large number of operations. Organoleptic properties are the strong point in purchasing a food product by the consumer. The moisture, the contents of sodium chloride, easily hydrolysable nitrogen and nitrites of the frankfurtes were below the legal limit.

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THE INFLUENCE OF THE CITRIC ACID ON THE PHYSICAL-CHEMICAL AND ORGANOLEPTICAL CHARACTERISTICS OF THE YOGURT STORED IN THE REFRIGERATION CONDITIONS

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ABSTRACT

Packages containing citric acid were prepared and their preserving activity of yogurt was tested. The yogurt samples were placed in plastic boxes and were mixed with solution of citric acid of different concentration (1%, 5%, 10%). The samples were stored in the refrigeration conditions (4^0C) for 14 days. At different time range, the following physical-chemical parameters were monitored: acidity, protein, dry mass and salt contents. The most accentuated decrease of the acidity was observed for yogurt samples deposited with 10% acid citric and the most accentuated decrease of the protein was observed for non-modified yogurt. The concentration of citric acid of 1% was established to be the most appropriate for the best preserving of the yogurt during refrigeration.

Keywords: *yogurt, preservation activity of citric acid, organoleptical and physical-chemical parameters*

1. Introduction

Citric acid is one of the most common acids in nature. So, it can be found in the plant, or produced as a result of metabolism of carbohydrates or lipids. Citric acid is also used for food preservation, being used in the preparation of refreshments as the "E 330" [1].

The calcium citrate is used as feed for the pets with the calcium deficit.

The two cultivars, which differ in anthocyanin and oxidative enzyme compositions, responded differently to the acid and chitosan treatment, with the result that the red color of Kwai may was better preserved during storage than that of Wai chee. In conclusion, the chitosan citric acid treatment increased the shelf-life of the fruit by at least 3 weeks compared to untreated control fruit that brown rapidly. [2]

In a general way, the association of citric acid and cassava starch coatings (CS and CSG) was efficient to reduce the respiration rate and maintain mechanical properties and color characteristics of mango slices during 15 days of storage. [3]

The aim of this study was to establish the influence of the citric acid on physical-chemical and organoleptical characteristics of the yogurt stored in the refrigeration conditions and if the citric acid can be used as preservative of the yogurt.

2. Materials and methods

2.1. Yogurt purchasing

The yogurt with a fat content of 3,5% was bought in Lidl`s supermarket in Baia Mare.

2.2. Samples preparation

The yogurt was mixed with citric acid powder to obtain different contents of citric acid in sample. Thus, three types of sample were obtained (1% wt, 5% wt and 10% wt). For reference, samples of non-modified yogurt were tested. The samples were stored in refrigeration conditions (4^0C) for 14 days. At different periods of time, the following characteristics were determined: organoleptical (color, aspect, consistence), acidity, protein and salt content.

2.3. Acidity measurements

A quantity of 5 ml yogurt was mixed with ultra pure water (25 ml) and 3-5 drops of phenolphthalein solution 1% (S.C.

Chemical Company S.A. Iasi, Romania) was added. The mixture was titrated with solution 0.1N NaOH (S.C. Chemical Company S.A. Iasi, Romania), until the pink color of the solution persisted for at least one minute [4].

The acidity was calculated using the Eq. 1:

Acidity (acidity degrees) = 20 × V (Eq. 1)
where: V - volume of NaOH 0.1 N solution used to titration (ml).

2.4. Protein content

In an Erlenmeyer vessel, 5 g of sample are mixed with 2 ml potassium oxalate solution 2%. Then, 1 ml phenolphthalein 2% was added. The mixture was titrated with NaOH solution 0.143 N until the pink colour appeared, for the free acidity neutralization. Then, 10 mL formaldehyde solution was added and the mixture was stirred. The pink colour disappeared. The mixture is let to rest for 30 seconds and then is titrated again with NaOH until the pink color appeared [4].

The protein content is calculated from the Eq. 2:

Protein (%) = V/2 (Eq. 2)
where: V- volume of NaOH 0,143 N (ml) used to the second titration.

2.5. Salt content

Was determined according to ref [5]. In a porcelain capsule weigh 5 g, to the nearest 0.01, cheese and mix with about 30 mL of distilled water, until they get a suspension as possible. Sample allow 10-15 minutes stirring several times during this time. Decant the liquid from the surface and pass through a quantitative and qualitative, in a conical flask. The filtrate is added by about 0.1 mL solution of potassium chromate and titrated with a solution of silver nitrate, liquid, stirring continuously until the switch to orange color.

Sodium chloride is calculated according to Eq. 3:

% sodium chloride =V/m (Eq. 3)
where: V-the volume of silver nitrate solution used in titration, in mL.

2.6. Dry mass

Was determined according to ref [6]. In a porcelain capsule, an amount of 10 g sand was inserted and a rod dry glass was added. A volume of 5 mL yogurt was added and the mass was weigh. The mixture was homogenized and was dried in oven at 105°C for 5 h, then the vial was weighted. Oven drying is repeated for 30 minutes to constant mass. The difference between the two weightings should not exceed 0,004g. Dry mass is calculated according to Eq 4:

$$\% \text{ dry mass} = \frac{m_2 - m_0}{m_1 - m_0} \times 100 \quad \text{(Eq.4)}$$

where: m_1 - mass of food sample and sand before drying (g), m_2 - mass of food sample and sand after drying (g), m_0 - mass of food sample (g)

3. Results and discussion

3.1. Organoleptical analyses

The samples have not present significantly organoleptical changes during storage at 4-5°C, for 14 days. In Table 1 are presented the organoleptic characteristics of the reference yogurt, at 0 day.

Table 1. Organoleptic examination of the reference yogurt, at 0 day.

Parameter	Characterization
Color	White, homogeneous
Aspect	Homogeneous, without whey drain
Consistence	Homogeneous, rough, creamy structure
Flavor	Nice

During the 14 days of storage, the organoleptical characteristics of different types of yogurt are similar with that of the reference.

This demonstrates the preservation capacity of the citric acid with regard to the colr, flavor and consistence.

3.2. Acidity

In figure 1 are presented the profiles of acidity variation of the yogurt modified with citric acid.

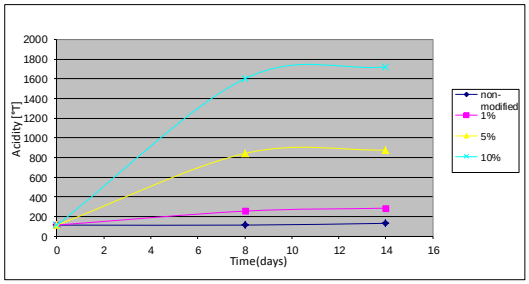


Figure 1. Variation of acidity in non-modified yogurt, yogurt with 1% acid citric, yogurt with 5% acid citric and yogurt with 10 % acid citric.

The acidity of all yogurt types increased during 14 days of storage. During the first 8 days of storage, the increase was accentuated (direct proportional with the citric acid content) and between 8 and 14 days of storage the increase was less pronounced. This behavior is explained by the acidity of the citric acid. The most accentuated increase was observed for the sample modified with 10%wt citric acid. The acidity increased as the citric acid content increases from 1%wt to 10%wt. The acidity of the yogurt kept with non-modified was almost constant during storage.

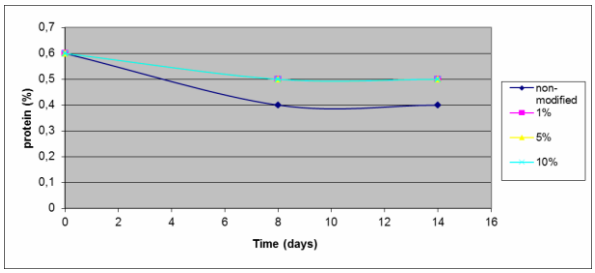


Figure 2. Variation of protein content in non-modified yogurt, yogurt with 1% acid citric, yogurt with 5% acid citric and yogurt with 10 % acid citric

3.3. Protein content

In figure 2 are presented the variation of the protein content for the samples modified with citric acid and for non-modified.

The protein content decreases during storage in all the investigated samples. The most reduced decrease of the protein content was observed in the modified yogurt. The variation of the protein content is similar for the samples modified with citric acid, thus demonstrating that the content of the preservation agent did not influence this parameter. Thus, the protein content in preserved by the presence of the citric acid. The most accentuated decrease was observed in the case of the non-modified sample, fact explained by the degradation of the proteins and aminoacids occuring during storage.

