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EFFECT OF FRYING TEMPERATURE AND RATIO OF *COCCINIA GRANDIS* POWDER ON NUTRITIONAL AND ANTIOXIDANT PROPERTIES OF FRIED INDIAN SNACK FOOD

Vinothini K[1], Sasikala S[2*], Anjana Bora[1], Sandra Aveena Monis[1]

[1]Department of Food Process Engineering, SRM University.
[2] Department of Food Process Engineering, SRM University, Kancheepuram, India- 603203.
[*]sasishan.shiva@gmail.com

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ABSTRACT

Coccinia grandis (Ivy gourd) is used for culinary purpose among Indian people. Polyprenol C60 is a bioactive compound which posse's antidyslipidemic activity, this compound has been isolated from ivy gourd fruit and quantified to be 242.548 ppm at retention time of 10 minutes. It has abundant health benefits with which we can prepare fried snack food by incorporating it with pulses for enhancing the taste, nutritive value and sensory properties. Analyzing the nutritional parameters, the snack fried at 190°C incorporated with 7g ivy gourd powder gave best results of 5.3% protein, 85% carbohydrate, and 23.2% of fat, 3% mineral, 0.104 % of antioxidant, 1% moisture content and the colour were found to be reddish yellow. When comparing the nutritional parameters with temperature, it was established that there was ($p<0.05$) significance between moisture content, protein, carbohydrate, fat, antioxidant and temperature, but no significance between colour, mineral and temperature. When compared with ratio of ivy gourd powder incorporated ($p>0.05$) there was no significance between amount of ivy gourd incorporated and nutrition of the developed snack, but mineral content was retained to certain limit.

1. Introduction

Coccinia grandis (Ivy gourd) from Cucurbitaceae family is widely grown in all parts of the world including Indian sub-continent. Ivy gourd also serves as functional food and can be consumed as diet for treatment of diabetes. This treatment using ivy gourd is being followed traditionally in India (Krishnaswamy, 2009). Terpenoids were found to have ant diabetic activity (Deokate et al., 2011). Ivy gourd has high nutritional profile of 1.4 g protein, 3.4 g carbohydrate, 0.2 g fat, 25 mg calcium and 0.9 mg iron per 100 grams of edible portion (Sanskriti et al., 2014). The unripened fruit also contains minerals like calcium, fibre and it is precursor of Vitamin A (Sushma, 2013). When screening for photochemical, it was found that the plant extract has alkaloids, carbohydrates, glycosides, phytosterols, saponins, fixed oils, fats, tannin, phenolic compounds, proteins, free amino acids, gums, mucilage, flavonoids, lignin and volatile oil (Elamathi et al., 2012).

Almost all the parts of ivy gourd have medicinal properties. Fruit has anti-inflammatory property, it can been used for treatment of eczema, root is used for treatment of urinary tract infection and for removing pain in joints, leaves for treatment of skin and used as cooling agent for eye (Goldy et al., 2014). The fruit also possess hepatoprotective activity (Vadivu et al., 2008). Plant extract also has biological properties like analgesic, antipyretic, antimicrobial, antiulcer, antidiabetic, antioxidant, hypoglycemic, hepatoprotective,

antimalarial, antidyslipidemic, anticancer, antitussive (Pekamwar et al., 2013). Ivy gourd also has excellent antimicrobial activity against all gram positive and gram negative bacteria like *Salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* except Proteus (Umbreen et al., 2008). When the fruit was screened for antioxidant property against standard BHA it was established that the fruit has free radical scavenging activity and there are many active compounds present in ivy gourd one such is polyprenol C60 which has antidyslipidemic activity (Goldy et al., 2014). Ivy gourd is also known as baby watermelon and it is generally used for culinary purpose among Indian people. Many recipes including chutney, vegetable curry, and dried powders can be prepared from ivy gourd.

Deep fried snack foods are preferred by all age groups. Many people are fond of foods like samosa and banana chips which are deep fried in oil. Frying is the oldest method of cooking; foods cooked by deep frying method are most commonly preferred by many people because of its taste. Not only because of its taste the time period of cooking is very rapid and heat transfer is more because of high temperature (Keliani, 2013). Deep fat frying is generally carried out at the temperature between 160 – 180°C. Even though the 160°C is suitable for frying for getting better products it is generally said the optimum frying temperature is above 160°C. For more crispy products the optimum temperature is 180°C (Wil van Loon et al., 2007). When frying was carried out at 120 °C, it was found that oil absorption was more and it was concluded that basic temperature for frying is 120-190°C (Vipul, 2014).

Frying time depends not only on frying temperature but also on various other factors like, type of oils used for frying, type of instrument (Fryer), oxygen present in frying environment and antioxidant content of product. High temperature reduces the time of cooking (Choe et al., 2007).

Even though there are increased risks for obesity, cardiac arrest, diabetes related to consumption of fried snack food, most of us are addicted to its taste and flavour. The developed snack food can give a solution for snack food lovers. The snack food has health benefits; at the same time it also satisfies the priority of the people who prefer healthier diet. Most of the people have less knowledge about the health benefits of ivy gourd and since less research have been done regarding developing products from ivy gourd, thus we can focus in developing a new product which will ensure health benefits and which can drive attraction among people of all age groups. Healthy fried snack food was developed by incorporating ivy gourd. The effect of frying temperature and the ratio of ivy gourd powder on the nutritional properties of fried snack food were analysed using one way ANOVA in this studies.

2. Materials and methods

This study was carried out in Department of Food Process Engineering, SRM University, Kattankulathur, Chennai, India.

2.1. Materials

The required ingredients for making fried snack food are freshly harvested ivy gourd (not fully ripen), rice flour, green gram, white lentils, salt, coconut milk, chilli powder and water. Refined sunflower oil was used for frying the product. The equipment used for frying was deep fat fryer (NOVA) with varying temperature ranges between 100-190 °C. Other facilities required for this study are tray drier, mixer grinder, electronic weighing balance, Soxhlet apparatus, muffle furnace, hot air oven, hunter colorimeter, spectrophotometer and measuring flask.

2.2. Methods

Ivy gourd was selected based on visual inspection. It should be thoroughly inspected that the ivy gourd fruit is not fully ripened. The selected ivy gourd was washed thoroughly with fresh water so as to remove unwanted dirt and dust which was sticking to the fruit. The ends of the fruit were trimmed and sliced into small pieces and the sliced pieces were placed in tray drier at the temperature of 40°C for 4 hours (hrs)

and the final moisture content was found to be 7.9 % (wb). The dried samples were ground into powder using mixer grinder. All the ingredients were mixed together to form a dough. The dough was extruded using mould into strips and frying studies was performed using sunflower oil in deep fat fryer at temperatures 140°C, 160°C and 190°C. The samples without incorporating ivy gourd powder were taken as control sample. Proximate analysis and antioxidant study were carried out for the developed product.

2.3. Nutritional Analysis

2.3.1. Moisture Content

Standard procedure (AOAC, 1999) at 105°C for hours until constant weight was obtained was followed to estimate the moisture content of the fried snack food.

2.3.2. Protein Analysis

Protein analysis for fried snack food was carried out using Lowry's method (Jakob, 2002) and the protein content was estimated based on spectrophotometer absorbance at 750 nm.

2.3.3. Carbohydrate Analysis

Carbohydrate content present in fried snack food was analyzed by anthrone method (Hansen et al., 1975) and total carbohydrate content was estimated based on spectrophotometer absorbance at 630 nm.

2.3.4. Fat Analysis

Amount of fat content present in the fried snack food was estimated using (AOAC, 1999) Soxhlet apparatus run for 6-8 hrs using petroleum ether. The solvent was evaporated and the residue was weighed.

2.3.5. Ash Content

Total mineral content in the sample was calculated using muffle furnace (AOAC, 1999). It was ignited in a muffle furnace at 550+/-250°C for 4 hrs and the weight of the ash was measured using electronic balance.

2.3.6. Colour Analysis

Colour analysis (SOP-PHYS-004 by Sarah Lanning) for snack food was carried out using hunter colorimeter. Based on L*, a*, b* value the colour of the sample was determined.

2.3.7. Antioxidant Assay

DPPH assay (Florence et al., 2014 and Ma Ma Lay et al., 2014) was done to estimate the amount of antioxidant present in fried snack food.

2.3.8. Statistical analysis

IBM SPSS statistics 19 was used to find out the significant difference between the independent variable and dependent variable.

2.4. Optimization of Sample by Sensory Analysis

Several trials were carried out by mixing all the ingredients with or without incorporating ivy gourd powder and the strips were fried in fryer at 180-190°C for 3-4 minutes for optimizing the control and fried snack food samples. The product was optimized (Table 1, Table 2) based on sensory analysis using 9 point hedonic scale method (Murray et al., 2001).

2.5. Frying Studies

Frying studies were carried out with independent and dependent variables (Vivek et al., 2014 & Fetuga et al., 2014). Ratio of ivy gourd powder (0g, 2g, 5g, 7g) and temperature for frying (140°C, 160°C, and 190°C) were taken as independent variable and moisture content, protein, carbohydrate, fat, mineral and antioxidant content were taken as dependent variables. The variables were analysed using IBM SPSS Statistics 19 (Indrani et al., 2014) and ANOVA (Sukhdev, 2014).

Table 1.Trial for Optimizing the Control Sample.

Ingredients	Weight
Rice flour	100g
Green gram	25g
White lentils	25g
Salt	2gm
Chilli powder	2g
Coconut milk	25ml
Water	75ml

Table 2. Trial for Optimizing the Fried Snack Food Samples.

Ingredients	Weight
Rice Flour	100g
Green gram	25g
White lentils	25g
Salt	2gm
Chilli powder	2g
Ivy gourd powder	0g / 2g / 5g / 7g
Coconut milk	25ml
Water	75ml

3. Results and discussions

3.1. Optimization of Control and Fried Snack Food Samples

In trial 1 (from the Table 3) the overall acceptability of the product was 8 but it was found that the taste, colour and frying time had to be optimized to get crispy and good quality product. In trial 2 the product was prepared by adding salt and chilli powder for improving taste of the product. When sensory analysis was performed it was found that the fried snack food prepared was good in terms of taste, colour, flavour, texture, mouth feel and was optimized with overall acceptability of 8.4 as control sample. Other trials were performed by incorporating ivy gourd powder in different ratio (0g, 2g, 5g and 7g). The three samples did not have any change in terms of taste when compared with control sample and it was accepted with overall acceptability of 7.2. The frying temperature for fried snack food was found to be between 140°C-190°C. For getting good quality snack food, the frying time and temperature has to be optimized. It is also clear that the nutritional parameters of the snack food play a vital role in quality of the product which has to be analysed.

3.2. Frying Studies and Nutritional Analysis for Fried Snack Food

Frying Studies was performed for fried snack food which was prepared using the ingredients from (Table 1) with three temperatures 140°C, 160°C, 190°C by incorporating 0g, 2g, 5g, 7g ivy gourd powder (Table 2) and nutritional analysis was carried out for all the samples. When frying was carried out at 140°C it was observed that it took nearly 16-20 minutes for frying, when frying was at 160°C the frying time was reduced to 8-10 minutes and when frying was carried out at 190°C the frying time was minimized to 4- 5 minutes. By increasing the temperature, we can reduce the time of cooking (Choe et al., 2007). When analysing the snack foods texture it was found that frying at 190°C gave more crispy products compared to snack food fried at 140°C and 160°C.

Table 3. Score Card for Sensory Analysis

Sensory Parameters	Trial 1 (0g)	Trial 2 (0g)	Trial 3 (2g)	Trial 4 (5g)	Trial 5 (7g)
Colour	7.6	8.5	7.1	7	6.8
Taste	8.5	8.5	7.4	7.5	7.7
Texture	7.7	8.5	7.4	7.1	7.7
Mouth feel	8.2	8.3	6.9	6.8	6.8
Overall acceptability	8	8.4	7.2	7.1	7.2

When nutritional analysis was carried out for the developed snack food it was found that (Table 4) there was significant decrease in the protein content (8.2-4.2%). The sample which was incorporated with 7g ivy gourd fried at 140°C showed highest protein content. Increasing the temperature will reduce the amount of protein content and the time taken for frying. The highest protein content in the sample may be because of frying at low temperature and longer time, since frying is also a process of dehydration. There was significant increase in carbohydrate (38-85%). Sample incorporated with 7g ivy gourd powder fried at 190°C had the highest carbohydrate content; this may be due to indigestible products present in the sample even though the snack food was fried in short time. When fat content was analysed, the fat content increased (16-23%) when the temperature was increased during frying. The sample incorporated with 7g ivy gourd powder fried at 190°C showed the highest fat content. Fat content generally depends not only in the frying temperature and time; it also depends on the oil used and instrument used for frying (Keliani et al., 2013). Analysing the mineral content of the snack food, it was found that some samples have retained mineral where as in some samples there was slight loss in mineral content. The mineral content ranged from (1.7-3.3%). Mineral content always depends on the temperature, time of frying and the amount of protein content present in the sample. This may be the reason for the highest mineral content in sample incorporated with 7g ivy gourd powder fried at 160°C (Ramasamy et al., 2011).

When antioxidant was analysed in the samples it ranged from (0.05-0.20%), antioxidant activity was more at 140°C but the scavenging activity was reduced when temperature was increased to 190°C. Increasing the temperature will reduce the antioxidant activity (Choe et al., 2007). The sample fried at 140°C incorporated with 7g ivy gourd powder showed highest antioxidant activity.

When moisture content was analysed there was significant loss, when the temperature was increased to 190°C. The fried snack food contains moisture content ranging from (3-1%). The reason for this may be because of high temperature and short time leading to water loss in the fried samples (Keliani et al., 2013). When colour of the sample was analysed using hunter colour meter reading showed that L* value ranging between (50.4 - 62.58), a* ranging between (10.87 - 13.23) and b* value between (19.54 - 27.97) which means that the fried snack food was found to be reddish yellow in colour (SOP-PHYS-004 by Sarah Lanning). Frying the snack food at 190°C for 4-5 minutes will result in more crisp products. The optimum temperature for frying the snack food was found to be 190°C and the optimum ratio of incorporation of ivy gourd powder was found to be 7g for preparing healthy fried snack food.

Table.4 Results of Nutritional Analysis per 100 g of Sample

Temperature °C	Amount of Ivy gourd powder g	Moisture Content %	Protein %	Carbo hydrate %	Fat %	Mineral %	L*	a*	b*	Anti oxidant %
140	0	3	7.5	38	16	1.7	61.41	10.87	27.13	0.163
160	0	2.5	5.3	53	19.6	2.1	62.58	11.92	27.97	0.110
190	0	1.8	4.9	69	19.8	2.3	60.64	13.19	27.96	0.052
140	2	2.4	6.9	54	16.4	2.7	61.06	12.11	27.40	0.164
160	2	2.2	6.5	78	20.1	2.7	60.34	12.92	27.60	0.112
190	2	1.6	4.2	77	21.4	2.9	54.89	13.23	23.94	0.071
140	5	2	6.5	55	16.8	3.3	54.56	12.75	23.90	0.186
160	5	2	5.5	79	20.8	2.7	57.95	11.99	26.88	0.120
190	5	1.4	4.7	82	21.6	2.1	50.40	12.29	19.54	0.099
140	7	1.8	8.2	67	17.4	3.1	50.88	11.61	21.16	0.202
160	7	1.8	6.9	80	21.4	3.3	52.31	13.17	21.91	0.146
190	7	1	5.3	85	23.2	3	53.76	12.94	23.53	0.104

Table 5. Effect of temperature on nutritional parameters of the Fried Snack Food (ANOVA)

Nutritional Parameters of the Fried Snack Food (ANOVA)		Sum of Squares	df	Mean Square	F	Sig.
MC %	Between Groups	1.612	2	.806	4.976	.035*
	Within Groups	1.458	9	.162		
	Total	3.069	11			
PROTEIN g	Between Groups	12.502	2	6.251	13.839	.002*
	Within Groups	4.065	9	.452		
	Total	16.567	11			
CARBOHYDRATE g	Between Groups	1342.167	2	671.083	5.588	.026*
	Within Groups	1080.750	9	120.083		
	Total	2422.917	11			
FAT %	Between Groups	52.272	2	26.136	26.921	.000*
	Within Groups	8.737	9	.971		
	Total	61.009	11			
L*	Between Groups	23.110	2	11.555	.541	.600**
	Within Groups	192.315	9	21.368		
	Total	215.426	11			
a*	Between Groups	2.365	2	1.182	2.889	.107**
	Within Groups	3.683	9	.409		
	Total	6.047	11			
b*	Between Groups	11.022	2	5.511	.579	.580**
	Within Groups	85.654	9	9.517		
	Total	96.676	11			
ANTIOXIDANT mg	Between Groups	1.910	2	.955	23.266	.000*
	Within Groups	.369	9	.041		
	Total	2.280	11			
MINERAL %	Between Groups	.042	2	.021	.066	.936**
	Within Groups	2.827	9	.314		
	Total	2.869	11			

3.3. Statistical Analysis of the Effect of Temperature and Effect of Amount of Ivy gourd Powder Incorporated on Nutritional Parameter of the Developed Product

One way analysis of variance was carried out using IBM SPSS Statistics 19 to find out the significance difference between the temperature and nutritional parameters of the fried snack food fried at 140°C, 160°C, and 190°C and to analyse the significant difference between the ratio of ivy gourd powder incorporated 0g, 2g, 5g and 7g and nutritional parameter of the fried snack food. It was concluded based on test of homogeneity of variance the ($p>0.05$) it means we have not violated the assumption variance (SPSS Survival Manual version 12). From the Table 5 it was evident that there was ($p<0.05$) significance between moisture content, protein, carbohydrate, fat, antioxidant and temperature, but there was ($p>0.05$) no significance between colour, mineral and temperature. This showed, colour does not depend on temperature (Indrani et al., 2014 and Sukhdev, 2014), when significant difference was analysed between temperature and nutritional parameters. From the Table 6 it was evident that ($p>0.05$) there was no significant difference between ratio of ivy gourd powder incorporated and nutritional parameters of the developed snack food. This may be because different ratios that was incorporated and fried at high temperature. It was also evident that only the mineral content had significant difference between the ratio of incorporation of ivy gourd powder and nutritional parameter of the developed snack food. This may be due to the amount of protein present in each sample analysed, since nutrition content also depends upon the amount of protein present in the sample (Keliani et al., 2013). It means that nutritional value does not depend on the amount of ivy gourd powder incorporated. But the sample incorporated with 7 g ivy gourd powder when combined with high temperature, short time will give best results (Sukhdev, 2014).

4. Conclusions

Ivy gourd is commonly used as vegetable among Indian people. A healthy fried snack food was prepared by incorporating ivy gourd powder in different ratios. When frying studies was performed it was found that 190°C was the optimum temperature and 4-5 minutes was the optimum time for frying the snack food. The snack food incorporated with 7g ivy gourd powder fried at 190°C gave best results of 5.3% protein, 85% carbohydrate, and 23.2% of fat, 3% mineral, 0.104 % of antioxidant, 1% moisture content and the colour of the snack food was found to be reddish yellow. When ANOVA was performed for comparing the nutritional parameters with temperature, it was concluded that there was ($p<0.05$) significance between moisture content, protein, carbohydrate, fat, antioxidant and temperature, but no significance between colour, mineral and temperature. When nutritional parameters were compared against the amount of ivy gourd powder incorporated it was analyzed that that ($p>0.05$) there was no significant difference between amount of ivy gourd incorporated and nutrition of the developed snack, but mineral content was retained to certain limit. Further analysis can be carried out for ensuring the shelf life of the fried snack food**.**

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RESEARCH ON PROXIMATE ANALYSIS OF FILLETS OF THREE MARINE SHRIMPS SPECIES FROM IRAN

Ali Aberoumand[1*], Saeed Ziaei-Nejad[1]

[1]Behbahan Khatam Alanbia University of Technology, Behbahan, Iran.
Tell: +98-916727718, Fax Number: +98-671-52729969,
Postal Addresses: No:22, second Alley of Ab va barg, Zolfegari, Behbahan , Khuzestan, Iran
[*]aberoumandali@yahoo.com

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ABSTRACT
Purpose: The present was investigated the comparison between the biochemical compositions of shrimps the Peanaeus semisulcatus, Metapenaeus affinis and Panalirus homareus. Materials and Methods: Main method used for the research was AOAC analysis. Results: The results showed that the percentage of protein in the P. homareus was higher (28.55% dry weight) than that of M. affinis protein (23.25%), but P. semisulcatus(32.38%) found the highest. The highest amount of lipid was found in P. homareus the value being 2.74% whereas in the higher level of moisture content was noticed in 64.2 % in M. affinis. The higher value of 6.61% of ash was noticed in the P. semisulcatus. Conclusion: It is concluded that P. homareus is a good source of proteins and metabolically energy and average mineral supply.

1. Introduction

Prawn contains good amount of organic and inorganic constituents. The main constituents are proteins, carbohydrates; lipids in addition to that prawn also contain a significant proportion of minerals (Ca, Mg, P, Mn and Cl) and vitamins A, C and D (Abulude et al., 2006).

The shrimps and fishes are an excellent source of protein and essential High-Unsaturated Fatty Acids (HUFA) such as eicosapentaenoic (20: 5n3, EPA) and docosahexaenoic (22: 6n3, DHA) acids (Feliz, et al., 2002) Besides, the white shrimp is a good source of minerals and vitamins such as calcium, iron, zinc, copper, vitamin B_{12} and essential amino acids (Yanar and Celik, 2006; Dong, 2001). Its biochemical composition may be affected by several factors as the species, environmental factors, size, age, natural diet and feed composition (Gruger, 1967; Bandarra et al., 1997; Sriket et al., 2007a). Shrimps are an extremely good source of protein, yet are very low in fat and calories, making them a very healthy choice of food. Although shrimps have high cholesterol content, they are low in saturated fat, which is the fat that raises cholesterol levels in the body. Minerals are essentials in shrimp nutrition (Vijayan and Diwan, 1996). Ravichandran and Rameshkumar (2009) reported maximum level of protein, carbohydrate and moisture content in the flesh tissues and higher level of lipid, fibre and ash content in the shell part of the shrimp *Penaeus indicus*. This work was undertaken to compare between the macro-nutrients composition of shrimps *Peanaeus semisulcatus, Metapenaeus affinis* and *Pana lirus homareus* collected from the southern waters in Iran.

2. Materials and methods

2.1. Shrimps preparation

The shrimps (*Peanaeus semisulcatus, Metapenaeus affinis* and *Panalirus homareus*) were collected from southern waters in Iran in April 2013. Samples were washed with de-ionized water to remove any adhering contamination, drained under folds of filter paper. Samples were then put in crushed ice in insulated containers and brought to the laboratory for preservation prior to analysis. The washed shrimps were wrapped in aluminum foil and frozen at -4°C for two days before samples were prepared for analysis. After defrosting, the shrimps were separated into the exoskeleton (head and the outer body shell) (*ie* shell) and the endoskeleton (*ie* flesh). The edible parts only were oven dried at 95-105°C until dried and ground into fine powder.

2.2.Determination of proximate composition

Estimation of moisture content by hot air oven method, lipid by (Bligh and Dyer, 1959), protein by (Lowry et al., 1951) and carbohydrate (Morris, 1984) were analyzed by AOAC (AOAC, 2000).

2.3.Statistical analysis

Experiments were performed in triplicate and results were expressed as mean ± SD and were analyzed by SPSS statistical programme.

3. Results and discussions

3.1. Proximate Composition

The proximate composition of the *Peanaeus semisulcatus, Metapenaeus affinis* and *Pana lirus homareus* is presented in (Table 1).The percentage of protein in *P. semisulcatus* was higher (32.38%) than *M. affinis* protein (23.25%) and *P. homareus* (28.55%). Carbohydrate content of *M. affinis* was higher (4.49%) than the others. The highest amount of fat was found in *P. homareus* (2.74%) whereas in the higher level of moisture content was noticed in 55.85 % in *P. semisulcatus*. The highest value of 6.61% of ash was noticed in the *P. semisulcatus*.

Biochemical parameters in *P. homareus* were generally more concentrated than others. The level of protein in the *P. semisulcatus* was of good comparison with the of *M. affinis*. Both the current and the literature references had low level of carbohydrate (Ravichandran and Rameshkumar, 2009). Some researchers found variation level of protein in shrimp waste, the level started from 39, 45 to 52, 70% (Adeyeye and Adubiaro, 2004; Fanimo et al., 2004). The results of the present investigation reported that of *P. homareus* and *M. affinis* shows maximum level of protein, carbohydrate and moisture content was reported in the edible parts respectively. Likewise higher level of lipid, and ash content was noticed in the shell part of the shrimps *P. homareus* and *P. semisulcatus* respectively. The results reported that *P. homareus* is a good source of proteins and metabolically energy and average mineral supply. Since shrimps are consumed as a major protein source in food, it is very important that the protein content should not be compromised during table preparation. It is significant to note, therefore that all the tables processing methods reduced the crude protein contents but the reduction did not follow a particular order or shrimp type (Adeyeye and Adubiaro, 2004; Okove et al., 2005).

The results of the proximate analysis (Table 1) shows that the protein content in the samples was high with respect to other nutrient composition. High level of protein was obtained in all of three species. The crude protein level of the *P. semisulcatus, M. affinis* and *P. homareus* in our study found 32.38 $\pm$ 1.07%, 23.25$\pm$ 1.08% and 28.55$\pm$ 1.09%, respectively. The results of the protein content was higher than that obtained by Bello-Olusoji et al., (2006) with protein content for *Caridina africana* was 18.98 ± 0.02%, Fasakin et al., (2000) for *M. vollenhovenii,* (16.99 ± 0.20), *M. macrobrachion* (17.30 ± 0.30); *Penaeus notialis* (20.57 ± 0.05) and *Bachrus niger* (18.52 ± 0.01).

Table 1. Biochemical composition of edible parts of *P.semisulcatus, M. affinis* and *P. homareus* (% dry weight)

Parameters	*P. semisulcatus*	*M. affinis*	*P. homareus*
Crude protein	32.38 ± 1.07[a]	23.25± 1.08[a]	28.55± 1.09[b]
Crude fat	1.84 ± 0.35[a]	2.02 ± 0.33[a]	2.74± 0.39[b]
Crude carbohydrates	3.32 ± 1.35 [a]	4.49 ± 10.38 [b]	1.71± 1.36[c]
Moisture	55.85 ± 3.45[a]	64.20 ±3.8 [b]	62.04±3.31[b]
Total ash	6.61 ± 0.55[a]	6.04 ±0.58[b]	4.96±0.67[c]
Energy (Kcal)	159.36 ± 3.67 [a]	129.14 ±3.23[a]	145.70±3.89[b]

Mean values are obtained from three samples analyzed in triplicate.
Different superscripts in the same row indicate significant differences ($p<0.05$).

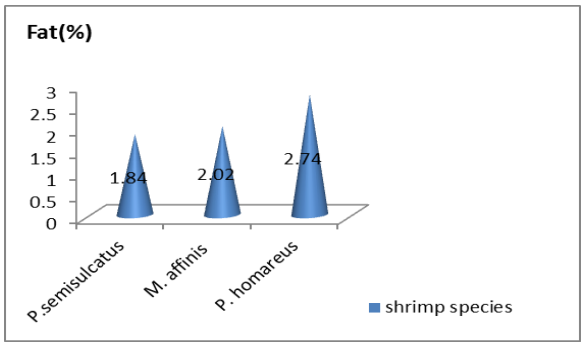

Figure 1. Showing the fat variation of the experimented shrimps

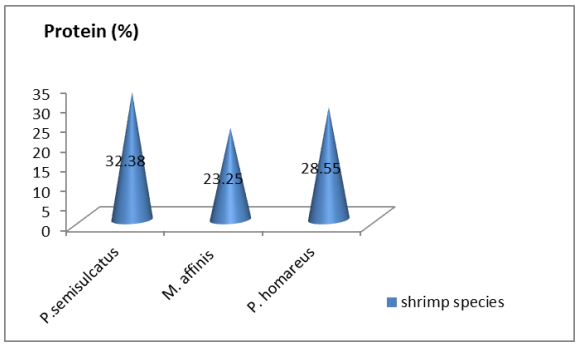

Figure 2. Showing the protein variation of the experimented shrimps

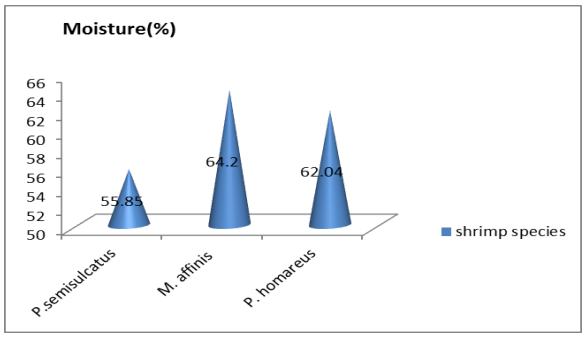

Figure 3. Showing the moisture variation of the experimented shrimps

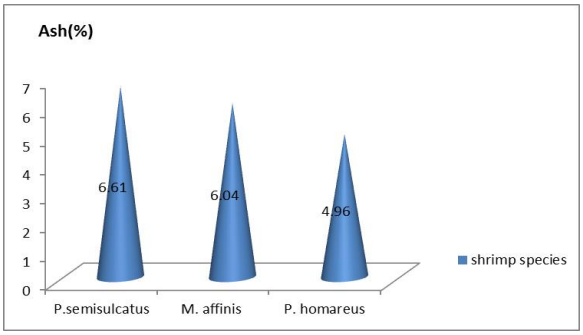

Figure 4. Showing the ash variation of the experimented shrimps

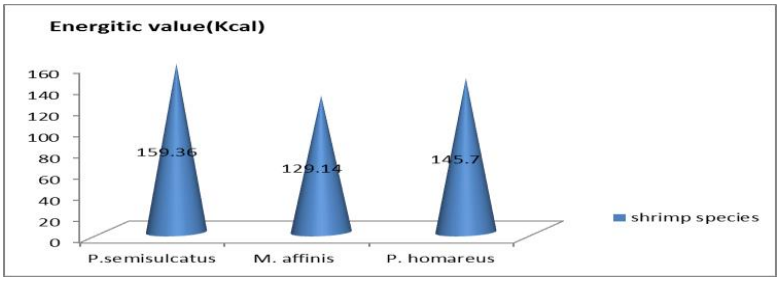

Figure 5. Showing the energetic values variation of the experimented shrimps

The little variation between this report and the work of these authors may be due to season prawn sizes. The high fat content of *P. homareus* will allow them to contribute significantly as a source of non- visible oil to any diet they may be present.

4.Conclusions

The results of our study suggested that muscles of analyzed shrimps and fishes are a good source of protein and lipid. The differences in biohemicalc compositions between samples might be associated with the origin and species. There were significant differences ($p<0.05$) between shrimps, but the *P. homareus* shrimp tend to have a better proximate composition than the others.

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FUNCTIONAL AND ANTIOXIDANT PROPERTIES OF DIFFERENT MILLING FRACTIONS OF INDIAN BARLEY CULTIVARS

Sneh Punia[1], Kawaljit Singh Sandhu[1*]

Department of Food Science and Technology, Chaudhary Devi Lal University, Sirsa, India
[*]kawsandhu@rediffmail.com

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ABSTRACT

Barley cultivars were dehusked, debranned to obtain different milling fractions: husk, bran, dehusked barley flour (barley after removal of husk, DBF) and polished barley flour (barley without husk and bran, PBF). Physical properties (husk, bulk density, 1000 kernel weight, l/b ratio), proximate composition, color characteristics, water absorption capacity (WAC), oil absorption capacity (OAC), and antioxidant property (total phenolic content, TPC; total flavonoids content, TFC; and antioxidant activity, AOA) among cultivars differed significantly ($p<0.05$). WAC and OAC of different fractions followed the order: PBF<DBF<bran<husk. TPC, TFC, and AOA for bran, PBF, and DBF were the highest for cv.BH-885 and the lowest for cv.PL-172. For husk fraction, cv.BH-393 showed the highest values for TPC and AOA whereas cv.BH-902 had the highest TFC value. Results revealed that husk content which is not economically important had significant higher antioxidant activity in comparison to other fractions.

1. Introduction

Barley (Hordeum vulgare L.) is an important functional cereal crop. According to FAO (2011) the world total production of barley was 134.2 Million tonnes (MT) and in India, about 0.7 lakh hectares of land is under barley cultivation with total production of 1.6 MT. India ranks first in the production of barley bran. Barley is gaining renewed interest as an ingredient for production of functional foods due to its high contents of bioactive compounds such as β-glucans (2-10%), tocopherols and tocotrienols. Moreover, there are many classes of phenolic compounds in barley, such as benzoic and cinnamic acid derivatives, proanthocyanidins, quinones, flavonols, chalcones, flavones, flavanones and amino phenolic compounds (Holtekjolen et al., 2008). All of these are known to have antioxidant activity and therefore, possibly health benefits (Andreasen et al., 2001; Beecher, 2004). Antioxidants have strong in vitro and in vivo activities and their ability to scavenge free radicals, break radical chain reactions and scavenging metals.

The outermost layers of the cereal grains possess a high phenolic content and antioxidant activity (Liyana-pathirana and Shahidi, 2007; Madhujith et al., 2006; Zhou et al., 2004.). However, the concentration of antioxidants present in the grains and their antioxidant activities may vary depending on the species, cultivar, and growing location and environmental conditions, among others (Adom et al., 2003; Bonoli et al., 2004; Zielinski and Kozlowska, 2000).

Barley is mainly used in the brewing process, where husk of the spent grains is a main by-product without any useful purpose

which accounts up to 15–20% of the dry weight of the grain (Pereira Al Abreu et al., 2012). Natural extracts of phenolic compounds with high antioxidant activity is obtained after delignification of barley husks. Husk contains vanillin, syringaldehyde, p-coumaric acid, p-hydroxybenzaldehyde, ferulic acid, syringic acid, p-hydroxibenzoic acid, vanillic acid and acetovanillone (Cruz et al., 2007). Barley husks show their efficacy as antioxidant and the potential usefulness of natural antioxidants extracted for food preservation (Pereira Al Abreu et al., 2012) and offers the valuable nutritional advantage as they contain antioxidant potency. The objective of the present study was to investigate the physico-chemical, functional and antioxidant properties of different milling fractions of barley cultivars grown in India.

2. Materials and methods

2.1. Barley samples and reagents

Common barley cultivars (cv.) BH-393, BH-932, BH-902, BH-885, DWR-52, PL-172, RD-2035 and RD-2552 were purchased from Chaudhary Charan Singh Haryana Agriculture University, Hissar, India. Cultivars BH-885 and DWR-52 were of two row barley type whereas others were of six row type. Standard Gallic acid, Folin-Ciocalteu's, DPPH and Catechin were purchased from Sigma-Aldrich, Germany. All chemicals were of analytical grade.

2.2. 1000 kernel weight, bulk density and l/b ratio of barley grains

Barley grains were randomly selected and 1000 kernels were counted. The counted grains were then weighed and expressed in gms. For measuring the bulk density, barley grains were gently filled in a 100 ml graduated cylinder, previously tared. The bottom of the cylinder was gently tapped on a laboratory bench, several times, until there was no further diminution of the sample level after filling to the 100 ml mark. Bulk density was calculated as weight of sample per unit volume of sample (g/100ml). l/b ratio was calculated using vernier-calliper. All the measurements were triplicated.

2.3. Dehusking and milling of barley

Dehusking and debranning of barley cultivars were carried out using a rice polisher (Khera Ltd., India). Barley grains were placed in the chamber of polisher, which was run till the husk was completely removed. The de-husked grains were then de-branned. Different milling fractions obtained was husk, bran, de-husked barley (barley without husk) and polished barley (barley without husk and bran). These milling fractions were then ground to obtain flour and those from de-husked barley and polished barley were designated as de-husked barley flour (DBF) and polished barley flour (PBF), respectively.

2.4. Proximate analysis

DBF from different cultivars was tested for their moisture, ash, fat, protein and fiber contents by employing the standards methods of analysis (AOAC, 1990). The carbohydrate content was calculated by difference. All the results were recorded on a dry weight basis (dwb).

2.5. Color characteristics of flours

Color measurements of barley flour samples were carried out using a Hunter colorimeter Model D 25 optical Sensor (Hunter Associates Laboratory Inc., Reston, VA, USA) on the basis of L*, a* and b* values. A glass cell containing flour from barley cultivars were placed above the light source, covered with a white plate and L*, a* and b* color values were recorded.

2.6. Water absorption capacity (WAC) and oil absorption capacity (OAC)

WAC and OAC of the flours were determined following methods of Sathe et al. (1981) with slight modifications. Briefly, flour sample (2 g) was mixed with 20 ml distilled water for water absorption and 20 ml of groundnut oil for oil absorption in a centrifuge tubes. Samples were then allowed to stand at

30°C for 30 min then centrifuged at 3,000 rpm for 10 min. The volume of supernatant in a graduated cylinder was noted. Means of triplicate determinations were reported.

2.7. Total phenolic content (TPC)

The total phenolic content was determined by following the Folin–Ciocalteu method as described by Gao et al. (2002). Barley samples (200 mg) were extracted with 4 ml acidified methanol (HCl/methanol/water, 1:80:10, v/v/v) at room temperature (25°C) for 2 h using wrist action shaker. The mixture was centrifuged at 3000 rpm for 10 min on a centrifuge (Remi, India). The supernatant was used for determination of total phenolic content. Aliquot of extract (200 µl) was added to 1.5 ml freshly diluted (20-fold) Folin-Ciocalteu reagent. The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 ml of sodium carbonate solution (60 g/l). After incubation at room temperature (25°C) for 90 min, the absorbance of the mixture was read at 725 nm. Acidified methanol was used as a blank. The results were expressed as µg of gallic acid equivalents (GAE)/g of sample. All analyses were performed in triplicates.

2.8. Total flavonoid content (TFC)

The total flavonoids content was determined by following the method described by Jia et al. (1998). Barley extract (250 µl) was diluted with 1.25 ml distilled water. Sodium nitrite (75 µl of 5% solution) was added and the mixture was allowed to stand for 6 min. Further, 150 µl of a 10% aluminium chloride was added and the mixture was allowed to stand for 5 min. After that, 0.5 ml of 1 M sodium hydroxide was added and solution was mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. Catechin was used as standard and the results were reported as µg catechin equivalents (CE)/g of sample.

2.9. Antioxidant activity (AOA)

Antioxidant activity was measured using a modified version of the method explained by Brand-Williams et al. (1995). This involved the use of free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution in the methanol. Ground barley samples (100 mg) were extracted with 1 ml methanol for 2 h and centrifuged at 3000 rpm for 10 min. The supernatant (100 µl) was reacted with 3.9 ml of a 6 x10-5mol/l of DPPH solution. Absorbance (A) at 515 nm was read at 0 and 30 min using a methanol blank. Antioxidant activity was calculated as % discoloration.

% AOA=(1 –(A of samplet=30/A of samplet=0))x100

2.10. Statistical analysis

The data reported in all the tables are an average of triplicate observations and were subjected to one way analysis of variance (ANOVA) using Minitab statistical software version 14 (Minitab Inc, USA).

3. Results and discussions

3.1.Physical property of barley cultivars

Physical properties of barley cultivars (cv.) are reported in Table 1. The husk content differed significantly ($p<0.05$) among cultivars and varied from 10.8 to 12.9% (Table 1). The husk content found in our cultivars were higher than that reported by Zielinski and Kozlowska (2000). They reported the husk content of barley in the range from 7.6 to 9.8%. The bulk density ranged between 0.567-0.643 g/ml with the highest and the lowest being observed for cv.BH-902 and cv.RD-2552, respectively. The 1000 kernel weight and l/b ratio of cultivars were in the range between 41.2-46.6 g, and 3.2-3.5, respectively. l/b among cultivars did not differ significantly ($p<0.05$). Bhatty and Rossnagel (1998) reported thousand kernel weight for Japanese and Canadian barley in the range from 27 to 40 g.

3.2. Proximate composition of DBF

The ash content among cultivars differed significantly ($p<0.05$) and ranged from 1.38 to 1.77% (Table 2). Sharma and Gujral (2010b) reported significant ($p<0.05$) difference in ash

contents among barley cultivars with range from 1.15 to 1.51%. Oscarsson et al. (1996) also observed the ash content ranging from 1.9 to 2.3% for different hulled barley cultivars. The protein content was found the highest for cv.BH-902 (13.6%) whereas the lowest was observed for cv.RD-2552 (9.1%). Anderson et al. (1999) reported the protein content in the range from 11.4 to 18.1% in different barley cultivars. The range for fat and fiber contents was from 3.2 to 4.2% and 1.1 to 1.5%, respectively. Jood and Kalra (2001) reported fat content ranging from 2.83 to 3.98% for different Indian hulled barley cultivars. The carbohydrate content calculated by difference was the highest for cv.RD-2552 (82.2%) whereas the lowest for cv.BH-885 (74.4%) was observed.

3.3.WAC and OAC

WAC represents the ability of a product to associate with water under conditions where water is limiting (Singh, 2001). WAC and OAC of different milling fractions of barley cultivars followed the following order: husk>bran>DBF>PBF (Table 3). WAC differed significantly ($p<0.05$) among cultivars with cv.BH-393 and cv.RD-2552 showing the highest and the lowest values for all milling fractions. OAC is of great importance from an industrial viewpoint, since it reflects the emulsifying capacity (Escamilla-Silva et al., 2003). Among cultivars, OAC also differed significantly ($p<0.05$) with cv.BH-393 having the highest value. Sharma and Gujral (2010b) reported the WAC and OAC in the range from 1.38 to 1.63 g/g and 1.5 to 1.68 g/g for barley.

Table 1. Physical property of different barley cultivars

Barley cultivars	Husk (%)	Bulk density (g/ml)	Thousand kernel weight (g)	l/b ratio
BH-393	12.9^{c}	0.619^{d}	45.8^{e}	3.4^{ab}
BH-932	12.8^{c}	0.599^{c}	43.9^{c}	3.2^{a}
BH-902	12.6^{c}	0.643^{e}	46.6^{f}	3.4^{ab}
BH-885	11.6^{b}	0.605^{cd}	44.7^{d}	3.5^{b}
DWR-52	10.8^{a}	0.587^{b}	41.7^{ab}	3.3^{a}
PL-172	10.9^{a}	0.592^{bc}	42.8^{b}	3.3^{a}
RD-2035	11.7^{b}	0.602^{cd}	44.6^{d}	3.2^{a}
RD-2552	12.6^{c}	0.567^{a}	41.2^{a}	3.2^{a}

Means followed by the similar superscript within the column do not differ significantly ($p<0.05$)

Table 2. Proximate composition of de-husked barley flour (DBF)

Cultivars	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	Carbohydrate (%)
BH-393	5.2^{d}	1.57^{b}	4.1^{bc}	13.3^{de}	1.4^{c}	74.5^{a}
BH-932	5.5^{d}	1.38^{a}	4.2^{c}	12.9^{d}	1.3^{b}	74.7^{a}
BH-902	3.3^{bc}	1.68^{c}	3.2^{a}	13.6^{e}	1.4^{c}	76.8^{b}
BH-885	5.3^{d}	1.77^{d}	4.0^{b}	13.1^{de}	1.4^{c}	74.4^{a}
DWR-52	3.7^{c}	1.58^{b}	3.4^{a}	12.8^{d}	1.5^{c}	77.1^{b}
PL-172	2.8^{b}	1.61^{bc}	3.3^{a}	11.6^{c}	1.1^{a}	79.5^{cd}
RD-2035	3.6^{c}	1.71^{cd}	3.9^{b}	10.9^{b}	1.3^{b}	78.6^{c}
RD-2552	2.4^{a}	1.66^{c}	3.2^{a}	9.1^{a}	1.4^{c}	82.2^{d}

Means followed by the similar superscript within the column do not differ significantly ($p<0.05$)

Color characteristics

Color parameters of DBF flour from different barley cultivars were evaluated using hunter color lab (Table 4). The L* value differed significantly ($p<0.05$) among cultivars with flour from cv.BH-902 lighter in color in comparison to others. The redness (a*) ranged from 0.59 to 1.38, the highest and the lowest values were observed for cv.BH-932 and cv.BH-393, respectively. All flours were yellowish in color with cv.BH-885 having the highest b* value (11.52).

3.4. Total phenolic content (TPC)

TPC of different milling fractions of barley cultivars followed the following order: bran>husk>DBF>PBF (Table 5). Butsat and Siriamornpun (2010) reported higher TPC of bran fraction (2.5–2.7 mg GAE/g) as compared milled rice (0.5–0.7 mg GAE/g). For bran, PBF and DBF fractions, the highest TPC was observed for cv.BH-885 whereas cv.PL-172 had the lowest value. Sharma and Gujral (2010a) reported TPC in the range from 3070 to 4439 µg/g, 3574 to 5021 µg/g and 2144 to2362 µg/g for whole flour, bran and refined barley flour. Among cultivars, TPC of husk fraction was found the highest for cv.BH-393. Gamel and Abdel (2012) reported that hull fractions of barley had the highest phenolics than other fractions; outer layers, middle pearlings and endosperm. The husk fraction of Thai rice also showed greater phenolic acids concentrations than the other fractions (Butsat and Siriamornpun, 2010). The husk of the barley grains is the main by-product without any useful purpose, however, our study revealed that husk fraction with higher phenolic compounds can be of immense value.

3.5.Total flavonoids content (TFC)

The antioxidants mechanism of flavonoids, may result from the interactions between flavonoids and metal ions especially iron and copper (Miller et al., 996). TFC in husk, bran, PBF and DBF among cultivars differed significantly ($p<0.05$) (Table 6). TFC of milling fractions followed the order: bran>husk>DBF>PBF. Among cultivars, cv.BH-885 showed the highest whereas cv.RD-2552 had the lowest TFC in bran, PBF and DBF fractions. The range TFC in bran fraction among cultivars was from 2668 to 3431µg CE/g. Sharma and Gujral (2011) reported values ranging from 732 to 1137 µg CE/g for barley flour. The husk fraction of cv.BH-902 had the highest flavonoids content in comparison to others. TFC was present in good amount in husk fraction which showed the potential value of this fraction.

Table 3. Water and oil absorption capacity of different milling fractions of barley cultivars

Barley cultivars	Water absorption capacity (%)				Oil absorption capacity (%)			
	Husk	Bran	PBF	DBF	Husk	Bran	PBF	DBF
BH-393	$365^{d,m}$	$315^{e,l}$	$214^{e,j}$	$224^{de,k}$	$275^{c,m}$	$238^{c,l}$	$159^{e,j}$	$187^{cd,k}$
BH-932	$333^{ab,m}$	$295^{c,l}$	$190^{c,j}$	$210^{c,k}$	$262^{bc,m}$	$224^{bc,l}$	$145^{d,j}$	$176^{c,k}$
BH-902	$327^{a,m}$	$276^{a,l}$	$171^{b,j}$	$197^{b,k}$	$250^{a,m}$	$205^{a,l}$	$159^{e,j}$	$182^{cd,k}$
BH-885	$346^{c,m}$	$306^{d,l}$	$215^{e,j}$	$229^{e,k}$	$261^{b,m}$	$224^{bc,l}$	$150^{de,j}$	$192^{d,k}$
DWR-52	$327^{a,m}$	$284^{b,l}$	$167^{b,j}$	$181^{ab,k}$	$260^{b,m}$	$200^{a,l}$	$139^{c,j}$	$178^{c,k}$
PL-172	$337^{b,m}$	$299^{c,l}$	$192^{c,j}$	$218^{d,k}$	$260^{b,m}$	$220^{b,l}$	$152^{de,j}$	$178^{c,k}$
RD-2035	$338^{b,m}$	$303^{d,l}$	$204^{d,j}$	$221^{de,k}$	$263^{bc,m}$	$221^{b,l}$	$106^{a,j}$	$139^{a,k}$
RD-2552	$325^{a,m}$	$274^{a,l}$	$138^{a,j}$	$172^{a,k}$	$259^{b,m}$	$226^{bc,l}$	$125^{b,j}$	$161^{b,k}$

a-e superscripts are significantly ($p<0.05$) different column wise within different cultivars and j-m superscripts are significantly ($p<0.05$) different row wise within a cultivar.

Table 4. Hunter color properties of de-husked barley flour from different cultivars

Barley cultivars	L*	a*	b*
BH-393	91.2^{c}	0.59^{a}	9.25^{d}
BH-932	89.5^{a}	1.38^{f}	11.35^{g}
BH-902	92.7^{d}	0.63^{ab}	7.75^{a}
BH-885	90.4^{b}	1.13^{e}	11.52^{h}
DWR-52	90.6^{b}	1.03^{d}	10.72^{f}
PL-172	89.2^{a}	1.08^{de}	9.81^{e}
RD-2035	91.2^{c}	0.78^{c}	8.19^{b}
RD-2552	92.5^{d}	0.70^{b}	8.29^{c}

Means followed by the similar superscript within the column do not differ significantly ($p<0.05$)

Table 5. Total phenolic content (TPC) of different milling fractions of barley cultivars

Barley Cultivars	Total phenolic content (µg GAE/g)			
	Husk	Bran	PBF	DBF
BH-393	$4257^{h,l}$	$4311^{d,m}$	$1956^{e,j}$	$3256^{d,k}$
BH-932	$4020^{g,l}$	$4256^{c,m}$	$2012^{f,j}$	$3056^{c,k}$
BH-902	$3822^{d,l}$	$4756^{g,m}$	$1834^{c,j}$	$3761^{f,k}$
BH-885	$3956^{f,l}$	$4911^{h,m}$	$2322^{g,j}$	$3922^{g,k}$
DWR-52	$3089^{a,l}$	$3984^{b,m}$	$1890^{d,j}$	$2922^{b,k}$
PL-172	$3855^{e,l}$	$3807^{a,m}$	$1567^{a,j}$	$2890^{a,k}$
RD-2035	$3456^{b,l}$	$4456^{e,m}$	$1787^{b,j}$	$3422^{d,k}$
RD-2552	$3691^{c,l}$	$4511^{f,m}$	$1860^{d,i}$	$3588^{e,k}$

a-h superscripts are significantly ($p<0.05$) different column wise within different cultivars and j-m superscripts are significantly ($p<0.05$) different row wise within a cultivar.

Table 6. Total flavonoid content (TFC) of different milling fractions of barley cultivars

Barley cultivars	Total flavonoids content (µg CE/g)			
	Husk	Bran	PBF	DBF
BH-393	$2411^{d,l}$	$3342^{g,m}$	$1655^{e,j}$	$2011^{de,k}$
BH-932	$2367^{c,l}$	$3186^{f,m}$	$1683^{f,j}$	$2024^{e,k}$
BH-902	$2489^{f,l}$	$2893^{b,m}$	$1494^{c,j}$	$1988^{cd,k}$
BH-885	$2456^{e,l}$	$3431^{g,m}$	$1783^{g,j}$	$2198^{f,k}$
DWR-52	$2355^{c,l}$	$3037^{e,m}$	$1622^{e,j}$	$2002^{d,k}$
PL-172	$2386^{d,l}$	$2973^{d,m}$	$1529^{d,j}$	$1968^{c,k}$
RD-2035	$2214^{b,l}$	$2914^{c,m}$	$1312^{b,j}$	$1835^{b,k}$
RD-2552	$2202^{a,l}$	$2668^{a,m}$	$1215^{a,j}$	$1736^{a,k}$

a-g superscripts are significantly ($p<0.05$) different column wise within different cultivars and i-l superscripts are significantly ($p<0.05$) different row wise within a cultivar.

Table 7. Antioxidant activity (%) of different milling fractions of barley cultivars

Barley cultivars	Antioxidant activity (%)			
	Husk	Bran	PBF	DBF
BH-393	$33.1^{f,l}$	$41.3^{c,m}$	$12.7^{c,j}$	$21.3^{c,k}$
BH-932	$30.6^{e,l}$	$36.9^{b,m}$	$12.1^{bc,j}$	$20.5^{bc,k}$
BH-902	$28.4^{d,l}$	$45.4^{e,m}$	$15.8^{f,j}$	$24.9^{e,k}$
BH-885	$30.1^{e,l}$	$46.4^{f,m}$	$16.8^{g,j}$	$25.8^{f,k}$
DWR-52	$22.3^{a,l}$	$36.5^{b,m}$	$11.4^{b,j}$	$19.8^{b,k}$
PL-172	$28.7^{d,l}$	$34.5^{a,m}$	$10.1^{a,j}$	$18.3^{a,k}$
RD-2035	$25.3^{b,l}$	$41.3^{c,m}$	$13.8^{d,j}$	$21.6^{c,k}$
RD-2552	$26.1^{c,l}$	$44.4^{d,m}$	$14.5^{e,j}$	$22.9^{d,k}$

a-g superscripts are significantly ($p<0.05$) different column wise within different cultivars and j-m superscripts are significantly ($p<0.05$) different row wise within a cultivar.

3.6. Antioxidant activity (AOA)

The free-radical scavenging activity of the extracts of milling fractions was evaluated using the DPPH, as DPPH is a stable free radical (Table 7). The antioxidant activity (AOA) of milling fractions differed significantly ($p<0.05$). The ability to scavenge DPPH radical by barley fractions was observed to be the highest for bran fraction. The bran fraction had significantly ($p<0.05$) higher AOA in comparison to husk, DBF and PBF. AOA observed for bran, PBF and DBF fractions showed the highest and the lowest for cv.BH-885 and cv.PL-172, respectively. Sharma and Gujral (2010a) reported the highest value of AOA for bran fraction among different fractions (34.2 to 57.6%). They also reported AOA values in the range from 17.01 to 24.92% in whole barley grain. Husk fraction showed good AOA, higher than PBF and DBF. The husk fraction of cv.BH-393 showed the highest AOA. Gamel and Abdel (2012) also observed the highest AOA in hull and outer layers (11.4-31.5 µmole/g and 42.6-64 µmole/g).

4. Conclusions

Barley cultivars differed significantly ($p<0.05$) in their husk content, proximate composition, color characteristics, and antioxidant compounds (TPC, TFC, and AOA content). TPC, TFC and AOA of different milling fractions of barley followed the order: bran>husk>DBF>PBF. Study revealed that husk had significant contents of TPC, TFC and AOA which can be of great economic importance. TPC, TFC, and AOA for bran, PBF, and DBF were the highest for cv.BH-885 and the lowest for cv.PL-172. WAC and OAC of different milling fractions followed the order: PBF>DBF>bran>husk content.

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EFFECT OF TEMPERATURE AND INOCULUM SIZE ON GOAT YOGURT FERMENTED BY *BIFIDOBACTERIUM BIFIDUM* AND *LACTOBACILLUS CASEI*

Guowei Shu[1*], Ni Lei[1], Hongchang Wan[2], He Chen[1], Hong Li[2]

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

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ABSTRACT

In order to obtain the optimum parameters of goat milk fermented by starter cultures containing *B. bifidum* and *L. casei*, the effect of incubation temperature and inoculum size on the fermentation were studied by single factor test. Incubation temperature was 35℃, 37℃, 39℃, 41℃and 43℃, the inoculum size was 1%, 3%, 5%, 7% and 9%, respectively. The results showed that the optimum incubation temperature was 39℃, the viable counts of *B. bifidum*, *L. casei*, the total viable counts, pH, acidity and sensory evaluation in BC-goat yogurt were 1.40×10^7cfu/ml, 1.30×10^7cfu/ml, 1.98×10^9cfu/ml, 4.58, 80.0^0T and 7.36, respectively. The optimum inoculum size was 7%, the viable counts of *B. bifidum*, *L. casei*, the total viable counts, pH, acidity and sensory evaluation in BC-goat yogurt were 8.40×10^7cfu/ml, 8.50×10^7cfu/ml, 2.30×10^9cfu/ml, 4.17, 102.2^0T and 7.13, respectively.

1. Introduction

Yogurt produced from cow milk is widely consumed throughout the world. However, there is a high demand for alternatives to cow milk due to problems associated with allergenicity, gastrointestinal disorders and desire for novel dairy products (Farnworth et al., 2007; Haenlein, 2004). Goat milk is reported to have higher digestibility and lower allergenic potential compared to cow milk (Barrionuevo et al. 2002; Haenlein, 2004; Martin-Diana et al.,2003), as well as a higher content of short chain fatty acids in the milk fat, higher content of zinc, iron and magnesium and antibacterial characteristics (Slacanac et al., 2010). In addition, these benefits might be further enhanced by using goat milk as a vehicle for delivering probiotics.

Probiotics are viable microorganisms that are beneficial to the host when administered in appropriate quantities (FAO/WHO, 2001). Probiotic cultures have been used in foods as an adjunct due to the beneficial effects on human health, such as modulating immunity, lowering cholesterol, improving lactose tolerance and preventing cancer (Sanders, 1999). Lactobacillus and Bifidobacterium are the most common genera of bacteria used as probiotics for the production of dairy products (Mohammadi et al., 2011).

Bifidobacteria are natural habitants of human gastrointestinal tract and can exert

several beneficial effects to the host (Julio et al.,2010). They have been incorporated into a variety of food products, mainly dairy products, such as fermented milk and yogurts (Sanchez, de los Reyes-Gavilan,Margolles, Gueimode, 2009).

L. casei is also a beneficial probiotic for human, it has been suggested that *L.casei* can tolerate defense mechanism of organism, including oral enzymes, low pH in gastric juice and bile acid in small intestine, etc., so when *L. casei* can live in intestine, can rise to adjust balance, promote human digestion and absorption, and so on (Vinderola et al., 2002).

