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OPTIMIZATION OF DRYING AND OSMOTIC DEHYDRATION OF *Asparagus Officinalis* IN MICROWAVE AND CONVENTIONAL HOT AIR OVEN USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

The main objective to this study was to determine of the drying time of asparagus slices in hot air oven and microwave oven and to optimize drying time and effective moisture diffusivity was done in terms of process parameters. The drying time of asparagus slices varied from 140 to1485 min for hot air oven and from 75 to 2580 sec for microwave drying. Effective moisture diffusivity of asparagus was between from 0.20×10^{-10} m^2/s to 2.90×10^{-10} m^2/s for hot air oven and 8.89×10^{-10} m^2/s to 75.26×10^{-10} m^2/s for microwave oven. The results presented indicated that process parameters were significantly important on drying time and effective moisture diffusivity for hot air oven drying. The less drying time was obtained in microwave oven compare to hot air oven. Drying time was affected by the concentration of $CaCl_2$ and thickness of slice and effective moisture diffusivity was significantly influenced by the microwave power and slice thickness during microwave drying.

Keywords:
Drying;
Osmotic dehydration;
Effective moisture diffusivity;
Response surface methodology.

1. Introduction

Asparagus officinalis is a spring vegetable and the flowering perennial plant species in the genus *Asparagus* in the lily family, like its allium cousins, onions and garlic, it is native to most of Europe, northern Africa and western Asia (Grubben and Denton, 2004). Green asparagus vegetables contain flavonoids, amino acid derivatives, and sulfur containing acids, glycolic acid, tyrosine, vitamins, saponins and essential oils. In addition, it also contains fluathione with antioxidant and anticarcinogenic properties and the plant also contains diuretic and laxative properties and antioxidants, such as rutin which is an important percentage of the antioxidant activity in asparagus, ascorbic acid, tocopherol, ferulic acid and glutathione (Bliss, 1973; Duke and Ayensu, 1985; Leung, 1980; Tsushida, 1994). It is used to treat parasitic diseases, cancer (antitumor and antioxidative activity), neuritis and rheumatism (Duke, 1984; Li, 2002) and it can

be used to treat cough, nose cancer, leukemia, lung cancer, breast cancer and lympthatic gland cancer (Je, 1999). In an in vitro study, hypotensive (Leung and Foster, 1996), antibacterial and antiviral effects of asparagus were declared (Pierce, 1999).

In osmotic dehydration process, vegetables or fruit slices immersed into highly osmotic solution, the water in the cells of the materials permeates into the osmotic solution through the cell membrane due to the high osmotic pressure and low water activity of the osmotic solution. Since osmotic dehydration is more economical than thermal drying methods, it is often used as a pretreatment for drying of biological materials (Pan et al., 2003). Osmotic dehydration method can be combined with other drying methods such as microwave and hot air oven. Microwave, relatively a new addition to the existing techniques, has been considered as a potential method for obtaining high quality dried food products, including fruits, vegetables and grains in this extent. Current studies have exhibited that drying of food material with microwave technology offers rapid, more uniform process and significant energy savings with a potential reduction in drying times of up to 50% and additionally avoiding undesired excessive surface temperature of treated material (Al-Harahsheh et al., 2009; Mcloughlin et al., 2003). Due to all these positive features of microwave drying some fruits and grains have been successfully dried by microwave technique and by a combination of microwave (Al-Harahsheh et al., 2009; Prasad, 2007; Prasad, 2006). Response surface methodology (RSM) is a statistical procedure frequently used for optimization studies. It uses quantitative data from an appropriate experimental design to determine and simultaneously solve multivariate problems. Equations describe the effect of test variables on responses, determine interrelationships among test variables and represent the combined effect of all test variables in any response. This approach enables an experimente to make efficient exploration of a process or system (Liyana-Pathirana and Shahidi, 2005). Therefore, RSM has been frequently used in the optimization of food processes (Eren and Kaymak-Ertekin, 2007; Corzo et al., 2008; Wani et al., 2008; Changrue et al., 2008; Mestgadh et al., 2008).

The aim of the study was to investigate drying of asparagus slices for two drying methods and to study the effects of slice thickness, microwave power, temperature of hot air oven and concentration of osmotic solution on drying time. In addition, optimization of drying time and effective moisture diffusivity was done in terms of process parameters by RSM.

2. Materials and methods

2.1. Sample Preparation

Fresh asparagus spears were supplied from market and stored in storage room at +4°C. The spears were thoroughly hand peeled prior to dehydration process and sliced to desired thickness by using an adjustable knife. All slices were sized in 30 mm 40 mm dimensions. Asparagus spears were sliced at 1, 2 and 3 mm in thickness and dried in the preheated oven to evaluate the influences of slice thickness on the drying characteristics of treated material. Initial moisture content of Asparagus was determined by placing spears in a conventional oven at 105°C till no further change in weight of sample was observed. The initial average moisture content of asparagus spears was determined as 94.19±0.89 %.

2.2. Osmotic Dehydration

For osmotic dehydration a solution of $CaCl_2$ was applied in a concentration of 0%, 20% and 40% w/w. Processing time was 60

min and constant bath temperature was 20°C. The ratio of material and osmotic solution is 1:10 w/w.

2.3. Drying

2.3.1.*Conventional Oven Drying*

Asparagus slices (2 mm in thickness) were placed into the preheated oven (Nüve, EN 400, Turkey) at air temperature of 70, 80, and, 90°C to evaluate the influences of temperature on drying process. Spears slices were spread as a single layer on the tray attached to the balance (KERN, EW-1500-2M with sensitivity of0.01g, Germany). During drying, weight of sample was recorded at a regular time interval. Drying process continued until desired moisture content was achieved (<10%, w/w).

2.3.2.*Microwave Drying*

A programmable domestic microwave oven (Samsung, MW71E, Malaysia) with maximum output of 800 W and wavelength of 2450 MHz was used for drying of slices. The dimensions of the microwave cavity were307x185x292 mm. Preweighed asparagus slices were spread in a glass dish (dried and weighed before use) as a single layer and placed on the center of microwave cavity. The sample was hold in the microwave oven under determined conditions. The samples were weighted taken out at every 60 s interval by switching off the microwave oven and after weight of sample was recorded, it was replaced in the oven. Drying process proceeded until desired moisture content was achieved (<10%, w/w). Three slice thicknesses (1, 2 and 3 mm) and three power level (100, 200 and 300 W) were examined to determine their effects on drying.

2.4. Effective moisture diffusivity

The effective moisture diffusivity (D_{eff}) was determined to obtain information about the mechanism of moisture transfer and the drying process. It was defined by Fick's second law with the assumption that diffusion is the only physical mechanism to control the transfer of water molecules to the surface. Asparagus slices prepared at different thicknesses were assumed to be an infinite slab, since other directions were large enough compared to the thickness. Thus, moisture movement was only throughout thickness. Fick's second law for moisture movement was solved with the following assumptions:

- The particle was homogenous and isotropic
- The material characteristics were constant, and the shrinkage was negligible
- Mass transfer was in one direction
- Moisture was initially uniformly distributed throughout the mass of a sample
- The pressure variations were negligible
- Evaporation occurred only at the surface
- Surface diffusion was ended, so the moisture equilibrium arises on the surface
- Effective moisture diffusivity was constant versus moisture content during drying
- Resistance to mass transfer at the surface was negligible compared to the internal resistance of the sample
- Mass transfer was represented by a diffusional mechanism the following analytical solution of Fick's second law proposed by Crank (1975) was used to calculate the effective moisture diffusivity.

$$MR = \frac{M_t - M_e}{M_i - M_e} = \frac{8}{\pi^2}\sum_{i=0}^{\infty}\frac{1}{(2i+1)^2}\exp\left(-\frac{(2i+1)^2 \cdot D_{eff} \cdot \pi^2}{4L^2}\cdot t\right) \quad (1)$$

where D_{eff} is the effective moisture diffusivity (m^2s^{-1}), L is the half thickness (drying from both sides) of the slab (m), MR is the fractional moisture ratio, t is the drying time (s), M_t is the moisture content of the material at any time, t; M_i is the initial moisture content of the material before drying and M_e is the equilibrium moisture content of a dehydrated asparagus slices, all moisture content values are in dry basis.

For long-term drying, only the first term of Eq.(1) was used to explain the drying procedure. The equilibrium moisture content (M_e) was assumed to be zero for microwave-assisted drying. The final equation to calculate the D_{eff} was as follows:

$$MR = \frac{M_t}{M_i} = \frac{8}{\pi^2}\exp\left(-\frac{D_{eff}\cdot\pi^2\cdot t}{4L^2}\right) \quad (2)$$

Further simplification of Eq. (2) resulted in a straight-line equation as Eq. (3);

$$\ln(MR) = \ln\left(\frac{8}{\pi^2}\right) - \left(\frac{D_{eff}\cdot\pi^2}{4L^2}\cdot t\right) \quad (3)$$

The effective moisture diffusivity was calculated by fitting Eq. (3) to the curve of ln(*MR*) vs. time (Figure 1 and 3), and the results are presented in Tables 1 and 3.

2.5. Experimental Design

Response Surface Methodology (RSM) was used to optimize drying and dehydration conditions. Box-Behnken model was selected for RSM analysis. Box-Behnken design requires three levels, coded as -1, 0, +1. The effect of three independent process parameters: thickness (X_1, mm), concentration of dehydration solution of $CaCl_2$ (X_2, %), applied microwave power (X_3, W) in microwave drying or oven temperature (X_3, °C) in hot air oven on weight of sample as dependent variables were investigated using RSM. The total number of microwave and oven drying experiments were 30, three replicates at the center point of design were done. Minitab 17.0 was used for the experimental design, data analysis and regression modeling. The independent variables were; X_1 (1, 2, 3 mm), X_2 (0, 20, 40 %), X_3 (100, 200, 300 W) in microwave drying and X_1 (1, 2, 3 mm), X_2 (0, 20, 40 %), X_3 (70, 80, 90°C) in hot air oven drying. The proposed model was as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_{11} + b_{22}X_{22} + b_{33}X_{33} + b_{12}X_{12} + b_{13}X_{13} + b_{23}X_{23} \quad (4)$$

where *Y* was the response of equation, b_0 was the constant coefficient, $\boldsymbol{b_1}$,$\boldsymbol{b_2}$,$\boldsymbol{b_3}$ were the linear coefficients, $\boldsymbol{b_{11}}$,$\boldsymbol{b_{22}}$, $\boldsymbol{b_{33}}$ were the quadratic coefficients, $\boldsymbol{b_{13}}$,$\boldsymbol{b_{23}}$,$\boldsymbol{b_{12}}$ were the interaction coefficients. The values of R^2, adjusted-R^2, and lack-of-fit of models were evaluated to check the model adequacies.

3. Results and discussions

The study was designed to evaluate drying conditions of *Asparagus officinalis* slices and to optimize the process conditions using response surface methodology (RSM). Drying time (DT) and effective moisture diffusivity (D_{eff}) were investigated for dried asparagus slices. Models developed by RSM to predict the experimental results determined for each interested response. Multiple linear analysis of experimental data yielded second order polynomial models for predicting DT and D_{eff}. Analysis of variance (ANOVA) was conducted to determine significant effects of process variables on each response and to fit second-order polynomial models to the experimental data. Regression equation coefficients of the proposed models and statistical significance of all main effects calculated for each response were obtained. The effects that were not significant ($p \geq 0.05$) were shown in Table 2 and 4. The ANOVA tables also showed that lack of fit was not significant for all response surface models at a 95% confidence level. On the other hand, R^2 and *Adj-*R^2 were calculated to check the model adequacy as a lack of fit ≥ 0.05; $R^2 \geq 0.95$; and *Adj-*$R^2 \geq 0.88$ (Tables 2 and 4).

Table 1. Experimental design of hot air oven drying and corresponded responses (drying time and effective moisture diffusivity)

RunOrder	X_1 Oven Temp. (°C)	X_2 $CaCl_2$Concentartion (%)	X_3 Thickness of slice (mm)	DT (min)		D_{eff} (m^2/sec) x 10^{10}	
				Observed	Predicted	Observed	Predicted
1	80	0	3	1015	1041.30	1.80	1.71
2	70	20	3	1485	1406.92	0.95	0.94
3	80	20	2	715	740.77	1.40	1.42
4	80	40	1	455	455.05	1.30	1.28
5	90	20	3	510	600.67	2.90	2.84
6	90	0	2	490	462.45	2.40	2.41
7	90	40	2	400	314.95	2.60	2.61
8	70	40	2	1110	1121.20	0.75	0.73
9	70	0	2	1280	1268.70	0.50	0.48
10	80	40	3	820	893.80	1.95	1.96
11	90	20	1	140	161.92	2.10	2.09
12	80	20	2	710	740.77	1.40	1.42
13	80	0	1	590	602.55	1.00	0.98
14	70	20	1	890	968.17	0.20	0.22
15	80	20	2	910	740.77	1.40	1.42

Table 2. Regression coefficients of predicted models for the investigated responses of hot air oven drying

Variables	Coefficients	
	DT (min)	D_{eff} x10^{10} (m^2/s)
b_0	5120	-79.4
b_1	929^{*+}	13.8^{*+}
b_2	-10.5^{*}	0.75^{*+}
b_3	-93.4^{*+}	-5.63^{*+}
b_{11}	-61^{ns}	4.37^{*}
b_{22}	0.007^{ns}	0.01719^{*+}
b_{33}	0.390^{ns}	0.0938^{*+}
b_{12}	-0.75^{ns}	-0.1875^{*}
b_{13}	-5.62^{ns}	0.125^{ns}
b_{23}	0.1^{ns}	-0.00625^{ns}
R^2	97.96	99.97
Adj-R^2	94.28	99.92
Lack of fit	0.842	0.863

^{ns}not significant $p \geq 0.05$; * significant at $p<0.05$; $^{+}$ significant at $p<0.01$

Table 3. Experimental design of microwave oven drying and corresponded responses (drying time and effective diffusivity)

RunOrder	X_1 MW Power (Watt)	X_2 $CaCl_2$ Concentration (%)	X_3 Thickness of slice (mm)	DT (sec)		D_{eff} (m^2/sec) x 10^{10}	
				Observed	Predicted	Observed	Predicted
1	200	40	1	120	43.13	35.64	34.8365
2	300	40	2	150	376.88	67.65	64.6111

3	200	20	2	420	290.00	48.88	47.2870
4	300	20	1	75	75.00	57.71	61.5684
5	300	20	3	180	153.75	72.49	74.6491
6	200	40	3	450	249.37	48.04	48.9237
7	200	0	1	450	650.63	40.80	39.9213
8	100	20	1	1020	1046.25	8.89	6.7359
9	200	20	2	240	290.00	47.66	47.2870
10	100	20	3	1350	1500.00	27.97	24.1266
11	200	20	2	210	290.00	45.31	47.2870
12	200	0	3	1050	1126.88	55.49	56.3055
13	100	40	2	750	800.63	10.41	13.3876
14	300	0	2	360	309.37	75.26	72.2984
15	100	0	2	2580	2353.13	15.12	18.1669

Table 4. Regression coefficients of predicted models for the investigated responses of microwave oven drying

Variables	Coefficients	
	DT (sec)	D_{eff} x10^{10} (m^2/s)
b_0	3734	- 47.5
b_1	503^{ns}	15.62^{*+}
b_2	$- 78.9^{*+}$	0.072^{ns}
b_3	$- 25.27^{*+}$	0.4602^{*+}
b_{11}	$- 38^{ns}$	$- 1.32^{ns}$
b_{22}	0.664^{ns}	$- 0.00243^{ns}$
b_{33}	$- 0.000420^{*}$	$- 0.000420^{ns}$
b_{12}	$- 3.37^{ns}$	$- 0.029^{ns}$
b_{13}	$- 0.0108^{ns}$	$- 0.0108^{ns}$
b_{23}	0.2025^{*}	$- 0.00036^{ns}$
R^2	95.59	98.65
Adj-R^2	88.64	96.21
Lack of fit	0.139	0.115

^{ns}not significant p≥0.05; * significant at p<0.05; $^{+}$ significant at p<0.01

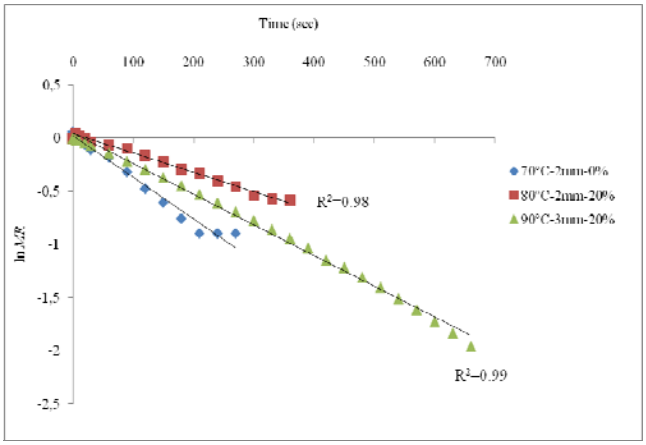

Figure 1. Linear relation between ln MR and drying time (sec) of asparagus slices and fitted proposed model for hot air oven drying $R^2 = 0.97$

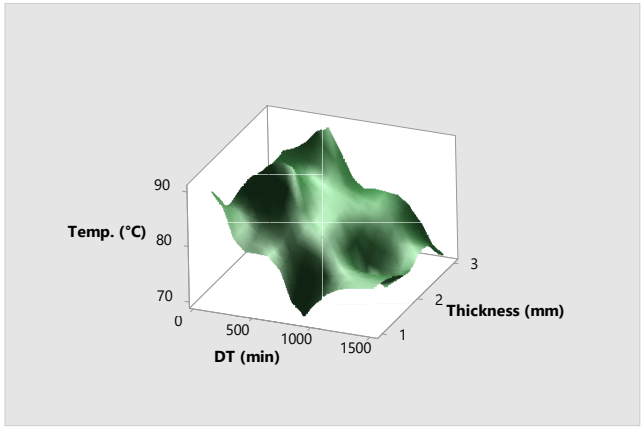

Figure 2. Response surface plot for the interaction effects of oven temperature and slice thickness on drying time (A). Response surface plot for the interaction effects of oven temperature and concentration of $CaCl_2$ one ffective moisture moisture diffusivity (B)

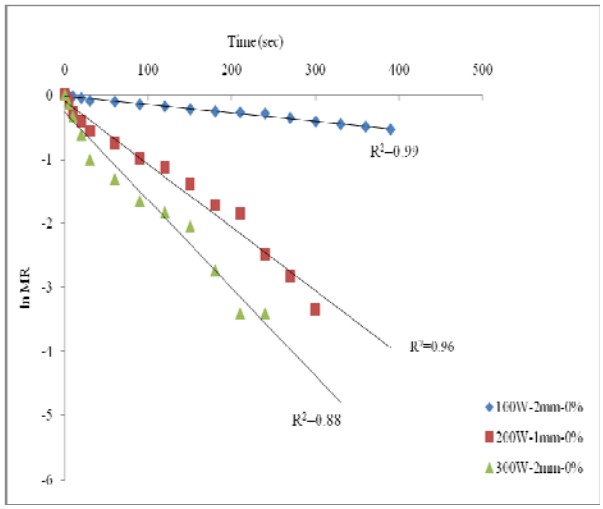

Figure 3. Linear relation between ln MR and drying time (sec) of asparagus slices and fitted proposed model for microwave oven drying

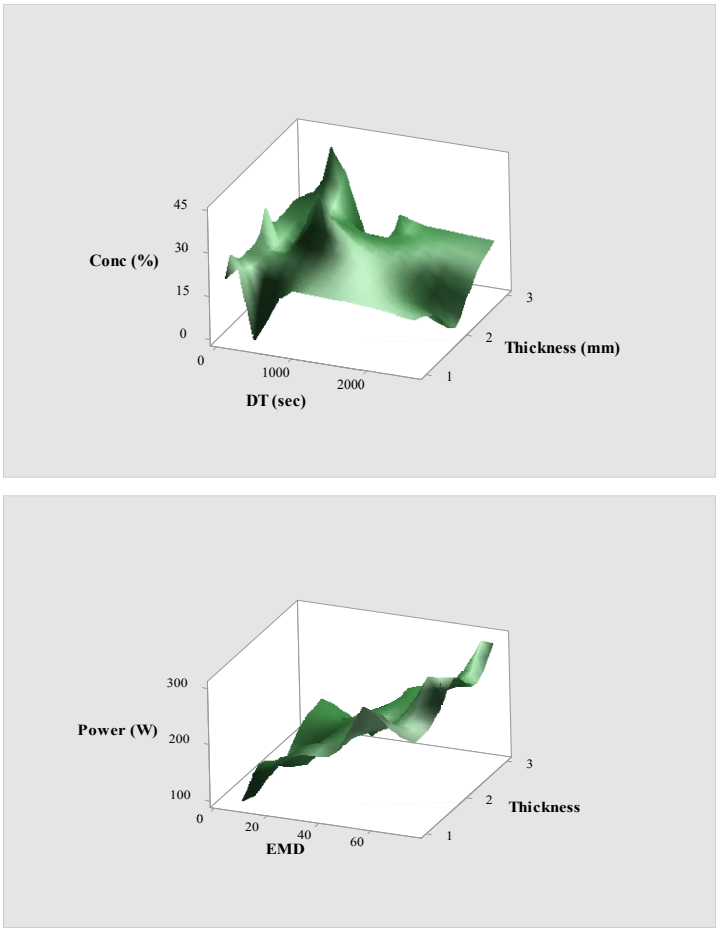

Figure 4. Response surface plot for the interaction effects of concentarion of $CaCl_2$ and slice thickness on drying time (A). Response surface plot for the interaction effects of microwave power slice thickness on effective moisture moisture diffusivity of asparagus slice (B)

3.1 Oven Drying

Temperature of hot air oven, concentration of $CaCl_2$ and thickness of asparagus slice were independent variables in this study. Observed and predicted values of DT and D_{eff} for hot air oven drying were given in Table 1. Temperature of hot air oven, concentration of $CaCl_2$ and thickness of asparagus slice were statistically important ($p<0.05$) on DT and D_{eff}. DT was increased with decreasing drying temperature. The highest DT value of asparagus slice was measured as 1485 min at 70°C oven temperature. The DT decreased as hot air oven temperature increased due to difference between temperature of sample and hot air (Figure 2). The influences of interaction between process factors (thickness of slice, temperature of oven and concentration of $CaCl_2$) were found insignificant (Table 2). Fitted model for drying time of asparagus slices was shown in Eq.5. Experimental results displayed high performance to predict drying time of asparagus slices within the studied range. Regression coefficient (R^2) and adjusted regression ($Adj\text{-}R^2$) coefficient of DT were calculated as 0.97, 0.94, respectively (Table 2).The model equation of DT was shown in Eq. 5. Figure 1 shows ln MR versus time (sec) in different values of oven temperature, slice thickness and concentration of $CaCl_2$. Pan et al. (2003) and Silva et al.

(2012) studied the effect of osmotic pretreatment before conventional drying of some fruit and vegetables. Reduction in total drying time by the application of osmotic pretreatment was reported.

The D_{eff} was calculated and used as an index of the rate of the drying process. The D_{eff} of a food material characterizes its intrinsic mass transfer properties of moisture (Karathanoset al., 1990). Increasing the D_{eff} was expected with temperature of hot air oven. Observed and predicted values of D_{eff} were given in Table 1. The fitted response surface plots and their corresponding contour plots for Dt and D_{eff} values of asparagus slices were given in Figure 2A/B. D_{eff} values of asparagus slices increased with oven temperature and concentration of $CaCl_2$ solution. Oven temperature, thickness of slice and $CaCl_2$ concentration were statistically important on D_{eff} values ($p<0.05$). Regression coefficient (R^2) and adjusted regression ($Adj\text{-}R^2$) coefficient of D_{eff} were evaluated as 0.99, 0.99, respectively (Table 2). The model equation of D_{eff} of asparagus slices was shown in Eq. 6. The D_{eff} for asparagus slices varied from 0.50 to 2.90 ($\times 10^{-10}$, m^2/s) for the different oven temperature, $CaCl_2$ concentration and slice thickness. The values lie within the general range of 10^{-11}–10^{-9} m^2/s for food materials (Zogzas et al., 1996). According the results of ANOVA of D_{eff}, slice thickness, $CaCl_2$ concentration and oven temperature affect the D_{eff} values of asparagus when hot air oven was used for drying. For experimental determination of effective moisture diffusivity in convective drying, the D_{eff} values increased from 1.45×10^{-10} to 10.3×10^{-10} m^2/s with temperature increases from 50 to 80°C (Erenturk et al., 2010).

Drying time (min) = $5120 - 93.4X_1 - 10.5X_2 + 929X_3 + 0.390X_1X_1 + 0.007X_2X_2 - 61.0X_3X_3 + 0.100\ X_1X_2 - 5.62\ X_1X_3 - 0.75\ X_2X_3$ (5)

Effectivemoisturediffusivity ($x10^{-10}$, m^2/s) = $-79.4 - 5.63\boldsymbol{X_1} + 0.750\boldsymbol{X_2} + 13.8\boldsymbol{X_3} + 0.0938\boldsymbol{X_1X_1} + 0.01719\boldsymbol{X_2X_2} + 4.37\boldsymbol{X_3X_3} - 0.00625\boldsymbol{X_1X_2} + 0.125\boldsymbol{X_1X_3} - 0.1875\boldsymbol{X_2\ X_3}$ (6)

3.2 Microwave Drying

Microwave drying was used in this study to investigate the usage of microwave on DT and D_{eff}. Reducing the DT for microwave drying was expected compare to hot air oven. DT values of asparagus decreased with increase in power level of microwave oven and thickness of slice. Less DT means less energy requirements for the drying process. DT values varied from 75 to 2580 sec for the different microwave powers, $CaCl_2$ concentrations and slice thickness'. The lowest value vas observed at 300W microwave power level, 1 mm thickness of slice and 20% $CaCl_2$ solution. Drying results are shown in Table 3 in terms of DT and D_{eff}. Slice thickness and concentration of $CaCl_2$ are significantly important on DT values ($p<0.05$). The interaction between factors of experiment (thickness of slice, microwave power and concentration of $CaCl_2$) were shown in Table 4 and only thickness and thickness factor significant on drying time ($p<0.05$). Square parameters of asparagus slice that is concentration of $CaCl_2$ and thickness of slice was statistically important ($p<0.05$) on DT. Ahrne et al. (2003) compared textural effects of two Ca pretreatments before microwave drying of apples and potatoes. Ca pretreatments influenced the strength of the plant tissue and producing products of hardness after rehydration. For apples and potatoes Ca pretreatments at 20°C increased the hardness of rehydrated samples compared with untreated ones. Figure 3 shows ln MR versus time (sec) in different values of microwave power, slice thickness and concentration of

$CaCl_2$). The model equation of DT was shown in Eq. 7. Regression coefficient (R^2) and adjusted regression ($Adj\text{-}R^2$) coefficient of DT were calculated as 0.95, 0.88, respectively (Table 4). Funebo et al. (2002) described microwave assisted drying for apple and mushroom and the drying time for apple and mushroom was reduced with the use of microwave oven. In addition, drying time of banana slices was decreased by about 64% in a study (Maskan, 2001).

The ranges and independent variables levels are shown in Table 4. Increasing the D_{eff} was desirable microwave process to drying the asparagus slices. D_{eff} values varied from 8.893 to 75.267 ($\times 10^{-10}$, m^2/s) for different drying conditions. D_{eff} values increased with studied parameters of asparagus drying. According to the results of ANOVA of D_{eff} microwave power and slice thickness were significantly affected ($p<0.05$) the D_{eff} values of asparagus slices whereas $CaCl_2$ pretreatment before drying was not affected D_{eff} values. Regression coefficients of predicted models for the drying responses of microwave oven drying of asparagus slices were given in Table 4. Fitted equation for D_{eff} value was shown in Eq.8 and the coefficients of fitted model were shown in Table 4. Regression coefficient (R^2) and adjusted regression ($Adj\text{-}R^2$) coefficient of D_{eff} were calculated as 0.98, 0.96, respectively (Table 4). The effects of process variables on DT and D_{eff} of asparagus slices are shown in Figure 4A/B. D_{eff} values of asparagus slices increased with microwave power and thickness of slice. Dak and Pareek (2014) studied the effect of microwave drying on D_{eff} of pomegranate and an increase of D_{eff} was clarified with increasing the microwave power. D_{eff} values increased with decreased in moisture content under drying conditions (Prasad, 2006).

Drying time (sec) = $3734 - 78.9 X_2 + 503 X_3 - 25.27 X_1 + 0.664 X_2X_2 - 38 X_3X_3 + 0.0404 X_1X_1 - 3.37 X_2X_3 + 0.2025 X_2X_1 - 0.56 X_3X_1$ (7)

Effectivemoisturediffusivity($x10^{-10}$, m^2/s) = $-47.5 + 0.072X_2 + 15.62X_3 + 0.4602X_1 - 0.00243X_2X_2 - 1.32 X_3X_3 - 0.000420X_1X_1 - 0.029X_2X_3 - 0.00036X_2X_1 - 0.0108X_3X_1$ (8)

4. Conclusions

In this study, the effect of slice thickness, power level/hot air oven temperature and concentration of $CaCl_2$ on the DT and D_{eff} values of asparagus slices were investigated. DT of asparagus slices decreased with the increase in microwave power and slice thickness for microwave oven and hot air oven. In addition, D_{eff} values of samples increased with temperature of oven and thickness of slices for hot air oven drying and power level and slice thickness for microwave oven drying. RSM was used to optimize the factors in order to obtain DT and maximum D_{eff} values of asparagus slices for drying in hot air oven and microwave oven. The lowest DT was observed as 400 min in 90°C of oven temperature, 40% of $CaCl_2$ concentration and 2 mm of slice thickness for hot air oven. In addition, 300 W of power level, 20% of $CaCl_2$ concentration and 1 mm of slice thickness gave the lowest DT for microwave oven. The highest D_{eff} value of asparagus slices hot air oven and microwave oven were measured as 2.90×10^{-10} m^2/s at 90°C oven temperature, %20 of $CaCl_2$, 3 mm slice thickness and 75.26×10^{-10} m^2/s at 300 W power, 0 % $CaCl_2$ and 2 mm slice thickness, respectively. When the two drying methods were examined lower DT and higher D_{eff} value were obtained in microwave oven drying of asparagus slices.

5. References

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STUDY DYNAMICS OF THE FREEZE DRYING PROCESS OF ROYAL JELLY IN VIET NAM

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ABSTRACT
Study dynamics of the freeze drying process of royal jelly in Viet Nam was building and solving mathematical models of heat and mass transfer during the freeze drying process in order to determine relationship between the residual water content of royal jelly product and time of the freeze drying process, $W_M = f(\tau)$. Results obtained were use to set up the technological mode of the freeze drying process of royal jelly in Viet Nam and kinetic parameters of the freeze drying process in order to use to calculate, design and manufacture the freeze drying system in Viet Nam. In addition, they were able to use to set up the technological mode of the freeze drying process for some foods that have high value as royal jelly in Viet Nam.

1. Introduction

The freeze drying is a dehydrate process inside drying material such as food product, pharmaceutical product and types of probiotics. This process is carried out in condition of low temperature and low pressure, under three phase point of water, O(0.0098^0C, 4.58mmHg). It can be exactly said that temperature of drying material is lower than the crystallization point of water inside drying material; pressure of drying chamber is lower than 4.58mmHg (Hammami, 1997). As a result, final products after freeze drying have very good quality as the same initial material. For example, protein of final product is not denaturated and be able to recover the original its quality; glucides are not hydrolysed; lipid is not oxidised; ours natural pigments, smells and flavours are not destroyed; bioactive compound and enzymes are not lost activities. In other words, the nutritional value, pharmaceutical value and cosmetics value of product is approximately constant. This is strong point of the freeze drying that have not any drying methods are able to create product as them. However, the freeze drying is quite complicatedly process. At the time, it is not only to happend the heat transfer but also to happend the mass transfer. For this reason, building and solving the mathematical model of heat and mass transfer during freeze drying process were one of difficult problems to answer. Up to now, there were many research on the mathematical models about the heat and mass transfer during freeze drying process to describe the different

physical models of drying materials such as flat – shaped model (Luikov, 1975), cylindrical model (Luikov et al., 1975) and spherical model (Luikov et al., 1975), Luikov equation applicable to sublimation – drying (Peng, 1994). Besides, there were also many authors that studied many the mathematical models to describe the complicated physical models such as Heat and mass transfer models for freeze drying of vegetable slices (George and Datta, 2002); Kinetic model for freeze drying process (Boss et al., 2004); Freeze drying pharmaceutical in vials on trays (Liapis et al., 2005); Exergy analysis of freeze drying pharmaceutical in vials on trays (Liapis and Bruttini, 2008), … etc. However, there were not any mathematical model of freeze drying process that was able to apply for the freeze drying of royal jelly; error between experimental data with calculating data of mathematical model is over 36.09%. Therefore, the aim of this study was building and solving heat and mass transfer models during the freeze drying process of royal jelly with parameters of optimal technological mode. Results obtained were used to determine moisture diffusion coefficient, kinetic parameters and calculate, design and manufacture the freeze drying system, establish the technological mode of the freeze drying process for some kind of high food.

2.Building and solving heat and mass transfer models

2.1.Hypotheses building mathematical models

- Royal jelly was frozen in trays, trays are made by glass, trays are rectangular parallelepiped that have area of f (m^2), length of a' (m), width of b' (m) and height of δ (m), with a', b' >> δ. Therefore, it was supposed that royal jelly in trays is infinite flat – shaped models. The heat and mass transfer during freeze drying process are only conformable to direction height (Oz), i.e. $\partial t/\partial x = \partial t/\partial y = 0$ (Figure 1) (Boss et al., 2004).

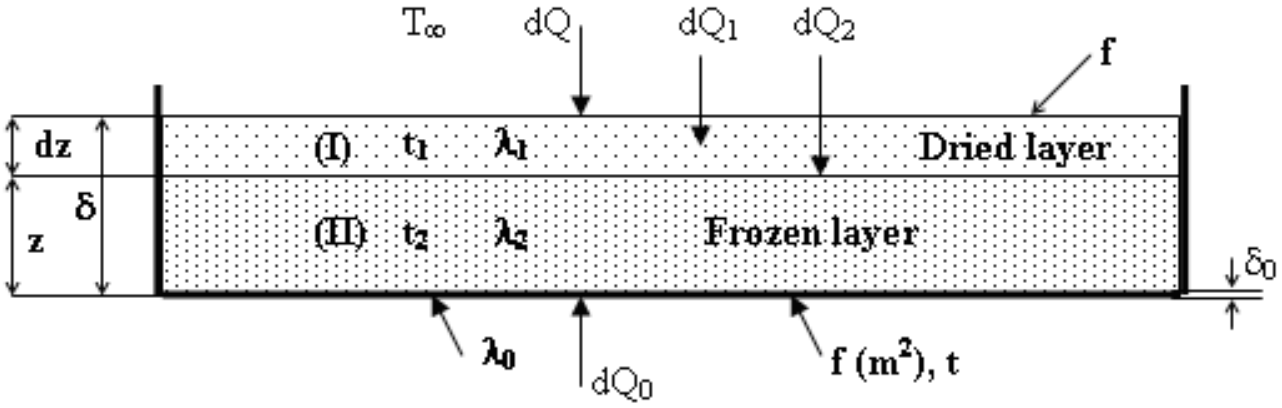

Figure 1. Trays content of royal jelly

- Thermophysical parameters of royal jelly such as initial residual water content of royal jelly W_0; specific heat c_1 (J/(kg.K)), density ρ, ρ_1 (kg/m^3); effective thermal conductivity λ_0, λ_1 (W/(m.K)); thermal diffusivity coefficient a_1 (m^2/s); heat transfer coefficient α_r ($W/(m^2.K)$); latent heat of sublimation r_{th} (J/kg); latent heat of evaporation r_{hh} (J/kg) were determined, (Liapis and Bruttini, 2008).

2.2.Building the mathematical models for freeze drying of royal jelly

2.2.1.Energy balance equation during the freeze drying of royal jelly

Energy supplied for the freeze drying of royal jelly is energy total of two parts: the first part is energy in order to sublimate ice in area (I); the second part is energy transmitted through area (I) in order to exchange heat with area (II). It can be illustrated as Figure 1

(George and Datta, 2002; Liapis and Bruttini, 2008).

$$dQ + dQ_0 = dQ_1 + dQ_2 \quad (1)$$

- $dQ + dQ_0$ (J): energy supplied for the freeze drying process of royal jelly.

$$dQ + dQ_0 = -\lambda_1\left(\frac{\partial t_1}{\partial z}\right).f.d\tau - \lambda_0\left(\frac{\partial t}{\partial z}\right).f.d\tau \quad (2)$$

- dQ_1 (J): energy in order to sublimate ice inside royal jelly in area (I).

$$dQ_1 = r_{th}.\frac{\partial G_w}{\partial \tau}d\tau \quad (3)$$

- dQ_2 (J): energy transmitted through area (I) in order to exchange heat with area (II).

$$dQ_2 = -\lambda_2\left(\frac{\partial t_2}{\partial z}\right).f.d\tau \quad (4)$$

Therefore:

$$-\lambda_1\left(\frac{\partial t_1}{\partial z}\right).f.d\tau - \lambda_0\left(\frac{\partial t}{\partial z}\right).f.d\tau = r_{th}.\frac{\partial G_w}{\partial \tau}d\tau - \lambda_2\left(\frac{\partial t_2}{\partial z}\right).f.d\tau \quad (5)$$

With $t_2 = T_{th} = const$ (Liapis and Bruttini, 2008), thus: $-\lambda_2\left(\frac{\partial t_2}{\partial z}\right) = 0$ (6)

Combination (5) and (6), result obtained was able to written as follow:

$$-\lambda_1\left(\frac{\partial t_1}{\partial z}\right).f - \lambda_0\left(\frac{\partial t}{\partial z}\right).f = r_{th}.\frac{\partial G_w}{\partial \tau} \quad (7)$$

2.2.2. Heat transfer equation of Fourier

When ice inside royal jelly is not sublimated, whole royal jelly in trays is frozen layer (II), its temperature is constant ($t_2 = T_{th} = const$). But ice inside royal jelly begins sublimation, surface layer of royal jelly in trays will be sublimated, it created two layers: dried layer (I) and frozen layer (II) is illustrated as Figure 1. Temperature of frozen layer (II) is still constant, temperature of dried layer (I) is root of Equation (8) as follow:

$$\frac{\partial t_1}{\partial \tau} = a_1\frac{\partial^2 t_1}{\partial z^2} \quad (8)$$

Initial condition:

$$\tau = 0;\ t_0 = t_2 = T_{th} = const. \quad (9)$$

Temperature of drying chamber is:

$$T_\infty = Z_1 = const; \quad (10)$$

Boundary conditions:

$$\begin{cases} -\lambda_1\left(\frac{\partial t_1}{\partial z}\right)_{z=\delta} = \alpha_{bx}\left(t_1 - T_\infty\right) \\ -\lambda_1\left(\frac{\partial t_1}{\partial z}\right)_{z=0} = 0 \end{cases} \quad (11)$$

Solving equation system from Eq. (8) to Eq. (11) was found root as follow:

$$t_1 = T_\infty + \left(T_{th} - T_\infty\right).\rightarrow \rightarrow \sum_{n=1}^{\infty} A_n.\cos\left(\mu_n.\frac{z}{\delta}\right).\exp\left(-\mu_n^2.Fo\right) \quad (12)$$

where: μ_n are roots of specific equation:

$$\cot g\mu_n = \frac{\mu_n}{Bi} \quad (13)$$

Bi is Biot number of dried layer (I):

$$Bi = \frac{\alpha_r.\delta}{\lambda_1} \quad (14)$$

Fo is Fourier number of dried layer (I):

$$Fo = \frac{a_1\tau}{\delta^2} \quad (15)$$

A_n is parameters root:

$$A_n = \frac{2\sin\mu_n}{\mu_n + \sin\mu_n \cos\mu_n} \quad (16)$$

with: $$q_0 = -\lambda_0\left(\frac{\partial t}{\partial z}\right) = \frac{\lambda_0}{\delta_0}\left(T_\infty - T_{th}\right) \quad (17)$$

$$\alpha_r = \frac{k.C_0.\varepsilon_{qd}}{T_\infty - T_{th}} \int_{T_{th}}^{T_\infty} \left(T^2 + T_{th}^2\right).\left(T + T_{th}\right)dT \quad (18)$$

with: $C_0 = 5.67\times10^{-8}$ W/(m^2.K^4) – thermal radiation coefficient of blackbody (Wei et al., 2005); ε_{qd} – conversion coefficient of dry material that they are transferred by radiation heat, (Holman, 1986); ε_1, ε_2: black level of dry material that that they are transferred by radiation heat, (Gebhart, 1992).

$$\varepsilon_{qd} = \frac{1}{\frac{1}{\varepsilon_1} + \frac{1}{\varepsilon_2} - 1} \quad (19)$$

2.2.3. Mass transfer equation of Fick 2

The mass transfer model for the freeze drying process of royal jelly can be written by second law of Fick (Boss et al., 2004) as follow:

$$\frac{\partial G_w}{\partial \tau} = D\frac{\partial^2 G_w}{\partial z^2} \quad (20)$$

where: D (m^2/s): moisture diffusion coefficient of royal jelly; $G_w = (G.W_0 - G.W)$ is ice mass inside royal jelly that sublimated, W is the residual water content of the royal jelly freeze drying. From Eq. (7) and Eq. (20), it can be written as follow:

$$-\lambda_1\left(\frac{\partial t_1}{\partial z}\right).f - \lambda_0\left(\frac{\partial t}{\partial z}\right).f = r_{th}.D.\frac{\partial^2 G_w}{\partial z^2} = r_{th}.D.\frac{\partial^2\left(G.W_0 - G.W\right)}{\partial z^2} = -r_{th}.D.\frac{\partial^2\left(f.\delta.\rho.W\right)}{\partial z^2}$$

As a result:

$$-\lambda_1\left(\frac{\partial t_1}{\partial z}\right).f - \lambda_0\left(\frac{\partial t}{\partial z}\right).f = -r_{th}.D.f.\delta.\rho.\frac{\partial^2 W}{\partial z^2} \quad (21)$$

Corresponding to (21):

$$\frac{\partial^2 W}{\partial z^2} = \frac{\lambda_1}{r_{th}.D.\delta.\rho}.\left(\frac{\partial t_1}{\partial z}\right) - \frac{\lambda_0\left(T_\infty - T_{th}\right)}{r_{th}.D.\delta.\rho.\delta_0} \quad (22)$$

Substituting Eq. (12) into Eq. (22), result received Eq. (23) as follow:

$$\frac{\partial^2 W}{\partial z^2} = -\frac{\lambda_1}{r_{th}.D.\rho.\delta}.\left(T_{th} - T_\infty\right)\sum_{n=1}^{\infty} A_n.\frac{\mu_n}{\delta}.\rightarrow \rightarrow \sin\left(\mu_n\frac{z}{\delta}\right).\exp\left(-\mu_n^2.Fo\right) - \frac{\lambda_0\left(T_\infty - T_{th}\right)}{r_{th}.D.\delta.\rho.\delta_0} \quad (23)$$

Integrate Eq. (23) found out Eq. (24) as follow:

$$W(z,\tau) = \frac{\lambda_1.\left(T_{th} - T_\infty\right)}{r_{th}.D.\rho}.\sum_{n=1}^{\infty}\frac{A_n}{\mu_n}.\sin\left(\mu_n\frac{z}{\delta}\right).\rightarrow \rightarrow \exp\left(-\mu_n^2.Fo\right) - \frac{\lambda_0\left(T_\infty - T_{th}\right)}{2.r_{th}.D.\delta.\rho.\delta_0}x^2 + C_1 z + C_2 \quad (24)$$

When $\tau = \tau_t$ and $z = 0$, $W = 0$ (the residual water content of the Final royal jelly freeze drying calculated by theory), substitute all these value into Eq. (24), result found $C_2 = 0$. For this reason, Eq. (24) can be written as follow:

$$W(z,\tau) = \frac{\lambda_1.\left(T_{th} - T_\infty\right)}{r_{th}.D.\rho}.\sum_{n=1}^{\infty}\frac{A_n}{\mu_n}.\sin\left(\mu_n\frac{z}{\delta}\right).\rightarrow \rightarrow \exp\left(-\mu_n^2.Fo\right) - \frac{\lambda_0\left(T_\infty - T_{th}\right)}{2.r_{th}.D.\delta.\rho.\delta_0}z^2 + C_1 z \quad (25)$$

When $\tau = 0$ and $z = \delta$, $W = W_0$ (Initial the residual water content of royal jelly), substituting all these value into Eq. (25), result found C_1 as follow:

$$C_1 = \left(W_0 - \frac{\lambda_1.(T_{th}-T_\infty)}{r_{th}.D.\rho}.\sum_{n=1}^{\infty}\frac{A_n}{\mu_n}.\sin\mu_n\right)/\delta + \frac{\lambda_0.(T_\infty - T_{th})}{2.r_{th}.D.\rho.\delta_0} \quad (26)$$

Substituting Eq. (26) into Eq. (25), result received as follow:

$$W(z,\tau) = \frac{\lambda_1.(T_{th}-T_\infty)}{r_{th}.D.\rho}.\sum_{n=1}^{\infty}\frac{A_n}{\mu_n}.\sin\left(\mu_n\frac{z}{\delta}\right).\rightarrow$$
$$\rightarrow \exp\left(-\mu_n^2.Fo\right) - \frac{\lambda_0(T_\infty - T_{th})}{2.r_{th}.D.\delta.\rho.\delta_0}\left(z^2-\delta.z\right) +$$
$$\left(W_0 - \frac{\lambda_1.(T_{th}-T_\infty)}{r_{th}.D.\rho}.\sum_{n=1}^{\infty}\frac{A_n}{\mu_n}.\sin\mu_n\right).\frac{z}{\delta} \quad (27)$$

2.2.4. Kinetic equation for the freeze drying of royal jelly

The residual water content of royal jelly varying time of the freeze drying process is always determined according to average value of water in volume unit of royal jelly.

$$W_M(\tau) = \frac{1}{\delta}.\int_0^{\delta}\left(\begin{array}{l}\frac{\lambda_1.(T_{th}-T_\infty)}{r_{th}.D.\rho}.\sum_{n=1}^{\infty}\frac{A_n}{\mu_n}.\rightarrow \\ \rightarrow\sin\left(\mu_n\frac{z}{\delta}\right).\exp\left(-\mu_n^2.Fo\right) \\ -\frac{\lambda_0(T_\infty-T_{th})}{2.r_{th}.D.\delta.\rho.\delta_0}z^2 + C_1 z\end{array}\right)dz \quad (28)$$

From Eq. (28), kinetic equation for the freeze drying of royal jelly can be written:

$$W_M(\tau) = \frac{\lambda_1(T_\infty - T_{th})}{r_{th}.D.\rho}.\sum_{n=1}^{\infty}\frac{2Bi}{\mu_n^2.\left(\mu_n^2+Bi+Bi^2\right)}.\rightarrow$$
$$\rightarrow \exp\left(-\mu_n^2.Fo\right) + \frac{1}{2}.\left(W_0 + \frac{\lambda_1.(T_\infty - T_{th})}{r_{th}.D.\rho}.\rightarrow\right.$$
$$\left.\rightarrow\sum_{n=1}^{\infty}\frac{A_n}{\mu_n}.\sin\mu_n + \frac{\lambda_0.\delta.(T_\infty - T_{th})}{12.r_{th}.D.\rho.\delta_0}\right) \quad (29)$$

3. Materials and methods

3.1. Materials

The royal jelly is harvested from bees's nest to grow up at Bao Loc area in Lam Dong province of Viet Nam. It is the pure natural product and does not mix any chemical composition. It is very thick solution (Sabatini et al, 2009). The basic composition of royal jelly is presented in Table 1 (Dzung, 2013). Before the freeze drying, royal jelly is frozen at the optimal freezing temperature in order that water in royal jelly is completely crystallized. According to research result of Dzung (2014), it was obvious that when royal jelly is frozen until temperature of royal jelly reach $T_{Fopt} = T_{th} = -18.33^0C$. At the time, water inside royal jelly was completely crystallized $\omega = 1$ or $\omega = 100\%$ (Dzung, 2014).