3.4. Salt content

In figure 3 are presented the variation of the salt content for the investigated samples.

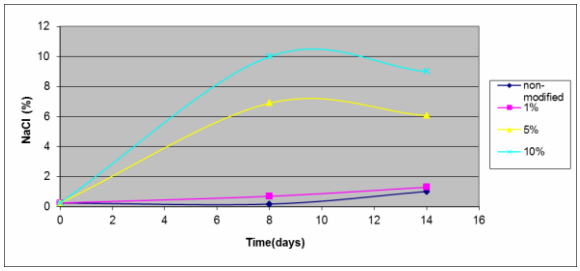


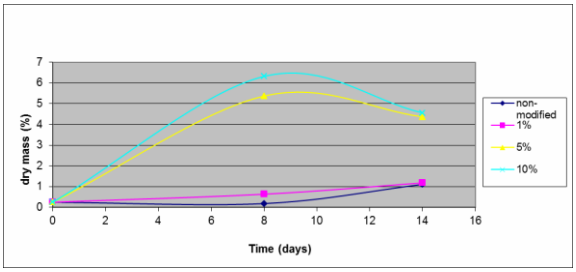
Figure 3. Variation of salt content in non-modified yogurt, yogurt with 1% acid citric, yogurt with 5% acid citric and yogurt with 10 % acid citric.

The salt content increased, after 8 days of storage, in all samples. The most accentuated increase was observed for the yogurt modified with 10% wt citric acid and 5%wt citric acid. The less pronounced increase was observed for the non-modified yogurt and for that modified with 1% wt citric acid. Between 8 and 14 days of storage, the salt content decreased in all samples. The most accentuated decrease was observed for the samples modified with 10%

wt and 5% wt citric acid and the most reduced for those modified with 1% wt and non-modified. The profile of variation for the sample modified with 1%wt citric acid is similar with that of the non-modified yogurt, sugesting that the 1%wt citric acid did not influence the salt content of the yogurt. This variation of the salt content is explained by the variation of the dry mass of the samples (section 3.5). The variation profiles are almost identical with those obtained for the dry mass. This sugests that, in fact, the variation of the salt content was significantly induced by the variation of the dry mass.

3.5. Dry mass

Figure 4 summarized the variation of the dry mass of the yogurt.



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4. Conclusions

Packages containing citric acid were prepared and their preserving activity of yogurt was tested. The yogurt samples were placed in plastic boxes and were mixed with citric acid powder to obtain three different contents (1%wt, 5%wt, 10%wt).

The optimal content of citric acid used to preserve the yogurt during storage in the refrigeration conditions was 1%wt, because this sample best preserved the propertied of the yogurt as compared with the values at 0 day.

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INFLUENCE STUDY OF FOOD CULTURE IN NATIONAL CUSTOMERS BASED ON LEAD CONTENT MEASURE OF PRESERVED EGGS

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ABSTRACT

Preserved eggs are people's favorite tradition food. In recent years, lead content of preserved eggs exceed national standard. Lead can damage various tissue organs. Long term eating of high lead content food will induce chronic cumulative poisoning Therefore; lead content measure is related to human health. In food measure, preprocessing of sampling is an important process. Measure of lead content can increase the guarantee of preserved eggs quality and ensure the national fame of traditional food of China. This paper discusses the influence study of food safety to food culture based on the experiment of lead content measure of preserved eggs.

Keywords: *preserved eggs; lead; power-spectral method; microwave*

1. Introduction

Preserved egg is also termed as Songhua eggs. It is a traditional food of Chinese people. It enjoys great popularity among people for its unique flavor, good taste, and long retention period. However, it was reported that preserved egg is the most serious one causing lead pollution among list of heavy metal food pollution. The average lead content of preserved egg exceeds 1.2 to 8.0 times of national standard limited value. High lead content of preserved eggs is mainly caused by utilization of lead oxide [1]. Lead can injure various tissue organs. Therefore, lead content measure of food is directly related to human health [2]. Lead is speculated as banded substances in food hygiene standard of China. And lead is the RII-required measure item of food safety risk monitoring. Lead measure method must be carried out analytical quality control before analysis in order to ensure the reliability of data analysis of preserved eggs, which can guarantee the accuracy and scientific feasibility.

Lead is an important limited indicator for food safety measure. Most lead is expelled from human body by faeces. However, there is still some residue in body. Long term accumulation can cause chronic intoxication. Lead combining with δ — amino enzyme acetyl acetone dehydratase and hydrosulphonyl in hemoglobin synthetase will cause hemachrome deficiency anemia. And "lead appearance" will appear on outlooking and "lead edge" will appear on teeth. And it can also cause vasospasm and waist pain, retinal arteriole spasm and high blood pressure. There are a lot of lead measure method such as spectrophotometric method and electrochemical techniques. Graphite furnace atomic absorption spectrometries have good sensibility, which have become the preferred method in lead content measure [3]. It is a method of international standard lead content measure. However,

the preprocessing of this method is complicated. It takes lots of time and labor and has a large consumption of reagent. In addition, it is easy to induce sample loss and pollution.

This paper explores combination of microwave assisted digestion and atomic spectroscopy to inspect lead in preserved egg. It solves the above drawback. In addition, the operations are easy, effective, and fast and have fewer disturbances, which is suitable for promotion. It can improve the quality of preserved eggs in the market, lower lead content, ensure safety eating and meet the demand of market at home and abroad. Lead measure of preserved eggs can also make continuation of traditional food in food culture.

2. Material and method

2.1 Instrument and reagent. Odulation, platform graphite tube, WX-4000 microwave digestion system. Lead standard stock solution: 1000 μ g/ml. Ammonium dihydrogen phosphate solution (20g/), nitric acid (1.0%), hydrogen peroxide (30.0%). Water for experiment is all distilled water. Nitric acid and ammonium dihydrogen phosphate are guaranteed reagent. Other reagent are analytical pure. Glassware instrument are soaked overnight by nitric acid (1+5), washed repeatedly and washed out by deionized water [4].

2.2 Graphite furnace atomic absorption spectrometry principal Sample was ashed or digested by nitric acid. Then it was injected into graphic oven of atomic absorption spectrophotometer and atomized by electric heating. Leads have specific absorption to spectral line sended by lead hollow cathode modulation in wave length of 283.3 nm. In certain range, absorption value and lead content was in direct proportion. Then it was compared with standard value and the lead content was obtained. Graphic furnace atomizer was made up by fixing graphite pipe between two electrodes. Large current went through graphite pipe in protection of inert gases. Graphite pipe was heated to high temperature and atomized sample. Compared with flame atomization, sample can all be atomized in graphite furnace atomizer. It has high measure sensitivity. It can be applied for element which is easy to form refractory oxide, low content of sample or few amount of sample. However, it also have drawback. Coexistence compound disturb a lot. And sample size and the change of injection location will lead to error because of the little sample size. Therefore the repeatability is not good. Principal of graphite furnace structure is shown in fig. 1.

2.3 Working condition of instrument

Wave length: 283.3 nm; lamp current: 6 mA; spectral bandwidth: 0.4 nm; correction mode: Zeeman background correction; shielding gas; argon; measurement mode: peak area. Heating up procedure of graphite furnace is shown in Table.1 [5].