In our previous study, the process of set goat yogurt fermentation was optimized for *S. thermophilus* and *L. bulgaricus* (Chen et al., 2010), the effect of inoculum and temperature on the fermentation of goat yogurt by L. bulgaricus and *S. thermophilus* (Shu et al., 2014) was investigated, the effect of the total inoculum size containing *L. acidophilus* or *L. casei* on the fermentation of goat milk was studied on the basis of *S. thermophilus* and *L. bulgaricus* as starter cultures (Chen et al., 2015). In this study the effect of the total inoculum size containing *L. acidophilus* or *L. casei* applied as adjuncts to the goat milk fermentation with *S. thermophilus* and *L. bulgaricus* was investigated. The aim of this study was to evaluate the influence of incubation temperature and inoculum size on the goat yogurt fermented with *Bifidobactrium bifidum* and *L. casei* as adjuncts for fermentation to which *S. thermophilus* and L. bulgaricus were added as starter cultures, for developing goat yogurt with probiotics (BC-goat yogurt).

2. Materials and methods

Bifidobacterium bifidum (BB), *S. thermophilus*, *L. bulgaricus* and *L. casei*(LC) were obtained from School of Food and Biological Engineering, Shaanxi University of Science and Technology. In order to obtain fresh culture, they were inoculated three successive times with MRS broth(for L. casei and *L. bulgaricus*), MRS broth with 0.5% Cysteine Hydrochloride (for B. *bifidum*) and M17 broth (for *S. thermophilus*) broth. The activated BB or LC was inoculated into treated goat milk at 5% inoculum size, mixed and cultivated at 37°C until coagulation, The activated *S. thermophilus* and *L. bulgaricus* were inoculated into sterilized goat milk at 5% inoculum size with 1:1 ratio, mixed and cultivated in the incubators until coagulation, They can be used for the production of probiotic goat yogurt.

Fermentation process

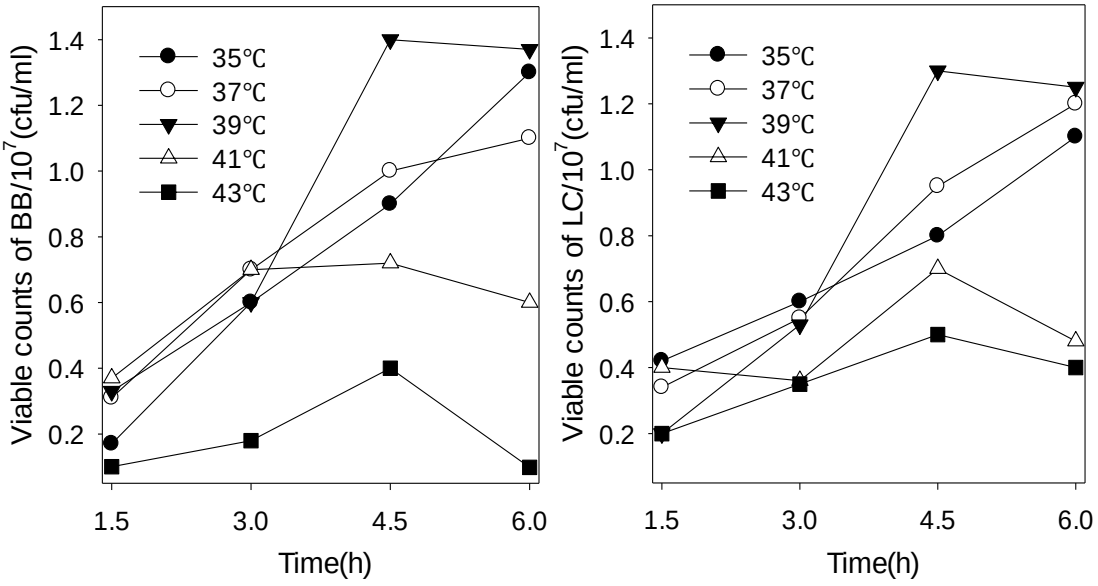
First of all, the fresh goat milk was treated at 95℃ for 10 min and cooled, then goat milk was fermented with different temperature (35℃, 37℃, 39℃, 41℃and 43℃) or inoculated different inoculum size (1%, 3%, 5%, 7% and 9%) with *B. bifidum*, *L. casei* and conventional starter cultures with 2:1:1 ratio, the viable counts of *B. bifidum*, *L. casei*, the total viable counts, pH and acidity were measured at different stage of fermentation, After 12h of cold storage a sensory evaluation was performed.

Analysis method

The viable counts were evaluated by plate counting method. The total viable counts were determinate by modified Tomato Juice medium, determination of *L.casei* by MRS agar containing 0.06% bile salt and determination of *B. bifidum* by MRS agar containing 0.10% LiCl (Chen et al, 2011; Shu et al, 2011). The pH of culture media was determined using a pH-meter（pHS-3c, Shanghai Precision Scientific Instrument Co., Ltd, Shanghai, China) at room temperature. The titrable acidity was conducted using 10g of sample titrated with 0.1mol/l NaOH, using phenolphthalein as an indicator. Five panelists would assessed the samples, tasted and described the texture of the product such as color, state, taste, smell, who were trained on the basis of normal sensory acuity and consistency.

3. Results and discussions

Effect of temperature on the goat milk ferment The effect of temperature on the in ***bifidum***



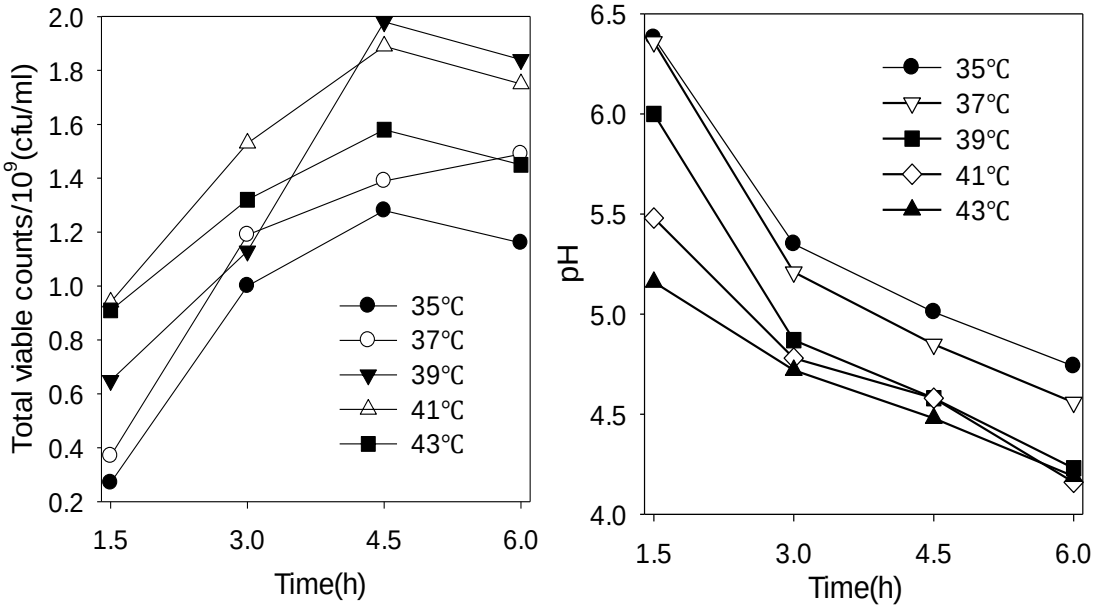


Figure 1 (c)

Figure 1 (d)

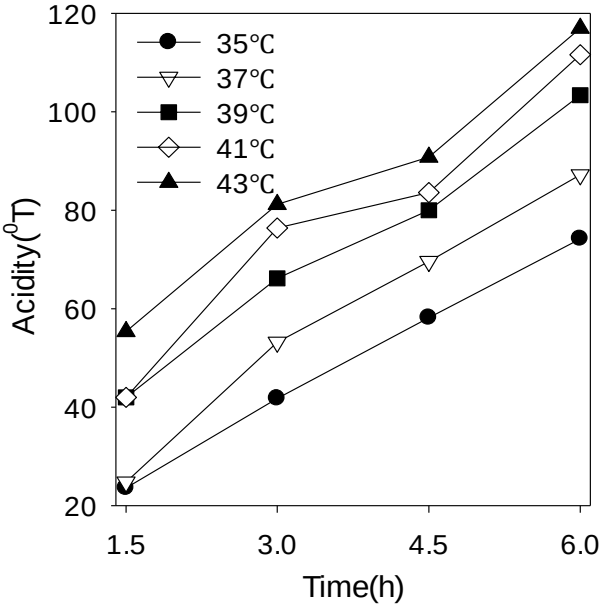


Figure 1 (e)

Figure 1. Effect of temperature on viable counts of *B. bifidum* and *L. casei*, total viable bacteria, pH and acidity in BC-goat yogurt

Figure 1(a) showed the viable counts of *B. bifidum* in BC-goat yogurt increased during the whole fermentation process at 35℃and 37℃, but first increased and then decreased at 39℃, 41℃ and 43℃, Which indicated the temperature had a significant effect on growth of *B. bifidum* in BC-goat yogurt. The viable counts of *B. bifidum* increased fast within 3 h except 43℃, the time of maximum viable counts of *B. bifidum* for 35℃, 37℃, 39℃, 41℃ and 43℃was 6 h, 6 h, 4.5 h, 3.0 h and 4.5 h. when the fermentation temperature was 39℃, the viable counts of *B. bifidum* in goat yogurt reached the maximum, 1.40×107cfu/ml, when the fermentation temperature was 43℃, the viable counts of *B. bifidum* was the lowest, 4.00×106cfu/ml.

Figure 1(b) illustrated changes of viable counts of *L. casei* between 1.5 h and 6 h, the change trend was similar to that of the *B. bifidum*. The time of maximum viable counts of *L.casei* was both 6 h for 35℃ and 37 and 4.5 h for 39℃ 41℃and 43℃, which indicated the temperature had a significant effect on growth of *L. casei* in BC-goat yogurt. The viable counts of *L.casei* had a increased growth trend at 35℃ and 37℃, reached the maximum at 6h, among then, the viable counts of *L. casei* at 39℃ presented the highest counts, 1.30×107cfu/ml, the viable counts of *L. casei* at 43℃ was the lowest, 5.00×106cfu/ml.

Figure1(c) showed the change of the total viable counts of BC-goat yogurt at different temperature. The total viable counts of BC-goat yogurt were all first increased and then decreased during the fermentation process. The time of maximum total viable counts was all 4.5 h under all selected temperatures. Among then, the total viable counts at 39℃ reached the maximum counts, 1.98×109cfu/ml, the total viable counts at 35℃ was the lowest, 1.28×109cfu/ml.

The figure 1(d) and 1(e) showed changes of pH and acidity in BC-goat yogurt, the changes between pH and acidity were in the opposite trend, the pH decreased and the acidity increased during the whole fermentation process under all selected temperatures. When the fermentation temperature was 39℃, the acidity and pH at 4.5h were 80 0T and 4.58, respectively.

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

Temperature (℃)	Color	Smell	Taste	State	CE*
35	0.99	2.09	1.68	2.17	6.92
37	0.99	2.10	1.98	2.16	7.23
39	0.99	2.18	2.01	2.18	7.36
41	0.99	2.21	2.13	2.24	7.57
43	0.99	2.29	2.18	2.34	7.80

*: Comprehensive evaluation

Table 1 showed the evaluation of color, taste, smell, state and comprehensive evaluation of BC-goat yogurt made from different fermentation temperature. Among then, the sour and sweet of goat yogurt had a little pale and had slight goaty flavor at 35℃ and 37℃, the sweet and sour of goat yogurt were moderate and no goaty flavor at 39℃, 41℃ and 43℃, and had a good coagulation texture. From above, the optimum temperature of goat yogurt was 39℃, The coagulation time was 4.5 h, the score of sensory evaluation was 7.36.

Effect of inoculum size on the goat milk fermented by starter cultures containing *B. bifidum* and *L. casei*

The effect of inoculum size on the fermentation of BC-goat yogurt was shown in figure 2 and table 2.

Fig.2 Effect of inoculum size on the viable counts of *B. bifidum* and *L. casei*, total viable bacteria, pH and acidity in BC-goat yogurt

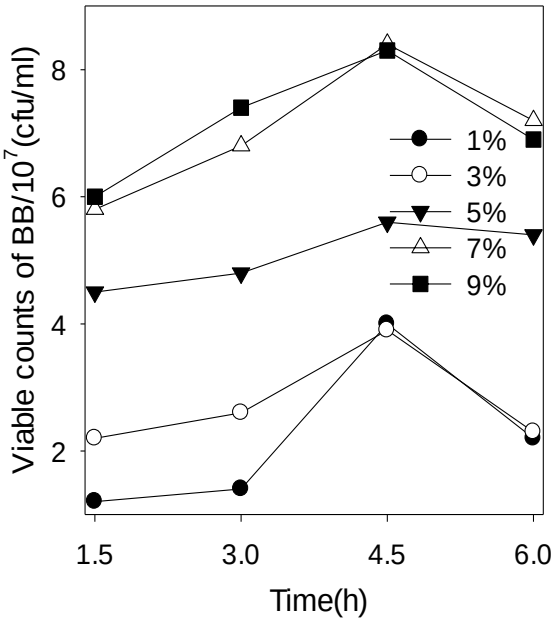


Figure 2 (a)

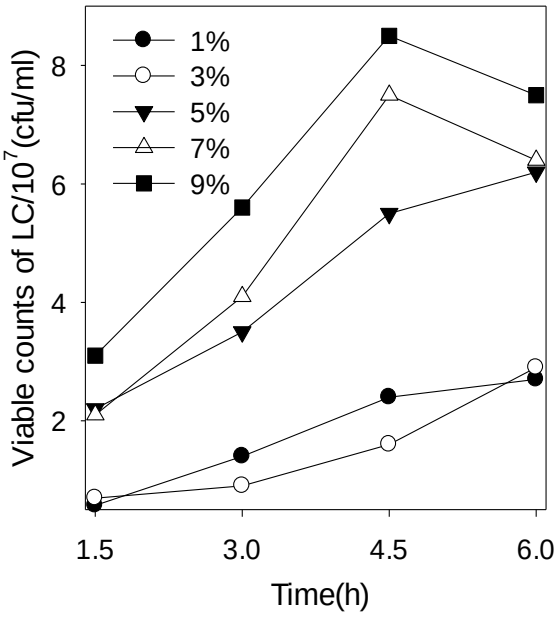


Figure 2 (b)

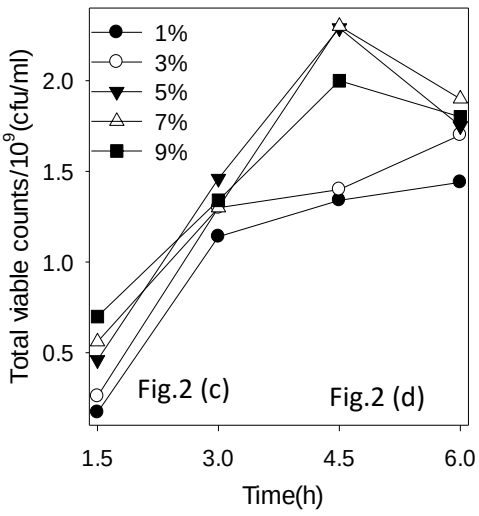


Figure 2 (c)

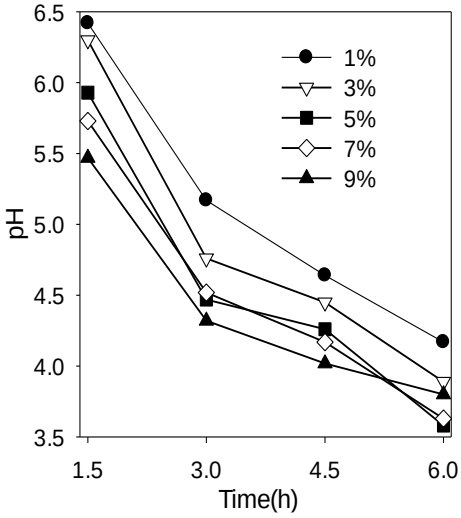


Figure 2 (d)

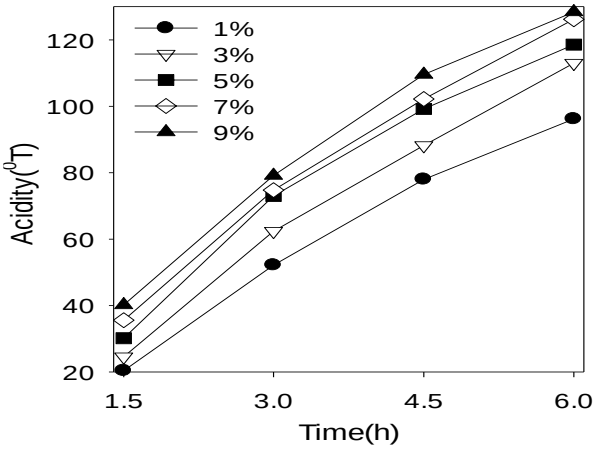


Figure 2 (e)

Figure 2. Effect of inoculum size on the viable counts of B. bifidum and L. casei, total viable bacteria, pH and acidity in BC-goat yogurt

Figure 2 (a) showed the viable counts of *B. bifidum* had a similar change trend at different inoculum size. The viable counts of *B. bifidum* in BC-goat yogurt were all first increased slowly at the initial stage of fermentation, then began to accelerate, reached the peak at 4.5 h, among then, the viable counts of *B. bifidum* in BC-goat yogurt at 7% inoculum size were the highest, 8.4×107cfu/ml. The viable counts of *B. bifidum* in goat milk at 1% inoculum size were the lowest, 3.9×107cfu/ml, which indicated the inoculum size had a significant effect on growth of *B. bifidum* in BC-goat yogurt.

Figure 2 (b) presented changes of the viable counts of *L.casei* in BC-goat yogurt at different inoculum size. The viable counts of *L.casei* in BC-goat yogurt increased during the whole fermentation process at 1% and 3% inoculum size, but first increased and then decreased at 5%, 7% and 9% inoculum size, which indicated the inoculum size had a significant effect on growth of *L.casei* in BC-goat yogurt. The viable counts of *L. casei* at 7% inoculum size reached the maximum, 8.50×107 cfu/ml, the

viable counts of *L.casei* at 1% inoculum size was the lowest, 2.40×107 cfu/ml.

Based on Figure 2(c), it is observed that the total viable counts in BC-goat yogurt at 1% and 3% inoculum size increased fast at the initial stage of fermentation, then increased slowly. The total viable counts at 5%, 7% and 9% inoculum size in BC-goat yogurt increased dramatically within 4.5h, and then began to decrease. Among then, the total viable counts at 5% and 7% inoculum size in BC-goat yogurt were higher, reached 2.29×109cfu/ml and 2.30×109cfu/ml, respectively. The total viable counts at 1% inoculum size in BC-goat yogurt was the lowest, 1.44×109cfu/ml.

The changes between pH and acidity were in the opposite trend from figure2 (d) and 2(e), the pH decreased and the acidity increased during the whole fermentation process at different inoculum size. The more the inoculum size was, the more quickly the pH decreased and the more quickly the acidity increased. Among then, the acidity and pH pf 5%, 7%, 9% inoculum size were 99.20T, 102.20T, 109.60T, 4.26, 4.17 and 4.02 respectively at 4.5h, the acidity and pH of 1% inoculum size were 78.00T, 4.64 for 4.5h, 96.20T, 4.17 at 6h.

Table 2. Sensory evaluation of goat BC-yogurt at different inoculum size

Inoculum size (%)	Color	Smell	Taste	State	CE*
1	0.97	1.78	1.48	2.12	6.34
3	0.97	1.89	1.65	2.20	6.70
5	0.97	1.88	1.70	2.22	6.76
7	0.97	2.15	1.78	2.23	7.13
9	0.97	2.16	1.63	2.24	7.00

*: Comprehensive evaluation

From table2, the inoculum size did not show obvious influence on the color and state of BC-goat yogurt, but had a significant influence on the smell and taste of BC-goat yogurt. Among then, the sour of goat yogurt was lighter and had an obvious goaty flavor in 1% 3% inoculum. The sweet and sour of goat yogurt was moderate in 5%, 7% and 9% inoculum size, however, there was slight goaty flavor at 5% and 9% inoculum size. From above, the optimum inoculum size of goat BC-yogurt was 7%, the time of coagulation was 4.5h, and the score of sensory evaluation was 7.13.

4. Conclusions

The temperature and inoculum size had a significant effect on fermentation of BC-goat yogurt. The optimum temperature for BC-goat yogurt was 39℃,the acidity, pH, the viable counts of *Bifidobactrium, L. casei* and the total viable counts were 80^0T,4.58,1.40×10^7cfu/ml, 1.30×10^7cfu/ml and 1.98×10^9cfu/ml, respectively, the score of sensory evaluation was 7.36.The optimum inoculum size for BC-goat yogurt was 7%, the time of coagulation was 4.5h, the acidity, pH, viable counts of *Bifidobactrium L. casei* and total viable counts were 102.2^0T, 4.17, 8.40×10^7cfu/ml, 8.50×10^7cfu/ml, 2.30×10^9cfu/ml, respectively, the score of sensory evaluation was 7.13.

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CONTENT OF BIOACTIVE SUBSTANCES IN CHERRY FRUIT BY DIFFERENT WAYS OF FREEZING

Elena Vasylyshyna [1*]

[1]Uman National University of Horticulture, Uman city, Ukraine.
[*]elenamila@i.ua

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ABSTRACT

Change of tannins and coloring substances and ascorbic acid in cherry fruit by different ways of freezing in plastic bags and plastic cups and six month storage in pieces in 20% sugar solution and adding 4% askorutin is shown.

During six month storage of frozen cherry fruit in pieces and packed in plastic bags reduction of tannins and coloring substances by 30-39% was recorded while in frozen fruit in sugar syrup and with addition of askorutin in plastic cups – by 27%. In general, the content of tannins and coloring substances in frozen fruit remained at the level of 0.92-1.1%.

For 6 months of storing frozen fruit content of ascorbic acid in them decreased in control by 56% while in previously soaked cherry fruit in sugar syrup and adding 4% askorutin and frozen in cups by 35%.

Correlation dependence is determined between content of tannins, coloring substances and ascorbic acid and the regression equation is given.

Expediency of freezing cherry fruit especially in 20% sugar solution with the addition of 4% askorutin is shown. Benefits of such fruit are in preserving ascorbic acid in 1.5 times, content reduction of tannins and coloring substances by 27% and tasting score in 5 points.

1. Introduction

Cherry fruit are traditional raw materials in Ukraine. Their chemical composition is a successful combination of sugars, acids and attractive color that is associated with a high content of polyphenolic compounds (flavonols, leucoanthocyanins and anthocyanins). Most of these compounds are represented by anthocyanins 700-2500 mg /100g. Qualitative composition of which is specific to fruit. Phenolic compounds, including anthocyanins, have protective functions: in case of damaging tissues their intensive growth begins which products of oxidation condensations form a protective layer.

The value of cherry fruit for a human is determined by the content of biologically active substances, including vitamins. Most of them account for vitamin C (ascorbic acid) 10…50mg/100g and vitamin P (rutin) 90...300 mg/100 g (Dzhafarov, 1985; Gudkovski, 2001; Tretiak et al., 1990). On the content of P-active substances cherry is not inferior to black chokeberry, ashberry, apples and black currants (Guidance on methods of quality and safety control of dietary supplements to food, 2004).

The content of ascorbic acid in cherry fruit depends on the meteorological conditions of the growing season and is a peculiarity of the variety.

Cherry varieties grown in southern Ukraine are characterized by 2 time lower content of ascorbic acid (2-15 mg/100g) than in St. Petersburg (15-30 mg/100g) (Naichenko et al., 1999; Gudkovski, 2001). The value of ascorbic

acid for the human body is quite significant and multifaceted. Very important are expressed protective properties on effects on a body of toxic substances of chemical nature, radionuclides, participation in processes of antioxidant protection (Kangina et al., 1992). In case of oxidation ascorbic acid turns to dehydroascorbic acid and undergoes degradation caused by ultraviolet radiation, presence of iron and copper as catalysts and heat treatment of production. During fruit storage its content gradually decreases. In time-sensitive cherry fruit its loss is more significant (Markh, 1996; Tserevitinov et al., 1949).

As period of storage of cherry fruit is limited by ripening period to 15 days, one way to extend their consumption is freezing. Freezing as a way of preserving retains as much as possible output taste properties of biologically active substances of the cherry. The basic requirement put forward to freezing is to provide conditions under which wild strawberries, blackberries, raspberries, cherries, etc. are not deformed, their integrity is kept, possibility of freezing some fruit is excluded, providing frozen product in pieces that is convenient to pack and process (Guidance on methods of quality and safety control of dietary supplements to food, 2004; Pleshkov, 1987).

Perspective is cryogenic freezing which is carried out in special liquids (liquefied nitrogen, carbon dioxide, freon) by method of irrigation or immersion. In such freezing structure, a high percentage of vitamins and other biologically active substances, taste, aroma and nutritional properties of the product are preserved. It is mostly used for soft berries. However, cryogenic freezing is the most costly, leads to environmental pollution and is not currently widely used (Dzhafarov, 1985; Tarusova et al., 1965).

The quality of frozen berries depends on the state in which production is received. According to this there are following ways: mass freezing in pieces and freezing berries in sugar syrup, including the addition of substances that preserve product quality.

Freezing berries in sugar syrup is carried out in a ratio of 1:2 or 1:3. The more concentrated sugar syrup is the slower process of freezing will be. Instead of water during preparation of syrup you can use juice and add biologically active substances such as askorutin (Gudkovski, 2001; Kangina et al., 1992).

The value of such a drug as askorutin is that its ingredients are ascorbic acid and rutin – biologically active substances that potentiate each other's action as a result of which inhibition of peroxidation processes is achieved thus preserving the high quality of the product. In addition, ascorbic acid and rutin are antioxidants that are recognized as synergists in relation to each other. Synergy is that rutin delays the oxidation of ascorbic acid and increases its antioxidant properties. Ascorbic acid at the same time increases activity of rutin. As a result, the accumulation of peroxides that cause browning of fruit is inhibited (Kangina et al., 1992).

2. Materials and methods

Considering the important biological and therapeutic value of cherry fruit which contain significant amounts of anthocyanins and vitamins, including vitamin C, it has been tasked to identify their content in frozen cherry fruit.

Studies were carried out during 2012-2013 with cherry fruit of variety Shpanka.

Research variants:

1) Frozen cherry fruit in pieces packed in plastic bags (control);

2) Cherry fruit previously sustained in 20% solution of sugar and frozen in pieces packed in plastic bags;

3) Cherry fruit previously sustained in 20% sugar solution with the addition of 4% askorutin and frozen in pieces packed in plastic bags;

4) Frozen cherry fruit in pieces packed in plastic cups with a capacity of 0.25 cm3;

5) Cherry fruit frozen in 20% solution of sugar in plastic cups with a capacity of 0.25 cm3;

6) Frozen cherry fruit in 20% sugar solution with the addition of 4% askorutin in plastic cups with a capacity of 0.25 cm3.

Preparation of cherry fruit included sorting, inspection, washing, removing remaining moisture with filter paper. Duration of holding fruit in sugar syrup and adding askorutin was 30 min. Thus, prepared fruit were frozen in pieces or in sugar syrup with the addition of askorutin in plastic cups at a temperature of –22-24°C. Frozen products were stored at a temperature no higher than –18°C up to 6 months.Quality assessment of cherry fruit was carried out in stages: before and after freezing – for two, four and six months of storage by the following indicators: ascorbic acid – by iodometric method (Naichenko V. et al., 1999), tanning and coloring substances – by Neubauer and Leventhal's method (Naichenko et. al., 1999).

3. Results and discussions

Production quality characterizes the content of tannins and coloring substances. Cherry fruit are particularly valuable for these substances which content is 1.5% (Figure 1).

During the whole period of storing frozen cherry fruit in pieces and packed in plastic bags reduction of tannins and coloring substances by 30-39% was recorded while in frozen fruit in sugar syrup and with addition of askorutin in plastic cups – by 27%. In general, the content of tannins and coloring substances in frozen fruit remained at the level of 0.92-1.1%. Changing content of tannins and coloring substances during storage of frozen cherry fruit is caused by peculiarities of most easily oxidated leucoanthocyanins and catechins.

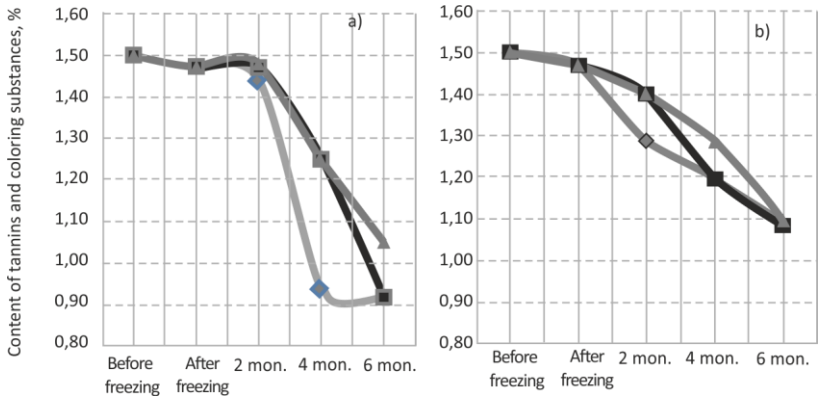

Figure 1. Changing content of tannins and coloring substances in cherry fruit during freezing and storage: a) plastic bags; b) plastic cups:

Metabolism in cherry fruit depends on the enzyme activity. When freezing due to destruction of enzymes balance and coordination of individual reactions, their synchronicity are disturbed, products of incomplete oxidation of energy substrates are accumulated, thus fruit quality deteriorates.

Cherry fruit quality during and after defrosting is determined by the activity of redox enzymes which include catalase. We found that the activity of catalase enzyme in cherry fruit – 2.5mol/min. After six months of storage it decreased in fruit of all variants twofold. This shows the progress of redox reactions in fruit after defrosting production which affects their quality changes.

For 6 months of storing frozen fruit content of ascorbic acid in them decreased in control by 56% while in previously soaked cherry fruit in sugar syrup and adding 4% askorutin and frozen in cups by 35% (Fig. 2).

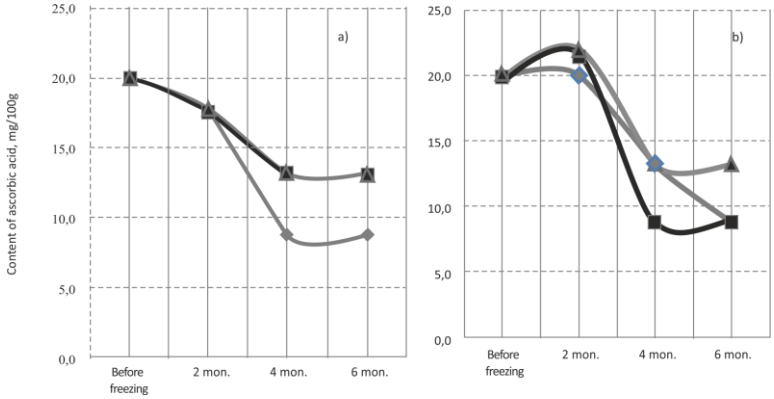

Figure 2. Changing content of ascorbic acid in cherry fruit during freezing and storage: a) plastic bags; b) plastic cups:

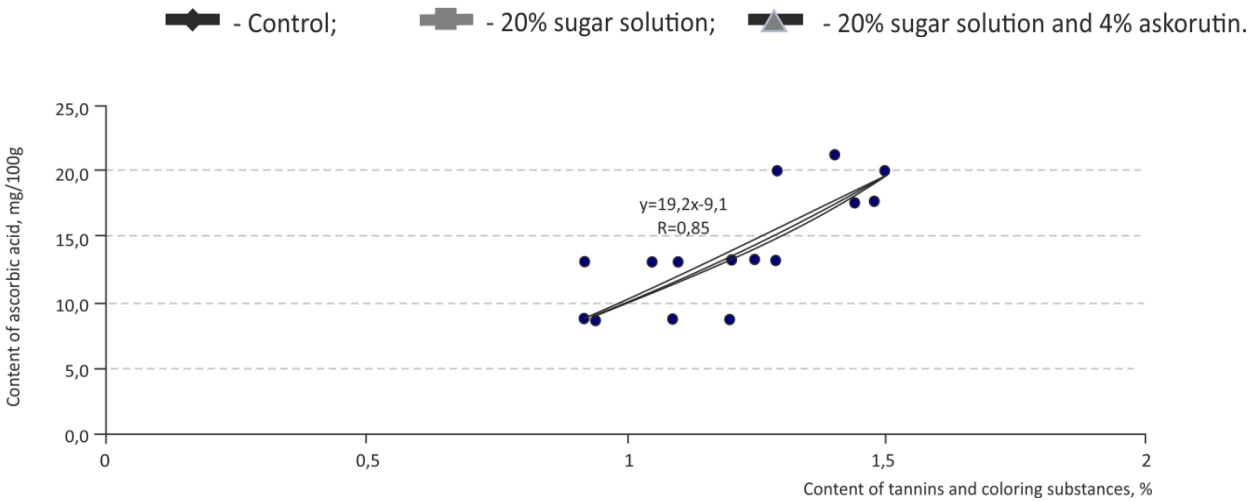

Figure 3. Point schedule and theoretical line of regression of straight dependence between content of tannins and coloring substances and ascorbic acid

Figure 3 shows a graph of depending content of tannins and coloring substances on the content of ascorbic acid and the regression equation is given.

As the correlation coefficient between these indicators − r=0.85 is average you can make the regression equation for correlation dependence y=19.2x−9.1 in which depending on the content of tannins and coloring agents you can determine the content of ascorbic acid.

Tasting assessment showed that frozen cherry fruit were estimated at 4.2-4.3 points. By color and visual aspect cherry fruit frozen in 20% sugar solution and with addition of askorutin received 5 points.

4. Conclusions

Experiments showed the expediency of freezing cherry fruit especially in 20% sugar solution with the addition of 4% askorutin. Benefits of such fruit are in preserving ascorbic acid in 1.5 times, content reduction of tannins

and coloring substances by 27% and testing score in 5 points.

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STUDY BY THERMAL METHODS OF PINHÃO STARCH MODIFIED WITH LACTIC ACID.

Layse do Prado Cordoba[1], Camila Delinski Bet[1], Egon Schnitzler[1]*

*[1]State University of Ponta Grossa - Av. Carlos Cavalcanti, 4748. ZIP 84030-900 – Ponta Grossa, Paraná, Brazil; * egons@uepg.br*

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ABSTRACT

The pinhão is a seed considered a potential source of starch with a starch content of 68-72%. In this work, the pinhão starch was modified with lactic acid at different concentrations (0.1 and 0.2 mol L-1) and its characterisation was performed by thermal analysis (TG-DTA and DSC), rheological behaviour (RVA) and structural analysis (NC-AFM). By TG-DTA was observed three characteristic mass losses of starchy samples, the modification with lactic acid resulted in a higher thermal stability before the thermal degradation. The DSC analysis showed a increase the gelatinisation temperature range of starches and a large variation of gelatinisation enthalpy involved in this process. The curves RVA showed a decrease in the viscosity of the modified samples as well as the tendency to retrogradation. The structural analysis showed an increase in the roughness of the granules for the treated starch with a higher concentration of lactic acid.

1. Introduction

The *Araucaria angustifolia*, is distributed in southern Brazil (states of Paraná, Santa Catarina and Rio Grande do Sul), Chile, Argentina and Paraguay (Silva et al., 2014). It seed, known as pinhão, is considered a potential source of starch (Cordenunsi et al., 2004). The pinhão has 68-72% starch, which makes it viable to develop new technologies and their industrial applications, preventing illegal logging of the *Araucaria angustifolia* (Daudt et al., 2014; Pinto et al., 2015).

Starch is a storage carbohydrate found in plants such as cereal grains (40-90%), vegetables (30-50%), tubers (65-85%) and immature fruits (40-70%). The starch granules are composed of two glucose polymers known as amylose and amylopectin. The amylose is composed primarily of linear chains of α-D-glycosyl units linked by glycosidic bonds α(1,4), while amylopectin is a highly branched polymer with linear chains of α(1,4) D-glycosyl and branch points α(1,6) (Thys et al., 2010).

The native starch form has technological limitations. Therefore, it has been genetically, chemically, physically or enzymatically modified in order to create or improve their properties (Hoover, 2010; Klein et al., 2013; Zhu, 2015). The lactic acid has been studied as a modifying agent which promotes cross-linking and confer new properties to starches, such as decreased intrinsic viscosity and the degree of crystallinity and increase the stability of starch against acidic conditions (Majzoobi and Beparva, 2014). It is used as viscosifiers and texturizers in soups, sauces, breads and dairy products (Singh et al., 2007).

The thermal decomposition, thermal stability, gelatinisation and retrogradation of starch can be studied by thermal methods of analysis, like: Thermogravimetry (TG) that

studies the behaviour when starch is heated to their degradation and differential scanning calorimetry (DSC) which is useful for studying the phenomenon of gelatinisation (Aggarwal and Dollimore, 1996).

In this study, the pinhão starch was modified with lactic acid at different concentrations (0.1 and 0.2 mol L^{-1}) and its characterisation was performed by thermal analysis (TG-DTA and DSC), rheological behaviour (RVA) and structural analysis (NC-AFM).

2. Materials and methods

The starch was isolated according to the method described by Ribeiro et al. (2014). The pinhão starch was obtained from healthy seeds, peeled and dried. The seeds were crushed and it was made a suspension with the addition of distilled water which was kept under constant stirring. After this step, the sample was passed through sieves (180 mesh) and after a rest period, the dispersion was centrifuged (9.500rpm for 5 min). It was discarded a thin surface layer, and the layer of starch was recovered and dried in an oven at 40ºC for 24h. The sample was maintained in a desiccator over anhydrous calcium chloride until constant mass.

The native starch was modified with lactic acid (0.1 and 0.2mol L^{-1}) (in the proportion of 10g of starch and 30mL acid solution) for 30 minutes at temperature of 25ºC in the presence of magnetic stirring.

The DSC analysis were made with DSC-Q200 (TA-Instruments, EUA) in the following conditions: air flow of 50 mL min^{-1}, heating rate of 10ºC min^{-1} and a suspension about 2.5mg of samples in 10µL of water in the aluminum crucibles sealed. The mixtures in the 1:4 ratio (starch:water w/w) were prepared and

maintained in order to equilibrate the moisture content for 60 minutes. The instrument was previously calibrated with Indium 99.99% purity, melting point with *m.p.* =156.6 ºC, ΔH = 28.56J g^{-1} (Ribeiro et al., 2014).

The thermogravimetric curves (TG) were obtained by TGA-50 (Shimadzu, Japan). The instrument was preliminarily calibrated with standard mass and with standard calcium oxalate monohydrate. The analysis was made in a heating from 30ºC to 600ºC, with approximately 5.0mg of each sample in open alumina crucibles under a synthetic air flow of 150mL min^{-1} and at a heating rate of 10ºC min^{-1} (Beninca et al., 2013; Adamovicz et al., 2015).

Using a rapid visco-analyser RVA-4 (Newport Sci., Australia) was obtained the pasting properties of the samples. A solution of 8% starch (about 2.24g) was prepared and after it was added distilled water until 28g. The prepared solution was subjected to a controlled heating and cooling cycle, where the sample was held at 50°C for two minutes, heated to 95°C at 6°C min^{-1}, kept at 95°C for 5 minutes, was cooled to 50°C at 6°C min^{-1} and maintained at 50°C for 2 minutes (Beninca et al., 2013; Costa et al., 2013).

An atomic force microscope SPM - 9600 (Shimadzu, Japan), the non-contact method (NC-AFM) was used to obtain the microimages with high resolution. It was calculated the average diameter (µm) and average roughness (nm) the samples (Ribeiro et al., 2014; Costa et al., 2013).

All analyzes were performed in triplicate. It was ascertained by Tukey's test to a 5% level of significance using analysis of variance (ANOVA) to comparison the averages of samples using SASM-Agri 8.2 Software (Brazil).

3. Results and discussions

From the DSC curves observes a broadening in the temperature range that occurs the gelatinization process. There was a shift to the right of the thermal event indicating an increase in the peak and conclusion temperature after modification (Figure 1).

The modified with lactic acid causes the hydrolysis of amylose and amylopectin, promoting the formation of cross-links which

can increase the starch resistance against the gelatinisation and this may explain the higher temperatures involved in this process (Hirashima et al., 2005).

As seen in Table 1, there were no major changes in the parameters T_o, T_p e T_c, behaviour similar that found for the wheat starch modified with lactic acid (Majzoobi and Beparva, 2014), after the modifying with lactic acid. Since the gelatinisation enthalpy involved in the process did not follow a standard, increasing when modified with lactic acid (0.1mol L^{-1}) and lowering when modified with lactic acid (0.2mol L^{-1}) relative to the native sample.

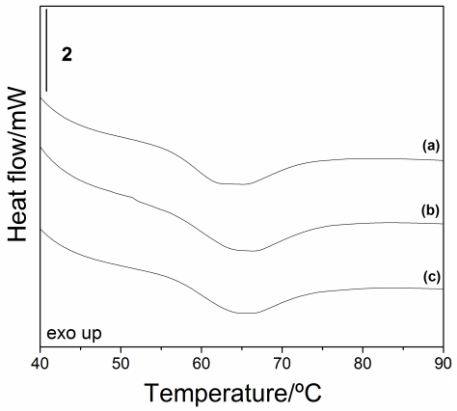

Figure 1. DSC gelatinization curves of: (a) untreated pinhão starch, untreated pinhão starch with: (b) lactic acid 0.l mol L^{-1} and (c) lactic acid 0.2 mol L^{-1}.

Table 1. TG and DTA results of: (a) untreated pinhão starch, untreated pinhão starch with: (b) lactic acid 0.l mol L^{-1} and (c) lactic acid 0.2 mol L^{-1}.

Sample	TG Results		DTA Results	
	Step	*Δm*/%	*ΔT*/ºC	*Tp*/°C
(a)	1st	11.22	30-150	85 (endo)
	stability	-	150-270	-
	2nd	75.63	270-463	305 (endo), 330 (exo), 345 (endo)
	3rd	11.21	463-570	523 (exo)
(b)	1st	13.01	30-145	90 (endo)
	stability	-	145-256	-
	2nd	68.39	256-405	297 (endo), 325 (endo), 382 (exo)
	3rd	16.28	405-580	520 (exo)
(c)	1st	12.34	30-152	93 (endo)
	stability	-	152-254	-
	2nd	72.19	254-448	296 (endo), 380 (exo)
	3rd	14.42	448-580	522 (exo)

(*) *Δm* mass loss (%), *ΔT* temperature range, T_p peak temperature

The TG-DTA curves show three characteristics starch loss (Figure 2), wherein the first referring to dehydration, which occurs at similar temperatures for the three samples (30-152ºC), and the decomposition and oxidation, which correspond to second and third mass losses occurring in consecutive steps. The ash content of the samples were: (a) 1.91%, (b) 2.32% and (c) 1.05%. The modified samples (b and c) had a decreased in stability period by lactic acid action, in comparison with the native pinhão starch sample (a), which suggests that the modified starch had a decrease in their stability before of the thermal degradation. This result was different than that found for the pinhão starch treated with glucose and fructose (Ribeiro et al., 2014), which presented greater stability after modification with sugars.

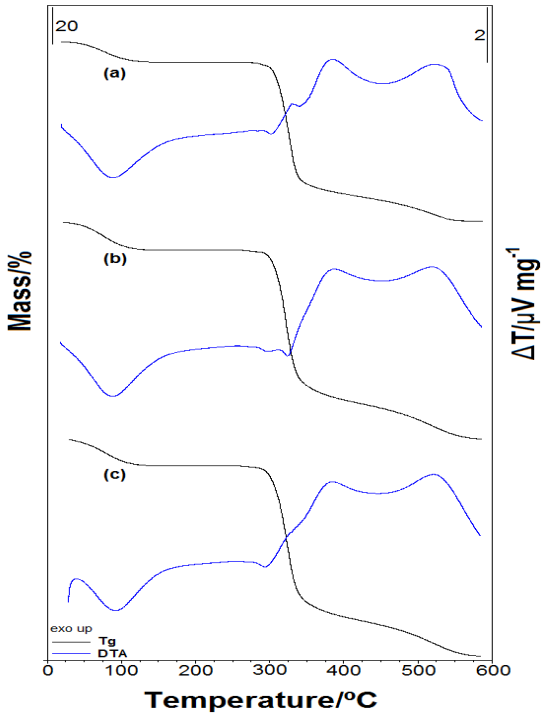

Figure 2. TG and DTG of: (a) untreated pinhão starch, untreated pinhão starch with: (b) lactic acid 0.l mol L^{-1} and (c) lactic acid 0.2 mol L^{-1}.

It was identified by DTA, an endothermic peak for all samples which refers to dehydration of the sample. During the second loss, the event is initially endothermic (except for the sample (a) who presented a shoulder endothermic), and after an exothermic peak occurs, when approaching the third loss which was predominantly exothermic due to the expulsion of energy by organic matter combustion. The cassava starch treated with HCl showed a similar behaviour, with endothermic event for the first and second loss and exothermic event for the third one (Beninca et al., 2013).

The RVA curves show a decrease in peak viscosity and final viscosity of the modified samples as well as a lower setback (tendency to retrogradation) (Figure 3).

The peak time and pasting temperature of the modified samples (b and c) were statistically similar by the Tukey test at 5% significance, differing to the native sample (a).

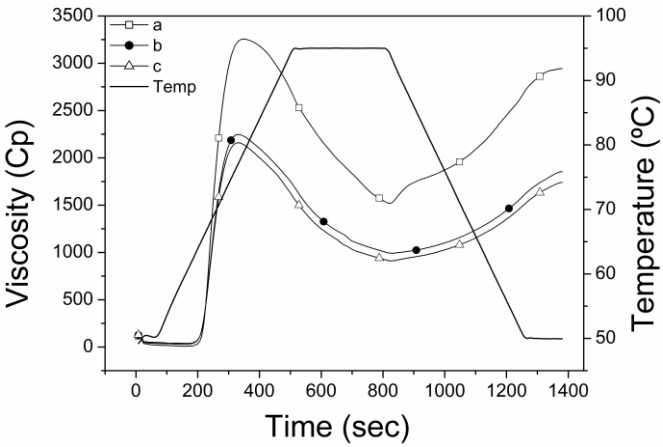

Figure 3. RVA curves of: (a) untreated pinhão starch, untreated pinhão starch with: (b) lactic acid 0.l mol L^{-1} and (c) lactic acid 0.2 mol L^{-1}.

The pasting temperature found (65.55ºC to 65.98ºC), is higher than that found by Pinto et al. (2015) of 60.7ºC and by Klein et al. (2013) of 64.6ºC. However, according to Costa et al. (2013) the values found change according to the botanical species of the pinhão used to extract starch.

Table 2. DSC gelatinization and NC-AFM results of: (a) untreated pinhão starch, untreated pinhão starch with: (b) lactic acid 0.l mol L^{-1} and (c) lactic acid 0.2 mol L^{-1}.

Sample	DSC gelatinisation				AFM	
	To/ºC	*Tp*/ºC	*Tc*/ºC	ΔH_{gel}/J g^{-1}	d_a/μm	r_a/nm
(a)	55.90±0.12^b	62.48±0.03^c	65.97±0.01^c	15.83±1.07^b	12.36±1.16^b	472.30
(b)	54.09±0.25^c	64.37±0.08^b	67.02±0.02^a	19.88±1.65^a	13.59±1.88^b	444.32
(c)	55.73±0.09^a	64.48±0.02^a	66.86±0.01^b	13.99±0.38^b	21.98±1.54^a	482.60

(*) T_o "onset" initial temperature, *Tp* peak temperature, T_c "endset" conclusion temperature, ΔH_{gel} gelatinisation enthalpy, (d_a) Average diameter, (r_a) Average roughness. The degree of crystallinity was calculated as a percentage, peaks are determined in 2θ. Values followed by the same letter are not significantly different ($p<0.05$).

Table 3. RVA results of: (a) untreated pinhão starch, untreated pinhão starch with: (b) lactic acid 0.l mol L^{-1} and (c) lactic acid 0.2 mol L^{-1}.

Sample	Pasting temperature/°C	Viscosity peak/cP	Peak time/sec	Setback/cP	Breakdown/cP	Final viscosity/cP
(a)	65.98±0.04^a	3258.50±0.71^a	344.05±0.07^a	1747.25±0.35^a	1432.40±0.57^a	2943.00±1.41^a
(b)	65.70±0.07^b	2252.50±3.54^b	331.00±1.41^b	865.50±0.71^b	1259.75±0.35^b	1854.80±0.28^b
(c)	65.55±0.01^b	2163.00±0.14^c	332.00±0.01^b	833.50±0.71^c	1254.05±0.07^c	1742.30±0.42^c

(*) cP "centipoises", sec "seconds". Values followed by the same letter are not significantly different ($p<0.05$).

By NC-AFM the data were obtained and are presented in Table 1. The granules showed an increase in their size after modification with lactic acid. This suggests that after the starch modification, new connections are established enabling the formation of cross-links which reinforces the hydrogen bonds in the starch granule, allowing greater absorption of water without breaking the bead, providing greater resistance to mechanical agitation and heat (Chatakanonda et al., 2000).

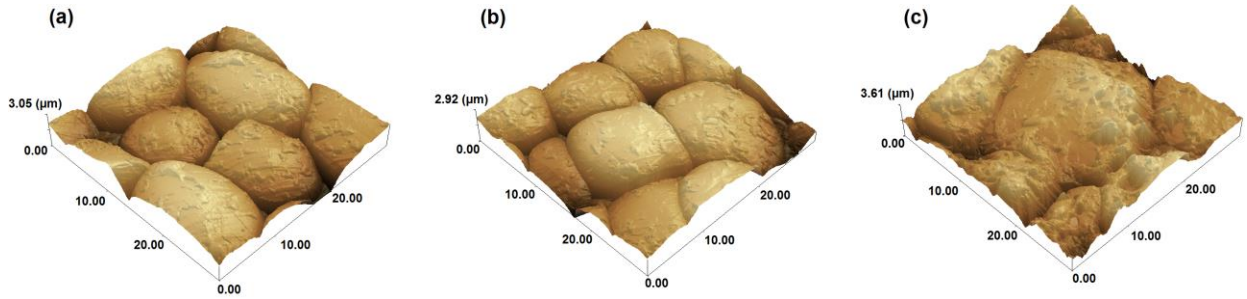

Figure 4. NC-AFM micro-images of: (a) untreated pinhão starch, untreated pinhão starch with: (b) lactic acid 0.l mol L^{-1} and (c) lactic acid 0.2 mol L^{-1}.

The roughness found in the granules was greater for higher concentrations of acid used. The sample (c) had the greatest roughness among samples as well as in other studies where the modified starch had an increased on the surface roughness: pinhão starch treated with glucose (Ribeiro et al., 2014), cassava starch treated with HCl 0.15mol L^{-1} at 50ºC (Beninca et al., 2013) and avocado starch modified with heat-moisture treatment (Lacerda et al., 2015).

By means of Figure 4, it was possible to calculate the average diameter of the samples. The average diameter of untreated pinhão starch (12 µm) was similar to the diameter of avocado starch oxidized with standard NaClO 0.5 % (Lacerda et al., 2015) and one of the samples of untreated pinhão starch studied by other authors (Costa et al., 2013).

4. Conclusions

The native pinhão starch showed a higher thermal stability before of its thermal decomposition, lower peak temperature in the gelatinisation process and lower granule size. With the modification is evident the formation of cross-linked allowing a higher heat stability before the thermal decomposition, resistance to stirring, lower values of peak and final viscosity, as well as a lower setback (tendency to retrogradation), favouring the use of modified starch with lactic acid in sauce, soups and bread industries.

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STUDIES ON SHELF LIFE ENHANCEMENT OF *CHHANA JALEBI* – A TRADITIONAL SWEET

Geetha Panneerselvam[1*], Arivazhagan Ramanathan[2], Nithyapriya[3]

[1]College of Food Science and Dairy Technology, Tamilnadu Veterinary and Animal Sciences University, Chennai, Tamilnadu, India
[2]School of Management, SRM University, Chennai, Tamilnadu, India
[3]Kumaraguru College of Technology, Coimbatore, Tamilnadu, India
[*]geetha.princy@gmail.com

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ABSTRACT

Chhana jalebi is a traditional Indian milk product prepared by frying coils of batter made of chhana (acid coagulum of milk), starch and water, and soaking the fried coils in sugar syrup. The final product has a pleasant sweet taste and chewy body and texture. However, shelf life of chhana jalebi is low owing to several factors which affects its marketability. Enhancement of shelf life will give boost its market potential. Two methods were adopted for enhancing the shelf life of chhana jalebi viz. use of potassium sorbate (a class II permitted preservative) and modified atmosphere of nitrogen and carbon dioxide. Potassium sorbate was dissolved in soaking syrup at 800 pm concentration before the fried jalebi pieces were soaked in it. Modified atmospheres used were: 100% N_2, 100% CO_2 and a mixture of these gases (1:1) within metalized polyester packages. The color and appearance, flavor, body and texture and overall acceptability scores of the jalebi samples during storage at 28°C containing preservatives were recorded on 9-point hedonic scale and the scores were analyzed by ANOVA. It was observed from the results that as storage progressed the overall acceptability score decreased irrespective of the presence of preservative used or gases tried. However, potassium sorbate and modified atmospheres inside the package controlled the growth of microbes to increase the shelf life of the product up to 20-40 days at 28°C.

1.Introduction

Indigenous milk sweets in India have played a vital role in the social, economic, religious and nutritional aspects since ancient period. Government of India lays emphasis on promoting indigenous technologies and encourages patenting and protection of these valuable technologies. *Chhanajalebi* making is one such technology on which no scientific and industrial works have been carried out so far. *Chhana jalebi* is like pretzel shaped sweet similar to those made in Iran and other countries. The key ingredients for preparing *chhana jalebi* are *chhana*(coagulum obtained by coagulation of hot milk by adding citric acid), *maida*(refined wheat flour), *suji* (coarse flour made from rice/wheat), corn flour, sugar and water. *Chhana* is mixed with the fermented mass of *maida*, *suji* and corn flour in proper composition to form the batter possessing semi solid consistency. About 100 g

of the batter is collected in a thickly woven cloth with an aperture at the centre, and extruded into hot oil with swift coiling action. The batter coils are thus deep fried till golden brown colour is attained, taken out of oil, soaked in hot sugar syrup for certain period of time, taken out of the syrup and served fresh. The fresh *jalebi* pieces are often kept in trays in the shelves of sweetmeat shops for sale. The major factors affecting the quality of the product are quality of *chhana*, type and quantity of *maida*, *suji* and corn flour as binding agent, frying time and temperature, sugar syrup concentration, temperature and time of soaking and storage conditions. The *jalebi* is being prepared by sheer culinary skills of homemakers and *halwais* since long periods of time in the northern parts of India. In the absence of any standards either with regard to physico-chemical quality or hygiene, the products like *chhana jalebi* have revealed alarmingly high incidence of microbial contamination besides large variations in chemical composition, flavour and texture. The shelf-life of the product without any packaging is about 2 days under ambient conditions which is limited by yeast and mold spoilage. This shelf life is not enough for large scale manufacture and marketing since the product is eaten at ambient temperatures and not refrigerated. Hence, in the present study, attempts were made to enhance the shelf life of *chhana jalebi* at ambient temperature by using the following techniques: (i) appropriate packaging material (ii) permitted preservative i.e. potassium sorbate and (iii) gas flushing. Such techniques were also successfully employed for preservation of other Indian sweets such as *burfi*(Palit et al., 2005), *kunda*(Mahalingaiah et al., 2012) and *khoai jalebi*(Bharat et al., 2012).

2. Materials and methods

2.1.Preparation of chhana*jalebi*

Fresh cow milk procured from Institute Dairy Farm and standardized to desired level of fat and SNF was heated to 90oC and cooled to about 80°C. Citric acid (SD Fine Chemicals, Mumbai) solution (2% concentration) was slowly added to the hot milk till coagulation was complete indicated by separation of a clear, green tinged whey. The mixture was filtered through muslin cloth and the coagulum obtained in muslin cloth was called chhana. The required quantities of maida and corn flour were separately mixed with water and left for some time for hydration. *Chhana* was then mixed with this hydrated mixture and kneaded thoroughly to a dough consistency. The batter so obtained was filled in a flexible plastic bottle and fitted with a cap having narrow mouth. The bottle was inverted and pressed to extrude the batter out of the bottle with a swift coiling actions into hot vegetable oil (Sunpure Brand). The *jalebi* coils were deep fried in hot oil for 1-2 min at 140° to 170°C and finally soaked in sugar syrup (68° Brix) for about 2 min (Geetha et al., 2005).The flow chart is shown in Fig 1.

2.2.Use of potassium sorbate

Potassium sorbate(LobaChemie Brand)was used at 800 ppm concentration in sugar syrup. This corresponded to the 0.8 gm of potassium sorbate per kg of sugar syrup. The sugar syrup was boiled and cooled before use. Then the preservative was dissolved in the warm syrup.