Table 1. The compositions of royal jelly in Viet Nam

No	Substance	Symbol	Value (% of material weight)	Value (% of dry weight)
1	Water	X_1	59.20	-
2	Proteins	X_2	14.26	34.95
3	Glucids	X_3	15.59	39.09
4	Lipids	X_4	4.00	9.80
5	Minerals	X_5	1.10	2.70
6	10-HDA & impurities	X_6	5.49	13.46

3.2. Apparatus

Equipments used in this study are listed as follow (Dzung, 2013 & 2014):

Figure 2. The freeze drying system DS-3 with the auto-freezing (-50 ÷ - 45)^{0}C

- Determining weigh of royal jelly by Satoriusbasic Type BA310S: range scale (0 ÷ 350)g, error: ± 0.1g = ± 0.0001 kg.
- Determining temperature of royal jelly Dual Digital Thermometer: range scale (-50 ÷ 70)^{0}C, error ± 0.05^{0}C.
- The Freeze Drying System DS-3 (Figure 2) that was controlled automatically by computer. It could reduce the temperature of freezing environment to (-50 ÷ -45)^{0}C. The temperature, pressure and time profile of freeze drying process are measured by computer.
-

3.3. Methods

- The residual water content of royal jelly of the freeze drying process (W_E, %) is determined by the mass sensor controlled via computer, (Dzung, 2013).

$$W_E = 100 - \frac{G_0}{G_e}(100 - W_0) \qquad (30)$$

- Using the mathematical tools and computer to solve the mathematical models of heat and mass transfer during freeze drying process of royal jelly (Dzung, 2014).

4. Results and discussions

4.1.Determining moisture diffusion coefficient of royal jelly in freeze drying process

The material properties used for freeze drying of royal jelly in Viet Nam in Table 2 are essential parameters for calculating and simulation Eq. (29), (Figura et al., 2007; Wytrychowski et al., 2013).

Table 2. Thermophysical parameters used for freeze drying of royal jelly in Viet Nam

Symbol	Value	References
W_0 (%)	59.2	Dzung, 2014
δ (m)	12.93×10^{-3}	Dzung, 2014
δ_0 (m)	3.0×10^{-3}	Dzung, 2014
T_{kt} (^{0}C)	-1.06	Dzung, 2014
T_{th} (^{0}C)	-18.33	Dzung, 2014
T_∞ (^{0}C)	20.58	Dzung, 2014

r_{th} (J/kg)	3231.78×10^3	Holman, 1986
r_{hh} (J/kg)	2555.65×10^3	Holman, 1986
C_0 W/(m^2.K^4)	5.67	Holman, 1986
ε_1	0.96	Heldman, 1992
ε_2	0.91	Heldman, 1992
ε_{qd}	0.8768	Calculation
k	0.9827	Heldman, 1992
ρ (kg/m^3)	1183.22	Heldman, 1992
ρ_1 (kg/m^3)	1328.07	Heldman, 1992
λ_0 (W/(m.K))	1.0183	Dzung, 2014
λ_1 (W/(m.K))	0.1790	Heldman, 1992
c_1 (J/(kg.K))	1681.577	Dzung, 2014
$A_1 = \lambda_1/(c_1.\rho_1)$ (m^2/s)	8.0167×10^{-8}	Calculation
α_r	4.4883	Calculation
$Bi = (\alpha_r.\delta)/\lambda_1$	0.3241	Calculation

Substituting thermophysical parameters in Table 2 into the specific Eq. (13). After that solving Eq. (13) was found roots and was presented in Table 3 as follows:

Table 3. Roots of specific equation (13) are μ_j (j = 1 ÷ 19)

Symbol	μ_1	μ_2	μ_3	μ_4
Value	0.5403	3.2413	6.3343	9.459
Symbol	μ_5	μ_6	μ_7	μ_8
Value	12.5921	15.7286	18.8667	22.0059
Symbol	μ_9	μ_{10}	μ_{11}	μ_{12}
Value	25.1456	28.2858	31.4262	34.5669
Symbol	μ_{13}	μ_{14}	μ_{15}	μ_{16}
Value	37.7077	40.8486	43.9897	47.1308
Symbol	μ_{17}	μ_{18}		
Value	50.2719	53.4131		

Eq. (29) can be described the kinetic for freeze drying of royal jelly, if moisture diffusion coefficient of royal jelly D (m^2/s) was determined. However, this kinetic parameter was not determined by the common method, it was determined by identify parameter of the mathematical model method as follow (Dzung, 2014):

Firstly, building the residual Root Mean Square Error (RMSR): between the residual water content of royal jelly from the experimental data and the mathematical model data. Correspondingly, RMSR was written by Eq. (31), (Dzung, 2014):

$$RMSR = f(D) = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}\left(W_E(\tau_i) - W_M(\tau_i)\right)^2} \quad (31)$$

where: D (m^2/s): moisture diffusion coefficient of royal jelly is variable;

RMSR = f(D) is function;

$W_E(\tau_i)$: the residual water content of royal jelly at time of τ_i from experiment in Table 4;

$W_M(\tau_i)$: the residual water content of royal jelly at time of τ_i from the mathematical model (29);

i = 1 ÷ N: the number of experiments;

τ_i: the time of the freeze drying of royal jelly in Table 4;

This much, the problem finding parameter D of mathematical model (29) was expressed as follows: Finding the root $D^{opt} = D$ in order to objective function of RMSR = f(D) reached the minimum value (Dzung, 2014):

$$RMSR_{min} = Min\{f(D)\} = Min\left\{\sqrt{\frac{1}{N-1}\sum_{i=1}^{N}\left(W_E(\tau_i) - W_M(\tau_i)\right)^2}\right\} \quad (32)$$

Secondly, finding the minimum value of RMSR by the meshing method: Setting up value of moisture diffusion coefficient of freeze drying of royal jelly varies in range of $D^{min} = D_0 = 10^{-10}$ to $D^{max} = D_n = 10^{-7}$, with jump is $\Delta D = 0.001\times10^{-10}$ and Step number jump determined is $n = (D^{max} - D^{min})/\Delta D$.

Setting up value of drying time varies in range of $\tau_0 = \tau_{min} = 0h$ to $\tau_n = \tau_{max} = 24h$, with jump is $\Delta\tau = 0.5$ and Step number jump determined is $m = (\tau_{max} - \tau_{min})/\Delta\tau$.

Finally, for $j_1 = 1$ to n, with $D = D_0 + j_1.\Delta D$; for $j_2 = 1$ to m, with $\tau = \tau_0 + j_2.\Delta\tau$. After that calculating $W_M(\tau_{j1})$ by Eq. (29) and RMSR by Eq. (32). A as result, the minimum value of RMSR could be determined by the meshing algorithm on above (Dzung, 2014) programmed in MatLab R2008a software. After solving and calculating, the root of Eq. (32) found the results:

$RMSR_{min} = 0.07676$

When: $D^{opt} = D = 9.50 \times 10^{-9}$ m^2/s

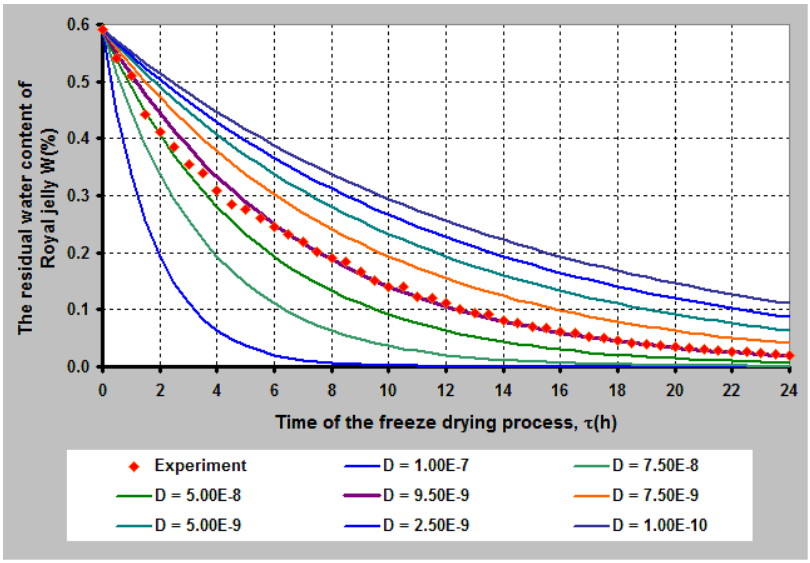

Fig 4. The relationship between residual water content of royal jelly with time of the freeze drying process

From Figure 4, it was obvious that moisture diffusion coefficient of royal jelly was determined by identify parameter of the mathematical model method that was quite complicated and difficult. Because it combined experimental data with mathematical model for freeze drying process of royal jelly. Result on above found out value of moisture diffusion coefficient of royal jelly of $D^{opt} = D = 9.50\times10^{-9}$ m^2/s was suitable to describe kinetic as well as calculate and set up technological mode for freeze drying process of royal jelly, (Akay and et al., 1997).

4.2. Test compatibility of the mathematical model

Substituting moisture diffusion coefficient of royal jelly $D = 9.50\times10^{-9}$ m^2/s, thermo-physical parameters in Table 2 and roots of specific equation (13) into Eq. (29) with drying time varying from $\tau_0 = 0$ to $\tau_n = 24h$, jump $\Delta\tau = 0.5h$. Results received were shown in Table 4a & b, (Akay et al., 1997; Ratti et al., 2004).

Table 4a. Experiment data and calculating data of the freeze drying of royal jelly

Time (h)	Fo	$W_E(\tau)$	$W_M(\tau)$
0.00	0.0000	0.5920	0.5920
0.50	0.8631	0.5412	0.5499
1.00	1.7262	0.5105	0.5116
1.50	2.5894	0.4419	0.4761
2.00	3.4525	0.4127	0.4430
2.50	4.3156	0.3848	0.4122
3.00	5.1787	0.3548	0.3836
3.50	6.0419	0.3389	0.3569
4.00	6.9050	0.3092	0.3321
4.50	7.7681	0.2845	0.3091
5.00	8.6312	0.2765	0.2876
5.50	9.4944	0.2612	0.2676
6.00	10.3575	0.2456	0.2490
6.50	11.2206	0.2314	0.2317
7.00	12.0837	0.2187	0.2156
7.50	12.9469	0.2013	0.2007
8.00	13.8100	0.1909	0.1867
8.50	14.6731	0.1842	0.1738
9.00	15.5362	0.1667	0.1617
9.50	16.3994	0.1502	0.1505
10.00	17.2625	0.1407	0.1400
10.50	18.1256	0.1392	0.1303
11.00	18.9887	0.1235	0.1212
11.50	19.8518	0.1205	0.1128
12.00	20.7150	0.1107	0.1050
12.50	21.5781	0.1003	0.0977
13.00	22.4412	0.0952	0.0909
13.50	23.3043	0.0914	0.0846
14.00	24.1675	0.0804	0.0787
14.50	25.0306	0.0756	0.0732
15.00	25.8937	0.0701	0.0682
15.50	26.7568	0.0673	0.0634
16.00	27.6200	0.0606	0.0590
16.50	28.4831	0.0593	0.0549
17.00	29.3462	0.0534	0.0511
17.50	30.2093	0.0494	0.0476
18.00	31.0725	0.0457	0.0442
18.50	31.9356	0.0408	0.0412
19.00	32.7987	0.0393	0.0383
19.50	33.6618	0.0379	0.0357
20.00	34.5250	0.0357	0.0332
20.50	35.3881	0.0332	0.0309
21.00	36.2512	0.0312	0.0287
21.50	37.1143	0.0291	0.0267
22.00	37.9774	0.0273	0.0249
22.50	38.8406	0.0252	0.0231
23.00	39.7037	0.0232	0.0215
23.50	40.5668	0.0221	0.0200
24.00	41.4299	0.0201	0.0187

Table 4b. Experiment data and calculating data of the freeze drying of royal jelly

$(W_E - W_M)^2$	$\|W_E - W_M\|$	Error (%)
1.2326E-32	1.1102E-16	0.00
7.50175E-05	0.00866127	1.60
1.20636E-06	0.00109834	0.22
0.001166728	0.0341574	7.73
0.000917481	0.03028995	7.34
0.000751842	0.02741974	7.13
0.000828683	0.02878686	8.11
0.000325544	0.01804283	5.32
0.000526679	0.0229495	7.42
0.000604094	0.02457831	8.64
0.000123425	0.01110966	4.02
4.13736E-05	0.00643223	2.46
1.18503E-05	0.00344243	1.40
1.18259E-07	0.00034389	0.15
9.32139E-06	0.0030531	1.40
3.99398E-07	0.00063198	0.31
1.73925E-05	0.00417043	2.18
0.000109008	0.01044071	5.67
2.51008E-05	0.00501007	3.01
6.70361E-08	0.00025891	0.17
4.78861E-07	0.000692	0.49
7.95126E-05	0.00891699	6.41
5.1369E-06	0.00226647	1.84
5.90959E-05	0.00768738	6.38
3.2757E-05	0.00572337	5.17
6.83861E-06	0.00261507	2.61
1.84925E-05	0.00430029	4.52
4.64334E-05	0.00681421	7.46
2.8546E-06	0.00168955	2.10
5.55451E-06	0.0023568	3.12
3.78026E-06	0.00194429	2.77
1.5042E-05	0.0038784	5.76
2.50804E-06	0.00158368	2.61
1.92104E-05	0.00438297	7.39
5.27859E-06	0.00229752	4.30
3.41181E-06	0.00184711	3.74

2.10291E-06	0.00145014	3.17
1.41566E-07	0.00037625	0.92
9.6798E-07	0.00098386	2.50
5.04141E-06	0.00224531	5.92
6.35993E-06	0.00252189	7.06
5.41237E-06	0.00232645	7.01
6.10551E-06	0.00247093	7.92
5.60014E-06	0.00236646	8.13
5.87277E-06	0.00242338	8.88
4.20789E-06	0.00205132	8.14
2.75304E-06	0.00165923	7.15
4.2249E-06	0.00205546	9.30
2.09599E-06	0.00144775	7.20

Test compatibility of the mathematical model (29) compared with experimental data is error of the mathematical model (29) with experimental data. It was examined by the Eq. (33) (Gebhart, 1992; Dzung, 2014):

$$Er = \frac{\left|W_E(\tau_i) - W_M(\tau_i)\right|}{\left|W_E(\tau_i)\right|} \qquad (33)$$

The maximum error of the mathematical model (29) is determined by Eq. (34)

$$Er_{max} = \frac{\max\left\{\left|W_E(\tau_i) - W_M(\tau_i)\right|\right\}}{\left|W_E(\tau_i)\right|} = \frac{\left|0.0221 - 0.02004\right|}{0.0221}.100\% = 9.30\% \qquad (34)$$

It was able to see that the mathematical model (29) was completely compatible with experimental results. Because of the maximum error of mathematical model (29) with experimental data Er_{max} = 9.30%, it is smaller than 10% (Heldman et al., 1992; Dzung, 2014). Therefore, the mathematical model (29) can completely use of describing kinetic for the freeze drying of royal jelly (Holman, 1986).

4.3.Describing kinetic for freeze drying process of royal jelly

From the data in Table 4, it can simulate relationship between residual water content of royal jelly with time of the freeze drying process, $W_E = f(\tau)$ and $W_M = f(\tau)$, (Ratti and et al., 2004). In other words, it can describe kinetic for the freeze drying process of royal jelly. Results obtained were presented in Figure 5. From Figure 5 it is obvious that calculation data from mathematical model (29) are completely suitable with experimental data for the freeze drying process of royal jelly. The average error of the mathematical model (29) compared with experimental data is lower than 9.30%. Therefore, the mathematical model (29) can be used to describe kinetic, calculate and set up technological mode for freeze drying process of royal jelly, (Barresi et al., 2008). The error of mathematical model (29) compared with experimental data was content of all causes as follow: Firstly, mathematical model (29) was calculated by thermophysical parameters of royal jelly of average value and constant, but in the fact that this parameters varied from temperature of royal jelly (Wytrychowski et al., 2013). Secondly, it was supposed that the sublimate temperature of ice inside royal jelly was constant ($t_2 = T_{th}$ = const) to apply for building and solving mathematical model (29), but in the fact that it changed according to time of the freeze drying process, (Barresi et al., 2008). In addition, it was also supposed that physical model of royal jelly in trays was infinite flat – shaped models, but in the fact that it was only approximate as infinite flat – shaped models. Finally, water inside royal jelly had to not be purity water that it was a solution of chemical compositions inside royal jelly and created chemical bonds, physicochemical bonds between water with chemical compositions. For this reason, its latent heat of sublimation and latent heat of evaporation was different compared with purity water (Wytrychowski et al., 2013). All the causes on above made error of mathematical model (29).

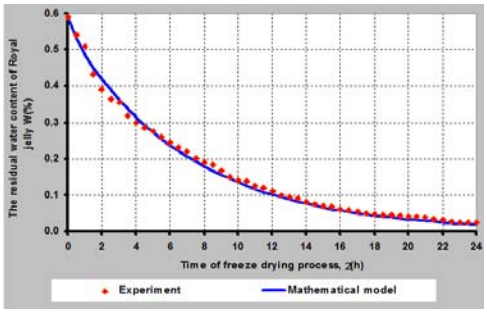

Fig 5. The kinetic for freeze drying process of royal jelly

5. Conclusions

Results of this study were obvious that moisture diffusion coefficient of royal jelly $D^{opt} = D = 9.50\times10^{-9}$ m^2/s was determined by identify parameter of the mathematical model method, this thermophysical parameter was important to calculate physical chemistry processes of the freeze drying (Marine Wytrychowski and et al., 2013). The above results could be concluded that the mathematical model (29) were built by heat and mass transfer models for the freeze drying process of royal jelly. It had well described relationships between the residual water content of royal jelly with time of the freeze drying process. In other words, it had well described kinetic for the freeze drying process of royal jelly. Because it was completely suitable with experimental data, the maximum error of the mathematical model (29) compared with experimental data was 9.30%, it was lower than 10%. Therefore, the mathematical model (29) was used to set up the technological mode of the freeze drying process of royal jelly (Boss et al., 2004; Barresi et al., 2008).

Nomenclature

W_0 (%): initial residual water content of royal jelly; W_E (%): residual water content of royal jelly to determine from experiment; W_M (%): residual water content of royal jelly to determine from mathematical model; G_0 (kg): weight of the initial material royal jelly used for freeze drying; G_e (kg): weight of the royal jelly freeze drying; X_j (%) ($j = 1 \div 6$): the chemical compositions of royal jelly such as water, porteins, glucids, lipids, minerals and impurities.

$c_1 = \sum_{j=2}^{6} c_j X_j = c_{pro}.X_2 + c_{glu}.X_3 + c_{lip}.X_4 + c_{ash}.X_5 + c_{im}.X_6$ (J/(kg.K)): specific heat of dried layer of royal jelly (Heldman, 1992); $c_w = 4167.2 - 9086.4\times10^{-5}\times T + 5473.1\times10^{-6}\times T^2$ (J/(kg.K)), when $0^0C < T < 150^0C$: specific heat of water (Heldman et al., 1992); $c_w = 4080.7 - 5306.2\times10^{-3}\times T + 9951.6\times10^{-4}\times T^2$ (J/(kg.K)), when $-40^0C < T < 0^0C$: specific heat of water (Heldman et al., 1992); $c_i = 2062.3 + 6076.9\times10^{-3}\times T$ (J/(kg.K): specific heat of ice (Heldman, 1992); $c_{pro} = 2008.2 + 1208.9\times10^{-3}\times T + 1312.9\times10^{-6}\times T^2$ (J/(kg.K)): specific heat of protein, (Heldman, 1992); $c_{lip} = 1984.2 + 1473.3\times10^{-3}\times T + 4800.8\times10^{-6}\times T^2$ (J/(kg.K)): specific heat of lipid, (Heldman, 1992); $c_{glu} = 1548.8 + 1962.5\times10^{-3}\times T + 5939.9\times10^{-6}\times T^2$ (J/(kg.K)): specific heat of glucid, (Heldman, 1992); $c_{ash} = 1092.6 + 1889.6\times10^{-3}\times T + 3681.7\times10^{-6}\times T^2$ (J/(kg.K)): specific heat of ash, (Heldman, 1992); $c_{mi} = 1296.78$ (J/(kg.K)): specific heat of impurities inside royal jelly, (Heldman, 1992); $\rho = 1/\sum_{j=1}^{6}\left(X_j/\rho_j\right) = 1/(X_1/\rho_n + X_2/\rho_{pro} + X_3/\rho_{glu} + X_4/\rho_{lip} + X_5/\rho_{ash} + X_6/\rho_{im})$ (kg/m^3): density of royal jelly (Heldman, 1992); $\rho_1 = 1/\sum_{j=2}^{5}\left(X_j/\rho_j\right) = 1/(X_2/\rho_{pro} + X_3/\rho_{glu} + X_4/\rho_{lip} + X_5/\rho_{ash} + X_6/\rho_{im})$ (kg/m^3): density of dried layer of royal jelly (Heldman, 1992); $\rho_w = 1001.75 - 0.4375\times T$ (kg/m^3), with $3.986^0C \le T \le 100^0C$: density of water, (Heldman, 1992); $\rho_i = 917\times(1 - 1.55\times10^{-4}\times T)$ (kg/m^3), with $T \le 0^0C$: density of ice, (Heldman, 1992); $\rho_{pro} = 1329.9 - 0.5184\times T$ (kg/m^3): density of protein (Heldman, 1992); $\rho_{glu} = 1599.1 - 0.31046\times T$ (kg/m^3): density of glucid (Heldman, 1992); $\rho_{lip} = 925.59 - 0.41757\times T$ (kg/m^3): density of lipid (Heldman, 1992); $\rho_{ash} = 2423.8 - 0.28063\times T$ (kg/m^3): density of mineral (Heldman, 1992); $\rho_{im} = 1017.29$ (kg/m^3): density of impurities

(Heldman, 1992); λ_0 (W/(m.K)): effective thermal conductivity of glass (trays), (Heldman, 1992); $\lambda_1 = \sum_{j=2}^{6} \lambda_j x_j$ (W/(m.K)), with $x_j = \left(X_j / \rho_j\right) / \sum_{j=2}^{5} \left(X_j / \rho_j\right)$: effective thermal conductivity of dried layer (Heldman, 1992); $\lambda_{pro} = 0.17881 + 1.1958\times10^{-3}\times T - 2.7178\times10^{-6}\times T^2$ (W/(m.K)): effective thermal conductivity of proteins (Heldman, 1992); $\lambda_{lip} = 0.18071 - 2.7604\times10^{-3}\times T - 1.7749\times10^{-7}\times T^2$ (W/(m.K)): effective thermal conductivity of lipids (Heldman, 1992); $\lambda_{glu} = 0.20141 + 1.3874\times10^{-3}\times T - 4.3312\times10^{-6}\times T^2$ (W/(m.K)): effective thermal conductivity of glucids (Heldman, 1992); $\lambda_{ash} = 1092.6 + 1889.6\times10^{-3}\times T + 3681.7\times10^{-6}\times T^2$ (W/(m.K)): effective thermal conductivity of minerals (Heldman, 1992); $\lambda_{im} = 1296.78$ (Heldman, 1992); $C_0 = 5.67\times10^{-8}$ W/(m^2.K^4): thermal radiation coefficient of backbody, (Holman, 1986); $\varepsilon_{qd} = 1/(1/\varepsilon_1 + 1/\varepsilon_2 - 1)$: conversion coefficient of dry material that they are transferred by radiation heat (Holman, 1986); ε_1, ε_2: black level of dry material that that they are transferred by radiation heat, (Holman, 1986); D (m^2/s): moisture diffusion coefficeint of royal jelly; $a_1 = \lambda_1/(c_1\times\rho_1)$ (m^2/s): thermal diffusivity coefficient of dried layer (Holman, 1986); α_r (W/(m^2.K): heat transfer coefficient. $r_{th} = (0.0024\times T^2 + 3.0606\times T + 3287.074)\times10^3$ (J/kg): latent heat of sublimation (Holman, 1986); $r_{hh} = (2509.64 - 2.51\times T)\times10^3$ (J/kg): latent heat of evaporation (Holman, 1986); T_{th} (^{0}C): temperature of ice sublimation; T_∞ (^{0}C): temperature of freeze drying chamber; t_1 (^{0}C): temperature of dried layer; t_2 (^{0}C): temperature of frozen layer; τ (s): time of freeze drying process; f (m^2): heat exchange area of trays; a', b' (m): length and width of trays; $Z_3 = \delta$ (m): height of royal jelly content of trays; δ_0 (m): depth of trays.

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PECTINASE-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM *POLYGONUM MULTIFLORUM* THUNB. ROOT

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ABSTRACT

The objective of this study was to figure out the optimal conditions for polyphenol extraction from *Polygonum multiflorum* Thunb. roots using pectinase-assisted extraction method. In this research, total phenolic contents (TPC) were determined by the Folin Ciocalteu method and antioxidant capacity (AC) was analyzed by free radical scavenging activity method with Trolox and DPPH as standard reagents. They were described by gallic acid equivalent (GAE) and Trolox equivalent (TE), respectively. The factors from extraction process were studied, gave the highest total phenolic content and strongest antioxidant capacity including material/solvent ratio (1/5 - 1/13), enzyme concentration (0.1% - 0.5%), extraction time (40 - 120 minutes), pH (3.5 - 5.5) and extraction temperature of (30°C-70°C). The optimal extraction conditions achieved such as the material/solvent of 1/11, enzyme concentration of 0.2%, extraction temperature of 50°C, pH value of 4.5 and extraction time of 80 minutes. TPC and AC peaked at 44.36 mg GAE/g DW (dry weight) and 80.43 µmol TE/g DW. After treatment, the materials were changed strongly and were also observed by scanning electron microscopy (SEM). Some extractive compounds of phenolic such as gallic acid and catechin were determined by HPLC method.

1. Introduction

From the late 20th century, the studies showed that long-term used of plant food containing many polyphenols can prevent cardiovascular diseases, chronic inflammation, degeneration, even though cancer diseases. Because of the great effect on health, polyphenols have been extracted into functional food to support human health. The plentiful polyphenols have been found in plants, especially in *Polygonum multiflorum* Thunb. (In Vietnam, it names Ha thu o do - HTOD). It was one of the precious plants that containing a considerable amount of phenolic compounds. The roofs of HTOD are used widely as an herb or tonic to treat hair loss, malaria and added essential substances in the blood, antioxidant liver cells. The extracted compound from HTOD had antioxidant effects, anti-ageing (Kwon et al., 2009) and anticancer (Milner et al., 1994). At the present, there are more than 100 chemical compounds that have been isolated from HTOD and major components with high biological activity have been identified such as stilbenes, quinones,

flavonoids, emodin and other substances (Lin et al., 2015).

There are many methods to extract phenolic compounds from plants such as using microwave, ultrasonic, supercritical fluid, high hydrostatic pressure, soxhlet extraction, etc. (Khoddami et al., 2013). Each extraction method has the advantage and weakness. Meanwhile, enzyme-assisted extraction is good effect for plants for instant pectinase. It has been widely applied in food technology to increase the extraction efficiency for phenolic compounds, as in apple (Zheng et al., 2009), grape seaweed (Ngô et al., 2014), mulberry (Nguyen et al., 2014), etc. Pectinase hydrolyzes pectin, breaks the cell wall and promotes the liberation of the components inside the material. In addition, using pectinase does not require complex equipment, environmentally friendly and high yield. Nowadays, there are no studies showed pectinase-assisted methods for the extractive compounds of phenolic from HTOD. Thus, determining the appropriate conditions for extractive process including the ratio material/solvent, enzyme concentration, pH, time and extraction temperature from HTOD were quite necessary.

2. Materials and methods

2.1. Materials

2.1.1 Plant material and sample preparation

Polygonum multiflorum Thunb. roots were collected from Cao Bang province (Vietnam). The fresh roots have the weight range from 0.5 to 1 kg, reddish brown color, no diseased or physical injuries. The cleaned roots were sliced into 2-3 mm thick pieces and dried at 60ºC until <12% of moisture. The dried samples were ground into a fine powder (<0.5 mm), packaged in vacuum condition and stored at room temperature for further use.

2.1.2 Chemical and reagents

Pectinase (Pectinex Ultra SP-L) was purchased from Novozyme Company (Denmark). Folin–Ciocalteu (FC) and DPPH (1,1–Diphenyl–2– pricrylhydrazyl) reagent was purchased from Merck (Germany). Trolox (6-hydroxy - 2, 5, 7, 8- tetramethylchroman-2-carboxylic acid) reagent was purchased from Sigma-Aldrich (USA) and other chemicals were of analytical reagent grade.

2.2. Methods

2.2.1 Extraction of phenolic compounds

The root powder (2 g) was extracted with the assistance of pectinase. Extraction of phenolic compounds was tested at different material/solvent ratios (1/5, 1/7, 1/9, 1/11 and 1/13), pectinase concentrations (0.1, 0.2, 0.3, 0.4 and 0.5%), extraction times (40, 60, 80, 100 and 120 minutes), pH (3.5, 4, 4.5, 5 and 5.5) and extraction temperatures (30, 40, 50, 60 and 70ºC). The mixture was filtered through Whatman No.4 filter paper in vacuum and then TPC and AC were analyzed.

2.2.2 Determination of total polyphenol content (TPC)

The TPC in the extracts was slightly modified and determined by the Folin-Ciocalteu colorimetric method (Siddiqua et al., 2010). The results were based on a standard curve obtained with gallic acid. TPC were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

2.2.3 Determination of antioxidant capacity (AC)

The AC in the extracts was determined by DPPH assay; this method was slightly modified and described by Soto et al. (2014). Trolox was used as the standard. AC was expressed in TEAC (Trolox equivalent antioxidant capacity) determined as μmol of Trolox per gram of dry weight (μmol TE/g DW).

2.2.4 Scanning electron micrographs (SEM)

Scanning electron microscope system (Jeol JSM-6480LV, Japan) was used to examine morphological of dried powder before and after enzyme treatment.

2.2.5 Determination of phenolic compounds by High Performance Liquid Chromatography method (HPLC)

HPLC analysis of phenolic compounds in extracts was carried out on an Agilent 1100 Series HPLC system equipped a diode-array UV-vis detector. The analysis was performed on a reversed-phase column (ACE C18, 4.6 × 150 mm, 3.5µm); the sample was injected into the injection port (loop 20 µL). The UV detector was set at a wavelength of 270 nm and 308 nm for gallic acid, catechin and resveratrol, respectively.

2.3. Data analysis

Results were analyzed by one-way analysis of variance (ANOVA) method and significant differences among means from triplicate analyses at $p_{value}<0.05$ were determined by Fisher's least significant difference (LSD) procedure using the Statgraphics software (Centurion XV).

3. Results and discussions

3.1. Effect of the ratios material/solvent on the extraction of phenolic compounds

Dried powder was extracted by the same extraction conditions including pectinase concentration of 0.2%, temperature of 50ºC, pH of 4.5, extraction time of 100 minutes and different material/solvent ratios. Figure 1 showed that using material/solvent ratio of 1/11 had the optimal results; TPC and TEAC of extracts had significant differences ($p_{value}<0.05$) and reached 41.62±0.98 mg GAE/g DW and 79.13±0.29 µmol TE/g DW, respectively.

During the extraction, the phenolic compounds moved into solvents that based on the diffusion. When the amount of solvent increases, the efficiency will also be improved (Vũ and Hà, 2009). If amount of solvent is too low, phenolic compounds will not diffuse completely into solvent. Thus, the extractive efficiency will decrease. However, the high volume of solvent can dilute the extract; the obtained yield is negligible and would not be cost effective. Based on the received results, the ratio of material/solvent 1/11 will use for the next experiment.

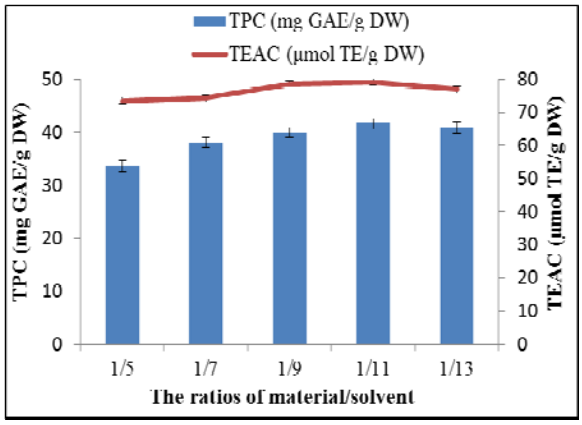

Figure 1. Effect of the material/solvent ratios on the extraction of phenolic compounds

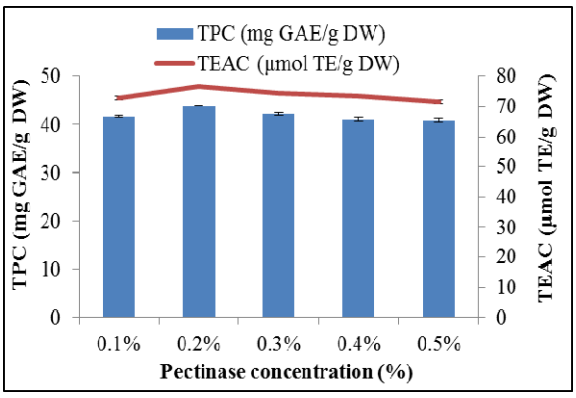

Figure 2. Effect of pectinase concentrations on the extraction of phenolic compounds

3.2. Effect of pectinase concentrations on the extraction of phenolic compounds

According to Figure 2, pectinase concentration of 0.2% was the best result, TPC and TEAC have the optimal values with 43.92±0.04 mg GAE/g DW and 76.96±0.26 µmol TE/g DW, respectively; and effect of pectinase concentration on the values of TPC and TEAC have a significant differences ($p_{value}<0.05$).

At first, pectinase hydrolyzes pectin in the materials, decomposes the structure of cell plants, reduces the viscosity of the solution and releases the substances inside the cell, especially phenolic compounds. Therefore, the yield of extraction can improve quickly. As enzyme concentration increases, the reaction

rate also increases, when the concentration of enzyme achieves the saturation, then the velocity the reaction does not change (Lê et al., 2002). Hence, TPC and TEAC increase insignificantly and maybe decrease because some phenolic compounds made the complexes with protein and inhibit enzymes (Begon et al., 1989). Pectinase concentration in this study is lower than some other studies, for instance Zheng et al. (2008) suggested that the level of pectinase up to 12% can increase the recovery yield of polyphenols from apple pomace, Ngô et al. (2014) used 5.63% pectinase concentration to extract polyphenol from seagrape (*Caulerpa lentillifera)*. Based on the above results, pectinase concentration of 0.2% was chosen for the evaluation of next steps.

3.3. Effect of extraction time on the extraction of phenolic compounds

The extraction time was researched from 40 to 120 minutes. The Figure 3 shows that the highest TPC and TEAC were 44.02±0.77 mg GAE/g DW and 77.99±0.12 µmol TE/g DW at the extraction time of 80 minutes. Effect of extraction times on the yield have a significant difference (p_{value}<0.05). TPC and TEAC tend to increase with increasing extraction time from 40 to 80 minutes then drops slightly from 80 to 120 minutes.

Extraction time is an important factor that influences on the extraction process. The optimal extraction time can save cost and time of process. In the other hand, increased extraction time is not effective but it can waste more time-consuming, reduces the economic and efficient use of the device. Most of the bioactive compounds are sensitive to high temperature, and their long storage will lead to the decomposition of bioactive compounds, especially phenolic compounds. They are oxidized by environmental factors such as light, temperature and oxygen. Extraction time depends on materials, extraction methods, devices and phenolic compounds. In this study, the extraction time was shorter than polyphenol extraction by pectinase from the seagrape *(Caulerpa lentillifera)* (102 minutes) (Ngô et al., 2014) and viscozyme L from unripe apples (12 hours) (Zheng et al., 2009), but it was longer than that of polyphenol extraction by microwave-assisted extraction (5 minutes) and ultrasound-assisted extraction (20 minutes) from melissa (Ince et al., 2013). Based on the achieved results, the optimal extraction time was 80 minutes for extraction process.