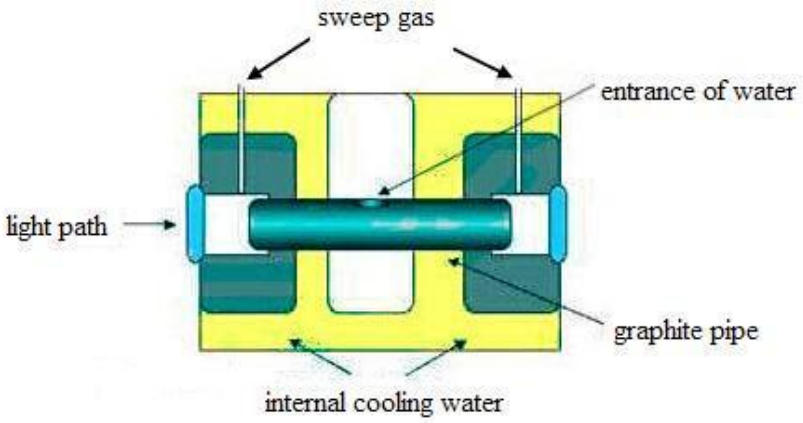

Figure 1. Principal of graphite furnace structure

Table 1. Heating up procedure of graphite furnace

Procedure	Temperature(℃)	Rate(℃/s)	Retention time (s)
Dry 1	90	5	20
Dry 2	110	3	20
ashing	500	250	15
atomization	1800	1400	4
erase residual	2300	500	5

2.4 Measurement method

0.500 g of uniform grinding sample was weighed and then placed into digestion pot. 5 ml nitric acid and 2 ml of hydrogen peroxide was added. It was put into the microwave digestion system for digestion after 15 min. Then it was placed on electric jacket to heat until boiling after the sharp reaction relaxed. Hydrogen peroxide was added dropwise until organic matter was all digested. Digestive juice was clear colorless or yellowish. It was moved into 50 ml volumetric flask after cooling. Flask was washed with little water repeatedly. Washing liquor was merged into volumetric flask and constant volumed to scale. It was mixed and stands by. Preprocessing by dry digestion has bigger error than by wet digestion. Therefore, we applied wet digestion here. The digestion condition of microwave was shown in Table 2. After digestion, digestion pot was put into boiling water bath to heat for 30 min and then cooled to indoor temperature. And it was washed for a few times by 1.0% nitric acid solution and constant volumed to 25 ml volumetric flask. Meanwhile, digestions blank was made and then wait for measure.

Table 2 Digestion condition of microwave

Procedure	Temperature(℃)	Pressure(atm)	Time(min)
1	130	25	5
2	180	30	5

Preprocessing by microwave digestion was adopted. And the procedure after optimizing microwave digestion condition is shown in Table 3.

Table 3 Digestion condition of microwave

Procedure	Heating up time(min)	Power(W)	Temperature (℃)	Retention time (min)
1	3	600	160	3
2	2	700	180	3
3	2	800	210	12

Bias is also termed as system error. It refers to the distinction between population mean and truth value of analysis result caused by some constant factor in the process of analysis. However, the system error is affected by random error. Therefore, bias is the overall error including random and bias test is system error source of analysis method. An effective bias experiment can add matters being tested into typical sample to become "spiked sample". Recycling value was calculated according to the analysis difference between spiked sample and non-spiked sample. Then accuracy of the method was estimated by recycling rate by the comparison of expected recycling value and recycling value.

1.0% nitric acid solution was applied to dilute standard stock solution (1000μg/L) to 50 μ g/L. Instrument performance was adjusted to the best state. Sample injection was automatically into the furnace furnace. Standard curve was appeared according to the ration of peak area. Regression equation is $x=369.1\gamma+0.003912$, $\gamma=0.9993$. Sample was fully mixed after constant volume and made a direct detection of sampling injection.

Result calculation $X= (C1-C0) \times V\times1000/m\times1000\times1000$

X-lead content in sample, mg/kg;

C1-measure of lead content in sample liquid, μg/L;

V-total volumes of sample digestion liquid ration, ml;

M-sample quality, g.

3. Result and discussion

3.1 Condition selection

Microwave digestion sample have different types. And the adopted digestion condition is also different. This experiment made repeated exploration on the digestion condition of preserved egg. The experiment shows that there will be a good digestion effect if the microwave digestion condition reaches Table 2. This experiment overcomes the volatilization loss of lead in the stage of ashing taking dihydrogen phosphate as collective improver [6]. After

repeated experiment, good effect can be achieved when the heating up procedure is shown in Table 1.

3.2 Test on experimental accuracy

Lead content in national standard substance GBW10016 (GSB-7 tea leaf) was 1.4mg/kg adopting this method, which conform to the standard value (1.5±0.2) mg/kg. Lead standard solutions with different concentration were added into standard substance sample. Then it was parallel determined for three times. And average recycling rate was calculated. The result is shown in Table 4.

3.3 Precision tests

Standard solution of 20.00μg/L was prepared and parallel determined for six times. The result is shown in Table 5.

Table 4 Test of recycling rate

Standard substance background value(μg/L)	Spiked amount(μg/L)	Determined average value(μg/L)	Recycling rate(%)
28.50	20.00	49.20	103.5
28.50	40.00	67.40	97.2
28.50	60.00	85.70	95.3

Table 5 Precision test

1	2	3	4	5	6	Average value	S	RSD
20.10	19.90	20.20	20.40	20.10	20.20	20.15	0.16	0.8%

Lead determination by microwave digestion-graphite furnace atomic absorption spectrometry can be controlled in the permitted range. The precision and accuracy of the method all conform to the requirement. The quality test found that there are four aspects that affect determination of the precision and accuracy of lead in preserved eggs:

①Selection of matrix improver

Matrix improver should make the analyte recycling meet requirement because of the complicated matrix of preserved egg food [7]. After repeat comparison, the experiment shows that single matrix improver of 5% ammonium dihydrogen phosphate has higher recycling rate than mixed matrix improver with magnesium nitrate, nickle nitrate and palladium nitrate. Moreover, double peak will not appear and atomization peak shape is ideal.

② Selection of ashing and atomization condition of graphite furnace

Ashing and atomization temperature affect lead a lot. The following procedures should be conducted before analytical test. Fix atomization temperature and select ashing temperature. Fix selected ashing temperature and optimize atomization temperature. Fix atomization temperature on the biggest characteristic absorption peak to optimize instrument condition. The optimal ashing and atomization condition of absorption photometry instrument

applied in the experiment is: 75 to 105℃of drying temperature and 105 s of drying time; 1900 ℃ of atomization temperature and 5.8 s of atomization time. Buckle background mode adopt Zeeman two magnetic fields in order to effectively eliminate the background interference in determination.

③Selection of quantitative mode

Experiments found that performance of standard solution series lines can be over 0.998. And the spike was sharp and had no trailing. However, the sample had serious trailing, low determined result and bad data repeatability [8]. The reason is that standard series is pure substance which is not conforming to sample matrix. Standard addition method was suggested to apply for matrix matching. Or lower the background interference by increasing dilution ratio. Then apply high sensibility detecting instruments to eliminate the matrix interference. By doing that, the determining result can be accurate and reliable.

④ preprocessing of sample

Preprocessing procedure of sample has an important effect on testing result. Full digestion and prevention pollution of other substance in the digestion process are all important factors to ensure accuracy of testing result. Glass dishes used in the test must be soaked overnight by dilute nitric acid, ultraphonic for 15 min by ultraonic cleaner, repeatedly washed according to the order of distilled water and deionized water. In the process of digestion, sputtering loss should be prevented. Complete digesting sample solution should be colorless and yellowish [9].

Trace element analysis includes error source of many factors and system error brought by some uncontrollable factor. Therefore, when some analysis method is pt into effect, testers should have full adaptivity on analysis method. In addition, internal quality control should also be done well for discovering the error brought by reagent, pure water, instrument and the immaturity of the method.

Analysis error in determining lead in preserved eggs microwave digestion atomic absorption spectrometry was predicted [10]. The experiment found that there are three aspects of errors and control measurement:

① **sample homogeneity:** Samplings have error because of the sample homogeneity, which make the determining result can not reflect the real composition of sample. So sampling should ensure the homogeneity of sample.

② **Capacity vessel and reagent:** inaccurate brightness and insufficient pure of reagent cause error. Error s caused by these factors often can not be controlled. Therefore, before simple determining, the glass dishes should be corrected and washed according to the requirement to eliminate the system error caused by uncontrolled factors to lower baseline value. Because the size of baseline value fully reflects the level of assay laboratory and analyst. In addition, variation brought by purity of reagent is mainly system error. Relative reagent should be selected according to the requirements of analytical method. ③Pollution and loss of sample: in the process of dilution, the loss and pollution of detected component should be effectively prevented. In trace analysis, pollution control is the most important point of quality control [11]. In addition, apparatus those are detected and in the

normal operation state should be applied. Standard substance that have traceability guarantee should be applied. Standard working curve should be correctly drawn. Application of standard sample similar to sample can effectively eliminate the effect of matrix to measure. Moreover, reliability analysis method that is international or through verification should be adopted. If the above laboratory analysis quality control work can be done well, error can be controlled within the range as small as possible.

6. Conclusion

We adopt microwave digestion-graphite furnace atomic absorption spectrometry to determine the lead content in preserved eggs.