2.3. Storage study

The *chhana jalebi* (20 gm in each packet) samples prepared by soaking in sugar syrup with and without preservative were packaged separately as follows: 1) LDPE, polystyrene cups with aluminium foil lid, metalized polyester, and butterpaper lined cardboard box 2)*Chhana jalebi* prepared without preservative was packed in metalised polyester packages and flushed with nitrogen, carbon dioxide and mixture of these gases and also packaged under vacuum using digital MAP machine.The packaging materials used can be a source of microbial contamination, hence were sterilized by exposing to UV light for 45 min before being used for packaging *chhana jalebi*.

All the packages containing the product were stored in incubator maintained at $28^{o}\pm2^{o}$C. Every alternate day, the *jalebi* samples were drawn from incubator and subjected to the following analyses: sensory characteristics (colour and

appearance, flavor, body and texture and overall acceptance), physical characteristics (pH and water activity), chemical characteristics (tyrosine value) and microbial characteristics (SPC and YMC).

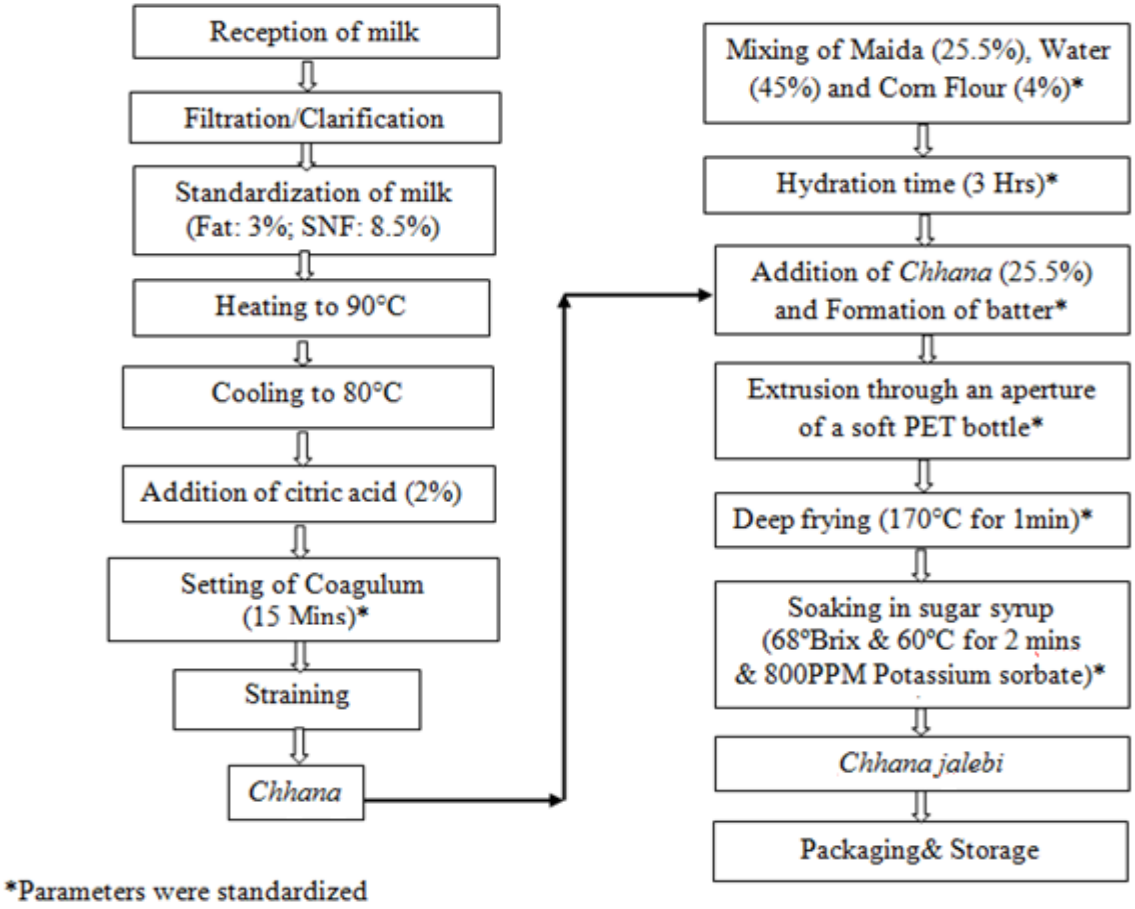

Figure 1. Flow chart for *chhana jalebi* preparation

2.4. Analytical methods

At every storage interval, the *jalebi* samples drawn from incubator were tempered to ambient temperatures and served to a panel of judges for evaluating the sensory acceptance level. The acceptance was scored on a 9- Hedonic scale (Amerine et al., 1965). The proteolytic changes in the product were monitored by estimating tyrosine value (mg/100g). The tyrosine value of *chhana jalebi* was determined by the method as described by Hull method and reported by (Navajeevan et al., 2005). The value of pH was determined using a digital pH-meter (Systronic Co., Bangalore). Water activity of the *jalebi* was determined using water activity meter (Rotronic GmbH, Switzwerland). SPC and YMC of the *jalebi* samples were estimated by standard methods of BIS (1981).

The data was analyzed by ANOVA to deduce the significant effect of the packaging material / preservative/modified atmosphere on storage changes in the *jalebi* using MS-Excel stastical package of 2007 version.

3. Results and discussions

3.1. Effect of different packaging materials on changes in sensory attributes of *chhana jalebi* during storage

The *jalebi* samples were initially very much acceptable with scores of 8.91, 8.91, 8.90 and 8.93, respectively for colour and appearance (CA), flavor, body and texture (BT) and overall acceptance (OA) (Table 1). These gradually decreased during storage irrespective of

packaging material used. The packet was cut open and the visual appearance recorded. It was observed that the original light brown colour was retained throughout the storage period, however CA scores decreased mainly because of visible mold growth after 4 days of storage. The scores at the end of 4th day were 7.21, 6.76, 7.23 and 6.43, respectively for LDPE, PS, MP and cardboard box (Table 1).

Table 1. Changes in sensory score of *chhana jalebi* samples packed in various packaging materials stored at 28±2°C

Attribute	Storage Day	Packaging material				Statistical means
		LDPE	PS	MP	Cardboard	
Colour and appearance	0	8.91	8.94	8.95	8.93	8.93^{d}
	2	8.21	7.47	8.31	7.45	7.86^{c}
	4	7.21	6.76	7.23	6.43	6.90^{b}
	6	4.33	3.33	4.54	3.54	3.93^{a}
Statistical means		7.17^{b}	7.26^{b}	6.63^{a}	6.59^{a}	-
Flavour	0	8.91	8.95	8.95	8.95	8.94^{d}
	2	8.32	7.43	8.32	7.41	7.87^{c}
	4	6.63	6.74	7.21	6.41	6.75^{b}
	6	4.32	3.62	3.22	3.46	3.66^{a}
Statistical means		7.05^{NS}	6.93^{NS}	6.69^{NS}	6.56^{NS}	-
Body and texture	0	8.90	8.93	8.80	8.95	8.90^{d}
	2	8.22	7.50	8.10	7.46	7.82^{c}
	4	6.59	6.58	7.01	6.43	6.61^{b}
	6	5.21	3.32	3.21	3.45	3.80^{a}
Statistical means		7.19^{NS}	6.78^{NS}	6.58^{NS}	6.57^{NS}	-
Overall acceptance	0	8.93	8.95	8.96	8.96	8.95^{d}
	2	8.31	7.50	8.45	7.45	7.9^{3c}
	4	7.00	6.75	7.21	6.45	6.85^{b}
	6	5.32	3.33	5.75	3.50	4.47^{a}
Statistical means		7.39^{b}	7.59^{ab}	6.63^{a}	6.59^{a}	-

Note: LDPE – Low density polyethylene; PS – Polystyrene; MP – Metalised polyester

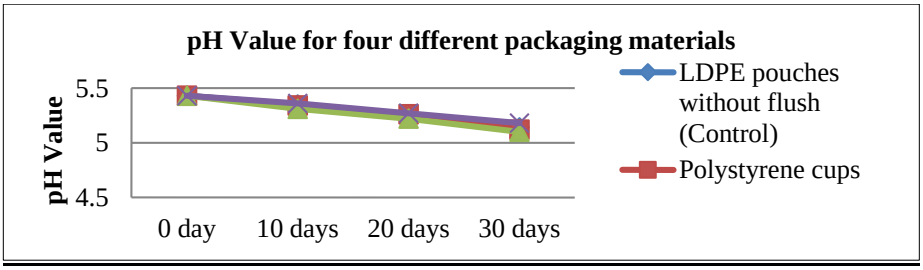

Figure 2.(a)

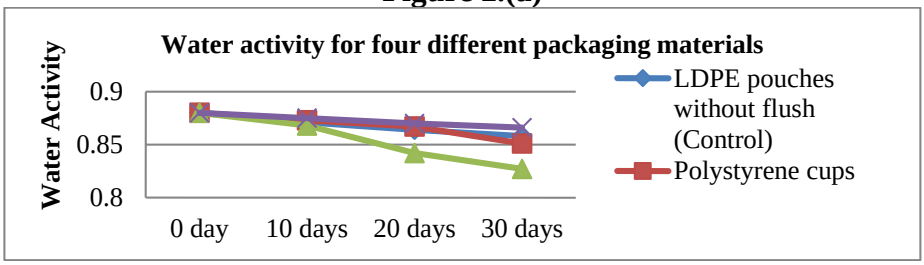

Figure 2.(b)

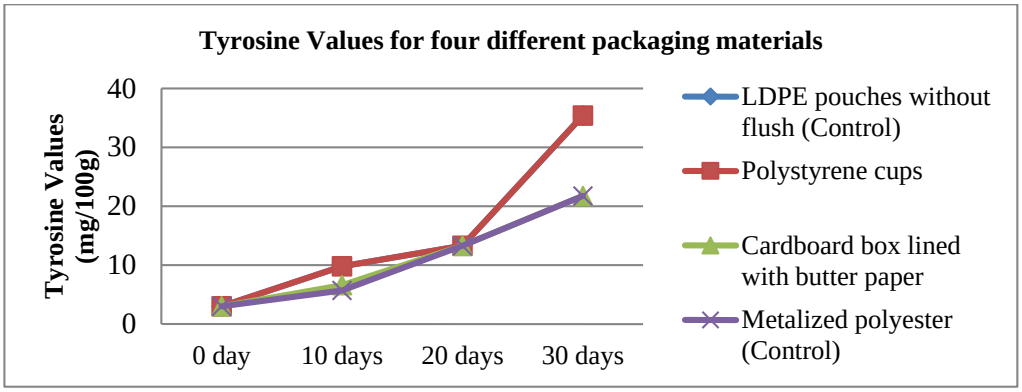

Figure 2.(c)

Figure 2. Effect of packaging materials on physic-chemical changes in*chhana jalebi* (treated with 800 ppm potassium sorbate) during storage at 28±20°C (a) pH (b) water activity and (c) tyrosine value

As the storage period progressed, the product appeared dry probably because of moisture evaporation through the packaging material itself as all the materials with the exception of MP are low barriers, but in case of MP which being a good barrier (Hirsch et al., 1991), evaporation might have occurred within the package; there was sufficient air space inside the package. With regard to the flavor characteristics, the *jalebi* possessed a pleasant sweet, nutty, flavor. This was maintained up to 4 days as indicated by acceptable scores (6.41 to 7.21), but thereafter the product developed off flavor, attributable to microbial growth, which also influenced BT characteristics. During storage the BT became firm, but turned slimy later because of mold growth. The overall changes are indicated by the changes in OA score. These scores remained within the acceptable range up to 4 days of storage (scores 7.00, 6.75, 7.21 and 6.45 respectively for the four packaging materials used). Thus, it can be understood that the *jalebi* packaged in LDPE or PS or MP or cardboard box remained well up to 4 days, which is slightly more than the shelf life of the *chhana jalebi* left without any packaging. The spoilage of the product may be attributed mainly to the growth of yeast and molds. There was no change in shelf life of the product packaged in the four packaging materials, though LDPE and MP showed higher scores at the end of 4 days. Statistical means of the packaging materials did not show statistical difference for flavour and BT scores, whereas storage period had significant influence on the change in sensory attributes ($P<0.05$).

3.2.Effect of preservative and packaging material on quality attributes of *chhana jalebi* during storage

Changes in physico-chemical characteristics:

There were changes in the physic-chemical characteristics of the jalebi during storage. The initial pH of the jaelbi was 5.43 which declined during storage (Fig. 2a). In cardboard box lined with butter paper packed samples, the rate of decrease was more rapid; the pH decreased from initial value of 5.43 to 5.14, 5.12, 5.10 and 5.18 for LDPE pouch, metallized polyester, polystyrene cups covered with aluminium foil and cardboard box lined with butter paper respectively on 30th day of storage at 28±2°C shown in Fig.2a. Relatively gradual decreasing trend was observed in all the packaging materials. (Kumar et al., 1997) also reported the decrease in pH of peda during storage for 180 days at 20°C. This was attributed to the growth of yeast and molds and a few water activity tolerant bacteria in the present study.

The changes in average value of water activity of chhana jalebi packed in four different packaged materials with preservative treated samples are illustrated in Fig. 2b. The curves in Fig.2b reveal that with progress in storage period, the water activity of chhana jalebi decreased from 0.88 to 0.858, 0.851, 0.827 and 0.866 for LDPE pouch, metalized polyester, polystyrene cups covered with aluminum foil and cardboard box lined with butter paper respectively on 30th day of storage at 28±2°C. This could be attributed to moisture loss through the packaging material or within the packaging material. This also had a bearing on the body and texture attribute.

Tyrosine value showed a gradual increase during storage. The chhana jalebi samples were analyzed for the value in terms of mg / 100g of product with a view to monitor proteolysis during storage. This increase in tyrosine value may be attributed to the breakdown of proteins by the surviving microflora or their enzymes. The proteolytic enzymes break the proteins down to simpler forms thereby increasing the amount of tyrosine in the product. It may also be attributed to heat stable proteolytic enzymes which might have survived the heat treatment. Microbial cells on lysis release enzymes from their cell walls, which may act on proteins (Bisht et al., 1994).

There was a gradual increase in tyrosine value in all type of treatment of chhana jalebi sample. The rate of increase of tyrosine value was higher in LDPE pouch and metallised polyester samples. The tyrosine value increased from initial value of 3.00 to 21.75mg /100g of product during 30 days of storage as shown in Fig.2c at 28±2°C. The rate of increase of tyrosine value in polyester cup and cardboard box lined with butter paper was less as compared to LDPE pouch and metalized polyester sample. Value of polyester cup and cardboard box lined with butter paper samples varied from initial value of 3.00 to 35.40mg /100g of product during 30days of storage shown in the figure.

Changes in microbial characteristics

The observation relating to the standard plate count (in $\log_{10}$ values) of *chhana jalebi* samples packed and stored at 28±2°C is illustrated in Fig.3a.The total bacterial count was found to increase with increase in storage time.

The standard plate count of *jalebi* samples for LDPE pouch, metalized polyester, polystyrene cups covered with aluminium foil and cardboard box lined with butter paper increased from 3.26 $\log_{10}$cfu/g on 0th day to 5.28, 4.62, 5.90 and 6.51 respectively on 20th day of storage at 28±2°C. During 30th day, the SPC count was found high in all the samples and spoiled due to contamination from packaging system and environment. The microorganisms are able to grow at the room temperature and increase their population. Majority of the yeasts and mold and bacteria got destroyed due to heat treatment. The survivors and contaminants acted on sugars and produced acids. This is the reason why the product pH decreased over the period of time. The microbial growth was higher in cardboard box lined with butter paper as compared to metalized polyester without gas flush treated sample. Earlier workers(Sachdeva, 1980), (Bhatele, 1983),(Reddy et al., 1993),(Garg et al., 1987),(Mishra et al., 1988) also reported an increasing trend in standard plate counts of burfi during storage. Yeast and mold growth tend to be major problem for most of the intermediate foods containing sugar (e.g. *khoai jalebi*, *peda*, *burfi*, *kalakand*). Often it is the most single factor limiting their shelf-life. The yeast and mold count multiplied as storage days increased. The result showed the increase in $\log_{10}$ counts during storage at 28±2°C and Fig.3b shows the trend in this respect. The initial count of yeast & mold was 0.41 for all the samples. The yeast and mold value increased from initial value of 0.41 to 1.21, 0.88, 1.46 and 1.67 $\log_{10}$cfu/g for LDPE pouch, metalized polyester, polystyrene cups covered with aluminium foil and cardboard box lined with butter paper respectively on 20th day of storage at 28±2°C.

Table 2. Changes in color and appearance score of 800ppm of potassium sorbate treated *chhana jalebi* samples packed in various packaging materials stored at ambient temperature (28±2°C)

Attribute	Storage Day	Packaging material				Statistical means
		LDPE	PS	MP	Cardboard	
Colour and appearance	0	8.91	8.94	8.95	8.93	8.93^{d}
	10	7.41	8.12	8.62	8.32	8.11^{c}
	20	6.50	6.53	6.67	5.53	6.30^{b}
	30	3.13	3.63	5.10	3.66	3.88^{a}
Statistical means		6.48^{a}	6.80^{a}	7.33^{ab}	6.61^{a}	
Flavour	0	8.95	8.95	8.94	8.95	8.95^{d}
	10	7.75	8.15	8.65	8.36	8.23^{c}
	20	6.51	7.55	6.69	7.56	7.08^{b}
	30	3.21	4.32	4.25	5.25	4.26^{a}
Statistical means		6.61^{a}	7.24^{b}	7.13^{ab}	7.53^{b}	
Body and texture	0	8.90	8.93	8.80	8.95	8.90^{d}
	10	7.45	8.17	8.10	8.35	8.02^{c}
	20	6.21	7.51	6.62	7.54	6.97^{b}
	30	3.15	5.44	4.21	5.34	4.54^{a}
Statistical means		6.43^{a}	7.51^{b}	6.93^{ab}	7.55^{b}	
Overall acceptance	0	8.97	8.95	8.95	8.95	8.96^{d}
	10	7.50	8.15	8.65	8.40	8.18^{c}
	20	6.50	6.50	6.69	6.50	6.55^{b}
	30	3.20	4.65	5.30	4.60	4.44^{a}
Statistical means		6.54^{a}	7.06^{a}	7.40^{ab}	7.11^{a}	

Note: LDPE – Low density polyethylene; PS – Polystyrene; MP – Metalised polyester

Table 3. Changes in sensory score of *chhana jalebi* samples packed in metalized polyester and flushed with nitrogen and carbon dioxide and stored at 28±2°C

Attribute	Storage Day	Atmosphere				Statistical means
		100% N_2	100% CO_2	50% N_2& 50% CO_2	Vacuum	
Colour and appearance	0	8.94	8.95	8.91	8.91	8.93^{d}
	20	8.25	8.31	8.25	8.25	8.27^{c}
	40	7.45	7.23	7.55	7.22	7.36^{b}
	60	4.22	4.54	4.44	3.32	4.13^{a}
Statistical means		7.22	7.26	7.29	6.93	
Flavour	0	8.91	8.96	8.92	8.95	8.94^{d}
	20	8.21	8.34	8.00	8.32	8.22^{c}
	40	7.20	7.46	7.24	7.21	7.28^{b}
	60	4.70	4.8	4.02	4.00	4.38^{a}
Statistical means		7.26	7.39	7.05	7.12	
Body and texture	0	8.91	8.93	8.93	8.80	8.89^{d}
	20	8.10	8.12	8.10	8.10	8.11^{c}
	40	7.33	7.43	7.64	6.64	7.26^{b}
	60	4.00	4.20	4.92	4.00	4.28^{a}
Statistical means		7.09	7.17	7.40	6.89	
Overall acceptance	0	8.96	8.95	8.95	8.95	8.95^{d}
	20	8.45	8.23	8.56	8.22	8.37^{c}
	40	7.21	7.18	7.34	7.14	7.22^{b}
	60	4.09	4.03	4.18	4.02	4.08^{a}
Statistical means		7.18	7.17	7.26	7.08	

Changes in sensory score

The sensory scores decreased during storage as indicated in Table-2. The initial C&A score was 8.91 – 8.95 which declined to 6.50 – 6.67 depending on the packaging material used (Table- 2). In case of card box, the score was 5.53 at the end of 20 days. Thereafter, all the C&A scores decreased to below acceptable limits. In general, the *jalebi* became dry because of evaporation of moisture either through the packaging material or within packaging material. But, after 20 days the visible mold growth was observed and the product had a dry appearance. The initial flavor scores were 8.94-8.95. The flavor was pleasant, but during storage the flavor score declined. Based on the scores which remained above 6.4, it can be observed that the flavor remained acceptable up to 20 days. Thereafter, off flavor developed which made the *jalebi* unacceptable. The body and texture score declined from an initial score of 8.8 – 8.95 to 6.21 – 7.54 at the end of 20 days. These decreased scores was because of loss of moisture and dryness. No mold growth was observed till this period. Overall, the scores remained acceptable up to a storage period of 20 days (overall acceptance score 6.5 – 6.69). After 20 days the overall acceptance scores fell to unacceptable limits because of off flavours caused by proteolyisis and oxidation. Researchers reported that by using 0.3% potassium sorbate *khoai*could be preserved up to 23 days at 30°C (Rajorhia et al., 1984). Potassium sorbate has been used in a number of products and is a useful preservative in food industry (FSSAI, 2006).

3.3.Storage studies in gas flushed in Metalized Polyester packaging material

The packages were exposed in UV light about 45 min. About 2- 3 pieces of *chhana jalebi* with gas flushing was packed in Metalized polyester pouch and stored at 28±2°C and 65% RH. The samples were analyzed for changes in sensory parameters during storage.

Changes in physico-chemical characteristics

Chhana jalebi is slightly acidic in nature as indicated by its pH value of5.43. This showed further decreasing trend during storage even in modified atmospheres. All the metalized polyester with gas flushed and vacuum treated samples also showed decreasing trend in pH. The pH decreased from initial value of 5.43 to 4.84, 4.86, 4.81 and 4.87 for metalized polyester pouch filled with 100% N_2, 100% CO_2, 50% N_2 with 50% CO_2 and vacuum, respectively on 60th day of storage at 28±2°C (Fig.4a). All the treated samples had shown the pH value between 4.81 to 4.87, thus indicating that nitrogen, carbon dioxide and vacuum hindered the growth of bacteria and mold. Researchers also observed decreasing trend of pH during storage period of *khoai jalebi*(Bharat et al., 2012).

During storage of *chhana jalebi* there was slight evaporation of moisture content therefore a declining trend was observed in water activity. From the graph (Fig.4b),it was observed that the water activity of *chhana jalebi* decreased from 0.88 to 0.685, 0.634, 0.657 and 0.721 for metalized polyester pouch filled with 100% N_2, 100% CO_2, 50% N_2 with 50% $CO_2$and vacuum respectively on 60th days of storage at 28±2°C.

The tyrosine value increased from initial value of 3.00 to 20.1, 16.3, 14.5 and 24.3 mg/100 g for samples packed in metalized polyester with gas combination of 100% N_2, 100% CO_2, 50% N_2 with 50% CO_2 and vacuum, respectively for 65 days of storage at 28±2°C. From the results (Fig4c), it was understood that samples packed in metalized polyester with gas combination of 50% N_2 with 50% CO_2 showed less tyrosine value compared to of 100% N_2, 100% CO_2 and vacuum treated samples. Hence, it is concluded that metalized polyester packaging material is suitable for packing the *chhana jalebi* modified atmosphere packaging. Proteolysis during storage of processed food products is a natural phenomenon due to surviving of microorganisms and their enzymes. Owing to proteolysis, protein gets broken into simpler form increasing the amount of tyrosine in the product which shown in Fig.4c. It may also be attributed to heat stable proteolytic enzymes which survived the heat treatment.

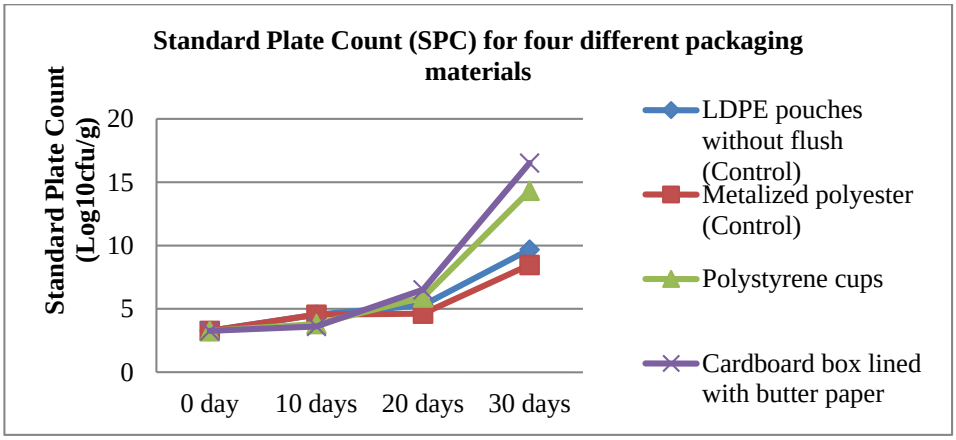

Figure 3 (a)

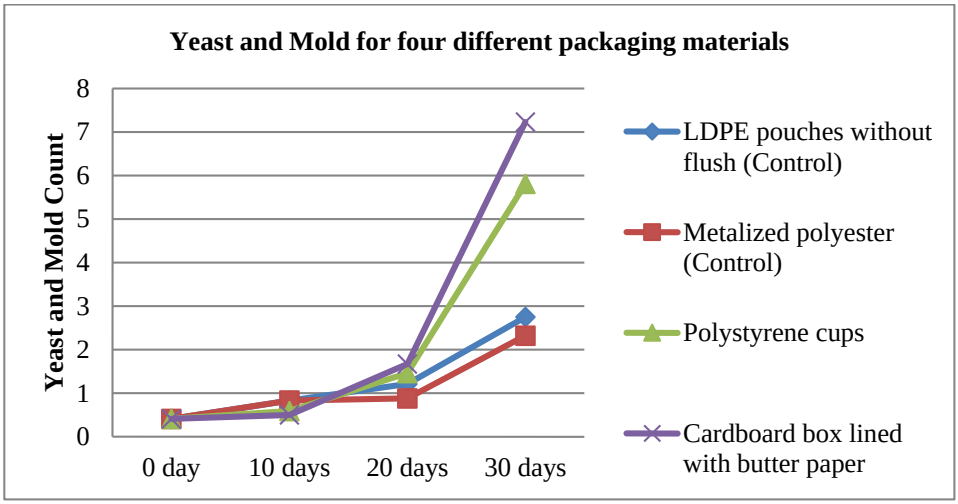

Figure 3 (b)

Figure 3. Effect of packaging materials on microbial counts of *chhana jalebi* (800 ppm potassium sorbate) during storage at 28±2°C (a) SPC and (b) YMC

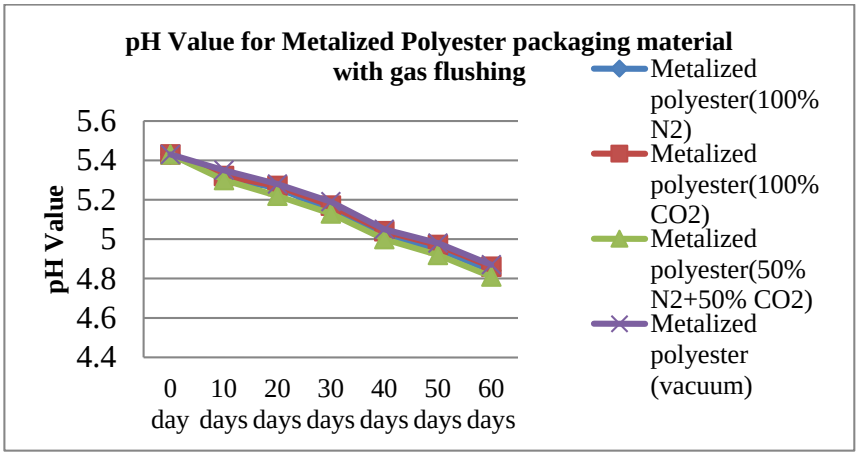

Figure 4 (a)

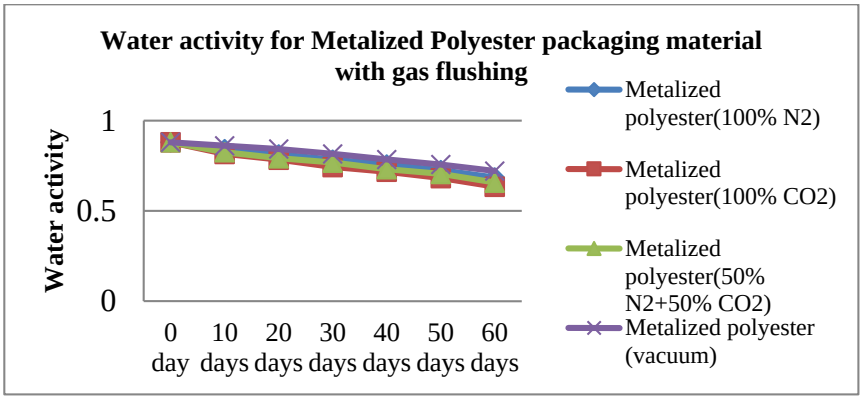

Figure 4 (b)

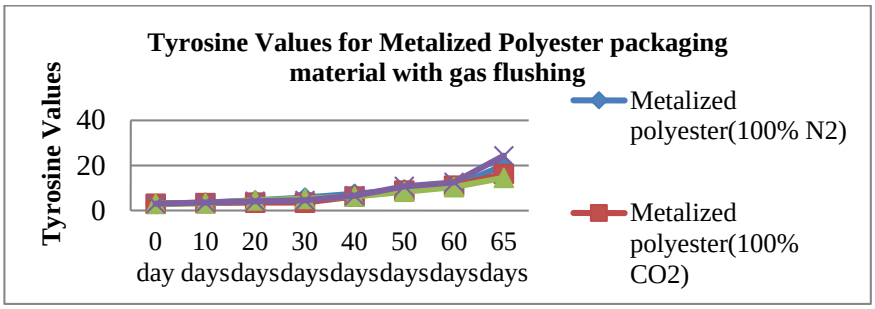

Figure 4 (c)

Figure 4. Effect of modified atmosphere on physic-chemical changes *chhana jalebi* during storage at 28±20°C (a) pH (b) water activity and (c) tyrosine value

Changes in microbial characteristics

The trend in standard plate count (in $\log_{10}$ values) in *chhana jalebi* stored at 28±2°C is shown in Fig. 5a . The total bacterial count was found to increase with increase in storage time.

The initial value of SPC was 3.26 which increased to 16.52, 11.47, 8.59 and 21.33 $\log_{10}$cfu/g for metalized polyester pouch filled with 100% N_2, 100% CO_2, 50% N_2 with 50% CO_2 and vacuum, respectively during 65 days of storage at 28±2°C (Fig. 5a). The counts were 4.92, 5.28, 4.76 and 6.26 $\log_{10}$cfu/g for metalized polyester pouch filled with 100% N_2, 100% CO_2, 50% N_2 with 50% CO_2 and vacuum, respectively on 60th days of storage. During 65th day, the SPC count was found higher in all the samples which were found with off flavor. Due to effect of gas flushing, the growth was slow in gas flushed and packed in metalized polyester samples compared to vacuum treated samples.

In the presence of oxygen, many of oxidation reactions and mold proliferation occur. Gas flushing in the present study was able to reduce the oxygen concentration and retarded microbial growth (Rooney, 1995), (Hurme et al., 1988), (Vermerien et al., 1999).

Figure 5b showed the gradual increase in $\log_{10}$ counts during storage and showed the trend in this respect. Yeast & mold growth tend to be major problem for high moisture food items. All the sample of *chhana jalebi* showed the presence of yeast & mold count which increased with the progress of storage period. The initial value of 0.41 increased to 7.23, 4.12, 2.43 and 12.41 and 1.67 $\log_{10}$cfu/g for metalized pouch filled with 100% N_2, 100% CO_2 and 50% N_2 with 50% CO_2 and vacuum respectively during 65 days of storage at 28±2°C shown in the figure.

It was found that the YM counts of *chhana jalebi* were 1.05, 1.21, 0.95 and 1.54 $\log_{10}$cfu/g respectively for metalized polyester pouch filled with 100% N_2, 100% CO_2, 50% N_2 with 50% $CO_2$and vacuum, respectively on 60th day of storage. During 65th day, the YMC count was found high in all the samples and found spoiled. Due to effect of gas flushing, the growth was slow

in gas flushed and packed in metalized polyester samples compared to vacuum treated samples. The yeast & mold count of samples increased during storage due to contamination from packaging system and environment on 65th day.

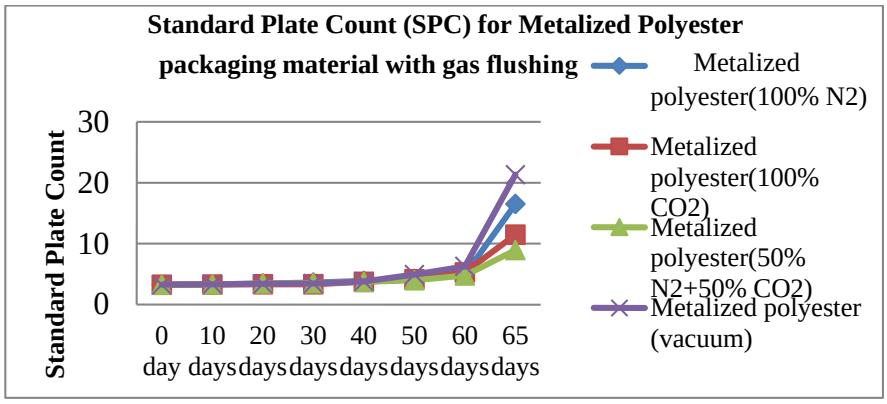

Figure 5 (a)

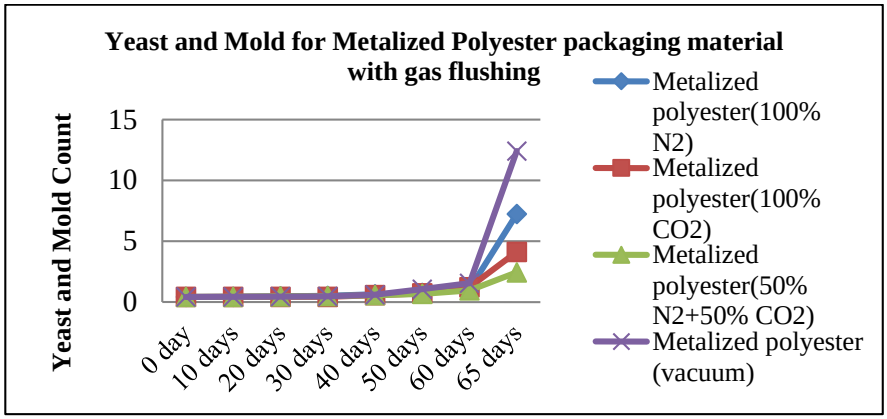

Figure 5 (b)

Figure 5. Effect of modified atmosphere on microbial counts of *chhana jalebi* during storage at 28±2°C (a) SPC and (b) YMC

Changes in sensory characteristics

There was gradual declining trend in sensory scores as indicated by the statistical means of the storage periods ($P<0.05$) (Table-3). The colour and appearance of the product was retained well up to 40 days of storage. The acceptability scores became lower because of dry appearance which could be attributed to moisture evaporation from the product within the package. Also, the product's initial moist appearance disappeared during storage imparting somewhat dull appearance because of surface moisture evaporation and hydration of proteins. It is known that protein hydration continues with storage time (Fox et al., 2003).

This affected the BT scores also because the product became firm during storage attributable not only to loss of moisture, but also to continued conformational changes of proteins (Navajeevan et al., 2008).

The flavor score also declined gradually, because of loss of aroma, and the product tended turn bland. This could be attributed to continued chemical changes in the product during storage (Fennema et al., 1996). The flavor scores were 7.20, 7.46, 7.24 and 7.21, respectively for 100% N_2, 100% CO_2, 50% N_2& 50% $CO_2$and vacuum packaging at the end of 40 days. The overall changes are reflected in changes in overall acceptance scores. These gradually decreased and

remained acceptable up to 40 days of storage at 28°C. The scores were 7.21, 7.18, 7.34 and 7.14, respectively for 100% N_2, 100% CO_2, 50% N_2& 50% CO_2 and vacuum packaging at the end of 40 days. But after 40 days, the scores fell to unacceptable limit because of visible mold growth, dry appearance and rancid flavor which could be attributed to chemical reactions. Thus, the shelf life of the product could be ascertained as 40 days irrespective of the type of gas used. The effect of the gas flushing and the interval of storage on overall acceptability of *chhana jalebi* were found significant ($P<0.05$).

From observations presented in Table-3, it was concluded that in all the gas flushed and vacuum filled packaging material samples overall acceptability score remained 6.5 and above for 60 days whereas the flavor score was turned unacceptable after 60 days. The decreasing score with advancement of storage period may be attributed mainly to the decline in aroma and flavor scores.

The ANOVA indicates the non- significant effect of gas flushing in metalized polyester packaging material, but significant effect on storage period and their interaction ($P<0.05$). MAP was found to enhance the sugar containing products by other workers as well. Kumar *et al.*(Kumar et al., 1997)reported that peda packed under 80% N_2 and 20% CO_2 stayed well up to 15 days at 37°C and 30 days at 20°C. *Malaipeda* shelf life was enhanced to 31 days at 11°C when packed under vacuum-nitrogen (Sharma et al., 2003).

3.4.Effect of vacuum packaging on shelf life of *chhana jalebi* packaged in low and high barrier material

Chhana jalebi samples were packed in low and high barrier packaging materials (LDPE and Metalized polyester) and packed with vacuum levels of (80%) 608 mm of Hg, (85%) 646 mm of Hg and (90%) 684 mm of Hg and (~100%) ~750 mm of Hg. It showed that all samples became crumpled and after cutting open the packets *chhana jalebi* samples were found with compressed appearance, ruptured the coils and oozed out sugar syrup. Hence, it is concluded that vacuum packaging treatment was not suitable for *chhana jalebi*.

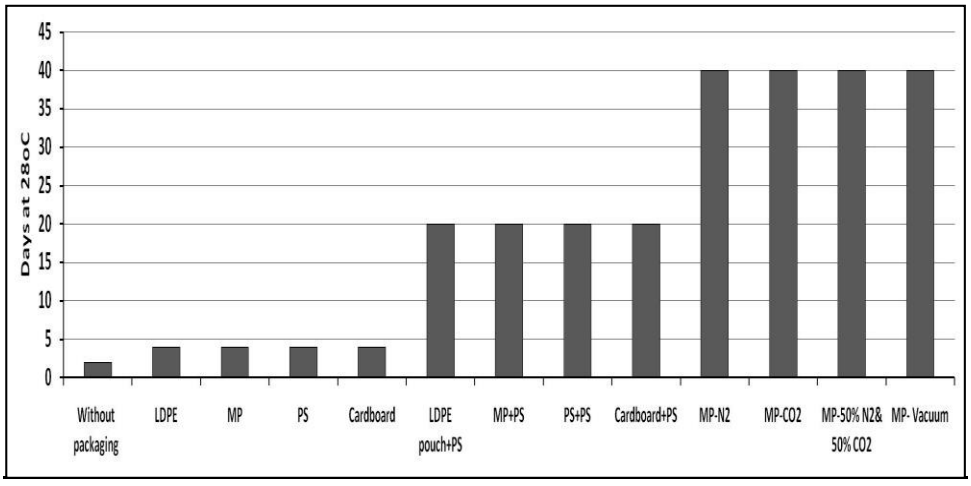

Figure 6. Shelf life of *chhana jalebi* at 28°C under various packaging conditions*[LDPE - LDPE pouch (Air);MP - Metalized Polyester (Air); PS - Polystyrene cups; Cardboard - Cardboard box lined with butter paper; MP+PS - Metalized Polyester+800 ppm potassium sorbate; PS+PS – Polystyrene cups+800 ppm potassium sorbate; Cardboard+PS - Cardboard box lined with butter paper+800 ppm potassium sorbate; MP – N2: Metalized Polyester (100% N_2); MP-CO2: Metalized Polyester (100% CO_2); MP-50% N_2& 50% CO_2:Metalized Polyester (50% N_2 + 50% CO_2); MP-Vacuum: Metalized Polyester with vacuum]*

4.Conclusions

Based on observations, it was concluded that *chhana jalebi* treated with 800 ppm of potassium sorbate, and packed in LDPE pouch, metalized polyester, polyester cups and cardboard box lined with butter paper and stored at ambient temperature of (28 ±2°C) showed 20 days of shelf life. Similarly, metalized polyester pack filled with 100% N_2, 100% CO_2,50% N_2&50% CO_2 and vacuum treated samples had shown 40 days shelf life at ambient temperatures (28 ±2°C) (Fig.6). Based on the chemical and microbial analyses, metalized polyester with 50% N_2& 50% CO_2 was found as more suitable for packing *chhana jalebi* samples to store at ambient temperatures. Researchers reported a shelf life of 13 days for *khoa* at 30°C when packed in four ply laminated pouches, but 75 days in cold storage(Rajorhia et al., 1984). Researchersreported a shelf life of 40 days at 30°C for brown *peda* under vacuum packaging(Londhe et al., 2012).

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INFLUENCE OF YEAST STRAINS ON AROMA AND TASTE COMPOSITION OF SAUVIGNON VERT WINE MATERIALS

Olga Kukharenko[1*], Tatiana Tanashchuk[2], Viktor Zagoruyko[2]

[1]Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, 03680, Academic Zabolotny St., 150, Kyiv, Ukraine
[2]National Institute of Grape and Wine "Magarach" of National Academy Agricultural Sciences of Ukraine, 98600, Kirova St., 31, Yalta, Autonomous Republic of Crimea, Ukraine.
[*]olinku@meta.ua

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ABSTRACT
A yeast strains selection could be helpful in improvement of Sauvignon vert grape variety wine materials. A comparative assessment of strains ability to make positive affect on the wine materials quality was given. Sauvignon vert must is fermented on four yeast strains: I-614, I-617, I-650, 47-K. The samples were analyzed by gas-chromatography. A comparison of tasting and gas-chromatography results allow to conclude, that increase of total number of volatile compounds (δ-dodecalactone, ethyl hexanoat, ethyl octanoat, ethyl decanoat, isoamil acetate, ethyl-4-oxybutirat, trance-2-heptenal, linalool, farnesol, hexen-1-ol), organic acids (octanoic, 9-decenoic, decenoic) and decrease of acetic acid and isoamylol improves quality of sample.

1. Introduction

An important field of science nowadays is production of high-quality wine materials for one-sort technology sparkling wines. The aroma of certain grape variety is responsible for the characteristics of future wine (Keyzers, 2011). Valuable properties of taste, its fine bouquet are forming not just by substances of grape berry, but also by fermentation products. The yeasts, in this case, take part in formation of aroma profile and its intensity. Because, as it well known, yeast strains have different fermentative systems that affect on variety of quantitative and qualitative fermentation products production (Wondra, 2001, Capece, 2010). In Sauvignon vert technology it has high importance, because this grape variety characterized by neutral aroma.

Given the weighty significance of this factor on wine materials quality, relevant is the study of yeast strains influence for the production of volatile and non-volatile fermentation products with a purpose to recommend best of them for the production of high-quality wine materials for sparkling wine Sauvignon vert grape variety with appreciable varietal aroma and taste.

2. Materials and methods

The subjects of our research were: must and wine materials Sauvignon vert grape variety, produced under production conditions of Zolota Balka winery (Sevastopol region, Autonomous Republic of Crimea, Ukraine) in 2010-2012 consecutive years.

The fermentation of wine materials had been performed by using four yeast strains

Sacch. cerevisiae (Kreger-van Rij N.J.W., 1984) numbered I-614, I-617, I-650, 47-K in the National Collection of Microorganisms National Institute of Grape and Wine (NIG&W) "Magarach". The yeast strain 47-K is industrial and are using for wine material production of Zolota Balka winery (a control strain).

A yeast starter of each strain had been activated using sterilized grape must with concentration of sugars 20 g/dm3. Before yeast starter had been added to must it contained: 130-140 mln. cells in 1 sm3 of medium; 45-50 % of gemmation cells; 1-2% of dead cells (Fugelksang et al., 2007).

The must of Sauvignon vert had been taken from production tanks after clarification (temperature 10-12ºC and SO_2 addition up to 70 mg/dm3). A temperature of must fermentation with usage of four yeast strain starters was 17±2ºC.

The samples were examined by production testing (the joint committee of panelists NIG&W "Magarach" and Zolota Balka winery).

Quantitative and qualitative analysis of volatile and non-volatile compounds is performed by gas-chromatography with mass selective detector (Agilent Technology 6890 (mass-selective detector 5973, column HP-INNOWAX, inner standard – amylol). Preparation of samples is executed by extraction (Виноградов et al., 1997)

3. Results and discussions

A method of gas chromatography let identify 34 volatile and non-volatile compounds, representatives of different chemical groups: aliphatic and aromatic alcohols, organic acids, esters, ethers of unsaturated fatty acids, terpenoids, lactones.

An analysis of obtained results had showed that sensory properties formation of wine material samples were caused by not all identified substances, but some of them, that were in higher concentrations then a threshold of human perception receptors (by aroma: for aromatic alcohols and δ-decalactone above 0,1 g/dm^3; for a volatile ether compounds – 1-5 g/dm^3; esters of unsaturated fatty acids – 0.2-20 g/dm^3; for higher alcohols 50-200 g/dm^3; by taste: for organic acids 5-10 g/dm^3.

Lactones (butirolactone, δ-decalctone) and some other cyclic esters of hydroxyacids in fewer concentrations were identified. These compounds bring Cheddar cheese, peach and raspberry fragrances and its increase is desirable, but in our samples butirolactone, δ-decalctone were in approximately equal concentrations (1.01-2.24 mg/dm^3 and 0.25-0.39 mg/dm^3 accordingly). So their affect of aroma wasn't significant in some certain sample.

An eleven different ethers and esters of fatty acids were identified. But high importance for the aroma because of large amount makes ethyl hexanoat (1.39-2.58 mg/dm^3), ethyl octanoat (1.67-4.27 mg/dm^3) and ethyl decanoat 1.06-2.67 mg/dm^3). These three esters have similar, but different by its intensity pleasant flavor of fruits and flowers (Edwards, 1990). Yeasts make significant affect on composition on these volatile compounds as they appear in fermentation process. Commending an affect of four experimental yeast strains is necessary to note, that highest total concentration of ether and ester compounds is observed in sample of wine material, produced by usage of I-650 yeast strain (Fig. 1).

In our samples of Sauvignon vert wine materials were identified acetal (trance-2-heptenal), aromatic alcohols (linalool, tyrosol, farnesol) - the compounds of grape berries but not appeared in result of fermentation. If trance-2-heptenal, linalool and farnesol are identified in lower concentrations in some samples only (trance-2-heptenal and linalool in 47-K sample – 0.13 mg/dm^3 and 0.32 accordingly; farnesol in I-614 sample – 0.32 mg/dm^3), tyrosol is present significantly in I-614, I-617 and I-650 in concentration within the limits 11.5-13.37 mg/dm^3, but it's not compound of high importance of strong and developed aroma of wine materials.

Another aliphatic alcohol (hexen-1-ol) had been identified in all samples. It brings an aroma of freshly cut grass. This compound because of higher concentration (1.35 mg/dm^3) affected more on aroma of 47-K sample.

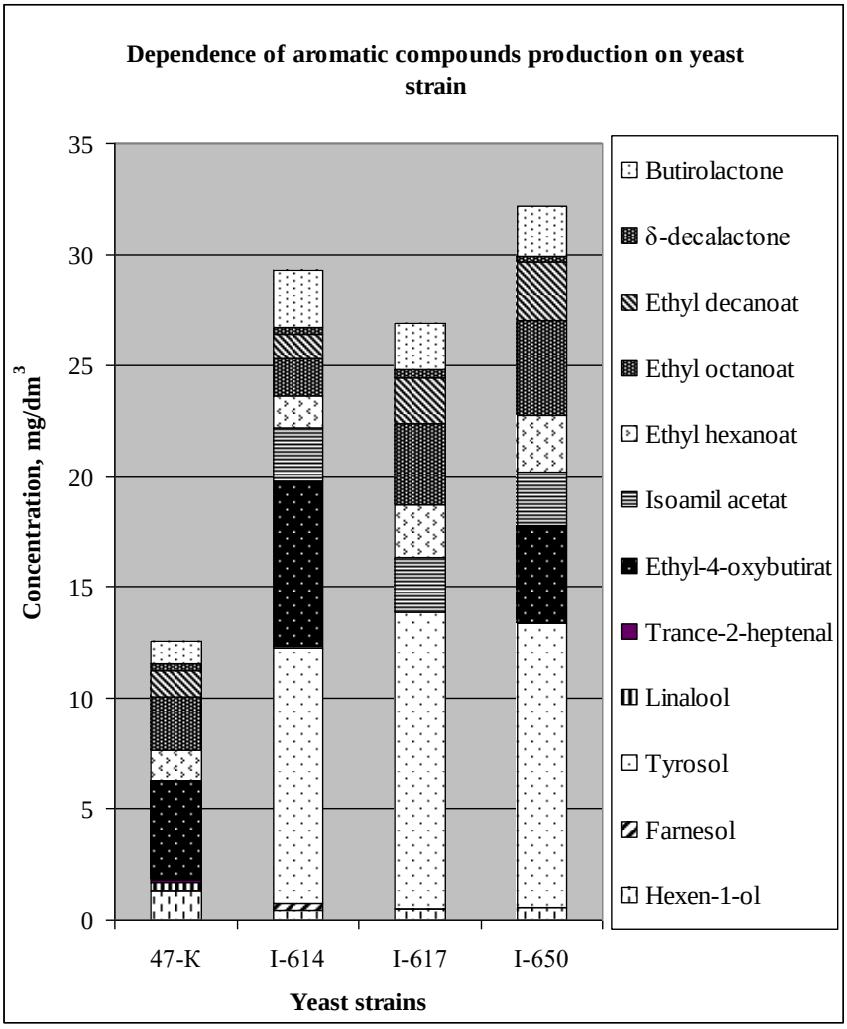

Figure 1. Dependence of aromatic compounds production on yeast strain

An important group of yeasts fermentation products is volatile esters. Eleven different esters were identified. But, analysis of received results let us conclude significant concentrations have been noted of ethyl-4-oxybutirat, isoamil acetate, ethyl hexanoat, ethyl octanoat and ethyl decanoat. An essential influence on wine material aroma makes three last of them. Ethyl-4-oxybutirat was present in all wine material samples in concentration 4.37-7.57 mg/dm^3, except I-617 sample.

Isoamil acetate, that gives to wine materials unripe apples aroma, was present in all samples within the limits 2.36-2.45 mg/dm^3. In sample of wine material, produced with usage 47-K yeast strain isoamil acetate wasn't identified.

Nine organic acids were identified in the samples of wine materials. A yeast strain I-650 is characterized by more high concentration of some organic acids (Fig. 2). These acids have a pleasant taste even in low concentrations. A tasting mark of wine material samples confirms positive affect on quality total increase of

organic acids concentration with long carbon chain (Table 1). Particularly, from all identified organic acids octanoic (in sample I-614 – 22.7 mg/dm^3; in I-650 sample – 45.75 mg/dm^3), decanoic (in sample 47-K – 18,2 mg/dm^3; in I-614 sample – 16.35 mg/dm^3; in sample I-617 – 25.04 mg/dm^3; in I-650 sample – 7,38 mg/dm^3) and 9-decenoic (in sample 47-K – 5.4 mg/dm^3; in I-614 sample – 5.0 mg/dm^3; in sample I-617 – 19,31 mg/dm^3; in I-650 sample – 6,25 mg/dm^3) were higher than threshold of perception and so they did main influence on the taste of wine materials.

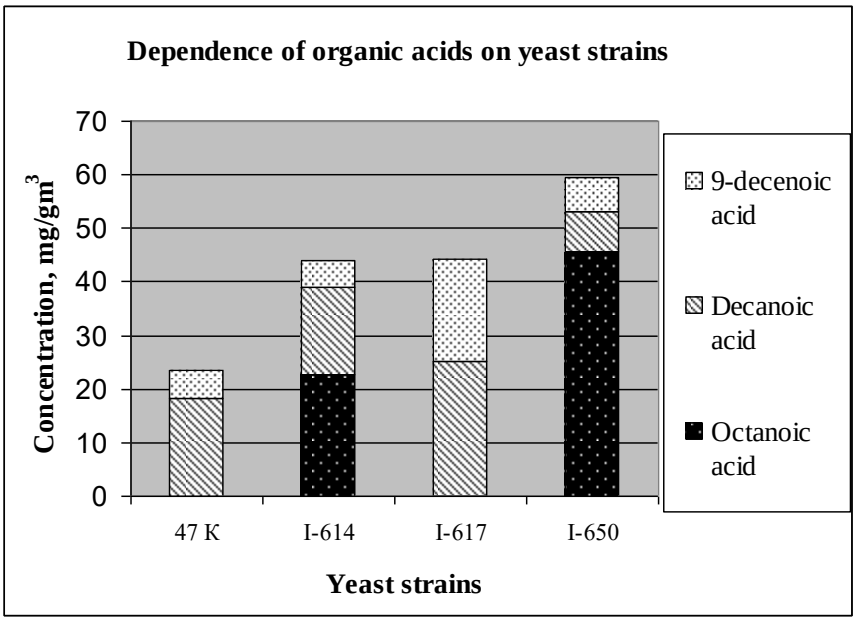

Figure 2. Dependence of organic acids on yeast strains

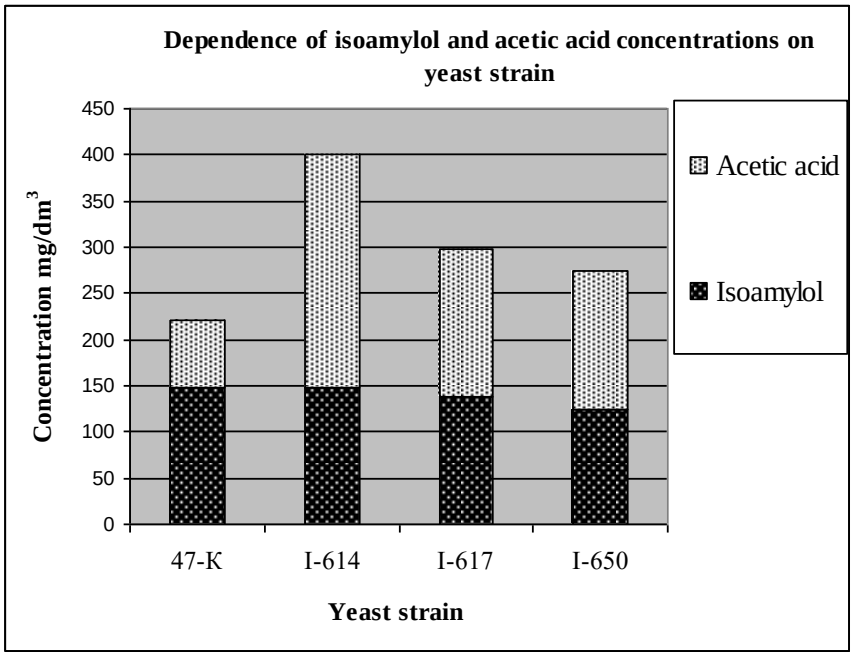

Figure 3. Dependence of isoamylol and acetic acid concentrations on yeast strain

Table 1. A sensory characteristic and testing mark of wine material samples

Yeast strain	Sensory characteristic	Testing mark
I-614	color: a pale-straw aroma: pure, floral taste: fresh, clean, acidic	7.8
I-617	color: a pale-straw aroma: pure, grape varietal taste: fresh, clean, underdeveloped	7.9
I-650	color: a pale-straw aroma: pure, developed, strong expressed fragrance of black currant leaf. taste: light, soft, fresh, strong expressed fragrance of black currant leaf	8.0
47-K	color: a pale-straw aroma: pure, floral not developed enough taste: fresh, clean	7.8

In the result of chromatographic analysis fermentation products (isoamylol and acetic acid) that make negative influence on sensory properties of wine materials in sufficient quantity were identified.

Higher alcohols with small quantity atoms of carbon affect on aroma and taste of wine materials (Ribereau-Gayon et al., 2006, Скурихин, 2005). Isoamylol had been identified in a largest percentage of all identified alcohols. Well-known its negative effect on bouquet of wine. Other alcohols were in concentrations that can not be identified by panelist. Analysis of aliphatic higher alcohol production let made a conclusion about different yeast strains production ability. In particular, yeast strain I-650 has ability to product lower concentration of isoamylol in comparison to I-614 and I-617 strains. An inverse relationship between tasting mark and concentration was identified (Fig. 3).

Significant percentage is occupying acetic acid that gives sharp odor and taste. It is the reason, why its low concentration in the sample of Sauvignon vert, produced with usage of I-650 strain, causes 96.6 mg/dm^3 in comparison to the I-614 and I-617 (199 and 208.4 mg/dm^3, accordingly), have a positive effect on taste of wine materials.