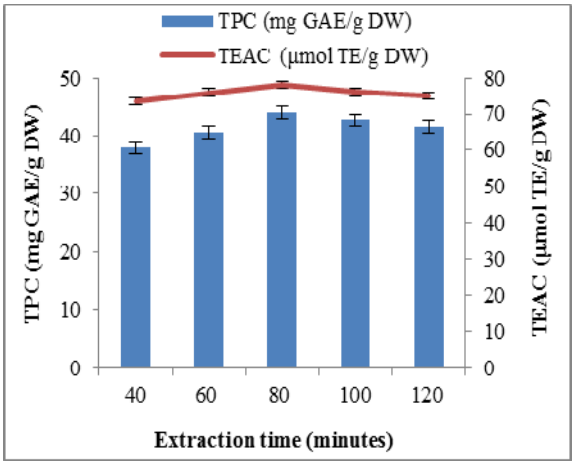

Figure 3. Effect of extraction time on the extraction of phenolic compounds

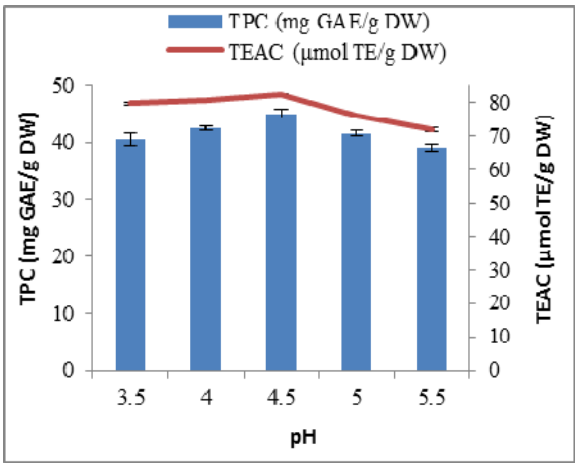

Figure 4. Effect of pH on the extraction of phenolic compounds

3.4. Effect of pH on the extraction of phenolic compounds

The optimal value of TPC and TEAC were shown in Figure 4 and effect of pH on TPC and TEAC had a significant difference (p_{value}<0.05). The highest TPC and TEAC obtained were approximately 45.05±0.63 mg GAE/g DW and 82.21±0.2 µmol TE/g DW at pH of 4.5, respectively.

The pH affected significantly the degree of ionization of substrates and enzymes (Lê et al., 2002). Besides that, due to the fact that the optimal operating conditions of pectinase are at pH of 4.5, TPC and TEAC were higher than the others, that means polyphenol in root of HTOD was stabled at pH<7 and could be unstable at pH>7. This results were similar with research of Zhu et al. (1997) who recognized that catechin in green tea was extremely unstable and decomposed almost completely in few minutes at pH> 8, while it was very stable at pH <4. Moreover, Friedman and Jurgens (2000) also demonstrated that caffeine, chlorogenic, and gallic acid of plants were unstable at high pH. Thus, the optimal pH of 4.5 is selected for the next survey.

3.5. Effect of temperature on the extraction of phenolic compounds

The analyzed results showed that the temperatures had a significant effect on extracting the phenolic compounds (p_{value}<0.05). According to Figure 5, the maximum values of TPC and TEAC were 44.36±1.87 mg GAE/g DW and 80.43±0.13 µmol TE/g DW at 50°C, respectively.

As extraction temperature increases from 30°C to 50°C, the values of TPC and AC also increase. The high temperature can promote the diffusion of phenolic compounds, reduces the viscosity of the solvent and easily releases phenolic compounds. However, when the temperature rose over 50°C, the TPC and TEAC decline extremely because they are quite sensitive with heat treatment. Besides that, the temperature also had a strong influence on enzyme reactions. Enzyme activity increased in a certain temperature limit; if it exceeds the limit, the enzyme activity will reduce (Nguyễn et al., 2004) and high temperature leads to a decrease in the value of TPC. Based on the achieved results, the optimal extraction temperature was 50°C.

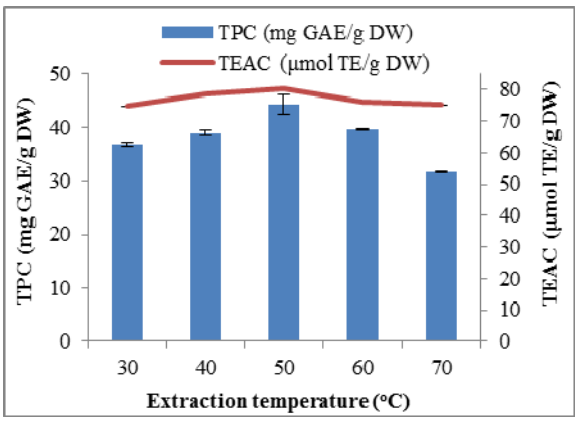

Figure 5. Effect of extraction temperature on the extraction of phenolic compounds

3.6. Effect of pectinase-assisted extraction on the structure of material and content of some phenolic compounds

Figure 6 shows that initial materials consist of many pieces of cell plants and starch that has many different diameter and egg (or oval) shape. After treatment by pectinase, almost of starch was not gelatinized at 50°C and a large number of wrinkles and fragments appear on surface of cell wall because the cell wall was destroyed by pectinase. It made the easy condition to release phenolic compounds.

Using HPLC method identified some phenolic compounds from the extracts at the optimal conditions. The detected phenolic compounds were gallic acid (3.65 mg/g), catechin (1.56 mg/g) (Figure 7, 8). Detecting these components are similar with research of Chen et al. (1999) but resveratrol in this study was not detected (Figure 9, 10) although there are many research proved that this compound also existed in HTOD extract (Quoc and Muoi, 2016; Chang et al., 2016). This difference was explained by many factors that could affect the content of phenolic compounds including extraction method, material, gene, climate, soil.

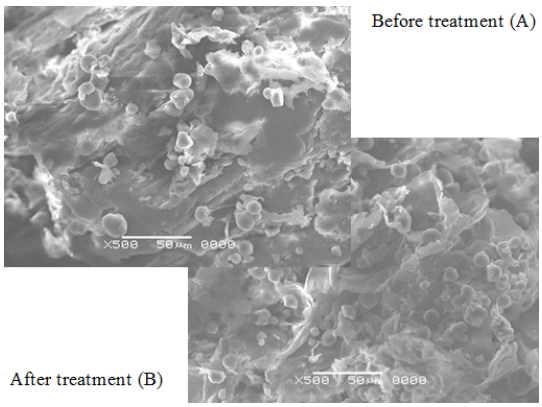

Figure 6. Structure of material before (A) and after (B) treatment by pectinase

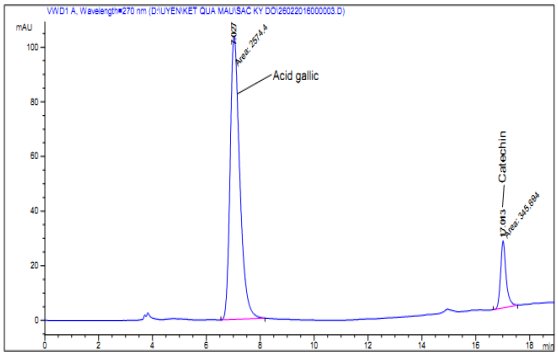

Figure 7. HPLC chromatograms of standard sample (Gallic acid and catechin) acquired at 270 nm

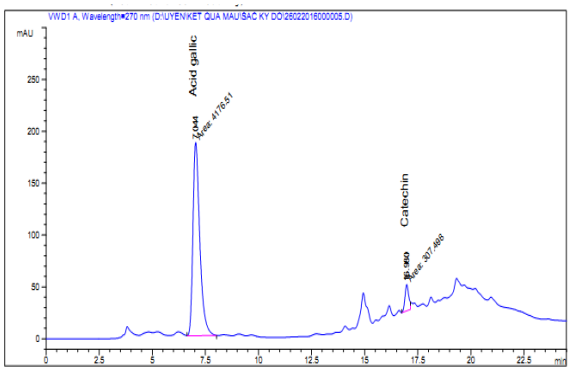

Figure 8. HPLC chromatograms of a sample of *Polygonum multiflorum* Thunb. root extracts acquired at 270 nm

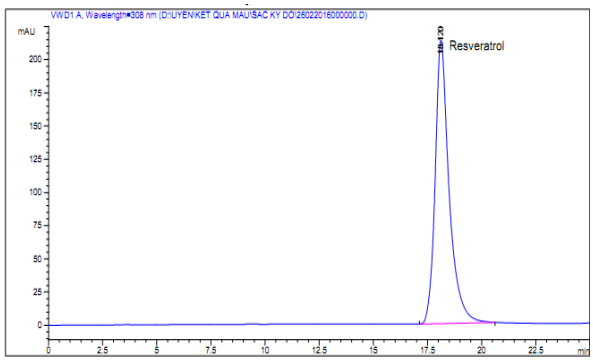

Figure 9. HPLC chromatograms of standard sample (Resveratrol) acquired at 308 nm

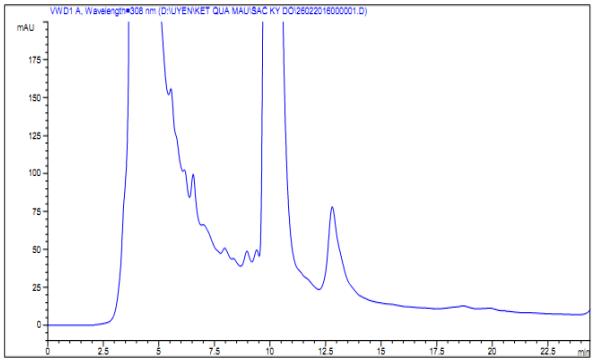

Figure 10. HPLC chromatograms of a sample of *Polygonum multiflorum* Thunb. root extracts acquired at 308 nm

4. Conclusions

The results pointed out that the support of pectinase can improve extraction process of phenolic compounds from *Polygonum multiflorum* Thunb. roots. The highest TPC and AC in the extract were 44.36±1.87 mg GAE/g DW and 80.43±0.13 µmol TE/g DW, respectively. The optimal conditions of extraction process were material/solvent of 1/11, pectinase concentration of 0.2%, pH of 4.5, extraction time of 80 minutes and extraction temperature of 50°C. Cells of material were destroyed by enzyme pectinase and there are some main phenolic compounds which were detected as catechin, gallic acid.

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EFFECT OF NISIN ON QUALITIES OF MILK PUDDING WITH FRUIT COCKTAIL DURING REFRIGERATED STORAGE

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ABSTRACT

The objective of this research was to investigate the efficacy of nisin (1.25-5.00 μg/g) on quality attributes of milk pudding with fruit cocktail, a gelled dairy dessert made of sweetened milk mixed with agar and topped with fruit cocktail, during storage at 4°C. The samples were analyzed for changes in microbial counts, syneresis, and texture profile analysis during storage, as well as chemical compositions and sensory acceptability at the beginning of storage. All samples containing nisin had significantly lower aerobic microbial counts when compared to that of the control ($p<0.05$). The sample with 5.00 μg/g had the fewer changes in syneresis, hardness, and chewiness when compared to those of the control during storage without affecting the springiness, cohesiveness, chemical compositions and sensory acceptability, including appearance, texture, flavor, and overall acceptance at the beginning of storage. Furthermore, the panelists could not discriminate the treated sample from the control in the duo-trio difference test.

1. Introduction

Dairy products contain a lot of essential nutrients such as calcium, essential amino acids, fatty acids, vitamins, and minerals. A recent study has shown that pudding and ice cream are the most frequently consumed dairy products at an average of 4.9 times/week (Colić Barić, 2001). Ready-to-eat creamy or gelled milk desserts with long shelf life have become popular in Europe and their popularity is growing in the USA. Milk pudding is one of commercially produced dairy desserts made of sweetened milk and starch or other hydrocolloids, such as agar, carrageenan, and low-methoxyl pectin (Hansen, 1993). According to Schmutz et al. (2007), milk pudding has a short shelf life of 5-6 days at 1-4°C.

Nisin is a low-molecular-weight polypeptide (3.3 kDa) produced by *Lactococcus lactis* subspecies *lactis*. It is effective against Gram-positive bacteria, including *Bacillus cereus*, *Bacillus sporothermodurans*, and *Clostridium botulinum* (Thomas and Delves-Broughton, 2005; Carballo et al., 2012), as well as some bacterial spores (Ray, 1992). In the United States, nisin has been approved as generally recognized as safe (GRAS) for inhibiting the outgrowth of *Clostridium botulinum* spores and toxin formation in pasteurized cheese spreads and pasteurized cheese spread with fruits, vegetables, or meats (US Food and Drug

Administration, 2015). In Abu Dhabi, Bahrain, and Dubai, nisin has been approved in pasteurized flavored milk with no limit level. In China, nisin is allowed in dairy products at permitted level of 12.5 μg/g food. In EU and Czech Republic, nisin is allowed in puddings at permitted level of 3 μg/g food (Thomas et al., 2000). In Argentina, Australia, France, Netherlands, USA, and Russia, nisin is permitted in processed cheese. In Mexico and Peru, nisin is also approved as a permitted additive in any food (Cleveland et al., 2001). According to Thomas and Delves-Broughton (2005), typical addition levels of nisin in dairy products and pasteurized chilled dairy desserts are 0.25-1.25 and 1.88-5.0 μg/g respectively. Recently, Martinez et al. (2016) have found that free and encapsulated (gum Arabic as a carrier agent) commercial nisin (Nisaplin®) were very effective against outgrowth and spore germination of *Bacillus cereus* in skimmed and whole milk during 21 days of storage at 6±1°C. Other *Bacillus* strains, such as *Bacillus pumilus* and *B. licheniformis*, were also inhibited by low concentrations of nisin in heat-treated cream (90°C for 15 min) during storage at 8°C (Nissen et al., 2001). Moreover, Wirjantoro et al. (2001) have reported that addition of nisin can prolong the shelf life of reduced heat-treated milk (117°C for 2 s) compared with UHT milk (processed at 142°C for 2 s) during storage at 10, 20, and 30°C.

The objective of this study was to investigate the efficacy of nisin on quality attributes of milk pudding with fruit cocktail during storage at 4°C.

2. Materials and methods

2.1. Materials

The following commercial food-grade ingredients and formulation were used for preparing milk pudding with fruit cocktail in this study: 49.87%(w/w) pasteurized fresh milk (CP-Meiji Co., Ltd., Saraburi, Thailand), 0.22%(w/w) agar (Phattanasin Enterprise Part., Ltd., Bangkok, Thailand), 26.60%(w/w) drinking water (Nestlé (Thai) Co., Ltd., Bangkok, Thailand), 2.49%(w/w) sucrose (Mitr Phol Sugar Co., Ltd., Bangkok, Thailand), 4.16%(w/w) non-dairy creamer (Nestlé (Thai) Co., Ltd., Bangkok, Thailand), and 16.66%(w/w) canned fruit cocktail in heavy syrup (Malee Sampraan Pub. Co., Ltd., Nakornpathom, Thailand).

2.2. Sample preparation

To prepare a control, agar was slowly dispersed in drinking water, which had been heated to 90°C and held for 1 min, and the mixture was continuously stirred until dissolved. Sucrose, milk (heated to 60±2°C and held for 10 min before used), and non-dairy creamer were slowly added to the agar solution. The sample was continuously stirred until dissolved and then cooled down to 40±2°C. The pudding was distributed in polyethylene hinged-lid containers (30 g each) and allowed to set at 4±1°C. The fruit cocktail was taken out of the heavy syrup and added on the top of the set pudding.

The procedures for preparing treated samples were similar to those for the control, except food-grade nisin (Shandong Freda Biotechnology Co., Ltd., China), which had been mixed into agar solution at three difference concentrations of 1.25, 2.50, 5.00 μg/g before sucrose, milk, and non-dairy creamer were added.

All samples were stored at 4°C for further analysis. The preparation process was independently repeated on 3 separate days as replication.

2.3. Microbiological analysis

Total viable counts (TVC) were determined by the pour-plate method (AOAC, 2000) at 0, 3, 6, 9, 12, 15, 18, and 21 days of storage. 10 g of each sample was aseptically weighted and homogenized with 90 ml of sterile peptone water (Merck, KGaA, Germany) for 1 min using a stomacher (Funke-Gerber, Germany) at a speed of 230 rpm. The homogenized samples were serially diluted (1:10) in sterile peptone water. 1 ml of sample from serial dilutions was added to each duplicate sterile Petri dish. Plate Count Agar (PCA) was added to each Petri dish

and samples were then incubated at 37±2 °C for 48 h. After incubation, colonies were counted and microbiological data was expressed as logarithms of number of colony-forming units (logCFU/g).

2.4. Syneresis

The determination of syneresis was carried out at 1, 8, 15, and 21 days of storage as described by Supavititpatana et al. (2008).

2.5. Texture profile analysis

Texture profile analysis (TPA) was measured by using a texture analyzer TA.XT2i (Stable Micro Systems Ltd., UK) as described by Liu et al. (2013) at 1, 8, 15, and 21 days of storage. The fruit cocktail was taken out from the top of each sample and liquid from syneresis was separated before analysis. The measurements were carried out at 10±1°C on three replicates. The following texture parameters of the samples were reported: hardness, springiness, cohesiveness, and chewiness.

2.6. Chemical analysis

Chemical analysis of the control and the best treatment (from microbiological analysis, syneresis and TPA) at the beginning of storage was performed following AOAC (2012) including moisture, lipid, protein, fat, ash, and carbohydrate contents.

2.7. Sensory evaluation

Two sets of sensory evaluation were performed to determine the difference between control and the best treated sample (from microbiological analysis, syneresis and TPA), as well as their acceptability, among 30 untrained panelists screening from juniors and seniors in the Department of Agro-Industrial, Food, and Environmental Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Thailand. In the first evaluation, duo-trio difference test was performed to indicate the difference between control and the treated sample at the beginning of storage. The panelists were initially asked to evaluate and remember the control, labeled with "S" as a standard. Then, they were given a pair of samples (the control and treated samples labeled with random 3-digit codes and presented in random order) and asked to indicate which the standard was. The number of panelists who indicated the standard correctly was reported.

In the second evaluation, the control and the treated sample at the beginning of storage were evaluated for acceptance (appearance, texture, flavor, and overall acceptance) using a nine-point hedonic scale, including 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely. All samples in both experiments were labeled with random 3-digit codes and presented in random order.

2.8. Statistical analysis

All analyses were run in triplicate, except microbiological analyses, which were run in duplicate. Data from microbiological analysis, syneresis, TPA, chemical analysis, and acceptance test were analyzed by analysis of variance (ANOVA) using IBM SPSS Statistics 21 (IBM Corporation, Armonk, NY). Duncan's multiple range test (DMRT) was used to determine significant differences among means. Data from the duo-trio test were analyzed by comparing the number of correct responses to the critical number by Meilgaard et al. (2007). Significance was defined at $p<0.05$.

3. Results and discussions

3.1 Microbiological analysis

Total viable counts of control and treated samples with different concentrations of nisin during storage at 4°C are presented in Fig. 1. The initial number of bacteria in all samples was less than 1.00 logCFU/g which indicated appropriate sanitation used in this study. Putthongsiri et al. (2011) also reported that there was less than 1.00 logCFU/g of initial total viable counts in milk pudding with and

without chitosan added in the recent study. Total viable counts increased with storage time in every sample ($p<0.05$) (Fig. 1). Compared with the control, all treatments significantly inhibited the growth of bacteria in milk pudding with fruit cocktail during storage ($p<0.05$). The sample treated with 5.00 µg/g nisin had the lowest total viable counts and maintained less than 4.00 logCFU/g until the end of study.

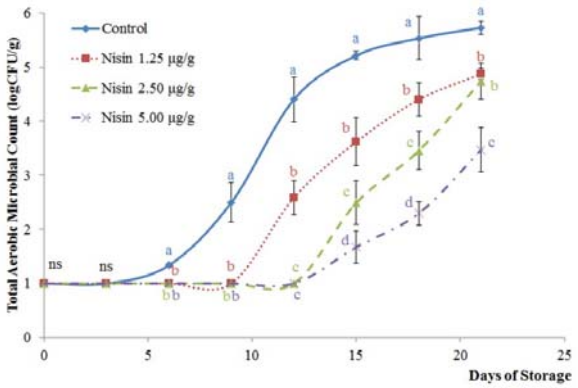

Figure 1. Total viable counts of control and treated samples with different concentrations of nisin during storage at 4°C. Different letters (a, b, c, d) indicate significant difference at the same day of storage at $p<0.05$. ns indicates no significant difference between samples at the same day of storage ($p\geq0.05$) (n=3, error bars: standard deviations).

Samples containing nisin showed slower growth of bacteria since nisin is effective against Gram-positive bacteria by forming pores at cytoplasmic membrane. These pores disrupt a proton motive force and pH equilibrium leading to leakage of ions, hydrolysis of ATP, and eventually cell death. Nisin can also inhibit cell wall biosynthesis by binding lipid II, a peptidoglycan precursor (Bauer and Dicks, 2005; de Arauz et al., 2009; Deegan et al., 2006).

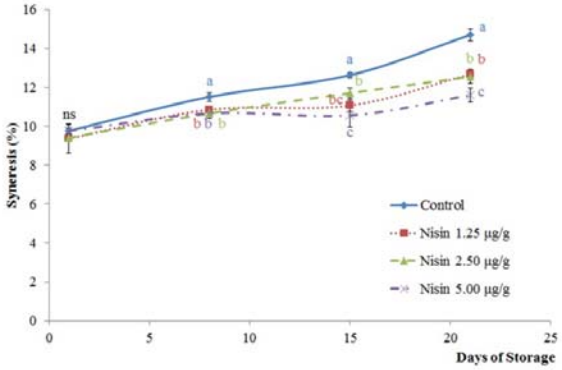

Figure 2. Syneresis of control and treated samples with different concentrations of nisin during storage at 4°C. Different letters (a, b, c) indicate significant difference between samples at the same day of storage at $p<0.05$. ns indicates no significant difference at the same day of storage ($p\geq0.05$) (n=3, error bars: standard deviations).

Recently, Ibrahim and Elbarbary (2012) have reported that bacteriocin from *Lactobacillus acidophilus* strain showed effective antimicrobial activity and extended shelf-life of pasteurized milk to 12 days during refrigerated storage. Nissen et al. (2001) also found inhibition of nisin against some bacteria, including 6 strains of *Bacillus*, in heat-treated cream (90°C for 15 min) during storage at 8°C. Arques et al. (2008) suggested that nisin combined with reuterin and lactoperoxidase system at low concentrations could improve microbial safety against *Listeria monocytogenes* and *Staphylococcus aureus* in "cuajada" (curdled milk), a semisolid dairy product manufactured in Spain, during storage at temperature abuse of 10°C.

3.2 Syneresis

Syneresis was expressed as percentage of water separated from the formed gel of pudding. The syneresis naturally occurs since gel water cannot be held in the gel structure during storage. Food containing higher concentration of gel, higher water holding capacity of gel, and higher gel strength tends to have lower rate of syneresis (Benerjee and Bhattacharya, 2011; Matsuhashi, 1990). Syneresis increased with storage time in every

sample ($p<0.05$) (Figure 2). Gels with low concentration of agar (1% or lower) can separate large amount of water over time (Matsuhashi, 1990). Abo-el-Fetoh (2010) also reported the increase of syneresis of pudding and white sauce produced from different starches, such as tiger nuts, sweet potato, and taro, during 5 days of storage at -10°C, 4°C, and room temperature. Samples containing nisin had significantly slower rate of syneresis during storage when compared with the control ($p<0.05$). The sample treated with 5.00 µg/g nisin had the lowest syneresis, especially at the end of storage. These results correlated with total viable counts presented in Fig. 1. As microbial counts increased, the gel structure might lose its strength, leading the separation of water during storage.

3.3 Texture profile analysis

Texture is another important factor that affects acceptability of consumers. Hardness and chewiness of control and treated samples with different concentrations of nisin during storage at 4°C are presented in Figure 3. TPA hardness is defined as the force needed for attaining a given deformation and chewiness is defined as the energy needed for masticating solid food to a state of readiness for swallowing (Bourne, 2002). In every sample, hardness and chewiness remained constant at the beginning of storage and increased at the end of the storage. The higher hardness and chewiness with time might occur due to the volumetric shrinkage of the pudding gel, influenced by syneresis (Matsuhashi, 1990). At the end of storage, the control sample had the highest hardness and chewiness while the sample with 5.00 µg/g nisin had the lowest hardness and chewiness ($p<0.05$). These results also correlated with total viable counts and syneresis presented in Fig. 1 and 2 respectively.

Springiness and cohesiveness of all samples did not show significant difference between samples throughout the storage ($p\geq0.05$) (data not shown).

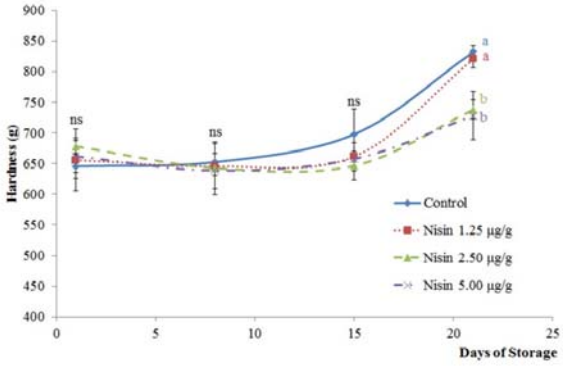

Figure 3. Hardness of control and treated samples with different concentrations of nisin during storage at 4°C. Different letters (a, b) indicate significant difference between samples at the same day of storage at *p<0.05*. ns indicates no significant difference at the same day of storage *(p≥0.05)* (n=3, error bars: standard deviations).

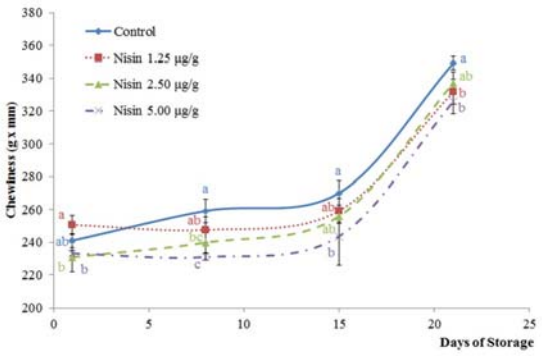

Figure 4. Chewiness of control and treated samples with different concentrations of nisin during storage at 4°C. Different letters (a, b, c) indicate significant difference between samples at the same day of storage at $p<0.05$ (n=3, error bars: standard deviations).

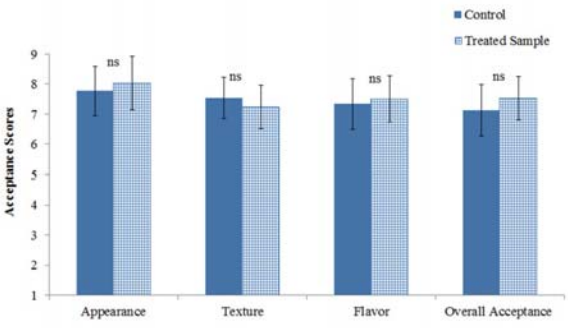

Figure 5. Acceptance scores of control and treated sample with 5.00 µg/g nisin at the beginning of storage (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely). ns indicates no significant difference between samples at the same attribute (*p≥0.05*) (n=30, error bars: standard deviations).

3.4. Chemical analysis

Since the sample containing 5.00 µg/g nisin showed the best results from microbiological analysis, syneresis, and TPA, it was selected for chemical analysis and sensory evaluation to compare with the control sample. Chemical compositions of control and treated sample with 5.00 µg/g nisin during storage at 4°C are presented in Table 1. There was no significant difference between control and treated sample in every chemical composition at the first day of storage (p≥0.05). Similarly, Benech et al. (2002) reported no significant difference between nisin-free cheddar cheese and *in situ* nisin-containing cheddar cheese on moisture content, total proteins and fat content after production.

3.5. Sensory evaluation

In the duo-trio test, the control was first provided to each untrained panelist as a standard. Then, both control and treated sample with 5.00 µg/g nisin were served and the panelists needed to indicate which the standard was. According to the table of critical number of correct responses in a duo - trio or one - sided directional difference test (Meilgaard et al., 2007), the minimum number of corrected responses required for significant difference at α-level of 0.05 (n=30) is 20. However, the number of corrected responses from this test was 16 (53%), indicating that the panelists could not discriminate the treated sample from the control sample. Similarly, the control and treated samples were not significantly different in microbiological analysis, syneresis, TPA, and chemical analysis at the beginning of storage (p≥0.05).

Table 1 Chemical compositions of control and treated sample with 5.00 µg/g nisin at the first day of storage (n=3).

Chemical composition (%w/w)	Control	Sample with 5.00 µg/g nisin
Moisture ^{ns}	83.22 ± 0.16	83.62 ± 0.56
Carbohydrate ^{ns}	11.44 ± 0.17	11.06 ± 0.55
Fat ^{ns}	3.16 ± 0.05	3.23 ± 0.07
Protein ^{ns}	1.66 ± 0.04	1.53 ± 0.17

ns indicates no significant difference (*p≥0.05*).

The acceptance test was conducted to evaluate the level of acceptability on the treated sample among untrained panelists (n=30), compared with the control sample. Scores from the acceptance test are presented in Fig. 5. There was no significant difference between the control and the treated sample in appearance, texture, flavor, and overall acceptance at the beginning of storage (p≥0.05). Both samples obtained scores at approximately '7' or 'like moderately'. These results could ensure that nisin did not affect the sensory qualities of the samples. In the application of nisin in buffalo meat sausage, Sureshkumar et al. (2010) also found that nisin did not affect the sensory qualities of samples, including appearance, flavor, juiciness, and overall acceptability at the beginning of storage. Moreover, Mahalingaiah et al. (2014) reported no significant difference between "kunda" (a sweet product prepared from milk and added sugar) with and without nisin added in sensory scores, including flavor, texture, and overall acceptance, at the beginning of storage (p≥0.05).

4. Conclusions

The recommended concentration of nisin in milk pudding with fruit cocktail was 5.00 µg/g since it efficiently reduced the changes in microbial growth, syneresis, hardness, and

chewiness of the sample while provided no effect on springiness, cohesiveness, chemical compositions and sensory acceptability.

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STUDIES REGARDING THE WATER CONTENT OF PORK HAM

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ABSTRACT
The aim of this research was to determine the water content from two samples of ham, made by a producer from the West Region of Romania. We obtained and analyzed two types of ham (Prague and Premier), during an experimental period of three years, 2013-2015, before implementing the HACCP system (2013) and after it's implementation (2014-2015), in order to trackthe food quality, regarding the nutritional value.

1. Introduction

Pork ham belonges to the category of of pasteurized meat specialties, being obtained from pork pulp and a brine consisting of: water, sugar, nitric salt, meat flavors and sodium tripolyphosphate. Analyzing the composition, pork leg contains heterogeneous unsaturated animal fats, water and salt (Mencinicopschi et al., 2006).

Determination of moisture is one of the basic methods used in food control, being a factor for assessing the nutritional value (the nutritional value decreases with higher water content), but also for the assessment of preservation strength (the preservation strength is better with a lower water content). The humidity is controlled for all food products either directly by assessing the amount of water or indirectly by measuring the dry matter remaining after removal of water (Neacsu and Chirigiu, 2006).

The water content represents the weight loss by heating at a temperature indicated by the analysis method to constant weight. In order to ensure a high protection level of consumers health it requires a thorough risk control, to prevent illnesses caused by food risk, especially by meat products (Law 150 regarding food safety).

The safety levels to admit chemicals and biological agents in meat products are established by Romanian law by Order no. 210 from 30 august 2006 concerning the admissibility conditions of physic-chemical properties for meat products sausage type.

2. Materials and methods

2.1. Materials

There where obtained and analyzed two types of pork ham (Prague and Premier), during three experimental years, 2013-2015.

2.2. Methods

Determinations were carried out according to the following standards:

SR ISO 1442:2010. Meat and meat products. Humidity content.

SR ISO 17025/2005. General requirements for the competence of laborators for testing and calibration.

3. Results and discussions

The manufacturing technology for Premier and Prague pressed ham followed where the same in all the three experimental years (2013-2015). Starting with 2014, it was implement the HACCP system n order to increase food safety and nutritional properties.

The studied ham samples were quarterly physic-chemical analyzed (I. Quarter, II. Quarter, III. Quarter andIV. Quarter), during the period 2013-2015.

The results regarding the nutritional properties of the studied ham samples, were reported at the admissibility conditions established by Romanian law.

The water content in ham has not been legislated by an order or regulation, but literature studies from Romania, highlight a water content of 70% in pressed pork ham (Mencinicopschi *et al.*, 2006).

The analysed samples of Premier hamstudied in 2013 record values of water content between 78.51% (I. Quarter) and 78.81% (II. Quarter) (Figure 1), higher than the values highlighted in literature studies of 70%.

Following the analysis performed in 2014 after implementing the HACCP system, the values of the water content in Premier pressed ham samples range between 71.3% (II. Quarter) and 74.81% (III. Quarter) (Figure 1), the results being in accordance with the values of 70% found in literature.

The water content registered in 2014 was lower than those recorded in 2013, resulting that the Premier pressed ham samples studied in 2014 have higher nutritional value becausewith lower water content, the nutritional value increases.

Following the analysis performedin 2015, the second year after the implementation of the HACCP system, the water content registred values between 72.18% (III. Quarter) and 72.63% (IV. Quarter), values according to otherliterature studies.

The values recorded in 2015 (Figure 1) are lower than those registered in 2013, between 78.51% (I. Quarter) and 78.81% (II. Quarter), and those from 2014, between 71.3% (II. Quarter) and 74.81% (III. Quarter),so we can conclude that the implementation of the HACCP has led to an improvement of the nutritional value of studied Premium ham samples. The water content registered in 2014 and 2015 decreased after implementation the HACCP system, that positively influences the nutritional value of the studied products.

Following the analysis performed for Prague pressed ham samples, produced in 2013, the water contentregistres values between 77.13% (I. Quarter) and 78.59% (II. Quarter) (Figure 2), higher than the value of 70%found in other literature studies.

Following the analysis on Prague pressed ham samples, studied in 2014, the water contentregistered values between 73.32% (I. Quarter)and 74.28% (II. Quarter) (Figure 2), higher than the value of 70% highlighted in literature studies, but lower than the values determined in 2013, before implementation the HACCP system.

The water content recorded in 2014 was lower than that recorded in 2013, with increasing nutritional value of the studied samples.

Following the analysis performed in 2015, the water content for the Prague ham samples range between 72.21% (III. Quarter) and 73.14% (II. Quarter).

The water content determined in 2015 was lower than the values determined in 2014, and these lower than those in 2013, which highlights a higher nutritional value of samples studied in 2015.

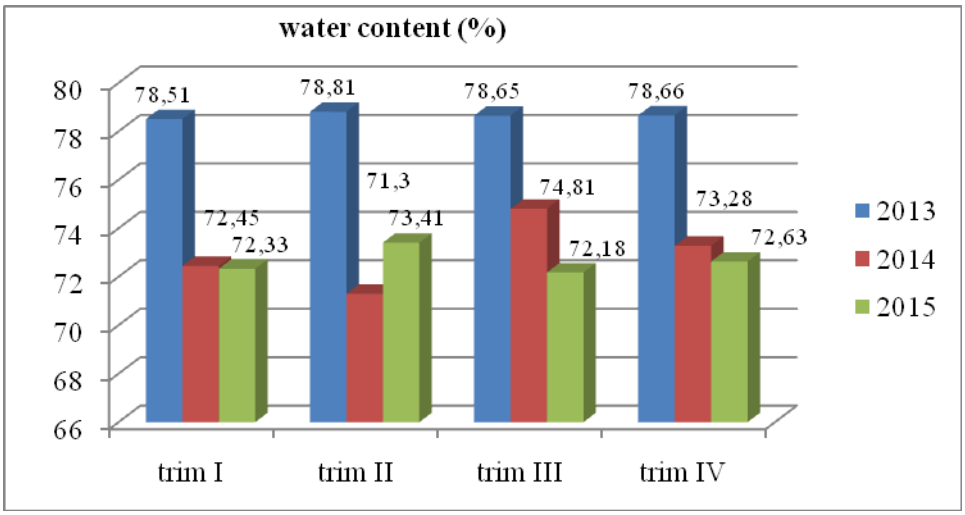

Figure 1. Water content (%) of Premier ham samples studied between 2013 and 2015

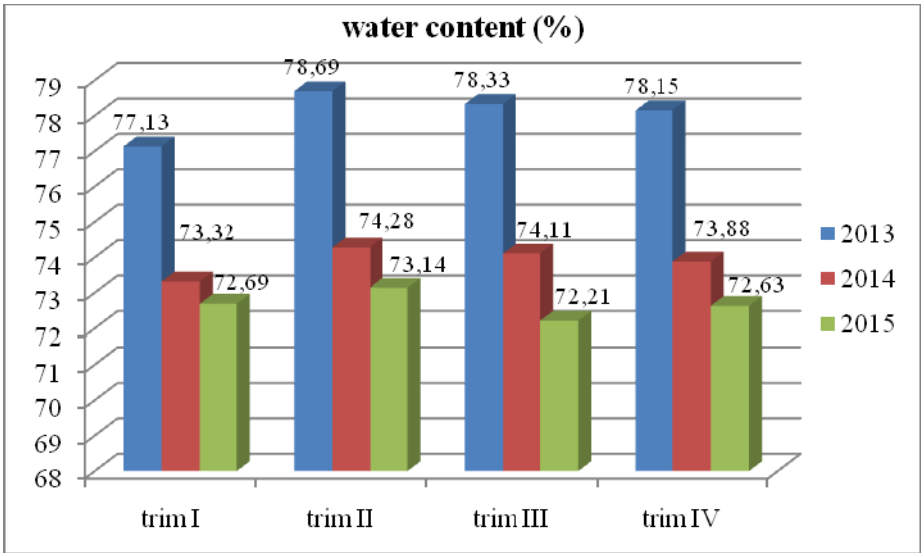

Figure 2. Water content (%) of Prague ham samples studied between 2013 and 2015

4. Conclusions

Implementation of the HACCP system in the technological process has led to an improvement of the nutritional value of studied ham samples.

The water content recorded in 2014 and 2015 decreased compared to samples studied in 2013, a fact that positively influences the nutritional value of the studied products.

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SR ISO 17025/2005. Cerințe generale pentru competența laboratoarelor de încercare și etalonare.

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EFFECT OF ACID-ALCOHOL TREATMENT ON PHYSICOCHEMICAL PROPERTIES OF *CARIOCA* BEAN (*Phaseolus vulgaris* L.) STARCH

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ABSTRACT
Starches from different sources present unknown potential for new technological applications mainly when modifications are considered. In the present study acid-alcoholic treatments of *Carioca* bean starch were evaluated considering selected physicochemical and thermal properties. Ethanol, methanol and their mixture (1:1) were employed as solvents for hydrochloric acid hydrolysis of bean starch. The results showed changes in DSC parameters, including gelatinisation temperatures that increased from around 70 to 80 °C and enthalpy changes that decreased from around 10 to 4 J g^{-1}. The viscographic behaviour analysed by RVA, L*, a* and b* colour parameters and also some changes in the granular morphology by SEM were observed. On the other hand, no changes were detected for the relative crystallinity by X ray diffraction. The acid alcoholic treatment resulted in different starch properties including the expected acid thinning due to depolymerisation.

1. Introduction

Starch is a natural biopolymer arranged as semi-crystalline microscopic granules comprising fractions of linear amylose and branched amylopectin (Zeeman et al., 2010; Dutta et al., 2011). Due to some limited properties of natural starch, chemical modification is widely used to enhance its functionality for application in the food industry(Xiao et al., 2012).

Common bean (*Phaseolus vulgaris* L.) is a very important starchy seed in human nutrition, because it presents high amounts of carbohydrates (50-60%) and proteins (20-25%). Starch is the most abundant carbohydrate (22-45%), presenting high levels of amylose which gives interesting properties for applications and uses (Hoover et al., 2010; Ovando-Martinez et al., 2011). In Brazil, this kind of common bean represents an important crop for small farmers and is a diary food for a population of almost 200 million people (Otsuboet al., 2013); the variety Carioca makes around 85 % of the Brazilian bean market (Silva et al., 2014).

Acid hydrolysis involves the suspension of granular starch in an aqueous solution of hydrochloric or sulfuric acid. The action of

the strong acid undercontrolled temperature results in partial cleavage of polysaccharides (Yiu et al., 2008).According to Shi et al. (2014), acid modification changes the physical and chemical properties of starch, but the granular structure remains the same.

Acid hydrolysis modifies the starch to be incorporated in foods in order to control viscosity, texture and to improve moisture retention, in addition to presenting low viscosity in aqueous solution (Gao et al., 2012). Alcohol-acid modifications were already described in the literature for several conditions as alcohol type, starch source and concentration, reaction time (Dutta et al., 2011; Luo et al., 2011). Those modifications were reportedforseveral starch sources such as lentils (Sodhi et al., 2009), barley (You and Izydorczyk, 2007) and sago starch (Yiu et al., 2008). Using acid hydrolysis in different alcohols, Ma and Robyt (1987) prepared and characterized soluble potato and waxcy maize starches having different molecular sizes and composition.

The aim of this study was to assess the effect of alcohol-acid hydrolysis on the main physicochemical and thermal properties of *Carioca* bean (*Phaseolus vulgaris* L.) starches, which is not found in literature.

2. Materials and methods

2.1. Materials

Starch, in its native form, was extracted from *Carioca* beans (*Phaseolus vulgaris* L.) acquired in a local retail market in Ponta Grossa, Paraná, Brazil. The reagents used for modification were anhydrous methanol, CH_3OH (J.T. Baker, Xalostoc, Mexico), ethanol, C_2H_5OH (Biotec, Porto Alegre, Brazil), hydrochloric acid, HCl 37 % (in weight), d=1.19 g mL^{-1} (Synth, São Paulo, Brazil) and sodium bicarbonate, $NaHCO_3$ (Caal, São Paulo, Brazil), all of analytical grade.

2.2. Methods

Acid-alcoholic starch treatment was performedaccording to Ma and Robyt(1987) and Lin et al. (2003) with some modifications: a quantity of starch (25 g) was suspended in 100 mL of methanol, ethanol, their mixture (1:1) or alcoholic-acid solutions (1 mL of HCl) in 400 mL flasks. Seven treatments were studied with varying methanol, ethanol and hydrochloric acid proportions (Table 1). The reaction was maintained at 65 °C for 1 hour(Ma and Robyt, 1987)in a Dubnoffshaker bath (TECNAL TE-053, Brazil). The reaction was quenched by the addition of 14 mL of 1 mol L^{-1} $NaHCO_3$ and then placed in an ice bath. The starch was centrifuged (ROTINA 420R, Hettich, Germany) at 3000 ×*g* for 5 minutes and then washed four times with 50 % ethanol (v/v). The precipitate was dried in an oven with air renewal and circulation (TECNAL TE394/2, Brazil) at 40 °C for 12 hours.