Compared to other method, it has advantages of little sampling, low baseline value, no pollution, small loss and complete digestion. In addition, the precision, accuracy, recycling rate and detection limit of the method is also good, which can meet the actual working requirement and have a good application value. Compared to traditional lead content detection method, graphite furnace atomic absorption spectrometry is featured by small size of sample. The absolute accuracy of the method is 10-14 g which is several orders of magnitudes higher than flame method. And the solid sample can be directly detected. Polarization zeeman graphite furnace atomic absorption spectrophotometer can be applied because of the complicated instruments and big interference of background absorption. This method is an efficient, rapid and practical method determining lead in preserved eggs with high sensibility, good precision and good accuracy. These methods have important meaning to ensure the quality safety and sales of preserved eggs, which can eliminate the worry of people because of the health problem.

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THE EVALUATION RESEARCH ABOUT THE FOOD SAFETY OF TOURISM HOTEL BASED ON HACCP SYSTEM

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ABSTRACT

In recent years, our country's food safety and health has been effectively controlled by the management of supervision department, but with the development of fast food demand, there is still flaw. Thus China began to promote a kind of hazard analysis critical control point (HACCP).This paper is to establish and implement effective mechanism for the food safety of hotel in tourism industry through HACCP system, and evaluate the existing hidden trouble through applying risk management prediction concept, then find out the critical control points of hotel food safety, finally the operating results of HACCP system will be comprehensively evaluated. And the typical case will be studied and analyzed through combing the practical situation; the conclusion would be derived after verification and discovery.

Keywords: *food safety, tourist industry, HACCP system, research and analysis*

1. Introduction

With the improvement of people's living standard and the progress of science and technology, the variety of modern food is diverse, people's health level has generally improved, and thus the problem of food safety has attracted more and more attention. Food safety is the great event related to national economy and people's livelihood, food safety has become an important constitution part of national security. Every country has taken legislative and administrative means etc to ensure the food safety supervision.

HACCP food safety control system has developed more than 40 years since its birth in 1960s.It has been widely recognized and generally accepted by the international community, then various countries in the world followed and implemented it. In many countries, especially the developed countries have taken HACCP as the basis of formulating laws and regulations about food, and finally made a huge success .HACCP system is recognized as a kind of most effective food safety management system in the current world. According to the existing information, there are more than 400 enterprises which have obtained HACCP authentication in China ,more than 9000 food enterprises have gotten registered certificate of export health, nearly thousands of food enterprises have passed ISO9000 quality system authentication or product quality certification .Recently, there are laws and regulations about food quality and safety, such as ‘the law of product quality’, ‘the inspection law of import and export goods’, ‘the law of food hygiene’, ‘licence regulations of industrial production’ and so on. In the aspect of standardization, 1050 national standard for food [1] have been formulated.

The tourism has six elements

including 'eating [2], ceasing, walking, touring, shopping, amusement', eating ranks the first among them. So food has significant status in tourism industry. The food produced and processed by restaurant will provides first-rate service for tourists in tourist destination. It is also the propaganda window of making the name for local characteristic food and promoting local food culture. But with the diffusing of public opinion, tourist hotel food has been labeled with less species, high price, low quality, insanitation, poor service attitude in the eyes of vast tourists. In order to keep pace with the rapidly developing tourism industry nowadays ,the food of tourism hotel is to be perfect, and a kind of advanced management system is urgently needed to standardize it [3].Only in this way, it could cater the development process of tourism industry and provide safety service with high quality for tourists.

2. The general review of HACCP system

HACCP [9] (Hazard Analysis Critical control Point) is the English abbreviations of 'hazard analysis and critical control point'. It is a kind of systematic method used to identify, evaluate and control the relevant significant hazards of food safety in specific food production process [4].Finally it develops into a set of quality management system later. HACCP pays the biggest attention to prevention, and there exists biological, chemical and physical hazard factors in each link and process of producing raw material, receiving, processing, packaging, storage, transportation, sales and consumption in food industry. Some analysis is performed on the existing harm and the possible injury degree to determine its precautionary measures, as well as necessary control points and control methods. Then procedural control is applied to eliminate the hazards or reduce harm to acceptable level [5.6].It emphasizes hazards recognition and implementing effective monitoring to the whole producing course of food processing, then would timely find and stop possible food safety problems, eliminate potentially significant harm through controlling critical limiting value or constraining the potential hazards within acceptable level, thereby effectively prevent and cure food poisoning accident[7.8] .

The advantages of implementing HACCP system includes :emphasizing the identifying and prevention of risk in food contamination, overcoming the restriction of traditional method in controlling food safety[10](through testing ,not preventing food safety problems);contributing to carrying out the investigation by authorities in law and regulations; making possible and reasonable potential hazard be identified, even though similar invalidation problems have not been experienced; more sufficient resilience allowing for change; coordinating more with quality management system, all those are conducive to enhancing the competitive power of food enterprise in global market, increasing the creditworthiness of food safety and promoting trade development.

It is precisely these features of HACCP that effective guarantee the smooth going of food safety in production process. Food safety incidents could be reduced in tourist hotel at uttermost through being in virtue of this system, thus the adverse risk in food production could

be reduced, and the occurring safety crisis of hotel food will be prevented.

3. The establishment and implementation of HACCP system in tourist hotel

3.1 The function of establishing HACCP in tourist hotel

The food safety of tourism hotel urgently requires the supervision and management of HACCP system, Table 1 represents the efficacy of HACCP system in supervising the food safety of tourism hotel.

Table 1. The function of establishing HACCP system in tourism hotel

	Function	Analysis
Internal function	The restraint in traditional test methods could be gotten rid of.	Identifying and preventing the risk of food contamination, overcoming the food safety control limit of traditional method is emphasized
	Complete scientific basis	The hotel's specification data records make investigators' efficiency in government department, the results are more effective, and that is beneficial to authorities in law and regulations to perform investigation.
	The participation of overall member could be promoted.	Hotel staff should have a better understanding of the production steps and security responsibility, optimizing production processes ,enhancing the staff's sense of responsibility and a sense of achievement
	The quality of the product could be improved.	The potency of improving food quality should be possessed, by which to potentially improve food quality.
	There is more sufficient resilience allowing for change	The hotel's food quality should be improved, and the processing technology and variety development relevant to food should be enhanced.
	The cost of management could be saved.	The capital could be controlled better, thus waste could be avoided.
External function	The needs of the international trade	The establishment of HACCP is beneficial to enhance the competitive power of food enterprise in global market, improve food safety credibility and promote the trade development.
	It is beneficial to the certification of other international management system	HACCP system is the internationally recognized to be the most safe system of food hygiene safety and quality management
	The investment risk could be reduced.	It is controlled by complaints and claims of food problems in hotel, thus the loss caused by major hazard event could be avoided.
	The image of tourist hotel could be enhanced.	Customers' confidence to food should be strengthened, consumers' satisfaction should be enhanced, and the relationship between tourism hotel and consumers could be improved.
	To the benefit of health registration	That HACCP passes the authentication is very beneficial to conduct hygiene licence for general food companies

3.2 The premise of establishing HACCP in tourism hotel

Good Manufacturing Practice (GMP) and sanitation standard operation procedure (SSOP) is the basis condition of effectively establishing and implementing HACCP system for enterprises. GMP refers to that government issues compulsory food sanitation law will makes the requirements and regulations for hard and soft conditions such as food production, processing, packaging storage, personnel's sanitation and health, building facilities, equipment, production, processing, and control and so on. SSOP refers to that the sanitation standard operation procedure (SSOP) is suitable for producers' forming documents compiled by HACCP group. In China, SSOP procedure has following 8 standards, namely the safety of water and ice, sanitation and hygiene of food contact surface, preventing cross-infection, hand disinfection and bathroom facilities, preventing the processing, storage and use of foreign contamination and toxic compounds, the removal and control of insects and morbid.

When implementing HACCP system in supervision and administration of food safety, we should pay attention to the combination of GMP\SSOP\HACCP，only in this way could the efficacy of HACCP be exerted. Good sanitation control could not replace the controlling of hazard analysis and key points， thus well implementing and managing these three points is also the important premise of establishing HACCP.

3.3 Establishing HACCP system in tourism hotel

Supervision and administration is performed on food safety of tourism hotel through applying HACCP system to realize the effective evaluation and management to safety and sanitation for food supervision department .Firstly, tourism hotel must be familiar with hotel's food production, processing, sale process. And the HACCP plan which guarantees food quality and health security could be formulated based on this process. Establishing HACCP system in tourism hotel contains five aspects, it is shown as Figure 1.