A testing of wine material samples, produced by usage of I-614, I-617, I-650 and 47-K revealed, that all they had been characterized by clean, fresh aroma. But only sample I-650 the developed hint of blackberry leaf was present. A tasting mark of this sample was the highest – 8.0 (Table 1).

4. Conclusions

Scientific studies of wine material samples aroma Sauvignon vert grape variety, let make next conclusions: big quantity of volatile and non-volatile compounds in wine material samples was detected. But only 17 of them were in concentrations over threshold of perception and took a part in general aroma and taste composition.

The comparative analysis of sensory and chromatographic results of wine material samples showed that tasting mark is dependable on level accumulation of substances in sufficient concentrations for panelist distinguishing.

The samples of wine materials (47-K and I-614) with low tasting marks (7.8 both) had some particularities of chemical composition. Namely, 47-K yeast strain sample had the lowest concentration of negative compound (acetic acid) either like a total concentration of aromatic volatile substances and organic acids;

I-614 yeast strain sample has enough high total concentration of aromatic compounds and organic acids, but the highest amount of undesirable isoamylol and acetic acid.

A sample of Sauvignon vert wine material, produced with I-650 yeast strain, had been characterized by high concentration of fatty unsaturated acids (octanoic, decanoic and 9-decenoic), ethers and esters – the products of fermentation process. Also this sample differs from other samples by lower amount of isoamyl alcohol and acetic acid.

The results of accomplished study are confirming a significant role of yeast strain in aroma and taste composition formation of wine material, which features is mainly based not on specific chemical substances, that are present in all barriers of different grape varieties, but on differences of their amounts in wine materials.

High tasting mark of experimental samples allow to recommend yeast strain I-650 for Sauvignon vert wine materials for one-sort technology sparkling wine in Zolota Balka winery conditions (Sevastopol region, Autonomous Republic of Crimea, Ukraine).

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THE COMMON SPOILAGE MICROORGANISMS OF BEER: OCCURRENCE, DEFECTS, AND DETERMINATION -A REVIEW

Saeideh Esmaeili[1], Marzieh Mogharrabi[2], Farzaneh Safi[2], Sara Sohrabvandi[1*], Amir Mohammad Mortazavian[1*], Niloofar Bagheripoor-Fallah[2]

[1]Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences, Food Science and Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Student Research Committee, Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences, Food Science and Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [*]mortazvn@sbmu.ac.ir.

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ABSTRACT

Beer is a favorite and highly drunk beverage, since it holds desirable sensory attributes as well as nutritional/medicinal characteristics. Although it is a fairly stable beverage from microbial spoilage point of view, any microbial spoilage occurred through malting and brewing processes or storage time can adversely affect the quality of beer and the brewing industry encounters detrimental financial consequences. Spoilage microorganisms include some Gram-positive and Gram-negative bacteria as well as wild yeast and moulds. Many traditional and advanced biotechnological techniques have been applied for qualitative and quantitative determination of mentioned microorganisms. The aim of this article is to generally review the specific spoilage organisms and the most important relevant methods of analysis.

1. Introduction

Beer is a brewed beverage consisting of malt, hop, water and yeast, which is drunk world-wide. In 2004, nearly 148 billion liters were produced, and on average, the per capita consumption was 72.9 liters annually (Sakamoto, 2002; Sohrabvandi et al., 2011). Beer is the world's third-most consumed beverage, because of its pleasant sensory and health attributes (Sohrabvandi et al., 2011). Surveys have shown that light to moderate consumption of beer can provide various impacts on humans' health, including nutritional benefits, anti-carcinogenic and anti-mutagenic effects (Sohrabvandi et al., 2012), reduction of cardiovascular disease (cardioprotective effect), immune system stimulation, hypolipidemic effect, anti-osteoporosis effect, and reduced risk of dementia (Sohrabvandi et al., 2012).

Microbial spoilage is a continuous challenge for the food industry, although beer is very restrictive to bacteria (due to its low pH, high acidity and different anti-microbial compounds) and the beer-spoiling organisms are limited to a few genera (Shabani and Devolli, 2010). The effects of the spoilage bacteria range from relatively minor changes in beer such as off-flavors and aroma defects (i. e., the buttery off-odor of diacetyl), turbidity problems, ropiness, abnormal attenuation rates and reduced yeast crops. These unwanted changes bring millions of dollars losses per year (March et al., 2005; Walkling-Ribeiro et al., 2011). Spoilage organisms are mainly lactic

be named such as high sensitivity, large dynamic range, rapid results, low cost and safety (Beck, 1990; Towbin et al., 1979).

3.2.6. Gas chromatography

The gas chromatographic tests of volatile and cellular fatty acids provide the chance to approve rapidly the growth of the *Megasphaera* and *Pectinatus* species directly from beer samples (Dworkin, 2000; Juvonen *et al.*, 2008). The growth kinetics of yeast cells during the fermentation process can be monitored by the assessment of optical density using a spectrophotometer at suitable wavelengths (Sohrabvandi et al., 2012). However, this method does not markedly reduce the total analysis time (Juvonen et al., 2008).

3.2.7. Microarray technology

Principles of nucleic acid hybridization are underlying the microarray technology. To detect the presence of base pairing complements in unknown samples, single stranded labeled 'probes' of known sequence are applied; since base pairing allows identification of complementary sequence within complex mixtures. To present microarray technologies, it is required that the solution phase nucleic acid hybridization to get adapted to solid supports (Southern et al., 1999).

For the identification of viable microorganisms, it is more reliable to analyze precursor ribosomal RNA (rRNA) rather than DNA. Microarrays are a useful method for the detection and identification of bacteria, also within mixed populations (Jin et al., 2005; Maynard et al., 2005). The microarray technique is specific, sensitive and fast, which allows the user to detect and identify variety of bacterial species simultaneously (Cho et al., 2001; Small et al., 2001; Al-Khaldi et al., 2002; Call et al., 2003; Weber et al., 2008).

3.2.8. Other Methods

Commercially, the amount of contaminating microorganisms in beer has been recommended to be assessed: in a hemocytometer (hemocytometer is a counting chamber loaded onto a microscope slide), electronically in a Coulter particle counter or using Abmeter, by optical procedure using NIR spectroscopy (Sohrabvandi et al., 2011; Jespersen and Jakobsen, 1996).

4. Conclusions

In modern breweries, the risk of microbial beer spoilage is mainly associated with growth of specific strains of *Lactobacillus* and *Pediococcus* spp., as well as a group of strictly anaerobic Gram-negative bacteria. In addition, wild yeasts, in particular *Saccharomyces* spp., constitute a significant risk. An important factor regarding the microbial spoilage of beer is the increased production of low-alcoholic and non-alcoholic beers that provide easier conditions for spoilage. Methods for microbial analysis of beer should have adequate selectivity, sensitivity, accuracy and precision. Therefore, still use of selective culture media is the most common method in breweries. Future investigations for adapting the advanced techniques for industrial applications from convenience and economical points of view are required.

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VARIANCE OF SOUR AND SWEET CREAM BUTTER ORGANOLEPTIC CHARACTERISTICS UNDER STORAGE

Bodnarchuk Oksana Vasylivna[1], Zhukova Yaroslava Fridrikhovna[1], Oliinichenko Oksana Volodymyrivna[1*]

[1]Institute of Food Resources, National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine;
[*]najahaja@gmail.com

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ABSTRACT
Influence of lactic flora starter culture on the formation of taste and aroma composition of sweet and sour cream butter and its transportation box storage qualities under the different temperature regimes were studied. It is shown that qualitative and quantitative composition of aroma-producing components also depends on the initial product plasma acidity mediated by the acid forming activity and added starter culture load (dose, amount). Influence of temperature condition (schedule) and storage time on the content of butter aroma producing components variation (spirits, lactones, aldehydes and volatile fat acids) were also estimated. Sour cream butter was shown to have different profile of taste and aroma compounds, smaller quantity of lactones and bigger amount of volatile organic acids throughout all storage stages, compared to sweet cream butter. Sweet cream butter contained comparatively higher amount of aldehydes (especially at the end of storage period at -(7-12) °C), that evidences about the presence of oxidative processes in product. It was shown that during long storage time (12 month) and low storage temperature -(12 – 18 °C) sour cream butter maintained its taste and flavor properties better than sweet cream butter.

1.Introduction

Currently, about 230 volatile substances are identified as natural components of butter, but only a small number of them plays a key role in the formation of aroma. The main compounds that cause taste and aroma of butter are volatile fatty acids, lactones, aldehydes, esters, ketones, sulfur compounds, free amino acids. Different combinations of these compounds determine the intensity of the characteristic aroma and aroma of butter (Mallia, 2008).

It is postulated that the main aroma forming substances of sweet butter are lactones such as δ-dodecalacton, δ-decalacton, γ-decalacton. The unique taste and aromatic characteristics of sour cream butter are resulting from substances present in sweet butter, and also from those produced by starter culture (Peterson, 2003).

Diacetyl (2,3-butandion) synthetized by lactic bacteria of the starter culture is the main aroma forming component of the sour cream butter (Mallia, 2008). It was identified diacetyl, acetic and lactic acids as key aroma forming components having microbial origin (Lindsay R. C, 1967). Seefeldt and Weimer found that catabolism of methionine by *L. lactis, L. cremoris, L. diacetilactis* results in production of sulfur-containing volatiles (metantiol, metyldysulfid, dymetyltrysulfid) that are involved in the formation of product aroma. Also L. diacetilactis was found to be able to synthesize volatile fatty acids (C2-C6) (Tungjaroenchai, 2004).

Accumulation of taste aromatic compounds of microbial origin in butter depends on the

species composition of starter cultures and speed of oxidative and biochemical reactions that occur in butter (Rieder, 2007).

Unlike lactones and diacetyl, a high content of volatile organic acids and acid oxidation products with a long carbon chain is undesirable because it provides an unpleasant aroma in butter (Mallia, 2007; Badings, 1970).

It is known that aromatic compounds are formed during the sour cream butter storage due to numerous biochemical processes such as hydrolysis, auto-oxidation, reduction, decarboxylation, deamination, esterification, and the intensity of these processes depend on butter type as well as storage time and conditions (Mallia, 2008). Recently a new technology for dairy industry was developed in Ukraine which represents a sour cream butter manufacturing technology involving specially designed starter culture. That is the reason why the study of taste bouquet formation and patterns of aromatic profile change during industrial storage of sour cream butter becomes significant and has a practical interest.

The aim of this work was to study the effect of long-term storage at low temperature on the profile of volatile organic acids, some lactones, aldehydes and alcohols that affect taste aromatic properties of sweet and sour cream butter.

2. Materials and methods

The objects of study were samples of fresh sour and sweet cream butter with fat mass concentration of 76-79%, stored as monolith in shipping container at a freezing chamber within a temperature range of - (7-11) °C for 9 months (mode I) and - (12-18) °C for 12 months (mode II) that meets the requirements of ISO 4399: 2005.

Butter products were produced from a high fat content cream by flow line method. Sour cream butter was produced on the basis of a starter culture, prepared by fermentation of sterile skim milk by bacterial preparations containing strains of lactic acid bacteria species *Lactococcus lactis ssp. diacetilactis, Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus*, at a rate of 1 g / dm^3. Starter cultures were injected by the dosing pump at the stage of product structure formation. This technology was applied at industrial conditions at PAT "Zhytomyr maslokombinat" to produce sour cream butter using 3.5% or 6% starter culture with acidity 98°T - (products №3.5%, 6% respectively) and 4% or 5% starter culture with acidity 90°T - (products № 4%, 5% respectively).

To generate butter product with required taste and aromatic characteristics it was subjected to maturation at a temperature of (10 ± 1) °C for 3 days.

The pattern of taste and aromatic compounds (volatile organic acids, free amino acids, esters, lactones, alcohols) of fresh and maturated butter was determined by gas-liquid chromatography according to DSTU 4222:2003 using chromatograph "Crystal-lux-4000M" (9); carbohydrates content was determined by high performance liquid chromatography according to ISO 11868: 2004 using chromatograph LC-5 ("Shimadzu").

3. Results and discussions

Given that the starter cultures used for the production of sour cream butter differed by their titrating acidity some distinctions of biochemical processes run were observed. Data analysis has shown that quantitative composition of all the volatile organic acids is changing over the storage time (table 1) but the greatest changes in both types of butter are due to acetic, butyric and isovaleric acids.

It was found that the initial level of volatile organic acids in products depends on acidity of butter plasma. resulting from the quantity and acidity of starter cultures applied. Thus, acetic acid content in fresh products with plasma acidity of 40-48ºT was 85.1-85.0%. in products with plasma acidity of 30-33ºT (4-5% of starter culture) it comprised 78.3-81.2%. while in sweet cream butter acetic acid made only 58.9%. This trend maintained until the end of the specified shelf life of the butter products.

The opposite pattern was found regarding the content of butyric acid. Thus, butter with higher plasma acidity (40-48ºT) contained 8.8-9.9% of butyric acid, while butter with lower plasma acidity (30-33ºT) characterized by relatively higher content of butyric acid - 12.6-19.7%.

Table 1. Variation of quantitative composition of volatile organic acids in sweet and sour cream butter over the storage time

Volatile organic acids	**Storage temperature -(7-11)^{0}C (mode I)**					**Storage temperature -(12-18)^{0}C (mode II)**				
	SoCB*				SwCB**	SoCB				SwCB
	3.5%	*4%*	*5%*	*6%*		*3.5%*	*4%*	*5%*	*6%*	
Fresh butter after 2 days of storage										
Acetic acid	16.00	12.23	17.16	23.24	6.28	16.00	12.23	17.16	23.24	6.28
Propionic acid	0.048	0.04	0.042	0.089	0.035	0.048	0.04	0.042	0.089	0.035
Isobutyric acid	0.027	0.024	0.026	0.028	0.032	0.027	0.024	0.026	0.028	0.032
Butyric acid	1.76	3.069	2.665	2.389	2.871	1.76	3.069	2.665	2.389	2.871
Isovaleric acid	0.836	0.701	1.001	1.128	1.199	0.836	0.701	1.001	1.128	1.199
Valeric acid	0.009	0.008	0.009	0.01	0.006	0.009	0.008	0.009	0.01	0.006
Capronic acid	0.11	0.21	0.193	0.201	0.203	0.11	0.21	0.193	0.201	0.203
Heptanoic acid	0.023	0.02	0.025	0.026	0.037	0.023	0.02	0.025	0.026	0.037
Total:	18.796	15.611	21.121	27.111	10.665	18.796	15.611	21.121	27.111	10.665
6 months of storage										
Acetic acid	18.034	17.761	0.237	25.560	15.942	17.019	12.474	18.297	17.997	11.940
Propionic acid	0.031	0.028	0.030	0.062	0.027	0.037	0.035	0.032	0.061	0.030
Isobutyric acid	0.051	0.045	0.055	0.067	0.011	0.045	0.040	0.045	0.061	0.010
Butyric acid	2.776	3.572	2.896	2.338	4.107	2.584	2.509	3.187	2.625	3.800
Isovaleric acid	0.902	0.952	1.296	1.340	0.914	0.837	0.696	0.910	0.779	0.897
Valeric acid	0.012	0.020	0.050	0.061	0.244	0.020	0.015	0.020	0.010	0.010
Capronic acid	0.136	0.250	0.218	0.177	0.100	0.177	0.201	0.228	0.222	0.140
Total:	21.940	22.637	28.195	29.604	21.345	20.720	15.970	22.720	21.753	16.827
12 months of storage										
Acetic acid	13.463	10.631	12.710	13.889	2.156	10.547	11.192	10.690	14.962	3.425
Propionic acid	0.031	0.023	0.052	0.025	0.045	0.220	0.019	0.022	0.043	0.052
Isobutyric acid	0.005	0.006	0.005	0.011	0.189	0.006	0.005	0.006	0.009	0.220
Butyric acid	1.392	1.758	0.068	0.827	0.050	0.566	2.482	1.768	0.942	0.055
Isovaleric acid	0.666	0.461	0.569	0.564	1.236	0.431	0.541	0.463	0.637	1.392
Valeric acid	0.007	0.007	0.012	0.008	0.005	0.009	0.008	0.008	0.009	0.007

Capronic acid	0.073	0.169	0.142	0.169	0.045	0.113	0.201	0.170	0.143	0.052
Total	15.636	13.055	13.558	15.492	3.726	11.892	14.447	13.239	16.745	5.204

*SoCB - sour cream butter produced with different starter cultures, ** SwCB - sweet cream butter

Monitoring of the total amount of volatile organic acids in sour cream butter held throughout 6 month of storage period has shown a slight increase of their content. except for propionic acid and valeric acids. Acetic acid was determined to dominate in all samples of the product at all stages studied. In particular, storage at mode I for six months resulted in its increase by 1.1-1.5 times reaching 17.76-25.56 mg / kg and at mode II by 1.1-1.3 times to the level of 12.47-18.30 mg/kg, respectively. Isobutyric acid concentration in sour cream butter increased by 11.9-2.4 times at mode I and by 1.6-2.1 times at mode II respectively. Differences in the quantitative composition of volatile organic acids of sour cream butter can be explained by intensification of biochemical processes due to lactic bacteria starter culture activation resulting from increase of the starter culture injection dose and storage temperature - (7-11) °C. The amount of total volatile organic acids in sweet cream butter increased for 6 months mainly due to acetic (2.5 times), butyric (1.4 times) and valeric acids (40.0 times), while the amount of other volatile organic acids, such as propionic, izobutyric, izovaleric and nylon decreased by 1.3, 2.9, 1.3, 2.0 times. respectively and showed only a weak dependence on storage temperature. It was also determined that the amount of butyric and valeric acids during this period was higher in sweet cream butter compared to sour cream butter by 13-32% and 75-95% respectively for the first and second modes of storage.

The amount of total volatile organic acids of sour and sweet cream butter samples stored for 12 months decreased compared to the initial level of fresh butter and showed no dependence on storage temperature. It was also observed that volatile organic acids content in sweet cream butter was lower compared to that in sour cream butter by 2.3-3.2 times for - (6-11)°C mode and 4.2-3.5 times for - (12-18)°C mode respectively.

Storage temperature conditions effect on composition variation of lactones, which are important for butter aroma, is represented in Fig. 1. It was found that the amount of lactones in fresh samples of sour cream butter depended on the initial plasma acidity of the products and ranged within 0.618-1.204 mg/kg that is by 4.6-2.3 times less than those in sweet cream butter. According to the data obtained, sour cream butter samples with higher plasma acidity stored under -(6-11)°C for 6 months accumulated lactones more intensively than other samples of butter.

Thus product enrichment with starter culture in amount of 3.5% and 6% during this period resulted in lactones quantity growth by 1.9 – 2.6 times relative to the original amount, while addition of 4.5% of starter culture mediated lactones quantity growth only by 1.6 - 1.6 times. However, during this period products with higher acidity (3.5% and 6% starter culture content) showed a decrease of δC12–lactone content by 0.02-0.06 mg/kg. At temperature mode of - (12-18)°C δC12–lactone content decreased in all samples of sour cream butter.

At the same time, a dramatic decrease of lactone content in sweet cream butter was observed after 6 month of storage. δC10- and δC12-lactones were shown to have the most rapid decrease that amounted 76.4% and 70.4% of the initial level, respectively. Subsequently, the content of these lactones slightly increased reaching 0.39 mg/kg (δC10) and 0.35 mg/kg (δC12) under mode I storage conditions and 0.45 mg/kg (δC10) and 0.32 mg/kg (δC12) under mode II storage conditions.

During the next 6 month of storage the content of lactones dropped in all samples of

sour cream butter. It is essential that the degree of lactones content decrease depended on the plasma acidity of the butter product. In particular, sour cream butter with acidity of 40-48ºT, derived with addition of 3.5% and 6% starter culture. characterized with decrease of lactones total content equaling 71% and 82%. respectively. In butter products with plasma acidity of 30-33ºT, obtained with addition of 4-5% starter culture. only 55-57% decrease was detected. Some authors connect these aromatic compounds loss with the intensification of hydrolytic processes leading to lactones transformation to hydroxy acids. Interestingly, after sour cream butter shelf life expiration no differences in lactones content were admitted regardless of the dose of the starter culture injected, though their quantity was low compared to the fresh products.

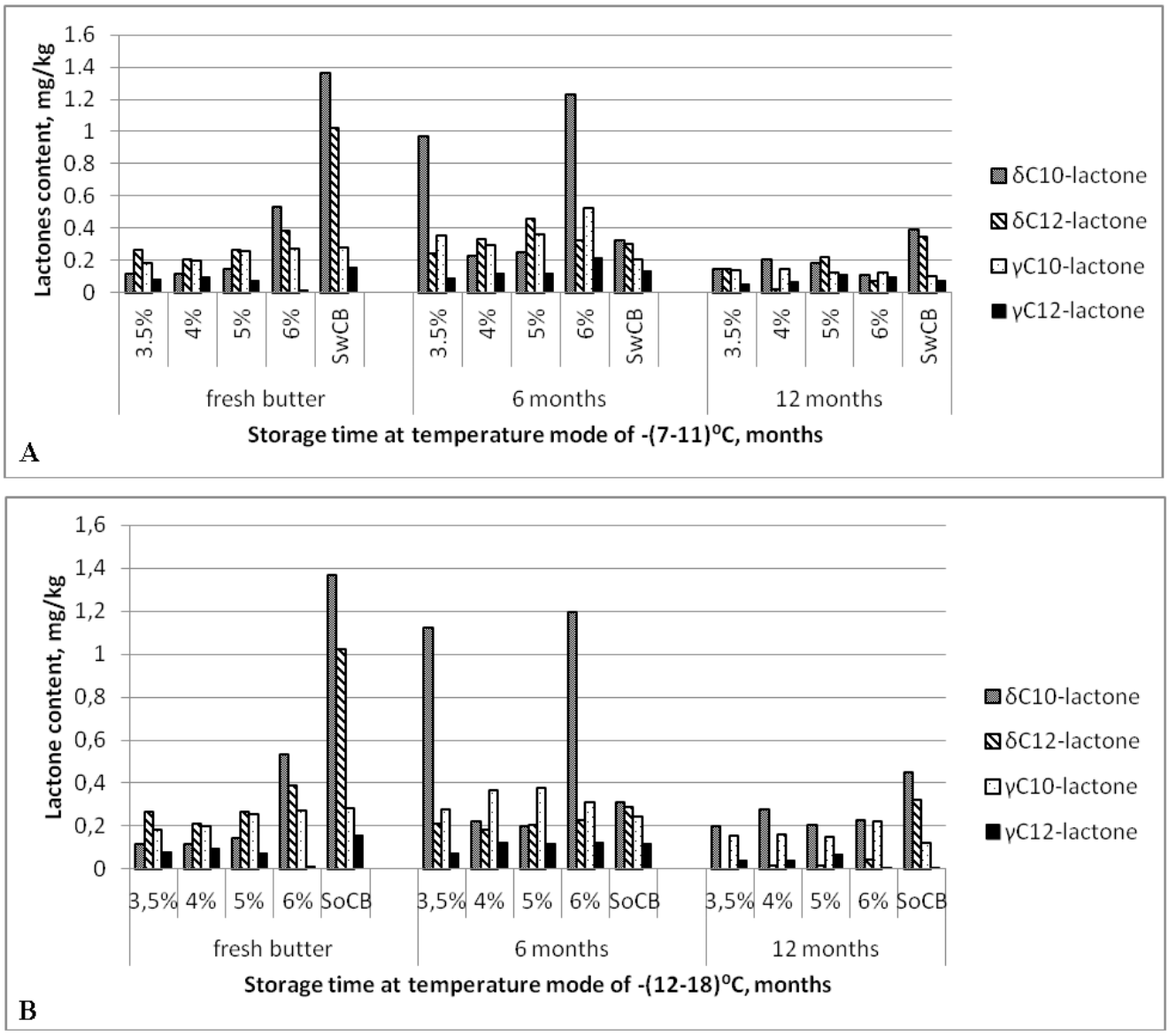


Figure 1. Lactones pattern variation in sour and sweet cream butter under low temperature storage conditions: A) -(7 - 11) °C; B) -(12 - 18) °C.

Lower storage temperatures showed some advantages concerning preservation of δC_{10}-. γC_{10}-lactones and had a positive effect on the product aroma intensity. After 9 months under -(6-11)ºC storage mode lactones content kept between 0.11-0.20 mg/kg and 0.12-0.15 mg/kg, while under - (11-18)ºC storage mode even after longer time of 12 month their content in all sour cream butter samples ranged from 0.20-0.28 mg/kg to 0.15-0.22 mg/kg, respectively. It was indicated, that at the end of shelf life all samples of butter contained higher quantities of mentioned above compounds compared to fresh products.

Therefore, the quantitative composition of lactones depends on acidity of the starter culture injected and the storage temperature mode applied. It means that the butter plasma acidity mediated by the starter culture injected has an impact on the lactones ratio, which explains the difference in lactone content between sour cream butter samples studied.

Carbonyl compounds variation under different storage temperature conditions is represented in Fig. 2. The study has shown that acetaldehyde content in sweet cream butter was 24.7-32.6% higher than that in sour cream butter, ranging between 1.5-1.7 mg/kg. After storage period of 6 month butter products, derived with 3.5-5% starter culture, accumulated only a little amount of acetaldehyde (only 1.1 times more compared to the initial level), independent of the storage temperature conditions. In butter products, derived with 6% starter culture, the content of acetaldehyde rose by 1.7 times and by the end of the shelf life it was somehow lower in samples stored at -(12-18)ºC

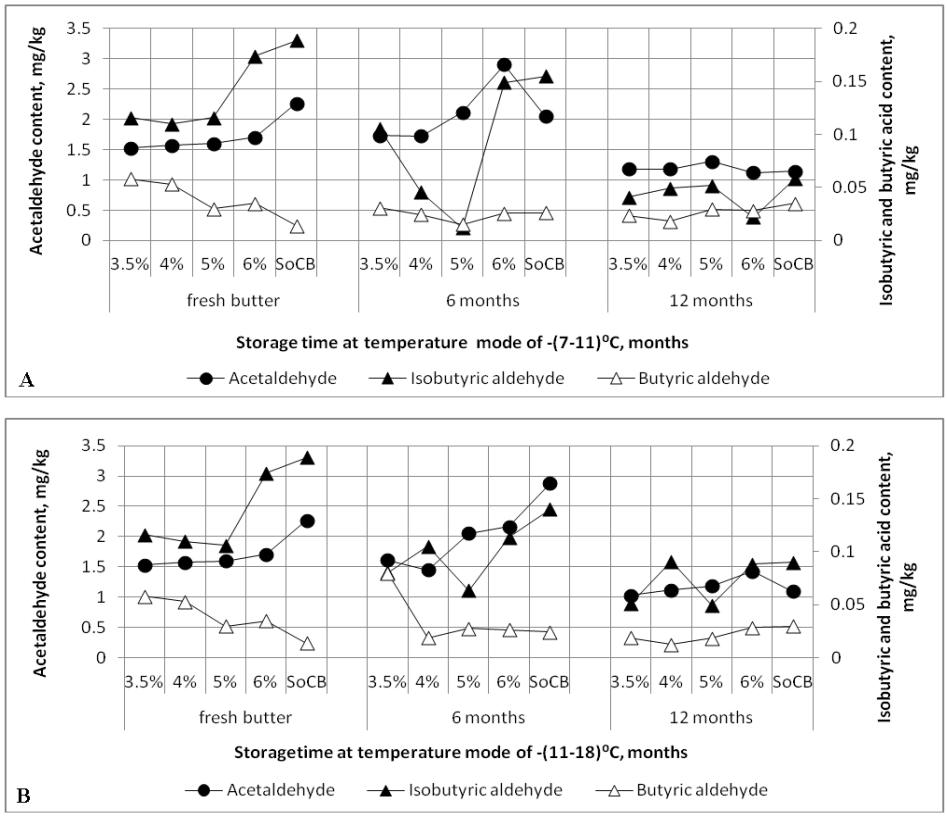


Figure 2. Aldehydes content variation in sour and sweet cream butter under low storage temperature conditions: A) -(7 - 11) °C; B) -(11 - 18) °C.

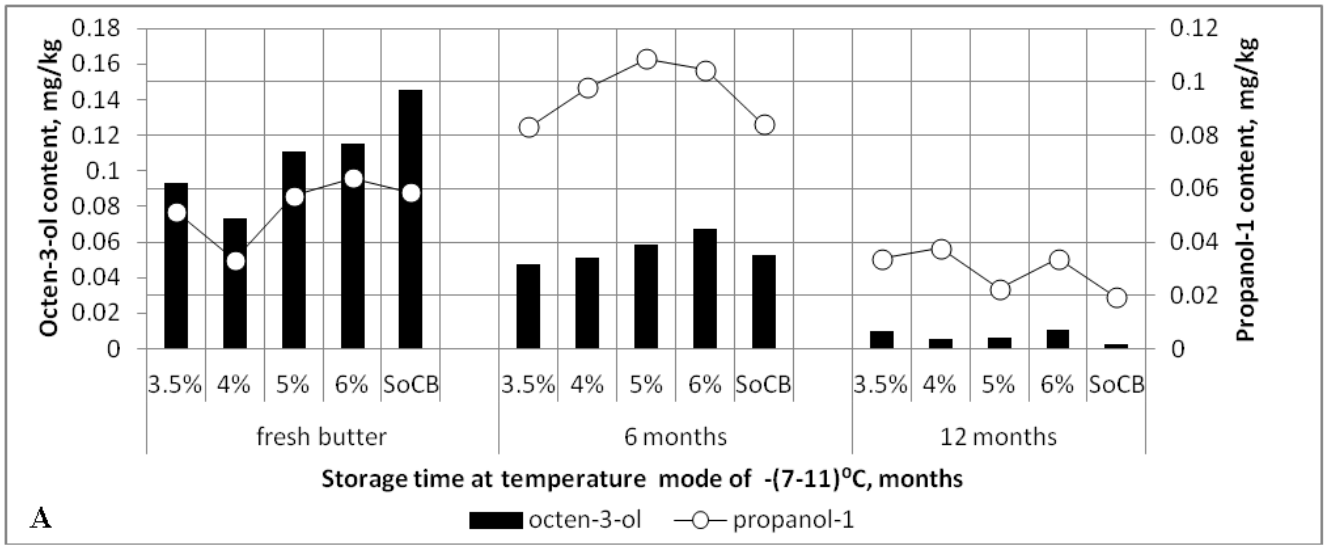
On the contrary, isobutyric aldehyde concentration reduced. and the most dramatic decrease was observed in sour cream butter with higher acidity, derived with the starter culture exhibiting the higher acid producing energy (the rate of lactic acid accumulation) of 98 ºT, after 6 month of storage. At the expiration of this period in butter products. derived from 3.5% and 6% starter cultures, isobutyric aldehyde concentration reduced by 2.9 and 8.7 times compared to their initial level. respectively and in butter products with lower plasma acidity. derived from 4% and 5% starter cultures. this reduction made 2.2 – 2.4 times.

It was found that after 12 month all samples of sour cream butter stored at temperature range of - (12-18)ºC contained higher levels of isobutyric aldehyde (up to 0.05-0.09 mg/kg), while when stored at temperature range of -(6-11)ºC it reached only 0.02-0.05 mg/kg, that approaches the levels characteristic for sweet cream butter. The other crucial fact is that in sweet cream butter samples isobutyric aldehyde dominated at all storage stages analyzed. On the contrary, within the initial storage stage in sour cream butter samples isobutyric aldehyde content amounted 0.03-0.06 mg/kg. that exceeds that in sweet butter by 2.1 – 4.1 times.

A gradual accumulation of butyric aldehyde was shown to take place both in butter, derived from 6% starter culture. and in control sample. regardless of the storage mode, that can be probably explained by the oxidative processes intensification. Variation of butyric aldehyde content in butter. derived from 3.5-5% starter culture, showed no distinct trend under storage mode II.

The data obtained demonstrate that accumulation of aldehydes in butter product depends both on storage temperature mode and on acidity of plasma.

Some alcohols content variations under different storage temperatures are presented in Fig.3.



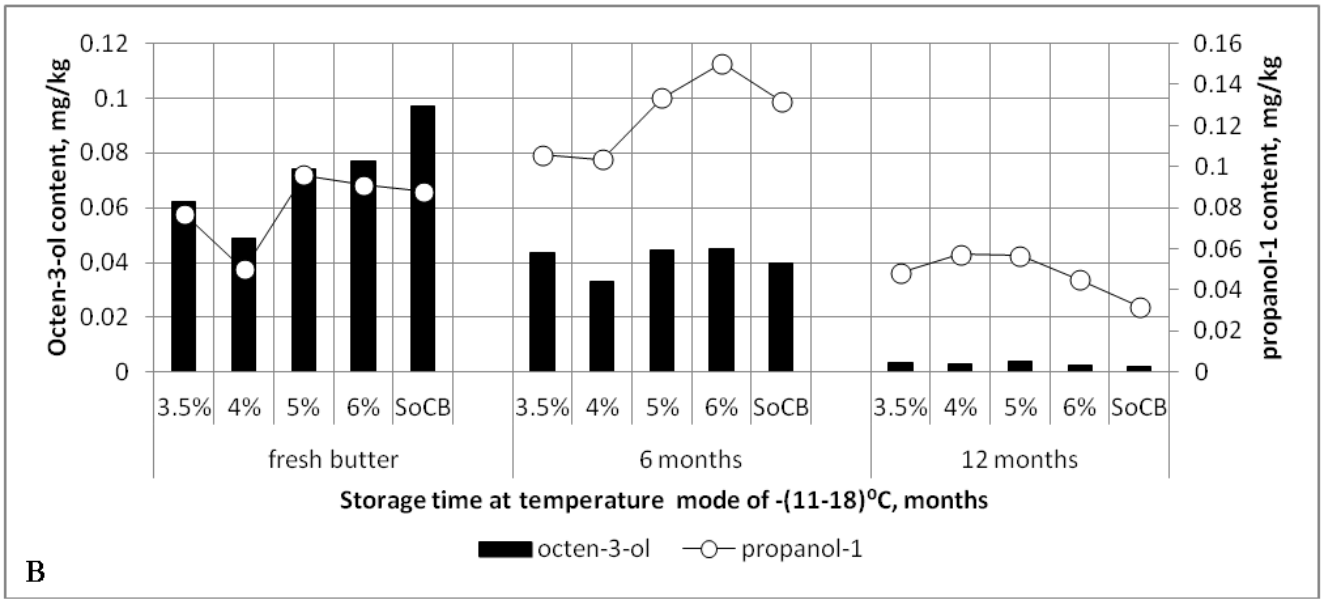


Figure 3. Alcohols content variation in sour and sweet cream butter under low storage temperature conditions: A) -(7 - 11) °C; B) -(11 - 18) °C.

The content of octen-3-ol ranges between 0.049-0.77 mg/kg in sour cream butter and makes up to 0.097 mg/kg in sweet cream butter, but there was not shown any strict dependence of this compound content and storage temperature or initial product acidity. In all samples of butter product only trace amounts of octen-3-ol were detected after 12 month of storage. However, a negative correlation was found between acidity & starter culture injection dose and accumulation of propanol-1. which was especially evident under storage mode I. Apparently, it is also induced by the shorter storage time. Thus. in butter products. derived from 3.5%, 4%, 5%, 6% starter cultures, it's amount increased by 1.6, 2.9, 1.8 and 1.6 times. respectively. On the contrary, in sweet cream butter samples under the same conditions the content of octen-3-ol rose by 1.4 times. At the same time, it was found that in sour cream butter samples at both storage temperature modes and throughout all storage stages the amount of octen-3-ol was higher than in sweet cream butter samples.

4. Conclusions

Sour cream butter was shown to have different profile of taste and aroma compounds, smaller quantity of lactones and bigger amount of volatile organic acids throughout all storage stages, compared to sweet cream butter. Sweet cream butter contained comparatively higher amount of aldehydes (especially at the end of storage period at -(7-12) ºC), that evidences about the presence of oxidative processes in product. It was shown that along with the variation of total amount of lactones, also the change of initial ratio between separate volatile organic acids takes place in butter during the storage period, affecting the taste and aroma characteristics of the product.

The studies conducted points to existence of direct correlation between the level of taste and aroma compounds and product plasma acidity, which in turn depends on the starter culture injection dose, acid producing energy and the storage temperature mode. It was established that the taste and aroma characteristics of butter essentially improve with the increase of starter culture dose and acidity.

Sweet and sour cream butter aimed for storage was estimated to have high quality according to the standard grading system of butter organoleptic characteristics. During the storage time a gradual degrease of butter quality occurred, thus butter samples with higher plasma acidity maintained its quality for a longer time and their taste and aroma characteristics were better expressed. Since plasma acidity affects the taste aroma compounds composition, it is clear that the plasma acidity regulation should have a practical application in production of high quality sour cream butter.

Therefore, it was established the effect of starter culture on the taste and aroma formation of sour cream butter monolith, as well as on its stability during transportation box storage under different temperature modes. In order to obtain a sour cream butter of high quality it is necessary to regulate its aromatic properties by the starter culture injection dose and acidity variation, and also by storage at low temperatures (-12ºC; -18ºC).

Data analysis allows making a conclusion that sour cream butter is more stable during storage period compared to sweet cream butter. It is evident, that stability of aromatic composition of sour cream butter is mediated not only by low storage temperatures, but also by starter culture properties and its ability to act as product preserving agent.

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COMPARISON OF VOLATILE PYRAZINE COMPOUNDS IN THREE DIFFERENT FERMENTED MYCELIUM FROM *PAECILOMYCES HEPIALI* BY THE ANALYSIS OF HS-SPME-GC-MS

Huawei Zeng[1,2*], Wenfeng Wang[1,2], Menghua Ma[1,2], Guohua Xu[1,2], Jianfan Sun[3], Wenchen Li[1,2], Yawei Yang[1,2]

[1]Jiangsu Shenhua Pharmaceutical Co. Ltd, Huaian, Jiangsu 211600, China
[2]Research Center of Biological Engineering Technology of Medicinal Fungi in Jiangsu Province,Huaian, Jiangsu 211600,China
[3]Institute of Environment and Ecology, School of the Environment and Safety Engineering Jiangsu University, Zhenjiang, Jiangsu 212013, China
**huaweizeng@163.com*

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ABSTRACT
Volatile pyrazine compounds in *Paecilomyces hepiali* fermented mycelium from three different main manufacturers were evaluated by using HS-SPME-GC-MS. Number of pyrazine compounds in C1, C2 and C3 amounted to twenty-eight, twelve, ten, respectively. Total pyrazine contents of C1 (10616.70μg/100g), C2 (241.89μg/100g) and C3 (955.56μg/100g) displayed significant difference among them. 2, 5-Dimethyl-pyrazine, 3-Ethyl-2, 5-dimethyl-pyrazine and 2-Ethyl-6-methyl-pyrazine were major volatile pyrazine compounds in all three samples. Tetramethyl-pyrazine in C2 and C3 and 2-Ethyl-5-methyl-pyrazine in C1 were main volatile pyrazine compounds. The results can help us to understand different flavor characteristics of pyrazine compounds and can be used in identifying manufacturers for quality controlling.

1. Introduction

Cordyceps sinensis called "Dong-Chong-Xia-Cao" in Chinese or "Tochukaso" in Japaneseis is the most well-known parasitic complex of Cordyceps. Recently, the yield of wild *C. sinensisis* declining in recent years because of its strict growing habitat and excessive harvesting (Wang et al., 2015). The strains of *Paecilomyces hepiali* have been isolated from *C. sinensis* (Zhu et al., 2010), and pharmacological studies on their fermented mycelium have revealed the effects of antioxidant, improving immune, antitumor (Yu et al., 2013; Xiang et. al., 2009; Wu J.F. et al., 2014). The *P. hepiali* mycelium from submerged culture is on of important substitutes for *C. sinensis*, and has been widely applied in functional foods industry base on similar functional effects between the substitute and *C. sinensis* (Wu Z. et al., 2014). However, those fermented mycelium of *P. hepiali* among different manufacturers have difference of functional effects due to certain reasons, such as different fermentation conditions and different drying technology, so it needs to seek for a method for identifying manufacturer of *P. hepiali* mycelium .

Pyrazine compounds, which are a group of nitrogen-containing heterocyclic compounds,

are important products of the Maillard reaction in processed foods, which starts with the condensation of a reducing carbohydrate with an amino compound (Koehler, 1970). Those compounds are directly related to food flavor (Wu, 2013). Because of the reason, pyrazine compounds are thought as an important analysis index .

Until now, volatile pyrazine compounds were not reported or determined in fermented mycelium of *P. hepiali*. In this paper, we aim at investigating of volatile pyrazine compounds in fermented mycelium of *P. hepialid* from three different main manufacturers by the analysis of HS-SPME-GC-MS. By comparison of the differences and similarities among the studied samples, a further knowledge of flavor characteristics among three different samples can be obtained and can be used in identifying manufacturers for quality controlling.

2.Materials and methods

2.1.Materials

One of dried mycelium from submerged cultivation of *P. hepialid* (C1) was produced in Jiangsu Shenhua Pharmaceutical Co. Ltd and the other two (C2 and C3) was purchased from Jiangxi State Drug Co., Ltd and Jiangsu Alphay Biological Technology Co., Ltd.

All standard samples and all reagents of analytical grade for following analysis were purchased from Sinopharm Chemical Reagent Co., Ltd in Shanghai.

2.2 The procedure of headspace SPME

The reaction liquid (containing dried mycelium of *P. hepiali*, 8mL distilled water, 2μl internal standard of 1.63g/L) was added to headspace bottle of 15 mL, and added NaCl (2.5g) to the reaction liquid for becoming saturated solution. The saturated solution was sealed by using lid and incubated at 40°C. Several minutes after the temperature (40°C) was achieved in the solution, SPME Fiber 75um Carbon molecular sieve/poly two methyl silane (Carboxen/PDMS) (Supelco, USA) was introduced into the sample bottle via siliconerubber septum cap and the fiber was exposed to the headspace for 30 min. After exposure, the fiber was immediately inserted into a GC injection port for desorption.

2.3. Gas Chromatography–mass Spectrometry

A Thermo Finnigan Trace GC 2000/MS (Thermo Electron. Corporation, USA) with single quadrupole and mass spectrometer was obtained with a PEG-20M capillarity column (30 m× 0.25 mm, 0.25 μm). The temperature of the injector was 250°C adjusted to splitless mode. The carrier gas (He) was helium at a flow of 0.8 mL min^{-1}. The temperature of the oven started at 40°C and held for 3 min. then, the temperature was increased at 6°C min^{-1} to achieve 120°C (total time15min) followed by 10°C $min^{-1}$to achieve 230°C (total time11min) and held for 7 min.

The MS conditions were as follows: ion-source temperature, 200 °C; ionization mode, EI+; ionizing voltage, 70 eV and mass spectrogram in full scan mode, m/z range of 33–450u.

The volatile pyrazine compounds were identified by comparison of their mass spectra database with those in National Institute of Standards and Technology (NIST) reference library and Wiley Mass Spectrometry Databases. According to the peak area corresponding to concentration of 2-octanol used as internal standard, concentration of the different pyrazine compounds was obtained.

3. Results and discussions

GC-MS total ion chromatograms of three different samples were presented in Figure1. The GC-MS total ion chromatograms were compared and analyzed with mass spectra database in NIST and Wiley, and then obtained those pyrazine compound compositions and contents, as are showed in table 1.

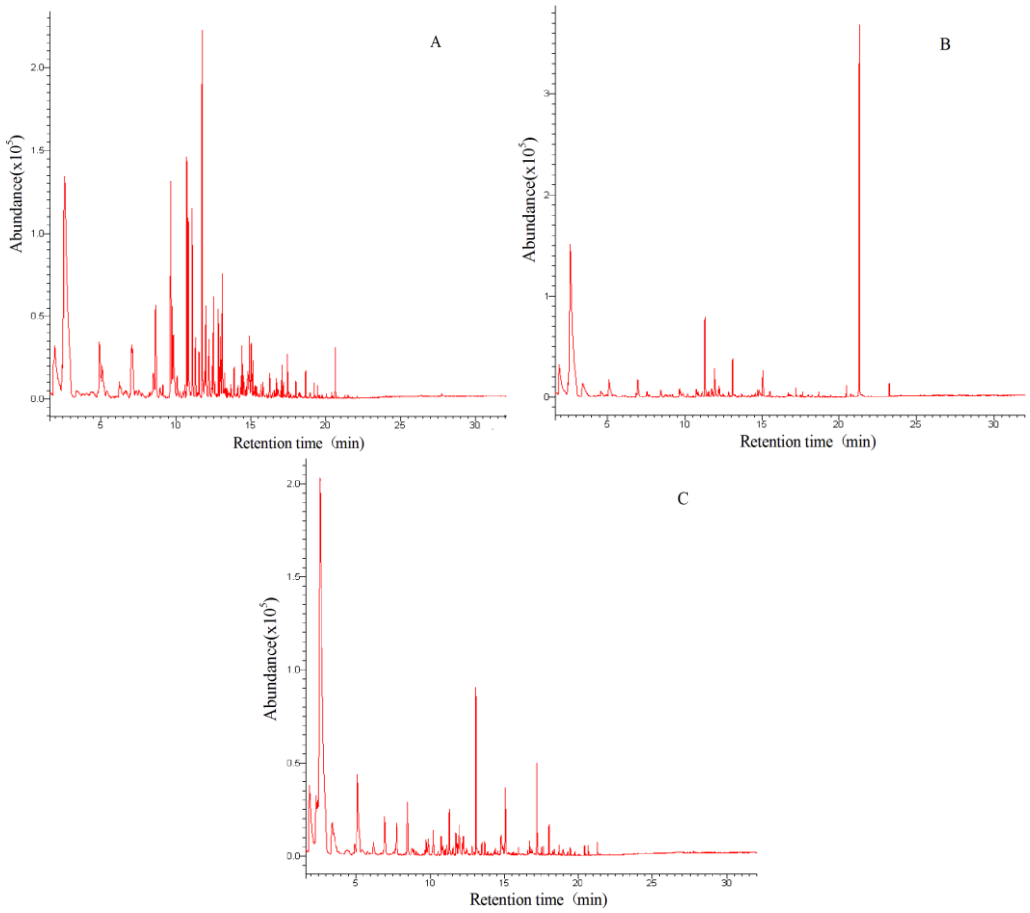

Figure 1. (A) GC-MS total ion chromatograms of C1; (B) GC-MS total ion chromatograms of C2; (C) GC-MS total ion chromatograms of C3

Number of pyrazine compounds identified in the headspace extract of C1, C2 and C3 amounted to twenty-eight, twelve, ten, respectively. Methyl-pyrazine, 2, 5-Dimethyl-pyrazine, 2-Ethyl-6-methyl-pyrazine, 2-Ethyl-5-methyl-pyrazine and 3-Ethyl-2, 5-dimethyl-pyrazine were found in all headspace extract of C1, C2 and C3. Total pyrazine compounds contents by analyzing the headspace extract of C1, C2 and C3 were 10616.70, 241.89 and 955.56 μg/100g, respectively. The data indicated that pyrazine compounds contents and pyrazine compositions were most abundant in C1 than C2 and C3. Although number of pyrazine compounds is very close between C2 and C3, pyrazines contents were higher in C3 (955.56 μg/100g) than those in C2 (241.89 μg/100g). Pyrazine compounds are generally thought to be generated by heating sugar and amino compounds by Maillard reaction, and the yield of pyrazine compounds can been influenced by reaction conditions (including temperature, time, pH value, amino acid composition, glucose compostion) (Koehler, 1970; Yu, 2012). The above results can attributed to comprehensive effect of died conditions.

The top four high pyrazines contents in C1, C2 and C3 were showed in Table 1;
C1:2-Ethyl-6-methyl-pyrazine (190μg/100g), 2,5-Dimethyl- pyrazine (162μg/100g), Tetramethyl-pyrazine (146μg/100g), 3-Ethyl-2,5-dimethyl-pyrazine (124μg/100g);
C2:3-Ethyl-2,5-dimethyl-pyrazine (1960μg/100g),
2,5-Dimethyl pyrazine(1270μg/100g),

2-Ethyl-6-methyl-pyrazine(1140μg/100g), 2-Ethyl-5-methyl-pyrazine(928μg/100g); C3:2,5-Dimethyl-pyrazine(37.9μg/100g), 2-Ethyl-6-methyl-pyrazine(29.4μg/100g), Tetramethyl-pyrazine(28.8μg/100g). 3-Ethyl-2, 5-dimethyl-pyrazine (27.2μg/100g). The result revealed that 2, 5-Dimethyl-pyrazine, 3-Ethyl-2, 5-dimethyl-pyrazine and 2-Ethyl-6-methyl-pyrazine were major volatile pyrazine compounds in all three samples. Tetramethyl-pyrazine in C2 and C3 and 2-Ethyl-5-methyl-pyrazine in C1 were main volatile pyrazine compounds.

Table 1. Volatile pyrazine compounds in C1, C2 and C3 (μg/100g)

	Compound	**C1**	**C2**	**C3**
1	Methyl-pyrazine	771	22.2	93.2
2	2,5-Dimethyl-pyrazine	1270	37.9	162
3	2,6-Dimethyl-pyrazine	508	12	-
4	Ethyl-pyrazine,	537	25	-
5	2,3-Dimethyl-pyrazine	164	-	-
6	2-Ethyl-6-methyl-pyrazine	1140	29.4	190
7	2-Ethyl-5-methyl-pyrazine	928	19.6	95.9
8	Trimethyl-pyrazine	-	27.2	-
9	2-Ethyl-3-methyl-pyrazine	869	-	109
10	2,6-Diethyl-pyrazine	246	-	-
11	3-Ethyl-2,5-dimethyl-pyrazine	1960	27.3	124
12	Tetramethyl-pyrazine	-	28.8	146
13	2,5-Diethylpyrazine	204	-	-
14	2-Methyl-5-propylpyrazine	1.95	-	-
15	2-Isobutyl-3-methylpyrazine	183	-	4．86
16	2,3-diethyl-5-methyl-Pyrazine	-	7.21	
17	2,3-Diethyl-6-methylpyrazine	514		-
18	3,5(3,6)-Dimethyl-2-n-propylpyrazine	4.61	-	-
19	2,5-Dimethyl-3-(2-methylpropyl)-pyrazine	160	-	-
20	2,5-Diethyl-3,6-dimethylpyrazine	35.6	-	19.3
21	2-(2-Methylpropyl)-3,5,6-trimethylpyrazi	21.2	-	-
22	2-(2'Methylbutyl)-3-methypyrazine	83.8	-	-
23	2-Isoamyl-6-methylpyrazine	-	2.97	-
24	2-Isopentyl-3-methylpyrazine	307	-	-
25	2,6-Dimethyl-3(2-methyl-1-butyl)pyrazine	69	-	-
26	2,3-Dimethyl-5-isopentylpyrazine	287	-	-
27	6,7-Dihydro-5-methyl-cyclopentapyrazine		2.31	11.3
28	5H-Cyclopentapyrazine, 6,7-dihydro-2,5-d	200	-	-
29	Trimethyl-propylpyrazine	39.7	-	-
30	2-methyl-5H-6,7-dihydrocyclopentapyrazin	77.8	-	-
31	2,5,7-trimethyl-6,7-dihydro-5H-cyclopent	15	-	-
32	2-ethyl-5H-6,7-dihydrocyclopentapyrazine	11.8	-	-
33	2-(2'-Furyl)-5-methylpyrazine and 2-(2'-	8.24	-	-
Total pyrazines in headspace		10616.70	241.89	955.56

"-"means not detected in sample

4. Conclusions

In the present work, we found that number of volatile pyrazine compounds and total pyrazine compounds content by investigation of HS-SPME-GC-MS was significantly different among the three samples. Tetramethyl-pyrazine in C2 and C3 and 2-Ethyl-5-methyl-pyrazine in C1 were major volatile pyrazine compounds. The result revealed that different characteristics of volatile pyrazine compounds among three samples.

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DEVELOPMENT OF Ag/TiO2-SiO2-COATED FOOD PACKAGING FILM AND ITS ROLE IN PRESERVATION OF GREEN LETTUCE DURING STORAGE

Anca Peter[1], Dora Tegla[1], Liviu Giurgiulescu[1], Anca Mihaly Cozmuta[1], Camelia Nicula[1], Leonard Mihaly Cozmuta[1], Ioannis Vagelas[2]

[1]Technical University Cluj Napoca, Department of Chemistry and Biology, Victor Babes 76, 430083, Baia Mare, Romania
[2]Technological Educational Institute of Thessaly, Department of Agronomy Technology, 41110, Larissa, Greece
[*]peteranca@yahoo.com

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ABSTRACT

Titanium dioxide unmodified or modified with noble metal particles and oxides has attracted attention as preserving material in food industry due to its antimicrobial effect as a result of its photoactivity. In this study, a Ag/TiO_2-SiO_2-coated food packaging film was developed in order to establish its ability to inactivate *Botrytis cinerea* during the storage of fresh lettuce. The packaging film was prepared by coating the Ag/TiO_2-SiO_2 ethanol suspension on polyethylene film. The preparation of Ag/TiO_2-SiO_2 composite consists in 4 steps: preparation of TiO_2-SiO_2 gel, deposition of silver ions on gel by reduction, drying and calcination. Commercial TiO_2 was also used to obtain reference packaging film. The as prepared packaging film was used for storage of green lettuce in a vegetation room, under continuous illumination (9 lamps = 1500 lm), at 20^0C, humidity 70%, ventilation. The spoilage of lettuce packed in the as obtained film during storage as a results of *Botrytis cinerea* development was established. A calibration scale for the spoilage of lettuce was also realized. The results showed that the shelf life of the lettuce stored in single- and double-layer layer film modified with TiO_2 was extended by 4 and 2 days, respectively as compared with the lettuce commercialized in normal atmosphere. After 5 days of storage, the spoilage of the lettuce in double-layer film modified with Ag/TiO_2-SiO_2 was lower than that of the lettuce stored in film modified with ethanol and reference (unmodified film).

1. Introduction

Fresh-cut vegetables have a very limited shelf-life due to the spoilage generated by microbial development as well as by physical-chemical processes. Lettuce is a popular salad ingredient with high perishability (Kim et al. 2011). The shelf life of lettuce commercialized in normal atmosphere is 3 days according to Romanian Law 2073/2005 completed by the Romanian Rule 1441/2007 for fresh-cut vegetables and fruits. The major processes occurring during lettuce storage are discoloration, flavour alteration and microbial contamination (Kim et al. 2011). Based on these, industry is searching for alternative environment-friendly solutions to preserve the quality of fresh-cut lettuce.

Titanium dioxide is already known as an efficient photocatalyst, that inactivates a wide spectrum of microorganisms when exposed at UV light (Chawengkijwanich and Hayata 2008,

Matsunaga et al., 1988; Fujishima et al., 1999; Kim et al., 2003; Duffy et al., 2004; Maneerat and Hayata, 2006). Chawengkijwanich and Hayata (2008) have demonstrated the ability of TiO_2 to inactivate *Escherichia coli.* Maneerat and Hayata (2006) showed that TiO_2 photocatalytic reaction reduced conidial germination of the fungal pathogen. Mihaly Cozmuta et al. (2015) have demonstrated that the Ag/TiO2-based packaging preserves the white bread during storage due to the inhibiting the proliferation of yeast/moulds, *B. cereus* and *B. subtilis*. Threepopnatkul et al. (2014) showed that PET-poly(ethylene terephthalate) and PBS-poly(butylene succinate) blend thin film modified with TiO_2 was active against both *E.coli* and *S.aureus* than the one with ZnO. Bodaghi et al. (2013) have successfully tested the ability of a low density polyethylene (LDPE) polymeric film modified with a combination of anatase and rutile titanium dioxide to inactivate *Pseudomonas spp*. and *Rhodotorula mucilaginosa.*

The aim of this study was to test the preservation activity of Ag/TiO_2-SiO_2-coated food packaging film during the lettuce storage in terms of ability to reduce botrytis blight caused by *Botrytis cinerea*.

2. Materials and methods

2.1. Preparation of Ag/TiO_2-SiO_2

First, TiO_2-SiO_2 gel was prepared by sol-gel method, by mixing 3.64%mol titanium tetraisoproxide (Merck, Germany), 1.82%mol tetraetoxisilane (Merck Germany), 21%mol ultrapure water, 73%mol absolute ethanol (Chemical Company, Romania) and 0.58%mol nitric acid (Merck, Germany). The gel were allowed to age for 4 weeks. Then, the gel was immersed in the needed volume of $AgNO_3$ solution (Silal Trading Romania) (5 mM) and $NaBH_4$ (Merck, Germany) solution (1 mM) at 4^0C. The mixture was filtrated and washed 5 times with absolute ethanol. The drying was performed in an oven at 80^0C, 24 h, and the obtained xerogel was calcined at 500°C, 2 h. Morpho-structural characterization of the composite was detailed in our previous study (Peter et al. 2015). Commercial TiO_2 (Carlo Erba, Spania) was used as reference. The

2.2. Film package preparation

The film package was obtained as single and double layer, respectively. The polyethylene film was purchased from a local supermarket in Baia Mare, Romania. For the single layer film package obtaining, a portion of 400 cm^2 was tailored and coated with a mixture of ethanol composite Ag/TiO_2-SiO_2 0.0005g/cm^2. For the double layer film package preparation, a second polyethylene film was deposited on the composite.