Table 1. Proportions of reagents used for each acid-alcohol hydrolysis treatment

Treatment	Volume (mL)		
	Methanol	Ethanol	HCl
1	-	-	-
2	100	-	-
3	100	-	1
4	-	100	-
5	-	100	1
6	50	50	-
7	50	50	1

2.2.1 Differential Scanning Calorimetry (DSC)

The DSC curves were obtained using a thermal analysis system model DSC-Q200 (TA-Instruments, USA). The DSC curves were recorded under the following conditions: air flow of 50 mL min^{-1}, heating rate of 10 ºC min^{-1} and samples weighing about 2.0 mg. A proportion 4:1 (water:starch, w/w) was considered and the

aluminum crucibles were sealed and rested for 60 minutes in order to equilibrate the moisture; after that, the curves were performed. The instrument was previously calibrated with indium standard 99.99% purity, with melting point T_p = 156.6 ºC, ΔH = 28.56 Jg^{-1}. Gelatinisation occurs as an endothermic event and the "onset", "peak" and "conclusion" transition temperatures (T_o, T_p and T_c) as well as the gelatinisation enthalpy change (ΔH_{gel}) were calculated using the "Universal Analysis 2000" software (Vatanansuchartet al., 2005; Granzaet al., 2014).

2.2.2 Pasting Properties (RVA)

The pasting properties of the starch samples were determined using the RVA-4 (Newport Scientific Pvt. Ltd., Narabeen, Australia). Anaqueous starch suspension (8 % w/w, dry basis) with 28 g of total mass underwent a controlled heating and cooling cycle under constant stirring (160 rpm) when it was held at 50 ºC for one minute; heating from 50 to 95 ºC at 6 ºC min^{-1}, and held at 95 ºC for 5 minutes; cooling until 50 °C at 6 ºC min^{-1}, and held at 50 ºC for 2 minutes (Franco, 2002). The apparent viscosity curve during the aqueous starch suspension heating, cooking and cooling was recorded producing the viscoamlylograms.

2.2.3 X-Ray Diffraction

The X-ray diffractograms were collected in theRigakuUltima IV (Rigaku, Tokyo, Japan) equipment with CuKα radiation (λ = 1.544 Å) at 40 kV and 20 mA (Beninca et al., 2008). The analysis was performed at 20 °C in a 2θ angle range of 7-30° with a measuring period of 5 s/2θ.

2.2.4 Colour Parameters

For determining the colour parameters of the starch, the MiniScan EZ 4500L (HunterLab, USA) was used. Three colour components were measured: L*,brightness ranging from 0 (black) to 100 (white); a* ranging from positive (red) to negative (green); and b*, which varies from positive (yellow) to negative (blue) (Falade and Onyeoziri, 2012).

2.2.5 Scanning electron microscopy

The morphology of the starch granules was examined using a scanning electron microscope (Tescan, VEGA 3, Kohoutovice, Czech Republic). The starch samples were coated with gold and examined in the scanning electron microscope under an acceleration voltage of 25 kV and magnification of 1,000 times.

2.2.6 Statistical Analysis

The normality of the experimental data was evaluated considering the Shapiro-Wilk test and thehomogeneity of variance, using Brown-Forsythe ($p>0.05$ was considered parametric). Then the parametric data were evaluated by analysis of variance (ANOVA) complemented with the Fisher LSD mean comparison test. A value of $p<0.05$ was considered significant. Statistical analyses were performed by Statistica software version 7.0 (Statsoft, Tulsa OK, USA).

3.Results and discussions

3.1. Differential Scanning Calorimetry (DSC)

The DSC curves were performed to study the gelatinisation process. They were obtained in a heating rate of 10 ºC min^{-1} with a 1:4 proportion of starch:water in sealed aluminum crucibles. Figure 1 shows the profile of the DSC curves.

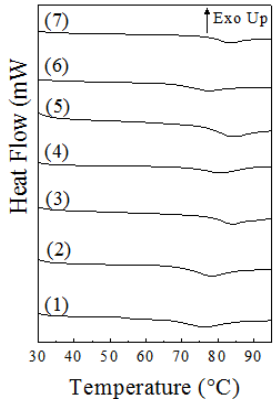

Figure 1. DSC curves of starches of *Carioca* beans after acid-alcohol treatment

Table 2 shows the DSC results and compared with the (N) native bean starch sample, the peak temperatures (T_p) of the modified samples did not show significant differences. These effects are dependent on the moisture level of the treatment, the starch source, and the amylose content(Lin *et al.*, 2012; Granza*et al.*, 2014). The calculated gelatinisation enthalpy change (ΔH_{gel}) presented an increase, contrasting with the result observed for sample from treatment 3 confirming that the modification occurred in this sample. Similar values for enthalpy changes arefound in the literature: 15.4, 14.3, 14.4 and 15.0 J g^{-1}, for annealed navy bean starch, for navy bean starch submitted to heat-moisture treatment and dual modified,i.e, annealed starch that was submitted to heat-moisture treatment (ANN–HMT) and heat-moisture treated starch that was submitted to annealing (HMT-ANN),respectively (Chung *et al.*, 2010). The differences between the results of ΔH_{gel} were due to the different treatments that were performed (reagent concentrations, time of treatment, genetic varieties and the employed physical methods).

The temperatures of gelatinisation (onset, peak and conclusion temperatures)of the starches are shown in Table 2 and Figure 1; the hydrolysis (treatments 2-7) increased the values of $T_o$andT_p, and reduced those of T_c and $\Delta H_{gel}$when compared with native starch (treatment 1). The increase and reduction of these parameters were more pronounced on the treatments with use of HCl (treatments 3, 5 and 7) than those with ethanol/methanol only.

The hydrolysis increased the gelatinisation temperature while ΔH_{gel}values were lower than that of native starch. These results are in line with other studies,as observed with potato and maize starches hydrolysedbyHCl in methanol. The modified starches presented higher gelatinisation temperature after treatment (Lin et al., 2003); the treatment with ethanol and HCl in waxy corn starch presented the same tendency for ΔH_{gel}.The reduction of ΔH_{gel}on treated starch may be attributed to its partial gelatinisation (Chang *et al.*, 2004).

3.2. Pasting properties

The RVA results are shown in Figure 2 and Table 3. When methanol alone was used to produce the starch suspension,in the absence of HCl (treatments 2 and 6), there was a significant increase in peak viscosity (V_p) and conclusion viscosity (V_c). However, when ethanol instead of methanol was used (treatment 4), a significant reduction of those viscosities occurred. That viscosity change was also reported previously (Lin et al., 2005) for potato starch suspended in methanol.

The addition of HCl (treatments 3, 5 and 7) had a marked effect on pasting properties of bean starch. Both viscosities (V_p and V_c) were significantly reduced and in some samples the peak viscosity values were undetectable. Similiar results were reported by Chang et al. (2004), Cavallini and Franco (2010) and Luo et al. (2011) when starches were treated with HCl in alcohol.

Table 2. DSC gelatinisation results of *Carioca* bean starches modified with different treatments

Treatments	DSC gelatinisation			
	T_0/ºC	Tp/ºC	Tc/ºC	ΔH_{gel}/J g^{-1}
1	67.5±0.13^{f}	76.6±0.00^{g}	92.2±0.69^{a}	10.2±0.11^{a}
2	73.1±0.00^{d}	80.1±0.00^{e}	87.7±0.69^{d}	8.3±0.03^{b}
3	78.6±0.10^{a}	83.6±0.00^{b}	91.2±0.16^{b}	5.0±0.48^{d}
4	73.4±0.15^{c}	81.1±0.00^{d}	87.8±0.24^{d}	7.7±0.28^{c}
5	77.5±0.02^{b}	84.0±0.00^{a}	89.2±0.35^{c}	3.7±0.03^{e}
6	72.7±0.18^{e}	79.6±0.00^{f}	86.4±0.30^{e}	4.8±0.07^{d}
7	78.5±0.04^{a}	83.1±0.00^{c}	87.5±0.28^{d}	3.9±0.09^{e}
p (Brown-Forsythe)*	0.73	1.00	0.72	0.41
p (Anova)**	<0.01	<0.01	<0.01	<0.01

T_0: on set temperature; Tp: peak temperature; T_c: endset temperature; ΔH_{gel}: gelatinisation enthalpy change.
Different letters in the same column represent significant difference according to Fisher LSD test ($p<0.05$)
* Probability value obtained by Brown–Forsythe test for homogeneity of variance
** Probability value obtained by Anova one-factor

Table 3. Pasting propertiesof *Carioca* bean starches modified with different treatments

Sample	T_p(°C)	V_p(cP)	V_c (cP)	Breakdown (cP)	Setback (cP)
1	80.5±0.10^{c}	1436.7±1.53^{c}	2134±2.00^{c}	342.7±2.52^{c}	1040.3±2.08^{b}
2	84.9±0.09^{b}	1677.3±1.15^{a}	2195±1.00^{b}	469.0±1.00^{b}	987.3±2.08^{c}
3	-	11.0±0.06^{e}	5.00±0.00^{e}	15.1±0.15^{f}	9.0±0.03^{e}
4	86.1±0.10^{b}	1000.3±2.89^{d}	1489.0±2.64^{d}	113.7±1.53^{d}	603.0±2.64^{d}
5	-	0	0	19.0±0.17^{e}	3.9±0.06^{g}
6	84.7±0.01^{b}	1656.7±0.15^{b}	2224.3±3.05^{a}	628.3±1.53^{a}	1195.3 ±3.21^{a}
7	-	0	0	9.0±0.00^{g}	7.0±0.06^{f}
p (Brown-Forsythe)*	0.47	0.39	0.30	0.19	0.82
P (Anova)**	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01

T_p: pasting temperature; V_p: peak viscosity; V_c: conclusion viscosity.
* Probability value obtained by Brown-Forsythe test for homogeneity of variance.
** Probability value obtained by Anova one-factor.
*** Different letters in the same column represent significant difference according to Fisher LSD test ($p<0.05$).

Table 4. Colour parameters of *Carioca* bean starches modified with different treatments

Treatment	L*	a*	b*
1	91.39±1.09^{a}	0.67±0.1^{e}	4.51±0.11^{g}
2	84.33±1.1^{c}	1.98±0.04^{f}	7.11±0.03^{b}
3	82.08±1.96^{d}	2.55±0.14^{c}	5.43±0.26^{f}
4	85.95±0.69^{c}	2.28±0.02^{d}	7.93±0.07^{a}
5	81.03±0.84^{d}	5.27±0.13^{a}	6.50±0.13^{d}
6	88.21±0.45^{b}	1.87±0.01^{f}	6.81±0.04^{c}
7	81.08±0.69^{d}	4.04±0.06^{b}	5.89±0.15^{e}

p (Brown-Forsythe)**	0.76	0.41	0.53
p (Anova)***	<0.01	<0.01	<0.01

** Probability value obtained by Brown-Forsythe test for homogeneity of variance.
*** Probability value obtained by Anova one-factor.
**** Different letters in the same column represent significant difference according to Fisher LSD test ($p<0.05$).

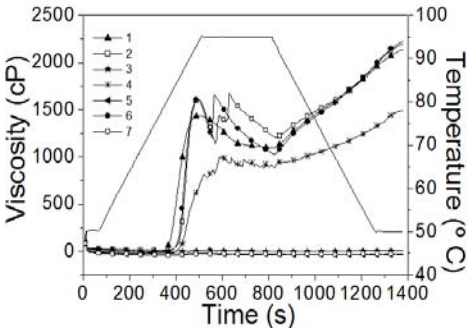

Figure 2. RVA curves of starches of *Carioca* bean modified by acid-alcohol treatment

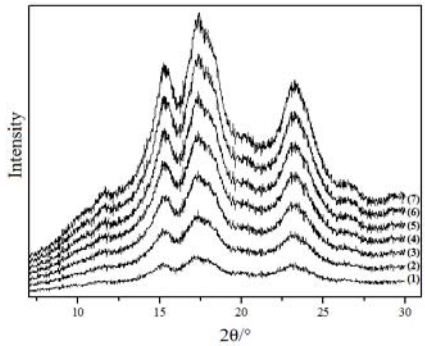

Figure 3. Diffractograms of starches of *Carioca* bean modified by acid-alcohol treatment

The reduction of the viscosities occurs due to degradation of the starch chains, caused by the presence of acid, with consequent molecular weight reduction (Cavallini and Franco, 2010). As the viscosities of the starch pastes that were submitted to acid-alcohol treatment were extremely low, the pasting temperature was not detected (Table 3).

Breakdown is the difference between the viscosity of the swollen starch granules and viscosity when the granules are disrupted (Han and Hamaker, 2001); setback is the difference between the viscosity after the disruption of the swollen granules after cooling and the hot paste viscosity (Shirai et al., 2007). Therefore, as a result of the reduction of V_p and V_c, the breakdown and setback values of the starches treated with alcohol and acid (treatments 3, 5 and 7) were significantly lower than those of the native bean starch. This reduction was also observed by Ferriniet al. (2008), Dutta et *al.*(2011) and Lin *et al.* (2012) for starches from cassava, jackfruit seed and maize, respectively.

3.3. X-Ray Diffraction

The X-ray diffractograms of the native starch and starches submitted to acid-alcohol hydrolysis are shown in Figure 3. The native starch and the modified starches presented diffraction peaks at 15°, 17° and 23°,characteristic of C-type starch (Wang and Ratnayake, 2014).

The native starch showed relative crystallinity of 24%, whereas treated starches showed values around 22.5%, without a significant difference between samples. The crystallinity revealed that the acid-alcohol treatment did not affect the crystalline structure of starches. In a study by Chang et al. (2004) native and modified waxy maize starches also did not present difference between the crystallinities. Luo et al. (2011) treated maize starches with different amylose contents (0%, 23% and 55%) in anhydrous methanol, ethanol, 2-propanol, 1-butanol with 0.36% HCl and concluded that the degradation occurred preferentially in the amorphous regions and the different changes depended on the crystal structure and amylose content of starch. Additionally the authors reported that

the extent of the changes caused by acid treatment in the same alcohol depended not only on the crystal structure of starch but also on its amylose content.

3.4. Colour Parameters

The L*, a* and b* colour parameters indicate the tendency of samples to white or black, green or red and yellow or blue, respectively. In Table 4 it is possible to verify the colorimetric variation among samples of starch from *Carioca* beans modified by acid alcoholic hydrolysis.

According to Table 4 the L* value was higher for native starch and a significant reduction in brightness for the samples modified by acid alcohol hydrolysis was found. As for the parameter a* the modified samples exhibited a greater tendency to red and native starch tended to neutrality between red and green.

The samples 5 and 7 showed higher a* due to treatment with ethanol and HCl and ethanol:methanol and HCl, respectively; ethanol and HCl was the treatment that most influenced the occurrence of this effect. Similarly, evaluating the parameter b*, the modified samples showed a greater tendency to yellow when compared to native starch. Thus, the acid-alcoholic hydrolysis promoted slight changes in the colour of the bean starches.

3.5. Scanning Electron Microscopy

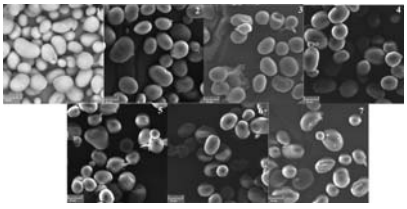

Figure 4. Scanning electron microscopy of native and modified starches of *Carioca* bean (1,000 x)

The native bean starch granules observed by SEM showed smooth surface without visible fissures, oval and spherical shapes according to Fig. 4 (treatment 1) and agreeing with data found in the literature (Ovando-Martinez et al., 2011; Granza et al., 2014; Demiate et al., 2016).Samples that were treated only with alcohol, according to Fig. 4 (treatments 2, 4, 6), showed no significant changes in shape and surface such as fragmentation or swelling, as reported for potato starch by Lin et al.(2005). On the other hand, samples 3, 5 and 7, that were modified with alcohol and acid (Fig. 4; 3, 5 and 7) showed some fissures randomly distributed as well as deformation and degradation in some granules, in line with previously published results for starches from other sources (Han et al., 2001; Lin et al., 2005).

4. Conclusions

The hydrolysis treatment resulted in decreased ΔH_{gel} as showed by DSC analysis and caused significant changes in pasting properties of bean starch due to depolymerisation of starch chains.

The modifications have not changed the X-ray diffraction pattern and relative crystallinity of the studied starches, but modified the colour of starches and the samples treated with ethanol and HCl presented a greater tendency to red, with substantial variation of the a*value.

By using SEM it was observed that the treatment with alcohol alone did not affect the morphology of the granules while the use of acid resulted in a partial deformation and apparent degradation.

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PROXIMATE COMPOSITION AND GELATINIZATION PROPERTIES OF RICE ANALOGUES FROM BREADFRUIT, MUNG BEAN AND CARROT FLOURS AT VARIOUS DOUGH COMPOSITIONS AND DRYING TIME

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Rice analogues.

ABSTRACT

The serious threat of rice supply in many parts of the world and high demand for more nutritious rice have driven the efforts on the production of rice analogues from other food sources. This research was aimed to study the effect of dough composition and drying time on the nutrition and physical characteristics of rice analogues from breadfruit flour with carrot and mung beans flours as nutrient fortificants. To achieve that aim, variations in the composition of breadfruit flour with the addition of carrots and mung beans flours during extrusion and drying time in rice analogues manufacturing were investigated. As quality indicators of the rice analogues, the nutrition contents, which include moisture, ash, protein, lipid, dietary fiber, carbohydrate and amylose contents were analyzed. In addition, the physical properties of the rice analogues which consist of hardness and gelatinization temperature were also determined. Based on those quality criteria, the best rice analogues could be obtained upon the extrusion of flour composite dough comprising breadfruit flour 80% w/w, mung bean flour 10% w/w and carrot flour 10% w/w, and followed by oven drying at 70°C for 7 hours. The consumption of such product is a highly promising especially for people residing in dry rural areas and those with high needs in protein and vitamin A.

1. Introduction

Rice is one of most important staple foods in the world as it sustains two-thirds of the world's population by providing 20% of the world's dietary energy supply. However, rice contains a low content of protein but the higher content of starch. This low protein content has triggered deficiencies of protein and some essential amino acids of people who take it as primary diet (Mishra et al., 2012). As the living standards of people improve from time to time, more attention has been devoted to providing better rice quality. Unfortunately, rice is commonly consumed in the form of grains without milling. Therefore, micronutrient fortification on rice is technically more difficult. Another problem on rice arises from climate change, which has been reported to badly impact the rice production and consequences on food security per se. Peng et al. (2004) reported that rice yields decreased by 10% for every 1°C increase in temperature. The temperature rise is predicted to cause rice yield falls by about 50% in 2100 relative to

the 1990 level in South East Asia, especially Indonesia, Philippines, Thailand and Vietnam. One approach to overcome the aforementioned problems is by preparing rice analogues (Mishra et al., 2012).

Rice analogues are imitation of conventional paddy rice, which has very close similarity in both appearance and nutrition content. Rice analogues could be developed as a new value added product using different types of carbohydrate sources, such as tubers and cereals with added nutrients and functionalities (Herawat et al., 2014). In addition to its main function as a substitute for paddy rice, rice analogue can be designed as a special food for patients of some diseases, such as diabetes, hypertension, and others. The nutritional content of the rice analogues can be enriched by incorporation of various types of foodstuffs through fortification process, which will allow consumers to benefit without experiencing major changes in their dietary habits. Several methods have been proposed to manufacture fortified rice analogues, however hot extrusion and cold extrusion are the most popular processes (Li et al., 2009). Various sources of carbohydrates have been used to manufacture rice analogues, such as soybean flour, corn flour and tapioca starch (Herawat *et al*., 2014), and low amylose rice and seeded banana flour (Borah *et al*., 2015). There have not been any reports on the use of bread fruit flour as a source of carbohydrate in the manufacture of rice analogues.

Breadfruit (*Artocarpus altilis*) is an essential source of carbohydrates,lipid, protein, fiber, vitamins and minerals such as vitamin C and potassium, respectively (Ma et al., 2012). Studies have been carried out in the utilization of breadfruit as a component of weaning diets and found to be a good source of carbohydrate with complementary protein content. Successful substitution of 10% w/w of wheat flour by breadfruit flour in bread making process has been reported by Ma et al. (2012). Based on their investigation on extrusion of breadfruit flour dough, Nochera and Moore (2001) concluded that the product formed is almost equal to the product produced from rice flour.

Carrot (*Daucus carota* L) is one of the most important sources of dietary carotenoids, and China is the world's leading carrot producing country. Carrot is rich in β-carotene, ascorbic acid and tocopherol, and is therefore classified as vitaminized food (Hashimoto and Nagayama 2004). Due to appreciable content of various bioactive compounds present, carrots are considered as a functional food with significant health-promoting properties (Hager and Howard 2006). Carrot intake may enhance the immune system, protect against stroke, high blood pressure, osteoporosis, cataracts arthritis, heart diseases, bronchial asthma and urinary tract infection (Hager and Howard 2006). With regard to those reports, the consumption of carrot and its products have increased steadily in the last few decades.

Mung bean (*Vigna radiate* (L.) Wilczek), also called green gram is an important short season summer-growing legume grown widely throughout the tropics and subtropics (Thomas et al., 2004). It constitutes important cereal-based diets to many people in Asia, particularly in Thailand, India, Pakistan, Indonesia, the Philippines, and China. It is a rich source of protein and amino acid, specifically lysine and thus can supplement cereal-based human diets. Coffmann and Garcia (1977) found mung bean flour to contain higher protein content (28.0%) than the whole seed (26.3%) or bran seed (11.0%). The high protein levels and high lysine/low methionine amino acid profile of mungbean complement the high carbohydrate and low

lysine/high methionine content of cereals to form a much balanced amino acid diet.

This study aimed to manufacture rice analogues from breadfruit flour via hot extrusion cooking process. To enrich the nutrient content in the rice analogues, carrot flour and mung bean flour were incorporated as fortificants. The study also aimed to investigate the effect of the dough composition (flour mixture) and drying time on proximate composition and gelatinization properties of the rice analogues.

2. Materials and methods

2.1. Materials

Breadfruit flour as the raw material for this study was obtained from Gunung Kidul, Yogyakarta-Indonesia, while carrots and mung beans were purchased from local market Peterongan in Semarang-Indonesia. Food grade sodium tripolyphosphate (STPP) and distilled water were purchased from an authorized supplier of cake and bakery materials in Semarang-Indonesia. Other chemicals of analytical grade with purity ≥ 98% w/w were purchased from Sigma-Aldrich Singapore Pte. Ltd. through an authorized chemicals distributor in Semarang-Indonesia.

2.2. Methods

2.2.1. Flour preparation

Mung beans as fortificant were washed, boiled, oven-dried, milled and sieved to obtain mung bean flours. Carrots tubers were washed, shredded, oven-dried, milled and sieved to obtain carrot flour.

2.2.2. Rice analogues preparation

The rice analogues were manufactured according to the method developed by Widara (2012) with slight modification. A set of experiments were conducted at various dough compositions to ensure sufficient content of nutrients in the rice analogues obtained. In addition, various drying times (3 to 8 hours) were investigated to find the best drying time, which results in nutritious and good shape rice analogues. To find the optimum dough composition, breadfruit flour, mung bean flour, and carrot flour were mixed to obtain flour composites according to the composition presented in Table 1. The flour composites with addition of a predetermined amount of sodium tripolyphosphate were then mixed with water (50% w/w) for 5 minutes to create approximately 33% w/w moisture content to facilitate complete gelatinization. The dough obtained was then heated at a temperature of 65-75ºC for 15 minutes to let the starch in the dough being pre-gelatinized. The pre-gelatinized dough was then molded into rice analogues by extrusion machine (Figure 1) at 65-75ºC, and immediately dried in an electric oven at 70ºC for 5 hours.

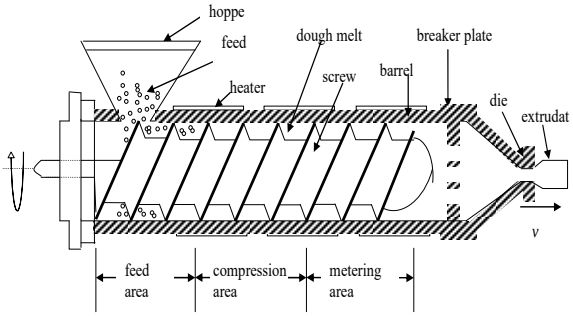

Figure 1. The Schematic Diagram of Extrusion Machine

After being equilibrated to ambient temperature, the rice analogues obtained was then stored in an air tight container for further chemical and physical analyses. To determine the optimum drying time, a variation of drying time (3 hours, 4 hours, 5 hours, 6 hours, 7 hours, and 8 hours) was investigated in the manufacture of rice analogues using optimum dough composition obtained from the preceding step.

2.2.3. Analysis

Proximate composition and physical characteristics of the rice analogues were used as quality parameters in the determination of optimum dough composition and drying time. Proximate composition analysis included moisture (AOAC, 2000), ash (AOAC, 2000), protein (AOAC, 2000), lipids (AOAC, 2000), dietary fiber (Bellucci, 1932), carbohydrates (AOAC, 2000), fatty acid (AOAC, 2000) and amylose (Juliano, 1972). While physical analysis included hardness test as the breaking force that acted on a unit area of the curved surface of the rice analogues under compression in accordance with ASAE Standards (ASAE, 2004) and gelatinization temperature determination by differential scanning calorimeter. A comparison with IR-36 paddy rice was subjected to proximate composition and physical characteristics of the rice analogues obtained. The IR-36 paddy rice was chosen as the representative characters of the rice consumed by the low-middle class family in Indonesia.

2.3.4. Statistical analysis

All of the experiments were performed in triplicates. One way analysis of variance (ANOVA) and Tukey's multiple range tests with a confidence interval of 95% were applied by means of statistical software (SAS v 9.2, The SAS Institute, USA) to report the significant differences between the obtained results.

3. Results and discussions

3.1. Effect of dough compositions

Starch, proteins, lipids, low molecular sugars, and fibers are food components that play an important role in the extrusion cooking processes. The proximate compositions of the rice analogues obtained from various dough compositions are presented in Table 1.

Free moisture contained in the spaces between cells, inter-granular and pores, and weakly bound moisture adsorbed on the surface of macromolecular colloids such as proteins, pectin starches, and cellulose are the main targets of drying process. Table 1 shows that the moisture contents of all of the rice analogues are lower than that of IR-36 paddy rice. The moisture contents of the rice analogue are also significantly different with that of IR-36 paddy rice ($p < 0.05$). This is because the grains of rice analogues are visually more porous than IR-36 paddy rice from which moisture removal is easier and faster during drying process. This observation agrees well with Cheftel (1986), who explained that extrusion cooking causes swelling and rupture of the granules as well as deterioration of the starch crystallinity. In addition, during extrusion of starches, factors such as temperature, moisture content before extrusion, amylose content and lipid content may all lead to structural modifications of starch granules. Increasing both moisture and lipid contents of the dough resulted in higher moisture content of the extrudate. To obtain extrudates with moisture content lower than 7% w/w, the initial moisture content of the dough should not higher than 12% w/w (Hoan et al., 2010). When the moisture content of the dough is higher than 12%, the rice analogues grains obtained by extrusion process are not in good shape. On the other hand, when the moisture content of the dough is below 12%, the extrusion process leads to the production of broken grains.

Table 1. Proximate composition of breadfruit flour, mung bean flour, carrot flour and rice analogues dried in an electric oven at 70°c for 5 hours.

Biomaterials	Moisture (% w/w)	Ash (% w/w)	Carbohydrates (% w/w)	Protein (% w/w)	Dietary Fiber (% w/w)	Lipid (% w/w)
Flours						
Breadfruit (BF)	5.09±0.07	2.50±0.04	87.21±0.21	2.10±0.13	2.80±0.06	0.30±0.02
Mung Beans MBF)	5.99±0.03	3.81±0.06	59.13±0.17	26.36±0.10	3.90±0.05	0.81±0.02
Carrot (CF)	2.44±0.03	5.37±0.04	69.85±0.21	0.68±0.02	20.39±0.15	1.27±0.01
Dough Compositions BF:MBF:CF						
100:0:0	10.53±0.03^{e}	5.69±0.06^{c}	75.92±0.20^{b}	3.84±0.09^{f}	2.90±0.06^{f}	1.11±0.15^{b}
90:5:5	10.41±0.02^{d}	5.37±0.02^{b}	72.45±0.02^{b}	6.72±0.10^{e}	3.83±0.04^{e}	1.20±0.10^{b}
80:10:10	10.36±0.03^{b}	5.35±0.03^{b}	69.87±0.21^{b}	8.63±0.06^{d}	4.76±0.20^{d}	1.03±0.02^{b}
70:15:15	10.33±0.07^{b}	5.36±0.06^{b}	68.17±0.28^{b}	9.07±0.08^{c}	5.85±0.15^{c}	1.20±0.03^{b}
60:20:20	9.45±0.10^{c}	5.39±0.05^{b}	66.58±0.65^{b}	10.89±0.17^{b}	6.61±0.10^{b}	1.18±0.02^{b}
IR-36 paddy rice[a]	12.58±0.10^{f}	0.20±0.01^{d}	78.86±0.26^{b}	7.39±0.01^{g}	0.78±0.05^{g}	0.19±0.02^{c}

[a](Purwaniet al., 2007). The values with the same superscript-small letter in the same column within each food product were not significantly different (P>0.05).The represented values are mean ± SD of three replicates.

Generally, extrusion cooking influences the existence of macromolecules. Smaller molecules may be affected upon by either the extrusion process itself or by the changes in larger molecules, which in turn alter the content other compounds present in the food (Singh et al., 2007). Table 1 shows that the ash contents of the rice analogues do not change significantly with dough compositions and are all higher than the IR-36 paddy rice. In addition, the ash contents of the rice analogue are also significantly different with that of IR-36 paddy rice ($p < 0.05$). The use of sodium tripolyphosphate as dough improver seems to affect the mineral content of the rice analogues. Sodium tripolyphosphate does its function by binding nutrients, which are soluble in saline solution such as protein, vitamins, and minerals. Minerals are heat stable and unlikely to become lost in the steam distillate at the die (Singh et al., 2007).

Generally, mild heat treatment of vegetable proteins improves digestibility due to inactivation of protease inhibitors and other antiphysiological substances and denaturation (Bjorck and Asp, 1983). During extrusion, protein structures may be

Table 2. The hardness and gelatinization temperature of rice analogues at various dough compositions

Biomaterials	Amylose Content (% w/w)	Hardness (MPa)	Onset Gelatinization Temperature (^oC)	Peak Gelatinization Temperature (^oC)	Conclusion Gelatinization Temperature (^oC)
Flours					
Breadfruit (BF)	25.00±0.10	n.a	67.00±0.09		
Mung Beans MBF)	41.43±0.04	n.a	70.10±0.28		
Carrot (CF)	15.64±0.01	n.a	57.70±0.13		
100:0:0	18.24±0.07^d	3.30±0.17^e	71.90±0.10^f	76.36±0.40^e	81.90±0.01^e
90:5:5	15.92±0.02^c	2.90±0.02^d	72.96±0.04^e	77.22±0.17^c	81.96±0.04^e
80:10:10	15.88±0.11^c	1.60±0.05^c	73.61±0.11^d	78.20±0.02^d	81.61±0.11^d
70:15:15	17.48±0.05^b	1.10±0.02^b	74.62±0.03^c	77.50±0.28^c	82.62±0.05^c
60:20:20	17.88±0.12^b	0.90±0.03^b	77.19±0.14^b	80.83±0.09^b	87.19±0.09^b
IR-36 Paddy rice[a]	27.30±0.05^e	6.53±0.12^f	81.96±0.10^g	n.a.	n.a.

[a] (Purwaniet al., 2007). The values with the same superscript-small letter in the same column within each food product were not significantly different (P>0.05).The represented values are mean ± SD of three replicates.

Table 3. Effect of drying time on proximate composition of rice analogues dried in an electric oven at 70°c

Drying Time (hours)	Moisture (% w/w)	Ash (% w/w)	Carbohydrates (% w/w)	Protein (% w/w)	Dietary Fiber (% w/w)	Lipid (% w/w)
3	19.63±0.04^e	6.04±0.07^d	61.12±0.34^e	8.28±0.10^d	4.10±0.11^f	0.83±0.02^d
4	10.37±0.03^d	5.90±0.02^d	69.52±0.61^d	8.60±0.05^c	4.58±0.06^e	1.03±0.04^c
5	10.36±0.09^d	5.35±0.06^c	69.87±0.16^d	8.63±0.01^c	4.76±0.20^d	1.03±0.02^c
6	10.44±0.04^d	5.48±0.05^c	69.54±0.15^d	8.73±0.05^b	4.77±0.13^d	1.04±0.06^c
7	9.30±0.09^c	5.65±0.07^b	69.28±0.32^c	8.77±0.04^b	6.03±0.02^c	0.97±0.02^c
8	7.02±0.03^b	5.74±0.01^b	69.03±0.18^b	8.81±0.01^b	8.45±0.01^b	0.95±0.06^b
IR-36 paddy rice[a]	12.58±0.10^f	0.20±0.01^e	78.86±0.26^f	7.39±0.01^e	0.78±0.05^g	0.19±0.02^e

[a] (Purwani et al., 2007). The values with the same superscript-small letter in the same column within each food product were not significantly different (P>0.05).The represented values are mean ± SD of three replicates.

disrupted and altered under high shear, pressure, and temperature (Perez et al., 2008). As a result, large molecules proteins may become dissociated into smaller subunits. Table 1 indicates that the protein content of the IR-36 paddy rice falls in the range of protein content of all rice analogues. However, the protein contents of the rice analogue are significantly different with that of IR-36 paddy rice ($p < 0.05$). The protein content of mung beans flour is higher than breadfruit and carrot flours. Therefore, rice analogues from dough of flour composite containing 20% w/w mung beans flour showed the highest protein content. In contrast, rice analogues manufactured from dough of breadfruit flour only has the lowest protein content. It can be concluded that the dough composition significantly affects the protein content of rice analogues. The increased protein content also showed no protein denaturation during the extrusion of dough flour composite to manufacture of rice analogues from bread fruit, mung beans and carrot flours.

During processing the nutritional value of lipids might be affected through different mechanisms such as oxidation, polymerization, cis-trans isomerization or hydrogenation (Rokey and Plattner, 1995). Table 1 shows that lipid contents of rice analogues are not significantly affected by dough composition and are higher than IR-36 paddy rice ($p > 0.05$). This fact can be linked to the use of lipid as lubricant in the extrusion process. Lipid reduces the friction between the particles in the mixture and between the surface of the thread and the sleeve with the dough. In addition, extrusion may inactivate lipase and lipoxidase present in foods, resulting in less oxidation of fatty acids during extrusion and storage (Stroucken et al., 1996). The lipid contents of the rice analogue are significantly different with that of IR-36 paddy rice ($p < 0.05$). Fatty acids composition analysis revealed that all of the rice analogues and IR-36 paddy rice contained five essential fatty acids for humans, which are palmitic, stearic, oleic, linoleic, and linolenic. However, rice analogues do not contain myristic, palmitoleic, gadoleic, eicosadienoic and lignoceric acids, which are found in IR-36 paddy rice. This fact supports the hypothesis that oxidation of fatty acids during extrusion is minimal (Stroucken et al., 1996).

Cooking and gelatinization of starch are known to increase the susceptibility to amylase hydrolysis, mainly due to hydration of starch granules and partial solubilization of starch molecules (Bjorck and Asp, 1983). Table 1 shows that carbohydrate contents of the rice analogues were lower than the IR-36 paddy rice. However, the values of carbohydrate content of the rice analogues are not significantly different ($p >0.05$) from the IR-36 rice. The reduction of breadfruit flour composition in the dough flour composite resulted in decreased of carbohydrate content of the rice analogues. Similar phenomenon was also reported by Anuonye *et al*. (2007) for effect of extrusion on amylose content. Due to extrusion, starch is gelatinized and therefore its structure is being degraded and more accessible to enzyme in stomach.

Very few studies have been made on the effect of extrusion cooking or other processes on dietary fiber. Table 1 reports a significant increase ($p < 0.05$) of dietary fiber content with the increase of mung bean and carrot flours in the dough flour composite. High dietary fiber contents of mung bean and carrot flours may be one of the reasons. However, severe extrusion-cooking of flours may also cause an apparent increase in dietary fiber due to the formation of amylase-resistant starch

fractions (Cheftel, 1986). Similar results were reported by Vasanthan et al. (2002) where extrusion cooking increased the total dietary fiber of barley flours. They suggested that the change in dietary fiber profile during extrusion of barley flour may be attributed, primarily, to a shift from insoluble dietary fiber to soluble dietary fiber, and the formation of resistant starch and enzyme-resistant indigestible glucans formed by transglycosidation.

Amylose content affects quality and texture of cooked rice. Rice with low amylose content produces rice, which is sticky, soft shiny, not expand, and still coagulate after cooled. In contrast, rice containing higher amylose content is normally more resistant to hydration and gelatinization (Yadav and Jindal, 2007). Therefore, starch that is high in amylose swells more slowly and exhibits a loss of order within the granule, followed by its destruction. This type of rice also gives more viscous flour dispersion during water cooking, and is firm and fluffy when cooked.

Table 1 shows that the amylose contents of the rice analogues were 31.19% to 41.83% lower than that of IR-36 rice, and these amylose contents are different significantly ($p < 0.05$). The low amylose content in the rice analogues has two possible causes. The first is due to the destruction of amylose during the extrusion process. Amylose degradation occurs due to breakdown of amylose chains to such an extent due to the pressure inside the extruder that helices could not be formed (Bhatnagar and Hanna, 1994). The second is the formation of amylose–lipid complex evident during extrusion. The amount of decrease was directly proportional to degree of complexing, which is dependent upon both starch and lipid type present in a food (Bhatnagar and Hanna, 1994). Monoglycerides and free fatty acids are more likely to form complexes than triglycerides, when added to high-amylose starch (Bhatnagar and Hanna, 1994). Addition of mung bean and carrot flours caused an increase in lipid content, which triggered more lipid-amylose interaction at elevated temperatures. This phenomenon leads to decrease the amylose content of the rice analogues.

Texture is an important factor in consumer acceptance of food products. The principal effect of the thermo mechanical treatment resulting from extrusion is to rupture the granular structure of starch. In addition, the complex formation between amylose and lipids during extrusion cooking plays important role in the development of structure, texture and other functional properties of the extrudate (de Pilli et al., 2011). The textural property (hardness) of the samples was determined by measuring the force required to break the rice analogues grain as reported in Table 2. The hardness values of the rice analogues obtained in this work are all far lower than the hardness of paddy rice IR-36, and therefore found to be significantly different ($p < 0.05$). Low strain and hardness values indicate weaker and more brittle particles that have a higher porosity.

In this study, the main source of carbohydrates is breadfruit flour, while mung beans flour and carrot flours are richer in lipid contents. The higher carbohydrate content in the dough should produce rice analogues with higher hardness value. The lower hardness value for rice analogues from dough flour composite with higher lipid content is in good agreement with Ruy et al. (1994) who demonstrated that emulsifier addition provided wheat flour extrudate with more uniform, larger cell (pore) sizes and therefore lower hardness and bulk density. The change in these properties is likely caused by an alteration in the ratio of free amylose to amylopectin by

the formation of amylose-lipid complexes. Ruy et al. (1994) also found that the breaking or shearing strength of the extrudate decreased as a result of lipid addition to the dough.

Table 2 shows that the rice analogues and IR-36 paddy rice have high gelatinization temperatures ranging from 71.90ºC to 81.96ºC. No significant difference between gelatinization temperature of the rice analogues and IR-36 paddy rice ($p > 0.05$). It is also clearly observed in Table 2 that rice analogues with high amylose content also have higher gelatinization temperature. Rice with higher amylose content may absorb more water during cooking resulting higher gelatinization temperatures so that the cooking time is longer than the rice with a low gelatinization temperature. Our investigation gave similar results as a study on maize starches with varying amylose content (0 to 66.1% w/w), which indicated that the gelatinization temperatures increased with increasing amounts of amylose (Park et al., 2007).

However, rice analogues manufactured from breadfruit flour without incorporation of mung bean flour and carrot flour has lower gelatinization temperature (71.9ºC) although it has higher amylose content (18.24% w/w) than any other rice analogues. This finding is contradictive to the positive correlation of amylose content to onset, peak and conclusion gelatinization temperatures reported by Park et al. (2007). Sasaki et al. (2000) explained that starch with higher amylose content contains more amorphous and less crystalline regions, leading to a lower gelatinization temperature and enthalpy. In addition, other factors such as starch structure and nutritional composition could have influenced the gelatinization temperature of rice analogues.

In comparison to paddy rice IR-36 nutrition quality, all of the rice analogues obtained in this work meets the standard for moisture, ash, lipid and dietary fiber contents. However, none of them meets the minimum carbohydrate content. If the rice analogues are being consumed by people and patients demanding to healthy diet, then the carbohydrate contents of the rice analogues are still acceptable. The protein content of paddy rice IR -36 is below protein content of rice analogues manufactured from dough flour composite comprising 80% w/w breadfruit flour, 10% w/w mung bean flour and 10% w/w carrot flour. The use of more mung bean flour and carrot flour definitely increases the protein content of the rice analogues, but it will be compensated by higher price. The texture of the rice analogues obtained from 80% w/w breadfruit flour as represented by hardness is still acceptable, whereas addition of more mung bean flour and carrot flour results in brittle rice analogues. It can be concluded that dough composed of breadfruit flour 80% w/w, mung bean flour 10% w/w and carrot flour 10% w/w is the best formulation over the other dough compositions. This dough composition is further used for the study of the effect of drying time.

3.1. Effect of dough compositions

It is already well known that drying process may affect the physicochemical properties of materials, as well as the functional properties of their active ingredients because of the distinction of temperature, drying time and other conditions. Rice analogues in this study were dried in an electric oven at 70°C with a variation of the drying time. Drying at temperatures higher than 70°C was not preferred as the gelatinization temperatures of the rice analogues were ranged from 71.90ºC to 77.19ºC. The proximate composition of the rice analogues can be seen in Table 3.

Table 3 shows that the moisture contents of all of the rice analogues are lower than those of IR-36 paddy rice. The moisture contents of the rice analogues are also significantly different with that of IR-36 paddy rice ($p < 0.05$). The values decreased with the increase of drying time. During oven drying process, moisture is mainly removed from the food in the form of water vapor. Longer drying time promotes higher intensity of the contact between the food material and the hot air. This condition leads to better removal of moisture from foodstuffs. Most of moisture in the foodstuff is contained in the form of free moisture, which resides in the spaces between cells, inter-granular and pores; therefore it is easily removed by drying process. While the rest of the moisture is contained in the foodstuff either being weakly bound on the surface of macromolecular colloids such as proteins, pectin starches, and cellulose, and/or strongly bound moisture as hydrate. The strongly bond moisture is relatively difficult to be removed or evaporated.

In practice, a moisture content of 10% w/w is generally specified for flours and other related products to increase storage stability. It should be pointed out that when these food products are allowed to equilibrate for periods of more than one week at 60% relative humidity and at room temperature, their moisture content might increase (Adegunwa et al., 2011). For that reason, drying of freshly extruded dough at 70^{o}C for 7 hours is the best drying method in this work as it may achieve 9.3% w/w moisture content (< 10% w/w).