3.3.1 Establishing HACCP group

HACCP group shoulder HACCP formulating plan of enacting premise condition such as GMP, SSOP. The group establishing is the important step of HACCP. It could reduce the risk of food hazards; avoid missing critical point and mistaking some operating process. For the group established by tourism hotel, its member should have experience about food processing, as well as basic knowledge about microbiology and food-borne disease, scientific understanding about good sanitation, operating instruction, specified food production. They also should know the prevention and control about tourism hotel's food contamination, as well as the basic knowledge of food processing equipment. Good expression ability and organization skills should be occupied, which guarantees that HACCP plan could be completely implemented.

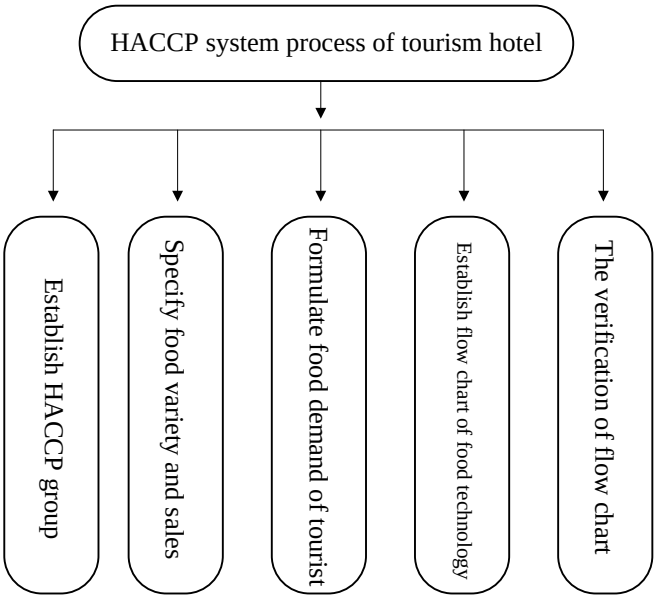

Figure 1. *HACCP system process of tourism hotel*

3.3.2The specification of food variety and sales

The ultimate goal of implementing HACCP supervision and administration to tourist hotel is to make each flow in food production process comply with formulated HACCP plan [11], thus reduce and eliminate food hazards. In order to better monitor food production and marketing process, superintendent office should register and understand the variety and sales of specific food in tourism hotel. The understanding of food should include the food designation, food processing line, the ingredients of additives, processing method at least. There are also some parameters including packaged form, as well as the approach of sales and storage etc.

3.3.3Formulate the food demand of tourists

What kind of food the passenger demand and what kind of tourist needs these foods will directly influence the hazard analysis results of food in HACCP system? Thus we must consider what kind of tourism food does tourists need, the food which could embody local tourism features, cultural connotation and novel food style will promote tourists' consuming .

3.3.4Establish the flow chart of food technology

The flow chart of food technology is to clearly and concisely describe the whole process from food's raw materials to ingredients' transportation, storage and processing, as well as the adding steps of food additives through using simple box and symbol .The flow diagram should cover all the steps and links of processing, such complete structure will provide important visual tools for HACCP group, verifier and reviewers. The supervision department needs to remind that the flow chart of tourist hotel should be drawn from

the source area of food additives, and then the hotel will enumerate the processing steps one by one when entering the market. HACCP group must compile a statement separately on the process and parameter tagging, which is beneficial to hazards analysis [12].

3.3.5 The verification of flow chart

The precision of flow chart is very critical to the accuracy and completeness of hazard analysis. The steps listed in flow chart should be verified in source area, if there is careless step, it maybe generate security flaw. HACCP group will determine whether formulating flow chart complies with actual production of current operation. Through investigation, all the group members could gain some understanding about the processing, transport, and storage sales of food material.

The established HACCP system of tourism hotel could dissect the food problems in such process in detail, it would provide help for future processing and sales reduce the occurrence probability of food hazards. In addition, once there occurs food safety problems, it is also convenient to find out the source of accident and timely control.

3.4 The implementation of HACCP system in tourism hotel

The operating of HACCP system is after establishing this system，according to the prospectus, the detection of every CCP would be performed in the process of actual production and processing, storage, sales in tourism hotel, thus the hazard numerical value will be controlled within limiting range .The food quality would be tracked with monitor routine [13], which helps to find out the problems and correct the deviation results timely，keep a detailed record of common problems, put forward solution as soon as possible, and adjust food technology.

The operation of HACCP system is the key of food safety supervision in tourist hotel. Firstly, HACCP system emphasizes the prevention of food contamination risk and overcoming the constraints of traditional regulation method in food safety which is used to apply detection method instead of preventing food safety problems; secondly, once there occurs food safety problems in producing process, some adjustment and improvement could be performed according to complete scientific basis; thirdly, as the tourism hotel has preserved the long time record complying with food safety law, instead of the satisfying degree in one day, which would make the supervision and managing efficiency of government department higher, the results will be more effective, thus it contributes to assisting authority in performing investigation; fourth, the possible and reasonable potential hazards will be identified ,which has the function of reference and precaution to new operating personnel; fifthly, there will be more sufficient resilience allowing for change, such as the improvement in equipment design, the enhancement of processing procedures and technology development relevant to products etc.

4. The evaluation research of HACCP system

The evaluation research of HACCP system could be divided into two stages. Due to the complexity of food safety in tourism hotel, we take the critical points of safety production as basis, and evaluate the

food safety and health of tourism hotel through applying analytic hierarchy process, then calculation is performed with the data obtaining from food safety and health through applying simulation and evaluation method.

4.1 Establishing hierarchical model for evaluation

As it is difficult to obtain statistical data of many indicators in hierarchical method mode，the matrix is constructed through applying 1-9 scaling method. According to analytic hierarchy process (ahp), A represents each project in food safety control of tourism hotel, the middle B layer denotes the critical point，the bottom layer represents each tourism hotel in HACCP system.

The judgment of scale 1, A_i and A_j is equally important. Scale 3, A_i are slightly more important than A_j .Scale 5, A_i are more important than A_j .Scale 7, A_i are much more important than A_j .Scale 9, A_i are absolutely more important than A_j .Scale 2, 4, 6, 8 are the determining median between above stated two dimensions.

If the importance ratio of element i and element j is A_{ij}, then the importance ratio of element j and element i will be $A_{ji} = 1/A_{ij}$.

The judgment matrix is:

$$\begin{bmatrix} A & B_i & \cdots & B_j & \cdots & B_n \\ B_i & b_{ii} & \cdots & b_{ij} & \cdots & b_{in} \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ B_j & b_{ji} & \cdots & b_{jj} & \cdots & b_{jn} \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ B_n & b_{ni} & \cdots & b_{nj} & \cdots & b_{nm} \end{bmatrix}$$

The quantization will be performed on the contrasting effect through applying 1-9 scaling method, then the relative weight between factors in a certain level and factors in above level could be calculated.

4.2 The weight calculation of critical point

For the weight calculation of critical control point, we adopt the simple method of weighted mean. That the numerical value of each item is divided by the summation of each column will be determined with analytic hierarchy process, then the new numerical value of each row will be added, and the number of critical control points will be divided by summation value, thus the obtained result is the control point weight of each critical point, the weight value of critical control point is 1.

4.3 Fuzzy assessment

The critical points of food safety management in tourism hotel are workplace sanitation U_1, sanitation in food storage areas U_2, the sanitation of hotel staff U_3 , ingredients procurement and acceptance U_4 , food storage and management U_5 , standard food

processing U_6, processing equipment U_7, sterilizing equipment U_8, hotel staff's health U_9, the grasping of food hygiene knowledge U_{10}, food safety training U_{11}, safety rules and regulations of hotel food U_{12}. Fuzzy evaluation assignment is

$H = \{H_1, H_2, H_3, H_4, H_5\} = \{100,80,60,30,0\}$

, the rating grade is obtained through the form of questionnaire survey. Multiplication processing is performed on the weights of critical control points and rating score，and then followed with adding, finally the comprehensive score of food safety critical control points in tourism hotel will be obtained.

5. Case analysis

5.1. Object and methods

A mid-sized tourist hotel is selected this time; the object is the producing and sale process of steak sauce which is commonly eaten by hotel's tourists. This tourism hotel has established good manufacturing practice (GMP) and sanitation standard operation procedure (SSOP).