Film packages with commercial TiO_2, without composite and ethanol, and without composite, respectively were prepared as references.

2.3. Experimental conditions

Letuce var. Butterhead was purchased from a supermarket in Larissa, Greece. In each package, an amount of 30 g was deposited. Before packaging, the leaves were washed with ultrapure water and dried. *B. cinerea* was isolated from naturally infected lettuce leaves and maintained at 20^0C on potato dextrose agar (PDA). Inoculation was carried out with PDA cultures of 14 day old conidia. The conidia were collected from sporulating colonies, prepared as described by Redmond et al. (1987) and used for inoculation the above lettuce dried leaves as described by Volpin and Elad (1991).

The as obtained packages were maintained in a Sanyo versatile environmental test chamber for 14 days, at 20^0C, humidity 70% and air circulation. The test chamber was permanently illuminated with 9 fluorescent lamps FL40SS W/37 (light intensity 27000 lms). The packages were analyzed at 3, 5, 7, 10 and 14 days in order to establish the level of lettuce spoilage as a result of the *Botrytis cinerea* presence.

The analyzed were repeated 6 times and the results were processed by using the Excel program. The reported results are accompanied by the deviation standard values. The

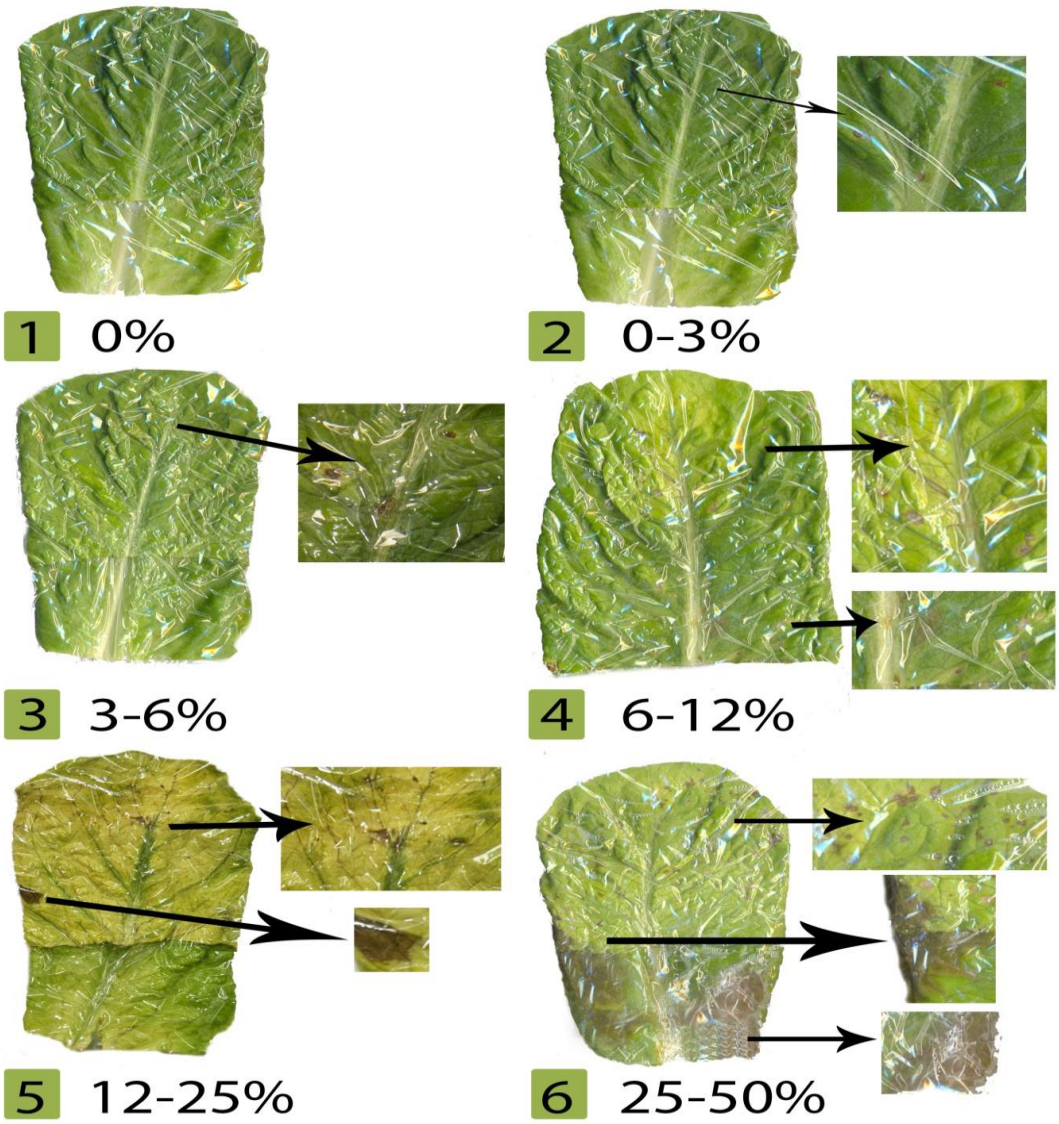
normalized standard deviation (Δq %) was calculated using the Eq. 1:

$$\Delta q(\%) = 100\sqrt{\frac{\sum_{i=1}^{n}\left[\frac{q_{\exp} - q_{calc}}{q_{\exp}}\right]^2}{n-1}} \quad (1)$$

where: n – number of the experimental values, q_{exp} – experimental value of spoilage, q_{calc} – calculated value of spoilage using the linear regression model.

In order to establish the spoilage, to visually estimate and to score the diseased area of the lettuce, a calibration scale was prepared, according to the method described by O'Brien et al. (1992). Each level of spoilage was correlated with a percent of lettuce degradation.

The diseased area on each lettuce leaf was estimated visually and a score was assigned to each leaf according to the percentage of the surface area covered with lesions on the scale 1-12, where 1 = 0%, 2 = >0-<3%, 3 = >3-<6%, 4 = >6-<12%, 5 = >12-<25%, 6 = >25-<50%, 7 = >50-<75%, 8 = >75-<87%, 9 = >87-<94%, 10 = >94-<97%, 11 = >97-<100% and 12 = lettuce leaf completely damage. The obtained calibration scale is presented in Figure 1.



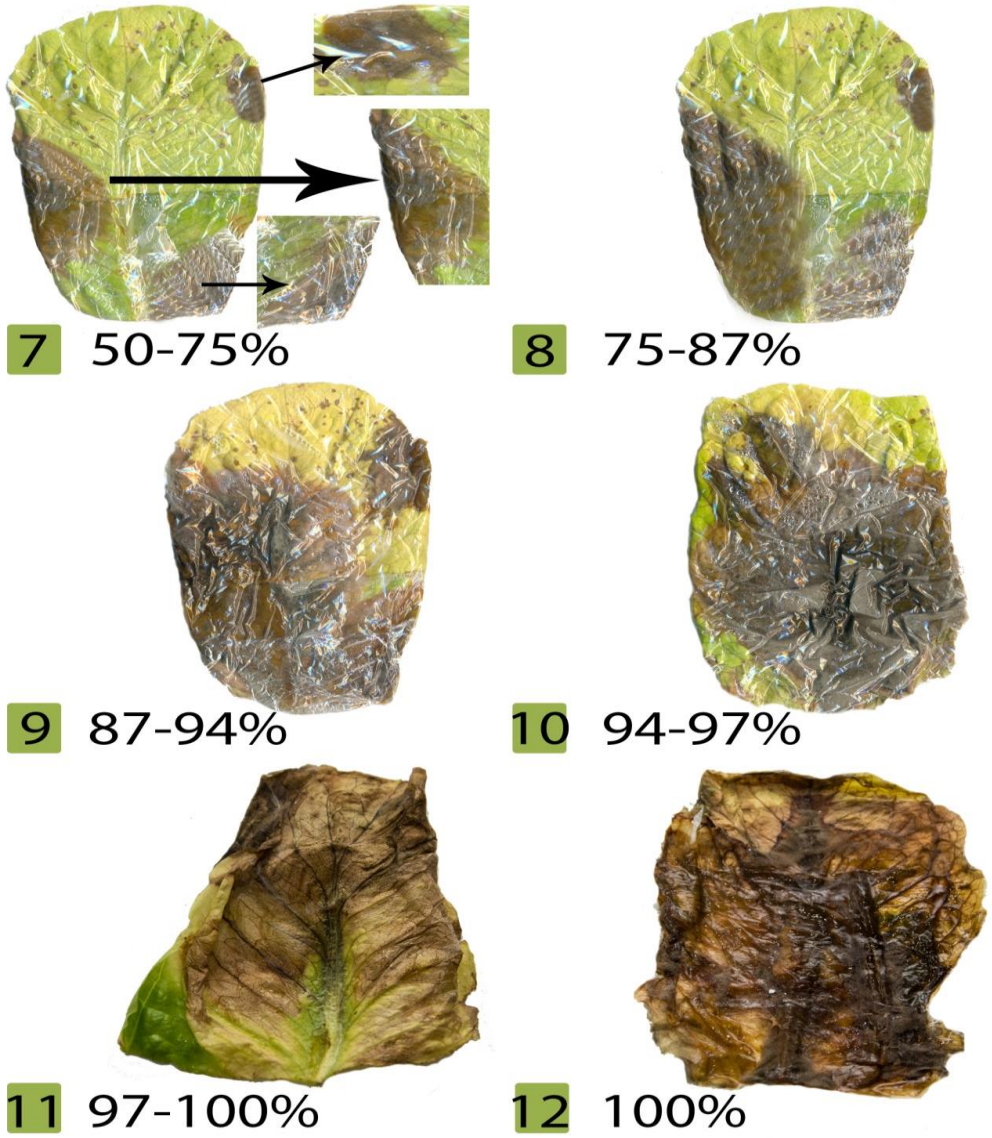


Figure 1. Calibration scale for spoilage of lettuce with *Botrytis cinerea*

3. Results and discussions

The results showed that the microorganism developed in the highest percentage was *Botrytis cinerea*. In Figure 2 are presented the aspect of infested lettuce leave and the microscopic images of this microorganism.

The spoilage of lettuce kept in single-layer packages and double-layer packages is presented in Figure 3 and Figure 4, respectively.

At the start of the experiments, the level of spoilage was 0 in both single and double layer packages. In the third day, only the lettuce deposited in single and double layer package with ethanol was spoiled in a percentage of 1.83% and 1%, respectively.

After 5 days, the lettuce packed in single-layer package with Ag-TiO_2-SiO_2 was spoiled by 3% than that stored in single-layer reference and single-layer package with ethanol. The spoilage degree of lettuce deposited in single-layer package with ethanol increased by 1% from day 3 to day 5. The lettuce stored in

double-layer package with Ag-TiO_2-SiO_2 was spoiled by 2% from day 3 to 5. After 5 days, the spoilage of lettuce in single layer package with Ag-TiO_2-SiO_2 was more intense than that in double layered package. The spoilage degree of lettuce in double layer package with ethanol slightly increased from day 3 to day 5. No spoilage of the lettuce in single-layer and double-layer package with TiO_2 was observed after 5 days of storage.

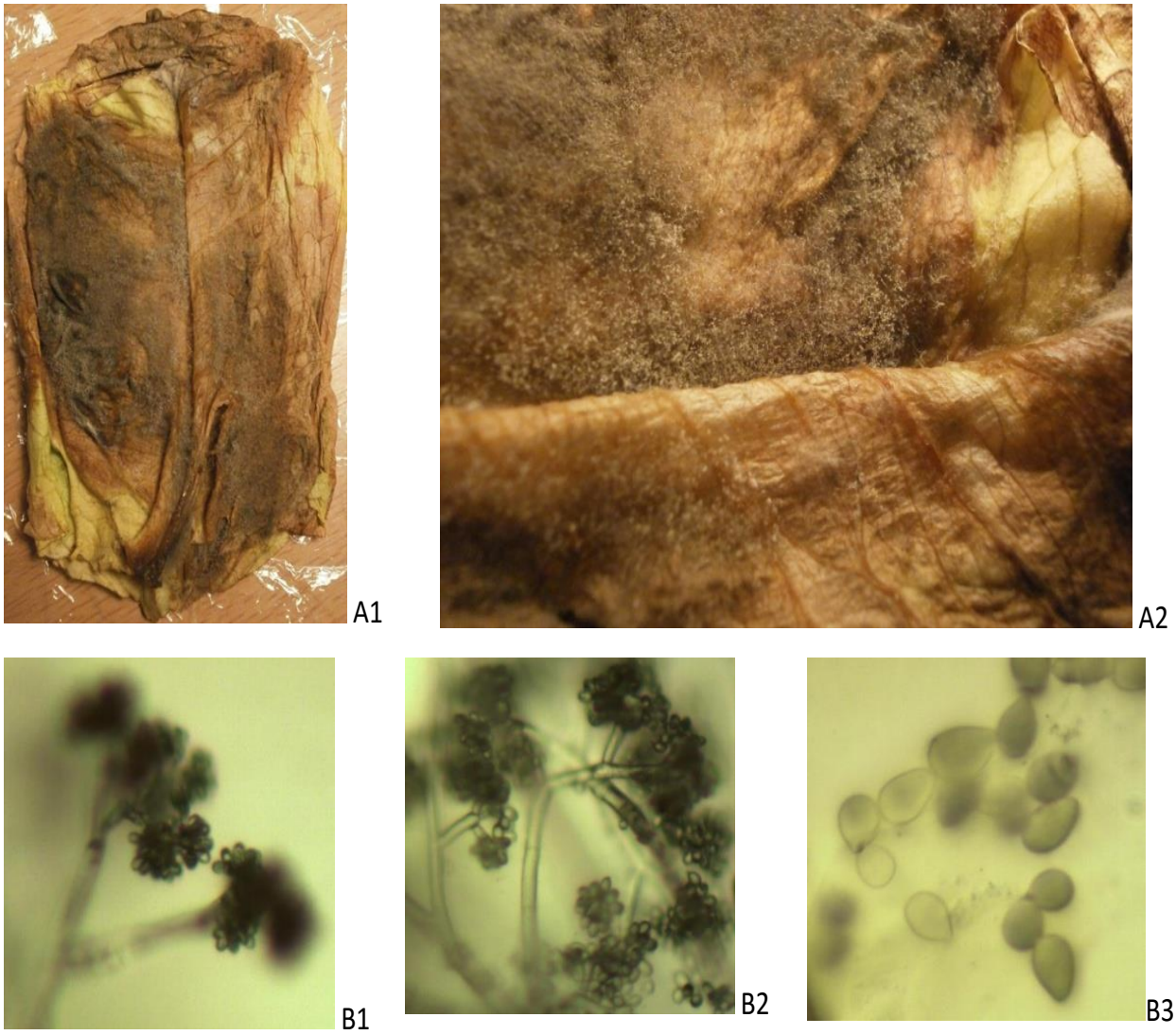


Figure 2. Infestation of lettuce with *Botrytis cinerea (Botrytis blight)* (A1), mold developed on the lettuce leave (A2), conidiospores and conidia of *Botrytis cinerea* x100 (B1), upper part of conidia x100 (B2), *Botrytis cinerea* conidia x 400 (B3)

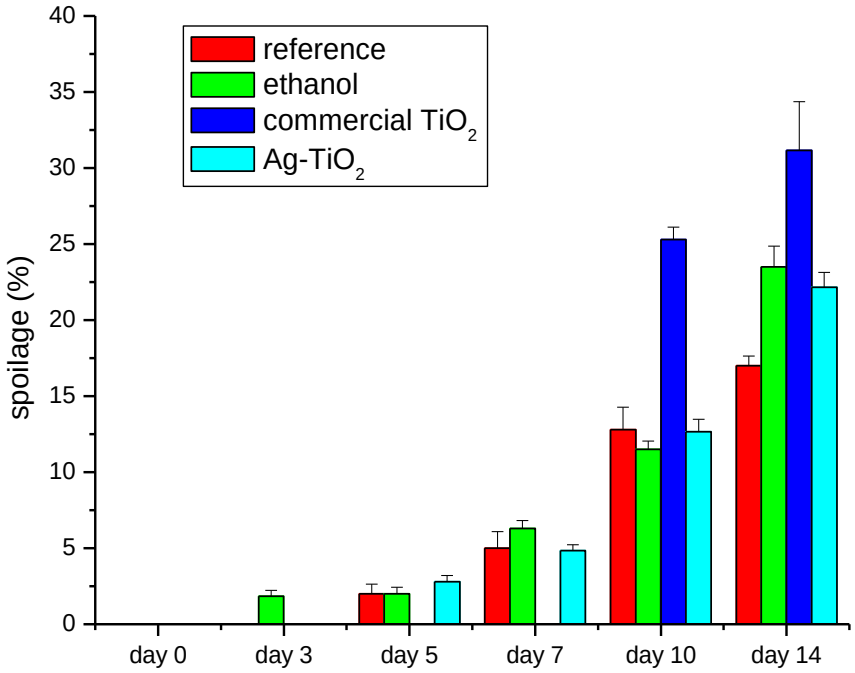


Figure 3. Spoilage of lettuce kept in the single-layer packages, during 14 days

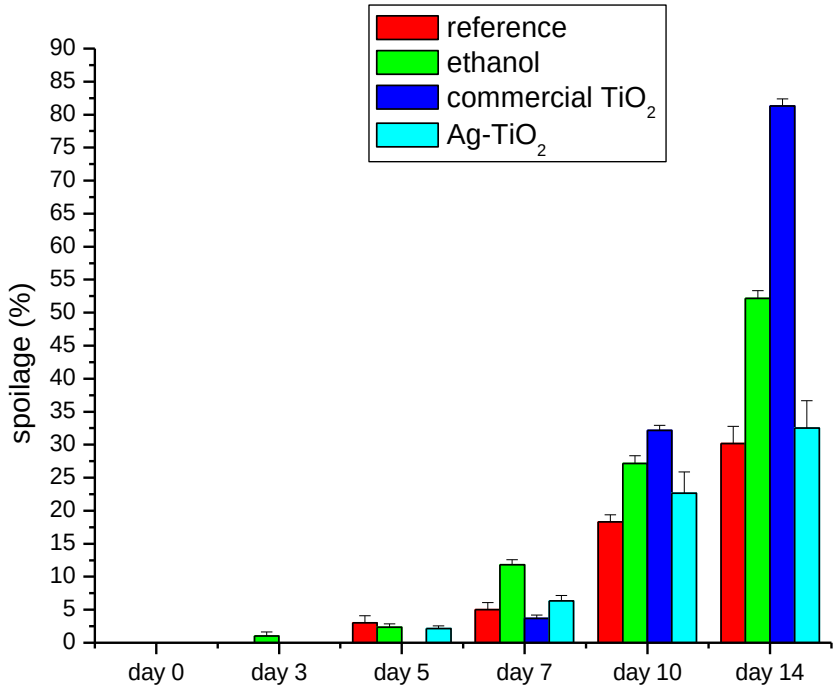


Figure 4. Spoilage of lettuce kept in the double-layer packages, during 14 days

After 7 days, the spoilage degree of lettuce in single-layer reference package was double that that at 5 days. The spoilage degree of single-layer package with ethanol was 7%. The spoilage degree of lettuce in single-layer package with Ag-TiO_2-SiO_2 increased by 5% from day 5. The spoilage of lettuce in single-layer package with Ag-TiO_2-SiO_2 is lower than that in reference package. After 7 days of storage, no spoilage of lettuce stored in single-layer package with commercial TiO_2 was observed, but 3.5% spoilage in double-layer package with TiO_2 was established. The spoilage degree of lettuce in double-layer package with ethanol and with Ag-TiO_2-SiO_2 significantly increased by 12% and by 6.5%, respectively from fifth day.

After 10 days, the spoilage of lettuce in single-layer reference package was 13% low as compared with than obtained in single-layer package with TiO_2 which significantly rises up to 25%. By comparing the single-layer package, the lowest spoilage was observed for lettuce in package with Ag-TiO_2-SiO_2 (8.5%). The spoilage of lettuce in single-layer package reference and with ethanol was doubled from day 7. The spoilage of lettuce in double-layer package with TiO_2 is high (35%) by 10% as compared with the value of single-layer same package and by 10 times higher than the value obtained after day 7. The spoilage of lettuce in double-layer package with ethanol was unchanged from day 5. In contrast, the spoilage of lettuce in double-layer package with Ag-TiO_2-SiO_2 was double than that at day 5.

After 14 days of storage, by comparing the results in the single-layer packages, the highest spoilage was observed for lettuce in package with commercial TiO_2 and the lowest value was determined for reference. The level of spoilage for lettuce in single-layer package with TiO_2 was higher than that in single-layer package with Ag-TiO_2-SiO_2. With regard to the double-layer packages, one can observe that the letucce in package with TiO_2 is the most intensely spoiled, as compared that in reference package and package with Ag-TiO_2-SiO_2 which was the less altered. By comparing the structure of the package with Ag-TiO_2-SiO_2, it can be observed that the degree of spoilage in single-layer package is 10% lower than that in double-layer package.

The microbial activity of TiO2 is given by its capacity to generate charge carriers (electrons and holes) when exposed to UV light. The charge carriers initiates redox reaction on the microbial cell surface that induces disruption of the cell wall, leakage of the intracellular constituents and, finally, cell lysis (Krishna et al. 2005). The degree of spoilage of lettuce stored in package with Ag-TiO2-SiO2 is higher than that stored in package with TiO2, fact that demonstrates the highest antimicrobial activity of Ag-TiO2-SiO2 as compared with TiO2 as a results of high porosity and presence of Ag particle and SiO2 phase.

The fact that the antimicrobial activity of package with Ag-TiO2-SiO2 was higher in single-layer that in double-layer is explained by the barrier assured by the presence of the second polyethylene film, than diminish the transfer of the photogenerated charge carriers to the lettuce in order to attack the mold.

4.Conclusions

The aim of this study was to establish the antimicrobial activity of polyethylene film modified with Ag-TiO_2-SiO_2 during storage of lettuce. The results showed that the shelf life of the lettuce stored in single- and double-layer layer film modified with TiO_2 was extended by 4 and 2 days, respectively as compared with the lettuce commercialized in normal atmosphere.

After 5 days of storage, the spoilage of the lettuce in double-layer film modified with Ag/TiO_2-SiO_2 was lower than that of the lettuce stored in film modified with ethanol and reference (unmodified film). After 5 days of storage, the lettuce stored, at 20^0C, in single-, as well as in double-layer package with commercial TiO_2 was not spoiled. After 14 days of storage, the lettuce stored in single-layer package with Ag-TiO_2-SiO_2 was less spoiled as compared with the other packages modified with TiO_2 or Ag-TiO_2-SiO_2.

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GEL PREPARATION FROM ALKALINE TREATMENT OF REFINED SAGO STARCH FROM TOP PART OF SAGO PALM

Abd Rashid Rinani Shima[1*], Ibrahim Hamizah[1], Hussin Farah Salina[1], Ahmad Noriza[1], Mohd Rosdan Faridatul Ain[1]

*[1]Universiti Kuala Lumpur - Malaysian Institute of Chemical & Bioengineering Technology, Alor Gajah, Melaka, Malaysia; * rinani@unikl.edu.my*

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ABSTRACT

The native sago starch has limited use in the food industry because of its undesirable physical and chemical properties. Therefore, modification of sago starch was needed to improve the undesirable properties. In this study, the effect modification with 1 % (v/v) potassium hydroxide (KOH) towards the sago starch gel was investigated. The phenolic compound reduction was also studied to see the effectiveness of enzyme treatment on sago starch by using 1U enzyme amyloglucosidase. The properties of gel that being determined were the texture, color, granule size and swelling power. The gel sago starch produced, resulting the texture which meets the requirement of starch in the food industry based on the gel hardness, cohesiveness, adhesiveness, gumminess, springiness and resilience. The color of the modified starch and enzymatic treatment starch was whiter with high L* value = 52.84 ± 0.20 than the native sago starch because of the decreasing in phenolic compound on sago starch which was 45.55 ± 0.17 mg/ml. Granule size was also larger compared to the native starch and shows the higher swelling power of sago starch reaching, 6.82 ± 0.1. Overall, sago starch gel produced after modification with KOH was better in terms of texture, color, and granule size and swelling power and thus allowed the modified sago starch used in food industry as a thickener, gelling agent and stabilizer.

1. Introduction

Starch modification is a process where the alteration of starch structure to overcome the unstable properties of native starch and improve its physical properties by affecting the hydrogen bond in a controllable manner. Usually, starch degradation can be done by several methods such as chemical degradation, physical alteration, genetic modification or enzymatic transformation. Chemically modified starches are of significant importance in many industrial applications. It can be used to improve functional properties of food products and used in the production of glue, coatings, chemicals and building materials (Yiu et al., 2008). These characteristics include viscosity, texture, clarity of forming pastes and binding properties (Keeling, 1997). The modification also somehow improves the gelatinization, pasting and retrogradation behavior (Singh et al., 2007). The effects of alkali on starch characteristics have not been studied as extensively as have other types of starch modification, such as acid and enzymatic hydrolysis. Therefore, this study was designed to determine the comparative susceptibilities of sago starch to alkali treatment. The results can be usefully applied to the development of products with improved textural attributes and

extended shelf life. In this study, the alkalizing agent used was potassium hydroxide (KOH) as a treatment to modify sago starch in order to improve the physicochemical properties of sago starch.

Different studies have been performed to determine the grade, quality and properties of starch extruded from different parts of the plant such as the base and mid heights of the sago palm. Moreover, particle size distribution, granule size and the pasting properties of starch have been found to vary from base to the top of bark from where starch exudates can be collected (Manan *et al*., 2008). In this study, the top part of sago palm was used to determine the physicochemical properties of sago starch. Native sago starch also undergoes enzymatic treatment to remove polyphenols on the surface of sago starch using amyloglucosidase.

2. Materials and methods

2.1. Materials

Raw sago palms of *Metroxylon rumphii* species were purchased locally in Batu Pahat, Johor and transported to the Food Technology laboratory for the project. The sago trunk was separated into top and bottom portions according to the method of Srioroth *et al.* (1999) as described by Hiroyuki *et al*., (2012). A stainless steel axe was used to separate the bark from the sago pith for each portion of the log. All other chemicals were analytical grade and obtained either from Merck, Darmstadt Co. or Sigma Chemical Co.

2.2. Extraction of Sago Starch

The top part sago pith has been cut into small cubes about 1-2cm. The cubes sago pith was ground with distilled water by using a blender. The slurry was filtered by using a sieve and squeezed to produce starch liquid. The resulting starch liquid has been left for 30 minutes to allow the sedimentation. The washing method was repeated several times until the clear water has been seen. Then, the sediment or starch paste was collected. The starch paste was drying in the drying oven at 60˚C for 24 hours and later was grounded into powder form by using mortar. The powder of sago starch was carefully stored at room temperature before further analysis.

2.3. Enzymatic Treatment of Sago Starch

Enzymatic treatment can be done after one unit enzyme of amyloglucosidase was determined. About 25 ml of acetate buffer was added to 10 grams of sago starch sample. Then, the sample was mixed with enzyme amyloglucosidase which consisting 1 Unit of enzyme concentration. A bottle of enzyme amyloglucisidase contains 300 U per 1 ml. The sample then was incubated at temperature 60 °C for 1 hour in the shaker water bath. The supernatant was collected after the incubation end. About 10 ml of distilled water was added to sample for washing purpose and was repeated for several times in order to remove the enzyme. The sample was drying using the drying oven at 60 °C for two days and lastly the dried starch was transferred to a Ziploic plastic bag used for further analysis.

2.4. Sago Starch Modification with Potassium hydroxide

20g of sample was dissolved in 250ml distilled water in a beaker. 5ml of potassium hydroxide (1% v/v) was added to the sample. Then, the sample was incubated in a water bath at 40˚C for 10 minutes. After the incubation, the sample has two layers which were supernatant and starch sediment. The supernatant was removed. In washing process, the starch sediment was added to distilled water, stirred the solution and left it for 30 minutes in order to let the starch sediment formation. The starch sediment was washed with distilled water until the pH was nearly neutral. The starch sediment was collected and dried in an oven at 60˚C for 24 hours. The dried modified starch was grounded with mortar until it turns into powder for further analysis.

2.5. Gel Preparation

The samples of sago starch were cooked in order to make it gelatinized and leave it to cool to form gels. 6g of sago starch sample was weighed and dissolved with 100ml distilled water in a beaker. The solution was cooked on a hot plate at 60-70˚C for 30 minutes and continuously stirred by using a magnetic stirrer. The solution was cooked until a shiny and clear gelatinized form of starch was formed. The gel was cooled at room temperature for 24 hours before further analysis.

2.6. Textural Profile Anaysis

The sample was prepared in gel formed to analysis the texture. Texture of gel was measured using the Texture Analyser (TA.XT.plus, Stable Micro System, UK). This instrument was coupled with easy to use of Exponent software. The sample was placed on container provided with a suitable probe for texture analysis. The probe used to be backward extrusion rig with the size of the disc was 45mm. The sample was placed on the platform to be tested by texture analyzer instrument. The gel was compressed at a speed of 5mm/s with a distance of 10mm. The parameters evaluated were gel hardness, gel cohesiveness, gel adhesiveness, gel gumminess, gel springiness and gel resilience.

2.7. Analysis of Colour

The sample was prepared in gel formed to analysis the color. The color characteristics were assessed using Chroma Meter CR-400/410 (Konica Minolta, Japan) to determine L* value (lightness or brightness), a* value (redness or greenness) and b* value (yellowness or blueness) of sago starch. Before analyzing the sample, the instrument was calibrated with a white standard tile: L* = 94.37, a* = -0.83 and b* = 0.02. The sample was placed on the cuvette of the Chromo Meter. Measurements were taken for triplicated and the average of L*, a* and b* value were obtained.

2.8. Microscopic Tests of Starch Granule Size

To observe the structure of the starch granules, the sample was prepared by 6g of sago starch was dissolved with 100ml distilled water. The solution was stirred homogeneously. The granule size was observed under a light microscope (Zeiss) which connected to a computer for better visual of microscopic. Two or three dropped of the solution was dispersed on the slide glass. The granule size was evaluated by using 100x of magnification power. By Motic Image Plus software, the parameters such as length and diameter of granule size have been measured and the image can be printed. The length and diameter of granule size were determined several times to obtain the average measurements.

2.9. Determination of swelling power

0.5g of sago starch sample was accurately weighed and quantitatively transferred into a clear dried centrifuge tube and re-weighed (W_1). The starch was then dispersed in 20ml of distilled water. The resultant slurry was heated at 95˚C for 30 minutes. The mixture was cooled to 30˚C and centrifuged (3000 rpm, 15 minutes). The supernatant obtained was carefully removed and the starch sediment was weighed as W_2. Analyses were performed in triplicate. Swelling power was calculated as follows:

$$\text{Swelling Power} = \frac{W_2 - W_1}{\text{weight of starch}} \quad (1)$$

2.10. Determination of Phenolic Compound

0.3g of the sample was placed in the filter paper and fitted it into extraction thimbles. Thimbles were transferred in soxhlet extraction (Soxhlet Extractor, Germany). The solvent used were methanol and acetone with a ratio of methanol to acetone was 20:80 which placed in solvent beaker. The soxhlet extraction was carried out for 4 hours to ensured complete extraction. Then, the solvent beaker was

transferred to rotary evaporator off the excess solvent at 50˚C. 1ml of sample was mixed with 7ml of distilled water and 0.5ml of Folin-Denis reagent in test tubes. The solution was thoroughly mixed by vortexing and incubated for 3 min at room temperature. 1ml of saturated sodium carbonate solution was added to the solution and made up to 10ml. The solution was incubated for one hour at ambient temperature. The absorbance was measured at 725nm using UV-VIS.

A series of standard was prepared by using galic acid (2mg/ml) into each of 50ml volumetric flasks and made up with distilled water. The concentration of polyphenols in the samples was derived from a standard curve of galic acid ranging from 20 to 120µg/mL. To obtain the phenolic compound standard curve, the concentrations of phenolic compound standards were plotted on the x-axis against absorbance was on the y-axis. The content of total phenols was expressed as galic acid equivalents in g/100 g extract.

3. Results and discussions

3.1. Texture Profile Analysis of modified sago starch

Overall, enzymatic treatment of sago starch with modification for the top palm of sago palm shows the highest gel hardness which were 3987.31 ± 0.28 (Table 1). Meanwhile, native of sago starch for top shows the lowest gel hardness. This is because amylopectin branching has negative effects on gel formation as acid hydrolysis attacks the amorphous region containing amylopectin. The factors affecting the mechanical properties of starch gels include the rheological properties of amylose matrix, interactions of gel constituents, and rigidity of starch granules. Mua and Jackson (1997) reported that higher amylose content or longer amylopectin chains result in stronger starch gels.

Cohesiveness was the quantity necessary to simulate the strength of the internal bonds, making up the sample or measurement of how well the structure of a product withstands compression. Meanwhile, the adhesiveness of gel was the work necessary to overcome the attractive forces between the surface of the food and the surface of other materials with which the food comes into contact (e.g. tongue, teeth, palate) or work required to pull food away from a surface. Springiness was the rate at which a deformed sample goes back to its undeformed condition after the deforming force was removed. Lastly, gumminess was the quantity necessary to simulate the energy required to disintegrate a semisolid sample to a steady state of swallowing. (So-Yoon Won et al., 2000). In this analysis, refined sago starch with modification for top part of sago palm shows the highest value for gel cohesiveness, adhesiveness, gumminess and springiness, while gel resilience was the lowest value as shown in Table 1.

3.2. Color analysis

The L* value gives an indication of the level whiteness of sago starch. The higher value of L*, the whiter the sago starch. Based on Table 2, top part of sago palm showed the highest value of L* for refined sago starch without modification and refined sago starch with modification (KOH) which were 45.54 ± 0.32 and 52.29 ± 0.20, respectively. Overall, native sago starch was less white than the others sample for top part of sago palm.

The low L* value obtained from native sago starch was due to oxidation of adsorbed catechin by polyphenol oxidase at the surface of the starch resulting in a dark greenish-blue complex. The presence of polyphenol oxidase enzyme of native sago starch, promotes enzymatic browning. During the oxidation process, aldehyde groups are formed and its presence increase the b* value or yellowness of starch color. Besides that, the natural color of some sago starch species and improper processing of the starch may lead to low value of L* (Tethool *et al.*, 2012).

3.3. Starch granules

Figure 1(a), (b) and (c) show the images of microscopic for native sago starch, refined sago starch without and with modification. The figure illustrated there was a change in arrangement of granules from native sago starch to refined sago starch with modification. The shapes of granules for all samples were similar but different in arrangement.

Figure 1(b) showed the granules of refined sago starch without modification. The arrangement of granules looks clearer compared than native sago starch and limited and isolated porous structures were observed. Besides that, hydrolyzed sago starch showed rough surface with some of the granules are still remained intact. Figure 1(c) showed that there was ruptured on the surface of sago starch. Modified sago starch with KOH showed more severe granule degradation than without modification. Alkaline solution probably reduces the rigidity and the stability of the molecular organization of the starch granule causing the loss of granule structure.

The average of starch granule for enzymatic treatment of sago starch with and without modification was bigger than native sago starch. This shows that the average size granule within the range 32.40 to 45.36μm. According to McCleary *et al.* (2006) sago starch grains have averaged 30 to 50μm in diameter. According to Hiroyuki et al., (2012), average particle size granule showed a decreasing trend from the base to the higher end of the sago trunk, thus indicating that potential exists for enhancing the value added potential of starch by segmenting the sections of the trunk from which it was extracted. The upper part of the trunk had the small particle size average of 32.75μm. Since, the starch was extracted from the top part of sago palm; the result was consistent with those reported by Hiroyuki et al., (2012).

3.4. Swelling Powers

Table 3 shows the swelling power of native sago starch, refined sago starch without and with modification. There was a progressive increase in swelling power for refined sago starch with modification (6.82 ± 0.09) compared to that refined sago starch without modification and native sago starch. The presence of KOH affected the swelling power of modified sago starch. It was investigated that the swelling power of sago starch increases with time of alkaline treatment and it has been reported that sago starch treated with 0.5% NaOH had a higher swelling power than those of starch treated with 0.1% NaOH (Karim *et al*., 2008). The amylose content also can affect the swelling power in starch. For example, high amylose corn starch containing more than 50% amylose has a very low swelling power (Pomeranz, 1991).

3.5. Phenolic Compound

The total phenolic compound was determined in order to observe the effect of enzyme amylogluside used on browning problem in starch. Table 4 shows the apparent total phenolic compound for native sago starch, refined sago starch without and with modification.

The total phenolic compounds for native sago starch, enzymatic treatment of sago starch without and with modification were 203.70 ± 0.26, 56.90 ± 0.17 and 45.55 ± 0.48 mg/ml, respectively. There was decreasing of total phenolic compound after the sample was treated with enzyme amyloglusidase. That shows, enzyme amyloglusidase was capable to remove the phenolic compound in sago starch. Treated under alkaline condition was shown the reducing of phenolic compound significantly, which might be due to the inactivity of phenolic compound. Coe S. *et al*., (2013) reported that polyphenols have an inhibitory effect on digestives enzymes amyloglucosidase. At higher concentrations of polyphenols, amyloglucosidase inhibition was increased. Hence, when the sago starch undergoes enzymatic treatment, the content polyphenol decreased. In contrast, native sago starch obtained the highest value phenolic compound

from the top part of sago palm. This is because the presence of high polyphenoloxidase activity during the extraction of these starch samples from the pith.

Table 1. Parameters of Texture Analysis for Top Part of Sago Starch

Sample	Gel Hardness	Gel Cohesiveness	Gel Adhesiveness	Gel Gumminess	Gel Springiness	Gel Resilience
Native sago starch	2200.54 ± 0.00	0.29 ± 0.00	- 153.95 ± 0.57	618.65 ± 0.31	0.55 ± 0.00	0.98 ± 0.00
Refined sago starch without modification	3574.40 ± 0.55	0.30 ± 0.00	- 142.90 ± 0.17	652.55 ± 0.45	0.62 ± 0.01	0.24 ± 0.00
Refined sago starch with modification	3987.31 ± 0.28	0.42 ± 0.00	- 20.27 ± 0.25	1105.76 ± 0.97	3.04 ± 0.04	0.15 ± 0.00

Table 2. Analysis Color of Sago Starch

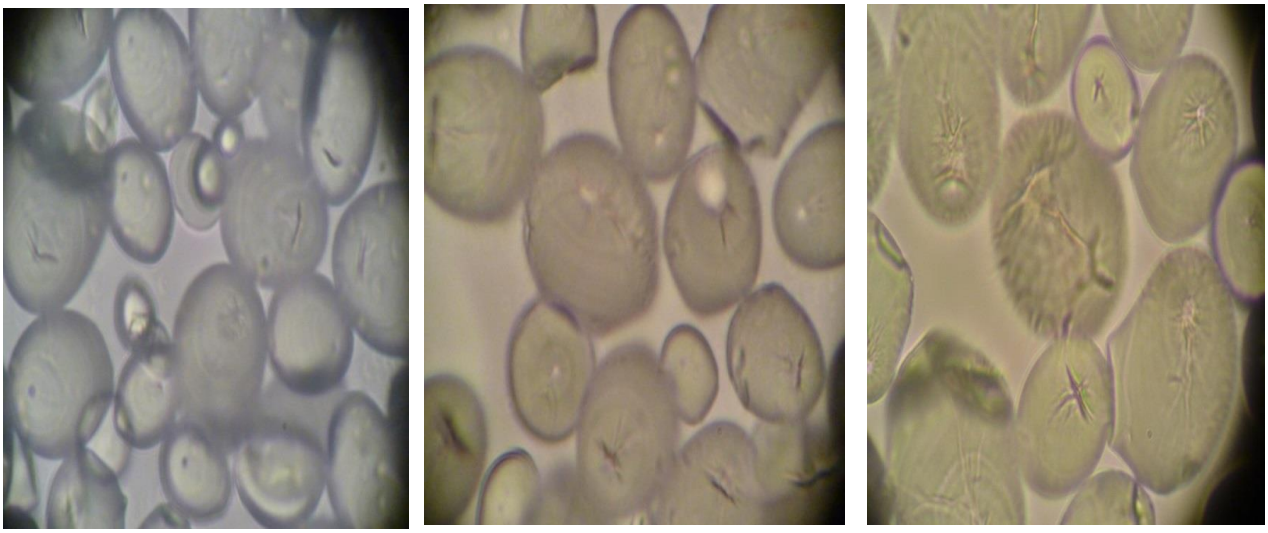
Sample	L*	a*	b*
Native sago starch	22.13 ± 0.12	0.57 ± 0.01	1.83 ± 0.02
Refined sago starch without modification	45.54 ± 0.32	0.16 ± 0.02	1.36 ± 0.00
Refined sago starch with modification	52.29 ± 0.20	0.12 ± 0.02	1.30 ± 0.00

Table 3. Swelling power of sago starch

Sample	Swelling Power
Native sago starch	3.27 ± 0.07
Refined sago starch without modification	3.58 ± 0.02
Refined sago starch with modification	6.82 ± 0.09

Table 4. Analysis of phenolic compound

Sample	Phenolic Compound (mg/ml)
Native sago starch	203.70 ± 0.26
Refined sago starch without modification	56.90 ± 0.17
Refined sago starch with modification	45.55 ± 0.48



(a) (b) (c)

Figure 1. Starch granules of (a) native sago starch; (b) refined sago starch without modification; (c) refined sago starch with modification at 40x magnification power.

4. Conclusions

The results have shown that the physicochemical properties of sago starch were affected due to potassium hydroxide (KOH) used acts as alkaline agent. The modified sago starch resulting the firm and elastic texture which acceptable in food industry in terms of hardness, cohesiveness, adhesiveness, gumminess, springiness and resilience. It can be said that KOH as the alkalizing agents influence the gelatinization behavior of sago starch. The phenolic compound in the sago pith was studied to examine the cause of browning of sago starch. The color of sago starch was whiter after treated with enzyme amyloglusidase due to the decreasing of phenolic compound contributed to the high of L* value was 52.84 ± 0.20. The granule of modified sago starch was bigger 46.16 ± 0.75 compared to the native sago starch and resulting the high of swelling power was 6.82 ± 0.09. There was progressive increasing in swelling power for modified sago starch compared to the native sago starch. As a conclusion, sago starch gel produced after modification with KOH was better in terms of texture, color, and granule size and swelling power and thus allowed the modified sago starch used in food industry as a thickener, gelling agent and stabilizer.

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EFFECT OF SUGAR SOLUTION ON MASS TRANSFER OF MANGO SLICES IN THE PROCESS OF OSMOTIC DEHYDRATION

S. M. Maruf Kabir[1*], Md. Ashraful Alam[2], M. B. Uddin[3]

[1]Department of Chemistry, Rajshahi University of Engineering and Technology, Rajshahi,
[2]Department of Chemistry, Rajshahi University of Engineering and Technology, Rajshahi and
[3]Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensigh-2202, Bangladesh,
** marufqc@gmail.com, pblqc@pip.prangroup.com*

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ABSTRACT

Osmotic drying is a partial dehydration process to give the product a quality improvement over the conventional drying process. The experiment was conducted for studying water loss(WL), sugar gain(SG), weight reduction(WR) and total solid(TS) during osmotic dehydration of mango slices (10 mm thick) in different concentration of sugar (40%, 50% and 60%) up to 6 hours at room temperature. It was found that increasing the concentration of the sugar solution used resulted in increased rates of water loss. The water loss at 40% sugar solution of mango slices were found to be significantly lower (p=0.05) than that of at 60% sugar solution. It was found there were rapid rates of water loss, sugar gain, and weight reduction for first four hour of the osmotic process.

1. Introduction

Mango (Mangifara Indica) is a delicious and popular fruit of our country. It is one of the most common tropical and sub tropical fruit, consumed largely because of its attractive flavour and refreshing sugar-acid balance and a very rich source of vitamin C and organic acids (Bartolomew et al., 1995). Mango contains sucrose, fructose and glucose in concentration that, in combination with acids and other compounds, determine the typical flavour of this fruit (Gherardi et al., 1994). The three varieties of mango grown in Bangladesh are Giankew, Calendar and Juldubi. The major mango growing areas in Bangladesh are Madhupur, Ghorashal and Chittangong Hill tracts. Osmotic dehydration is a method for the partial dehydration of water-rich foods, such as fruits and vegetables by immersing them in a concentration of sugar and salt. It results in two simultaneous crossed flows: a water out flow, from the food to the solution and a solute inflow from the solution into the food (Hough et al., 1993: Raoult-Wack et al., 1994; Spiazzi, 1997). The main problem is availability of adequate and sufficient processing technologies. Development of new technologies to process the mango products may promote the round year availability of mango and also serve the interest of the farmers.

The objectives of this study were to investigate the influence on water loss, sugar gain, weight reduction, normalized solids content during osmotic dehydration.

2. Materials and methods

The experiment was conducted in the Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh. The mangoes, having 10-30% coloration from base was collected from local market. Collected samples (*Ananas comosus*) were washed thoroughly in clean water to remove the adhering soil and organism, outer skin were peeled manually by using knife and cut into pieces of 10 mm thickness. The core of the slices was also removed by using core remover.

2.1.Osmotic dehydration

The dehydration solute used was sucrose (food grade, commercial granulated cane sugar manufactured by Lenart and Flink in 1984. Solutions of 40%, 50% and 60% (w/w) sucrose concentrations were prepared by blending an amount of sucrose with distilled water on a weight to weight basis. The weight mango slices were dipped in different concentration of sugar. The ratio of the fruits and sugar solution was 1: 6 in order to ensure proper soaking of the samples. Samples were taken at half an hour interval up to six hours, drained quickly and wiped gently with tissue paper and analyzed for different process variables like water loss, sugar gain, weight reduction, and total solid.

2.2.Effect of sugar solution

2.2.1.Calculation of water loss

Percent Water Loss (%WL) was defined as the net loss of water from the fresh mangoes after osmotic dehydration based on the initial sample weight and was calculated from the following equation:

$$\% \text{ WL} = \frac{(M_1)(1-TS_i)-(M_0)(1-TS_0)}{(M_1)} \times 100 \quad (1)$$

2.2.2.Calculation sugar gain

Sugar Gain (%SG) was defined as the net uptake of sugar by the osmosed mangoes based on the initial sample weight and was calculated by the following equation:

$$\% \text{ SG} = \frac{(M_0)(TS_0)-(M_i)(TS_i)}{(M_i)} \times 100 \quad (2)$$

2.2.4.Calculation of weight reduction

Weight Reduction (WR) was defined as the net difference in weight between the initial sample weight of the mangoes and the weight of the osmosed fruit based on the initial sample weight and was calculated by the following equation:

$$\% \text{ WR} = \frac{(M_i)-(M_0)}{M_i} \times 100 \quad (3)$$

where, M_i = initial weight of the raw mangoes; M_0 = weight of the osmosed mangoes; TS_i = initial total solids content of the raw mangoes; and TS_0 = total solids content of osmosed mangoes (% dry weight basis).

2.2.3.Calculation of total solid

Amount of total solids in the fruit was determined gravimetrically by vacuum oven drying at 70°C for 24 hours. The slices were transferred to a reweighed aluminum dish, weighed and dried in the vacuum oven. After cooling in a desiccators, the dish and the dried samples were reweighed. The percentage total solids (% TS) was calculated as follows:

$$\% \text{ TS} = \left(\frac{W_3 - W_1}{W_2 - W_1}\right) \times 100 \quad (4)$$

where, W_1 = Weight of aluminum dish; W_2 = Weight of dish and sample; W_3 = Weight of dish and the vacuum dried samples.

3. Results and discussions

3.1. Influence of osmotic solution concentration on mass transfer

The study of the influence of osmotic solution concentrations on mass transfer behavior during osmotic dehydration of mango slices was carried out using three different sugar solution concentrations of 40%, 50% and 60% (w/w). This experiment was carried out at temperature of 40°C and using of 10 mm mango slices throughout the experiment.

Fig. 1 to 4 illustrate plots of each of the respective mass transfer parameters (% WL, % SG, % WR and % TS) as a function of osmotic times due to the influence of three different sugar solution concentrations. It was observed from these figures that for all the three sugar solution concentrations (40%, 50% and 60%) studied, there were rapid rates of water loss (WL), sugar gain (SG) and weight reduction (WR) for the first hour of the osmotic process, after which the rate of these mass transfer parameters gradually slowed Khanom et al., down with time towards equilibrium end-point. Contreras and Smyrl (1981) as well as Lu and Brennan (1987) have also reported rapid removal of water and uptake of solids in the early stages of the osmotic process for apples. It was also found that both sugar solution concentration and osmotic time have highly significant effects ($p = 0.001$) on values of water loss, sugar gain, weight reduction and total solids of the osmosed mango.

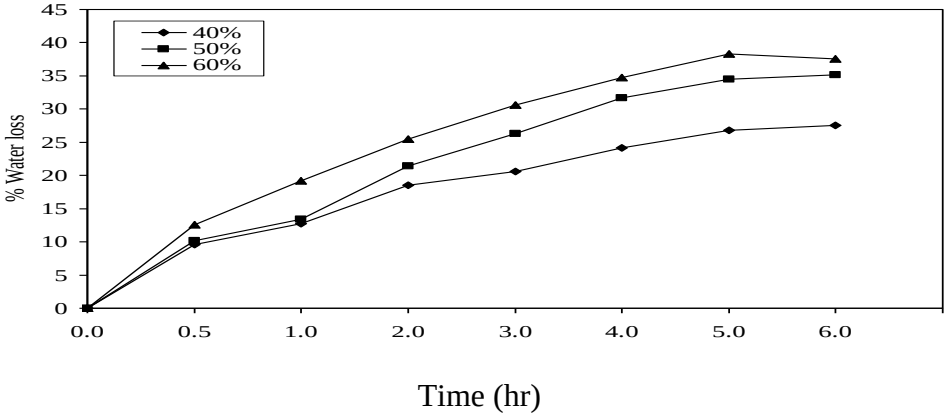

Figure 1. Effect of different sugar solution at 40°C on %water loss (WL) of mango slices of 10mm thickness

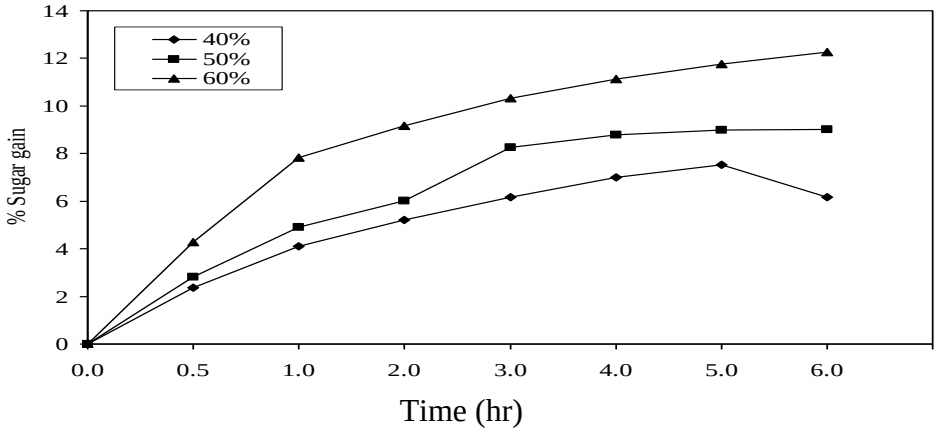

Figure 2. Effect of different sugar solution at 40°C on % sugar gain (SG) of mango slices of 10mm thickness

For water loss, the higher the concentration of the sugar solution used, the greater was its rate of water loss. When 40% sugar solution was used, the water loss values of the mango were found to be significantly lower ($p = 0.05$) than those of 60% sugar solution.

In the case of sugar uptake, Fig. 2 shows that sugar gain significantly increased ($p = 0.05$) with increasing sugar solution concentration. However, values sugar gain of the fruit obtained by using 40% sugar solution was not significantly different ($p = 0.05$) from that obtained when 50% sugar solution was used. This was observed in Figure 2 by the closeness of the two curves depicting changes of sugar gain of mango with osmotic time due to the 40% and 50% sugar solution concentrations.

3.2. Effect of sugar solution

Fig. 3 shows that increasing the sugar concentration of the osmotic solution from 40% to 60% increased the weight reduction values of the osmotically-dried fruit. Ponting et al. (1966) using apples, noticed that at high sugar concentrations (above 65%) additional increase in concentration did not promote further weight loss. Similar responses to concentration increases were observed by Contreras and Smyrl (1981) although there was a difference regarding the concentration cut point; that is, the point above which an increase in concentration was not followed by a significant increase in weight loss. This difference can be explained on the basis of differences in experimental setup among the above workers (Lazarides, 1994).

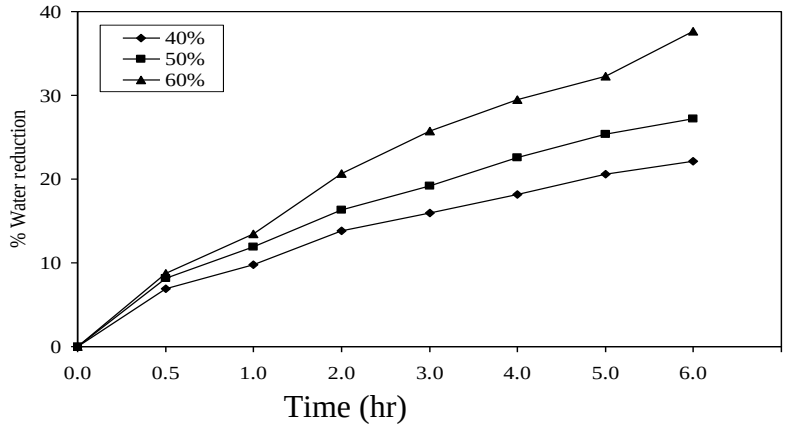

Figure 3. Effect of different sugar solution at 40°C on %weight reduction (WR) of mango slices of 10mm thickness

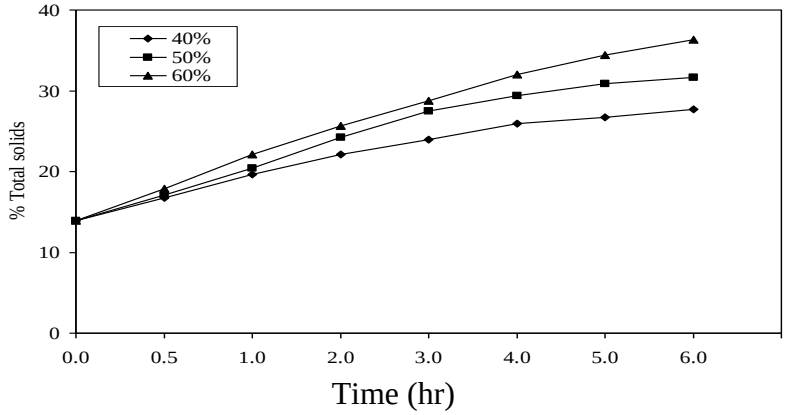

Figure 4. Effect of different sugar solution at 40°C on % total solids (TS) of mango slices of 10mm thickness

The higher amount of sugar uptake probably resulted in rapid development of a concentrated sugar layer under the surface of the fruit pieces, upsetting the osmotic pressure gradient across the fruit-sugar solution interface and therefore decreasing the driving force for water flow (Hawkes and Flink, (1978). Hughes *et al.,* (1958) working on the penetration of maltosaccharides in processed Clingstone peaches, reported that the rate of solute penetration was directly related to the solution concentration and inversely.

Khanom et al.,(2015*),* related to the size of the sugar molecule. At lower concentrations of sugar (40 to 50% sugar), WL/SG ratios were the highest for the first 30 minutes of osmosis indicating the rate of water loss from the mango was the highest during the first half hour of the osmotic process before the ratios began to drop and then increased or decreased slightly with time (Table 1). The TSo/TSi ratio (NSC) of the three sugar solution concentrations studied increased with increase in both sugar concentrations and osmotic time.

Furthermore, the WL/SG and TSo/TSi ratios (Table 1) for the 60% sugar concentration after 6 hours of osmotic process were 3.79 and 2.73, respectively (second highest in both cases). Sankat (1992) found that in the case of banana slices immersed in 50° Brix sugar solution for 36 hours, the reduction in weight of the fruit slices was only 24.2%.

Table 1.WL/SG ratio and normalised solids content (NSC) of osmosed mango as a function of fruit blanching treatments and osmotic time and sugar solutions

Osmotic Time (hours)	WL/SG ratio			NSC = TSo/TSi		
	Different Sugar Solution			Different Sugar solution		
	40%	50%	60%	40%	50%	60%
0.5	4.04	3.61	2.94	1.20	1.22	1.28
1.0	3.10	2.73	2.46	1.41	1.46	1.58
2.0	3.55	3.55	2.78	1.59	1.74	1.84
3.0	3.34	3.18	2.96	1.72	1.97	2.06
4.0	3.45	3.61	3.12	1.86	2.11	2.29
5.0	3.56	3.84	3.26	1.91	2.21	2.47
6.0	3.46	3.90	3.22	1.98	2.27	2.60
Mean*	3.5	3.48	2.96	1.66	1.85	2.01

* Mean of WL/SG ratios determined at seven osmotic times

Several researchers working with other fruits and vegetables (Hawkes and Flink, 1978; Moy et al., 1978; Islam and Flink, 1982; Conway et al., 1983; Lenart and Flink, 1984a and Pavasovic et al., 1986) have also reported increased water loss with increase in osmotic solution concentrations. However, they did not point out the occurrence of reduced WL/SG ratio when high osmotic solution concentration ($\geq$ 60% sugar solution) was used.