The ash content reflects the total amount of minerals present within a food. Table 3 shows that the ash content of the rice analogues does not change significantly with drying time. Long drying time does not affect the ash content because minerals contained in food are heat stable (Singh et al., 2007). Minerals also have a low volatility compared to other food components, which makes them unlikely to become lost during drying process. However, the ash contents of the rice analogues are higher than that of IR-36 paddy rice and all are also significantly different with that of IR-36 paddy rice ($p < 0.05$).. This is due to the mineral content of the IR-36 paddy rice is affected by pre-harvest conditions and varieties, while the mineral content in rice analogues affected by raw materials that contain different type and level of minerals. The increase in mineral content in this study could be as a result of moisture removal, which leads to increase the concentration of other nutrients.

In Table 3, protein content of rice analogues is higher than the IR-36 paddy rice. The protein contents of the rice analogues are also significantly different with that of IR-36 paddy rice ($p < 0.05$). Table 3 also shows an increase in protein content and proves that no protein is denatured. Hot air drying could significantly reduce the drying time and improve the crude protein content of the dried products. High temperatures can reduce enzyme activity, preventing protein from enzymatic decomposition (Duan et al., 2014).

As presented in Table 3, lipid content increased with drying time from 3 hours to 6 hours. However, lipid content started to decrease as drying time exceeds 6 hours. The lipid contents of the rice analogues are different significantly with that of IR-36 paddy rice ($p < 0.05$).The lower lipid content observed during long period of drying is associated to oxidation of lipid. The lipid oxidation is known to increase as influenced by many factors such as heat, light and radiation (Savage et al., 2002). The low fat content is desirable because it will enhance the storage life of the flour due to the lower chance of rancid flavor development. Lipid content in all of the rice analogues is still higher than that of IR-36

paddy rice. The use of lipid in the extrusion process is likely the cause of this phenomenon. In the extrusion process, the lipid acts as a lubricant because it is able to reduce the friction between the particles in the mixture and between the surface of the thread and the sleeve with molten mixture.

Table 3 shows no significant increase of carbohydrate content of the rice analogues with the increase of drying time ($p > 0.05$). However, the carbohydrate contents of the rice analogues are significantly different with that of IR-36 paddy rice ($p < 0.05$). The reduction of the moisture content will result in the concentration of compounds contained in such as carbohydrates, proteins, and minerals into higher. However, after 6 hours drying time, the carbohydrate content decreased gradually. Such decrease in carbohydrate content may be attributed by degradation of polysaccharides and Maillard reaction, which results in complex changes in food due to the reaction between carbohydrate and protein (Wiriya et al., 2009).

The dietary fiber content is significantly affected by drying time. Table 3 presents significant increase of dietary fiber content with the increase of drying time ($p < 0.05$). in addition, the dietary fiber contents of the rice analogues are also significantly different with that of IR-36 paddy rice ($p < 0.05$). The increase in dietary fiber content in this study could be as a result of moisture removal, which leads to increase the concentration of other nutrients, such as dietary fiber and ash.

Economic consideration in term of energy used and technical consideration in term of moisture content were counted for the determination of optimum drying time. Longer drying process will consume more energy and higher operation cost. A moisture content of 10% is generally specified for flours and other related products to increase storage stability. Therefore, drying of freshly extruded flour composite dough at 70ºC for 7 hours is the best drying method in this work as it may achieve 9.3% w/w moisture content (< 10% w/w) and lower lipid content (0.97% w/w).

4. Conclusions

Nutritious rice analogues have been successfully made from breadfruit flour, mung bean flour and carrot flour composites dough through extrusion cooking process. The fortification process has also been proven to be applicable in the process of rice analogues manufacturing. The dough composition and drying time were found to significantly influence the nutrition and gelatinization properties of rice analogues. Based on the results of this study, the best rice analogue was obtained upon extrusion of flour composite dough consisting of 80% w/w breadfruit flour, 10% w/w mung bean flour and 10% w/w carrots flour and drying in an electric oven at 70°C for 7 hours.

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RHEOLOGICAL AND FUNCTIONAL PROPERTIES OF WHEAT AND GREEN GRAM COMPOSITE FLOURS

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ABSTRACT
In the present study composite flour blends of wheat (WF) and green gram flour (GGF) were prepared to investigate their proximate, functional, rheological and antioxidant properties. The composite flours with varied mass ratios (WF: GGF) were prepared in six variants, namely T1 (100:0), T2 (80:20), T3 (60:40), T4 (40:60), T5 (20:80) and T6 (0:100). Results showed that the level of GGF in the composite flour contributed to total flour protein, while carbohydrate, moisture, and fiber were found at high levels in WF enriched flour blends. Other properties such as ash, crude fat, and energy were similar across the blends. Furthermore, minerals (K, Ca, Mg, Fe, and Zn) in the composite flours were observed at high levels in the GGF enriched flours (T4-T6). The major functional properties (pH, aw, water and fat absorption capacities, foam capacity and stability, gelatinization temperature, least gelation capacity, swelling capacity and bulk density) were enhanced by a high proportion of GGF in the blend. On the other hand, the rheological performance of the blend gradually degraded with the GGF content. In addition, GGF improved the antioxidant properties (radical scavenging ability and metal chelating activity) of the flour blend, whereas WF only had minute antioxidant activity. Overall, the addition of GGF in the flour mixture with WF tended to provide potential health benefits and improve flour functional properties.

1. Introduction

Flour is a major ingredient in the majority of ready to eat snack foods, especially in bakery products. Nutritious flour can be made from a variety of pulses, legumes, nuts, root, and tubers. Bakery industries around the world use wheat flour as the predominant component in bakery products because of its viscoelastic properties when mixed with water that makes it uniquely fit for many food applications; in the bakery industries, it dominates over other cereal flours. Wheat gluten protein is mainly responsible for the special viscoelastic properties and strengthens the dough (Shewry et al., 2000; Song and Zheng, 2007). However, the wheat dough is considered nutritionally poor. Wheat flour contains low levels of protein and essential amino acids, such as lysine, tryptophan or methionine (Yadav et al.,

2012). Additionally, the consumption of gluten can lead to celiac disease (CD), which is an autoimmune disorder from damage to the small intestine caused by gluten intake. Therefore, non-gluten and composite flours have been widely studied, to restrict the consumption of gluten and to avoid unpleasant disease symptoms in the consumers (Bourekoua et al., 2016).

Composite flour is a mixture of various flours usually acquired from different sources, such as roots, tubers, cereals, and legumes, and may or may not contain also wheat. These have been widely studied in recent times and are applied in the bakery industries. Additionally, the composite flours can have nutritional benefits than the individual flours lack. Although composite flours can be beneficial, the dough's rheological properties play a vital role in the final quality of bakery products. These properties can be affected or "optimized" by adjusting the composition of the flour blend (Oladunmoye et al., 2010). Rapid Visco Analyzer (RVA) is primarily used to analyze the rheological behavior of flour, as it relates to food applications. The suitability of an individual or composite flour for producing a cake, cookie, bread or snack products can be evaluated using the RVA (Itagi and Singh, 2012). Thailand is produced only small amounts of wheat flour, so the demand for bakery products is met by imports from nearby countries in Asia, mainly India. Therefore, composite flours provide opportunities to increase the use of domestic agricultural crops in flours for bakery products. Presently, rice flour and potato flour are commonly blended into the bakery products found in Thailand. Though there are plenty of healthy legumes produced throughout the country, their use in bakery products is very rare.

Among such legumes, green gram or mung bean is a staple legume produced widely in Asia. Flour made from green gram is starchy and a non-gluten fine-grained flour mostly used in South Asia, and in South-East Asia, it is used as a whole grain and/or starchy flour to produce local sweet desserts, such as Luuk Chuup, Med Kanun, and Tua Pap in Thailand. Green gram flour is, however, rarely used in bakery products in South-East Asia. Several studies have proven that green gram provides both good functional properties and human health benefits. A few studies have shown that the dough made from composite flours containing green gram can have excellent functional and rheological properties for bakery products (Chandra and Samsher, 2013; Chandra et al., 2015). However, most such scientific information concerns multi-flour composites with only a tiny portion of green gram. Therefore, the present study focused on the binary blends of wheat and green gram flours, over the whole range of mixture proportions, examining the rheological and functional properties that predict suitability for bakery products.

2. Materials and methods

2.1. Materials and flour blend preparation

The whole wheat grains and green gram legumes were purchased in a single batch from a local market in Muang, Surat Thani, and Thailand. They were sorted, cleaned (destoned) and stored separately in airtight containers, and kept in a dry and cool storage prior to use. The preparation of flour blends started by grinding the materials separately in an electric grain mill, and sieving (44 mesh) them. After that, components were weighed for mixing in desired proportions to the blends T1 (100:0), T2 (80:20), T3 (60:40), T4 (40:60), T5 (20:80) and T6 (0:100). The composite flours were checked with the following determinations.

2.2. Determinations

2.2.1. Proximate composition

The proximate analysis of protein, crude fat, moisture, ash, fiber, and gluten index in the composite flours was based on standard methods (AOAC, 2000). The results are reported as mass percentages (%). The carbohydrate contents of the flours were estimated by subtraction. Gross energy content (kcal) of each flour was measured using a ballistic bomb calorimeter. Mineral contents

(K, Ca, Mg, Fe and Zn) of the composite flour was measured using an inductively coupled plasma optical emission spectrophotometer (ICP-OES, Vista Pro, Australia).

2.2.2. Functional properties

Water activity (a_w) of the samples was measured using a water activity analyzer (Aqua lab, USA). pH of the composite flours was measured using a digital handheld pH meter (Clean pH30, 30 Series Tester, China). The viscosity of the flour was measured using the Brabender amylograph. Water (WAC) and fat absorption capacities (FAC) of the composite flours were measured following the method of Yadav et al. (2012). Swelling capacity (SC) was measured by the method of Okaka and Potter (1979). Bulk density (BD) was measured based on the method of Wang and Kinsella (1976). Foaming capacity (FC) and foam stability (FS) were measured based on the method of Narayana and Narasinga Rao (1982). Least gelation concentration (LGC) of the flours was determined based on the method of Coffmann and Garcia (1977). The emulsion capacity (EMC) and stability (EMS) of the composite flours were measured using the method of Yasumatsu et al. (1972).

2.2.3. Rheological properties

The rheological properties of composite flours were evaluated by the method of Julianti et al. (2015) using Rapid Visco Analyzer (RVA, Newport Scientific, Model RVA-4, Australia). A suspension of 3 g flour in 25 g of distilled water underwent controlled heating and cooling under constant shear, with the flour held at 50ºC for 1 min, heated from 50 to 95ºC at 6ºC/min and held at 95ºC for 5 min. During this measurement process, the following data were recorded: pasting temperature (P_{temp}), peak viscosity (PV), hot paste viscosity (HPV), breakdown viscosity (BPV), setback viscosity (SBV) and stability ratio (SR).

2.2.4. Extraction and antioxidant properties of extracts

A flour sample (10g) was mixed with 50 ml of acidified methanol solvent in an amber colored bottle under nitrogen. The sample mixture was mixed thoroughly and subjected to extraction. The extraction was continued in a temperature controlled water bath with an electrical shaker for 8 hr at 30 °C. Then, the extract was centrifuged (50 ml tube size at fixed rotor angle of 40°) at 7800 x g for 15 min, and the supernatant was collected and stored in dark, in a sealed container at -4°C, until use in further analysis.

The total chlorophyll content (TCC) in the composite flours was measured according to AOAC (2000). The results are expressed as mg chlorophyll/g. Total phenolic content (TPC) of the composite flour was determined according to the method of Singleton et al. (1999). The results are expressed as mg equivalents of Gallic acid/g (mg Eq GA/g). Total flavonoid content (TFC) in the samples was determined based on the method of Zhishen et al. (1999). The results are expressed as mg equivalents of Catechin/g (mg Eq CE/g). The capacities of the composite flours to scavenge DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals (DPPH-RSC) were measured by the method of Sánchez-Moreno et al. (1998). The results are expressed as percentages. The ferric reducing power (FRAP) of each flour was measured according to the method of Benzie and Strain (1996). The results are expressed as percentages. The metal chelating activities (MCA) of the flours were measured by the method in Aktumsek et al. (2013). The results are expressed as mg equivalents of EDTA/g (mg EDTA/g).

2.2.5. Statistical analysis

Data from the completely randomized experimental design are expressed as Mean ± SD (n=6). These data were subjected to one-way analysis of variance (ANOVA) followed by testing for Least Significant Differences (LSD) at 95% confidence level. The threshold $P \leq 0.05$ was required for statistical significance.

3. Results and discussions

3.1. Proximate composition

The proximate compositions of the WF and GGF blends are shown in Table 1. The protein contents ranged from 10.1 to 21.51%, with significant differences between blends. The pure component T6 (100%, GGF) flour had the highest protein content, while at the other extreme T1 (100%, WF) flour had the lowest. Carbohydrate content in the composite flour ranged from 8.12 to 10.12%, with T1 having the most (10.12%) carbohydrates while adding GGF tended to decrease the carbohydrate content. The moisture content of the blends had a similar trend as their carbohydrate content, with GGF decreasing the total moisture level. The moisture content ranged from 8.6% to 10.10%, with the highest level observed in T1 and the lowest in T6. The ash contents of the blends had significant differences, ranging from 0.97% to 3.12%. The highest level of ash was in T6 and the lowest in T1: GGF contributed to the ash content. The gluten content mostly came from the WF component of the blend, so GGF content decreased the gluten level as expected. The composite flours T4, T5, and T6 are suitable for the bakery products with low gluten profile. On the other hand, the crude fat (0.970-0.977%), total fiber (0.27-0.40%) and energy (359.22-395.25 kcal) in the flour blends did not vary significantly. The fiber content decreased with GGF content. The micronutrients or mineral in terms of metals (K, Ca, Mg, Fe, and Zn) were significantly affected by the blend ratio (Table 1). The data show that GGF enriched composite flours contained elevated levels of minerals relative to the WF; in particular, K, Ca and Mg, but also Fe and Zn.

3.2. Functional properties

The functional properties of WF and GGF blends are shown in Table 2. Overall, flour pH was found to be slightly acidic (6.15; T1) and increasing the percentage of GGF brought it towards neutral (6.71; T6). However, the pH did not significantly differ between the blends ($P>0.05$). Adeleke and Odedeji (2010) reported that the shelf life of flour could be prolonged by acidic pH. Water activity (a_w) decreased slightly from 0.639 to 0.598. The highest a_w was found in T1, and the lowest in T6: increasing GGF content decreased the water activity. However, the a_w variations were not significant ($P>0.05$). Water absorption capacity (WAC) and fat absorption capacity (FAC) showed that GGF had a stronger affinity to water while WF had a stronger affinity to fat. Itagi and Singh (2012) reported that WAC of composite flours mainly depends on the content of polar amino groups in proteins and polysaccharides. The changes in affinities to water and fat were significant ($P\leq0.05$). WAC ranged from 84 (T1) to 97% (T6) and, whereas FAC ranged from 101.72 (T6) to 124.22% (T1). Chandra and Samsher (2013) reported similar findings, in that WF absorbed more fat than GGF or other flours in that study. Emulsion capacity (EMC) and emulsion stability (EMS) exposed the better emulsion properties of WF relative to GGF. EMC ranged from 39.63 (T6) to 44.87% (T1) and, on the other hand, EMS ranged from 36.46 (T6) to 39.99% (T1). The differences in EMC and EMS were significant between T1 and the rest (T2 to T6). However, the EMC and EMS did not significantly vary within the latter group of cases. Emulsions play a crucial role in bakery products, in which proteins interact with fats, and this improves the quality and stability of the products (Sathe and Salunkhe, 1981). Although GGF had higher protein content than WF (Table 1), the latter had stronger emulsion properties. Kaushal et al. (2012) reported that emulsion properties are mainly influenced by protein solubility in the flours.

Table 1. Proximate analysis of the composite flours

Proximate analysis		Flour Composite (% WF: GGF)					
		T1	**T2**	**T3**	**T4**	**T5**	**T6**
		100 WF	**80 WF: 20 GGF**	**60 WF: 40 GGF**	**40 WF: 60 GGF**	**20 WF: 80 GGF**	**100 GGF**
Macro Nutrients	Protein (%)	10.10 ± 0.38^{f}	12.44 ± 0.29^{e}	15.06 ± 0.02^{d}	17.20 ± 0.01^{c}	19.40 ± 0.03^{b}	21.51 ± 0.28^{a}
	Crude fat (%)	0.970 ± 0.01^{a}	0.977 ± 0.00^{a}	0.977 ± 0.00^{a}	0.974 ± 0.00^{a}	0.973 ± 0.00^{a}	0.970 ± 0.00^{a}
	Carbohydrate (%)	10.12 ± 0.02^{a}	9.76 ± 0.02^{b}	9.44 ± 0.04^{b}	9.10 ± 0.02^{b}	8.70 ± 0.00^{c}	8.40 ± 0.03^{c}
	Moisture (%)	10.10 ± 0.03^{a}	9.70 ± 0.05^{b}	9.60 ± 0.09^{b}	9.06 ± 0.02^{b}	8.90 ± 0.11^{bc}	8.60 ± 0.13^{c}
	Ash (%)	0.57 ± 0.04^{d}	1.01 ± 0.05^{c}	1.5 ± 0.05^{c}	2.10 ± 0.09^{b}	2.50 ± 0.05^{b}	3.12 ± 0.04^{a}
	Fiber (%)	0.40 ± 0.05^{a}	0.31 ± 0.01^{b}	0.29 ± 0.33^{b}	0.28 ± 0.00^{b}	0.27 ± 0.00^{b}	0.27 ± 0.01^{b}
	Gluten Index (%)	89 ± 0.26^{a}	73 ± 1.04^{b}	51 ± 1.88^{c}	36 ± 0.61^{d}	17 ± 0.78^{e}	0 ± 0.00^{f}
	Energy (kcal)	359.22 ± 0.01^{a}	359.22 ± 0.13^{a}	359.23 ± 0.53^{a}	359.23 ± 0.13^{a}	359.24 ± 0.52^{a}	359.25 ± 0.48^{a}
Micro Nutrients	K (%)	0.18 ± 0.00^{e}	0.352 ± 0.01^{d}	0.524 ± 0.05^{c}	0.696 ± 0.00^{c}	0.868 ± 0.08^{b}	1.04 ± 0.00^{a}
	Ca (%)	0.17 ± 0.00^{a}	0.186 ± 0.00^{a}	0.202 ± 0.00^{a}	0.218 ± 0.00^{a}	0.234 ± 0.05^{a}	0.25 ± 0.00^{a}
	Mg (%)	0.06 ± 0.00^{c}	0.078 ± 0.02^{c}	0.106 ± 0.01^{b}	0.124 ± 0.00^{a}	0.138 ± 0.04^{a}	0.16 ± 0.01^{a}
	Fe (mg/kg)	26.79 ± 0.10^{f}	35.58 ± 0.50^{e}	44.38 ± 0.00^{d}	53.18 ± 0.12^{c}	61.98 ± 0.88^{b}	70.78 ± 0.88^{a}
	Zn (mg/kg)	12.72 ± 0.01^{c}	14.67 ± 0.01^{bc}	16.56 ± 0.70^{b}	18.48 ± 0.05^{ab}	20.4 ± 0.54^{ab}	22.33 ± 0.40^{a}

Note: The values are shown as mean ± standard deviation (n=6). Different superscripts indicate statistically significant differences within one row ($P \leq 0.05$).

Table 2. Functional properties of the composite flours

Functional Properties	Flour Composite (% WF: GGF)					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
	100 WF	**80 WF: 20 GGF**	**60 WF: 40 GGF**	**40 WF: 60 GGF**	**20 WF: 80 GGF**	**100 GGF**
pH	6.15±0.05^{a}	6.46±0.02^{a}	6.56±0.02^{a}	6.66±0.04^{a}	6.71±0.08^{a}	6.71±0.03^{a}
a_w	0.639±0.00^{a}	0.587±0.01^{a}	0.595±0.01^{a}	0.602±0.00^{a}	0.619±0.00^{a}	0.598±0.00^{a}
WAC (%)	84±1.73^{f}	86.6±1.11^{e}	89.2±3.66^{d}	91.8±3.08^{c}	94.4±5.13^{b}	97±1.14^{a}
FAC (%)	124.22±1.21^{a}	119.4±1.14^{b}	117.8±0.81^{c}	110.2±1.05^{d}	105.6±1.40^{e}	101.72±1.77^{f}
EMC (%)	44.87±1.54^{a}	43.57±0.84^{b}	42.00±0.88^{b}	41.88±0.51^{b}	40.10±0.14^{bc}	39.63±0.22^{bc}
EMS (%)	39.99±0.67^{a}	39.10±1.44^{a}	38.55±0.80^{b}	37.48±2.10^{b}	36.88±3.80^{bc}	36.46±1.30^{bc}
FS (%)	10.87±0.36^{f}	15.66±0.61^{e}	21±0.68^{d}	25.91±0.80^{c}	30.51±0.47^{b}	35.11±0.71^{a}
FC (%)	13±0.20^{f}	17.8±0.42^{e}	23.7±0.20^{d}	27.5±0.46^{c}	33.9±0.31^{b}	36.57±0.21^{a}
GT (ºC)	61.27±2.27^{a}	59.84±1.24^{b}	60.47±0.85^{b}	61.09±1.10^{a}	61.72±2.00^{a}	62.27±1.84^{a}
LGC (%)	10±0.78^{f}	11.2±0.51^{e}	12.4±0.91^{d}	13.8±0.13^{c}	15.2±0.41^{b}	16±0.12^{a}
SC (%)	18.60±0.85^{ab}	18.4±0.71^{ab}	19.16±0.22^{a}	19.44±0.57^{a}	19.72±1.12^{a}	20±0.48^{a}
BD (g/cm^3)	36.32±0.81^{c}	37.42±0.53^{b}	37.45±0.62^{b}	36.84±0.74^{c}	36.59±0.59^{c}	38.08±0.56^{a}

Note: The values are shown as mean ± standard deviation (n=6). Different superscripts indicate statistically significant differences within one row (P≤0.05).

Table 3. Rheological properties of the composite flours

Rheological properties	Flour Composite (% WF: GGF)					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
	100 WF	**80 WF: 20 GGF**	**60 WF: 40 GGF**	**40 WF: 60 GGF**	**20 WF: 80 GGF**	**100 GGF**
P_{temp}	83.6 ± 0.02^{a}	82.8 ± 2.10^{a}	80.9 ± 0.80^{b}	75.7 ± 0.40^{c}	75.1 ± 1.20^{c}	70.2 ± 0.50^{d}
PV (Cp)	1040 ± 50.50^{a}	934 ± 71.00^{b}	826 ± 18.20^{c}	722 ± 8.20^{d}	616 ± 12.70^{e}	510 ± 52.12^{f}
HPV (Cp)	793 ± 10.00^{a}	755 ± 8.50^{a}	717 ± 10.20^{a}	680 ± 13.00^{ab}	642.6 ± 5.50^{b}	605 ± 11.70^{c}
BDV (Cp)	295 ± 10.00^{a}	270 ± 11.50^{a}	268 ± 12.00^{a}	244 ± 10.00^{a}	240 ± 9.00^{a}	236 ± 10.00^{a}
SBV (Cp)	950 ± 12.00^{a}	840 ± 15.00^{b}	730 ± 11.00^{c}	620 ± 14.00^{d}	580 ± 10.00^{d}	450 ± 7.00^{e}
SR	1794 ± 12.00^{a}	1562 ± 11.00^{b}	1257 ± 12.00^{c}	1109 ± 11.00^{d}	1050 ± 14.00^{d}	879 ± 5.00^{e}

Note: The values are shown as mean ± standard deviation (n=6). Different superscripts indicate statistically significant differences within one row (P≤0.05).

Table 4. Antioxidant capacities of the composite flours

Antioxidant capacities	Flour Composite (% WF: GGF)					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
	100 WF	**80 WF: 20 GGF**	**60 WF: 40 GGF**	**40 WF: 60 GGF**	**20 WF: 80 GGF**	**100 GGF**
TCC (mg Chl/g)	0.160 ± 0.05^{e}	1.654 ± 0.03^{d}	2.720 ± 0.10^{c}	3.617 ± 0.01^{c}	4.293 ± 0.04^{b}	6.450 ± 0.32^{a}
TPC (mg GAE/g)	0.019 ± 0.00^{b}	0.020 ± 0.00^{ab}	0.020 ± 0.00^{ab}	0.021 ± 0.00^{a}	0.022 ± 0.00^{a}	0.024 ± 0.00^{a}
TFC (mg CAE/g)	0.023 ± 0.00^{a}	0.024 ± 0.00^{a}	0.024 ± 0.00^{a}	0.024 ± 0.00^{a}	0.024 ± 0.00^{a}	0.025 ± 0.00^{a}
DPPH-RSC (%)	44.58 ± 0.46^{f}	56.64 ± 0.05^{e}	62.39 ± 0.05^{d}	66.32 ± 0.05^{c}	71.10 ± 0.11^{b}	88.07 ± 0.60^{a}
FRAP (%)	54.69 ± 0.27^{e}	55.90 ± 0.17^{d}	56.71 ± 0.04^{d}	58.51 ± 1.22^{c}	60.59 ± 0.17^{b}	62.35 ± 0.53^{a}
MCA (mg EDTA/g)	2.20 ± 0.10^{e}	3.70 ± 0.20^{d}	4.20 ± 0.05^{d}	5.50 ± 0.01^{c}	6.90 ± 0.02^{b}	7.80 ± 0.30^{a}

Note: The values are shown as mean ± standard deviation (n=6).
Different superscripts indicate statistically significant differences within one row ($P \leq 0.05$).

Foam stability (FS) and foam capacity (FC) values ranged from 10.87 to 35.11% and from 13 to 36.57%. T1 flour had the least FS and FC among the flours in this study. The GGF increased FS and FC of the flour blends significantly ($P \leq 0.05$). Chandra and Samsher (2013) reported that the foaming properties of GGF were better than those of other flours they tested. Acuña (2012) found that the protein content in legume plants always induces high foaming abilities. Gelatinization temperature (GT) slightly increased with the GGF content, ranging from 59.84 to 62.27 ºC. The lowest GT was observed in T1 and the highest in T6. However, the differences in GT were not significant between the flours. Generally, GT of flour is increased by high contents of proteins and carbohydrates that might promote physical competition for water between protein gelation and carbohydrate gelatinization when the flour is heated. Least gelation capacity (LGC) was highest in the GGF containing flour blends, ranging from 10 to 16%. T1 had the least LGC, followed by T2 to T6. The differences in LGC between the composite flours were minimal but still statistically significant. High level of protein in the flour possibly increased the LGC. It can be seen that the T6 flour contained the most proteins. In addition, the gelation of flour is primarily guided by the balance between hydrophobic and repulsive electrostatic interactions by the proteins. The highest value of swelling capacity (SC) was observed for the T6 flour (GGF), whereas the flours rich in WF (T1, T2 & T3) had low values. However, the actual flour bends between these extremes did not differ much mutually ($P > 0.05$). SC ranged from 18.4 to 20%. The bulk density (BD) of T6 was high, while that of T1 was comparatively low. BD ranged from 36.32 to 38.08 g/cm^3, showing no significant effects from mixture proportions ($P \geq 0.05$). GGF rich composite flours had slightly increased BD values relative to other cases. The BD of flour is affected by density and particle size of the flour. High levels of BD enable further applications in food preparation (Akpata and Akubor, 1999; Karuna et al., 1996).

3.3. Rheological properties

Rheological properties of the composite flours are shown in Table 3. The results demonstrate that WF had higher values of the rheological characteristics (PV, HPV, BDV, SBV, SR, and P_{temp}) than GGF. Julianti et al. (2015) reported that normally composite flours show poorer rheological properties than wheat flour, represented by the case T1 in the current study. Increasing the GGF level in the composite flour diminished the rheological properties in a consistent gradual manner. PV is an indicator of starch water binding capacity and of the granules' peak swelling during cooking (Itagi and Singh, 2012; Julianti et al., 2015). PV of the composite flours ranged from 510 to 1040 Cp, with the highest value for the WF flour (T1) and for the GGF flour (T6). The size of starch granules plays a major role in determining the physicochemical properties, especially swelling power, paste clarity and water binding capacity (Singh et al., 2003). Additionally, the starch content in WF is higher than in GGF (we used flours, not purified starches); this could also decrease the PV of GGF containing composite flours. HPV of the composite flour ranged from 605 to 793 Cp, with the similar trend as found in PV. BDV ranged from 236 to 295 Cp, and the lowest BDV values were observed for the GGF enriched flour blends. However, the BDV values did not differ significantly ($P > 0.05$). SBV indicates retrogradation of starch, and it ranged from 450 to 950 Cp. T1 showed the highest SBV with the consistent trend across the blends. SR ranged from 879 to 1794 Cp, and the WF rich composite flours had the highest values with a consistent decrease by GGF content. Low SR indicates good stability against retrogradation after gelatinization of starch. The results indicate that GGF content in the composite flour is reduced retrogradation. P_{temp} is indicative of the minimum temperature required to cook the flour (Kaur and Singh, 2005), and it ranged between 70.2 and 83.6ºC.

Cases T1 and T2 had the highest P_{temp}, followed by the other composite flours. In particular, pure WF (T1) had the highest P_{temp}. This result is in agreement with the results of Wani et al. (2016). Kesarwani et al. (2016) reported that high protein content in the flour could thicken the walls around starch granules, and as a result could reduce the rheological properties. In the present study the GGF had high protein content (Table 1), and in addition the WF component had higher starch content: both aspects could induce higher viscosity values of the WF.

3.4. Antioxidant properties

The antioxidant properties of WF and GGF composites are shown in Table 4. TPC (0.0227 to 0.0246 mg/g) and TFC (0.0193 to 0.024 mg/g) varied insignificantly across the cases, although T1 had lower observed TPC and TFC than the other flours. Generally, the whole green gram pulses are rich sources of polyphenolics and have high antioxidant activities; however, the processing into flour may decrease the phytochemical and antioxidant abilities (Guo et al., 2012; Wei-Yu and Wang, 2015). Total chlorophyll (TC) of the composite flours increased with the GGF content, ranging from 0.015 to 6.448, with significant variation ($P \leq 0.05$). DPPH radical is a stable free radical that accepts electrons to form a stable diamagnetic molecule. The results showed that DPPH radical scavenging ability was enhanced with GGF content in the composite flours. It ranged from 44.58 to 88.07% ($P \leq 0.05$). Normally, antioxidant activity in plant originated materials is mainly contributed by the polyphenolics and vitamins. The TPC and TFC results exposed that the GGF had more activity than the WF. The activities may be influenced by amino acids that can interfere with the phytochemicals in the flour, improving the antioxidant capacity (Itagi and Singh, 2012). However, the ferric reducing power (FRAP) did not significantly differ against the tested composite flours, ranging from 54.69 to 62.35%. Although without statistical significance, these values slightly increased with the content of GGF. On the other hand, metal chelating activity (MCA) significantly differed between the blends. MCA is predominant as it decreases the transition metal concentration in the lipid peroxidation. The blends with GGF had a higher level of MCA than pure WF, and MCA ranged from 2.2 to 7.8 mg/g. Increased GGF content overall tended to increase the antioxidant properties. Bhattacharya and Malleshi (2012) reported that GGF with higher chlorophyll and carotenoid pigment contents possesses higher antioxidant activities. In addition, GGF also contains antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase.

4. Conclusions

The combinations of cereal grain and legume flours can facilitate producing nutritionally rich and low gluten profile bakery products. In the present study, the results showed that blends of wheat flour (WF) and green gram flour (GGF) allowed significant control of the proximate, functional and antioxidant properties. However, the added GGF tended to reduce the pasting properties (RVA viscosities) of these composite flours. Overall, the flours with a high content of WF could be more suitable for softer bakery products, such as bread and cakes, due to high pasting viscosities, while those with dominant GGF fraction could be more appropriate for the harder bakery products such as cookies or crackers, due to the lower pasting properties. However, further studies are required to develop actual bakery products and test them, using such flour blends.

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EFFECT OF UNGERMINATED AND GERMINATED FLAXSEED ADDITION ON THE RHEOLOGICAL PROPERTIES OF WHOLE WHEATMEAL AND WHEAT FLOUR

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ABSTRACT

Rheological properties of composite flour prepared by addition of ungerminated and germinated flaxseed flour in wheat flour and whole wheat meal were studied. The ungerminated and germinated flaxseed flour was at added at different levels 5%, 10%, 15% and 20%. It was observed that as the levels increased the development time increased significantly whereas stability time and mixing tolerance index decreased. A significant increase in paste temperature, peak viscosity, final viscosity, final viscosity upto 10% addition and later on started decreasing. Farinograph results showed that as the levels of incorporation of ungerminated and germinated flaxseed meal increased in wheat flour and whole wheat meal, the development time increased significantly whereas the stability time decreased.

1. Introduction

The globalization scenario in the new millennium has increased the demand for value-added bakery products due to change in perception, economic consideration, westernization, urbanization, busy lifestyle, increased women employment and increased per capita income. Due to health promoting properties and excellent nutrient profile of flaxseed, it has become a popular candidate for incorporation in human diet. The components of flaxseed, identified to exhibit the health benefits are fiber, lignans and linolenic acid (Omega-3 fatty acid). Moreover flaxseed is a good source of high quality protein, soluble fibers and phenolic compounds (Oomah and Mazza, 2008).

The process of germination fundamentally changes the nutrient composition of the seed. Nutrients such as enzymes, amino acids, and vitamins are substantially increased and become more bioavailable, allowing for better absorption. For example, sprouting doubles the antioxidant (ORAC) value of flaxseed. The "anti-nutrients" such as phytic acid, enzyme inhibitors and insoluble fibers are decreased; allowing for increased bioavailability and nutrient absorption. The dough rheological properties are influenced by the structure of the aggregates and their tendency to interact with

each other. Quality and quantity of the proteins affect the water absorption capacity of the dough (Finney, 1984). The mechanical and rheological properties of the dough exert promising effect on the overall quality of baked products (Blokshma and Bushuk, 1988). The arrangement and interaction of constituents (especially proteins) and the structure of materials are the responsible factors affecting the rheological properties (Bushuk, 1985).

Flaxseed mucilage is composed of mainly polymeric carbohydrates while galacturonic acid, rhammose, galactose, fructose, glucose are also present in small quantities. It can help to improve the water absorption characteristics of the dough (Fedeniuk and Biliaderis, 1994).

There is more information on nutritional and physiological properties than on its use in food. However, the knowledge about effect of supplementation of flaxseed on the rheological properties of dough for bread making is scanty. This research was undertaken to examine effect of replacement of wheat flour and whole wheat meal with germinated and ungerminated flaxseed flour on the rheological properties of dough used for bread making.

2. Materials and methods

2.1. Raw materials

Commercial wheat flour and whole wheat meal were procured from the local market. Flaxseed variety LC 2063 was procured from Narayangarh farms (Khanna), Punjab Agricultural University, Ludhiana.

2.2. Germination of flaxseeds

Flax seeds were germinated using different time-temperature combination after soaking in water followed by drying.

2.3. Chemical analysis of raw material

2.3.1. Fatty acid profile analysis

Lipids were extracted from sample using methanol/chloroform (Christie, 1989). The lipid fraction was quantified gravimetrically. For the isolation of triglycerides (TAG), the lipid extract was dissolve with hexane and passed through a Pasteur pipette containing florisil retained by glass wool. The sample was eluted with hexane/diethyl ether 4:1 (v/v) (10 ml) and the solution was collected. The TAG was trans esterified into fatty acid methyl esters (FAME) with a sodium methoxide catalysis method. The FAME was analyzed using a Shimadzu GC-17A gas chromatography coupled with a flame ionization detector (FID) and equipped with a HP-Innowax column (30m X 0.32mm i.d., 0.25µm film thickness). Helium was used as the carrier gas with a spilt ratio of 15:1. The flow rate through column was 1.5ml/min. The injector temperature was 225°C and the detector temperature was 240°C. The initial column temperature 120°C was increased to 200°C at a rate of 4°C/min and then to 240°C at a rate of 10°C/min. Fatty acids were identified using a mixture of commercial methyl esters 68D. The proportion of each fatty acid was determined without correction factors. Fatty acids were analyzed in two replicates.

2.3.2. Total phenolics

Total phenolics were determined by colorimetric method. A known amount of sample was taken and extracted with 80 percent methanol in a volumetric flask and made to 100 ml with 80% methanol and filtered. 0.5 ml of filtrate was taken into a test tube containing 0.5 ml water. The Folin- Ciocalteau reagent (0.5 ml) then kept for 5 min, and saturated solution of sodium carbonate (1 ml) was mixed. Absorbance of the developed color after 60 minutes was measured at 760 nm using Spectronic-20 Spectrophotometer. A standard curve was plotted by taking known amount of Gallic acid as reference standard (Swain and Hillis, 1959).

2.3.3. Antioxidant activity

Free radical scavenging activity was determined by DPPH (diphenyl picrylhydrazyl) method. Five hundred micro litres of 0.5 mM DPPH solution and 2 ml of 80% methanol aqueous solution were mixed with 25 µL of

methanolic extract of sample, and absorbance was determined under 517 nm (blank as 80 percent methanol and tris buffer) after maintaining at 20°C for 30 minutes. The free radical scavenging activity was evaluated by comparing the absorbance of the sample solution with control solution to which distilled water was added instead of sample (Koga et al., 2007).

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control OD(0min)} - \text{Sample OD (30min)}}{\text{Control OD (0min)}} \text{x100} \quad (1)$$

2.3.4. Mineral analysis

Mineral analysis was done using Atomic Absorption Spectrophotometer by AOAC (2005) method.

2.3.5. Proximate composition of raw materials

Moisture, protein, ash and fat were determined by AACC methods 44.15 A, 46-11 A, 08-01 and 30-10 respectively.

2.3.6. Crude fibers

Crude fiber of raw grains and multigrain porridge was estimated using Fibertec (Foss Company). Switched on the instrument and preheated the hot plate. Dried capsules kept in hot air oven at 100ºC for 20 minutes. Cooled and weighed 1 g Formulation in capsules. Fix the capsules to the rotating stand. Defatting of breads was done if necessary. Added 250-275 ml of 1.25% H_2SO_4 to the large extraction cup and immersed the stand into the beaker. Acid extraction was done by boiling it for 30-40 minutes followed by its washing with hot water. Then alkali washing was done with 1.25% NaOH for the same time duration followed by hot water washing. Finally, acetone washing was given and the capsules were dried in oven for 2 hours at 130ºC. Cooled and weighed for crude fiber estimation.

2.3.7. Dietary fibers

The contents of total soluble and insoluble dietary fibers were determined using an enzymatic-gravimetric method (Lee et al. 1992).

2.3.8. Calorific value

Calorific value was determined by using Bomb Calorimeter, Parr Calorimeter assembly 6100 (Parr Instrument Company, Moline, Ilinois 61265, USA).

2.3.9. Rheological properties

Farinographic properties were determined using the Brabender farinograph (AACC 2000) and flour pasting properties were determined using a rapid viscoanalyser (RVA) starch master R & D pack V 3.0 (Newport Scientific Narrabeen, Australia).

3. Results and discussion

3.1. Standardization of germination time-temperature

Various trials of germination of flaxseed were conducted by varying the soaking and germination time. The different trials conducted for germination are given as follows:

A- Soaking- 4hrs; Germination-1 day; Drying-24 hrs.

B- Soaking-2 hrs; Germination-2 day; Drying-36 hrs.

C- Soaking- 2 hrs; Germination-1 day; Drying-36hrs.

On the basis of fatty acid analysis, best time-temperature combination i.e. G-C for germination was selected, which was soaking flaxseeds for 2 hours in water, germination for 1 day and at drying for 36 hours at a temperature of 40±5ºC. This combination gave the unique and healthy fatty acid profile from nutrition and stability point of view. It has low amounts of saturated fatty acids (approximately 6%); moderate amounts of monounsaturated fatty acids (approximately 41%) and beneficial omega-6 (linoleic acid) and omega-3(linolenic acid) fatty acid were 13% and 40%, respectively.

In the selected combination, on germination, palmittic acid, oleic acid, linoleic acid content were increased by 0.7%, 44% and 57% respectively as compared to ungerminated

flaxseed, whereas, linolenic acid content decreased by 20% as compared to ungerminated flaxseed.

Increase in oleic acid content is desirable, as it has many health benefits associated with it. High concentration of oleic acid can lower the blood cholesterol level and lowers the risk of heart problems (Rickman, 2004). Decrease in linolenic acid content adds to stability of the product. As linolenic acid contains three double bonds in it, that is why, it is more prone to oxidation as compared to other unsaturated fatty acids and decreases the shelf life of the product. Thus, slight decrease in linolenic content is also beneficial. Although linolenic acid content decreased upon germination, but its beneficial effects didn't alter much. This is supported by increase in linoleic acid content, which makes the omega6 to omega 3 ratio unaltered.

3.2. Analysis of raw material

3.2.1. Proximate composition of raw material

The proximate composition of raw material was shown in Table 1. The germination of flaxseed resulted in an increase in protein, fat and fibers content.

3.2.2. Mineral analysis of raw materials

The mineral analysis of the raw materials like wheat flour, whole wheat meal, ungerminated and germinated flaxseed were carried out as shown in Table 2. The minerals like Zn, Ca and Mg were found to increase on germination of flaxseed whereas minerals like Cu, Fe and Mn decreased.

3.2.3. Farinographic properties of flour used for preparation of bread

A. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in wheat flour on the farinographic properties

The effect of incorporation of germinated and ungerminated flaxseed meal at 0, 5, 10, 15, 20% levels in wheat flour on farinograph has been discussed in the Table 3, Figures 1 and 2. Water absorption increased significantly with the addition of the flaxseed meal. The stability time of dough decreased significantly with the addition of ungerminated and germinated flaxseed meal as compared to control. The change in stability due to the flaxseed meal might have been attributed to the dilution of gluten forming proteins that caused weakening of dough. This value gives some indication of the tolerance to mixing or strength of the flour and gluten breakdown the flour will have. The mixing tolerance index of the flaxseed containing breads was found to be less as compared to control breads. Flours that had a low mixing tolerance index tend to have a good tolerance to mixing; whereas, the higher the tolerance index, the weaker is the flour (Shuey et al., 1972). The results agreed with those obtained by Garden (1993) who reported that incorporating ground flaxseed into wheat flour significantly increased water absorption and dough development time but decreased stability. The longer dough development time could have resulted from the dilution of gluten and difficulty of mixing of flax seed flour and wheat flour homogenously.

B. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in whole wheat meal on the farinographic properties

The water absorption of whole wheat-flaxseed meal was found to be more than that of wheat flour as represented by Table 4 and Figures 3 and 4.This might have been due to the high fibers content in the whole wheat meal.

Table 1. Proximate composition of raw material

Constituent	Wheat flour (Mean ± SD)	Whole wheat meal (Mean ± SD)	Ungerminated flaxseed (Mean ± SD)	Germinated flaxseed (Mean ± SD)
Moisture content (%)	14.93± 0.21	8.20±0.10	8.76±0.04	3.22±0.06
Ash content (%)	0.38±0.05	1.13±0.01	3.16±0.01	3.01±0.09
Protein (%)	11.47±0.24	12.03±0.14	20.33±0.25	27.69±0.27
Fat (%)	1.43±0.09	1.64±0.07	35.17±0.82	38.9±0.38
Crude fiber (%)	0.50±0.04	1.80±0.01	22.15±0.28	26.6±0.32
Dietary fiber (%)	0.50±0.07	1.40±0.03	19.70±0.19	24.90±0.24
Calorific Value (Kcal/g)	4.21±0.14	4.58±0.13	6.69±0.11	6.75±0.23
Antioxidant activity (%)	-	-	92.05±0.15	91.18±0.18
Total phenols (mg/ 100g)	-	-	307.36±0.29	238.56±0.31

Table 2. Mineral content (mg/Kg) of raw materials

Minerals (mg/Kg)	Wheat flour	Whole wheat meal	Ungerminated flaxseed	Germinated flaxseed
Cu	BDL*	0.90	9.85	9.40
Fe	16.92	25.64	88.02	82.36
Mn	4.26	14.81	31.47	30.42
Zn	5.55	13.91	34.03	42.53
Ca	183.33	281.05	1723.38	2532.86
Mg	917.31	1056.70	2737.97	3235.36

BDL*=Below Detection Limit

Table 3. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in wheat flour on the farinographic properties

Formulation	Level (%)	Water Absorption (%)	Dough Development Time (minutes)	Stability Time (minutes)	Mixing Tolerance Index (BU)
Control	0	61.3	3.5	13.6	80
Ungerminated	5	62.9	6.9	6.2	32
	10	63.8	7.7	6.7	30
	15	62.4	8.0	6.4	45
	20	62.1	8.2	4.9	60
Germinated	5	62.1	70	7.7	26
	10	61.6	7.2	9.1	36
	15	61.6	8.9	5.6	30
	20	62.2	8.5	4.6	49
Critical Difference ($p \leq 0.05$)		0.30	0.27	0.39	3.76

Table 4. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in whole wheat meal on the farinographic properties

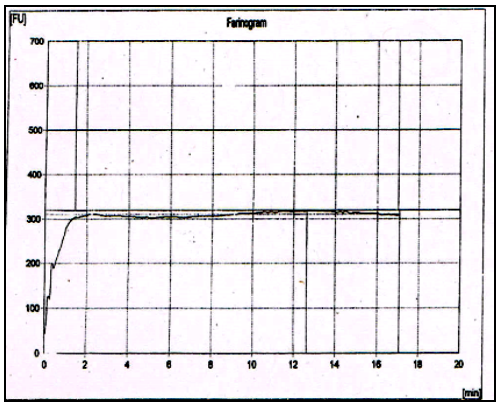
Formulation	Level (%)	Water Absorption (%)	Dough Development Time (minutes)	Stability Time (minutes)	Mixing Tolerance Index (BU)
Control	0	70.2	4.0	4.8	70
Ungerminated	5	72.9	3.9	4.9	59
	10	71.1	4.5	2.9	60
	15	70.9	4.8	4.9	55
	20	69.5	6.4	3.2	59
Germinated	5	70.2	4.2	5	68
	10	70.9	3.9	5.3	51
	15	69.7	4.7	3.3	70
	20	70.1	4.8	3.3	76
Critical Difference ($p \leq 0.05$)		0.46	0.19	0.24	1.71

Table 5. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in wheat flour on the pasting properties

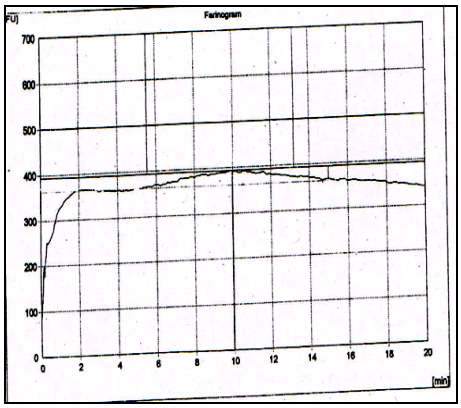
Breads	Level (%)	Parameters					
		Paste Temperature (ºC)	Peak Viscosity (cP)	Hold Viscosity (cP)	Final Viscosity (cP)	Breakdown Viscosity (cP)	Setback Viscosity (cP)
Control	0	92.80	1317	847	1658	470	811
Ungerminated	5	94.10	1555	1039	1869	470	759
	10	94.60	1436	961	1822	468	742
	15	94.85	1337	884	1758	454	738
	20	95.00	1156	826	1570	329	702
Germinated	5	94.75	1376	939	1714	438	775
	10	94.90	1386	938	1754	448	816
	15	94.90	1274	886	1662	388	775
	20	95.00	1132	806	1520	326	715
Critical Difference ($p \leq 0.05$)		0.32	4.35	2.28	3.95	2.42	4.16

Table 6. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in whole wheat meal on the pasting properties

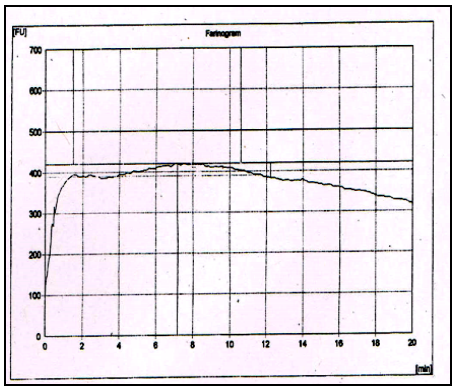
Breads	Level (%)	Parameters					
		Paste Temperature (ºC)	Peak Viscosity (cP)	Hold Viscosity (cP)	Final Viscosity (cP)	Breakdown Viscosity (cP)	Setback Viscosity (cP)
Control	0	91.9	756	538	1192	258	654
Ungerminated	5	93	856	598	1279	218	680
	10	95.6	800	588	1236	212	648
	15	95.2	718	543	1166	174	624
	20	0	648	500	1059	148	559
Germinated	5	92.85	752	536	1180	215	644
	10	94.55	737	538	1176	199	638
	15	95.6	734	552	1177	182	623
	20	0	686	537	1132	134	595
Critical Difference ($p \leq 0.05$)		0.44	3.07	9.89	2.28	3.83	3.87



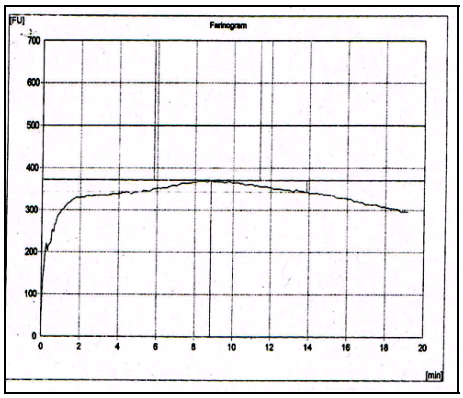
Control-whole wheat meal



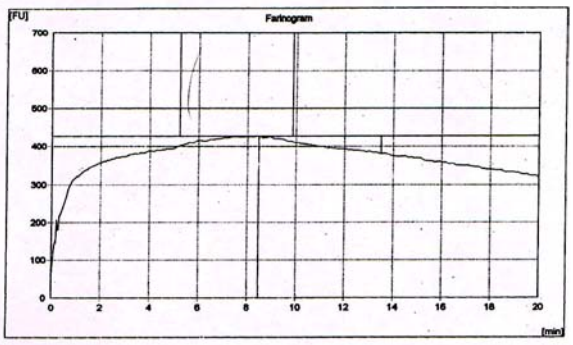
Whole wheat meal + 5 % germinated meal



Wheat flour +10 % Germinated meal



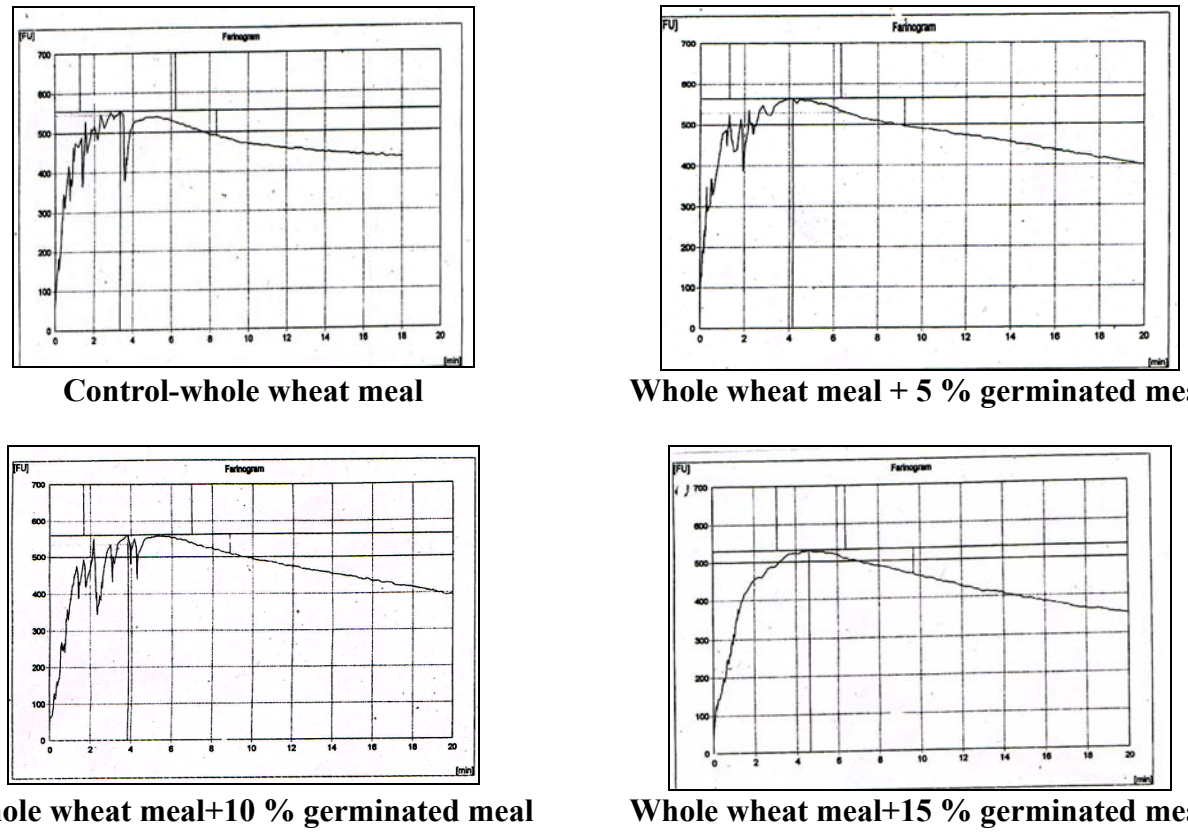
Wheat flour + 15 % Germinated meal

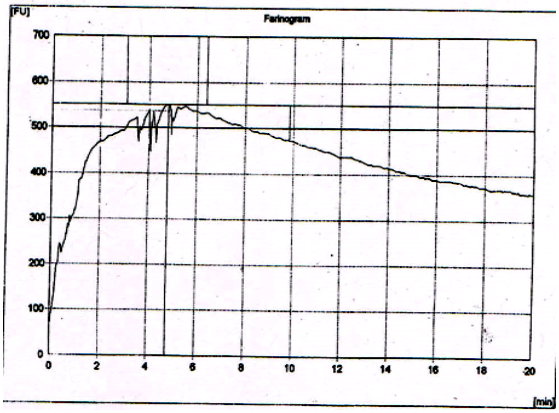


Wheat flour + 20 % germinated meal

Figure 1. Farinographs showing effect of incorporation of different levels of germinated flaxeed meal

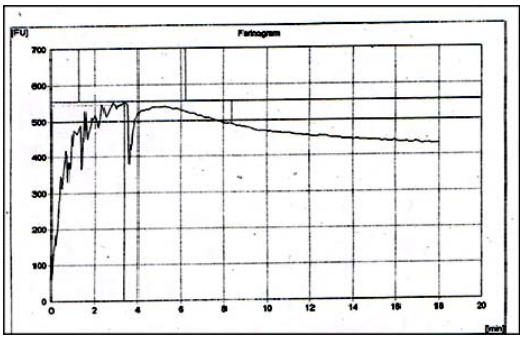
Figure 2. Farinographs showing effect of incorporation of different levels of ungerminated flaxeed meal



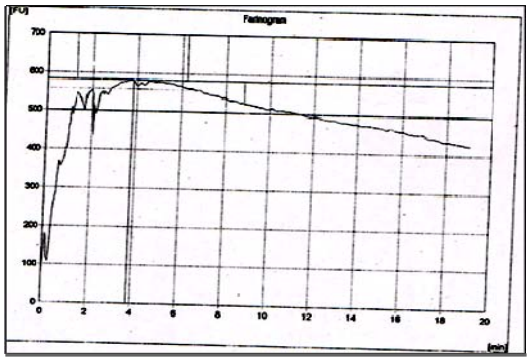


Whole wheat meal + 20 % germinated meal

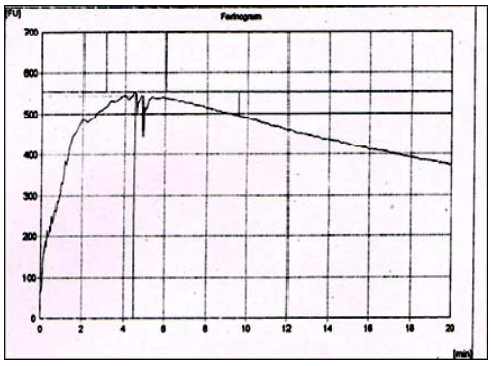
Figure 3. Farinographs showing effect of incorporation of different levels of germinated flaxseed meal



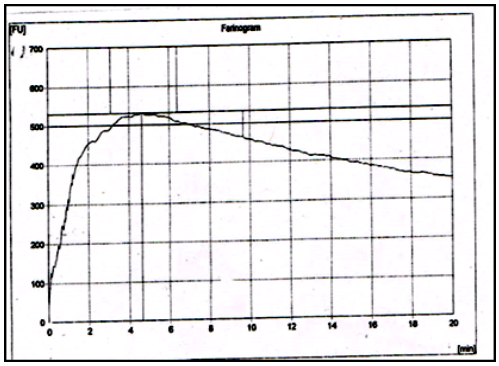
Control-whole wheat meal



Whole wheat meal + 5 % ungerminated meal



whole wheat meal+10 % ungerminated meal



Whole wheat meal +15%ungerminated meal

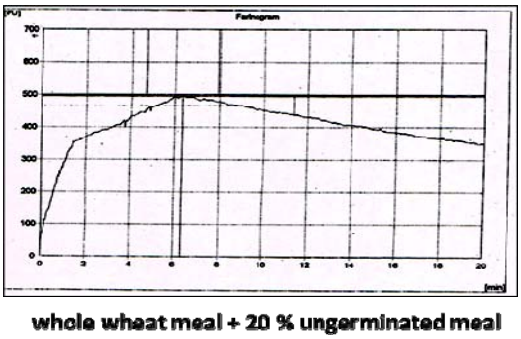


Figure 4. Farinographs showing effect of incorporation of different levels of ungerminated flaxseed meal

The water absorption in the flaxseed breads was found to be significantly more than that of control breads at 5 and 10 percent level of substitution. The increased water absorption might have been due to the gum present in the flaxseed which possesses excellent water binding capacity (Konessni et al., 2005). Also, the flaxseed mucilage was found to be composed of polymeric carbohydrates that can help in improving the water absorption characteristics of the dough (Fedenuik and Biliaderis, 1994).

3.2.4. Pasting properties of flour used for preparation of bread

A. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in wheat flour on the pasting properties

Pasting is one of the most important properties of the starch, which often occurs in various types of flour during processing. The pasting properties of various blends prepared by the addition of ungerminated and germinated flaxseed meal were determined by use of rapid visco-analyser and are represented in the Table 5.

The pasting temperature of the flaxseed containing breads was found to increase significantly with the increasing level of addition of the ungerminated and germinated flaxseed meal. It increased to 95ºC at 20% level of incorporation of ungerminated flaxseed meal whereas in 10% germinated flaxseed meal incorporated breads, it increased to 95.1ºC as compared to the control which was 92.8ºC. Too much pasted starch will cause stickiness, small volume and prone to the stale of bread. On the other hand too little pasted starch cannot form a continuous phase to be involved in the gas wall of bread.

The peak viscosity for the ungerminated flaxseed meal added wheat flour was found between 1555 cP at 5% level of addition and 1436 cP at 10% level of addition after which it started to decline. Peak viscosities attained during the heating portions of tests indicate the water binding capacity of starch. Similar trend was observed in the hold viscosity and final viscosity which first increased significantly upto 10% level of addition of ungerminated and germinated flaxseed meal and then started decreasing.

Final viscosity was minimum at 20% level of addition (1570 cP) and maximum at 5% level of addition (1869 cP) in the flour incorporated with ungerminated flaxseed meal. In case of germinated flaxseed meal incorporated Formulation, it was found to be in the range of 1714 cP at 5% level of addition and 1520 at 20% level of addition.

Breakdown and setback viscosity were found to decrease with the increase in level of addition of flaxseed meal. The setback viscosity decreased to 702 cP at 20% cent level of addition of ungerminated flaxseed meal whereas it decreased to 715 cP at 20% cent level of addition of germinated flaxseed meal as compared to control which was 811 cP. Set back values have been reported to correlate

with the ability of starch to gel into solid pastes.

The results are in accordance with Chetana et al. (2010) who found that on cooling to 50ºC, the viscosity decreased from 1147 to 680 BU and 499 for 40% incorporation of raw and roasted flaxseed powder whereas breakdown values decreased from 286 to 56 BU and 286 to 54 BU with an increase in level of incorporation of raw and roasted flaxseed powder. This indicated that the fiber fraction interacted with the wheat starch.

B. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in whole wheat meal on the pasting properties

The pasting temperature was found to increase significantly with the increase in level of addition of ungerminated and germinated flaxseed meal (Table 6). The peak viscosity was found to increase significantly upto 10% level of addition of both ungerminated and germinated flaxseed meal and then started decreasing whereas in case of germinated flaxseed meal addition, it significantly decreased with the increasing level of addition. The hold viscosity showed a significant decrease to 500cP after the addition of 20% of ungerminated flaxseed meal and 537 cP in germinated flaxseed meal added breads as compared to control which was 538 cP. Breakdown viscosity was also found to decrease significantly with the increase in level of addition of ungerminated and germinated flaxseed meal. It was found between 218 cP at 5% cent level of addition and 149 cP at 20% cent level of addition in case of ungerminated flaxseed meal incorporated breads.

Setback viscosity also showed a significant decrease with the increasing level of addition of flaxseed. The final viscosity is the most commonly used parameter to determine a particular starch based quality. It gives an idea of a material to gel after cooking. The decrease in pasting properties might have been due to the fibre present competing with the starch in sample for water. As viscosity of paste were directly related to the degree and extent of starch gelatinization and hence realignment during subsequent cooling. Any material that competes for water will restrict the amount of water available for the starch granules during starch gelatinization.

The pasting temperature of whole wheat meal was found to be less than that of wheat flour. Huang et al. (2007) also found lower pasting temperature and viscosities in whole wheat meal as compared to the commercial white flour. Breakdown is a measure of susceptibility of cooked starch granules to disintegration whereas setback is a measure of recrystallization of gelatinized starch during cooling (Beta and Corke, 2001). A low breakdown of wheat flour blends suggests that they are more stable under hot conditions than wheat flour. Moreover, wheat flour blends exhibit lower setback value indicating less amylose retrogradation as the system is cooled. In addition, the difference in pasting properties of wheat flour and wheat flour blends also could be due to other factors, such as particle size, enzyme activity or water-holding capacity.

4. Conclusions

It was observed that on germination of flaxseed, the fibre content and the protein content was found to increase. Farinograph results showed that as the levels of incorporation of ungerminated and germinated flaxseed meal increased in wheat flour and whole wheat meal, the development time increased significantly whereas the stability time decreased. Pasting properties like the breakdown viscosity and set back viscosity was found to decrease with increasing levels. However, stability time of germinated flaxseed added dough was more than that of ungerminated and can be further increased by adding improvers.

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PHYSICOCHEMICAL CHARACTERISTICS AND PHENOTYPIC DIVERSITY OF SICILIAN WINTER PUMPKIN (*CUCURBITA MAXIMA*) POPULATIONS

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ABSTRACT

Six pumpkin genotypes (G1, G2, G3, G4, G5 and G6) were evaluated for their morphological, agronomical and physico-chemical properties under ecological conditions of Sicily (Italy). Considering the agronomical, morphological and physicochemical data including fiber, humidity, pH, total acidity, soluble solids, carotenoids, phenolic and ascorbic acid content, it was possible to differentiate among genotypes. The morphological analysis showed that the pumpkin genotypes did not have a homogenous morphology and present a large physico-chemical characteristic variability. Furthermore, high contents of carotenoids and dietary fiber, soluble solids, phenolic and ascorbic acid were observed with the maximum values in G1 and G5 landraces. The correlation between the thirty four parameters and the genotypes showed a wide range of variability in both positive and negative direction. The variability was statistically accumulated with the considered parameters following a significant characterization. In conclusion, we found a wide range of genotype variability among Sicilian winter pumpkin populations. This germplasm may represent a valuable genetic source for future breeding studies.

1. Introduction

Cucurbita maxima Duch. (winter pumpkin) belongs to the *Cucurbitaceae* family. The species, is originated in South America and was introduced early into Europe (Ferriol et al., 2003). It represents one of the most cultivated and economically important vegetable, characterized by high production standards (Taylor and Brant, 2002). It can be used for human consumption (Ferriol et al., 2003), as fodder for livestock, medicinal and cosmetic purposes, containers, or ornamental objects, and as rootstock (Lira-Saade, 1995). In addition, it is suitable to realize cross with the *Cucurbita moschata* species, that allows to obtain hybrid of *Cucurbita maxima* x *Cucurbita moschata* that confer tolerant or resistance of soilborne disease (Ekaterini et al., 2000).

Sicily is the largest Mediterranean island located in southern Italy. It is a cultural and a commercial port, and one of the most important centre of origin and differentiation of vegetables. During the centuries, the farmers obtained many genotypes for each species, adapting them to the pedoclimatic requirements, and don't caring them to the genetic purity. For this reason, it was estimated a presence of 2650 taxa (Raimondo et al., 1992) in Sicily on an extension of 26000 Km^2. This selection criteria allowed to

obtain an inter-specific variability that brought other genotypes perfectly integrated with the cultural environment and with positive effects on the qualitative and organoleptic characteristics (Schiavi et al., 1991). Breeding activity is always depended on the availability of genetic variability, and thanks to the selection criteria applied by the farmers the biodiversity was saved (Schippmann et al., 2002). The local populations are genotypes of high intrinsic value with a particular capacity of adaptability to their environment (D'Anna and Sabatino 2013; Sabatino et al., 2013; Sabatino et al., 2014; Sabatino et al., 2016). These characters might allow easier cultivation in Mediterranean basin compared to the varieties selected in different environments. The antioxidant capacity of different vegetables is one of the most features of food product (Dragsted et al., 2006). Plants contain high concentrations of numerous redox-active secondary metabolites (ie, antioxidants), such as polyphenols, carotenoids, ascorbic acid, and enzymes with antioxidant activity (Torunn et al., 2009), which help them from hazardous oxidative damage to plant cell components. It is well known that carotenoids are synthesized only by plants and micro-organisms, therefore, the interest on their content increased the attention on pumpkin fruits (Murkovic et al., 2002). This interest was justified by cancer prevention (Astorg, 1997), atherosclerosis, cataracts, age-related macular degeneration and an array of other degenerative diseases (Murkovic et al., 2002). Moreover, the quality and the quantity of antioxidants in fruits and vegetables, change by cultivar, environment, soil type, growing and storage conditions (Achouri et al., 2005; Antolovich et al., 2000; Griffith and Collison, 2001; Lee et al., 2004; Luthria, 2006; Naczk and Shahidi, 2004; Robbins, 2003).

The purpose of the present study was the discovery, recovery, and characterization of five Sicilian winter pumpkin local populations by using morphological and chemical characteristics such as water content, pH, acidity, soluble solid, carotenoid, polyphenol, dietary fiber, ascorbic acid and colour, to reveal trait significant differences between them.

2. Materials and methods

2.1.Plants material and cultivation technique

The experimental field was carried out in open field during spring-summer 2012 and repeated during spring-summer 2013 at the experimental farm of the Palermo University (Italy). Five landraces were taken (G1), (G2), (G3), (G4), and (G5) from the Buonfornello area (37°98'39''N, 13°69'63''E) (Palermo, Sicily). In addition, one variety 'Butternut' (G6) was also tested as control sample.

Thirty seeds per genotype were seeded into 66 cells plug trays, containing a substrate mix characterized by blond peat moss and black peat moss (1:1, v/v). After sowing, the seeds were covered with a layer of the same substrate, and the trays were placed in greenhouse with a target of air temperature 25/18 °C (day/night). All trays were sub-irrigated using a nutrient solution containing the following elements in $mg \cdot L^{-1}$: 178.5 N (164.5 NO_3-N, 14 NH_4), 38.71 P, 254.15 K, 110 Ca, 24.31 Mg, 32.07 S, 0.84 Fe, 0.55 Mn, 0.33 Zn, 0.27 B, and 0.048 Mo (Sonneveld and Straver, 1994; De Kreij et al., 1999). After 15 days from the sowing, the seedlings achieved the plant phenological stage. Planting of all the genotypes took place on 20th April 2014, and repeated on 25th April 2015. In our study, a typical Sicilian cultivation technique in open field was used for the growth of winter pumpkin. Seedling bed was prepared through medium-deep ploughing (35 cm) and declodding using a rotary harrow. Aged manure was added as a soil amendment at a rate of 40 t ha^{-1}. Seedlings with their root ball were used and planted with a 2 m planting distance, and a 3.5 minter-row distance layout was adopted. A drip irrigation system was installed to provide water and nutrient solution containing

the following elements in mg L^{-1} 241.5 N (224 NO_3^-, 17.5 NH_4^+), 38.71 P, 312.8 K, 160 Ca, 33.42 Mg, 44.09 S, 0.84 Fe, 0.55 Mn, 0.33 Zn, 0.27 B, and 0.048 Mo (Sonneveld and Straver, 1994; De Kreij et al., 1999). All genotypes were planted with a distance between the plots containing the different genotypes of 25 m. At the flowering moment, all female flowers were submitted to the manual pollination and were applied one clip insulator to avoid the crossing between the genotypes.

2.2. Morphological characterization and data production

The morphological characterization was carried out upon 30 plants per accession, using over the IPGRI descriptor for *Cucurbitaceae* (Esquinas-Alcázar and Gulick, 1983). In addition, other morphological characters for a more complete description were evaluated. Twenty characters were evaluated: average total production per plant (kg), average number fruits per plant, average weight fruit (kg), thickness epicarp (cm), thickness mesocarp (apical, median, and distal part) (cm), leaf longitudinal length (cm), leaf transverse length (cm), petiole length (cm), thickness petiole (cm), peduncle length (cm), thickness peduncle (cm), fruit length (cm), fruit width (cm), fruit length/width ratio, scar flower diameter (cm), flesh colour (apical, median, and distal part)(L*, a*, b*). The flesh colour of raw pumpkin samples was assessed by a tristimulus colorimeter (Chroma Meter - CR-400, Konica Minolta) which is suggested by Mendoza et al. (2006), and evaluated along the longitudinal section of fruits (apical, median, and distal part).

2.3. Preparation of sample and chemical analysis

All reagents were analytical grade Sigma of Fluke products used without any purification. Pumpkin fruits were peeled, the seeds were removed, then chopped into small sections with a kitchen knife, and the juice was extracted using a blender. All the samples of winter pumpkin juices were immediately analyzed within the extraction day for the both years production 2012 and 2013. pH were performed on the homogenized samples using a pH meter (METTLER TOLEDO mod. MP 220) following NMKL 179 Method (2005), the samples temperature was standardized at 25 °C. Pumpkin's water content is calculated as the ratio of (wet - dry) relative to the dry winter pumpkins. The dry matter content is then calculated as (100 – moisture content). It was obtained by placing 2 g of sample into a small china bowl of known weight, and putting all samples inside of oven set at 70 °C for 3 days. Determination of the soluble solids was made by homogenizing and filtering the juice of pumpkin samples. Then, the filtered samples were subjecting at the reading with an Optical Refractometer (ATAGO Hand Refractometer N-50 E). In order to evaluate total acidity, the samples were diluted with 250 ml of distilled water, and titrated with a sodium hydroxide solution (0.1 M) in presence of the indicator (alcoholic solution of phenolphthalein: 1% in ethyl alcohol at 95%), until the colour indicator changes (the pink colour of phenolphthalein persist for 30 seconds). The results were expressed as grams of citric acid monohydrate per 100 g of product, following the conversion index. The total carotenoids content was determined according to the method of Lee and Castle (2001) which was modified for pumpkin (Nur and Gülsah, 2012). Two ml of a winter pumpkin sample was mixed with 38 ml extraction solvent (hexane-acetone-ethanol (50:25:25 v/v/v)) with an agitator vortex mod. RX3 (constant speed 2400 rpm). Then, centrifuged (4000 rpm, 10 min) (HERAEUS, MULTIFUGE 3S+ Centrifuge), the supernatant was collected, and the absorption was measured with a SHIMADZU UV mini- 1240 spectrophotometer at 450 nm. Total carotenoid content was calculated as milligrams of carotene per gram of sample by using the following equation:

$$A = (\varepsilon bc)/1000 \quad (1)$$

were A is the absorbance value, **ε** = molar absorbance coefficient **ε** (1cm) = 2505, b = extent of unit light way, 1 cm, c = mg g^{-1} total carotenoid content.

To determine the total phenolic content, two grams of homogenized sample were added with 10 ml of pure ethanol. The extraction was done by using a vortex mixer mod. RX3 for 60 seconds. The mixture was filtered and the filtrate was taken into a test tube. The Folin-Ciocalteau micro method of Waterhouse (Brand-William et al., 1995) was used to determinate the total phenolic content (TPC). Three hundred µl of the filtrate were diluted in 4.8 mL of Milli-Q grade water, and 300 µl of Folin-Ciocalteau reagent was added and shaken. After 8 min, 900 µl of 20 % sodium carbonate solution was added with mixing. After reaction at 40 °C for 30 min, absorbance was measured at 765nm using SHIMADZU UV mini- 1240 spectrophotometer. A calibration curve of gallic acid (3, 4, 5- trihydroxybenzoic acid) was prepared (0-50 µg) and used as standards. The results were given as mg gallic acid equivalent per gram of fresh weight.

The measurement of ascorbic acid content was determined as described by Lee and Coates (1999) using an HPLC photodiode array detection approach. About 10 g of winter pumpkin sample was homogenized with 10 mL of 10 % metaphosphoric acid and sea sand in a mortar. The slurry obtained was pipetted and was transferred into a centrifuge tubes containing 20 mL of 5 % metaphosphoric acid and centrifuged (HERAEUS, MULTIFUGE 3S+ Centrifuge) for 20 min at 3000 rpm. The supernatant was filtered through 0.45 mm PTFE syringe filters and diluted 20 fold with distilled water. Ten mL of diluted supernatant were diluted with 0.13 mL of 2.5 M K_2HP0_4 to give a final pH of 7.0, and 20 µl of this solution was injected into C18 (15 cm 4.6 cm, pore size 5 mm) coupled with HyperODS guard column. The mobile phase was 8 mM phosphate buffer, pH 6.8, containing 3 mM tetra-n-butylammonium bromide with a flow rate of 1 mL min^{-1}. Eluate was monitored by UV detection at 245 nm. Chromatograms were recorded and processed with EZ Start Chromatography Software V.7.2.1. The identification of peak corresponding to ascorbic acid detected by their co-elution with standard.

Total dietary fibre was determined using an enzymatic-chemical method (Willem et al., 2010). Samples and blanks used to be tested for dietary fibres content were quadruplicate. One gram amounts of the sample being analysed was incubated in 250 mL Duran bottles in a shaking incubation bath at 150 rev/min in orbital mode, after adding 50 mL of pH 6 phosphate buffer to each bottle with 0,1 mL α- amylase (Product code A 3306) for 15 min at 95 °C. After pH correction at 8 by adding 10 mL of 0.275 N NaOH with 0.1 mL (5 mg Protease) (Product Code P 3910). The solutions reaction were incubated at 60 °C for 30 min to obtain the inactivation of α-amylase and denaturation proteins after adjusting the pH at 4.5 with 10 mL of 0.325 M HCL. In addition, 0.1 mL of amyloglucosidase (Product Code A 9913) was added and incubated for 30 min at 60 °C. One mL of diethylene glycol was added followed by four volumes of ethanol with mixing to precipitate soluble polymeric dietary fibre. The suspension was filtered and washed sequentially with 76% ethanol, 96% ethanol and acetone. Furthermore, it was dried and weighed. One duplicate was used to determine the proteins Kjeldahl nitrogen analysis as specified in the Official Methods of Analysis of AOAC 1997, and the other was incubated at 525 °C to determine ash. Finally, the total dietary fibre was calculated as the difference between the weight of the filtered and dried residue, and the weight of the proteins and ash.

2.4. Experimental design and statistical analysis

Treatments were defined by a completely randomized design with three replicates per

treatment, each consisting of 10 plants. Statistical analysis were performed using ANOVA and mean separation was carried out by Duncan multiple range test by the statistical program SPSS 14.0 (StatSoft, Inc., Chicago, USA). Percentages were subjected to angular transformation prior to perform statistical analysis ($\Phi = \arcsin(p/100)^{1/2}$). Investigation of multi-character variation was conducted by Principal Component Analysis (PCA).

3. Results and Discussion

The results demonstrated that winter pumpkin genotypes differed in respect of physical and chemical characteristics which are confirmed in literature (Sztangret et al., 2004; Gajc-Wolska et al., 2005; Paulauskiene et al., 2006). Recently, in other vegetable crops, such as in eggplant, the chromosomal region and QTL (Quantitave Trait Loci) associated to the content of anthocyanin, dry matter, solamargine glycoalkaloid, chlorogenic and other organic acid have been identified (Gramazio et al, 2014; Toppino et al, 2016).

For the colorimetric evaluation, significant differences ($P \leq 0.001$) were observed among genotypes for flesh colour represented by the three color space values: L*, a*, b*. This demonstrates that genetic variation for flesh color is present among the tested samples. The lowest value of L* (lightness) and highest value of a* were found in G2 and G6 ('Butternet' variety) genotype samples respectively (Table 1). Along the longitudinal section, from apical to distal part, the lowest L*, a* coordinates values were obtained in the median part, therefore, seem to be a longitudinal flesh colour gradient.

Table 2 indicates that the dry matter content in the pumpkin fruit samples differed significantly ($P \leq 0.001$) between the samples. It ranged from 5.93 to 10.35 %. The highest and the lowest proportion of dry matter was measured in the sample G1 and G3, respectively. Such high variation in dry matter content of winter pumpkin fruit has also been reported by Paulauskiene et al. (2006). This response could be due to variation in starch content of the genotype (Hazzard, 2006). The changes in the pH of the fruit flesh influences the activities of ripening related enzymes and antioxidant system, ultimately affects the sensory quality (McCollum et al., 1988). The pH value of the sixth fresh winter pumpkin fruit samples was significantly different ($P<0.001$). It ranged between 6.12 and 6.48. The highest pH value was recorded in winter pumpkin sample G1 and the lowest in sample G4. These results are similar to those reported by Paulauskiene et al. (2006).

Significant ($P \leq 0.001$) difference in fruit total soluble solids content was obtained among the winter pumpkin samples with a range varying between 4.89 and 9.28^{o}Brix (Tab. 2). Pumpkin sample G1 had the highest TSS (9.28^{o}Brix.), followed by sample G2 (7.26 ^{o}Brix) while, sample G3 had significantly lower TSS (4.89^{o}Brix). These variations in TSS are due to the genetic differences among the genotypes that affect fruit quality (Stepansky et al., 1999; Burger et al., 2006, Burger and Schaffer, 2007). More than 85% of pumpkin fruit TSS content is sugar and it is highly related to the sensory quality of pumpkin fruit and it is used to screen pumpkin fruit (Cantwell and Suslow, 1998).

The biosynthesis and metabolism of carotenoids in vegetables can significantly be affected by the differences in growing environment, such as temperature, nutrient availability, soil, intensity of sunlight, ripening stage, and post harvesting (Rodriguez-Amaya, 1999; Cazzonelli and Pogson, 2010). According to Murkovic et al. (2002); Gajc-Wolska et al. (2005) the genotypes significantly influence carotenoids content in pumpkin fruits. The content of total carotenoids in the samples examined varied between 0.25 and 0.48 mg g^{-1}, the highest value being measured in the control 'Butternut' sample G6 (Table 2).

From the nutritional point of view polyphenols are important determinant factors for the quality trait of winter pumpkin. The content of polyphenols was found to differ considerably among the samples, ranging from 28.57 to 93.70 mg/100g (Table 2). The lowest value was measured in the control 'Butternut' sample G6 (28.57 mg/100 g).

Table 1. Flesh colour measurement of winter pumpkin samples.

Genotype	L apical part	a apical part	b apical part	L median part	a median part	b median part	L distal part	a distal part	b distal part
G1	59.13 bc	15.36 d	56.70 n.s.	60.23 a	15.89 c	59.77 a	62.1 a	16.55 cd	59.56 ab
G2	66.98 a	16.60 cd	55.47 n.s.	62.00 a	13.99 d	53.51 ab	63.2 a	15.65 d	53.53 d
G3	63.85 a	21.23 b	55.81 n.s.	61.51 a	20.82 ab	54.96 a	63.1 a	21.76 b	56.64 c
G4	55.92 c	17.80 c	50.77 n.s.	53.56 b	16.47 c	46.90 b	51.7 c	17.20 c	49.70 e
G5	57.87 c	17.50 cd	54.56 n.s.	58.37 ab	20.26 b	59.44 a	57.3 b	20.61 b	60.20 a
G6	63.35 ab	24.04 a	57.67 n.s.	61.98 a	22.53 a	55.88 a	59.7 ab	25.86 a	57.76 bc

Results indicate mean value of two years (2014 and 2015). In each column, figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 2. Physico-chemical characteristics of winter pumpkin samples.

Genotype	Dry matter [%]	pH	Total acidity [g/100g citric acid monohydrate]	Soluble solid [°Brix]	Carotenoids [mg g^{-1}]	Total phenolics [mg/100]	Total dietary fibre [g/100g]	Ascorbic acid [mg/Kg]
G1	10.35 a	6.48 a	0.09 n.s.	9.28 a	0.27 de	66.36 d	1.50 a	30.00 a
G2	8.18 c	6.45 a	0.10 n.s.	7.26 b	0.29 d	66.67 c	1.40 b	26.50 b
G3	5.93 f	6.27 c	0.10 n.s.	4.89 e	0.38 c	41.52 e	1.40 b	23.00 d
G4	7.07 e	6.12 d	0.09 n.s.	6.20 c	0.25 e	76.20 b	1.20 d	24.90 c
G5	9.27 b	6.15 d	0.09 n.s.	5.30 d	0.43 b	93.70 a	1.30 c	15.63 f
G6	7.49 d	6.39 b	0.15 n.s.	6.39 c	0.48 a	28.57 f	1.40 b	21.30 e

Results indicate mean value of two years (2014 and 2015). In each column, figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 3. Factor loadings, eigen values, and percentages of variance and cumulative variance that can be explained by the first 3 PCs.

Variable	PC1	PC2	PC3
Average total production/plant	0.799	-0.015	-0.316
Average number fruits/plant	-0.682	0.453	-0.166
Average weight fruit	0.695	-0.086	-0.214
Thick epicarp	-0.172	-0.479	-0.409
Thick mesocarp (apical part)	0.224	0.079	0.856
Thick mesocarp (median part)	0.709	0.096	-0.603
Thick mesocarp (distal part)	0.320	0.328	-0.011
Average weight fruit	0.695	-0.086	-0.214
Leaf transverse length	0.004	-0.701	0.008
Petiole length	0.209	-0.750	0.024
Thickness petiole	0.095	-0.502	0.519
Peduncle length	0.538	0.032	-0.379

Thickness peduncle	0.526	0.277	-0.276
Fruit length	0.220	0.382	0.687
Fruit width	0.934	-0.099	-0.128
Fruit length/width ratio	-0.510	0.550	0.593
Scar flower diameter	0.819	0.004	0.097
Humidity	0.608	-0.631	-0.439
pH	0.183	0.838	-0.307
Total acidity	0.676	-0.013	0.051
Soluble solid	-0.501	0.736	-0.262
Total carotenoid	0.700	-0.105	0.644
Total phenolic content	-0.783	-0.147	0.361
Total dietary fiber	0.299	0.933	-0.072
Ascorbic acid content	-0.317	0.504	-0.771
Flesh colour L* (apical part)	0.637	0.321	-0.291
Flesh colour a* (apical part)	0.774	-0.297	0.041
Flesh colour b* (apical part)	0.390	0.511	0.124
Flesh colour L* (median part)	0.598	0.555	0.049
Flesh colour a* (median part)	0.654	-0.234	0.512
Flesh colour b* (median part)	0.153	0.610	0.587
Flesh colour L* (distal part)	0.495	0.736	-0.119
Flesh colour a* (distal part)	0.727	-0.209	0.390
Flesh colour b* (distal part)	0.218	0.584	0.716
Eigen value	9.955	7.863	5.689
Variance/%	29.278	23.126	16.731
Cumulative/%	29.278	52.405	69.136

Results indicate mean value of two years (2014 and 2015).