Before establishing HACCP system, HACCP working group should be firstly set up to formulate plan, verification and maintenance work. The hotel will perform training to group member, which contains hotel manager, executive chef, foreman and so on.

The steak sauce is the food of cold drinks and snacks after hot processing. It is suitable for edible of tourists. The processing method includes: purchaser buys the beef, then stores it in -20℃ freezer; cleaning preparation is taken out for cooking, condiments has green onion, ginger, garlic; seasoning has soy sauce, monosodium glutamate, salt, sugar, grape wine; then it is of cold storage after sabot, thus tourists could order and eat them at any time.

5.2 Determine critical control point and perform hazard analysis

The critical points in HACCP plan has following process of purchasing raw material(beef), ingredients additives, cold storage etc，the hazard factors are the pathogenic bacteria pollution, parasite pollution, heavy metal residues ,pathogens residue after death in food material, and microbial contamination of ingredients, organic pesticide's residues mildew; chemical contamination in food additives when purchasing raw material; microbial cross-contamination during cooking; microbial breeding during cold storage; tableware's microbial contamination during serving the dishes.

HACCP group member have onerous work, they must check beef character and make simple test in the purchasing office of raw material and ask supplier for qualified certificate of food hygiene quality detection. The ingredients and additives also have to get quality qualification in procurement source, and the temperature control in transporting storage environment should be emphasized, mildew or corruption should be prevented. Cleaning work should be accomplished in preparation process of beefsteak, the sanitation situation of hotel's staff, environment, and tools' hygiene should all

pass SSOP for control. HACCP group member should make good record for the objects, method, relevant staff and controlled time in critical control point, thus it is convenient to timely correct and find out the cause of accident.

5.3 Case summary

Tourism hotel could accept tourists from different age and has extremely high number of customers in fastigium. This is different from the mechanized production of factory, the processing of much food is through manual operation, the randomness is larger, and the variety is extremely numerous. Not every food has established HACCP system, such kind of food could be divided into cold food after hot processing, uncooked food, and eating food after hot processing and so on through case analysis. We could establish the HACCP system of food belonging to the same category and implement controlling measures in different stage to reduce the hazards to the minimum, only the HACCP system constructed by this way could play its biggest role.

6. Conclusion

The evaluation research for food safety in tourism hotel is a systematic and complex process due to the exploitation of current tourism and springing up of hotels around many tourist attractions. While in such catering market, the food quality safety is irregular, and tourists determine the advantages and disadvantages through primary impression. Thus we establish HACCP system to effectively guarantee the food safety of tourists. Through analyzing the effect of HACCP, it proposes the necessity of establishing HACCP. Starting from establishing HACCP group, the producing processing of food should be specified, tourists' demand should be understood, the process of technology should be set up, and its integrity should be verified. The monitoring on food through implementing the whole set of plan must be matched exactly. Through our evaluation research for HACCP system, it not only could specify the producing processing of tourism hotel, but also make accurate rating evaluation for hotel, thus compare the advantages and disadvantages of hotel to achieve the purpose finally. In some individual cases, we could obtain from analysis that it is necessary to establish HACCP group, and find out management experience from work. We could not sum up some food into the same category generally, but instead start from the perspective of convenient control and perform unified control to food according to the similarity of production and processing，thus to be strict and precise.

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COMPARATIVE COLOR ANALYSIS OF RED, ROSÉ AND WHITE WINES BY SPECTROPHOTOMETRIC METHOD

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ABSTRACT

The paper presents the chromatic differences of three types of wine (white, rosé and red) related to the color intensity, color hue and chromatic structure. The absorbance spectra of wines were presented and compared. In addition, we present how these chromatic dimensions are affected by exposure to air (48 or 96 hours) compared to the unexposed wines.

Key words: *Color measurement, wine color, color stability*

1. Introduction

Color is an important characteristic of wine that can be assessed both by sensory and instrumental analysis.

The color of wine is due to some color components such as polyphenols, tannins and anthocyanins and is influenced by the pH of wine, total acidity, grape variety, the degree of maturity of the grapes, winemaking technology, the fining agents used etc. [1-2].

During the aging process of wine, the pigments (color component) may undergo a series of chemical transformations which influence the color shade of the wine [2-5].

The antioxidant capacity of wine tends to decrease along the aging period due to the reducing of the polyphenol content, especially of the monomeric forms of anthocyanin [4]. Another factor that influences the composition of the wine pigments is the contact with the oxygen that is involved in several chemical processes like polymerization of phenolic compounds, condensation reaction that lead to the formation of more stable compounds [6]. In the presence of oxygen, reactions of oxidation, polymerization and condensation of the color compounds take place leading to changes in the chromatic characteristics of wine. Experimentally, these chromatic characteristics can be measured by the

method of Glories that entails the absorbance measurement of wine samples at wavelengths 420, 520 and 620 nm [4-5,7-8].

The paper highlights the differences among the chromatic indicators of white wine, rosé and red wines and also the differential effects of air exposure on each of these chromatic indicators.

2. Materials and methods

2.1. Analyzed wines

The analyzed wines were two types of red wines, two types of rosé wines and two types of white wines, obtained from black and white grapes, varieties of Merlot (dry red 2008), Merlot and Cabernet (sweet red 2013), Merlot (dry rosé 2012), Pinot Noir and Merlot (sweet rosé 2012) and Riesling (sweet and dry white 2010). These wines were purchased from the commercial market.

2.2. Reagents and equipment

All reagents were analytical grade purchased from Merck and Chimiopar Bucharest.

Absorption measurements were made using a T60U Spectrophotometer, PG Instrument.

2.3. Spectrophotometric measurement of wine color

The spectrophotometric absorbance of each wine sample was determined using a 1 cm cuvette made of optic glass. The blank sample was distilled water. The absorbance at specific wavelengths (λ) of 420, 520 and 620 nm was measured and registered to assess the chromatic parameters (color intensity, color hue and chromatic structure of wine). Also the absorbance of wine samples was registered in the range of 420-700 nm and the wines spectra A=f(λ) was recorded.

2.3.1. Color intensity of wine

Color intensity is the sum of absorbance at 420 nm and 520 nm [4, 8]:

$$IC = A_{420} + A_{520} \text{ (Sudrand index)} \quad (1)$$

2.3.2. Color hue

Color hue, named also tint or tonality, is the ratio of A_{420} to A_{520} [7-9].

2.3.3 Chromatic structure of wine

IC' according to Glories method [7] is expressed as the sum of the absorbance at 420 nm, 520 nm and 620 nm (equation 2).

$$IC' = A_{420} + A_{520} + A_{620} \quad (2)$$

The chromatic structure of wine is expressed as the ratio of absorbance at 420, 520 and 620 nm to the color intensity, IC' (equations 3-5):

$$\text{yellow \% pigments} = \frac{A_{420}}{IC'} \cdot 100 \quad (3)$$

$$\text{Red \% pigments} = \frac{A_{520}}{IC'} \cdot 100 \quad (4)$$

$$\text{Blue \% pigments} = \frac{A_{620}}{IC'} \cdot 100 \quad (5)$$

2.4. pH and total acidity measurement

The pH was measured using a Consort pH-meter. The titratable acidity was determined titrimetrically using the Romanian standard method with NaOH 0,1N as titrating agent to pH end point of 8.1. Total titratable acidity was expressed in tartaric acid (g/L).

2.5. The color shift of wine samples exposed to air for 48h and 96 h

A 100 mL wine sample of each studied variety was exposed to air for 48h and 96h. The wine samples were placed in glass beakers. Then the color characteristics (color intensity, color hue, chromatic structure) were analyzed as in the case of unexposed wine samples.

3. Results and disscussion

3.1. Characteristics of analyzed wines

In Table 1 are presented some of the characteristics of analyzed wines: pH, total acidity and ethylic alcohol content. The values of these characteristics correspond to the standard requirements [9].

The concentrations of etylic alcohol of dry wines were higher than of the sweet ones with 10-13 %.

3.2. Wines absorbance spectra

The wine absorbance spectra were drawn and presented in Figure 1. The white wine and rosé wine spectra do not show sharp absorption peaks, and for red wines the peaks are not well defined because of the complex composition of wines with many colored substances [9-11].

By comparing the spectra it is observed that in the case of sweet white wine the absorbance is higher in comparison to the dry one at 420-470 nm, and at 470-430 nm the optical absorption properties are almost identical.