Hawkes and Flink (1978) in investigating the mass transport in the osmotic concentration of apples found that the mass transfer coefficient increased with sucrose concentration and was also influenced by agitation when the concentration of the sugar solution was 50% or greater. It should also be pointed out that, if the levels of sugar uptake into the fruit slices need to be a certain desired level, the sugar solution concentration that can maximize the rates of water loss and weight reduction and at the same time minimize the rate of sugar uptake had to be found.

4. Conclusions

Six hours of osmotic process, using 60% sugar solution is recommended for the osmotic dehydration of mangoes due to its high rates of water loss (39.56%) and weight reduction (37.63%) as compared to those of 50% sugar solution (35.18% water loss; 27.18% weight reduction) and 40% sugar solution (27.58% water loss; 22.15% weight reduction).

The level of sugar uptake in 6 hours of osmotic process in 60%, 50% and 40% sugar solutions were 12.26%, 9.02% and 6.18%, respectively.

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PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF TRADITIONAL *KOOZEH* CHEESE, RIPENED IN CLAY JUG AND PLASTIC CONTAINER

Babak Pakbin[1], Seyyed Hadi Razavi[2], Razzagh Mahmoudi[3*]

[1]Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
[2]Department of Food Science and Technology, Faculty of Biosystems Engineering, Campus of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.
[3]Department of Food Hygiene & safety, School of Health, Qazvin University of Medical Sciences, Qazvin, Iran.[*]r.mahmodi@yahoo.com

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ABSTRACT
Background: Studying physico-chemical and microbiological properties of traditional food products such as domestic cheeses is essential to standardize manufacturing method. Materials & methods: In this research phisyco-chemical and microbiological characteristics of traditional Koozeh cheese ripened in clay jug and plastic container, and produced domestically in Qazvin have been investigated. Result and discussion: Different phsico-chemical and microbiological properties observed between Koozeh cheese samples ripened in clay jug and plastic container, because of varied moisture content that leads to be different other characteristics of cheese. Moisture content of cheeses ripened in plastic container found higher than that in clay jug so other properties were different in samples. According to microbiological analyses, coliform and proteolytic bacteria counts in Koozeh cheese ripened in plastic container are higher than those in cheese ripened in clay jug, however moulds and yeasts, and Staph. aureus counts were lower in cheese ripened in plastic container. Also, Pearson correlation coefficient ($p < 0.05$) corresponded with obtained results. Based on our results, Low level of hygienic producing conditions and high variability found between physico-chemical and microbiological properties of Koozeh cheese samples taken from domestic producers in Qazvin. Finally, it is essential that a standardized production process on industrial scale be developed and hygienic quality be improved. Also, raw material that can be used for production of this traditional cheese (raw cow milk) must be pasteurized sufficiently to produce high level hygienic final product.

1. Introduction

The majority of traditional dairy products are domestic cheeses, the result of accumulated empirical science passed from generation to generation. It would be natural for the local traditional cheese to be made from the milk of autochthonous breeds such as domestic cow, sheep, ewe and goat (Alichanidis et al., 2008). Traditional cheese production methods eventually lead to using unstandardized process and causing variability in physicochemical, technological and sensory properties (Queiroga et al., 2013). Oliszewski et al. (2007) reported significant variability in physicochemical and

microbiological characteristics of Quesillo, an Argentinean traditional cheese. Cheese as primarily concentrated form of milk is rich in protein and calcium (Walther et al., 2008). As a recently studied nutritional aspect of traditional cheeses, bioactive peptides produces due to proteolytic activity during ripening period of cheese making and some other functional components (Choi et al., 2012) probably present through proteolysis in traditional cheeses (Moatsou et al., 2002; Khosrowshahi et al., 2006). Most important traditional cheeses produced from about 8000-9000 years ago until now in Iran, Iraq and Turkey area (Hayaloglu et al., 2002), include *Lighvan*, *Koozeh* (Edalatian Dovom et al., 2001), *Koopeh* (Madadlou et al., 2005) and Iranian white cheese (Madadlou et al., 2005) in Iran; *Beyaz, Tulum* and *Civil* in Turkey (Hayaloglu et al., 2002); *Kishfa* and *Gaymer* in Iran.

Koozeh cheese (Named in some area *Koopeh* cheese) is now produced domestically in east and west Azerbaijan (Edalatian Dovom et al., 20011), Kurdistan (Hassanzadazar and Ehsani, 2013) and Alamout area of Qazvin (Hosseini et al., 2010). Koozeh cheese is produced usually similar in mentioned areas of Iran but in ripening container; time and origin of employed milk is difference. For producing *Koozeh* cheese first milk with origin of area production (Raw sheep and cow milk in Azerbaijan and Kurdistan, also just raw cow milk in Qazvin area) must be tempering to 36-38°C then rennet is added to for coagulation of milk curd. After grinding of milk curd, 3-5% salt is added to be mixed with cheese granules. Salted cheese granules are impacted in a Jug (Clay jug in Azerbaijan and Kurdistan, also clay jug and plastic container in Qazvin area) whose door then would be sealed with clean cloths and clay. The impacted jugs are transferred underground in a shadow, a place with a balanced of air and moisture around the jug for ripening in an anaerobic condition in jug (3 months in Azerbaijan and Kurdistan, also 3-6 months in Qazvin area). After ripening period *Koozeh* cheese with granular shape is ready to consume (Edalatian Dovom et al., 2011; Hassanzadazar and Ehsani, 2013). Similar domestic cheeses are produced in neighbor countries of Iran such as Turkey and Iraq (Hassanzadazar and Ehsani, 2013). *Carra*, Literally meaning earthenware jug, is a traditional cheese most commonly manufactured and consumed in Turkey. It is produced from mostly goat`s milk and ripened in clay jug (Aygun et al., 2005) similar to *Koozeh* cheese manufactured in Iran. *Streptococcus parauberis, Lactococcus lactis*, *Enterococcus faecium* (Edalatian Dovom et al., 2011; Hassanzadazar and Ehsani, 2013), *Acinetobacter* (Aygun et al., 2005) and *Lactobacillus plantarum* (Hassanzadazar and Ehsani, 2013) were found in *Koozeh* cheese manufactured in Azerbaijan during and after the ripening period. Overall, physico-chemical and microbiological properties of *Koozeh* cheese specially manufactured in Qazvin area have not studied yet.

Studying physico-chemical and microbiological properties of traditional food products such as domestic cheeses is essential to standardize manufacturing method, limit variability in sensory and technological characteristics and improve hygienic condition of production (Alichanidis et al., 2008). Some preliminary work was carried out in the year 2007 by Oliszewski et al. to investigate chemical and microbiological properties of *Quesillo*, a traditional Argentinean cheese. They found shelf life of *Quesillo* produced domestically less than the product manufactured experimentally. Queiroga et al. (2013) also studied nutritional and textural aspects of *Coalho*, a Brazilian traditional cheese and found that reduction of goat milk during manufacturing of cheese did not lead to any changes in physico-chemical and textural characteristics of the final product. Aygun et al. (2005) investigated microbiological quality of *Carra* and reported poor hygienic quality of this domestic cheese produced in Turkey. However, there has been no study carried out on the properties of *Koozeh* cheese manufactured in Qazvin, the aim of our

research is to broaden current knowledge of physico-chemical and microbiological characteristics of this traditional cheese (Ripened in clay jug and plastic container) domestically produced in Qazvin, Iran.

2.Materials and methods

2.1.Cheese samples

Twenty *Koozeh* cheese samples (ten samples ripened in clay jug and ten samples ripened in plastic container) were taken according to standard FIL-IDF 50B (IDF, 1985) from ten domestic *Koozeh* cheese major producers in Qazvin. Samples then transferred to the laboratory under refrigerated conditions for physic-chemical and microbiological analysis.

2.2. Chemical and physical analyses

Titrable acidity of cheese samples were determined by the Dohrnic method (James, 1995). The pH value of cheese samples was measured using a digital pH-meter (Methrohm 744, Netherland). Moisture content of cheese also was determined by Vacuum-oven method (IDF, 1958). Lactose content was measured according to method reported in IDF (1982). Cheese analyzed for ash content according to the method in AOAC (2000). Salt content also determined by using Mohr method (Johnson and Olson, 1985). Cheese samples were analyzed for fat content by the Gerber method (James, 1995). Total protein (Total nitrogen) content of cheese samples was determined by measuring total nitrogen using the Kjeldahl method (AOAC, 2000) and converting it to protein content by multiplying by 6.38. Proteolysis index (Water Soluble Nitrogen/Total Nitrogen WSN/TN) measured according to method suggested by Ling (1963). Density and porosity measurements determined for all granular cheese samples with the method suggested by Mohsenin (Mohsenin, 1986).

2.3. Microbiological analyses

Eleven gram of each *Koozeh* samples (ripened in clay jug and plastic container) was homogenized for 2.0 min with a Lab-blender 400 Stomacher in 99 ml 2.0% sodium citrate solution. Serial dilutions were prepared in 0.1% sterile peptone water, and duplicate plates were used for all determinations. The following analyses were made on cheese samples: total colony counts on plate count agar (PCA) after incubation at 30 °C for 72 h; coliforms on violet red bile agar (VRBA) after incubation at 32 °C for 24 h; *Staphylococcus aureus* on Baird-Parker egg yolk-tellurite medium (BPM) incubated at 37 °C for 48 h; lactic acid bacteria (LAB) on MRS agar (Bergamini et al., 2009) incubated anaerobically at 30 °C for 3 days (Aneorobic jars (Biolab and Oxoid) with Anaeocult A kits (Merck IVD CE) were used for anaerobic incubation); moulds and yeasts on oxytetracycline glucose yeasts extract (OGYE) agar (v, 1992) (Oxoid) incubated at 22 °C for 5 days; proteolytic bacteria on Milk agar (Bergamini et al., 2009) incubated at 30 °C for 48 h.

2.4. Statistical analyses

All calculations were carried out in duplicate and all chemical and physical measurements were done in triplicate for each sample. All data expressed as mean values. The Standard Deviation of the results was evaluated for obtained physico-chemical and microbiological data. Pearson correlation coefficient was measured separately between analyzed properties of *Koozeh* cheese samples ripened in clay jug and plastic container. All statistical analyses were conducted using the software SPSS (v. 17, Chicago IL, USA).

3. Results and discussions

3.1.Physico-chemical analyses of *Koozeh* cheese samples

Physico-chemical characteristics of *Koozeh* cheese samples ripened in clay jug and plastic container that were produced in Qazvin are shown in Table 1. Also Pearson correlation coefficient between phisyco-chemical and microbiological properties of *Koozeh* cheeses ripened in clay jug and plastic container presented in Table 3 and 4, respectively.

Accordingly, obtained results demonstrated significant differences in chemical and physical properties of *Koozeh* cheese samples ripened in clay jug and plastic container and all characteristics in all cheese samples that had been taken from different producers in Qazvin. There are two major factors lead to variations between samples: different ripening condition (in clay jug and plastic container) and different producing conditions between the producers. While cheese granules placed into the clay jug containers under anaerobic condition for ripening, to permit the removal of moisture is higher than that in compared with plastic containers (Hassanzadazar et al., 2012). Also, Hassanzadazar and Ehsani (2013) reported that the initial microbiological properties of *Koozeh* cheese according to its ripening container are significantly different. Moisture contents influenced the physico-chemical and especially microbiological properties of cheese during ripening (Simal et al., 2001; Zalazar et al., 2002). Different microbiological characteristics also had significantly impact on chemical properties of ripened cheese (Menéndez et al., 2001) with the same initial used milk (Park et al., 2007). According to Table 1, moisture content of cheese samples ripened in plastic container is higher than that in clay jug, 60.37 and 53.81% in clay jug and plastic container respectively. Consequently, other chemical characteristics have been affected and were different between samples with varied moisture content. While moisture content of cheese is increased, therefore fat and protein contents is lowered (Oner et al., 2006) that it can be observed in Table 1 between cheese samples. A significant (p<0.05) negative correlation was found between fat and moisture contents in both kind of *Koozeh* cheese samples (Table 3 and 4). Water soluble nitrogen is a fraction of cheese protein [Total nitrogen] released during ripening of cheese by proteolysis of microbial activity (Madadlou). Nevertheless, different moisture content and other chemical composition of cheese lead to different proteolytic microbial ripening conditions, so proteolysis index should be varied between cheese samples ripened in clay jug and plastic container, as it is indicated from Table 1, proteolysis index is higher in cheese ripened in plastic container. It was interesting that a significant (p<0.05) positive correlation was recorded between proteolysis index and proteolytic bacteria counts in *Koozeh* cheese samples ripened in clay jug and plastic container (Table 3 and 4). Proteolytic activity during ripening of cheese attributes to releasing of bioactive peptides that have many health benefits for consumers (Settanni et al., 2010). Density of cheeses declines with enhancement of moisture content and decrease in protein content in cheese composition with lower fat content (McMahon et al., 2005) as Table 1 demonstrated higher density for cheese samples ripened in clay jug. The significant (p<0.05) negative correlation was found between both moisture content and porosity, and density of cheese samples. Also, significant (p<0.05) positive correlation was observed between density and protein content of cheese samples (Table 3 and 4); finally, these Pearson correlation coefficients have proved obtained results. As indicated through standard deviation of 10 *Koozeh* cheese producer groups, drastic variability could be observed in all characteristics between all samples. Differences in domestic producing conditions and initial chemical and microbiological properties of employed milk make variability in characteristics of final product (Ozer et al., 2002). Innocente et al. (2007) reported high variability in physic-chemical properties of semi-hard Italian traditional cheese samples produced domestically. Tarakci et al. (2004) also reported drastic variability in properties of herby cheese, a traditional cheese produced in Turkey.

3.2.Microbiological analyses of *Koozeh* cheese samples

Microbiological properties of *Koozeh* cheese samples ripened in clay jug and plastic container that were domestically produced in Qazvin are presented in Table 2. Also Pearson correlation coefficient between phisyco-

chemical and microbiological properties of *Koozeh* cheeses ripened in clay jug and plastic container presented in Table 3 and 4, respectively. The coliform and proteolytic bacteria counts in *Koozeh* cheese ripened in plastic container are higher than those in cheese ripened in clay jug, however moulds and yeasts and *Staph. aureus* counts were lower in cheese ripened in plastic container. Hassanzadazar and Ehsani (2012)reported 9.6×10^6 CFU/g as microbial total count in *Koozeh* cheese ripened in clay jug that produced in Azerbaijan and we found in this study over than 10^7 CFU/g microbial total count in *Koozeh* cheese samples domestically produced in Qazvin. High microbial total count indicates poor hygienic conditions of cheese production (Beresford et al., 2001). The number of yeasts and molds in our study should be accounted for by the explanation of Beresford et al. (2001) that low pH, moisture and high salt level during ripening of cheese contribute to the growth of yeasts. As demonstrated in Table 2, yeasts and molds count are higher in cheese ripened in clay jug considering low moisture content of them that were shown in Table 1. Aygun et al. (2005) found *Staph. Aureus* in *carra* cheese, a traditional Turkish cheese, ranging from 10^2 to 10^4 CFU/g and most traditional cheeses produced domestically such as Tulum, Herby and White cheeses reported to be contaminated with *Staph. aureus* over than 10^2 CFU/g microbial count (Aygun et al., 2005). *Staph. aureus* microbial count is ranging in *Koozeh* cheese samples in this study from <10 CFU/g in cheese ripened in plastic container to over than 10^2 CFU/g in samples ripened in clay jug. Moisture and salt contents influence the growth of *Staph. aureus* in cheeses (Beresford et al., 2001).

Table 1. Physico-chemical properties of 10 *Koozeh* cheese samples produced domestically in Qazvin, and ripened in clay jug and plastic container.

	Ripened in clay jug				Ripened in plastic container			
	Mean	SD[A]	Minimum	Maximum	Mean	SD	Minimum	Maximum
Titrable Acidity[B]	1.24	0.289	0.87	1.69	1.1	0.243	0.79	1.43
pH	4.36	0.231	4.11	4.86	4.28	0.155	4.07	4.55
%Moisture	53.81	3.12	49.57	59.48	60.37	4.08	53.42	66.65
%Lactose	0.524	0.161	0.29	0.75	0.523	0.355	0.12	1.16
%Ash	2.69	0.749	1.63	3.73	1.65	0.460	0.97	2.42
%Salt	3.08	0.59	2.23	4.17	2.94	0.76	1.95	4.95
%Fat	20.17	1.48	18.39	22.18	15.93	2.99	11.47	20.32
%Protein	24.61	1.39	22.32	26.63	18.68	1.88	15.45	21.12
Proteolysis index[C]	0.049	0.018	0.028	0.083	0.117	0.039	0.053	0.182
Density [g/cm^3]	1.898	0.0346	1.828	1.947	1.838	0.0161	1.814	1.867
Porosity[D]	0.429	0.009	0.417	0.448	0.517	0.022	0.470	0.551

[A] SD, Standard Deviation, [B] Titrable Acidity = % lactic acid, [C] Proteolysis index = WSN/TN [Water Soluble Nitrogen/Total Nitrogen], [D] Porosity = 1-[$p_{bulk}/p_{particle}$]

Table 2. The microbial counts of 10 *Koozeh* cheese samples produced domestically in Qazvin, and ripened in clay jug and plastic container [CFU/g].

Microorganisms	Ripened in clay jug				Ripened in plastic container			
	Mean	SD[A]	Minimum	Maximum	Mean	SD	Minimum	Maximum
Total Bacteria	1.84×10^7	2.55×10^7	1.30×10^6	8.4×10^7	8.40×10^7	1.40×10^8	3.4×10^6	4.4×10^8
Coliforms	1.14×10^2	1.19×10^2	1.4×10	3.60×10^2	9.39×10^3	2.25×10^4	8.3×10	7.3×10^4
Staph. aureus	7.91×10^2	1.01×10^3	3.4×10	2.70×10^3	1.8	1.13	0	4
LAB[B]	3.59×10^5	3.76×10^5	3.40×10^4	9.80×10^5	5.69×10^5	1.32×10^6	1.60×10^4	4.30×10^6
Yeasts and Molds	6.69×10^4	9.77×10^4	1.60×10^3	3.2×10^5	1.12×10^3	1.63×10^3	9.8×10	5.5×10^3
Proteolytic bacteria	5.41×10^3	4.96×10^3	7.40×10^2	1.50×10^4	1.00×10^5	1.65×10^5	4.20×10^3	4.30×10^5

[A] SD, Standard Deviatio, [B] LAB=Lactic Acid Bacteria

It was interesting that a significant (p<0.05) positive correlation was recorded between *Staph. aureus* counts and salt content in *Koozeh* cheese samples ripened in clay (Table 3). Psoni et al. (2003) found 10^6 CFU/g *Staph. aureus* in *Batzos* samples, a traditional Greek cheese, produced in Greece. They stated that low level hygienic condition of cheese production contributes to contamination with *Staph. aureus*. Coliform microbial counts in *Koozeh* cheese ripened in plastic container is higher than those in cheese ripened in clay jug and overall approximately same coliform microbial counts in compared with that we found in this research, reported by Aygun et al. [14] for *Carra* traditional cheese samples. Hassanzadazar and Ehsani (2013) reported LAB counts ranging from 10^4 to 10^5 CFU/g in *Koozeh* cheese manufactured in Azarbaijan and in this study also we found the same range of LAB counts in *Koozeh* cheese samples produced in Qazvin. As indicated in Table 2, proteolytic bacteria counts is higher in *Koozeh* cheese ripened in plastic container than that in clay jug. Proteolysis is one of the principal biochemical events during the ripening of cheese acted by proteolytic bacteria and proteolysis index also indicates proteolysis level in ripening of cheese (Madadlou et al., 2005) As it was shown in Table 1 *Koozeh* cheese ripened in plastic container had higher proteolysis index than that in clay jug that this corresponded with result obtained for proteolytic bacteria counts in Table 2 between cheeses ripened in clay jug and plastic container. Also, significant (p<0.05) positive correlation coefficient between proteolysis index and proteolytic bacteria counts that mentioned in physico-chemical analyses section corresponds with this result.

High variability also found in microbiological properties between *Koozeh* cheese samples taken from 10 domestic producers. Psoni et al. (2003) on *Batzos* (traditional Greek cheese), Aygun et al. [14] on *Carra* (Traditional Turkish cheese) reported drastic variability in microbiological characteristics of samples produced domestically.

Table 3. Pearson correlation coefficients between physico-chemical and microbiological properties of 10 *Koozeh* cheese samples ripened in clay jug.

	TA	pH	Moisture	Lactose	Ash	Salt	Fat	Protein	Pi	Density	Porosity	TC	Coliforms	*S. aureus*	LAB	Y & M	Proteolytic
TA[A]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH	0.088	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
%Moisture	-0.194	0.144	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
%Lactose	-0.524	-0.527	-0.255	-	-	-	-	-	-	-	-	-	-	-	-	-	-
%Ash	-0.303	0.259	-0.609	0.153	-	-	-	-	-	-	-	-	-	-	-	-	-
%Salt	-0.203	0.443	-0.042	-0.033	0.560	-	-	-	-	-	-	-	-	-	-	-	-
%Fat	0.082	-0.633*	-0.623	0.370	0.331	-0.066	-	-	-	-	-	-	-	-	-	-	-
%Protein	-0.021	0.366	0.373	-0.177	0.095	0.642	-0.076	-	-	-	-	-	-	-	-	-	-
Pi[B]	-0.277	-0.122	-0.445	0.548	0.469	0.383	0.137	0.066	-	-	-	-	-	-	-	-	-
Density[C]	0.017	0.311	0.542	-0.342	-0.093	0.292	-0.093	0.854*	-0.206	-	-	-	-	-	-	-	-
Porosity	0.209	-0.072	-0.796*	0.117	0.369	-0.374	0.313	-0.698	0.118	-0.722*	-	-	-	-	-	-	-
TC[D]**	0.182	-0.210	-0.176	-0.153	-0.152	0.058	-0.064	-0.242	0.387	-0.380	0.019	-	-	-	-	-	-
Coliforms**	0.275	-.077	-0.495	0.057	0.091	-0.131	-0.100	-0.535	0.407	-0.777*	0.630	0.620	-	-	-	-	-
*S. aureus***	-0.060	0.320	-0.126	-0.153	0.445	0.774*	-0.206	0.250	0.568	-0.015	-0.197	0.559	0.284	-	-	-	-
LAB[E]**	-0.108	0.166	0.009	-0.186	0.170	0.256	-0.386	-0.252	0.400	-0.241	-0.047	0.622	0.355	0.068	-	-	-
Y & M[F]**	0.385	-0.185	-0.025	0.232	-0.263	0.121	0.247	0.129	-0.138	0.060	-0.208	-0.229	-0.257	-0.104	-0.275	-	-
Proteolytic**	-0.138	-0.048	-0.549	0.360	0.449	0.385	0.196	0.081	0.941**	-0.173	0.148	0.521	0.409	0.594	0.396	-0.158	-

* p < 0.0, ** Microbial count as CFU/g,[A] TA, Titrable Acidity as % lactic acid,[B] Pi=Proteolysis index,[C] Density [g/cm^3], [D] TC=Total Count,[E] LAB=Lactic Acid Bacteria, [F] Y & M=Yeasts and Molds

Table 4. Pearson correlation coefficients between physico-chemical and microbiological properties of 10 *Koozeh* cheese samples ripened in plastic container.

	TA	pH	Moisture	Lactose	Ash	Salt	Fat	Protein	Pi	Density	Porosity	TC	Coliforms	*S. aureus*	LAB	Y & M	Proteolytic
TA[A]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH	-0.935*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
%Moisture	0.015	-0.025	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
%Lactose	0.373	-0.303	-0.442	-	-	-	-	-	-	-	-	-	-	-	-	-	-
%Ash	-0.343	0.380	0.390	-0.350	-	-	-	-	-	-	-	-	-	-	-	-	-
%Salt	-0.336	0.337	0.555	-0.609	0.921*	-	-	-	-	-	-	-	-	-	-	-	-
%Fat	-0.264	0.230	-0.696	-0.132	-0.078	-0.257	-	-	-	-	-	-	-	-	-	-	-
%Protein	-0.432	0.320	-0.372	0.049	-0.064	-0.173	0.170	-	-	-	-	-	-	-	-	-	-
Pi[B]	-0.293	0.298	0.403	-0.544	0.671	0.414	0.056	0.089	-	-	-	-	-	-	-	-	-
Density[C]	-0.001	-0.139	-0.258	-0.171	0.004	-0.241	0.184	0.735*	-0.233	-	-	-	-	-	-	-	-
Porosity	0.075	-0.009	0.255	0.113	-0.026	0.230	-0.060	-0.426	0.453	-0.912*	-	-	-	-	-	-	-
TC[D]**	0.555	-0.623	-0.310	0.195	-0.182	-0.197	-0.050	-0.198	-0.501	0.030	-0.090	-	-	-	-	-	-
Coliforms**	-0.388	0.528	0.188	-0.235	0.606	0.078	-0.058	-0.357	0.407	-0.293	0.513	-0.081	-	-	-	-	-
*S. aureus***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LAB[E]**	-0.435	0.500	-0.243	0.162	0.054	-0.071	-0.127	0.429	-0.425	-0.411	-0.390	-0.145	0.016	-	-	-	-
Y & M[F]**	-0.203	0.312	-0.339	0.344	-0.214	-0.347	0.020	0.228	-0.606	0.408	-0.375	-0.147	-0.167	-	0.089	-	-
Proteolytic**	-0.250	0.418	0.055	-0.436	0.722	0.620	0.228	0.081	0.770*	-0.222	0.268	-0.309	0.586	-	-0.105	-0.231	-

* $p < 0.05$, ** Microbial count as CFU/g,[A] TA, Titrable Acidity as % lactic acid,[B] Pi=Proteolysis index [C] Density [g/cm^3],[D] TC=Total Count,[E] LAB=Lactic Acid Bacteria,[F] Y & M=Yeasts and Molds

4. Conclusions

Based on our results, Low level of hygienic producing conditions and high variability found between physico-chemical and microbiological properties of Koozeh cheese samples taken from domestic producers in Qazvin. Finally, it is essential that a standardized production process on industrial scale be developed and hygienic quality be improved. Also, raw material that can be used for production of this traditional cheese (raw cow milk) must be pasteurized sufficiently to produce high level hygienic final product.

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EVALUATION OF POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY OF TWO TABLE GRAPE VARIETIES UNDER ENVIRONMENTAL CONDITIONS OF THESSALY

Nikolaos Gougoulias[1*], Panagiotis Vyrlas[1], Liviu Giurgiulescu[2], Dimitrios Kalfountzos[1], Fola Eugenia[1]

[1] Department of Agronomy Technology, Technological Educational Institute of Thessaly, 41110 Larissa, Greece

[1]Technical University Cluj Napoca, Department of Chemistry and Biology, Victor Babes 76, 430083, Baia Mare, Romania

[*]ngougoulias@teilar.gr

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ABSTRACT

This paper examines the seeds, the skin and the flesh of two table grape varieties grown in Larissa, Greece, namely, the white variety Victoria and the colorful variety Muscat Hamburg, as to the content of total phenols (TP), non-flavonoid phenols (NFP), flavonoid phenols (FP), tannin flavonoids (TF), non-tannin phenols (NTP), total flavanols (F-3-ols), antiradical (DPPH$^{\bullet}$) and ferric reducing power (FRAP). The content of (TP) in the flesh, the skin and the seeds of grapes varied in a range from 0.64 mg (GAE)/g f.m. for the flesh of the variety Victoria to 11.14 mg (GAE)/g f.m. for the seeds of Muscat Hamburg variety. The (FP) and (TF) was the major phenolic fraction. The content of total F-3-ols which include catechin and epicatechin (monomers) and procyanidins (dimers, trimers and tetramers) is varying from 0.118 mg (CE)/g f.m. for the flesh of the variety Victoria to 2.40 mg (CE)/g f.m. for seeds of the variety Muscat Hamburg. The antiradical power (DPPH$^{\bullet}$) ranges from 0.61 μmol DPPH$^{\bullet}$/g f.m. for the flesh of Victoria variety to 16.83 μmol DPPH$^{\bullet}$/g for the seeds of the same variety and the antioxidant capacity ranges from 1.88 μmol FRAP/g f.m. for the flesh of Victoria to 150.3 μmol FRAP/g f.m. for the seeds of Muscat Hamburg.

1. Introduction

For a healthy diet it should be paid particular intentness to natural foods, including table grapes, which are rich in chemical substances which have nutritional effects in the human body. Such substances are the polyphenols which are known as powerful natural antioxidants (Kanner et al., 1994). The intake of polyphenols with food reduces the risk of cardiovascular, carcinogens and other diseases, because of the commitment of the oxygen free radicals (ROS), which damage the cells of the human body (Kanner et al., 1994; Bravo, 1998).

The biosynthesis of polyphenols in plants and grapes is associated with many physiological and biochemical processes (Moore,1989). Many studies have shown that exogenous application of phytohormones in grapevine can improve the quality and yield of grapes (Basra, 2000; Gougoulias and Masheva, 2010; 2011).

The purpose of this study is to quantify and evaluate polyphenols, phenolic fractions and

antioxidant properties in the flesh, skin and seeds of two table grape varieties grown in Larissa, Greece.

2. Materials and methods

The experiment was conducted in the vineyard Gkarani located at the outskirts of Larissa in 2014. Planting took place 15 years ago with two table-grape varieties, the Victoria and Muscat Hamburg. The vine layout is 1.5m x 1.5m (plant spacing x row spacing). The white grape Victoria variety originated from the crossbreed of Cardinal variety and Afuz Ali variety in Romania, while the colored variety Muscat Hamburg grapes came from the crossbreed of the Italian variety Schiava Grossa and the Egyptian Muscat of Alexandria, in England. The cultivation include the application of 30 kg of fungicide (sulfur), 42.5 kg nitrogen, 18 kg P_2O_5 and 25.5 kg K_2O per hectare while 6500 m^3/ha of irrigation water were added.

The grape was collected in the phase of technological maturity from which 100 grape berries were randomly selected for chemical analysis. The grape extracts were obtained after twofold treatment of 20 g sample with 60%-methanol solution after 1-hour storage at dark and room temperature. The collected extracts after centrifugation (filtration) were brought to 50 ml with aqueous methanol and used for chemical analysis. The grape skins and seeds were separated manually and the extracts were prepared after extraction of the corresponding amount of fresh material (Meyeret al., 1997).

Total polyphenols (TP) contents were determined with the Folin–Ciocalteu reagent according to the method of (Singleton and Rossi, 1965) and were expressed as gallic acid equivalent (GAE).

The non-flavonoid phenols (NFP) were determined with the Folin–Ciocalteu reagent after the removal of flavonoid phenols (FP) with formaldehyde according to the method of (Kramling and Singleton, 1969). FP content was determined as a difference between the content of TP and NFP.

The content of nontannin phenols was determined by removing the tannins using the method of (Peri and Pompei, 1971) by precipitation with quinine sulphate. The amount of NTP was estimated with the Folin–Ciocalteu reagent and expressed in the same units (GAE). The tannin flavonoids (TF) content was evaluated indirectly as a difference from the total phenols (TP).

The total flavanols (F-3-ols, catechins and procyanidins) were assayed using p-DMACA reagent after the method of (Li, et al. 1996) and were presented as catechin equivalent (CE).

The antiradical activity of the methanol extracts was determined according to the method of (Brand-Williams, et al. 1995) applying the stable radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH). The activity was evaluated in μmol DPPH/kg fresh matter.

The ferric reducing antioxidant power (FRAP) was evaluated according to the method of (Benzie, I.F.F. and Strain, J.J. 1999) and was expressed as μmol FRAP reagent/kg fresh matter.

Soil samples were analysed using the following methods which are referred by (Page, et al. 1982).

Organic matter was analyzed by chemical oxidation with 1 mol L^{-1} $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol L^{-1} $FeSO_4$.

Inorganic forms of nitrogen were extracted with 0.5 mol L^{-1} $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively.

Available P forms (Olsen P) were extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured by spectroscopy.

Exchangeable forms of potassium and sodium were extracted with 1 mol L^{-1} CH_3COONH_4 and measured by flame Photometer (Essex, UK).

Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triamine pentaacetic acid 0.005 mol L-1 + $CaCl_2$ 0.01 mol L-1 + triethanolamine 0.1 mol L-1) and measured by atomic absorption.

The pH, the Brix degrees and the total acidity were measured in grape must. The Brix degrees by a Zeiss refract meter, while the total acidity by titration with 0.1N NaOH solution and expressed in g of citric acid/100 ml grape must.

The chemical analyses were the mean of four parallel samples. Data analysis was made using the MINITAB (Ryan et. al., 2005) statistical package. Analysis of variance was used to assess treatments effect. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

3. Results and discussions

The soil where the grapes are grown has a low salinity and a high content of organic matter, available K and P (Table 1).

Table 1. Chemical soil properties at the beginning of crops

Soil properties	**Soil depth (0-30) cm**	**Soil depth (30-60) cm**
Texture	Sandy clay loam	Sandy clay loam
pH (1part soil :5parts H_2O)	7.88 ± 0.18	7.92 ± 0.16
Electrical conductivity, (1part:5parts H_2O) dS m^{-1}	0.38 ± 0.01	0.37 ± 0.01
$CaCO_3$ (%)	2.36 ± 0.09	1.95 ± 0.10
Organic matter (%)	3.38 ± 0.17	3.22 ± 0.15
N-inorganic (mg kg^{-1})	66.5 ± 6.43	70.0 ± 7.38
K-exchangeable (mg kg^{-1})	800.2 ± 11.4	750.8 ± 9.9
Na-exchangeable (mg kg^{-1})	126.5 ± 1.80	161.0 ± 2.23
CEC (cmol kg^{-1})	41.5 ± 0.86	38.6 ± 0.59
P -Olsen (mg kg^{-1})	60.6 ± 3.4	47.8 ± 2.69
Cu-DTPA (mg kg^{-1})	11.2 ± 0.18	12.6 ± 0.20
Zn-DTPA (mg kg^{-1})	2.1 ± 0.5	2.8 ± 0.07
Mn-DTPA(mg kg^{-1})	14.9 ± 0.50	17.1 ± 0.58

Data represent average and SE deviation. (n)=4

At the stage of maturation, the grape Victoria compared with Hamburg Muscat grape gave a lower sugar content and total acidity (Table 2). The weight of the Victoria variety grapes was 321 g/100 berries, while the Muscat Hamburg grapes gave 240 g/100 berries. Production reached 38 tons/ha for the Victoria table grape variety, while for the Muscat Hamburg variety was 29 tons/ha.

The content of total phenols (TP) in the flesh, the skin and seeds of grapes ranges from 642 mg (GAE)/kg f.m. up to 11605 mg (GAE)/kg f.m. (Table 3).

In the seeds of grapes studied, the concentration of total phenols (TP) is greater by 8% in the colored grape variety Muscat Hamburg compared to white grape variety Victoria ($p < 0.05$).

The skin and the flesh of the variety Muscat Hamburg has 20% and 60% higher concentration (TP) from the skin and flesh of the Victoria variety, respectively ($p < 0.05$). Many researchers have reported that the level of total phenols in the seeds can be greater by 5% on dry matter (Muir, 1996). The content of total phenols in the seeds of grapes is a specific characteristic of the variety of grape that is not dependent on the color of grapes but on the agro-climatic conditions (Bourzeix, M. et al. 1986; Gougoulias and Mashev, 2008).

Table 2. Chemical properties of the grape must

properties	**Grape variety Victoria**	**Grape variety Muscat Hambourg**
pH	4.63 ± 0.12	4.37 ± 0.15
Brix degrees	19.2 ± 0.32	21.5 ± 0.36
Total acidity (g tartaric acid /L)	2.88 ± 0.07	3.85 ± 0.10

Data represent average and SE deviation. (n)=4

Table 3. Content of the total phenols (TP) in seeds, skins and flesh of the grapes

	TP (mg GAE/kg fresh material)		
Grape variety	Flesh	Skin	Seeds
Victoria	642 ± 9.2	4618 ± 67.9	10.623 ± 132.8
Muscat Hambourg	1028 ± 14.9	5773 ± 81.3	11.605 ± 158.9

Data represent average and SE deviation. (n)=4

Table 4. Polyphenol fractional composition (mg/kg f. m.)

Grape organs	**NFP**	**FP**	**NTP**	**TF**	**F-3-ols**
	(GAE)				(CE)
Grape variety Victoria (White)					
Flesh	172 ± 2.46	470 ± 6.7	184 ± 3.1	458 ± 7.6	118 ± 1.7
Skins	1006 ± 13.4	3612 ± 45.1	1050 ± 13.1	3568 ± 44.5	805 ± 10.7
Seeds	2337 ± 29.2	8286 ± 98.3	2687 ± 31.6	7936 ± 93.4	2400 ± 32.8
Grape variety Muscat Hamburg (Colored)					
Flesh	178 ± 2.5	850 ± 12.1	170 ± 2.4	858 ± 12.3	142 ± 3.6
Skins	1090 ± 13.6	4683 ± 58.5	1100 ± 13.8	4673 ± 58.4	925 ± 18.5
Seeds	1857 ± 18.6	9748 ± 97.5	2566 ± 27.6	9039 ± 97.2	2196 ± 36.6

Data represent average and SE deviation. (n)=4

The results of Table 4 reveal that in the seeds, the flesh and the skin of the two grape cultivars tested, the fraction of FP prevails against the fraction of the NFP. The NFP are represented mainly by gallic and other phenolic acids. The flavonoid fraction, contains the monomers catechin and epicatechin forming the plant tannins, particularly procyanidins (Muir, 1996).

The data in Table 4 also show that the fraction of TF in different organs of the grape prevails against the fraction of NTP for both grape varieties studied. Many researchers have reported that the content of tannin phenols in the seeds of grapes ranges between 2-5% on dry matter, depending on the variety and the climatic conditions (Bourzeix et al., 1986).

The results on the content of total F-3-ols, which represent the sum of catechin and procyanidins are given in Table 4, were presented as catechin equivalent (CE). The procyanidins, which react with p-DMACA, represent dimers, trimers and tetramers of the monomers catechin and epicatechin. The amount of catechins and procyanidins, depends on the grape variety and the climatic conditions and varies in wide limits (Bourzeix et al., 1986). The content of total F-3-ols in the flesh, the skin and the seeds of grapes ranges from 118 mg (CE)/kg f.m. up to 2400 mg (CE)/kg f.m. The greatest concentration of total F-3-ols was recorded in the seeds of the grape variety Victoria with a value equal to 2400 mg (CE)/kg f.m., while the lower content of total F-3-ols

was measured in the flesh of the grape variety Victoria with a value equal to 118 mg (CE)/kg f.m.

The seeds of the Victoria grape variety compared with the seeds of Muscat Hamburg variety, have a higher content of NFP, NTP and F-3-ols (Table 4) ($p < 0.05$). While the skin and the flesh of variety Muscat Hambourg compared to the skin and flesh of the grape Victoria, have a higher content of NFP, NTP and F-3-ols (Table 4) ($p < 0.05$). Also the seeds, the skin and the flesh of the variety Muscat Hamburg compared with seeds, skin and flesh of the variety Victoria have a higher content of FP and TF (Table 4) ($p < 0.05$).

The data in Table 5 also confirm that the nonflavonoid phenols (NFP) in the seeds, skin and flesh of both varieties of grapes examined, represent on average the 19%, the 20.35% and the 22.05%, respectively, of the total phenols content. While nontannin phenols (NTP) in seeds, skin and flesh of both grape varieties studied, represent an average of 23.7%, the 20.9% and 22.6%, respectively, of the total phenols content.

Table 5. Percentage composition of Phenolic fractions in terms of composition of total phenols in the seeds, skin and flesh of the grapes

Flesh				
	% of TP			
Grape variety	NFP	FP	NTP	TF
Victoria	26.8	73.2	28.7	71.3
Muscat Hamburg	17.3	82.7	16.5	83.5
Skins				
	% of TP			
Victoria	21.8	78.2	22.7	77.3
Muscat Hamburg	18.9	81.1	19.1	80.9
Seeds				
	% of TP			
Victoria	22.0	78	25.3	74.7
Muscat Hamburg	16	84	22.1	77.9

Table 6. Antiradical capacity in μmol DPPH/g fresh material

Grape variety	**Flesh**	**Skins**	**Seeds**
Victoria	0.61 ± 0.03	3.24 ± 0.11	16.83 ± 0.34
Muscat Hambourg	1.07 ± 0.05	4.04 ± 0.11	15.36 ± 0.32

Data represent average and SE deviation. (n)=4

Table 7. Antioxidant capacity in μmol FRAP/g fresh material

Grape variety	Flesh	Skins	Seeds
Victoria	1.88 ± 0.05	10.86 ± 0.18	134.4 ± 1.92
Muscat Hambourg	4.08 ± 0.09	18.65 ± 0.21	150.3 ± 2.08

Data represent average and SE deviation. (n)=4

The highest antiradical capacity (16.83 μmol DPPH/g f.m.) has been found in the seeds of the grape Victoria variety, while the lowest antiradical capacity (0.61 μmol DPPH/g f.m.) was found in the flesh of the same variety (Table 6).

The ferric reducing antioxidant power (FRAP) of the phenol compounds in the grape

flesh, skins and seeds demonstrate changes depending on the grape organ and variety. The seeds of the grape variety Muscat Hamburg showed the highest antioxidant capacity (150.3 μmol FRAP/g f.m.), while the lowest antioxidant capacity (1.88 μmol FRAP/g f.m.) was recorded in the flesh of grape Victoria (Table 7).

4. Conclusions

Of the two varieties of table grapes under study, the colored grape Muscat Hamburg compared to the white variety Victoria, in terms of the flesh, skin and seeds, is distinguished by a higher content of total phenols. While the variety of grape Victoria compared with Muscat Hamburg grape, as regards the seeds, revealed a higher content in NFP, NTP and F-3-ols.

Between the flesh, the skin and seeds of grapes under investigation the seeds contain the highest content of total phenols, exercising a strong antioxidant activity to in vitro tests we carried out.

Particularly dominant is the fraction of flavan-3-ols (catechin and procyanidins) exhibiting biological effects. The seeds of grapes are an excellent source of procyanidins, which are connected with the property of reducing the cholesterol content in the blood (Bourzeix et al., 1986). The grape seeds are an excellent source for the production of pharmaceutical preparations with antioxidant action for human purposes (Bravo, 1998).

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NUTRITIONAL-QUALITATIVE FACTORS CHANGES DURING RIPENING OF CANNED OLIVES AT DIFFERENT TEMPERATURES STORAGE

Neda Sallak[1], Parisa Sadighara[1*]

[1]Department of Environmental Health, Food Safety Division, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[*]sadighara@farabi.tums.ac.ir

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ABSTRACT

Background: Olive oil and other olive products are regarded as an important part of many people diet, the huge value of which can be the result of olive antioxidants that contribute to the oxidative balance in vivo. Due to the fact that olive (Oleaeuropaea) can provide prominent components of the different countries diet, it seems vital to establish evident characterization and quantitation of the total antioxidant compounds it contains. The objective of this study is to determine some nutritional ingredients in brine of olive drupes which are kept in room (24˚C) and refrigerator temperatures (4˚C). Methods: Total flavonoid compounds, carotenoids, antioxidant activity and total chlorophylls were examined for some nutritional ingredients in brine of olive drupes in 3 interval times (30, 60 and 90 days). The samples were kept in room (24˚C) and refrigerator (4˚C) temperature. Results: The brine of olive drupes which were kept in room temperature (24˚C) indicated more nutritional ingredients at the end of 30, 60 and 90 days, but the amount of chlorophyll b had no significant difference in both sample types at the end of 90 days. Likewise, the total antioxidant capacity which was determined by the ferric reducing antioxidant power (FRAP) showed no significant difference in both sample types after 90 days. Conclusion: Findings suggest that cold temperature (refrigerator) can provide more appropriate situation for storage of brined olive drupes as certain nutritional ingredients immigrated less from drupes to brine.

1. Introduction

It is known that fruit and vegetables play role against cancers and cardiovascular disease. These protective effects to different antioxidant constituent such as vitamin C and E, carotenoids, flavonoids and phenolic acids (Mazzeo et al., 2006).

Olive fruit includes various types of polyphenols, mainly tyrosols, phenolic acids, flavonols and flavones. Some phenolic compounds such as oleuropein and oleocanthal are responsible for its bitter and pungent taste. Oleocanthal, oleuropein, and its derivative hydroxytyrosol are nature's strongest anti-oxidants. Together with vitamin E and carotenoids, they play an important role combating against cancer, inflammation, coronary artery disease, degenerative nerve diseases, diabetes, etc. (Cicerale, et al., 2012, Perez-Martinez, et al., 2011, Covas, 2008, Leger et al., 2005).

The olive drupe, its oil and the leaves of the olive tree have a substantial background of

nutritional, medicinal and ceremonial usages. The processing of olives fruit especially affects their phenolic content, but other parts of the plant also contain phenolic, such as leaves and bark.

The olive drupe from which olive oil is obtained has not been essayed to such an extent. Clearly, olive drupes can be used fresh, but they have a very bitter taste. Therefore, to make them tastier, they are brined for a few months to discard the bitterness, a procedure which also separates a portion of the phenolic antioxidants. To get a more perfect picture of the amount to which olives may subscribe to dietary antioxidant intake, it is required to get an unmistakable sketch of the major phenolic ingredients in brined olive drupes. It should be noted that high levels of polyphenolic antioxidants supplies greater resilience to oxidative stress (Valko, et al 2006).

Many people cannot access to fresh olive and they use brined olive drupe. Therefore, in this work, the effects of room and refrigerator temperature on the change in several nutritional ingredients related to brine of olive drupes were studied in 3 interval times. Total flavonoid compounds, carotenoids, total antioxidant activity and total Chlorophylls were examined in every 30 days in both sample types which were kept in room (24˚C) and refrigerator (4˚C) temperatures.

2. Materials and methods

Chemicals: FeCl3, AlCl3.6H2O (Merk Company,Germany) and Neocuproine (Sigma Company,USA) were used in this study.

Samples Preparation: Brined olive drupes were supplied by Medical Science of Tehran University. The samples contained 100 cc brine +60 gr olive drupe without stone (11% salt) which were kept in two different temperatures (24˚C and 4˚C). After 30, 60 and 90 days only the brine of samples was examined separately for determination of immigrated nutritional ingredients from drupes to brine. The pH of brine for total samples was 3.7-3.8.

2.1. Determination of total flavonoid compound

Total flavonoid content was assayed according to previous methods (Qiu–Lin et al., 2006). Diluted extracts were mixed with reagent; ALCl3.6H2O 2% in methanol flavonoids could make complex with trivalent aluminum ion. Then, the samples were incubated in room condition for 10 minute. The absorbance of the samples was measured at 430 nm.

2.2. Determination of total carotenoids

Total carotenoids were determined by B carotene standard curve and by spectrophotometric method at 470 nm. The total carotenoids content of samples were calculated on the basis of the standard curve of B carotene (Thaipong, et al 2005).

2.3. Determination of total chlorophylls

The Chlorophyllcontent was assayed according to the previous method (Dere et al.,1998). The supernatant was separated and the absorbances were read on Schimadzu UV-260 spectrophotometer. It was recorded that Chlorophyll a showed the maximum absorbance at 663 nm, and chlorophyll b at 645 nm. The amount of these pigments was calculated according to following formulas:

Chlorophyll a = (19.3 ×A663 - 0.86 × A645) V/100W

Chlorophyll b = (19.3 ×A645 - 3.6 ×A663) V/100W

2.4. Determination of total antioxidant compound

2.4.1 The ferric reducing/antioxidant power (FRAP)

The total antioxidant capacity was determined by the ferric reducing antioxidant power (FRAP). Briefly, the stocks solutions included 300 Mm acetate buffer 10 mM TPTZ (2,4.6-tripyridyl-s-triazine) solution in 40 mMHCl, and 20 mM $FeCl_3.6H2O$ solution were prepared. The fresh working solution (FRAP reagent) was prepared by mixing acetate fuffer, TPTZ solution, and $FeCl_3.6H_2O$

solution. The samples and deionized water were mixed with 3 mL of the FRAP reagent and allow to react for 5 min in the dark. The changes in absorbance at 593 nm are related to the total reducing power of antioxidants of tissues (Morales and Paredes, 2014).

2.4.2. Determination of cupric ion reducing assay (cupric assay)

The cupric ion reducing capacity assay measures the cupric reducing capacity. The samples were mixed with solutions of $CuCl_2$, neocuproine reagent in ammonium acetate buffer. The resulting absorbance at 450 nm is recorded directly after incubation at 50 degrees C for 20 min (Apak et al., 2008).

3. Results and discussions

Levels of nutritional ingredients in different temperatures (4 ˚C & 24˚C) are shown in Tables 1, 2 and 3. The data values were expressed as mean ±SD. The evaluation was made by comparing groups by T-Test analysis in SPSS software. The level of total Carotenoids and total Flavonoids were significantly different in room temperature (24˚C) compared to refrigerator temperature (4 ˚C). The P values are not more than 0.05 in total Carotenoids and total Flavonoids after three interval times (Table 1).

The total antioxidant capacity was determined by Ferric reducing /antioxidant power (FRAP) and Cupric ion reducing assay (cupric assay). Level of total antioxidant which was measured by these two methods was shown in Table 2. The total antioxidant capacity in FRAP method was significantly more in samples which were kept in refrigerator temperature (4 ˚C) in comparison with those that were kept in room temperature (24˚C) after 30 and 60 days but after 90 days the difference was not meaningful (P > 0.05).

Table 1. Results of total carotenoids and total flavonoids

	After 30 days		After 60 days		After 90 days	
	Total Carotenoids	**Total flavonoids**	**Total Carotenoids**	**Total flavonoids**	**Total Carotenoids**	**Total flavonoids**
Samples stored at (24˚C)	0.026 ± 0.002	0.001 ± 0.000	0.068 ± 0.005	0.0019 ± 0.000	0.141 ± 0.002	0.178 ± 0.011
Samples stored at (4˚C)	0.018 ± 0.002	0.0004 ± 0.000	0.059 ± 0.004	0.0015 ± 0.000	0.093 ± 0.001	0.125 ± 0.008

Each value represents the Mean ± SD per group. The total carotenoids & total flavonoids were significantly more in samples stored at 24˚C compared to those stored at 4˚C.

Table 2. Results of total antioxidant capacity (Cupric & FRAP methods)

	After 30 days		After 60 days		After 90 days	
	cupric	**FRAP**	**cupric**	**FRAP**	**cupric**	**FRAP**
Samples stored at room temperature (24˚C)	0.38 ± 0. 01	2.77±0.1	0.19 ± 0.007	2.89±0.2	0.184 ± 0.006	0.59±.006
Samples stored at refrigerator temperature (4˚C)	0.27 ± 0.01	3.08±0.2	0.14 ± 0.008	3.38±0.0	0.146 ± 0.003	0.55±0.03

Each value represents the Mean ± SD per group .P <0.05 for Cupric assay, but P value was greater than 0.05 for FRAP only at the end of 90 days.

Table3. The results of Chlorophyll a and Chlorophyll b

	After 30 days		After 60 days		After 90 days	
	Chlorophyll a	**Chlorophyll b**	**Chlorophyll a**	**Chlorophyll b**	**Chlorophyll a**	**Chlorophyll b**
Samples stored at (24˚C)	0.015 ± 0.000	0.013 ± 0. 001	0.034 ± 0.008	0.029 ± 0.02	0.027 ± 0. 02	0.038 ± 0.00
Samples stored at (4˚C)	0.019 ± 0.006	0.014 ± 0. 003	0.142 ± 0.164	0.025 ± 0.00	0.018 ± 0. 01	0.028 ± 0.00

Each value represents the Mean ± SD per group. The changes in level of Chlorophyll b were significant after 90 days.

Total antioxidant components which were examined by Cupric assay were significantly different in both sample types, it should be noted that these components were found more in brine which were kept in room temperature (24˚C) compared to those at refrigerator temperature (4˚C).

Chlorophyll a and b were measured for total Chlorophylls (Table 3). The changes in level of Chlorophyll a and b were not observed in both sample types, but the amount of Chlorophyll b was significantly more in brine of olive drupes kept in room temperature ($P<0.05$) after 90 days.

In this study the total carotenoids, flavonoids, antioxidants and Chlorophylls of brine of olive drupes were measured and compared in two different temperatures and three intervals. To date, there are few studies about the correlation between immigration of some nutritional ingredients to brine of olive drupe and their storage temperature. Total Flavonoids as Polyphenolic components in fruits and vegetables vary according to numerous genetic, environmental, and technological factors such as canning, packaging, heating…etc. In recent study, the amount of total Flavonoids was noticeably more in brine of samples kept in room temperature (24˚C) in comparison with those kept at refrigerator temperature (4˚C), although the amount of flavonoids increased in both types of samples with the duration of storage (Table 1). It means that the temperature of storage can influence the maintenance of these components in foods, even their immigration. Briefly Tarozzi, et al (2004) has indicated that cold storage clearly reduced the total phenolic concentration and the total antioxidant activity, but not the vitamin C concentration of apple with skin. This is not compatible with our results in terms of the effect of cold storage on maintenance of these components. Bach, et al., (2013) have investigated the migration of some non nutritional ingredients in various bottled waters exposed to different temperatures and shown that at 60˚C the migration of these compounds was highly accelerated. This could be explained by the storage temperature increases the mobility of polymeric chains directly linked to the migration (Bach et.al, 2011). This finding is in agreement with our results regarding of the effect of storage temperature on migration of some nutritional factors.

The present study showed that the amount of total carotenoids increased according to our findings about total flavonoids. Thus, we can imply that the brine of samples stored in room temperature (24˚C) contain more total carotenoids (Table 1). Rivera-Pastrana et al., (2010) have indicated that ripe Papaya stored at 25˚C had more carotenoids than those stored at 1˚C, as low temperature (chilling) negatively affected the content of major carotenoids except β –caroten.

In our finding, total antioxidant capacity was investigated by two different methods: ferric reducing antioxidant power (FRAP) and cupric ion reducing capacity assay (Cupric assay). our results have shown that the amount of antioxidants was considerably more in brine

of samples stored at refrigerator temperature (4˚C) by FRAP method after 30 and 60 days but after 90 days the changes were not meaningful. However, the antioxidant trend was found to be opposite Cupric assay (Table 2). Interestingly, the levels of antioxidants were more in brine of samples stored in room temperature (24˚C) after 30, 60 and 90 days.

It would be notable to evaluate the high FRAP values related to the tendency of polyphenols to turning to pro-oxidants under some conditions. This has been shown for some flavones and flavanones which also have marked FRAP values (Cao et al.1993). On the other hand, not all antioxidants reduce Fe3+ at a rate fast enough to allow its measurement within the time of observation (Ilhami, 2012). In this method, the reducing ability of thiols and carotenoids is not be determined (Pulido et al.2000; Ou et al.2002a, b).

Cupric assay is applicable for a variety of antioxidants (Hydrophilic and Lipophilic) regardless of chemical type or structure and is carried out at a pH (7.0). The method is suitable for measuring thiol-type antioxidants unlike the widely applied FRAP test, which is non-responsive to -SH group antioxidants (Ilhami, 2012). Therefore, these two methods may indicate different results in terms of measuring antioxidant activity.

Our findings have demonstrated that the changes of chlorophyll a and b were not significant in both types of samples after three interval times, but the amount of chlorophyll b was more in brine of samples stored at room temperature after 90 days. The decline in the chlorophyll content during storage is depended to temperature and this process has been shown in many fresh vegetables such as rocket, Swiss chard and spinach (Vina and Chaves, 2003 and Ferrante et al., 2007). In our experiments, the measurement of chlorophyll a and b were not good markers for estimating the migration of these components from olive drupes to brine in different temperatures, unfortunately they were not able to differentiate the storage temperatures on their migration.

Collectively, brined olive drupes are kept in the non-cold part of stores. Our results confirmed that the bioaccesibility of their composition is dependent on the storage temperature. This product should be stored at a cool temperature. Also, it's best to store it away from oven or stove in home and it should be refrigerated.

4. Conclusions

Findings suggest that cold temperature (refrigerator) can provide more appropriate situation for storage of brined olive drupes as certain nutritional ingredients immigrated less from drupes to brine.

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EFFECT OF FERMENTED AND UNFERMENTED COCOA BEAN ON SOME LIVER ENZYMES, CREATININE AND ANTIOXIDANT IN WISTAR ALBINO RATS

Ejiofor U. Emmanuel[1]*, Ebhohon Shirley Onagbonfeoana[1], Omeh Yusuf Ndukaku[1]

[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, PMB, 7267, Umuahia, Abia State, Nigeria.
[*]ejioforemmanuelbiz@gmail.com

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ABSTRACT
The study investigated the effect of cocoa bean on the antioxidant status, creatinine and liver enzymes in wistar albino rats. Twenty five animals were randomly distributed into five groups of five animals each. Group A served as the control group, Group B and C received 15 and 30% of unfermented cocoa bean incorporated in their diet respectively while group D and E received 15 and 30% fermented cocoa bean incorporated in their diet. Experimental period lasted for 27 days, after which animals were fasted overnight and blood obtained through ocular puncture for liver enzymes and creatinine analysis. Liver organ obtained from animals were used for antioxidant assay. Result of the study showed a significant ($P<0.01$) decrease in GSH concentration in the 15% fermented and unfermented fed group when compared to the control group. Catalase concentration was significantly ($P<0.01$) higher in the 15% fermented, 15% unfermented and 30% fermented fed group when compared to the control group. For SOD concentration, the 30% fermented fed group was significantly ($P<0.01$) higher than the control group. MDA concentration showed a significant ($P<0.01$) increase in the control group when compared to the Cocoa fed group. Result for AST showed a significant ($P<0.01$) increase in the control group when compared to the cocoa bean fed group. The study showed that cocoa possess antioxidant activity and also hepato-protective ability. Also, fermentation of cocoa improved the antioxidant ability of the plant. This indicates that fermented cocoa can be useful in the fight against free radical and oxidative stress.