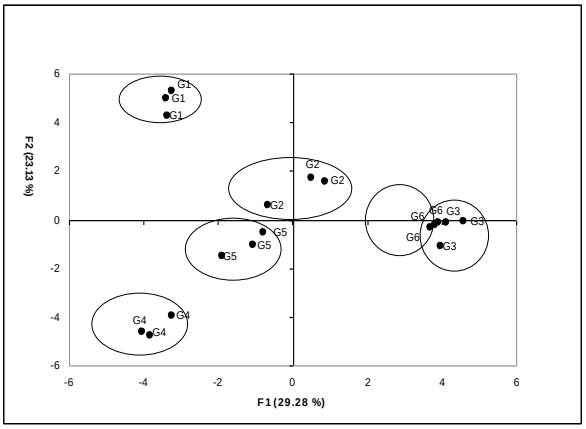

Figure 1. Results indicate mean value of two years (2014 and 2015). Principal component score plots of the separation of winter pumpkin samples (PC1 and PC2). Abbreviated variables are explained in Table 3

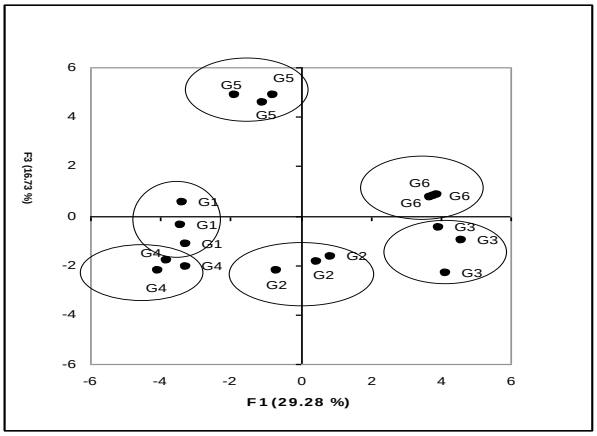

Figure 2. Results indicate mean value of two years (2014 and 2015). Principal component score plots of the separation of winter pumpkin samples (PC1 and PC3). Abbreviated variables are explained in Table 3.

The highest total phenol content (93.70 mg/ 100g) was recorded in G5. These results are in accord to those reported by Sharma and Ramana Rao (2013).

Winter pumpkin fruit samples dietary fibre content showed variations among landraces. The sample G1 showed the highest value (1.5 g/100 g), and the sample G4 showed the lowest value (1.2 g/100 g). These variations reflect the genotype effect on dietary fibre content. This variability was already detected from other fruits by Mrabet et al. (2013). Furthermore, both factors (genotype and environment) have been shown to contribute significantly to the dietary fibre content of cereals such as wheat (Gebruers et al., 2010) and leguminous like peas (Stoughton-Ens et al., 2010).

Many researchers reported that pumpkin provides a valuable source of ascorbic acid that have a major role in nutrition in the form of vitamin C as antioxidants (Duke and Ayensu, 1985; Sudhakar et al., 2003). Winter pumpkin fruit samples examined showed highly significant ($P < 0.001$) variation in ascorbic acid content (Tab. 2). The ascorbic acid content ranged between 15.63 mg/Kg for the sample G5 and 30 mg/Kg for the sample G1. Our results confirmed those obtained by Sudhakar et al. (2003) who indicated that ascorbic acid content of pumpkin varies among genotypes.

Principal Component Analyses was performed to evaluate the global effect of agronomical, morphological, and physicochemical properties on the winter pumpkin landraces (Tab. 3). The factor loadings are shown in the Table 3. The first axis accounted for 29.28 % of the variance, the second 23.13 % and the third 16.73 %, making a total of 69.14% of variance with the three axis together.

The first two factors (PC1 and PC2) represent 52.40 % of the initial data variability. However, some information still might be hidden behind the third factor, PC3, 16.73 %. Figure 1 represents the clustering differences between the sixth winter pumpkin genotypes; all of the G3 samples are positioned on the right side of the PC1, and other samples are positioned on its left side, with G2 samples situated in the up-right position. The first principal component (PC1) explains 29.28 % of the total variance, and was positively correlated with average total production per plant, thickness mesocarp (apical, median and distal part), average weight fruit, leaf transverse length, petiole length, thickness petiole, peduncle length, thickness peduncle, fruit (length, width), scar flower diameter, humidity, pH, total acidity, total, carotenoids, total dietary fibers, and

flesh color (Table 3). The second principal component (PC2) explains 23.13 % of the variance, and was positively correlated with average fruits number per plant, thickness mesocarp (apical, medium and distal part), peduncle length, thickness peduncle, fruit (length and length/width ratio), scarf flower diameter, pH, soluble solid, total dietary fibre, ascorbic acid content, flesh color except flesh color a, and negatively correlated with average total production per plant, thickness epicarp, average weight fruit, leaf (longitudinal and transversal length), petiole (length and thickness), fruit width, humidity, total acidity, total carotenoids, total phenolic content (Table 3). The second two factors (PC1 and PC3) represent 69.14 % of the initial data variability. In the figure 2 all of the G2, G3 and G6 samples are positioned on the right side of the PC1 expect one sample (G2), and other samples (G1, G4 and G5) are located on its left side, particularly G5 samples is situated in the up-left position, and G4 sample in the down-left position. While G3 samples is located in down-right position and G6 in the up-right position. The first and the third factor (PC1 and PC3) were also presented graphically (Fig. 2), they stand for the cumulative 69.14 % of the initial data variability. Thick epicarp and mesocarp (median part), humidity and ascorbic acid content were strongly negatively correlated with PC3, while the thickness petiole, fruit (length and length/width ratio), total carotenoid, flesh color a, and b (median and distal part) were strongly positively correlated with PC3 (Table 3). It is possible to observe that for the PC1, the average total production per plant, thickness mesocarp (median part), flesh color a* (apical and distal part), and total carotenoids content were the most important variables that explain the separation in the winter pumpkin samples. The loading in PC2 the most important variables that explain the separation between the winter pumpkin samples were the average number fruits per plant, the fruit length/width ratio, the flesh colour L* and b* (median and distal part), pH, and total dietary fibre, while in PC3 the most important variables were both the thickness mesocarp (apical part) and total carotenoids content. For most of the variations it is more likely that flesh colour and total carotenoids content parameters had a high influence in explaining the separation among samples. The correlation between colour parameters in CIE Lab system and carotenoids content in vegetables were found also in some other works (Paulauskiene et al., 2006).

4. Conclusions

Our results demonstrated the possible differentiation among the chosen pumpkin genotypes by their agronomical, morphological, and physico-chemical characteristics. The data reveal great deal of diversity for morphological traits. Among the sixth tested pumpkin genotypes, sample G3 showed the highest total production per plant followed by both samples G2 and G6 (control sample) which represent the biggest fruit sizes. By contrast, the sample G1 demonstrated small fruit sizes but with the highest fruit number per plant, soluble solids, and ascorbic acid content. Growers must realize that greater fruit number will result in a smaller average fruit size. The sample G5 showed the highest carotenoid and total phenolic compounds contents. In terms of nutritional value both samples G1 and G5 were the most valuable genotypes. Qualitative characteristics showed also variation in flesh color. The results of this study demonstrate a wide range of genotype variability among Sicilian winter pumpkin populations and might represent a valuable source of information for future *Cucurbita maxima* breeding improvement.

5.References

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CHEMICAL AND ANTIOXIDANT PROPERTIES OF *CERATOTHECA SESAMOIDES* ENDL. LEAVES

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ABSTRACT

Chemical and antioxidant properties of *Ceratotheca sesamoides* vegetables were investigated. The leaves were freshly harvested, dried, milled into powder and packaged. Proximate, antioxidant, amino acids profiles, mineral and carotene contents were analyzed. The proximate compositions of the vegetable were fat (1.79%), crude fibre (6.21%), ash (9.15%), protein (28.92%) and carbohydrate (44.11%). The free radical scavenging activity of the vegetable extract using 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) showed higher antioxidant activities (72000 µ moles TE / 100g) in the vegetable. Amino acids profile showed glutamic acid (2.010 %) and aspartic acid (1.927 %) as the major non-essential amino acids while leucine (1.436 %) was the major essential amino acid. Potassium and calcium were the major mineral contents in the vegetable with appreciable amount of iron. Total carotene found in *C. sesamoides* was 32000 IU/100g with trans beta carotene having higher value. *C. sesamoides* vegetable serves as source of nutrient and antioxidant which aids body metabolism and fight against diseases.

1. Introduction

Nigeria is naturally endowed with numerous vegetables and according to Izuogu et al. (2012), most of which are unexploited beyond traditional localities where they are found and eaten. They are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, minerals, vitamins, fibers and other nutrients which are usually in short supply in daily diets (Mohammed and Sharif, 2011; Asaolu et al., 2012). *Ceratotheca sesamoides* Endl. is closely related to *Sesamumindica* and is commonly referred to as 'false sesame' (Falusi et al., 2002). It is native to the northern parts of West Africa (Zeven and de Wet, 1982; Fasakin 2004). It is widely distributed in variable forms and consumed as a leafy vegetable in the savanna ecological zones of Nigeria (Nura et al., 2012). *Ceratotheca sesamoides* is found in tropical Africa like the open savanna woodlands across the region from Senegal to Northern and Southern part of Nigeria. It is known by various names such as eku (Yoruba-Western Nigeria), (False Sesame-English) (Adegoke et al., 1968; Fasakin and Olofintoye,

2005; Yakubu et al., 2012). It is an erect or sub-erect herb of about 60 cm tall (Yakubu et al., 2012). Its leaves and tender shoot portions are used for imparting a mucilaginous consistency on soup that is intended to be taken with starchy morsel prepared from cereal, cassava and yam (Fasakin and Olofintoye, 2005). The vegetables are cooked directly after shaking off the stones and unwanted materials in Western Nigeria. Washing of the vegetable is difficult as the mucilaginous compounds are released into the washing medium. Thus, utilization of the vegetable is limited due to poor farming techniques and handling. This vegetable had not been studied extensively like other vegetable species such as *Amarantus* species and *Corchorus olitorius* (Onwordi et al., 2009; Olayinka et al., 2012). African leafy vegetables had been reported to play a significant role in food security of the underprivileged and are also vital for income generation (Orech et al., 2005). Studying the properties of *Ceratotheca sesamoides* leaves could improve the utilization of the vegetable and also provide necessary information on the feasible uses of the crop. Therefore, this paper presents the chemical and antioxidant properties of *Ceratotheca sesamoides* leaves.

2. Materials and methods

Ceratotheca sesamoides leaves were harvested in October, 2013 on a farm at Iree, Osun State, Nigeria. The *Ceratotheca sesamoides* leaves were removed from the stems and dried at 50ºC for 24 hrs. The dried leaves were milled and packed in plastic container for analyses.

2.1. Proximate analyses

Proximate analysis (moisture content, ash, crude fiber, protein and fat) were determined using standard method of AOAC (1990). Carbohydrate was calculated by difference.

2.2. Radical DPPH Scavenging Activity

DPPH-free radical scavenging capacity of the vegetable extracts was evaluated according to the method of Chen and Ho (1995) and Enujiugha et al. (2012). A dose of 0.2 ml of the leave extracts was added to 3.8 ml ethanol solution of DPPH radical (final concentration was 0.1 mM). The mixture was shaken vigorously for 1min and left to stand at room temperature in the dark for 30 min. Thereafter, the absorbance for the sample (A_{sample}) was measured using the UV 160 spectrophotometer at 517 nm against ethanol blank. A negative control ($A_{control}$) was taken after adding DPPH solution to 0.2 ml of the respective extraction solvent. The percent of DPPH discoloration of the sample was calculated according to the equation:

$$\% \text{ Discoloration} = [1 - (A_{sample}/A_{control})] \times 100 \quad (1)$$

The free radical scavenging capacity of the seed extracts was expressed as an equivalent of Trolox. Every sample was extracted in triplicate and the results were calculated and expressed as micromoles curve of Trolox equivalents (TE) per 100 gram of leaves using the calibration curve of Trolox.

2.3. Amino acid determination

Amino acid determination was carried out using ion-exchange chromatography with Technicon Sequential Multi sample Amino Acid Analyzer, TSM (Technicon Instruments Corporation, Dubin, Ireland), as outlined in Adeyeye and Afolabi (2004) and Chinyere and Obasi (2011). 2.0 g of each of the processed samples were defatted with petroleum ether using Soxhlet extraction methods. The defatted samples were re-dried and milled into fine powder using porcelain pestle and mortar. 30.0 mg sample in triplicate were weighed into a glass ampoules to which 5.0 cm^3 of 6 M HCl and 5.0 µmol leucine were added. The ampoules were evacuated by passing nitrogen gas (to remove oxygen so as to avoid possible oxidation of some amino acid during hydrolysis), sealed with Bunsen burner flame and hydrolyzed in an oven at 110°C for 24 h. The ampoules were cooled, broken at the tip and the content filtered. The filtrates were

evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residues were dissolved to 5.0 µl (for acid and neutral amino acids) or 10.0 µl (for basic amino acids) with buffer; pH 2.2 and the solutions were dispensed into the cartridge of TSM. The chromatograms (amino acids peaks) obtained from automatic pen recorder correspond to the quantity of each amino acid present. Quantification was performed by comparing the peak of each amino acid in the sample to the area of the corresponding amino acid standard of the protein hydrolysis.

2.4. Mineral contents

Ceratotheca sesamoides vegetable (0.5 g) was weighed into a clean ceramic crucible. A blank was prepared with empty crucible. The crucible was placed in a muffle furnace at 500ºC for 4 hr. The sample was allowed to cool down in the oven after which it was removed carefully. The sample was poured into already labelled 50 ml centrifuge tube. The crucible was rinsed with 5 ml of distilled water into the centrifuge tube. The crucible was rinsed again with 5 ml of aqua regia. This was repeated to make a total volume of 20 ml. The sample was mixed properly and centrifuged (IEC Centra GP8) for 10 min at 301.86 g. The supernatant was decanted into clean vials for mineral determination. The absorbance was read on atomic absorption spectrophotometer (Buck Scientific Model 200A) at different wavelength for each mineral element (Cu-324.8 nm, Zn-213.9 nm, Ca-422.7nm, Fe-248.3 nm, Mg-285.2 nm, Mn-279.5 nm, Na-589 nm and K-766.5 nm) (Novozamsky et al., 1983).

2.5.Extraction and chromatographic analysis of carotenoids

Carotenoids were extracted according to the method described by Cardoso et al. (2009), with some modifications. About 5 g of each sample was titrated with cold acetone in a bench grinder and vacuum filtered through a Buchner funnel containing ash-free filter paper. The extract was transferred in a stepwise fashion to a separation funnel containing cold petroleum ether and then washed in distilled water until complete removal of the acetone. Anhydrous sodium sulfate was added to remove any residual water. The extract was concentrated in a rotary evaporator at 35 ºC. All chemical analyses were performed in the dark, with care taken to protect the pigments from light and oxygen by using amber glassware or aluminum foil to wrap the glassware.

The presence of carotenoids in the samples was analyzed by HPLC. 2 mL of the extract stored in petroleum ether was evaporated under a nitrogen flow and re-suspended in acetone. The extracts were then filtered through a 0.45-µm filter unit and 20 to 30 µL of the sample was injected into the chromatographic column. For high-performance liquid chromatography (HPLC), a Shimadzu LC chromatograph equipped with a high-pressure pump, manual injector with a 50-µL size loop, Lichrospher 100 column RP-18 (Merck; 5 µm, 250 mm long x 4 mm internal diameter), and UV-visible spectrophotometer detector (model SPD 10 AV, Shimadzu) was used. Mobile phase consisting of methanol:acetonitrile:ethyl acetate (80:10:10); flow rate of the mobile phase: 2.0 mL/min; run time: 13 min.

2.6. Statistical analyses

The analyses were carried out in triplicate. The mean and standard deviation of the data obtained were calculated using SPSS (17.0).

3. Results and discussions

3.1.Proximate composition of *Ceratotheca sesamoides* leaves

Proximate and antioxidant compositions of *Ceratotheca sesamoides* are shown in Table 1. The fat content of the vegetable was 1.79%. This value was low when compared to the values (4.00-5.25%) reported by Fasakin (2004) for *Ceratotheca sesamoides* vegetable. The crude fibers (6.21 %) and protein (28.92 %) contents observed in the vegetable were slightly lower than the values reported by Fasakin (2004) while the ash and carbohydrate contents were within the range reported.

Differences in the values obtained may be due to season of harvest, time, variety, maturity and methods of analyses. The *Ceratotheca sesamoides* vegetable had higher protein contents than *Melochia corchorifolia* (23.31%), *Amaranthus cruentus*(12.66%), *Celusia argenta* (9.35%) and *Corchorus olitorius* (11.24%)vegetables (Umar et al., 2007; Onwordi et al., 2009). Likewise, the protein contents of the vegetable were higher than the three vegetables (*Cnidoscolus chayamansa*, *Solanium nodiflorum*, and *Senecio biafrae)* determined by Adeleke and Abiodun (2010). The fat content of *Ceratotheca sesamoides* vegetable was higher than *Amaranthus cruentus, Celusia argenta* and *Corchorus olitorius* but had lower ash contents than these vegetables.

Table 1.Proximate and antioxidant composition of *Ceratotheca sesamoides*

Parameter	Value
Fat (%)	1.79± 0.86
Moisture (%)	9.82±0.43
Crude fiber (%)	6.21±0.22
Ash (%)	9.15±0.17
Protein (%)	28.92±0.57
Carbohydrate (%)	44.11±0.66
Antioxidant (µ moles TE / 100g)	72000 ±0.49

Mean±SD

The protein, crude fiber, ash and fat contents of bitter leaf (*Veronia amygdalina* L), India spinach (*Basella alba* L), bush buck (*Gongronema latifolium*), scent leaf (*Ocimium grastissimum*), Smooth amaranth (*Amaranthus hybridus*), Roselle plant (*Hibiscus sabdariffa*) and fluted pumpkin (*Telfaria occidentali*) vegetables analyzed by Asaolu et al. (2012) were higher than *Ceratotheca sesamoides* leaves. *Ceratotheca sesamoides* leaves contained appreciable amount of protein, ash and crude fiber which are used for growth, digestion and aid bowel movement in human.

3.2. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of C. sesamoides vegetable

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of *C. sesamoides* was presented in Table 1. In DPPH scavenging assay, the antioxidant activities of the leave extract was found to be 72000 µ moles TE / 100g. Higher antioxidant activities were observed in the *C. sesamoides* vegetable than extracts of *Mimusopselengi, Amaranthus* species and African yam beans (Saha et al., 2008; Enujiugha et al., 2012; Olayinka et al., 2012). Unlike other vegetables, such as *Amaranthus* species where blanching and boiling reduced the antioxidant properties (Olayinka et al., 2012), *C. sesamoides* vegetables are boiled directly into soup for short period of time thereby reducing loss of nutrients and antioxidants properties. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants (Saha et al., 2008). Antioxidants intercept free radical and protect cells from the oxidative damage that leads to aging and disease (Olayinka et al., 2012).

3.3. Amino acid profile of *Ceratotheca sesamoides* leaves

Amino acids profile of *Ceratotheca sesamoides* vegetables are shown in Table 2. The vegetable contained higher percentage of glutamic acid (2.010%) followed by aspartic acid (1.927%) and leucine (1.436%). Among the essential amino acids (EAA), leucine, arginine and the aromatic phenylalanine were the predominant amino acids while glutamic acid was the major non-essential amino acid (NEAA) in the *Ceratotheca sesamoides* vegetable.

Table 2. Amino acid profile of *Ceratotheca sesamoides* vegetable

Amino acid (%)	Value
HydroxyProline	0.00
Aspartic acid	1.927
Threonine	0.786
Serine	0.686
Glutamic acid	2.010
Proline	0.802

Glycine	0.768
Alanine	0.937
Valine	0.942
Isoleucine	0.816
Leucine	1.436
Tyrosine	0.708
Phenylalanine	0.982
Lysine	0.811
Histidine	0.362
Arginine	0.999
Total Hydrolyzed Amino acid	14.972
EAA	6.481
NEAA	8.491

Although, the amino acids of *Ceratotheca sesamoides* were lower than *Veronica amygdaline, Gnetum africana, Gongronemala tifolium* and *Ocimum gratissimum* reported by Chinyere and Obasi (2011) but similar trend was observed in the amino acids profile with highest value in glutamic acid (NEAA) and leucine (EAA). Likewise, amino acids composition of the leaves of *S. indicum* and *B. aegyptiaca* reported by Kubmarawa et al. (2008) were higher than the value obtained for *Ceratotheca sesamoides* vegetable**.** Hydroxyl proline and histidine were the limiting amino acid in the vegetable. The amino acids observed were lower than the reference standard amino acids profiles for food established for pre-school children and adults (WHO/FAO/UNU, 1985). The results showed that *Ceratotheca sesamoides* vegetable could not provide adequate amino acids for growth and repair of worn out tissues.

3.4.Mineral composition of *Ceratotheca sesamoides* leaves

Table 3 showed the mineral composition of *Ceratotheca sesamoides* vegetable. Potassium was the predominant mineral in the vegetable followed by calcium (1.08 %) with lowest value in sodium (0.04 %). The vegetable contained appreciable amount of iron (261.00 ppm), manganese (76.50 ppm), zinc (62.00 ppm) and copper (13.50 ppm). Higher calcium contents ranging from 2.65-3.15% were reported for *Ceratotheca sesamoides* leaves and seeds (Fasakin, 2004).

Table 3. Mineral composition of *Ceratotheca sesamoides*

Parameter	Value
Ca (%)	1.08±0.12
Mg (%)	0.36±0.15
Na (%)	0.04±0.09
K (%)	1.78±0.10
Mn (ppm)	76.50±0.11
Fe (ppm)	261.00±0.16
Cu (ppm)	13.50±0.11
Zn (ppm)	62.00±0.21

Mean±SD

The mineral compositions of *Ceratotheca sesamoides* leaves determined were higher than *Solanium nodiflorum and Senecio biafrae* reported by Adeleke and Abiodun (2010). Variations in the mineral values may be due the maturity, species, locations, soil type, climatic conditions, period of harvesting and cultural practices adopted during planting.

3.5.Carotenoid profile of *Ceratotheca sesamoides* vegetable

Table 4 showed the carotenoid contents of *Ceratotheca sesamoides* vegetable. The total carotene found in *C. sesamoides* was 32000 IU/100g. The major carotenoids detected were trans and cis beta carotene (Figure 1). Trans beta carotene (28700 IU/100g) was the predominant carotene detected in the vegetable. The least value was in alpha carotene which was present in insignificant amount. The vegetable are rich source of carotene which is a vitamin precursor and also act as antioxidant in the body thereby preventing disease (IITA, 2008). Carotenoids are stable in their natural environments but when food is heated they become much more labile (Coultate, 2002). On heating in the absence of air, there is a tendency for some of the *trans* double bonds of carotenes to isomerise to *cis*. In the presence of oxygen, particularly in dried foods, oxidation and bleaching occur rapidly.

Table 4. Carotenoid profile of *Ceratotheca sesamoides* vegetable

Carotenoid (IU/100 g)	Name
< 0.5	Alpha carotene
28700	Trans beta carotene
3320	Cis beta carotene
32000	Total beta carotene
32000	Total carotene

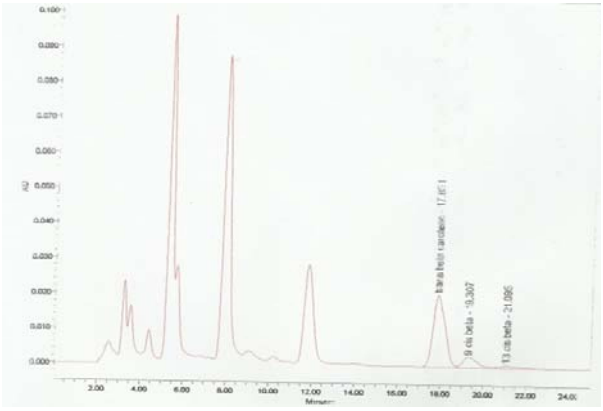

Figure 1. Carotenoids in *Ceratotheca sesamoides*

The carotenoid composition of foods are affected by factors such as cultivar or variety, part of the plant consumed, stage of maturity, climate or geographic site of production, harvesting and postharvest handling, processing and storage (Rodriguez-Amaya 1993, Rodriguez-Amaya, 2001).

4. Conclusions

C. sesamoides vegetable is among the underutilized vegetables in Nigeria with high potentials to curb nutritional deficiencies in the consumer. The properties of the vegetable revealed it to be a source of protein, mineral and possessed higher antioxidant activities coupled with the presence of carotene. Utilization of *C. sesamoides* vegetable could also add variety to our diet and reduce the incidence of vitamin A deficiency in our society.

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APPLICATION OF STARCH PROCESSING ENZYMES IN FOOD TECHNOLOGY-A REVIEW

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ABSTRACT

Starch is an important storage compound in plants that has many benefits for human beings. It has complex structure and made of two polymers: amylose and amylopectin; so the combination of enzymes is needed for its decomposi.tion Many of these enzymes exist in natural resources.

In industry, starch is used in producing different compounds. Some of them are made by chemical methods but others are produced by just enzymes. Starch processing enzymes are categorized into two groups based on their operation. The first group are hydrolases that hydrolyze glycoside bonds by water as endo and exo. The second group are glocanohydrolases which break one glycoside α-1.4 bond and creates a new glycoside α-1.4 or α-1.6 linkages. The aim of this article is a general review of these enzymes operation and application in food industry.

1. Introduction

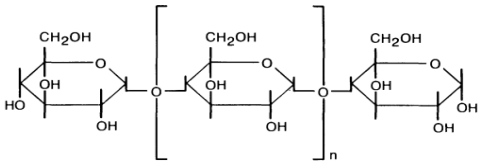
The starch is an important nutrition carbohydrate for human being which is stored in leaves, glands, seeds and roots of plants. This compound is the second plentiful heterogeneous polysaccharide after cellulose that produced by the plants (Yamada et al., 2009, Zeeman et al., 2010, Roy Goswamiet al., 2015). The most known sources of starch are cereals, potato, and tapioca which are used for starch extraction moreover direct consumption (Whitehurst and van Oort, 2010). Corn, wheat and potato are mostly used for producing industrial starch in Europe and America, whereas in Asia, casava, rice, sweat potato, yam and arrow root are used too. The annual production of starch is around 48 $\times 10^6$ ton in the world which nearly 70% of this is used in food industry (Daiuto et al., 2005).

Starch is stored in granolas. The shape and size of the granolas are varying in different sources. There are several methods to extract starch. Each method has different efficiency of extraction and the obtained starch has different practical properties too. During extraction, every damages to the crystalline phase of starch and depolymerizing of it is undesirable (Lee et al., 2007). The most common method of starch extraction is grinding the soaked raw substances for breaking cells' wall, separating substances by different sieves and finally separating starch from slurry by decanters or centrifuges (Daiutoet al., 2005; Zhang et al., 2009). In another method, fractionation is used for separating starch from protein. In this method, grinding is done for dry substances and then air is used for separating starch. This method is efficient and easy and there is no need to moving plenty amount of starch solution (Lee et al., 2007) but the produced starch shows weaker practical properties comparing with the starch from moist method

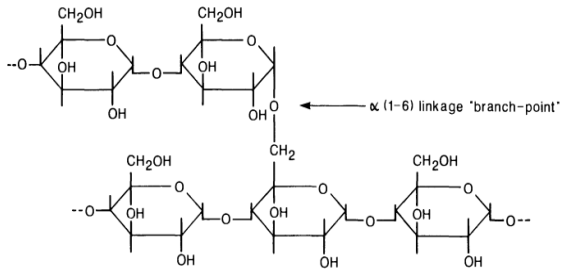
(Tian et al., 1999). In some methods, alkali is used for solubilizationof protein and extraction of starch (Cardosoa et al., 2007). The produced starch from this method has got high purity (Puchongkavarinet al., 2005) but comparing with the one produced from moist method, it shows lower pasting temperature and higher pasting viscosity (Dokicet al., 2010; Lai et al., 2004; Han and Hamaker, 2002).

2. Types of starch processing enzymes

The natural starch is made of about 20% amylose and 80% amylopectin. Amylose is linear polymer of glucose with α-1.4 bounds and its molecular weight is around 10^6 Dalton whereas amylopectin has 5% α-1.6 links in addition to mentioned bonds and its weigh is about 10^8 Dalton. In this way amylopectin has a lot of small branches but amylose is composed of fewer long branches (Srichuwong et al., 2005; Yoo and Jan., 2002). The structure of starch components is shown in Figure 1. The construct, molecular weight and proportion of amylose to amylopectin is different in extracted starch from different plant sources. This leads to diverse characteristics invarious types of starch (Syaharizaet al., 2010; Srichuwong et al., 2005). The waxy starches consist of plenty amount of amylopectin but the hylon consists of more than 50% of amylose (van der Maarel and Leemhuis. 2013). At the moment, near 30% of worldwide enzyme production has allocated to starch processing enzymes.In Table 1 most commercial applications of starch processing enzymes in food industry has been .shown



Amylose: linear polymer of glucose with α -1,4 links



Amylopectin; glucose polymer with α -1,4 links and branched with α -1,6 links

Figure 1. Chemical structure of amylose and amylopectin (Tucker and Woods, 1995)

Table 1. The most common commercial application of starch processing enzymes in food industry

Application	Enzyme/Enzymes
Starch liquefaction	α-amylase
Starch saccharification	α-amylase, glucoamylas,pullulanase,isoamylase, maltogenic amylase
Baking industry	α-amylase,

Anti-staling	α-amylase, β-amylase, pullulanase, debranched enzymes, branching enzymes, maltogenic amylase, glucoamylase, cyclodextrin glucanotransferases
Cycloamylose production	α-glucanotransferases

As starch polymer has complex constructer, combination of enzymes is needed for decomposing it to smaller parts or transferring oligoglycosidic bonds or creating new bonds (Bertoldo and Antranikian, 2002). The important enzymes which are usually used for biotechnology field and also starch process are called amylase. In one category, starch enzymes are classified in to two groups: a) hydrolases which hydrolyze glycosidic bonds by water in two ways endo and exo; b) glocanotransferases which break one α-1.4 bound and create new α-1.4 or α-1.6 bound.

Generally, the effective enzymes on starch can be categorized into four groups: endo-amylases, exo-amylases, de-branched enzymes and transferses (Hii et al., 2012). The best-known enzymes affecting on starch belong to glycoside hydrolases families 13, 15 and 28 (Whitehurst and van Oort, 2010). Specificationsof starch processing enzymes have been shownin Table 2. Table 3alsoincludes enzymessubstrates and products derived from the effects of these.

Table 2. Characteristics of starch processing enzymes

Activity	Enzyme name	EC	Enzyme Group
Hydrolysis of α-1,4 linkages	α-amylase	3.2.1.1	Endoamylases
Hydrolysis of α-1,4 and α-1,6 linkages	Glucoamylase	3.2.1.3	Exoamylases
Hydrolysis of α-1,4 linkages	α-Glucosidase	3.2.1.20	
Hydrolysis of α-1,4 linkages	β-amylase	3.2.1.2	
Hydrolysis of α-1,6 linkages	Amylo-1-6-glucosidase	3.2.1.33	De-branched enzymes
Hydrolysis of α-1,6 linkages	Pullulanase type I	3.2.1.41	
Hydrolysis of α-1,4 and α-1,6 linkages	Pullulanase type II	3.2.1.41	
Hydrolysis of α-1,4 linkages	Pullulan hydrolase types I (neopullulanase)	3.2.1.135	
Hydrolysis of α-1,4 linkages	Pullulan hydrolase types II (isopullulanase)	3.2.1.57	
Hydrolysis of α-1,6 linkages	Isoamylase	3.2.1.68	
Hydrolysis of α-1,4 linkages and formation of new α -1,4 linkages	Cyclodextrin glucanotransferases	2.4.1.19	α-glucanotransferases
Hydrolysis of α-1,4 linkages and formation of new α -1,4 linkages	4-α-glucanotransferase	2.4.1.25	
Hydrolysis of α-1,4 linkages and formation of new α -1,6	Branching enzyme (Q-enzyme)	2.4.1.18	

linkages			

Table 3. Substrates and products obtained from acting ofstarch processing enzymes

Product/products	Mainsubstrate	Enzyme name
Linear and branched oligosaccharides, α-limit dextrins	Starch	α-amylase
High glucose syrup	Starch	Glucoamylase
1- Glucose, isomaltose, isomaltortiose 2-Isomalto-oligosaccharides (IMO)	1-Starch 2-Oligosaccharidescontainingmaltose 3-Branched Oligosaccharides likely panose and isopanose	α-Glucosidase
β-limit dextrins	Starch	β-amylase
	Side chain containing of one glucose (product of transglycosylase enzyme)	amylo-1-6-glucosidase
Maltotriose	Pullulan, Amylopectin	Pullulanase type I
Maltose, maltotriose	Pullulan, Amylopectin	Pullulanase type II
Maltose, maltotriose	Amylopectin, glycogen	Isoamylase
α، β و ϒ-cyclodextrins, gluco-oligosaccharides with cycle α-1,4 bonds (scchardinger sugars)	Compounds containing of someconsecutive α-1,4 glycosidic bond	Cyclodextrin glucanotransferases
Glycogen, amylopectin	Compounds containing of someconsecutive α-1,4 glycosidic bond	4-α-glucanotransferase
Amylopectins and glycogen with more side chains	Starch, glycogen	Branching enzyme (Q-enzyme)

2.1. Endo-amylases

These enzymes can break α-1.4 glucosidic bonds that exist in inner part of amylase or amylopectin chain. Among these enzymes, there is α-amylase (EC1.1.2.3) which breaks mentioned bonds randomly but when reaches to α-1.6 linkages, these operation is stopped. In this way, they produce linear and branched oligosaccharides and α-limit dextrins (Kammoun et al., 2008; Van der Maarel et al., 2002). These enzymes are industrially obtained from bacterial and fungal sources. The bacterial types of α-amylases are really resistant to high temperature, react in normal pH and need to calcium ion for their activity. While the fungal α-amylases are nearly sensitive to temperature. These enzymes are maltogenic and their main product are maltose and other oligomers; so they are not used for liquefaction but are used as substitute of β-amylase for producing high maltose syrups (Souza and Magalhaes, 2010; Tucker and Woods, 1995).

2.2.Exo-amylases

These enzymes which consist of glucoamylase (EC 3.2.1.3) and α glucosidase (EC 3.2.1.2) break α-1.4 and α-1.6 bonds between glycosides from non-reducing end of amylase and amylopectin and therefore, they produce glucose. β-amylases (EC 3.2.1.2), which also belong to this group, break α-1.6 bonds exclusively from the end of non-reducing and therefore produce maltose and β-limit dextrins (Haki and Rakshit, 2003; Bertoldo and Antranikian, 2002).

2.2.1. β-amylases

These enzymes are exo-acting. They cannot separate α-1.6 branches like α-amylases, and can't turn the obstacles; so they produce high molecular weight dextrin that is well-known as β-limit (Li and Yu, 2011). These enzymes accompany with de-branched

enzymes are used for producing syrups in industry. The maltose syrups are hygroscopic and have more constant color than glucose syrups. Moreover, they are used in confectionary industry and producing frozen desserts because of less crystallization and adhesion (Tucker and Woods, 1995).

2.2.2. Glucoamylase

These enzymes which are known as amylo-glucosidase or sugar-making amylase separate α-1.4 bonds and in lower extent α-1.6 bonds from non-reducing end, similar pullulanase type II. (Whitehurst and van Oort, 2010). The high activity of this enzymewould produce high glucose syrups. This activity is done by using a lot of enzymes but this amount of enzyme may cause undesirable side reactions such as return reaction and producing isomaltose and maltose and therefore may decrease final output. These enzymes are acid like and sensitive to temperature. In industry, they are used for hydrolysis dextrins to glucose syrup in saccharification (Hii et al., 2012; Tucker and Woods, 1995).

2.2.3. α-Glucosidase

α-glucosidases are also exo-acting enzymes that separate amylose, amylopectin and oligosaccharides with maltose from non-reducing end and produce glucose (Latorre-Garcia *et al*., 2005; Tatsumi and Katano, 2005). When the maltose concentration is high enough, transglycosylation reaction is done by this enzyme and therefore maltose changes to isomaltose (two glucose with 1, 6 linkage). In the next stage isomaltose will be glycosylated and will produce isomaltotriose. Branched oligosaccharides like panose and isopanose can also be glycosylated by this enzyme. These compounds are generally well- known as isomalto-oligosaccharides (IMO) which are produced as prebiotic fibers in China and Japan (Whitehurst and van Oort, 2010).

2.3. De-branched enzymes

These enzymes catalyze hydrolysis of α-1.6 bonds and tend to separate amylopectin branches.This tendency is the main difference between de-branched enzymes and other hydrolysis enzymes because the main place of other hydrolysis enzymes is α-1.4 bonds (Hii*et al*., 2012). These enzymes are categorized into two main groups: direct de-branched and indirect de-branched enzymes (Fogarty and Kelly, 1990).

2.3.1. Indirect de-branched enzymes

Amylo-1,6-glucosidase (EC 3.2.1.33) is an indirect de-branched enzyme. In the beginning , the substrate of this enzyme has to be reformed by another enzyme because this enzyme can hydrolyze just the side chain which consists of one glucose molecule. For this reason, at first oligosaccharide was taken with transglucosylase enzyme (4-α-glucanotransferase, EC 2.4.1.25) and substrate was ready for acting of this enzyme (Nakamura, 1996). This enzyme does not widelyused in industry (Hii et al., 2012).

2.3.2. Direct de-branched enzymes

These enzymes directly hydrolyze α-1.6 bonds in unreformed substrates. Among these enzymes are pullulanases and isoamylases.

2.3.2.1. Pullulanases

Other names ofpullulanases EC 3.2.1.41 are α-dextrine 6-glucanohydrolase, amylopectine 6-glucanohydrolase, pullulan 6-glucanohydrolase and limit dextrinase. These enzymes are extracted from microbial sources and arehydrolyzed α-1.6 bonds in pullulan (the repeated units contain of three glucose residues with α-1.4 bonds which are linked together by one α-1.6 bond) (Zareian et al., 2010; Kuroiwa*et al*., 2005; Roy and Gupta, 2004). The final product by these enzymes is maltotriose (Kunamneni and Singh, 2006). Pullulanases type I hydrolyze α-1.6 bonds and produce branched dextrins. These enzymes are like isoamylases. Pullulanases type II hydrolyze both α-1.4 and α-1.6 bonds andoften

generate maltose and maltotriose. Recent enzymes are similar toglucoamylase and are commercially used in saccharification (Whitehurst and van Oort, 2010).

,So far five groups of pullulan hydrolyzing enzymes are reported that are categorized based on characteristics of the substrate and reaction of products:

Pullulanases type I: they hydrolyze α-1.6 bonds in pullulan and branched polysaccharides. Pullulanases type II (amylopullulanase): they hydrolyze α-1.4 or α-1.6 linkages and are used vastly in starch processing industry. Both enzymes don't affect on cyclodextrins (Roy et al., 2003; Ben Messaoudet al., 2002; Duffner et al., 2000). Neopullulanases (pullulan hydrolase type I) and isopullulanases (pullulan hydrolase type II) can break α-1.4 bonds and affect on cyclodextrins but not starch. The enzymes which analyze cyclodextrins faster than starch are called cyclodextrinase (Kaharet al., 2013). Since these enzymes are able to recognize the structural differences between α-1.4 bonds and α-1.6 bonds exactly, they are used vastly for analyzing polysaccharide and oligosaccharide structures (Roy et al., 2003). Pullulan hydrolase type III which were known by Niehause et al, unlike other pullulan hydrolyzing enzymes, can hydrolyze α-1.4 and α-1.6 bonds in pullulan that produce a mixture of maltotriose, panose, maltose, and glucose (Niehause et al., 1999). These enzymes can also analyze starch, amylose and amylopectin and produce maltose and maltotriose (Hii et al., 2012).

2.3.2.2. Isoamylase

Isoamylases (EC 3.2.1.68) can hydrolyze α-1.6 bonds. These enzymes are the only known ones which can debranche glycogen completely. They are made of two subunits with 45000 molecule weight (Zobel, 1992).

2.3.2.3. Comparing pullulanase and isoamylase

The main difference between these enzymes is in their substrates so that pullulanase hydrolyzes α-1.6 bonds in pullulan and amylopectin whereas isoamylase hydrolyzes these linkages in amylopectin and glycogen (Mikami et al., 2006). In other words, pullulanase needs two chains for actions containing at least two glucose residues with α-1.4 bonds which are joined with α-1.6 bonds whereas isoamylase prefers high molecule weight substrates. Another difference is their source so that the de-branched enzymes in yeasts and molds are mostly isoamylase but bacterial de-branched are mostly pullulanases (Hii et al., 2012; Van der Maarel et al., 2002).

Application of only α-amylase or β-amylase during starch saccharification processes produces plenty of α-limit and β-limit dextrins respectively and reduces the final viscosity of glucose. For this reason, the use of de-branched enzymes increases efficiency of glucose production. The hydrolysis of amylopectin and limit dexterins like produced dexterins by *Klebsiella planticola* and *Bacillus acidopullyticus* can be done by using bacterial pullulanase, but using fungal isoamylase is limited because of the lower temperature stability and lower pH adaptation with β-amylase.

Another problem for using isoamylase in saccharification is its inability in hydrolysis of side chains consisted of 2 and 3 glucose unit in α-limit and β-limit dextrins. Therefore the simultaneous action of β-amylase and isoamylase cannot change amylopectin to maltose. On the other hand, β-amylase is considered as isoamylse inhibitor. The existence of maltotrios and maltotetraose also inhibits isoamylse action competitively (Hii et al., 2012).

The biggest advantage of using pullulanase instead of isoamylase in saccharification is that adding stages of isoamylse is very critical. For example adding this enzyme to the hydrolysis system before amyloglucosidase can cause depolymerisation of amylopectin and therefore acceleration of the retrogradation (Guzman-Maldonado and Paredes-Lopez, 1995).

2.3.3. Application of de-branched enzymes

During saccharification process, α-1.6 bonds in starch act as an inhibitor for action of starch hydrolyzing enzymes and for this reason, hydrolysis continues with the usage of de-branched enzymes and efficiency of glucose production increases.

Application of glucoamylase alone can produce plenty of isomaltose. Since this compound is resistant to hydrolysi,s the efficiency of final production decreases (Crabb and Shetty, 1999; Uhlig, 1998). Using glucoamylase and pullulanase simultaneously can prevent by-product isomaltose. In this situation, pullulanase hydrolyses branch points specifically and then glucoamylase hydrolyses α-1.4 bonds in line chain (Hii et al., 2012). With utilization of de-branched enzymes together with glucoamylase, the speed of saccharification increases, the amount of needed glucoamylase decreases and less activity of glucoamulase is needed. In this way, the cost of pullulanase usage will be compensated by saving in vaporization cost and glucoamylase cost. Application of these two enzymes in fructose syrup process decreases the isomerization expenses too because of increasing capacity of factory as the result of decreasing saccharification time (Poliakoff and and Licence, 2007).