The dry rosé and red wines show absorbance's that are higher than those of the sweet varieties. The absorbance is higher for dry wines because these wines, by having higher alcohol content, they have a higher power to extract colored compounds [1, 9].

In fact, the routine chromatic analysis consists in measuring the

absorbance at wavelengths 420, 520 and 620 nm based on which the chromatic indicators are calculated.

Table 2 shows the chromatic characteristics of the analyzed wines and the changes in them after exposure to air for 48h and 96h.

The values of color intensity (IC') of red wines vary in the range of 5-15 [1, 4, 8, 11].

Table 1. pH and total acidity of the analyzed wines

Type of wine		Alcohol content, V %	pH	Total acidity, tartaric acid g /L
White wine (Riesling)	Sweet	10	3.16	8.55
	Dry	11	3.03	6.59
Rosé wine (Merlot)	Sweet	11.5	2.87	6.825
	Dry	13	3.08	5.625
Red wine (Merlot)	Sweet	11.5	3.37	5.25
	Dry	13	3.43	5.25

Table 2. Chromatic proprieties of wines through Glories method and their evolution under air exposure

Type of wine		Time of air exposure/Absorbance (A_{420}, A_{520}, A_{620}) and Color intensity											
		(initial) 0 h				48 h				96 h			
		A_{420}	A_{520}	A_{620}	IC	A_{420}	A_{520}	A_{620}	IC	A_{420}	A_{520}	A_{620}	IC
WW	Sweet	0.14	0.02	0.003	0.163	0.143	0.035	0.006	0.184	0.149	0.038	0.017	0.204
	Dry	0.051	0.009	0.00	0.06	0.056	0.027	0.006	0.089	0.06	0.03	0.017	0.107
RW	Sweet	0.250	0.211	0.022	0.483	0.274	0.277	0.006	0.557	0.292	0.322	0.017	0.631
	Dry	0.390	0.358	0.050	0.798	0.442	0.421	0.006	0.869	0.449	0.425	0.017	0.891
RdW	Sweet	2.093	2.047	0.454	4.594	2.249	2.283	0.325	4.857	2.320	2.444	0.374	5.138
	Dry	3.281	4.069	0.938	8.288	3.520	3.853	0.812	8.185	3.206	3.810	0.879	7.895

WW- white whine, RW-rose wine, RdW-red wine

Figure 2 shows the ratios of the colors in terms of percent of yellow, red and blues pigments for the unexposed wine samples and their modifications after the exposure to air for 48 h (Figure 3) and for 96 h (Figure 4).

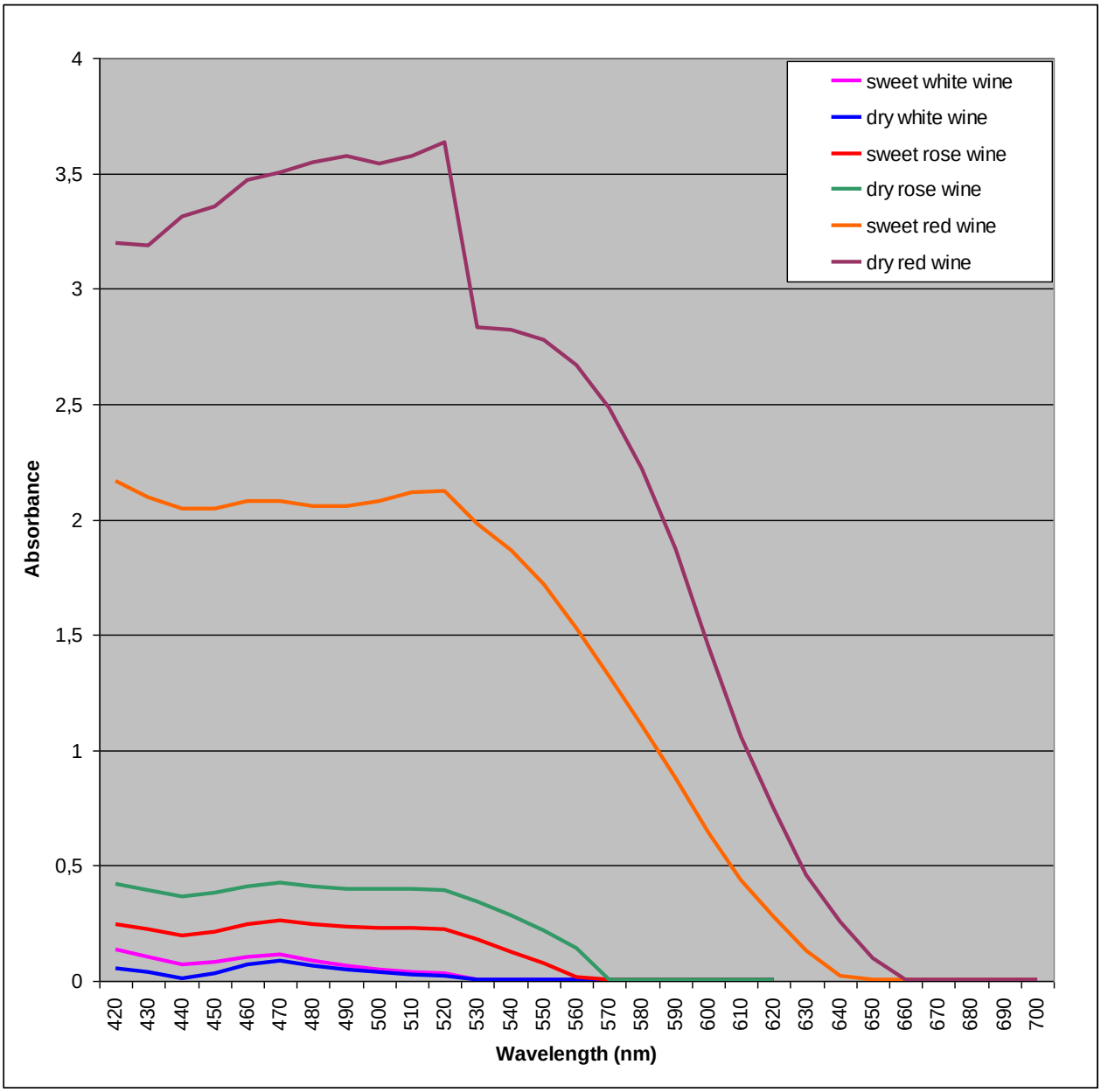

Figure 1. Comparative absorption spectra of red, rosé and white wines

3.3. Chromatic structure of wines and the influence of air exposure

In the sweet white wine a decrease of the yellow color ratio from 85.89% to 73.04% is observed. The red color ratio increases, and then slightly decreases, and the blue color ratio increases continuously from 1.84% to 8.33%.

In the dry white wine a continuous decrease of the yellow color ratio from 85% to 56.07% is observed, also an increase and then a slight decrease of the red color ratio, and the blue color ratio increases continuously from 0% to 15.89%.

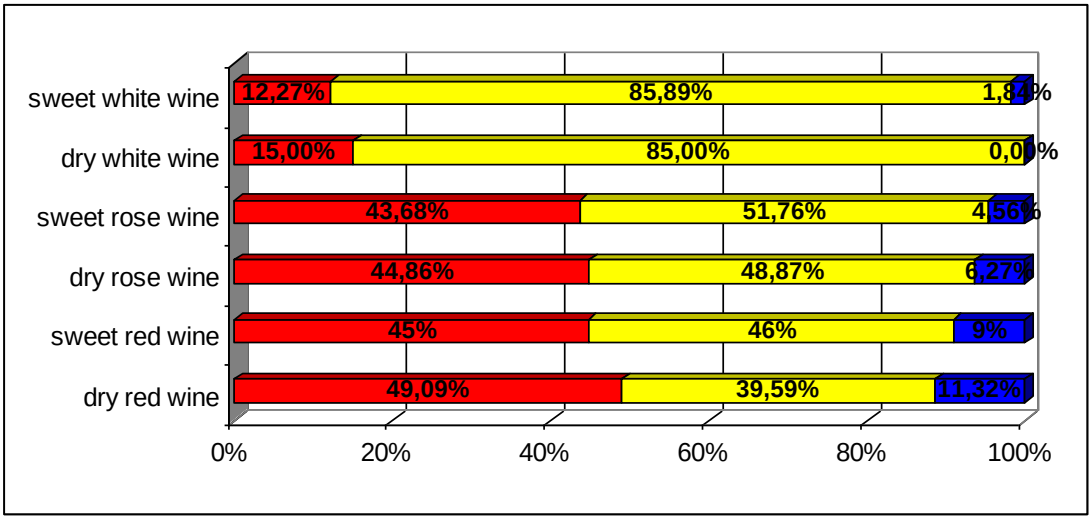

Figure.2 Chromatic structure of wines unexposed to air (0 h)