1. Introduction

The cocoa an ancient American crop is the mature fruit of the cocoa tree. The cocoa pod houses the cocoa bean which is a rich source of Saponin, tannin, phenol, flavonoid and alkaloid (Izuka and Mbagwu, 2013). Flavonoid in cocoa entails it polyphenol content which has attracted significant response because of it antioxidant activity and health benefits (Wollgast and Anklam, 2000). The polyphenols found in cocoa bean can be grouped into three groups which are catechins, anthocyanins and proanthocyanidins (Rusconi and Conti, 2010) however, Francene (2003) had earlier reported that catechins which is a flavonoid is the major antioxidant in cooca bean and widely used in chocolate preparation. Cocoa has been described as food since it is useful as a beverage (Mbagwu, 2009). Naturally, cocoa has a bitter taste and thus is fermented to develop it sweet taste and enticing flavor.

Fapohunda (2012) reported reduction in phenolic, tannin and lipid after fermentation.

Previous studies have reported the pharmacological and nutritional benefits of cocoa and it derived products on living system. Kurosawa et al., (2005) reported cocoa and it products suppress the development of atherosclerotics lesion, inhibit breast cancer cell proliferation (Ramljak et al., 2005), hypoglycemic ability (Tomaru et al., 2007). These ability of cocoa has been attributed to it high phenolic content. Polyphenol in cocoa have shown strong antioxidant ability both *in vitro* and *in vivo* (Adamson et al., 1999). Studies by Amin et al., (2004) showed that cocoa polyphenols reduced the severity of hepato-carcinogenesis.

Reactive oxygen species (ROS) are always present in living system especially those that rely on aerobic respiration. Antioxidant on the other hand plays a defensive role in the fight against oxidative stress generated from ROS and diseases. They achieve this by scavenging free radicals before they carry out their hazardous actions which leads to various diseases such as cancer, aging disorders, atherosclerosis, obesity (Chisolm, 2001). Enzymatic antioxidant such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) are present in living system and carry out this scavenging role. However, antioxidants derived from diet and other external sources such as carotenoids, flavonoids, vitamin C can improve the ability of these enzymatic antioxidants.

Food processing is useful in removing unwanted materials from food. However, processing have been shown to affect plant materials. The study however tried to determine if fermentation would affect the antioxidant and hepato-protective potentials of cocoa bean.

2. Materials and methods

2.1.Plant sampling

Ripe cocoa pod were obtained from a cocoa plantation in Umuariaga Village, Ikwuano Local Government Area of Abia State, Nigeria. The cocoa pod were identified by Dr. Garuba Omosun, a taxonomist in the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike.

2.2.Plant material preparation

The cocoa pod were washed in running tap water to remove sands and debris on the body. It was dried with a clean towel and the pod broken using a pestle to obtain the cocoa bean. The bean were divided into two groups. Group A was air-dried for four days under shade and milled using an electronic blender to fine powder. Group B was fermented by placing the bean in plantain leaves, wrapped carefully to avoid entry of air and tied in a black polythene bag. This was buried underground for seven days for complete fermentation to occur. After fermentation period, the fermented bean pod was allowed to air dry, followed by milling. The both samples were stored in airtight container.

2.3.Animals.

Twenty five male Wistar albino rats (103-110g) were obtained from the Veterinary Department, Michael Okpara University of Agriculture, Umudike. The animals were housed in stainless steel cages with a rubber base and allowed one week acclimatization period. The animals were fed ad libitum with standard commercial pelleted grower feed (Vital Feed, Nigeria) and free access to clean drinking water. Ethical guidelines for use of laboratory animals were maintained (DHHS, 1985).

2.4.Experimental Design

The animals were randomly distributed into five groups (A-E) of five animals each. Group A, served as control, Group B and C received 15% and 30% unfermented cocoa bean in their diet respectively, while Group D and E had 15% and 30% fermented cocoa bean respectively in their diet. Experimental period lasted for 28 days, after which the animals were fasted overnight and blood obtained through

ocular puncture for serum liver enzymes and creatinine estimation. The animals were further euthanized after cervical dislocation and the liver obtained was homogenized for antioxidant assay. Blood was spun using a centrifuge at 895 x g for 10mins and serum obtained was transferred into a serum bottle.

2.5.*In vivo* antioxidant

- Catalase activity was determined using the modified method described by Atawodi (2011)
- Superoxide dismutase activity was determined by the method described by Sun et al., (1998)
- The level of thiobarbituric acid reactive substance (TBARs) and malondialdehyde (MDA) production was determined by the method described by Draper and Hadley (1990).
- GSH activity was determined using method described by Owen and Belcher (1965)

2.6.Liver enzymes and creatinine assay

- Alanine Amino Transferase, Aspartate Amino Transferase and Creatinine in serum was determined using Randox commercial kits.

2.7.Statistical Analyis

Data obtained were statistically analyzed using one way analysis of variance (SPSS software). The variant mean were separated by least significance difference. Significance was accepted at 99% confidence level. The result was reported as Mean± SD using tables.

3. Results and discussions

Table 1. Antioxidant activity of fermented and unfermented cocoa bean

	MDA (µMole /mg protein)	SOD (Unit/g protein)	CATALASE (µMole/g protein)	GSH (µMole/g protein)
Control	0.72±0.07	18.58±5.36	61.56±1.29	24.04±0.58
15% Unfer.	0.68±0.08	20.28±5.76	64.02±4.90	22.58±0.71[a]
30% Unfer.	0.58±0.04[a]	23.18±2.71	75.62±2.41[ab]	24.16±0.65[bc]
15% Fer.	0.56±0.02[ab]	24.20±6.98	82.64±5.10[ab]	22.80±0.59[a]
30% Fer.	0.55±0.04[ab]	29.46±1.70[ab]	74.32±9.52[ab]	22.28±0.20[bc]

[a] significantly *(P<0.01)* different when compared to control group
[ab] significantly *(P<0.01)* different when compared to control and 15% unfermented group
[b] significantly *(P<0.01)* different when compared to 15% unfermented group
[bc] significantly *(P<0.01)* different when compared to 15% unfermented group and 15% fermented

Table 2. Liver enzymes and creatinine concentration

	AST (U/L)	ALT (U/L)	CREATININE (mg/dL)
Control	27.00±4.00	28.04±7.69	0.88±0.44
15% Unfer.	29.40±3.57	30.88±9.28	0.78±0.53
30% Unfer.	13.60±2.51[ab]	36.56±6.24	0.70±0.18
15% Fer.	13.00±2.12[ab]	32.20±11.38	0.88±0.20
30% Fer.	12.40±2.51[ab]	30.60±6.87	0.58±0.19

[ab] significantly *(P<0.01)* different when compared to control and 15% unfermented group

Result for MDA showed a significant *(P<0.01)* increase in the control group when compared to the 30% fermented and unfermented group and also 15% fermented group. Also, MDA concentration was significantly *(P<0.01)* higher in the 15% unfermented group when compared to the 15% fermented group. Result for SOD showed a significant *(P<0.01)*

increase in the 30% fermented group when compared with the control group and 15% unfermented group. Result for catalase indicated a significant *(P<0.01)* increase in the 15% fermented, 30% fermented and 30% unfermented group when compared to the control group. GSH concentration showed a significant *(P<0.01)* decrease in the 15% unfermented and fermented group when compared to the control group. Also, GSH concentration showed a significant *(P<0.01)* increase in the 30% unfermented group when compared to the 15% fermented and unfermented group. Result for AST showed a significant *(P<0.01)* decrease in the 30% fermented, 30% unfermented and 15% fermented group when compared to the control group.

Antioxidants play vital role in biological system. They are useful in the mopping up of free radicals, removing them from cells and also inhibit oxidation reaction caused by these free radicals thereby preventing cells and membrane damage. In animals, animals can be enzymatic or non-enzymatic. Catalase, SOD and GSH are enzymatic antioxidant found in humans and animals.

Result for antioxidant assay in this study presented in Tab. 1 above showed that both the fermented and unfermented cocoa bean pod improved the enzymatic antioxidant system in the studied animals. However, the fermented cocoa bean fed group showed a better effect than the unfermented bean fed group. This indicated that fermentation improved the antioxidant activity of cocoa bean *in vivo*. Cocoa bean are rich in polyphenol compounds which is responsible for it antioxidant capacity (Aikpokpodion and Dongo, 2010), and during fermentation antioxidant and polyphenol compounds are increased (Afoakwa et al., 2015). Fermentation is also known to remove undesired bitter and astringent taste followed by increase in sweet desired taste (Biehl et al., 1985).

Result showed that SOD was significantly *(P<0.01)* higher in the group fed 30% fermented cocoa bean when compared to the control group and group fed 15% unfermented cocoa bean. Dietary polyphenols have been shown to increase the activity of SOD *in vivo* (Shen *et al.,* 2007). Stated earlier, polyphenols make up the major phytochemical constituent of the cocoa bean, and from this study, it increased consumption (30% fermented) showed a stronger activity of SOD. Polyphenols has also been shown to scavenge free radicals such as superoxide anion, hydroxyl and peroxyl radicals (Huang et al., 2004). Due to the scavenging ability of dietary polyphenol on superoxide anion in biological system, SOD activity will be kept at its peak as superoxide anion might not be present in the cell or be reduced to the minimum by polyphenol. SOD catalyzes the dis-mutation of superoxide to hydrogen peroxide and oxygen.

From this study, catalase concentration was significantly *(P<0.01)* higher in the 30% unfermented group, 30% fermented and 15% fermented group when compared to the control group. In rat model, dietary polyphenols have been shown to increase catalase activity (Shen *et al.,* 2007). Catalase catalyze the breakdown of hydrogen peroxide which is a product of SOD activity to hydrogen and water (Chelikani *et al.,* 2004). Antioxidant polyphenols such as quercetin and procyanidins have been shown to increase catalase expression and also increase intracellular antioxidant activity (Akazome, 2004).

GSH was significantly *(P<0.01)* higher in the 30% unfermented fed group when compared to the 15% fermented and unfermented fed group. GSH also an antioxidant is important for cellular defense against ROS and lipid peroxidation. GSH works actively by donating it electron there by putting GSH in it oxidized form. NADPH from the pentose phosphate pathway (PPP) serve as electron donor which reduces the oxidized glutathione to enable it carry out more scavenging activity. Dietary polyphenols has been shown to modulate expression of the enzyme gamma glutamylcysteine synthetase which is important in cellular antioxidant defenses. This enzyme catalyze the rate

limiting step in the synthesis of glutathione (Jan et al., 2005).

Malondialdehyde (MDA) has been widely used as a biomarker for lipid peroxidation. Increase in MDA concentration has been associated with increased lipid peroxidation which in turn leads to oxidative stress, cellular and DNA damage. MDA concentration was significantly *(P<0.01)* lower in the 30% fermented, 30% unfermented group and 15% fermented group when compared to the control group. From this study, the fermented cocoa reduced MDA concentration in the 15% fed group when compared to the 15% unfermented fed group. This gives an indication that fermentation increase phenolic compounds in the cocoa bean pod and also inhibits lipid peroxidation. Previous studies have reported that cocoa inhibits lipid peroxidation (Schinella *et al.*, 2010), Shen et al., (2007) reported that polyphenols decreased MDA concentration in animal studies. Martorell et al., (2011) showed that cocoa polyphenols could induce resistance to oxidative stress in organism.

Result for liver enzymes and creatinine presented in Tab. 2 showed that the concentration of AST in the 30% fermented, 30% unfermented and 15% fermented group was significantly *(P<0.01)* lower when compared to the control group and 15% unfermented group. Concentration of ALP and creatinine revealed no significant difference. AST catalyzes the transfer of an amino group between aspartate and glutamate while ALT catalyze the transfer of an amino group from alanine to α- ketoglutarate. During liver damage and certain disease condition such as hepatitis, myocardial infarction concentration of AST may increase in blood serum (Burtis and Ashwood, 1994).

Polyphenols has been shown to possess hepato-protective ability. Studies by Saradeya (2014), showed that polyphenols reduce concentration of AST and ALT in animals treated with cadmium sulphate. Hanaa *et al.*, (2015) also reported the hepato-protective and antioxidant ability of polyphenols as it improved blood GSH concentration, reduced MDA concentration and lowered the concentration of AST, ALT and ALP. Cui et al., (2014) also reported the hepato-protective effect of polyphenols on mice treated with carbon tetrachloride. Zhang et al., (2005) suggested the protective effect of polyphenols on alcoholic liver injury.

Creatinine a breakdown product of creatine phosphate in muscle and can serve as biomarker for kidney injury. An increase in creatinine indicates kidney and nephron damage. Studies by Rezzani et al., (2009) showed that red wine polyphenols reduced creatinine concentration in cyclosporine-induced nephrotoxicity in male rats.

The liver plays a role in the metabolism of toxic compounds especially the phase two biotransformation. GSH conjugation is important in phase two xenobiotic biotransformation. Through conjugation, xenobiotic and toxic compounds are excreted through the kidney and out of biological system. This indicates that GSH is very useful in protecting living system and also liver organ against damage. Polyphenols may exert their hepato-protective ability by increasing concentration of GSH as seen in this study, thus aiding GSH conjugation making xenobiotic more hydrophilic and easily excreted.

4. Conclusions

The study showed that in the two processed form the cocoa bean was administered it showed significant hepato-protective and antioxidant ability. However, the fermented cocoa bean showed a better antioxidant activity in *vivo* which is due to it phenolic content. The hapato-protective activity of the fermented cocoa bean can be attributed to degradation of toxic component through fermentation.

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THE MAIN PARAMETERS AND INDICATORS THAT DEFINE THE QUALITY AND AUTHENTICITY OF WHITE WINES FROM VINEYARD DRĂGĂŞANI - ROMANIA

Felicia Stoica[1*]

[1]*University of Craiova, Faculty of Horticulture, Department of Horticulture & Food Science, A.I.Cuza Street, no.13, Craiova, Dolj,*
[*]*feliciastoica@yahoo.com*

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ABSTRACT
Always, especially lately, it lays emphasis on achieving food quality, natural and authentic that does not affect human health. Besides the analyzes modern, last generation HPLC, GC MS, GC FTIR, IRMS, NIR, quality and naturalness of the wines can be determined by analyzing the main parameters (alcohol, total acidity, extract, ash) and calculating the most important quality indicators (ratio alcohol / glycerol, alcohol / extract, extract / ash Halphen and Gautier). For this purpose were analyzed neutral white wines (Crâmpoşie selecţionată, Fetească regală, Riesling italian) and the semi flavored and flavored wines (Sauvignon, Sauvignonasse, Muscat Ottonel, Tămâioasă românească) from the famous vineyard Drăgăşani.

1.Introduction

20% of wines sold around the world are fake, presenting business risk for companies, but also the risk to consumer health (Loubry, 2015).

Attributes authentication such as origin is a critical element in wine certifying compliance with national and international regulations. Basing on the fact that the term 'origin' is of considerable importance directly correlated with the quality of wines, ranking them in terms of geographical origin and variety became an issue of significant interest to the community producers and consumers (Banu et al., 2013).

Knowledge of the chemical composition of wine and its association with the grape variety/cultivar is of paramount importance in oenology and a necessary tool for marketing (Muntean et al, 2015).

The chemical composition of wine is affected by multiple factors, including production area, grape variety, soil type, climate (terroir) and viticole and oenological practices. These factors play an important role in differentiating the wines according to their geographical origin and year of harvest (Cichi et al., 2007, 2009, Schlesier K et al., 2009).

In white winesin addition to the main chemical constituents defined for each category and type of fruitiness characteristics, flavor and expressiveness typical varieties and areas of origin shall be constituted in basic quality criteria. Moreover, these products must be offered to consumers in a state of perfect clarity and lasting stability over time (Băducă Cîmpeanu et al., 2007).

Aromatic wines are made from grape varieties that have some capacity to accumulate and biosynthesis in the skin beans flavored terpene (linalool, geraniol, terpineol), which confers "Muscat". A specific technology development requires for aromatic substances in grapes are stored in the superficial layers or deeper of the skin (Stoica, 2008, 2015).

At aromatic wines, with all other quality parameters, great importance submits aromatic profile which impresses favorable the consumer (Boidron and Torres, 1982; Stoica, 2008)

2.Materials and methods

For this study had the following objectives:

- Analyze the main quality parameters attesting naturalness of neutral and semiaromate white wines and aromatic – pure variety
- Analyze the main quality parameters attesting naturalness of wine - technological mixtures.

2.1.The material used

For this study were analyzed neutral white wines – pure varieties - Crâmpoşie selecţionată, Fetească regală şi Riesling italian , semi-flavored and flavored wines – pure varieties Sauvignon, Sauvignonasse, Muscat Ottonel and Tămâioasă românească and mixtures technological Muscat Ottonel + Tămâioasă românească (V1), Sauvignon+ Muscat Ottonel (V2), Sauvignon 50% + Sauvignonasse 40% + Muscat Ottonel 10% (V3). All wines were obtained in Drăgăşani vineyard, 2014.

To calculate ratios alcohol - glycerol extract - ash, alcohol - extract, Halphen, Gautier and the main parameters that define the flavor of wines were determined in the laboratory of oenology at the Faculty of Horticulture alcoholic contents in wines, glycerol contents, ash and dry extract using OIV methods and flavor characteristics of the INCC Rm. Valcea, gas-cromatography.

With these data were calculated ratios weight dosed alcohol and glycerol, alcohol and extract relationships, the relationship between the weight of non-reducing dry extract and ashes. There have also been calculated and the sum Linalol + Nerol + Geraniol expressing parameter called quality aromatic wines by flavor profile.

For determining the ratio alcohol - glycerol, alcohol degree is multiplied by 10 to obtain the alcohol by volume. The amount of alcohol by volume are then multiplied by 0.79 (the molecular weight) to obtain the alcohol by weight. Then, the mathematical calculation is determined the ratio of the weights of the two elements. The limits of variation of this ratio is between 5.5 and 13.5, with the average of 8.5 for Romanian wines.

For determining the ratio extract - ash extract is considered 100% and ash is as a percentage of extract. Between extract reduced and there is no linear relationship ash.

For determining the R_R ratio alcohol – extract,, alcohol degree is multiplied by 10 to obtain the alcohol by volume. The amount of alcohol by volume are then multiplied by 0.79 (the molecular weight) to obtain the alcohol by weight. The limits of variation of R_R ratio is 4.3 to 5.5 for white wines and 3.6 to 4.5 for red wines.

The Halphen ratio is Total acidity (g / L) / Alcohol (% vol.) Values of this report are between 0.2-0.8 for natural wines.

Gautier index representing the amount of alcohol% vol. total acidity g / L presents legal limits range from 13-17 (Banu et al, 2013).

These ratiosand quality indicators are taken into particular consider in determining the degree of naturalness of wine products and compositional balance.

3.Results and discussions

The main characteristics of the composition of neutral white wines and semi-flavored and flavored wines obtained in vineyard Drăgăşani are presented in Table 1 and Table 2.

According to data listed in Table 1 and 2 from analyzed wines have alcohol levels of between 10.4% vol. (Crâmpoşie selecţionată) and 13.1% vol. (Sauvignon). Riesling italian and Fetească regală wines exceeded by 0.1 to 0.2% vol. while the flavored with 0.5-0.7% vol. the threshold of 12.0% vol.

Total acidity other than Muscat Ottonel wine, presented values of more than 4 g / L H_2SO_4 or more than 6 g / L in $C_2H_6O_6$, all other varieties to comply with the rules for applying LVV current.

The close relationship between Gay-Lussac and glycero- pyruvate fermentation faithfully

reflected the contents of glycerol. This explains the greater proportions of glycerol (10.23 g / L and 10.05 g / L) of wines Sauvignon and Tămâioasă românească to which degrees alcohol had values of 13.1% vol. vol and 12.7% respectively. For the other wines in glycerol content ranged from 7.0 g / L (Crâmpoşie selecţionată), 9.2 g / L (I Riesling italian was 9.66 g / L (Sauvignonasse).

Non-reducing extract presented values determined by the genetic nature of the variety, and some interventions in winemaking. The quality of raw material and slight maceration applied to winemaking grapes Sauvignon, Muscat Ottonel and Tămâioasă românească have determined the largest extract content in wines (23.26 g / L and 23.17 g / L). For the other wines extract presented values between 19.08 g / L (Crâmpoşie) and 22.18 to 22.7 g / L (Fetească regală respectively Riesling italian).

Table1. The main characteristics of the composition of the neutral white wines

Composition characteristics	The wine variety		
	Crâmpoşie selecţionată	Fetească regală	Riesling italian
Alcohol % vol.	10.4	12.0	12.2
Total acidity, g/L H_2SO_4	4.05	4.22	4.23
Glycerol, g/L	6.84	9.11	9.2
Non-reducing extract g/L	19.08	22.18	22.70
Ash, g/L	1.79	2.06	1.92
Glycerol x100/Alcohol	8.39	9.62	9.55
Alcohol/Extract	4.3	4.27	4.24
Total acidity / Alcohol	0.38	0.35	0.34
Ash x100/ Non-reducing extract	7.81	9.29	8.46
Gautier index %vol Alcohol+ Total acidity, g/L	14.05	16.22	16.25

The contents of mineral substances (ash) generally follow non-reducing extract proportional sizes. There are more than 2 g / l in Sauvignon and Tămâioasă românească wines, less than 2 g / L to Crâmpoşie selecţionată wines (1.79 g / L), Italian Riesling (1.92 g / L), Sauvignonasse (1.99 g / L).The proportions of glycerol to alcohol dispensers, except Crâmpoşie selecţionată are a little under 10% or even above this threshold considered ideal (Tămâioasă românească wine).

The proportions of non-reducing extract to ash ranging between 8.46 (Riesling italian) 9.37 (Sauvignon) and 9.75 (Tămâioasă românească) are considered as expressing favorable relations between the two oenological sizes.

Gautier index representing the amount of alcohol% vol. total acidity g / L has values between 13-17, legal limits. Sauvignon wine only, the index exceeds the maximum due to higher in alcohol and the total acidity content.

Natural character and high-quality of semi-flavored and flavored wines, emerged from the ratios values glycerol × 100/alcohol and ashes× 100 / extract non-reducing. Ratios glycerol × 100/alcohol and ashes100 ×/ non-reducing extract were close considerably by ideal level (10%) Tămâioasă românească, Muscat Ottonel and Sauvignon wines, them presenting values between 9.34 and 9.88 for the ratio glycerol × 100 / alcohol and between 9.29 and 9.75 the ratio ash × 100 / non-reducing extract, signifying important criteria for quality and compositional equilibrium of products.

Regarding the ratio alcohol / extract, all the wines analyzed is between 4.3 to 5.5 legal limits, are not suspected of falsification of alcohol and Halphen ratio values fall within the range 0.2-0.8.

It can be concluded from all the values for quality indices, that all wines are authentic, natural, without illegal added alcohol.

Table 2. The main characteristics of the composition of the semi-flavoured and flavoured wines

Composition characteristics	The wine variety			
	Sauvignonasse	Sauvignon	Muscat Ottonel	Tămâioasă românească
Alcohol % vol.	12.1	13.10	12.4	12.7
Total acidity. g/L H_2SO_4	4.16	4.13	3.95	4.26
Glycerol. g/L	9.63	10.23	9.81	10.05
Non-reducing extract g/L	22.10	23.26	22.18	23.17
Ash. g/L	1.99	2.18	2.06	2.26
Glycerol x100/Alcohol	9.48	9.89	9.98	9.98
Alcohol/Extract	4.14	4.44	4.41	4.33
Total acidity / Alcohol	0.34	0.31	0.31	0.33
Ash x100/ Non-reducing extract	9.00	9.37	9.28	9.53
Gautier index %vol Alcohol+ Total acidity. g/L	16.26	17.23	16.35	16.96

According to the table 3 it is found that the sum of three terpene is higher at V1 wine mixture Tămâiosă românească + Muscat Ottonel as well Tămâioasă românească and Muscat Ottonel wines and tasting confirms a gustativ advantage.

The V3 wine, Sauvignon 50% Sauvignonasse + 40% + Muscat Ottonel 10% , amount Linalool, Geraniol, Neroli i the lowest recorded reading because in technological mixture were used 90% semi-flavored wines (Sauvignon 50% and 40 Sauvignonasse %) and only 10% flavored wine made from Muscat Ottonel.

Also, an intermediate value of the sum of three terpene are registered to a technological mixture consisting of Muscat Ottonel and Sauvignon.

Terpene fraction of Muscat Ottonel and Tămâioasă românească wine was at considerable levels. In total flavored fund were identified high content of linalool, geraniol and nerol.

Table 4 presents the other quality parameters of technological mixture wines and parameters of naturalness and authenticityof wines quality indicators along with amount of linalool, geraniol, nerol.

Table 3. Free terpene content of flavored wines and mixtures technological

Variant	Linalol, µg/L	Geraniol, µg/L	Nerol, µg/L	L+G+N
V_1- Tămâiosă românească+Muscat Ottonel	1435	720	388	2543
V_2- Muscat Ottonel+Sauvignon	1289	734	416	1434
V_3- Sauvignonasse 50% + Sauvignon blanc 40% + Muscat Ottonel 10%	986	493	271	1750
V_4- Muscat Ottonel	1430	812	414	2656
V_5-Tămâioasă românească	1580	950	523	3053

Table 4. The main characteristics of the composition of the white wines – technological mixture

Composition characteristics	The wine vaeiety				
	V_1-	V_2	V_3	V_4	V_5
Alcohol % vol.	12.4	12.5	12.2	12.4	12.7
Total acidity, g/L H_2SO_4	4.05	4.0	4.11	3.95	4.26
Glycerol, g/L	9.90	9.90	9.76	9.81	10.05
Non-reducing extract g/L	22.50	22.26	21.84	22.18	23.17
Ash, g/L	2.16	2.10	2.02	2.06	2.26
Glycerol x100/Alcohol	9.89	9.97	9.87	9.98	9.98
Alcohol/Extract	4.35	4.40	4.41	4.41	4.33
Total acidity / Alcohol	0.32	0.32	0.33	0.31	0.33
Ash x100/ Non-reducing extract	9.6	9.43	9.24	9.28	9.53
Gautier index %vol Alcohol+ Total acidity, g/L	16.45	16.5		16.35	16.96
L+G+N	2543	1434	1750	2656	3053

Like the analysis of wine - pure variety, the essential composition of wines produced with technological mixtures fairly approaching the wines analyzed above, it is influenced by the percentage of particiapre of each wine - pure variety in part to obtain the desired wine.

Thus, alcohol content does not fall below 12.2% vol. to any one of the wines analyzed in terms of all of thetotal acidity of more than 4 g / L H_2SO_4 all wines.

Glycerol contents are consistent with those in alcohol, ranging between 9.76 g / L at V3 and 9.90 g / L at V1 (technological mixture of Tămâioasă românească and Muscat Ottonel). Neither the other two wines analyzed glycerol content has not dropped below 10 g / L which reflects that all analyzed wines are natural, from the alcoholic fermentation of grape must.

The best extractivitate was recorded in V1, which is consistent with the extract values of the two wines that participated in the technological mixture.

Corresponding non-reducing extract and glycerol content increased olso, ratio Glycerol x100 / alcohol and alcohol / extract, guarantee quality indicators of naturalness and legal methods of production.

Also, all the other indicators of quality and authenticity of wines have values evidencing this.

Comparing the pure variety wines with wines made by technological mixtures concluded that the wines pure varieties have a significantly higher aromatic substance, analytically. These wines present a fruitiness and obvious floral nuances, with a typical aroma, very clearly marked.

Quality of product oenology (wine) are not only a gift of nature, as it was thought often in the past, but the "fruit" and a wide range of technological intervention, together with the granting of a rigorous attention on hygiene winemaking throughout the entire production process.

4. Conclusions

Studies showed convincingly that all wines analyzed in Drăgăşani vineyard are of exceptional quality, emphasized by chemical composition harmonious and very pleasant organoleptic through.

Wines neutral proved both by the balanced composition and the satisfactory qualities and varieties from which they are able to use the excellent natural conditions in the area, and as such should be extended to in culture.

Studies in vineyard Drăgăşani on semi-flavored and flavored wines wines have shown that Sauvignon, Muscat Ottonel and Tămâioasă românească achieved exceptional oenological potential.

All control parameters falsification, ratios Alcohol / Glycerol, alcohol / Extract Extract /

ash index Gautier and ratio Halphen by the values obtained from all analyzed wines from the vineyard Drăgăşani indicates that we are in the presence of natural wines, authentic, high quality, according to the category they belong.

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ASSESSMENT ON THE SEA CUCUMBER FRESHNESS BASED ON THE FUSION OF TACTILE FEATURES AND VISUAL TEXTURES

Huihui Wang[1,2*], Yuansong Zheng[1,2], Wei Gu[1,2], Shiyuan Xing[1,2], Yan Lv[1,2], Xu Zhang[1,2], Xueheng Tao[1,2]

[1]*School of Mechanical Engineering and Automation, Dalian Polytechnic University, Dalian, 116034, China*

[2] *National Engineering Research Center of Seafood, Dalian, 116034, China*

**whh419@126.com*

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ABSTRACT

The detections on the sea cucumber freshness mainly depend on the combination of finger touch and eye observations, with strong subjectivity and certain potential dangers. In this article, a humanoid tactile & visual seafood freshness detection system was constructed, i.e., the pressure was detected using a humanoid finger and the texture analysis was conducted using the machine-vision-based observations. The tactile and visual data were fused for assessing the sea cucumber freshness. Pressure-time response model during the pressuring process was established, and the maximum pressure value, the counting ratio and the elastic potential energy were selected as the tactile features. The large-gradient advantage and inverse moment in texture analysis were selected as the visual features. Finally, measured tactical and visual features were fused and a probabilistic neural network was constructed for assessment. The results demonstrate that the grading accuracy of the freshness can be up to 93.51%.

1.Introduction

For ready-to-eat seafoods, freshness is one of the most important indexes for quality assessment. During processing, transportation and consumption processes, ready-to-eat seafoods will suffer from enzymatic action, microbial action and the effects from the external environmental factors, and their freshness degrees gradually decrease [Cianti et al., 2013; Erdilal et al., 2014; Adriano et al., 2007; Hong Hanmei et al., 2014]. Even more seriously, the seafoods may decay and deteriorate. The common freshness detection methods for meat products include the sensory evaluation and many laboratory methods such as chemical detection, physical detection and electrochemical detection [Zhang Zhuomin et al., 2010; Gamal ElMasry et al., 2015]. The sensory evaluation method lacks of universality in applications, and moreover, this manual detection methods potentially dangerous and has strong subjectivity. The laboratory detection methods have been widely applied in the previous studies [Qiong Daia et al., 2015; Majid Dowlati et al., 2013; Huang Xingyi et al,2011], which mainly rely on some bionic detection devices such as electronic tongue, electronic nose and texture analyzer [Gonzalez-Martin A et al., 2010;Hong Xuezhen et al., 2012; Majid Dowlati et al., 2015;Amy Loutfi et al., 2015]. These devices are generally expensive and set high requirements on the

operators as well as the shape and properties of the materials. Currently, in most of online detections of the ready-to-eat seafoods, the quality assessments are implemented depending heavily on human sense such as finger touch and eye observations. Using the bionic sensing methods in which the multiple senses are digitalized for detection, not only the subjectivity of manual detection can be overcome, but also the objective and quantitative data comparable with the laboratory detection can be acquired. Moreover, owing to the combination of multiple senses, the information shortage in multi-feature detection when using a single type of sensor can be effectively avoided.

In the present study, based on the rottenness mechanisms of the seafoods, a humanoid tactile and visual seafood freshness detection system was constructed for detecting the mechanical and visual texture information. By fusing tactile and visual data, a freshness assessment model for seafoods was established with the content of taotal volatile basic nitrogen (TVB-N) as the standards. The present study can provide data supports for food detection and the development of service robots.

2. Materials and methods

2.1. Basic structure and working principle of the system

Firstly, the three-dimensional (3D) model of the detection system was constructed and the feasibility of the motion mechanisms was proved. As shown in Fig.1, the constructed humanoid tactile and visual detection system for seafoods freshness mainly consists of a motion module, a control module, an end effector for tactile detection and an visual detection module.

The motion module is composed of a motor, a coupling and a lead screw, i.e., the vertical movement of the lead screw is driven by the motor via the coupling. In the control module, the control system was programmed with Visual C++ for the implementation of the detection actions such as press and the withdrawal of force as well as the collection and processing of images and sensor data. The sensor component of tactile-detection end effector is a S-type pressure sensor. To ensure that the samples remain intact after touch, the end of the pressure head was designed to be similar to a finger pulp. As shown in Fig.2, the end of the pressure head is spherical. The vision detection module mainly includes a dome diffuse-reflection light source and an industrial color camera. As displayed in Fig. 3, the samples were placed under the dome light source for visual detection. This closed data acquisition mode can ensure that all the images were collected under the same environment.

2.2. Experimental procedures and method

The ready-to-eat sea cucumbers of the same bath purchased from the Dalian Changxing Aquatic Product Market, China, were adopted as the materials in the present experiments. As shown in Fig. 4, in order to avoid the effects of the shape differences on the experimental results, we selected the middle of the sea cucumbers and chopped them into the samples with the length of 20 mm. Moreover, to guarantee the reliability of the experiments, every sea cucumber was implemented in tactile and visual detection continuously twice a day until they have already deteriorated. 116 samples were obtained at last. During the detection, the environmental temperatures were maintained between 25 to 30 ℃. According to the hygienic standards for the fresh and frozen livestock meat (GB 2707-2005), for the first-

level fresh meat, the content of total volatile basic nitrogen (TVB-N) should be less than or equal to 15 mg/100g; for the second-level fresh meat, the TVB-N content ranges from 15 to 25 mg/100g; when the TVB-N content exceeds 30 mg/100g, it can be considered that the seafood has gone bad. On the other hand, the samples were qualitatively and rapidly detected using the TVB-N kits prepared by Beijing Zhongnuotaian Science and Technology Ltd. Based on the detected TVB-N contents, the samples were classified into the following three levels—fresh, less fresh and deteriorated.

2.2.1.Tactile detection

Firstly, in order to eliminate high difference among the samples, the pressing displacement experiments were conducted, and the results demonstrate that the detection performance is most favorable when the sample is pressed to 40% of its height by the pressure head. Accordingly, a complete pressing period can be defined. Specifically, the pressure moves downward, reaches the surface of the sample and then begins to press the sample at the velocity of 2 mm/s. The moment when the pressure reaches the surface of the sample is denoted as the zero point and then the sensor begins to collect the useful pressure data; subsequently, when the pressure reaches 40% of the sample height, the detection returns back to zero point, i.e., a complete pressing detection is finished. During the detection process, the tactile data were collected by the control module. After angle-digital (A/D) conversion and data filtering, the pressure-time response model was constructed and thereby the characteristics of the response curve were extracted for conducting comprehensive assessment on the freshness of the seafoods.

2.2.2.Visual detection

On account of enzymatic and bacterial actions, the muscular tissues in seafoods gradually degrade. At the initial stage of degradation, the surface textures of the muscles are clear and ordered; with the prolongation of storage time, the textures gradually become disorder and blurred [Jesús Chamorro-Martínez et al., 2015; Soleiman Hosseinpour et al., 2014]. Accordingly, in the present work, the 24-bit color images of the samples in the format of BMP were collected for texture analysis. To ensure the correspondence and accuracy in the fusion of tactile and visual data, for each sample, the textural image should be collected before the tactile detection.

3. Results and discussions

3.1. Tactile detection feature extraction and the related analyses

Using the mechanical information detected by the tactile detection control module, the pressure-time response model was established and presented in Fig. 5, in which the horizontal and vertical coordinates denote the time points and the corresponding pressure values in a detection period, respectively. As shown in Fig. 5, Curve 1 and Curve 2 represent the mechanical characteristics of a fresh ready-to-eat sea cucumber under the action of pressure on the first day, Curve 3 and Curve 4 reflect the mechanical characteristics of the same sea cucumber under the pressure on the second day, and Curve 5 and Curve 6 reflect its mechanical characteristics on the third day.

According to the TVB-N detection results, the sea cucumber represented by Curve 1 and Curve 2 is fresh; the samples represented by Curve 3 and Curve 4 are less fresh and the samples represented by Curve 5 and Curve 6 are deteriorated. To further analyze the

response models of the sea cucumber measured on different days (i.e., corresponding to three different levels), the following three characteristics can be concluded.

1. To achieve the same displacements, a larger force should be applied on the fresher sea cucumber. This rule is most obvious when the pressing displacement reaches the maximum in design.

2. At the initial stage of tactile detection, the pressure varies slowly. As the pressing continues, the pressure value increases. For the fresh and less fresh sea cucumber, the pressure increases slightly. For the deteriorated sea cucumber, the response curves are almost level, i.e., the pressure value remains almost unchanged during the initial pressing process; however, at the moment when approaching the largest displacement point, the response curves present step-type increases, and the flat curves instantly reach the maxima. This is mainly due to the fact that the muscular tissues were damaged because of the deterioration. Accordingly, the pressures at the neighboring time points were compared. To avoid the effects induced by mechanical vibration, the average pressure values at the neighboring three time points were averaged and then pressure value differences were calculated. The number of the pressure differences exceeding 0.03 N was counted, as shown in the following Eq. (1) and Eq. (2).

$$E(x_i)=\begin{cases} 1 & Mi-Mi-1\geq 0.03 \\ 0 & Mi-Mi-1<0.03 \end{cases}$$
$$i=6/3,9/3\cdots\cdots,\quad n/3\cdots\cdots n_{\max}/3 \tag{1}$$

In which $n_{\max}$ denotes the time point which is an integral multiple of 3 and closest to the time point with a maximum pressure value.

$$Num=\sum_{i=2}^{n_{\max}/3}E(x_i) \tag{2}$$

To eliminate the effects of different shapes, the curve feature was finally denoted by the counting ratio, which is defined as

$$P=\frac{Num}{n_{\max}} \tag{3}$$

In which M_i denotes the average of the pressure values at three neighboring time points and *Num* denotes the total count. A greater *Num* value indicates that the pressure value should take longer to increase to the maximum, and thereby a higher freshness degree. On the contrary, the smaller the *Num* value, the pressure value takes less time to reach the maximum and the poorer the freshness degree.

3. The constructed pressure/time response models are actually the smooth curves composed of multiple points. According to the definition of power, the work done by the pressure head on the sea cucumber can be calculated by:

$$\Delta W=F_i\times ds \tag{4}$$

In which F_i denotes the pressure value at any one point on the curve and *ds* denotes the distance between two time points. Then the products of all the forces on the curve and *ds* were integrated,

$$W=\sum_{i=1}^{n}Fi\times ds \tag{5}$$

In which n denotes the number of time points when the pressure reaches the maximum.

Therefore, the area of the shaded part as shown in Fig. 6 denotes the work done on the sea cucumber during the downward pressuring process, which is also referred to as the elastic potential energy of the sea cucumber. As shown in Fig. 5, for the sea cucumber with poorer freshness, the area of the shade parts is smaller and therefore the elastic potential is smaller.

3.2. Texture analysis based on visual detection and feature extraction

As the freshness varies, the obvious sensory changes occur on the surface of the sea cucumbers due to the reproduction of the microorganisms and bacterial colonies as well as the proteolysis and autolysis. In particular, the originally clear textures on the body wall then tend to be blurry. Therefore, based on the analysis of the combined statistical distribution of the grey value and edge gradient, a grey-gradient co-occurrence matrix was constructed in this article,

$$\{ f(x,y); x = 0, 1, \ldots, M_f - 1; \quad y = 0, 1 \ldots M_g - 1 \} \tag{6}$$

In which x and y denote the grey value and gradient of the sea cucumber image, and $f(x,y)$ denotes the total number of pixels. By taking the image size and processing time into overall consideration, M_f and M_g were set as 16 in this article, which represent the specified grey level and gradient, respectively.

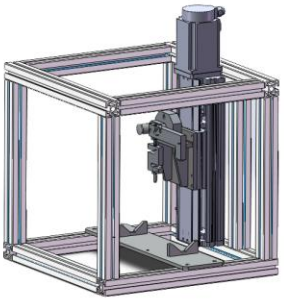
Then the correlation, the uneven characterization of grey distribution, the grey level entropy, the inverse moment, the lager gradient advantage, the mean square error, the gradient, the average gradient, the uneven characterization of gradient distribution, the gradient entropy, the average grey value, the entropy of mixing, the mean square error of the grey value and the inertia were adopted as the texture parameters, and their correlations with the freshness degree were calculated. The correlation coefficients between these texture parameters and the freshness are 14.8%, 57.8%, 63.9%, 70.8%, 70.1%, 64%, 60.3%, 62.1%, 61.9%, 61.8%, 55.8%, 60.9% and 60.9%, respectively. As demonstrated, most of the texture parameters are moderately correlated with the freshness of the sea cucumber. In view of the model learning and data process time, the parameters which have relatively stronger correlations with the freshness, i.e., the inverse moment and the large gradient advantage, were selected as the features for data fusion and quality assessment. The inverse moment ($G(x, y)$), and the large gradient ($Q(x, y)$), can be calculation according to the following Eq. (6) and Eq. (7)

$$G(x,y) = \sum_{x=0}^{M_f-1} \sum_{y=0}^{M_g-1} \frac{f(x,y)'}{1+(i+j)^2} \tag{7}$$

$$Q(x,y) = \frac{\sum_{y=0}^{M_g-1} \sum_{x=0}^{M_f-1} f(x,y)' \times y^2}{\sum_{x=0}^{M_f-1} \sum_{y=0}^{M_g-1} f(x,y)'} \tag{8}$$

In which $f(x,y)'$ denotes the grey-gradient matrix after normalization

$$f(x,y)' = \frac{f(x,y)}{M_f \bullet M_g}. \tag{9}$$



(a) Three-dimensional model of the system

(b) Picture of the system prototype

Figure 1. Illustration of the humanoid tactile and vision seafood detection system

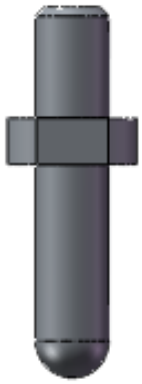


Figure 2. Spherical pressure head in the humanoid finger

Figure 3. Visual detection module

Figure 4. a sea cucumber sample

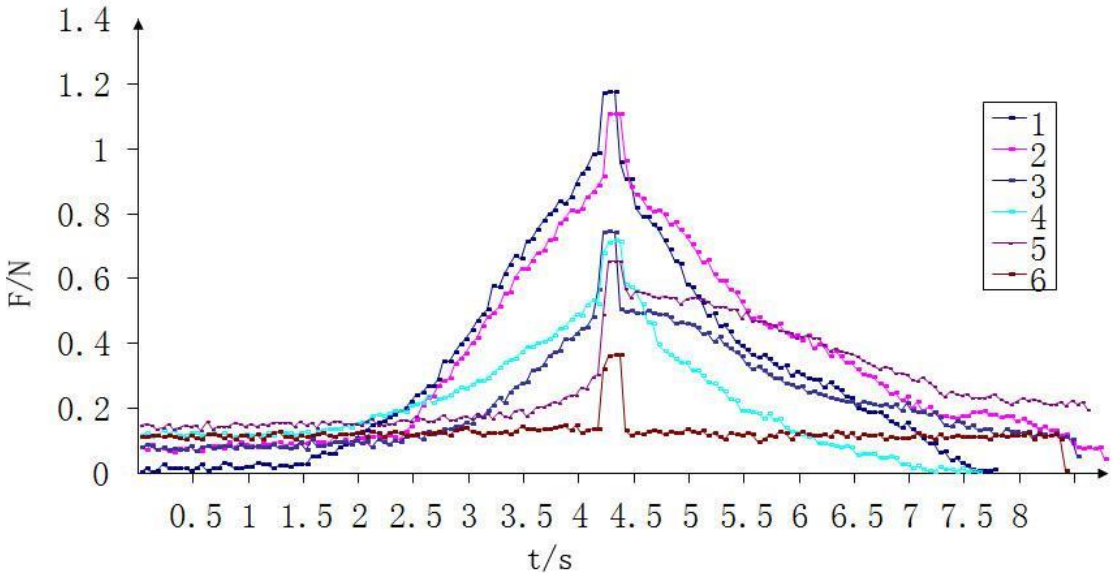


Figure 5. Pressure/time response model

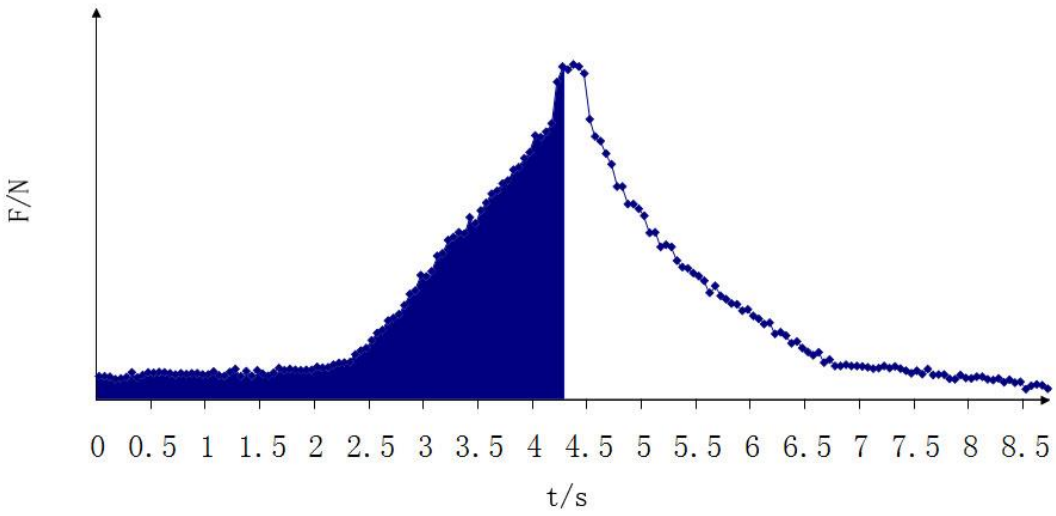


Figure 6. Work done on the sea cucumber during the downward pressuring process of the pressure head

Table 1. Quality assessment results based on the fusion of tactile and visual data

Grading based on the measured TVB-N content	Number of samples in the test set	Grading results based on the constructed PNN			Grading accuracy/%	Average grading accuracy/%
		Fresh	Less fresh	deteriorated		
Fresh	33	31	0	2	93.94	93.51
Less fresh	20	1	18	1	90	
Deteriorated	24	0	1	23	95.83	

3.3. Fusion of tactile and visual detection data and assessment

To effectively overcome the limitations of the single-mode biological feature identification and make the assessment results more close to manual detection, the tactile and visual information were fused in this article for acquiring the comprehensive sensing results. Considering that the selected features vary greatly in acquisition method and types, these parameters were firstly normalized, i.e., the detected tactile information including maximum pressure value, the P value, the elastic potential energy *W*, and the visual information including the inverse moment and the large gradient advantage, were converted to be dimensionless. The tactile and visual data were fused and then the quality was assessment by means of artificial neutral network.

In this article, a probabilistic neutral network (PNN) was constructed, in which the neurons in hidden layer were adaptively acquired through the network. In the input layer, there are totally 5 neurons, i.e., the above-described 5 tactile and visual features. 3 neurons are included in the output layer, which represent the three levels of the freshness, i.e., fresh, less fresh and deteriorated. The experimental results demonstrated that the expansion coefficient was preferably set as 1.25. The constructed PNN was trained using 39 sea cucumber samples as the training set and 77 samples as the test set. The test results are listed in Table

1, and the average accuracy can be as high as 93.51%.

4. Conclusions

A humanoid tactile and visual seafood detection system was constructed in this article. According to the habits in manual detection of the seafood freshness, the tactile and visual detection were performed and fused. Firstly, the pressure-time response model was established, and the maximum pressure value, the counting ratio (i.e., the P value) and the elastic potential energy *W* were adopted as the tactile features; subsequently, the texture analysis was performed on the collected BMP images. The inverse moment and the large gradient advantage were adopted as the visual features. Using these tactile and visual features as the characteristic parameters in detection, the constructed model has relatively strong distinguishing cap ability on the freshness of sea cucumber.

(2).To avoid the information shortage when using the single sensor, the tactile and visual features were fused. A PNN network was constructed for assessing the freshness degree of sea cucumbers. The test results demonstrate that the grading accuracy can reach up to 93.51%.

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RESPONSE OF TOMATO YIELD AND QUALITY TO ALTERNATE PARTIAL ROOT-ZONE IRRIGATION AT DIFFERENT WATER AND NITROGEN LEVELS

Xiaogang Liu [1], Xinle Wang[2], Fusheng Li[3*], Fucang Zhang[4], Huanjie Cai[4], Qiliang Yang[1].

[1]Faculty of Modern Agricultural Engineering, Kunming University of Science and Technology, Kunmng, Yunnan, 650500, China
[2]Faculty of Foreign Language and Culture, Kunming University of Science and Technology, Kunmng, Yunnan, 650500, China
[3]College of Agriculture, Guangxi University, Nanning, Guangxi, 530004, China
[4]Key Laboratory of Agricultural Soil and Water Engineering in Arid and Semiarid Areas, Ministry of Education, Northwest A&F University, Yangling, Shanxi, 712100, China
[*] lpfu6@163.com

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ABSTRACT

To investigate the benefits of alternate partial root-zone irrigation (AI) on fruit yield and quality of tomato modified by water and nitrogen (N) management, taking conventional irrigation (CI) as the control, the effects of AI at different water levels and growth stages, i.e. AI_1 (high water (W_1) from the blooming to fruiting stages), AI_2 (W_1 at the blooming to fruit setting stage (BFS) and low water (W_2) at the fruiting stage (FS)), AI_3 (W_2 at BFS and W_1 at FS) and AI_4 (W_2 from the blooming to fruiting stages), on fruit yield and quality and WUE of tomato were studied under the three N levels, i.e. low N (N_L), middle N (N_M) and high N (N_H). Irrigation quota for W_1 and W_2 in AI at BFS or FS was 80% and 60% of that in CI, respectively. AI increased tomato soluble solids content in fruits, but reduced organic acids content compared with CI. AI at N_L and N_M increased WUE by 7.4%-24.4%, and AI_1 did not reduce fruit yield significantly. Compared to N_L, N_M increased fruit yield, WUE and the contents of soluble solids, soluble sugars, lycopene and organic acids. Compared to CIN_L treatment, AI_1N_M treatment increased fruit yield, WUE and the contents of soluble sugars, lycopene, vitamin C and sugar/acid ratio, respectively. Thus AI under high water from the blooming to fruiting stages can guarantee fruit yield and improve fruit quality and WUE under middle nitrogen level. In addition, AI can improve WUE under proper water and nitrogen management.

1.Introduction

Partial root-zone irrigation (PRI), including alternate PRI (AI) and partial root-zone drying (PRD), is a water-saving irrigation technique (Kang et al., 2002; Loveys et al., 2000). There are many studies about the effects of PRI on tomato yield, quality and water use efficiency (WUE). Compared to full irrigation, PRI can guarantee tomato fruit number, mean fruit mass, fruit dry mass and harvest index yield, but reduce water consumption greatly, thus it

increases the WUE(Kirda et al., 2004; Yang et al., 2012). Meanwhile, PRI can also improve tomato fruit quality to some extent. In some cases, PRI improved fruit quality in terms of total soluble solids contents as well as titratable acidity and juice pH (Casa et al., 2014).

There are some studies about the interactive effects of PRI and fertilizer on tomato. Yang et al. indicated that PRI with 0.8% of Ca supplied as calcium chloride is a water-saving, quality-improving and practical irrigation technology (Yang et al., 2012). Wang et al. indicated that PRI contributed to the improved fertilizer-N use efficiency relative to the deficit irrigation (Wang et al., 2013). However, more studies are still needed to investigate the effects of PRI on tomato yield, quality and WUE in improving water and fertilizer management of tomato under different water and fertilizer conditions.

Tomato has various water requirements and responses at different growth stages (Zegbe-Domınguez et al., 2003; Zegbe et al., 2006; Ngouajio et al., 2007). Whether PRI can increase WUE and yield and improve tomato quality or not needs further study under different water and fertilization management. Therefore, the hypothesis of this study was that alternate partial root-zone irrigation increased fruit yield and quality and water use efficiency of tomato under proper water and nitrogen management, and the objective was to obtain the optimal mode of water and fertilizer supply of tomato plants.

2. Materials and methods

2.1. Experimental site and materials

The pot experiment was conducted in a greenhouse (370 m^2, 4 m high) in Northwest A&F University in Yangling, Shaanxi, China (latitude 34°18′N, longitude 108°24′ E, 521 m altitude). During the experimental period, mean day and night temperatures were 15-27 ^{0}C, the photon flux density ranged from 450 to 800 μmol/(m^2·s) and relative humidity ranged from 30 to 70% in the greenhouse. Experimental soil was alluvial soil (Fluvisols) and had a field capacity (FC) of 24% by weight, organic matter content of 6.2 g/kg, available N content of 50.5 mg/kg, available P content of 14.7 mg/kg and available K content of 140.5 mg/kg. Experimental crop was tomato (*Solanum lycopersicum*, var. Maofen-802).

2.2. Experimental method

Five irrigation treatments and three nitrogen (N) levels were designed in complete combinatorial pot experiment, and each treatment was replicated six times. Five irrigation treatments were showed in Table 1. Irrigation methods included conventional irrigation (CI, both sides of the pot irrigated simultaneously at each watering) and four AI methods (AI, alternate watering on both sides of the pot) under different irrigation levels at various growth stages. Irrigation in CI was controlled by weighing method and soil water content was kept from 70 to 85% FC. Irrigation quota for W_1 and W_2 in AI at the blooming to fruit setting stage (BFS) or fruiting stage (FS) was 80% and 60% of that in CI, respectively. Three N levels included low N level (N_L, 0.15 g N /kg soil), middle N level (N_M, 0.30 g N /kg soil) and high N level (N_H, 0.45 g N /kg soil). Nitrogen was supplied as urea and equal N solutions were applied three times, i.e. at BFS (July 12), early FS (August 20) and later FS (September 6). P and K were applied in KH_2PO_4 form, and mixed into the soil in powdered form at the commencement of the experiment, and all treatments were applied with 0.15 g K_2O /kg soil and 0.12 g P_2O_5 /kg soil. Because water content in soil was lower than field capacity, water could not lead to the leakage.

Experiments were conducted in plastic pots (32.5 cm in diameter at the top edge, 26.5 cm at the bottom and 33 cm in depth). The inside of all pots was evenly separated into two compartments with plastic sheets in the middle, so that water exchange among two parts of the pots was prevented. U-shaped notches were made in the center of plastic sheets for planting tomato. One PVC tube (2 cm in diameter) with

holes wrapped with gauze was installed in each container to supply irrigation water.

Each part of the pots was filled with 10.5 kg air-dried soil after sieving 2 mm with mean bulk density of 1.25 g/cm^3. One twenty-day old tomato seedlings was transplanted to each pot on June 27, 2013. Before the irrigation level was controlled, soil water regimes in all pots were kept FC. Weighing the pots controlled soil water regime in CI during the experimental period. For AI treatments, at 10 d after transplanting, irrigation was controlled at the two growth stages according to Table 1. Irrigation water is from tap water (pH 7.2).

Table 1. Irrigation treatment for pot experiment

Irrigation method	Irrigation level and stage	
	Blooming to fruit setting stage	Fruit setting to fruiting stage
CI	70 to 85% FC	70 to 85% FC
AI_1	W_1	W_1
AI_2	W_1	W_2
AI_3	W_2	W_1
AI_4	W_2	W_2

CI and AI represent conventional irrigation and alternate partial root-zone irrigation. Irrigation quota for high and low water in AI at the blooming to fruit setting and fruiting stages was 80% and 60% of that in CI, respectively.

2.3. Fruit sampling and measurements

Tomato fruits were harvested when approximately 80% of the fruits were red or orange. From the fruits harvested on September 10, samples were taken and passed through a 0.8 mm mesh sieve to separate seeds and epidermis from the juice. Soluble solids content, soluble sugar, lycopene contents, organic acid and V_C contents were measured using the homogenized juice by hand-held refractometer, anthrone colorimetry, UV-visible spectrophotometer, titration method with 0.1 mol/L NaOH and 2, 6-dichlorophenol indophenol sodium, respectively (Gao et al., 2006).

Crop water consumption was calculated from the pot water balance, and water use efficiency (WUE) was calculated as the ratio of fresh fruit mass to total water used.

2.4. Statistical analysis

Analysis of variance (ANOVA) was performed using two-way ANOVA from SAS software. All treatment means were compared for any significant differences using the Duncan's multiple range tests at significant level of $P_{0.05}$ using the SAS software.

3. Results and discussions

3.1. Tomato fruit yield

Irrigation method and N level had significant effect on tomato fruit yield (Fig. 1). Compared to CI, AI_2, AI_3 and AI_4 reduced fruit yield by 13.5-34.6, 7.5-23.4 and 17.6-36.9%, which showed that AI had the risk of yield reduction under low watering level. AI_1 at N_H reduced it by 22.5%, but AI_1 at N_L increased it by 5.0%, AI_1 at N_M did not increase it significantly. N_M increased fruit yield by 14.5-35.5%, but N_H at AI reduced it by 3.8-16.2% except that N_H at CI increased it by 13.6% when compared to N_L. And AI_1N_M treatment had the highest fruit yield and increased it by 35.3% when compared to CIN_L treatment, indicating that AI can guarantee yield under certain water and nitrogen supply.

3.2. Tomato fruit quality

Tomato fruit taste and nutritional value are mainly determined by the contents of soluble solids, soluble sugars, organic acids, vitamin C, and sugar/acid ratio etc. (Dorais et al., 2001). Irrigation method and N level had significant effect on soluble solids content in fruits (Table 2). Compared to CI, AI_1, AI_2, AI_3 and AI_4 increased soluble solids content by 7.6%-26.0%, 20.9%-45.4%, 12.1%-28.9% and 28.1%-46.7%, respectively. Compared to N_L, N_M and N_H increased soluble solids content by 2.4%-14.2% and 6.4%-28.0% respectively.

There were significant effects of N level and the interactions of N level and irrigation method on soluble sugars content in fruits (Table 2). N_M and N_H respectively increased the soluble sugars content by 11.3%-22.6% and 2.7%-44.4% as compared to N_L.

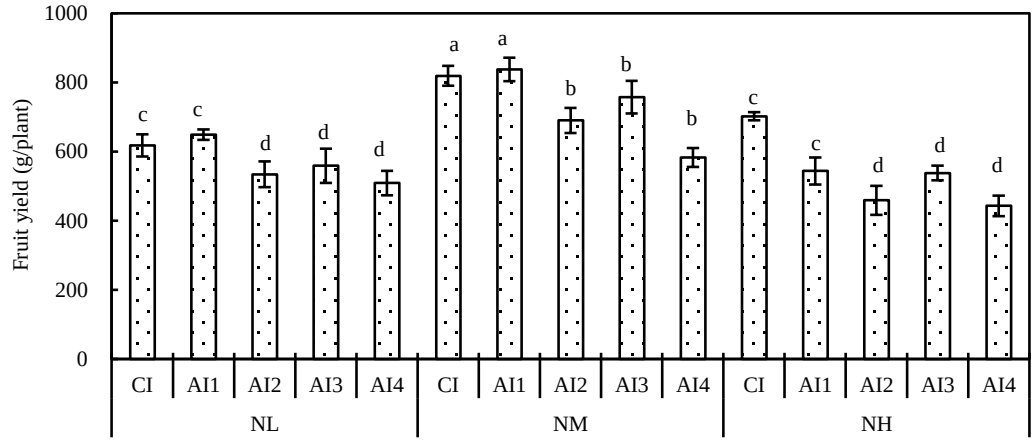

Figure 1. Effect of irrigation treatment and nitrogen level on fruit yield of tomato
(Values are means ± standard errors (n=6). Different letters in the same column indicate significant difference at $P<0.05$.)

Compared to CIN_L treatment, the other treatments increased soluble sugars content by 15.9-66.4%, and AI_4N_M treatment had the maximum soluble sugars content, with the value of 5.6%, and increased it by 66.4% as compared to CIN_L treatment.

Table 2 shows that there were significant effects of N level and the interactions of N level and irrigation method on lycopene content in fruits. Lycopene content in CI was less than that of AI under the same N level, indicating that AI could promote lycopene accumulation. N_M and N_H increased the lycopene content by 23.9%-86.3% and 5.3%-45.9%, respectively, when compared to N_L. Compared to CIN_L treatment, the other treatments increased lycopene content by 45.9%-178.0%. AI_2N_M treatment had the maximum lycopene content, with the value of 90.62 mg/kg, and increased it by 178.0% compared to CIN_L treatment.

Irrigation method, N level and their interaction had significant effects on organic acids content in fruits (Table 2). Compared to CI, AI_1, AI_2, AI_3 and AI_4 reduced the organic acids content by 24.1%-25.5%, 23.4%-30.0%, 20.8%-21.8% and 27.0%-31.4%, respectively. AI could increase soluble solids content significantly, but decrease organic acid content. Possible reason is that water stress induced by AI treatment strengthens the activity and content of soluble acid invertase and cell wall invertase, and promote the accumulation of soluble sugars such as hexose and sucrose (Baselga et al., 1993), thus AI increases soluble solids content in fruits. Compared to N_L, N_M and N_H increased the organic acids content by 21.4%-35.2% and 43.6%-53.5%, respectively. Compared to CIN_L treatment, AIN_L reduced the organic acids content by 21.8-28.2%, CIN_M increased it by 32.9%, AIN_M did not affect it obviously, but N_H in CI and AI increased it by 5.2%-53.3%.

Table 2 shows that there were significant effects of N level and the interaction of irrigation method and N level on V_C content in fruits Compared to N_L, N_M increased V_C content by 0.3%-5.7% except that N_M at AI_4 did not reduce it. N_H did not affect V_C content significantly except that N_H at CI increased it by 6.7% and N_H at AI_4 reduced it by 7.0%. Compared to CIN_L treatment, the other treatments increased V_C content by 2.3%-12.5%. AI_3N_M treatment had the maximum V_C content, with the value of 128.55 mg/kg, and increased it by 14.27 mg/kg when compared to CIN_L treatment.

The interactions of irrigation method and N level had significant effect on sugar/acid ratio in fruits (Table 2). Compared to CIN_L

treatment, CIN_M and CIN_H treatments respectively reduced sugar/acid ratio by 8.8 and 5.7%, but AI at the three N levels increased it by 11.5-95.1%, indicating that AI could increase sugar/acid ratio and improve tomato taste. Compared to CIN_L treatment, AI_1N_M treatment increased the contents of soluble sugar, lycopene and V_C and sugar/acid ratio, indicating a better synergistic effect of water and N.

Table 2. Effect of irrigation treatment and nitrogen level on tomato fruit quality

N level	Irrigation treatment	Soluble solids (%)	Soluble sugars (%)	Lycopene (mg/kg)	Organic acids (%)	Vitamin C (mg/kg)	Sugar/acid ratio
N_L	CI	4.98±0.71g	3.34±0.34g	32.60±6.75g	0.48±0.02def	114.28±2.76e	6.91±0.57efg
	AI_1	6.27±0.61f	4.22±0.76def	63.78±9.96cde	0.37±0.03h	122.00±3.99bc	11.64±2.64abc
	AI_2	7.24±0.43bcd	4.41±0.44def	57.17±7.40def	0.37±0.06h	123.96±4.75ab	12.25±2.78ab
	AI_3	6.15±0.31f	3.87±0.53fg	62.5±5.16cde	0.38±0.05gh	121.58±3.74bcd	10.34±1.40bcd
	AI_4	7.31±0.77bcd	4.53±0.41dcef	53.75±8.97cde	0.35±0.07h	125.75±4.74ab	13.48±2.44a
N_M	CI	5.45±0.50g	4.04±0.25ef	60.72±4.15cde	0.64±0.03b	118.04±1.98cde	6.30±0.32g
	AI_1	6.68±0.36def	4.70±0.39bcde	78.99±12.74ab	0.48±0.03def	123.52±3.79b	9.72±1.07cde
	AI_2	7.48±0.33abc	5.27±0.54ab	90.62±10.13a	0.45±0.01fg	124.37±2.03ab	11.73±0.96abc
	AI_3	7.03±0.67bcde	4.55±0.44cde	79.04±7.59ab	0.51±0.08cdef	128.55±3.41a	9.16±1.80def
	AI_4	7.48±0.55abc	5.56±0.53a	70.77±8.94bc	0.47±0.05ef	122.17±3.26bc	12.00±1.08abc
N_H	CI	6.37±0.25ef	4.82±0.12bcd	47.55±7.34f	0.74±0.03a	121.9±1.08bc	6.52±0.22fg
	AI_1	6.86±0.33cdef	4.48±0.40def	67.17±4.64bcd	0.55±0.08cd	121.20±3.78bcd	8.31±1.66defgh
	AI_2	7.71±0.28ab	4.53±0.44dcef	73.62±7.65bc	0.53±0.11cde	122.40±3.20bc	8.86±2.12deg
	AI_3	7.15±0.77bcd	4.47±0.38def	69.76±5.35bcd	0.58±0.02bc	121.58±3.10bcd	7.71±0.52efgh
	AI_4	8.17±0.40a	5.16±0.54abc	61.13±13.70cde	0.51±0.04cdef	116.94±2.60de	10.21±1.24bcd

(Values are means ± standard errors (n=6). Different letters in the same column indicate significant difference at $P<0.05$.)

3.3. Tomato water use

Irrigation method and N level had significant effects on tomato water consumption (Fig. 2). Compared to CI, AI_1, AI_2, AI_3 and AI_4 reduced water consumption by 15.6%-16.5%, 28.2%-30.2%, 19.5%-19.9% and 32.2%-33.7%, respectively. N_H reduced water consumption by 9.5%-11.2% but N_M did not affect it significantly as compared to N_L.

There were significant effects of irrigation method and N level and their interaction on tomato water use efficiency (WUE) (Fig. 3). Compared to CI, AI_1, AI_2, AI_3 and AI_4 at N_L and N_M increased the WUE by 22.4%-24.4%, 20.7%-21.8%, 12.5%-15.6% and 7.4%-23.5%, respectively, but AI at N_H reduced it by 4.7%-9.0%, indicating that AI at N_L and N_M can greatly reduce water consumption and increase WUE simultaneously, which agreed with previous results(Kang et al., 2012). N_M and N_H at CI increased the WUE by 26.2% and 27.8% and N_M at AI increased WUE by 9.7%-29.6% as compared to N_L. The WUE increased firstly and then decreased at AI with the increase of N level. AI did not increase the WUE significantly at higher N level, which might be associated with lower soil water availability in AI. Thus suitable N rate in AI is lower than that of CI, suggesting that AI can increase N use efficiency, which agreed with previous result (Topcu et al., 2007). Compared to CIN_L treatment, other treatments increased the WUE by 12.5%-54.5%. In addition, AI_1N_M treatment had the highest WUE and increased it 54.4% compared to CIN_L treatment.

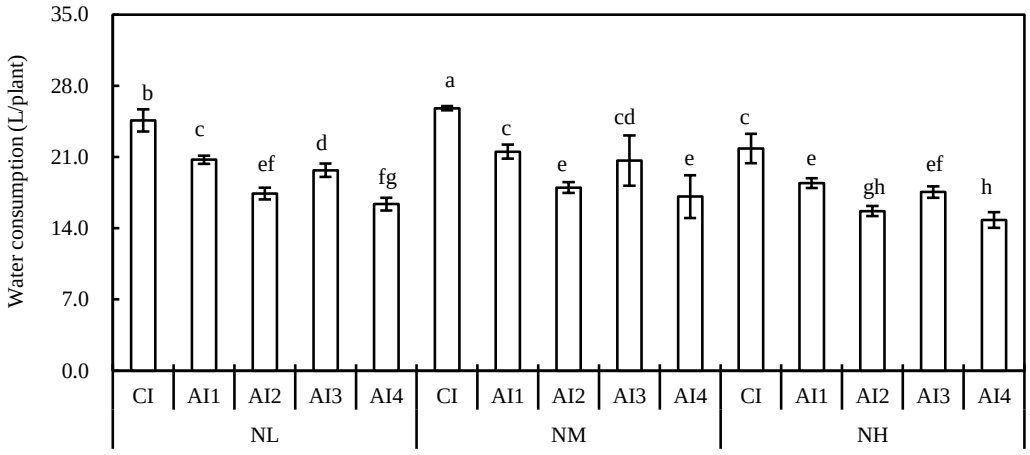

Figure 2. Effect of irrigation treatment and nitrogen level on water consumption of tomato

(Values are means ± standard errors (n=6). Different letters in the same column indicate significant difference at P<0.05.)

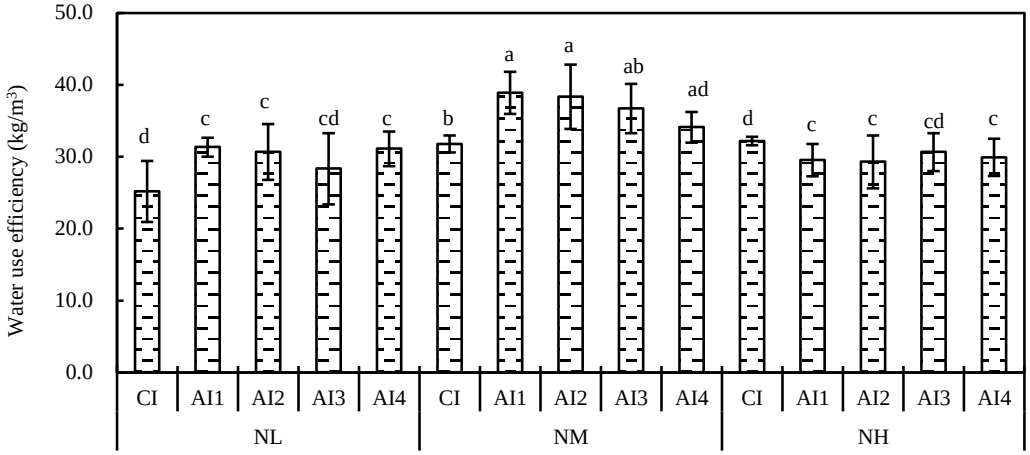

Figure 3. Effect of irrigation treatment and nitrogen level on water use efficiency of tomato

(Values are means ± standard errors (n=6). Different letters in the same column indicate significant difference at P<0.05.)

4. Conclusions

(1) Alternate partial root-zone irrigation (AI) at low N level (N_L) and middle N level (N_M) could improve fruit soluble solids content, reduce water consumption greatly and increased tomato water use efficiency (WUE), and AI under high water from the blooming to fruiting stage (AI_1) at N_L and N_M did not decrease fruit yield significantly as compared to conventional irrigation.

(2) Compared to low N level, N_M could increase fruit yield, WUE, and the contents of soluble solids, soluble sugars, lycopene and organic acids in fruits.

(3) The combination of AI_1 and N_M was the optimal mode of water and N supply for tomato.

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STUDIES ON EFFECT OF COARSE CEREAL SOLID BEVERAGE ON PHYSICAL HEALTH AND ITS RHEOLOGICAL PROPERTIES

Wei Wu[1*]

[1]School of Physical and Health Education, Mianyang Normal University, Mianyang, Sichuan, 621000, China;
[*] wei_wu1981@163.com

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ABSTRACT
Solid beverage is a kind of beverage which takes one certain kind of raw material as the main raw material like fruit juice, dairy products, bean flour, sugar or plant extract, etc., and is also added with defined amount of food additives or auxiliary materials. Coarse cereals have rough taste and are difficult to be digested and absorbed by body; however, production techniques of coarse cereal solid beverages can solve these problems and such kind of beverages are beneficial for balanced diet, blood pressure reduction as well as blood fat reduction. In the experiment of this study, we mixed six kinds of flour according to a certain proportion as the raw material, including red bean flour, sweet rice flour, corn flour, adlay seed flour, oat flour and buckwheat flour. In this study, we also built a quadratic polynomial model of water adsorption index (WAI) of extruded powder and four varying factors of the twin screw extruder through observing effect of four indexes of twin screw extruder on extruded powder WAI as well as the central composite design of response surface analysis, thus to determine the optimal extrusion process parameters of coarse cereal solid beverages. Besides, rheological properties of extruded powder suspension liquid were studied using digital viscometer; viscosity variation curves of extruded powder suspension liquid in different conditions were measured and a mathematical model of effect of temperature on viscosity of suspension liquid was established, thus to provide theoretical basis for equipment selection and processing techniques.

1. Introduction

Coarse cereal solid beverages use cereals as ingredients, which do not only contain nutritional ingredients that are beneficial for physical health, but also taste better and are easy to be digested and absorbed. Thus, coarse cereals solid beverage has extensive market prospect. Among common beverages in market, carbonated beverages mainly contain carbonated water, citric acid, sugar, spices and even caffeine and artificial color, etc. Except for sugar which can provide body with energy, almost no nutrients can be found in carbonated beverages. Tea beverages contain rich tea polyphenol which has protective effect on intestines and stomach of body; however, tea beverages also contain tannin which will reduce absorption of ion, and long-term drinking of tea beverages can result in ischemic anemia, especially for young women. Milk tea is a kind of high-glucose, high-oil and high-calorie beverages which can cause weight gaining and cholesterol increasing. Juice beverages contain various kinds of vitamins

and sugar, and appropriate amount of intake of juice beverages can provide vitamins and inorganic salt for body; however, for people whose digestive systems are not fully developed like children, excessive amount of juice beverage can lead to some diseases.

With the changing of people's concept of diet, development of solid beverages closely centers on consumption habits of people which converts from pursuit of taste to nutrition and health. Some new technologies are gradually applied to processing techniques of solid beverages, such as spray drying, enzymolysis and superfine grinding, etc., which significantly enrich varieties of solid beverages (Paudel et al., 2013; Kaur et al., 2014; Zhao et al., 2014). Although coarse cereal solid beverages contain macromolecules like protein molecules and starch molecules which have poor dissolving property, non-water soluble proteins, water-soluble proteins and starch molecules will have complicated interaction in suspension liquid system, thus a suspension liquid reaction system which contains solution as well as associated compounds or complex compounds of above substances is formed (Shin et al., 2004; Meillot et al., 2013). Coarse cereals solid beverages sold in current market are usually produced by spray drying technique (Tan et al., 2011)which has disadvantages like complicated process method, low use ratio of coarse cereal materials and loss of nutrients. This study adopted response surface analysis method (Yi-Jun et al., 2011; Zhang, Hu and Gao, 2005) and took coarse cereals as raw materials to have optimization analysis on influencing factors and describe significance of influencing factors as well as mutual effects between factors, thus to finally build a complete quadratic polynomial model.

It is of great significance for development of coarse cereals industrialization to produce new-type solid beverages using extrusion technology which are rich in nutrients and are easy to be absorbed by body. Most instant coarse cereal beverages in current market have poor stability and phenomena like stratification and caking are common. Some researches indicate that change of viscosity of suspension liquid is probably the reason that causes caking (Deng et al., 2010; Chou, Liao and Hsiau, 2011); however, the specific mechanism is still unknown. Food rheology is a branch of rheology (Zhong and Daubert, 2013; Moschakis, 2013); rheological properties of food are closely related to processing techniques, dispersed state and texture stability of food, especially to the stability of beverages.

One of the research objects of food rheology is change of viscosity and studies about viscosity changes of extruded powder suspension liquid are beneficial for understanding of interaction between macromolecules during mixing process of extruded powder with water.

2. Materials and methods

2.1. Experimental materials

Following materials are used in the experiment: red bean flour, sweet rice flour, corn flour, adlay seed flour, oat flour and buckwheat flour; soybean lecithin; glycerin monostearate; laboratory-prepared extruded powder of coarse cereals; sodium carboxyl methyl cellulose (CMC-Na); xanthan gum; sugar esters; carrageenan; locust bean gum. All materials used in experiment are food-grade.

2.2. Experimental Instruments

DV-79+PRO digital viscometer; SMS texture analyzer; DS32-II twin screw extruder; MB45 halogen moisture tester; KC-500 small-size high-speed disintegrating machine; DSC-60 differential scanning calorimeter; RS600 rotational rheometer.

2.3. Experimental methods

Selection of extruded powder: water adsorption indexes (WAI) were used in the experiment as indexes of response surface analysis (Jackson et al., 2011; Singh, Gamlath and Wakeling, 2007; Cerezal et al., 2012); in mixing process of extruded powder, permeation

rate of moisture as well as dispersity of extruded powder can be affected by granularity, and appropriate granularity of extruded powder was determined by single factor experiment. Parameters of twin screw extruder like machine barrel temperature, screw speed, feed rate and material moisture content were 170 °C, 170 r/min, 25 Hz and 15% respectively. Coarse cereal flour was mixed according to a certain proportion and after moisture adjustment, extrusion experiment was performed. Then obtained extruded particles were put into a dryer until moisture content of extruded particles was less than 5%. After that, extruded particles were put into a high-speed disintegrating machine and sizes of obtained extruded particles were from 20~100-mesh. Then extruded powder was sieved using sample sieves in 20-mesh, 40-mesh, 60-mesh, 80-mesh and 100-mesh respectively. Then different extruded powder samples were marked and sealed for preservation. WAI of extruded powder samples in different granularity were measured.

Response surface analysis: proper ranges of extrusion parameters were determined by single factor experiment that material moisture content should be 15%~21%, machine barrel temperature should be 150 °C~190 °C, screw speed should be 140 r/min~180 r/min and feed rate should be 15 Hz~25 Hz. Using central composite design (Gonçalves et al., 2006) of Design-expert 7.0 software, taking screw speed (group A), extrusion temperature (group B), material moisture content (group C) and feed rate (group D) as response surface analysis objects and taking WAI of extruded powder as response values, response surface design list as well as optimal composition conditions of extrusion technologies of coarse cereal beverages were determined.

Building of temperature-viscosity model (ByungIn, Moon and Kun, 2014; Sohn and Chang, 2006): extruded powder sieved through 80 mesh was mixed with water in 85 °C to obtained suspension liquid whose mass fraction was 12.5%; viscosities of the suspension liquid were measured using texture analyzer in 65 °C, 55 °C, 45 °C, 35 °C, 25 °C and 15 °C respectively. Probe P/50 was adopted in the experiment for texture profile analysis (TPA): pre-test speed was 5 mm/s, speed during test was 5 mm/s and post-test speed was 10 mm/s; test distance was 10 mm and trigger force was 3 g; each group had three times of parallel tests. Experimental results had one-dimensional linear and nonlinear regression analysis using SPSS17.0 software.

3. Results and discussions

3.1. Effect of Granularity on WAI of Extruded Powder

The finer the extruded powder is, the higher the mesh number should be. As shown in figure 1, when granularities of extruded coarse cereal powder were 20~100-mesh, with the increase of granularity, WAI of extruded powder decreased first and then increased; WAI of extruded powder was the lowest when granularity was 80-mesh. Therefore, 80-mesh extruded powder was better in consideration of resolvent properties of extruded powder (Carvalho et al., 2010).

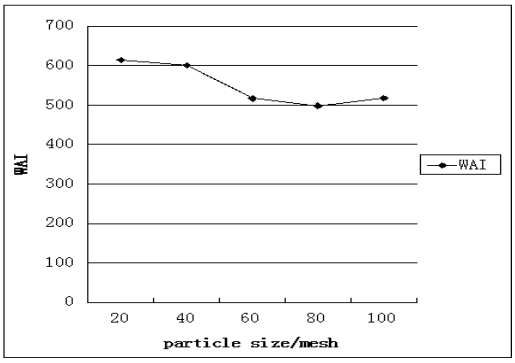

Figure 1. Effect of granularity of coarse cereal powder on WAI

3.2. Response Surface Coding

Response surface coding designs of screw speed (group A), extrusion temperature (group B), material moisture content (group C) and feed rate (group D) are shown in table 1 (Hee and Sung, 2010).

Table 1. Code table of response surface design

Names of variables	**Groups**	**Levels**				
screw speed (r/min)	A	126.47	140	160	180	193.42
Extrusion temperature (°C)	B	137.24	150	170	190	201.48
Material moisture content (%)	C	12.86	15	18	21	23.17
Feed rate (Hz)	D	11.27	15	20	25	28.30
Coding		-1.682	-1	0	1	1.682

3.3. Response Surface Regression Equation and Analysis Results

Design-Expert 7.0 software was used in the experiment and variance analysis and regression model coefficients of screw speed (group A), extrusion temperature (group B), material moisture content (group C) and feed rate (group D) are shown in table 2; quadratic polynomial regression equation of WAI is as follows (Annadurai and Sheeja , 1998):

WAI=27.87262-0.060808A-0.13044B-0.6384C-0.14138D+0.00011AB-0.00008AC-0.00033AD+0.00183BC+0.00039BD-0.001CD+0.00017A2+0.00021B2+0.01089C2+0.00371D2

Table 2. Variance analysis for response surface design

Sources of variance	**Total variance**	**Degree of freedom**	**Mean square error**	**P value**	**F value**
Models	0.6197	14	0.0443	<0.0001	224.47
A	0.0085	1	0.0085	0.0006	42.85
B	0.0061	1	0.0061	0.0015	30.68
C	0.1266	1	0.1266	<0.0001	642.13
D	0.0008	1	0.0008	0.0905	4.06
AB	0.0058	1	0.0058	0.0016	29.49
AC	0.0002	1	0.0002	0.3528	1.01
AD	0.0035	1	0.0035	0.0054	17.97
BC	0.0968	1	0.0968	<0.0001	490.91
BD	0.0050	1	0.0050	0.0024	25.10
CD	0.0018	1	0.0018	0.0234	9.13
A2	0.0655	1	0.0655	<0.0001	332.35
B2	0.1052	1	0.1052	<0.0001	533.45
C2	0.1437	1	0.1437	<0.0001	728.47
D2	0.1285	1	0.1285	<0.0001	651.79
Residual error	0.0012	6	0.0002	——	——

Therefore, as shown in table 2, p value of regression item of model was less than 0.0001 which was far less than 0.05, thus the selected model was highly significant; lack of fit p = 0.0732 which was bigger than 0.05, thus lack of fit of model was insignificant; model coefficient R2=0.9981. Above three parameters indicated that the quadratic regression model significantly fitted influence of screw speed, extrusion temperature, material moisture content and feed rate on WAI; thus the selected model was appropriate. Determination coefficient AdjR2 of the regression model was 0.9936, thus 99.36% of response value change could be explained by the model while only 0.64% of changes could not be explained, which further verified significance of the model. F values reflected influence degrees of different factors on WAI of extruded powder: the bigger the F value was, the more significant influence of corresponding factor was on WAI. Order of single factor influence was material moisture content (C) > extrusion temperature (B) > screw speed (A) > feed rate (D). Therefore, changes of material moisture content and extrusion temperature played an important role in WAI control of extruded powder.

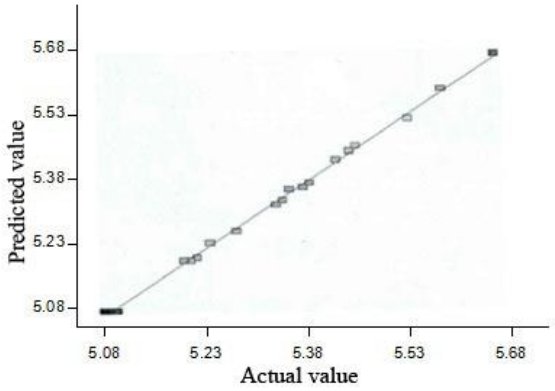

Figure 2. Residual graph of response surface quadratic regression equation model

As shown in figure 2, actual values and predicted values are in the same line, which indicates that the model greatly fits the relationship between four extrusion indexes and WAI. Using Design-Expert 7.0 software as well as minimum value optimizing method, the optimum conditions of twin screw extruder were that screw speed was 150.84 r/min, extrusion temperature was 184.46 °C, moisture content was 15.53%, feed speed was 18.30 Hz and predicted value of WAI was 5. The average WAI value of actual obtained extruded powder was 5.03, thus the error between actual value and predicted value was 0.59%. Therefore, response surface method was effective to control of WAI of extruded powder.

3.4. Establishment of Temperature-viscosity Model

As shown in figure 3, when the temperature was from 15 °C to 75 °C, viscosity of suspension liquid decreased with the increase of temperature, and experimental results were the same as test results of digital viscometer. The reason was that with the increase of temperature, movement of water molecules aggravated and acting forces between amylase molecules were weakened, thus viscosity of suspension liquid decreased.

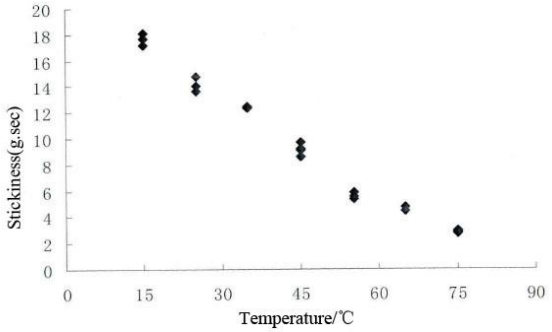

Figure 3. Effect of temperature on viscosity of suspension liquid

Table 3 shows linear and nonlinear fitting results using SPSS17.0 software. Results indicate that the higher the R2, the higher fit degree of predicted values and actual values of the model, Fitting degrees order of models is parabola model > quadratic model > linear model > (exponential model, compound model, growth model and logistic model) > logarithmic model > power function model > reciprocal model > S curve model.

Table 3. Model fitting

Equations	Model summary			Estimate of parameters
	R2	F	Constant term	
Linear model	0.973	845.472	20.736	-0.253
Logarithmic model	0.964	510.786	44.308	-9.425
Quadratic model	0.986	684.276	23.176	-0.362
Parabola model	0.993	515.780	20.289	-0.142
Compound model	0.973	593.272	31.621	0.968
Power function model	0.847	117.285	438.045	-1.080
S curve model	0.668	41.201	1.214	28.342
Growth model	0.963	593.698	3.450	-0.326
Exponential model	0.963	593.698	31.255	-0.362
Reciprocal model	0.871	109.583	1.876	263.891
Logistic model	0.965	593.698	0.032	1.029

Therefore, equation of parabola model can be obtained as follows:

Y=20.454-0.144X-0.004X2+0.000041X3, R2=0.989

Quadratic model equation is:

Y=23.007- 0.375X+0.001X2, R2=0.986

4. Conclusions

When granularities of extruded coarse cereal powder are 20~100-mesh, WAI of extruded powder decrease first and then increase with the decrease of granularities; when granularity is 80-mesh, WAI of extruded powder is the lowest. Quadratic polynomial regression equation of screw speed, extrusion temperature, material moisture content and feed rate is established based on response surface analysis. The obtained optimal extrusion parameters are that crew speed is 150.84 r/min, extrusion temperature is 184.46 °C, moisture content is 15.53%, feed speed is 18.30 Hz and WAI is 5.03. Order of single factor influence on WAI of extruded powder is material moisture content (C) > extrusion temperature (B) > screw speed (A) > feed rate (D).

TPA experiment indicates that when temperature is at 15 °C to 65 °C, viscosity of suspension liquid decreases with the increase of temperature. Temperature-viscosity models with appropriate fitting are as follows:

Parabola model: Y=20.454-0.144X-0.004X2+0.000041X3, R2=0.989

Quadratic model: Y=23.007-0.375X+0.001X2, R2=0.986

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REGULATORY EFFECT OF SHORT WAVE ULTRAVIOLET AND HEAT TREATMENT ON STRAWBERRY DURING TRANSPORTATION

Huwei Song[1], Xinfeng Wang[1], Weiming Yuan[2*]

[1] Jiangsu Collaborative Innovation Center of Regional Modern Agriculture & Environmental Protection, College of Life Science, Huaiyin Normal University, Huaian 223300, Jiangsu, China;
[2] Suzhou Polytechnic Institute of Agriculture, Suzhou 215008, Jiangsu, China.
** yuanweimingywm@163.com*

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ABSTRACT
Strawberry is characterized by heart shape, bright color, juicy pulp, sour and sweet taste and full-bodied aroma. But strawberry is easy to have mechanical damage during transportation, leading to quality deterioration. This study explores regulation mechanism of short wave ultraviolet (UV-C) and hot heat treatment on physiological damage and quality of strawberry. We found UV-C could significantly lower rotting rate of the damaged site of strawberry, respiration intensity and generation of ethene. Moreover, hot heat treatment also lowered rotting rate, respiration intensity, specific conductance and generation of ethene, indicating heat treatment is effective in lowering rotting rate and consumption speed of nutritional substances of fruits, restraining membrane lipid peroxidation and reducing mechanical damage. All the findings suggest that, heat treatment and UV-C both can regulate physiological and antioxidant substance metabolism, reduce damage on quality of strawberry caused by damage stress and ensure good quality.

1. Introduction

Strawberry with high edible value is called the queue of fruits. But due to thin pericarp, 90%~95% water content and soft tissue, it is easy to be damaged during collection and transportation, leading to rapid rotting, deteriorated quality and reduced value (Ribeiro and Peretto et al., 2014; Sofia et al., 2010). Research on preservation of strawberry now focuses on storage of complete strawberry and concerns little about physiology and quality of damaged strawberry. It is of great importance to explore physiological and quality characteristics of strawberry collected for storage of strawberry (Li, 2014; Qing et al., 2014).

Short wave ultraviolet (UV-C) (Marquenie et al., 2003) as a safe and effective physical method with no toxicity, no pollution and no residue avoids the unbeneficial influence of chemical bactericide and its residue, satisfying consumers' pursuit for health, safe and natural food. In recent years, storage of fruits and vegetables are attached more importance by researchers. UV-C can effectively ensure quality of fruits and vegetables collected and lower rotting rate. But effect of UV-C on physiological damage and quality of strawberry has not been researched thoroughly. Heat treatment (Hauptman et al., 2013, Jeong et al., 2004) as a physical method with no toxicity and residue will not cause bad influence on food safety like chemical disinfectant and its residue. Heat treatment can effectively lower incidence of pest and disease damage, lower rotting rate, delay ageing and ensure good quality of fruits and vegetables. But research on

regulatory effect of heat treatment on physiological damage and quality of strawberry is few.

2. Materials and methods

2.1. Reagents and materials:

Regents used included ethylenediamine tetraacetic acid (EDTA), polyvinylpyrrolidone (PVP), polyvinyl-polypyrrolidone (PVPP), methoxypolyethylene glycols (MPEG), ascorbic acid (ASE), titanium tetrachloride and guaiacol. Instruments used included gaschromato-graph, ultraviolet / visible ultraviolet spectrophoto-meter (UV/VISS), high-speed freezing centrifuge, -70 ℃ ultra cold storage freezer, color difference meter and sugar refractometer.

2.2. Processing of materials

Strawberry from strawberry base, Pizhou, Jiangsu, China was selected. The materials were sent to laboratory within two hours after picking. Fruits with no mechanical damage, pest and disease damage, even size and consistent maturity were selected and disinfected by 0.21% sodium hypochlorite solution (Taharaguchi et al., 2014).

Ordinary UV disinfection lamp (Lyn, 2014) (diameter of tube: 2.4 cm; length: 89 cm; output power: 30 W; 95% UV launch wave energy at wavelength of 254 nm) was used as radiation source. Fruits and vegetables which have been preprocessed were placed 12 cm below UV lamp. UV intensity was measured as 0.847mW/cm2 by digital radiometer. When half time passed, it was overturned. Radiation dosage was determined by processing time under certain radiation intensity. Control group was not given radiation.

Strawberry was randomly divided into three groups, UV-C group, heat treatment group and control group, 300 in each group, according to preliminary experiment. Strawberry in UV-C group was cut into two parts along fruit stem line and then the wound surface was radiated under UV lamp (dosage 4.1KJ/m2). Strawberry in heat treatment group was processed by 45 ℃ hot air. Then strawberry was stored in constant temperature humidity chamber (temperature 20±1℃, relative humidity: 85% ~ 90%). Finally rotting situation of strawberry was observed.

Relevant indexes were measured 0d, 1d, 2d, 3d, 4d and 5d after radiation. Forty samples were selected every time, 10 for detection of hardness and specific conductance, 10 for detection of respiration intensity and ethene and the others for detection of antioxidant substance. Pulp on wound site was take and quick-frozen by liquid nitrogen. Then pulp was grinded into powder with beater under low-temperature environment and stored in ultra cold storage freezer (-70℃).

2.3. Experimental method

Detection of respiratory rate (Linchun et al., 2007): strawberry samples were weighed and then placed in closed container at 20 ℃ for 2 h. Then CheckMate O2-CO2 tester was used to measure content of CO2. The detection was repeated thrice.

Detection of release amount of ethene: strawberry was placed in closed container for 2 h. Then injector was used to extract sample, 2.5ml every time. Ethene was detected under the environment of 0.05mPa carrier gas pressure, 0.05mPa hydrogen pressure, 0.1mPa nitrogen pressure, 80℃column temperature, 80 ℃injection temperature and 120℃detector temperature. External standard method was used for quantification, peak area was taken as parameter and standard gas was set as 10.0μl/L. Detection was repeated for three times.

Detection of hardness (Vicente, 2005): hardness of pulp in incision position was measured with texture analyzer. Detection depth was set as 5 mm, diameter of probe was 5mm and detection speed was 5mm/s. Maximum was taken. Detection was repeated for 10 times. Finally, the average value was taken and the final result was expressed as N.

Detection of rotting rate (Shuttle et al., 2008): rotting degree was divided into four levels: level 0: no rotting; level 1: 25% rotting

area; level 2: 25% ~ 50% rotting area; level 3: 50% over rotting area. Rotting index was calculated according to the following formula.

Rotting index (%) = $\sum$ [(Rotting level × Number of fruits at that level)/ (The highest rotting level × Total number of fruits)] × 100%

Rotting situation was observed 1d, 3d and 5d later.

Weight loss ratio (%) = (Initial weight of strawberry − Weight when sampling)/ Initial weight of strawberry × 100%

Detection of content of soluble solid and weight loss ratio (Liu et al., 2010): strawberry was fully crushed and mixed. Then filter liquor was squeezed with four layers of gauze, followed by filtration of double-layer filter paper. The filter liquor was collected. WYT-32 type handheld sugar refractometer was used to measure content of soluble solid. Weight of fruits before storage and at different time points was measured respectively. Weight loss ratio can be calculated using the following formula:

2.4. Statistical method

Measurement data were expressed by mean ± SD. All data were processed by SPSS19.0 software. Difference was considered as statistically significant if $P<0.05$.

3. Results

3.1.Effect of UV-C on respiratory action and yield of ethene of damaged strawberry

Figure 1 shows that, UV-C had positive influence on respiration intensity of damaged strawberry; treatment group and control had little difference from day 0 ~ 2; however, respiration intensity of control group at 3th day was 43.80% higher than at 2nd day, while that of treatment group remained stable; respiration intensity of control group was 40.42% higher than treatment group at day 3 ($P<0.05$); from day 3 to day 5, respiration intensity of damaged strawberry showed increasing tendency, but the increasing speed in control group was significantly higher than treatment group ($P<0.05$); respiration intensity reached maximum at day 4 in both groups, but at day 5, control group had a slightly decreased respiration intensity; respiration intensity remained stable from day 4 to day 5.

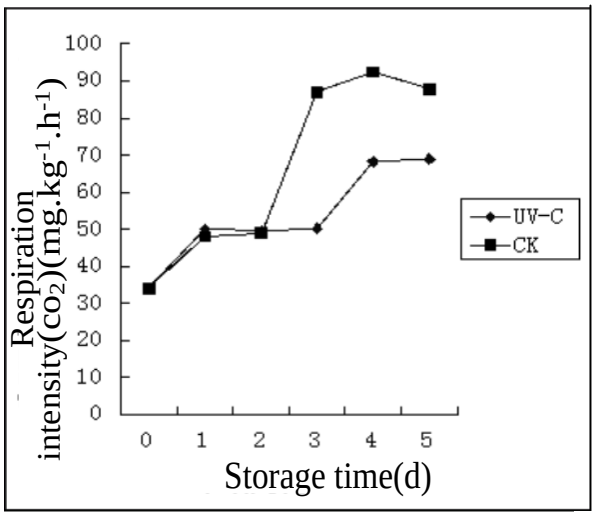

Figure 1. Effect of UV-C on respiration intensity of damaged strawberry

Figure 2 shows that, UV-C could keep the content of ethene at a relatively low level; from day 0 to day 4, content of ethene in control group was quite low, showing a decreasing tendency, but reached maximum at day 5; content of ethene in treatment group was much lower than control group ($P<0.05$); content of ethene in control group kept a relatively high increase in other time except for day 2.

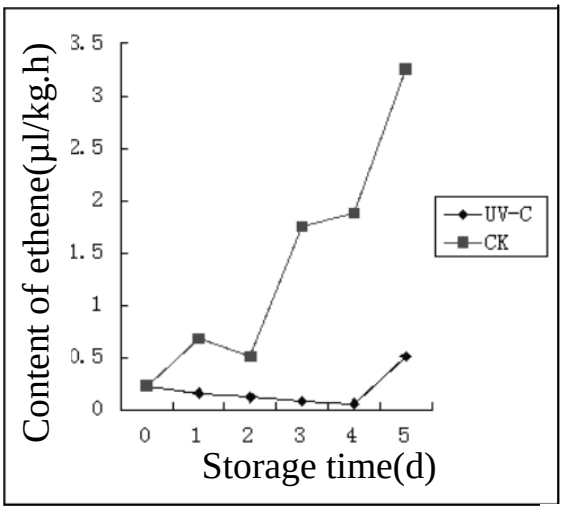

Figure 2. Effect of UV-C on content of ethene in damaged strawberry

3.2.Effect of UV-C on hardness and rotting index of strawberry

Figure 3 suggests that, damaged strawberry which has been treated by UC-V had significant different with control group in hardness of fruits; hardness in storage period had a consistent tendency, rising first and then decrease; there was no remarkable different from day 0 to 1 (P>0.05); control group was harder than treatment group from day 2 to day 5 (P<0.05); both groups reached maximum at day 3, control group was 22% higher than treatment group; after day 3, both groups tended to have lower hardness and control group was in a faster decreasing speed.

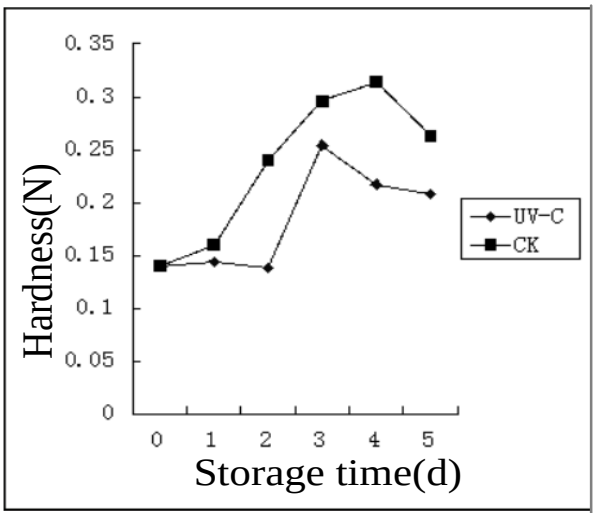

Figure 3. Effect of UV-C on hardness of damaged strawberry

Figure 4 shows that, rotting index showed a rising tendency during storage period; rotting index in treatment group was always lower than control group; difference between two groups was the largest at day 5.

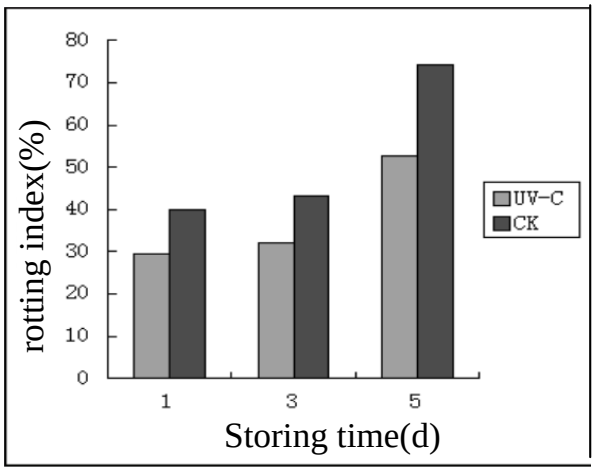

Figure 4. Effect of UV-C on rotting index of damaged strawberry

3.3.Effect of UV-C on soluble solid content and weight loss ratio of damaged strawberry

Figure 5 suggests that, content of soluble solid showed up a decreasing tendency overall; the decreasing amplitude was large from day 0 to day 2, 51.72% in treatment group and 47.13% in control group; from day 2 to day 5, decreasing tendency in treatment group and control group slowed down; content of soluble solid in control group was higher than control group at day 1, and treatment group was 1.29 times of control group.

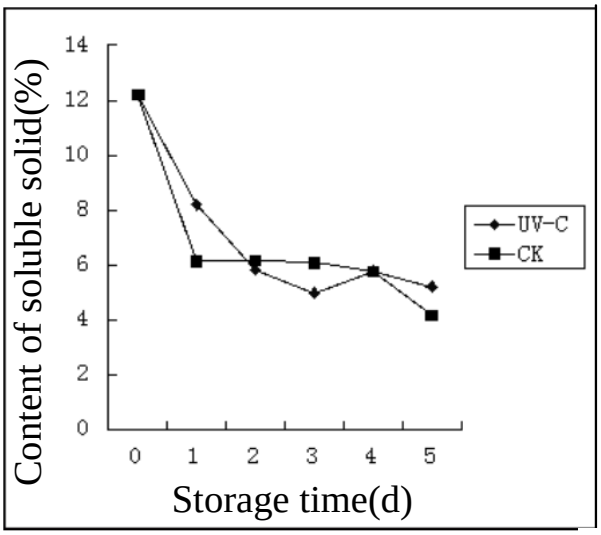

Figure 5. Effect of UV-C on soluble solid content of damaged strawberry

Figure 6 demonstrates that, weight loss ratio of damaged strawberry showed a consistent tendency in both groups during storage period, both increased; from day 0 to day 3, weight loss ratio in treatment group was much lower than control group from day 0 to day 3 (P<0.05); weight loss ratio in treatment group at day 1, 2 and 3 was 65.59%, 30.23% and 8.31% lower than control group; difference of weight loss ratio between treatment group and control group at day 4 and 5 was not obvious (P>0.05).

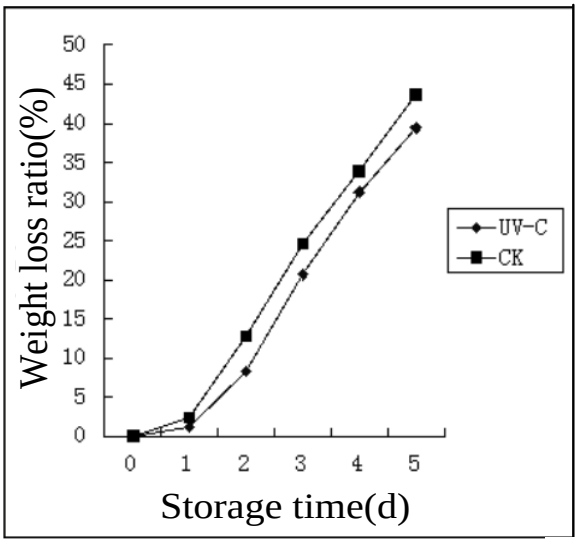

Figure 6. Effect of UV-C on weight loss ratio of damaged strawberry

3.4.Effect of heat treatment on respiration intensity and content of ethene of damaged strawberry

Figure 7 demonstrates that, respiration intensity in both groups rose first and then decreased during storage period. From day 1 to day 2, treatment group and control group had a slow variation and treatment group showed a slight decrease. Difference between two groups was not obvious at day 2, but became significant at day 3, 4 and 5 (P<0.05); respiration intensity in control group was 24.87%, 15.94% and 10.11% higher than treatment group at day 3, 4 and 5.

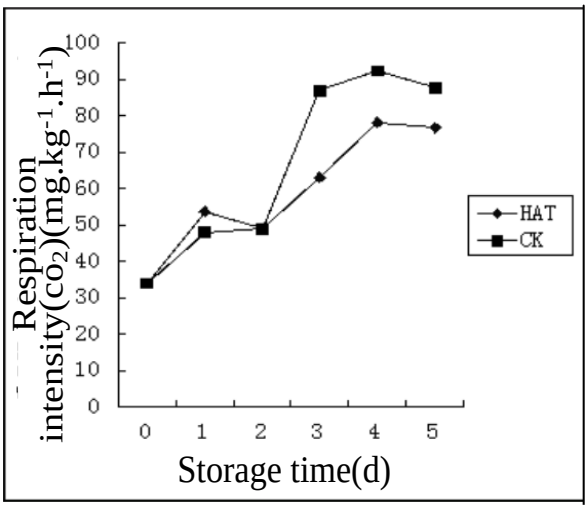

Figure 7. Effect of heat treatment on respiration intensity of damaged strawberry

Figure 8 suggests that, content of ethene in strawberry gradually increased during storage period. Content at day 2 was slightly lower than day 1. Content of ethene in heat treatment group increased slow first, then decreased and then rose. To be specific, content of ethene in treatment group gradually increased from day 0 to day 3, reached maximum at day 3, decreased at day 4 and slightly increased at day 5. Except for day 2, content of ethene in heat treatment group was lower than control group at other time points, and the difference was significant (P<0.05).

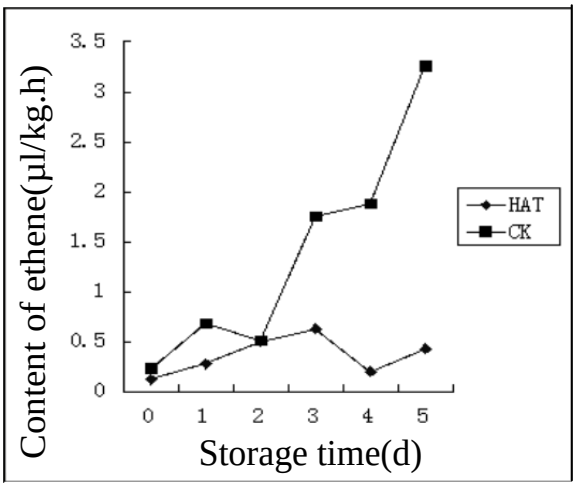

Figure 8. Effect of heat treatment on ethene content of damaged strawberry

Effect of heat treatment on hardness and rotting index of damaged strawberry

Hardness of strawberry rose first and then decreased slowly. Figure 9 suggests that, strawberry was the hardest at day 4 in control group; hardness of strawberry in heat treatment group reached maximum at day 2, 2 days earlier than control group; hardness value of heat treatment group was 41.57% higher than control group at day 2; hardness in heat treatment group tended to decrease after day 2; moreover, hardness of strawberry in heat treatment group was much higher than control group ($P<0.05$).

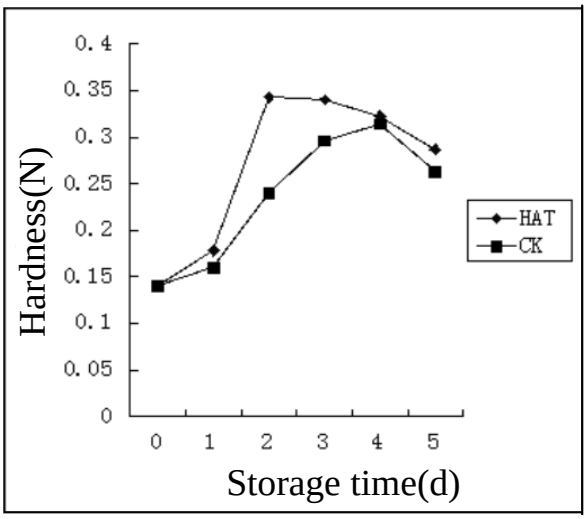

Figure 9. Effect of heat treatment on hardness of damaged strawberry

Figure 10 demonstrates that, rotting index of damaged strawberry gradually increased. Rotting index of heat treatment group was always lower than control group. At day 5, rotting index in treatment group and control group both reached maximum, 66.66% and 75.83% respectively.

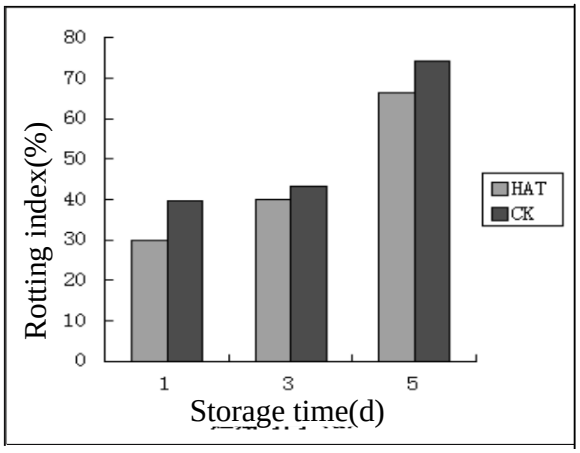

Figure 10. Effect of heat treatment on rotting index of damaged strawberry

3.5.Effect of heat treatment on soluble solid content and weight loss ratio

Figure 11 suggests that, soluble solid content showed a decreasing tendency during storage period. It decreased in a high speed at day 1. Variation from day 1 to day 3 is little; after day 3, it decreased slowly. Soluble solid content in heat treatment group showed a decreasing tendency. But the content in heat treatment group was 36.90% higher than control group at day 1; from day 2 to day 5, the content in heat treatment group was lower than control group.

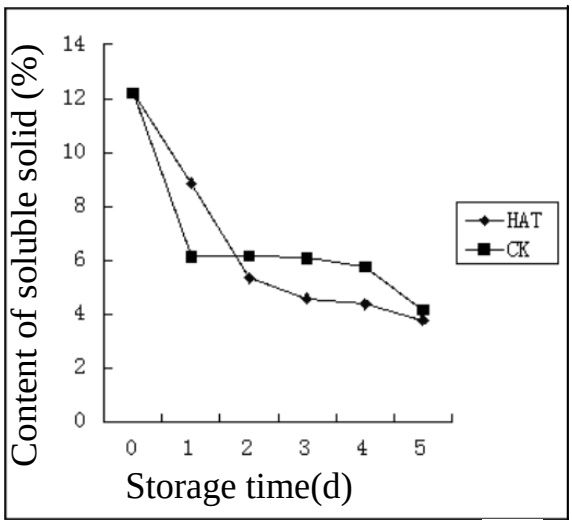

Figure 11. Effect of heat treatment on content of soluble solid of damaged strawberry

Figure 12 shows that, weight loss ratio of strawberry showed an increasing tendency during storage period. Weight loss ratio was low at day1, but showed an increasing tendency from day 1 to day 5, indicating longer storage time led to rapid weight loss. During storage period, weight loss ratio of strawberry in heat treatment group was always lower than control group, suggesting heat treatment could effectively restrain increase of weight loss ratio and reduce loss of organic matter.

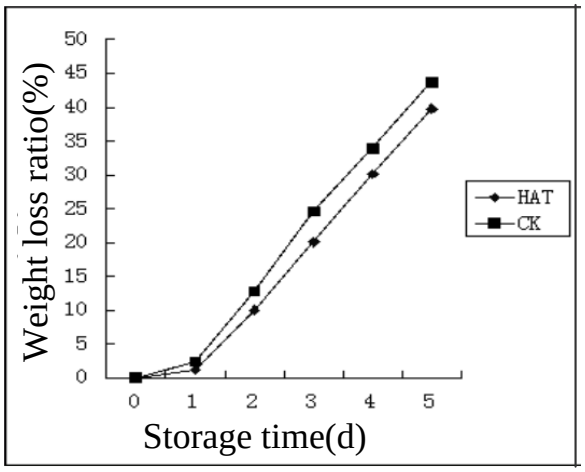

Figure 12. Effect of heat treatment on weight loss ratio of damaged strawberry

4. Conclusions

UV-C has been proved to be able to regulate respiratory intensity and content of ethene of damaged strawberry. Experimental results indicate that, UV-C can effectively lower respiration intensity of strawberry in the late storage period, thus to reduce consumption of organic matters on incision site and ensure quality; content of ethene of strawberry which has been treated by UV-C is much lower than control group, suggesting UV-C can effectively restrain generation of ethene and delay aging and deterioration of quality. Freshness is in a close correlation with its quality and weight loss ratio has importance influence on content of organic matters of fruits. UV-C treatment can keep freshment of damaged strawberry and delay air drying speed of wound, thus control weight loss ratio of strawberry at a relatively low level.

Processing strawberry with 45℃ hot air for 3 h can regulate the quality of strawberry and inhibit deterioration of damaged strawberry. Heat treatment can lower respiration intensity, rotting rate and content of ethene, thus slow consumption of organic matters, delay maturity and aging of fruits and improve healthy fruit rate. Weight loss ratio of strawberry in heat treatment group is lower than control group, but content of soluble solid in heat treatment group is higher than control group in early stage and lower than control group in late stage. That may be because that, soluble solid in treatment group involves respiratory activity excessively in late period.

Foreign researches (Stevens et al., 2007; Aghdam et al., 2014; Nardi et al., 2012; Alexandre et al., 2012) have explored treating fruits and vegetables with heat treatment and UV-C; however, few focus on regulatory mechanism of heat treatment and UV-C on physiology and quality of fruits which receive mechanical damage. Therefore, this work is of great significance.

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In the paper: "FOOD PACKAGING DESIGN AND ITS APPLICATIONIN THE BRAND MARKETING" by Aifeng Wu, publish in Carpathian Journal of Food Science and Technology Volume 7, issue 3, pages 5-15 at the author affiliation, page 5, paragraph 4, "*Sciences*" will be replace with "*Science*".