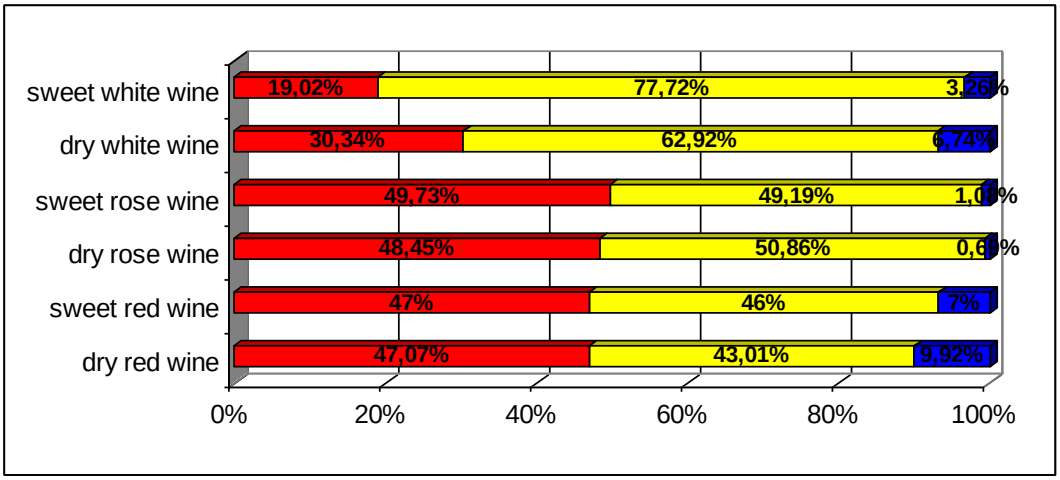

Figure.3 Chromatic structure of wines unexposed to air for 48 hours

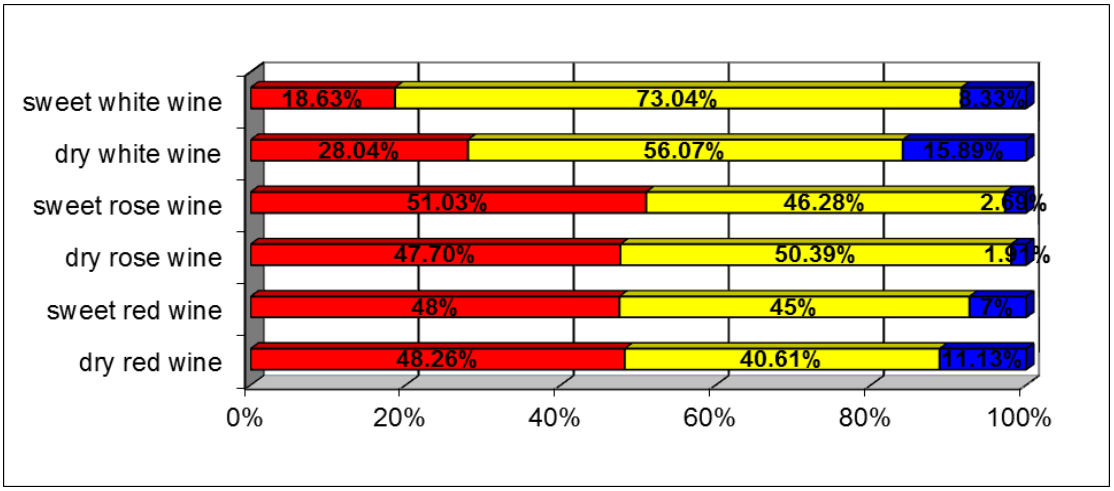

Figure.4 Chromatic structure of wines unexposed to air for 96 hours

The sweet rosé wine yellow color ratio decreases continuously, while the red and blue color ratios increase

The dry rosé wine yellow color ratio increases from 48.87% to 50.39%, while the red color ratio increases and then slightly decreases, and the blue color ratio first decreases and then slightly increases.

In the sweet red wine an increase followed by a decrease of the yellow color ratio is observed, the red color ratio increases continuously from 44.56% to 47.57%, and the blue color ratio decreases followed by a slight increase.

In the dry red wine an increase followed by a decrease of the yellow color ratio is observed, while the red and blue color ratios show a decrease followed by a slight increase.

These changes are explained by the reactions the color compounds undergo under the action of the oxygen in the air: oxidation, condensation and polymerization of the free anthocyanins. [5, 11-12].

The sweet rosé wine was most affected by the exposure to air (Table 1, Figure 2), it's color intensity IC' increased by 30.64% (after 96h exposure to air), followed by dry rosé wine to which the change of IC was of 11.65% (after 96h of exposure to air).

3.4. Wine color hue

The wine color hue is an indicator used to assess the quality of red and rosé wines and it is observed in the process of maturation and aging of these types of wines. Its decrease with the aging of the wine is due to the oxidation process of the red pigments [5, 11, 13]. In Figure 5 are presented the hue values for the analyzed wines unexposed to air and after the air contact for 48 h and for 96 h.

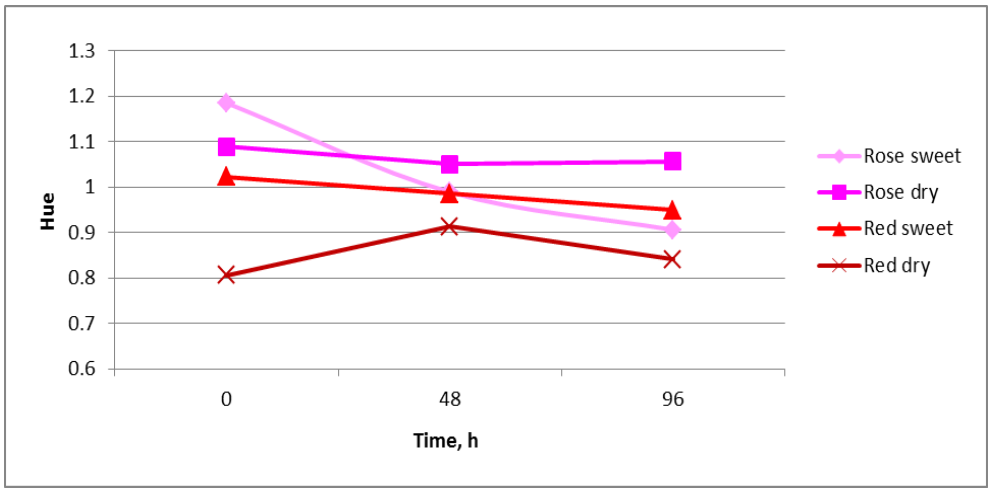

Figure 5. Hue variation during the exposure to air of rosé and red wines

The wine color hue of red wines shifts towards close shades of orange [4-5, 13]. Generally, these changes are due to the changes of anthocyanins in wine in new pigments through different oxidation-reduction reactions and the formation of complex combinations with other wine compounds like carbohydrates, proteins, metals or flavonols [13-15]. Cliff reported a hue of 0.88 for Merlot and 1.02 for Pinot [15] close to our findings of 0.806 for the red dry wine –Merlot and Cabernet.

The hue which expresses the ratio of red and yellow colorants decreases over time

for sweet rosé and red wines, and increased after 48h for the dry red wine sample. But, the tendency to increase was not maintained, and at 96h a decrease was observed due to the oxidation and polymerization suffered by the color component.

4. Conclusions

Samples of red, rosé and white wines were analyzed in terms of color. The analysis of white, rosé and red wine spectra showed similar profiles for these types of wines. Higher absorbance values were observed in dry red and rosé wines, probably due to the higher alcohol content which provides a better extraction of colored compounds.

The chromatic analysis of wines is a useful method to determine the quality of finished wines and also during the technological production stages.

When exposed to air wines show changes in color due to the reactions with the oxygen in the air. These changes are more obvious in rosé wines, in which the color is less intense than in the case of red wines.

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