Nowadays de-branched enzymes are used in saccharification of starch, production of high maltose corn syrup, production of detergents and with CGTAs in production of cyclodextrins (Hii et al., 2012).

2-4. α-glucanotransferases

Application of a glucanotrnsferases or AGTAs (EC 2.4.2.XX) is made prevalent in two recent decades. These enzymes do not hydrolyze starch as amylases but at first break some parts of amylose and amylopectin molecules and then makes new bonds again (Van der Maarel and Leemhuis, 2013).

Substrates of α glucanotrnsferases are the compounds consist of some consecutiveα-1.4 glycosidic linkages like amylose, amylopectin, maltodextrins and glycogen. α-glucanotransferases belong to glycoside hydrolases (GH) family. Members of this family break glycosidic bonds and move them to acceptor (usually water). These enzymes can also link separate parts to other sugar and make new glycosidic bonds (so-called disproportionation reaction) (Oh et al., 2008). α-glucanotransferase used in industry belong to GH 13,57,77 families. All these enzymes are as α-retaining; it means that anomeric configuration of new made glycoside bonds is similar to broken bonds in substrate (Vocadlo and Davies, 2008). Reaction of these enzymes at first is started by breaking one α-1.4 glycosidic linkage in substrate and an enzyme-glycosyl intermediate is produced. In the next stage and after existing the fragmented sugar part from acceptor, non-reducing end from another glucan can enter acceptor site and the new bond is made. On the base that whether non-reducing end attack from 4-hydroxyle or 6-hydroxyle, the new bonds are as α-1.4- and α-1.6 respectively (Stam*et al*., 2006; Zona et al., 2004).

2.4.1. The types of α-glucanotransferases

Tree types of α-glucanotransferases have been known so far: I. Cyclodextrin α-glucanotransferase (CGTAs; EC 2.4.1.19), II. 4-α-glucanotransferase (4αGT; EC 2.4.1.25) which is sometimes shown as amylo-maltase, disproportionating or D-enzyme too, III. Branching enzyme (BE; EC 2.4.1.18) which is known as Q-enzyme (Van der Maarel and Leemhuis, 2013; Leemhuis et al., 2010; Kaper et al., 2004). In Table 4 there is industrial application of these enzymes briefly.

2.4.1.1. Cyclodextrin glucanotransferases (CGTAs)

For this group of enzymes the dominant reaction is intra-molecular transglycosylation which causes cycle product with 6, 7, 0r 8

glucose residues with α-1.4 bonds that are called α-, β- and γ-cyclodextrins (Vollu et al., 2008; Qi and Zimmermann, 2005). When the amount of glucan acceptor containing one free 4-hydroxile group ishigh , CGTAs can shift the glucan intermediate to another glucan chain and do inter molecular transglycosylation. In this way, a circular product is created (Whitehurst and van Oort, 2013; Van der Maarel and Leemhuis, 2013).

2.4.1.1.1. Application of cyclodextrin glucanotransferases (CGTAs)

These enzymes exist in bacteria and archaeas which areactive in high temperature. They are considered asthe first industrial AGTAs (Biwer*et al*., 2002; Leemhuis *et al*., 2010). The main usage of these enzymes is production of industrial cyclodextrins. α-, β-, and γ-cyclodextrins are considered asgluco oligosaccharides with cyclic α-1.4 bonds which are known as sacchardinger. These compounds have hydrophobic inner part and hydrophilic outer part because the sequence of their glucose residues. Therefore, they can complex with hydrophobic compounds and change their physical and chemical properties. These compounds are used as antiseptic factor (Whitehurst and van Oort, 2010). In food industry, α-, β-, and γ-cyclodextrins are also used vastly for taking cholesterol, stabilizing aromatic and sensitive compounds, solubilizing hydrophobic compounds in water, and eliminating undesirable taste and odor (Astray et al., 2009; Szente and Szejtli, 2004; Reineccius et al., 2003).

Wacker Chemie Company has offered α-cyclodextrins as indigestible diet fiber. This product is obtained from the effect of cyclodextrin glucanotransferases enzyme on the liquefied starch.Mentioned enzyme is extracted from *K.oxytoca*. This product has been produced with KAWAMAX W6 trade name and is used as a diet fiber in carbonated and noncarbonated soft drinks, dairy products, and bakery products (Whitehurst and van Oort, 2010).

CGTAs are also used for preventing of bread staling. Since the structure of this enzyme is highly similar to the structure of anti-stalingenzyme, Novomyl, the structural change in later enzyme changes it to a CGTAs. There are some reports which show that the mutant CGATs types improve the quality of bakery production too (Shim et al., 2007; Kelly et al., 2009).

2.4.1.2. Amylo-maltases or 4αGT

This enzyme in contrast to CGTAs does intermolecular transglycosylation. In this way, they *break* α-1.4 bonds and create new bonds. These new bonds are in the form of α-1.4 too. If the concentration of glucan acceptor containing a free 4-hydroxile group is little, these enzymes do intermolecular transglycosylation and create cyclic molecules containing 15 glucose residues with -1,4α bonds which are known as Large-ring cyclodextrins LR-CD (Srisimarat et al., 2011; Kaperet al., 2004; Terada et al., 1999).

2.4.1.2.1. Application of Amylo-maltase or 4αGT

In nature, these enzymes participate in glycogen metabolism in bacteria and also foundation of amylopectin in plants (Kaper et al., 2004). But in industry, the amylo-maltases of thermal resistant bacteria are used to transfer a part of amylose to non-reducing end of amylopectin. The obtained substance is composed of the amylopectins with long chains which can produce a white opaque gel with ability of thermal returning. Therefore, this substance is a suitable vegetal gel and can be used instead of gelatin which is an animal compound (Euverink and Binnema, 1998). The starch which is used in this purpose has to contain amylose (Hansen et al., 2009; Hansen et al., 2008). In this process, α-1.4 linkage between two glucose is broken and a new α-1.4 linkage is formed (Oh et al., 2008). The new substance is similar to primary substance in reducing power, percebranches, and average of molecular weight (van der Maarel et al., 2005).

Etenia is a trade name for a thermal reversible gel which is produced by AVEBE, a Dutch company. This substance is obtained by the effect of extracted amylo-maltase enzyme from *Thermus thermophiles* bacteria on potato starch. It is free of amylose and unlike starch, produces thermal reversible gel starch because it has amylopectin with side chains containing more than 35 glucose. The only difference between this gel and starch is its opal. This compound is used for making creamy texture in low fat products. Altinget *al.* applied this enzyme for increasing ceramin in yogurt. The mentioned enzyme is produced from B.amyloquefaciens bacteria by DSM Company (Altinget al., 2009; Munet al., 2009). In contrast to most enzymes which are used for starch processing, this enzyme doesn't secreted out of cells so the downstream process of its extraction is difficult (Whitehurst and van Oort, 2010). All modified starch with this enzyme contain amylopectins with long chains (Hansen et al., 2008).

When the high amount of amylose with high molecule weight is incubated with high amount of amylo-maltase, some compounds called cycloamyloses are produced. Cycloamyloses are large cycle compounds containing at least 16 glucose residues. Because of the large size of these compounds, they make two or more unparalleled spiral. In this way a hydrophobic channel is made. This channel is used for conservation of different compounds like drugs and also protection of proteins from deforming (Tomonoet al., 2002; Machida et al., 2000).

The starch that is resistant to digestion have been produced by using of amylo-maltase accompany with a de-branched enzyme like pullulanas. These products are called Promitor RS60 and RS75 and are linearmalto-oligosaccharids with different polymerization degrees which are made in crystal form. Pancreatic and saliva α-amylases has no effect on these crystals so usage of these compounds comparing with normal starch increases blood insulin and glycemic index less (Van der Maarel and Leemhuis, 2013).

2.4.1.3. Q- Enzymes (branched enzymes)

This group of enzymes is completely different as they break α-1.4 bonds and link produced α-glucan to 6-hydroxile groupin a linear glucan chain which has α-1.4 bonds itself. Therefore, one branch is made (van der Maarel and Leemhuis, 2013; Whitehurst and van Oort, 2010). These enzymes are in GH 13 and GH 57 families. GH 13 family affects on amylopectin and amylose whereas the members of GH 57 family affect on just amylose (Palomo*et al*., 2009; Palomo*et al*., 2011).

2.4.1.3.1. Application of Q- Enzymes (Branched enzymes)

Innature these enzymes are involved in constructingof side branches in amylopectin and glycogen (Zeeman et al., 2010; Murakami et al., 2006). Glycogen branched enzymes differs from starch branched ones in number of synthesized α-1.6bonds so that all types of starch branched enzymes make about 3.5 to 5% of α-1.6 bonds whereas the construction of these bonds in glycogen is more than 10 % (Whitehurst and van Oort, 2010).

The ability of these enzymes in breaking α-1.4 bonds and creating α-1.6 instead is lead to products which lack long α-1.4 chains. Therefore, they produce dextrins contain short side chains and as a result do not retrograde and have low viscosity (Spendler and Jorgensen, 1997). Novozym company has extracted a glycogen branched enzyme from *Rhodothermus obamensi s*thermophile bacteria that produces branched dextrin. Since this enzyme is stable up to 80°C, it seemed to be suitable for starch process (Shinohara et al., 2001). So far no branched dextrin has been produced as anti-stalling factor because of non-commercial production of enzymes (Van der Maarel and Leemhuis, 2013). The constant viscosity derivatives of starch are used for paper and texture industry (Bisgaard-Frantzen et al., 1999). Kim et al. in 2008 extracted a

glycogen branched enzyme from *Streptococcus mutans* that delayed retrogradation by changing the starch to more branched structure.

Q- Enzymes arealso used for producing branched dextrins which are digested slowly. The higherthe amount of branch in starch, the harder pancreatic amylose can degrade starch, therefore the level of blood glucose remains low (Whitehurst and van Oort, 2010). *Deinococcus radiodorans* is able to produce branched enzyme which changes amylopectin to a branched maltodextrin. This enzyme accompany with pancreatic amylase releases glucose slowly in vitro condition (Palomo et al., 2009). Roquete company has also produced a starch with low digestibility by a branched enzyme accompany with β-amylase (EC 3.2.1.2) (Dermaux et al., 2007). Using maltogenic amylase instead of β-amylose showed similar effect (Le et al., 2009).

Other products yielded by branched enzymes are cluster cyclodextrins (CCD). These compounds are used in sport drinks. This substance increases the time of being empty in stomach and can affect positively on body perseverance. Ezaki Glico Company extracted a branched enzyme from *Aquifexaeolicus* thermophile bacteria and applied it for converting corn starch to a cluster cyclodextrins. At present, this enzyme is produced by Nagase Company (Whitehurst and van Oort, 2010).

3. Conclusions

In this research the starch processing enzymes that are used for modification and conversion of starch and production of various compounds were studied. Some of these enzymes as alpha amylase and glocoamylase are used in large scale but the others are applied in smaller scale. Producing ofsweeteners is the most common application of these enzymes. Some other produced compounds by these enzymes have functional properties and their usage will have high potential influence on users' health. Despite performance ofvide researches and production ofvarious compounds, all starch processing enzymes have not been known thoroughly. Therefore many studying in the field of recognition and application of these enzymes for new products is being continued so that production of new refined and practical starch will be seen in future.

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EFFECTS OF *CITRUS AURANTIUM* EXTRACT AND PACKAGING UNDER VACUUM ON CHEMICAL, MICROBIOLOGICAL AND SENSORY CHARACTERISTICS OF RAINBOW TROUT DURING STORAGE AT REFRIGERATOR TEMPERATURE

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ABSTRACT
The intent of vacuum packing is usually to remove oxygen from the container to extend the shelf life of foods. The aim of this study was to determine the effects of *Citrus aurantium* extract and packaging under vacuum on shelf life of Rainbow trout during storage at refrigerator temperature. Fish fillets were immersed in traditional marinades and stored at -18 °C for 56 days. Some chemical and microbial characteristics like total volatile basic nitrogen (TVN), thiobarbituric acid (TBA), water holding capacity (WHC), pH, mesophilic, and psychrophilic bacterial count were performed with 7 days interval. The results were divided into four categories including the first control sample (without extract and packing), the second treatment (packed in the vacuum without extract), the third treatment (immersed in a solution of 1.5% extract for 30 min) and the fourth treatment (immersed in a solution of 1.5% extract for 30 min and packed in vacuum) were stored in a refrigerator (4±1°C). Sensory and some chemical and microbial characteristics like total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), and free fatty acids (FFA) were measured at days zero, 5, 10, 15, and 20. It seems that the *Citrus aurantium* extract and packaging under vacuum reduce the oxidation process and increases the shelf life of rainbow trout and can be an appropriate alternative for artificial preservatives.

1. Introduction

The rainbow trout (*Oncorhynchus mykiss*) is one of the most widely introduced fishes on a global basis. This fish has the ability to culture in most parts of Iran and has been one of the most desirable farmed fish in the last two decades (Salazar and el., 2016). Rainbow trout is very closely related to salmon which having the highest content f polyunsaturated fatty acids such as eicosapentaenoic acid and decosahexaenoic acid compared to other fish and seafood (LaPatra et al., 2015). Fatty fish such as rainbow trout has limited shelf life and quality deterioration of this species is mainly caused by rapid growth of microorganisms and lipid oxidation (Fontagné-Dicharry et al., 2014). So, the off-odor and off-taste of the products affect the consumer acceptability (Secci et al., 2016). Today, the addition of synthetic preservatives, antioxidants, colorants to extend shelf life has been revised by authorities due to certain health problems. Use

of natural preservative has been the subject of many investigations (Raeisi et al., 2016).

These days consumers are more willing to consume foods that are free of chemicals and artificial antioxidant. The use of natural antioxidants is recommended to replace synthetic antioxidants *Citrus aurantium* is as a natural antioxidant that is one of larges species among plant; it consists of 40 species which are distributed in all continents (Parhiz et al., 2015). Citrus is one of the most important fruits, which is consumed mostly fresh and has been used as an herbal medicine or additive or food supplement. Citrus is believed to possess bioactivities such as antioxidant, anti-inflammatory, antimicrobial, and is suggested to be responsible for the prevention of cancer and degenerative diseases (Abirami et al., 2014). Those bioactivities of citrus are due to the present of bioactive compound such as phenolics, flavonoids, essential oil, and vitamins (Park et al., 2015). It is a hybrid between *Citrus maxima* (pomelo) and *Citrus reticulata* (mandarin). Many varieties of bitter orange are used for their essential oil, and are found in perfume, used as a flavoring or as a solvent. The Seville orange variety is used in the production of marmalade. Bitter orange is also employed in herbal medicine as a stimulant and appetite suppressant, due to its active ingredient, synephrine (Marzouk, 2013). Bitter orange supplements have been linked to a number of serious side effects and deaths, and consumer groups advocate that people avoid using the fruit medically (Al-Juhaimi, 2014).

Vacuum packing is a method of packaging that removes air from the package prior to sealing. This method involves (manually or automatically) placing items in a plastic film package, removing air from inside, and sealing the package. Shrink film is sometimes used to have a tight fit to the contents. The intent of vacuum packing is usually to remove oxygen from the container to extend the shelf life of foods and, with flexible package forms, to reduce the volume of the contents and package Vacuum packing reduces atmospheric oxygen, limiting the growth of aerobic bacteria or fungi, and preventing the evaporation of volatile components. It is also commonly used to store dry foods over a long period of time, such as cereals, nuts, cured meats, cheese, smoked fish, coffee, and potato chips (crisps). On a shorter term basis, vacuum packing can also be used to store fresh foods, such as vegetables, meats, and liquids, because it inhibits bacterial growth. Vacuum packing greatly reduces the bulk of non-food items. For example, clothing and bedding can be stored in bags evacuated with a domestic vacuum cleaner or a dedicated vacuum sealer. This technique is sometimes used to compact household waste, for example where a charge is made for each full bag collected. In an oxygen-depleted environment, anaerobic bacteria can proliferate, potentially causing food-safety issues. Vacuum packing is often used in combination with other packaging and food processing techniques (Mohan et al., 2016). This study was to determine the effects of *Citrus aurantium* extract and packaging under vacuum on shelf life of rainbow trout during storage at refrigerator temperature.

2. Materials and methods

2.1. Sample preparation

Rainbow trout weighing 25 ± 250 were purchased from a trout farm located in "Noor" city in north of Iran. It transferred to Tehran university laboratory, then washed with drinkable water, and also abdominal drain and scaling were done and it cut off into 3-27 pieces. For the first day, 3 pieces were used as sample of first day and the rest of them were divided into 4 sections. Section one was placed in vacuum packing as control or blank sample. Another part of sample (section 2) was placed in *Citrus aurantium* which was extracted by GC Mass Chromatography at 4° C for 20 days. Section 3 was stored under vacuum section and the last sample was stored in vacuum packing with the *Citrus aurantium* which was extracted by GC Mass Chromatography. Chemical tests (TBA, TVB, FFA), Sensory evaluation (taste, odor, taste, color, appearance and texture),

microbial tests (Coliforms, psychrophilic, mold, yeast and total count of aerobic mesophilic bacteria) were carried out on the 4 samples of rainbow trout for 3 times within 20 days (zero, five, tenth, fifteenth and twentieth).

2.2. Determination of Thiobarbituric Acid (TBA) and Free Fatty Acid (FFA)

The TBA value (mg malonaldehyde (MDA) kg-1of fish flesh) was determined colorimetrically by the Porkony and Dieffenbacher method. Free fatty acid (%) was determined by the Kirk and Sawyer method (Binsi et al., 2016).

2.3. Determination of Total Volatile Base Nitrogen (TVB-N)

The amount of TVB-N (mg 100 g-1fish flesh) was determined by the direct water distillation method according to Goudlas and Kontoinas (Binsi et al., 2016).

2.4. Microbiological analysis

Psychrotrophic (TAPC) counts were performed using standard microbiological methods (Wu et al., 2014). Enumeration of Coliform was determined by pour technique according to AOAC, 1 mL of diluents was mixed with10 - 12 ml of VRBA (Violet Red Bile Salts Agar)/Mac-Conkey agar, then after solidification over layed with VRBA With MUG (5 - 10 ml) and incubated at 37C for 24 hours, all theca-lonies having 0.5 Mm in diameter were presumed as coliforms (Wu et al., 2014).

2.5. Sensory evaluation

The samples in each sampling period by panelist trained sensory parameters in accordance with the grading of the Council of Europe (EC) was graded. Sample of fish from each of the randomly in the same containers for evaluating the sensory properties Such as appearance, texture, color and odor part of the same fish that to evaluate the taste were Steamed. In panelist were put at the disposal of grading scheme EC, the excellent quality (E), good quality (A), medium quality (B) bad quality (C) respectively are given scores 4,3,2,1 and finally 3 as it was considered acceptable for human consumption (Yang et al., 2014).

2.6. Statistical procedure

The results were analyzed by repeated measure ANOVA test using SPSS Inc. software (v. 16.0, Chicago, IL).

3. Results and discussions

3.1. Chemical analysis

Amount of (TBA), (TVB) (FFA) had increasing trend over time in all Treatments But this increase in control samples was higher than others samples and the lowest increase in samples simultaneous application of packaging under vacuum and *Citrus aurantium* extract was observed and this increase in the sample *Citrus aurantium* extract was lower than samples under vacuum packed Due to the low free fatty acids in treatments containing *Citrus aurantium* extract may live on Antibacterial activity may be *Citrus aurantium* extract and thus reduce microbial activity as well as the enzymes secreted in fat fillet attributed (Table 3).Also The difference increases the amount of TBA treatments during the period Can be Attributed to lipid oxidation and peroxide converted into substances such as aldehyde (Table 2). TBA low in treatments containing *Citrus aurantium* and packed under vacuum can be attributed to a ntioxidant properties *Citrus aurantium* and prevent fat oxidation by packaging under vacuum (Al-Juhaimi, 2014). These results are according to the previous researches performed by kang et al, 2006 which is showing samples contained *Citrus aurantium* extract were significantly protective lipid oxidation. Increase in TVB during storage e can relate associated with activity spoilage bacteria TVB consisting of trimethylamine, ammonia and other nitrogenous compounds escape seafood is associated with corruption and by bacterial spoilage, autolytic enzymes, D-amino acids and nucleotides generated are produced (Wu et al., 2014). Low levels of TVB in

treatments containing *Citrus aurantium* and application of vacuum packaging and *Citrus aurantium* extract compared to the control can be due to antibacterial properties and decreased activity of phenolic compounds *Citrus aurantium* and a population of bacteria for amine oxidation and removal of non-protein nitrogen compounds due to less oxygen for vacuum packaging or both (Yang et al., 2014). Through inhibition of proteolytic bacteria that cause spoilage, inhibit the activity of these bacteria and break down protein and thus prevent their release nitrogen compounds. In general, the results of this study are consistent with previous studies howed that the use of different types of antioxidants, including vitamin C, alone or in combination with packaging mullet fillets stored oxidation process frozen delay (Table 1).

3.2. Microbiological analysis

Microbial test results showed that the number of psychrophilic microorganisms, mesophilic aerobic microbes, total count, coliform] had increased over time in all treatments but this increase was higher than other treatments in control samples and in samples containing *Citrus aurantium* extract and packaged under vacuum. The lowest increase was observed. As well as increasing the number of microbes in the samples with *Citrus aurantium* extract have been lower than control sample and vacuum packed samples. Generally, the reduction number of microbes that can be attributed to antimicrobial phenolic compounds in the extract. Also microbial counts in the samples packaged under vacuum can be attributed to the lack of sufficient oxygen for bacteria. The results were consistent with previous research results, as studies have shown that extracts of lemon, cloves, cinnamon and rosemary, especially cinnamon has an antibacterial activity against Gram positive bacteria and Gram- negative bacteria (Diao et al., 2014), *Citrus* fruits is a rich source of flavonoid glycosides, coumarins and glycosides (Shahnan et al., 2007) and the citrus flavonoids have a large spectrum of biological activity including antibacterial, antioxidant and antifungal activities. Manthey and Grohmann (2001) reported that polyphenol compounds such as *p*-coumaric, ferulic, and sinapic acids and narirutin were present in citrus extract.

Table 1. Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both onThe TVB during storage at refrigeration temperatures for 20 days (mea±SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
30/79±0/39^{a}	24/21±0/27^{d}	16/83±0/16^{g}	10/9±0/08^{n}	7/75±0/06^{m}	Control sample (T1)
27/57±0/09^{f}	22/52±0/20^{e}	16/52±0/06^{d}	10/40±0/04c	7/31±0/07^{b}	Vacuum packaging rainbow trout (T)
26/54±0/05^{t}	22/32±0/08^{f}	16/04±0/03^{s}	10/17±0/07	7/15±0/04^{i}	*Citrus aurantium* extract 1.5% (T3)
24/62±0/12^{d}	21/18±0/44^{g}	14/50±0/02^{k}	10/38±0/03^{i}	6/22±0/10^{n}	*Citrus aurantium* extract 1.5% percent and vacuum packaging (T4)

Means in each column followed by different letters are significantly different ($p<0.05$).

Table 2. Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both onThe TBA during storage at refrigeration temperatures for 20 days (mean ± SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
$0/76\pm0/01^{a}$	$08^{m}0\pm/0/19$	$0/29\pm0/01^{g}$	$0/26\pm0/01^{n}$	$08^{m}0\pm/0/19$	Control sample (T1)
$0/65\pm0/02^{f}$	$0/11\pm0/02^{b}$	$0/24\pm0/01^{d}$	$0/21\pm0/01^{c}$	$0/11\pm0/02^{b}$	Vacuum packaging rainbow trout (T_2)
$0/51\pm0/02^{t}$	$0/09\pm0/01^{i}$	$0/22\pm0/05^{s}$	$0/14\pm0/03^{k}$	$0/09\pm0/01^{i}$	*Citrus aurantium* extract 1.5% (T3)

Table 3. Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both onThe FFA during storage at refrigeration temperatures for 20 days (mean±SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
$2/6\pm0/01^{a}$	$1/8\pm0/01^{d}$	$1/02\pm0/01^{g}$	$0/6\pm0/005^{n}$	$0/4\pm0/01^{m}$	Control sample (T1)
$2/2\pm0/01^{f}$	$1/6\pm0/01^{e}$	$0/9\pm0/005^{d}$	$0/4\pm0/005^{c}$	$0/4\pm0/01^{b}$	Vacuum packaging rainbow trout (T_2)
$2/003\pm0.005^{t}$	$1/1\pm0/01^{f}$	$0/7\pm0/01^{s}$	$0/4\pm0.01^{k}$	$0/2\pm0/01^{j}$	*Citrus aurantium* extract 1.5% (T3)
$1/8\pm0/01^{d}$	$1/03\pm0/01^{g}$	$0/5\pm0/01^{k}$	$0/2\pm00/01^{i}$	$0/1\pm0/01^{n}$	*Citrus aurantium* extract 1.5% and vacuum packaging (T4)

Table 4. Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both on the total count of aerobic mesophilic bacteria during storage at refrigeration temperatures for 20 days (mean ± SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
$8/25\pm0/31^{a}$	$7/35\pm0/23^{d}$	$5/54\pm0/28^{g}$	$4/25\pm0/20^{n}$	$3/65\pm0/2^{m}$	Control sample (T1)
$7/57\pm0/27^{f}$	5/56±0/25e	$4/60\pm0/26^{d}$	$3/75\pm0/41^{c}$	$3/24\pm0/27^{b}$	Vacuum packaging rainbow trout (T_2)
$5/57\pm0/24^{t}$	$4/25\pm0/13^{f}$	$3/38\pm0/15^{s}$	$2/65\pm0/25^{k}$	$2/07\pm0/14^{i}$	*Citrus aurantium* extract 1.5% (T3)
$2/02\pm0/12^{n}$	$2/28\pm0/17^{i}$	$3/07\pm0/17^{k}$	$4/11\pm0/22^{g}$	$5/26\pm0/28^{d}$	*Citrus aurantium* extract 1.5 % and vacuum packaging (T4)

Means in each column followed by different letters are significantly different ($p<0.05$).

Table 5. Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both on the *Coliforms* during storage at refrigeration temperatures for 20 days (mean ± SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
8/433±1/32^{a}	8/29±0/38^{d}	6/57±0/25 ^{g}	5/35±0/46 ^{n}	3/85±0/13^{m}	Control sample (T1)
6/79±0/61^{f}	5/59±0/38^{e}	4/59±1/38^{d}	3/53±0/49 ^{c}	3/23±0/20^{b}	Vacuum packaging rainbow trout (T_2)
6/68±0/37^{t}	4/46±0/45^{f}	4/20±0/18^{s}	3/33±0/41^{k}	2/88±0/16^{i}	*Citrus aurantium* extract 1.5% (T3)
4/19±0/27^{d}	2/10±1/82^{g}	3/84±0/15 ^{k}	2/12±0/15^{i}	0/92±0/20^{n}	*Citrus aurantium* extract 1.5 % and vacuum packaging (T4)

Table 6. Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both onthe psychrophilic microorganisms during storage at refrigeration temperatures for 20 days (mean ± SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
8/66±0/39^{a}	7/68±0/32^{d}	6/11±0/26^{g}	4/73±0/24^{n}	4/02±0/20^{m}	Control sample (T1)
7/01±0/26^{f}	5/87±0/34e	5/01±0/17^{d}	4/56±0/21c	4/01±/15^{b}	Vacuum packaging rainbow trout (T_2)
5/95±0/28^{t}	4/70±0/22f	3/65±0/25^{s}	3/20±0/25^{k}	2/65±0/16i	*Citrus aurantium* extract 1.5% (T3)
5/70±0/23^{d}	4/61±0/17^{g}	3/57±0/22^{k}	2/84±0/15i	2/26±0/19^{n}	*Citrus aurantium* extract 1.5% and vacuum packaging (T4)

Table 7. Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both the sensory characteristics

Reception	Flavor	Texture	Odor	Color	Treatment
3±0/000^{a}	3±0/000^{a}	1±0/000^{a}	3±0/000^{a}	1±0/000^{a}	Control sample (T1)
2±0/000^{b}	2±0/000^{b}	1±0/000^{a}	2±0/000^{b}	1±0/000^{a}	Vacuum packaging rainbow trout (T_2)
2±0/000^{b}	2±0/000^{b}	1±0/000^{a}	2±0/000^{b}	1±0/000^{a}	*Citrus aurantium* extract 1.5% (T3(
1±0/000^{c}	1±0/000^{c}	1±0/000^{a}	1±0/000^{c}	1±0/000^{a}	*Citrus aurantium* extract 1.5% and vacuum packaging (T4)

Means in each column followed by different letters are significantly different ($p<0.05$)

Singh et al. (2010) stated that constituents such as gamma-terpinene, terpinolene, alpha-terpinene, hesperidin, neohesperidin etc. are responsible for the preservative action in

Citrus aurantium extract. The result of the present study indicated a positive role of. *Citrus aurantium* extractin on rainbow trout during storage at refrigerator temperature.

3.3. Sensory evaluation

As can be seen in Table 7. The use of *Citrus aurantium* extract and packaged under vacuum in rainbow trout have no effect on color and texture and treatments containing *Citrus aurantium* extract and packaged under vacuum are similar in terms of color and texture with control treatment. The factor of odor and flavor samples containing *Citrus aurantium* extract and vacuum packaging have had odor and flavor better than the control samples and the samples vacuum packed. The overall acceptability comments containing *Citrus aurantium* extract and packed under vacuum gained points better than the control and vacuum packed Sha'banpour et al. (2012) using thyme extract with vacuum packing in a refrigerated temperature on Rainbow trout and their results coincided the sensory properties of this study. Also, the results are consistent with the study of Chouliara et al. (2011) for Shank Fish.

4. Conclusions

The effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of vacuum packaging and *Citrus aurantium* extract to evaluate the shelf-life and anti-oxidant rainbow trout to examine in the refrigerator for up to 20 days were studied. The results showed that all samples compared to control samples have had higher shelf life and antioxidant properties as well as the simultaneous application of vacuum packaging and orange extract antimicrobial and antioxidant properties have been more and better compared to the control samples and other specimens also sensory evaluation of all the samples have been acceptable But samples containing combined use *Citrus aurantium* extract and packed under vacuum to gained the more points. Therefore orange juice and packaged under vacuum, especially using their combined can be used for preservation of Rainbow trout for human consumption.

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A COMPARATIVE STUDY ON THE PHYSICOCHEMICAL CHARACTERISTICS, ORGANIC ACID PROFILES, MINERAL COMPOSITIONS AND SENSORY PROPERTIES OF ICE CREAMS PRODUCED WITH DIFFERENT TYPES OF NUTS

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ABSTRACT

The aim of this study was to evaluate the selected physicochemical properties, organic acid profiles, mineral compositions and sensory properties of ice creams produced with hazelnut (HIC), almond (AIC), walnut (WIC) and pistachio (PIC). Obtained results showed that all physicochemical properties, and organic acid profiles of the ice creams affected by the addition of different types of nuts at the level of $p<0.01$. Ca, Cu, Mg, K, Zn and Na were determined in all ice cream samples, while Al was not found in any of the samples. However, Fe was determined in the WIC and PIC. Observing the sensory evaluations, colour and appearance ($p<0.05$), flavour ($p<0.05$) and overall acceptability ($p<0.01$) scores of the samples showed statistically significant differences from each other. The highest overall acceptability score belonged to HIC and it was followed by WIC, AIC, control and PIC samples, respectively.

1. Introduction

Ice cream is a complex colloidal frozen system and it consists of a lot of ingredients and components including milk, fat, sweeteners, emulsifiers, stabilizers, fruits and flavouring agents, air cells, ice crystals and a continuous aqueous phase (Goff et al., 1999; Koxholt et al., 2001; Frost et al., 2005). This product is consumed fondly worldwide especially during summer months. For that reason, production of different kinds of ice cream is of great importance. In recent years, natural, functional and aromatic additives such as fruits, nuts, plants and species are used for ice cream production (Diplock et al., 1999; Granger et al., 2005).

In recent years, nuts especially hazelnut, almond, walnut and pistachio are consumed popularly by the people due to their high unsaturated fatty acid compositions, phenolic compounds, mineral contents and functional properties (Gonzalez and Salas-Salvado 2006; Shahidi and Alasalvar, 2007). Nuts are known as a nutraceutical food sources and they have relatively high amount of lipid, fibre, Vitamin E and polyphenols. The regular consumption of them can significantly reduce the total LDL cholesterol level and cardiovascular disease risk of humans. Also, they reduce the risk of coronary heart disease, some types of cancer, physiological conditions and syndromes

(Abbey et al., 1994; Yurttas et al., 2000; Monagas et al., 2007).

Hazelnut (*Corylusavellana* L.) is a member of Betulaceae family and grows in temperate climates and humid regions. It is generally located along the coasts of the Black Sea Region of Turkey, in some areas of the United States (Washington and Oregon) and in southern Europe (Italy, Spain, Portugal, and France). Also, it is cultivated in Azerbaijan, Iran, China, New Zealand, Georgia and Chile (FAO, 2008). Hazelnut is evaluated in various ways by consumers such as raw fruit or snack and the production of various foods including chocolates, bakeries, cereals, dairy products, ice creams and candies. Hazelnut is an important source of unsaturated fatty acids, fibres, vitamins, minerals and phenolic compounds. It contains approximately 50–73% fat that is consisted of linoleic, linolenic, oleic, palmitic and stearic acids. For that reason, hazelnut and its oil is an important nutrient for human diet (Alphan et al., 1997; Özdemir and Akıncı, 2004; Köksal et al., 2006; Oliveira et al., 2008).

Almond (*Amygdaluscommunis* L.) is a member of Prunus belonging to the Rosaceae family. It is a perennial plant and grown in cold and temperate regions. The production of almond is focused in three regions of the world. These are Asian and Mediterranean countries, California and in limited amounts in Australia, South Africa, Chile and Argentina (Aslanta and Güleryüz, 2001). Lipid is the main component of almond seeds constituting the approximately 50% of the total weight of the seeds (Ren et al., 2001). Moreover, almond is rich with respect to phytochemicals, vitamin E, monounsaturated fatty acids, polyunsaturated fatty acids, arginine and potassium (Mandalari et al., 2010).

Walnut (*Juglansregia* L.) is a member of Juglandaceae family and it is one of the most important types of nuts in temperate regions (Ozcan, 2009). Walnut is an important nut for human nutrition and food industry in terms of organoleptic characteristics and health benefits. Walnut had beneficial effects on blood lipid and cholesterol levels of human due to its high poly unsaturated fatty acids content. These fatty acids reduce the risk of heart disease by decreasing total and LDL-cholesterol and increasing HDL-cholesterol level of blood. Health benefits of walnut stem from its chemical composition and they are important sources of essential fatty acids. The major fatty acid of walnuts is linoleic acid and is followed by oleic, linolenic, palmitic and stearic acids, respectively. It also contains important components including protein, carbohydrates, vitamins, pectic substances, minerals and phytochemicals (Amaral et al., 2003; Tapsell et al., 2004; Davis et al., 2007; Arranz et al., 2008; Pereira et al., 2008).

Pistachio nut (*Pistaciavera* L.) is one of the favourite kinds of nuts and only edible product of 11 genus of Pistacia. It is widely grown in the Middle East, Mediterranean countries and United States. On the other hand, Turkey is one of the important pistachio nut producers of the world (Shokraii and Esen, 1988; Kashaninejad et al., 2005). Pistachio nut is consumed by the consumers in different ways. It is generally consumed directly as snack, while it can be used for the preparation of confectionery, cakes, deserts, ice creams and chocolates (Peyman et al., 2013).

Organic acids are important components of dairy products and other foods adding to their flavour, stability and other quality characteristics. They occur in dairy products and foods during fermentation of carbohydrates, hydrolysis of fat, microbial activity during production or storage and addition of organic acids (Fernandez-Garcia and McGregor, 1994; Tormo and Izco, 2004). The determination of organic acids found in foods is extremely important with respect to determination of negative microbial activity

and changes in quality parameters of final product (Gonzalez de Liano et al., 1996; Adhikari et al., 2002).

Minerals are important food components for human nutrition and health. Mineral deficiency may cause many important diseases in humans. On the other hand, minerals have beneficial effects in small quantities, while they can be harmful if they exceed the limit values must be present in foods (Rodriguez Rodriguez et al., 2002; Caggiano et al., 2005).

The aim of this research was to quantify the changes in physicochemical properties, colour values, organic acid profiles, mineral compositions and organoleptic qualities of ice creams produced with different types of nuts. For this purpose, hazelnut, almond, walnut, and pistachio were used for ice cream production and ice creams were analysed in terms of specified parameters. As a result of this study, tried to determine that the advantage of different types of nuts against each other and to determine which one is the best for ice cream production in terms of quality parameters.

2. Materials and methods

2.1. Materials

Bovine milk and cream were supplied from the dairy farm of Atatürk University located in Erzurum, Turkey. Hazelnut, almond, walnut, and pistachio were used as the materials in ice cream production and they were purchased from a local shop in Erzurum, Turkey. Skim Milk Powder (SMP) was provided by Pınar Dairy Products Co., Turkey, while sugar, emulsifier (mono- and diglycerides) and sahlep or salep were purchased from local markets.In this study, sahlep was obtained by powdering of dried tubers of wild orchids from Kahramanmaraş city. It was used as a stabilizer in ice creamproduction for obtaining high water holding capacity, high consistencyand viscosity.

2.2. Manufacture of experimental ice creams

In this study, ice cream preparation was done as duplicate. For the production of each party of ice cream mix, milk was divided into five equal parts to make 3 kg of ice cream mix. Mixes were produced including 5% fat, 4.7% skim milk powder, 18% sugar, 0.6% sahlep (stabilizer) and 0.2% emulsifier (mono- and diglycerides). They were pasteurized at 85°C for 25 s and cooled to 4°C, and stored for 24 hours at 4°C for maturation. Then, all nuts were minced into small pieces (almost equal size)using a cutter at the same speed and time in order to add to the mixes and were added to each mix at the level of 7%, separately. The first mix was taken as the control and the remaining batches were prepared with hazelnut (HIC), almond (AIC), walnut (WIC) and pistachio (PIC). After all these steps, ice cream mixes were iced in ice cream machinery (-5°C; Ugur Cooling Machineries Co., Nazilli, Turkey) and hardened at -22°C for 24 h and then stored at -20°C for analyses.

2.3. Physicochemical analysis of experimental ice creams

Total solid, ash, fat, titratable acidity and pH values of experimental ice creams were determined by the method of Demirci and Gündüz (1994). The pH was measured with a pH meter (model WTW pH-340-A, Weilheim, Germany) fitted with a combined glass electrode (Demirci and Gündüz, 1994). Viscosity value was measured at 15°C by a digital Brookfield Viscometer, using a spindle LV-4, Model DV-II (Brookfield Engineering Laboratories, Stoughton, MA, USA).The temperature control of the samples was performed using a cooler (±15°C) during the measurement. Overrun was found by the equation [(weight of mix)-(weight of ice cream)/weight of mix×100]. For this purpose, a standard 100 mL cup was used to measure both the weight of ice cream mix and ice

cream (Jimenez-Florez et al., 1993). First dripping and complete melting times of ice cream samples were determined according to the method by Guven and Karaca (2002). For this analysis, hardened ice cream sample (25 g) was left on a 0.2 cm wire mesh screen above a beaker at room temperature (approximately 20°C) and first dripping and complete melting times of samples were found as seconds.

2.4. Colour analysis of experimental ice creams

Colour values of ice cream samples were determined in triplicate using a Minolta colorimeter (CR-200; Minolta Co., Osaka, Japan). These parameters were measured in port size of 20x15x10 cm with pulsed xenon arc lamp built into measuring head by CIE standard observer curves at room temperature and found *L**(lightness; 100=white, 0=black), *a** (redness; ±, red; -, green), and *b** (yellowness; ±, yellow; -, blue) colour parameters of the ice cream samples.

2.5. Organic acid analysis of experimental ice creams

Organic acid profiles of the ice cream samples were determined by the modified methods of Fernandez-Garcia and McGregor (1994) and Akalın et al. (2004) and for this purpose, a high-performance liquid chromatography (Agilent HPLC 1100 series G 1322 A, Germany) was used. At first, 4g ice cream sample was dissolved with 0.001 N H_2SO_4(25 mL) and centrifuged at the 5000x*g* for 10 min. Then, supernatant was filtered from Whatman No.1 filter paper and 0.45 µm membrane filter (PALL, USA), respectively. For each sample, 2 mL aliquot was stored in HPLC vials at -20ºC for HPLC analysis. 0.001 N H_2SO_4 was used as mobile phase at a flow rate of 0.6 mL/minute and the wavelength of detection was 210 nm for the quantification of organic acids. Duplicate injections (approximately 10 µL) were performed for each sample and the standard solutions of citric, orotic, malic, lactic, acetic, propionic and butyric acids were prepared in 0.001 N $H_2SO_4$for the detection of elution times and to prepare the calibration curves.

2.6. Mineral analysis of experimental ice creams

Mineral contents of the ice cream samples were determined by an Inductively Coupled Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT, USA) (Rodriguez Rodriguez et al., 2002, Caggiano et al., 2005; Güler, 2007). For this purpose, the ice cream samples were dried in microwave oven (Berghof speed wave, Germany) at 70ºC until the dry matter contents of the samples were at a constant value. At first, approximately 0.5 g of ice cream samples were weighed to the vessels and 10 mL (9: 1 v/v) of nitric acid (%65 HNO_3)/ perchloric acid (70-72% $HClO_4$) were added, and then samples were kept overnight in this way. After this period, the temperature of the samples was increased slowly to 160-170ºC by a hot-plate until the white smoke appeared. Finally, the samples were diluted with 50 mL with distilled water and filtered through the Whatman no. 42 filter paper in flask and the volumes of the samples were completed to 50 mL. All samples were analysed using an Inductively Coupled Plasma Spectrophotometer (ICP-OES) and the results were expressed as ppm.

2.7. Statistical analysis

The obtained data were analysed using the SPSS statistical software program version 13 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine the differences between results.

3.Results and discussions

3.1. General physicochemical characteristics of experimental ice creams

The results of total solids, ash, fat and protein values of the ice cream samples are presented in Table 1. The highest mean values of fat and protein were found in sample AIC and it was followed by WIC, HIC, PIC and control samples, while the highest mean total solids value was determined in PIC and followed by AIC, WIC, HIC and control samples. According to the statistical evaluations, there were statistically significant differences among the samples ($p<0.01$) with respect to quality characteristics. These determined differences among the ice creams can be explained by using different types of nuts.

As seen in Table 1, there were statistically significant differences ($p<0.01$) among the ice cream samples in terms of pH values. The pH values of control and WIC samples showed a similar trend with respect to statistical evaluations, while, HIC, AIC and PIC samples showed differences from each other and other ice cream samples (control and WIC) at the level of $p<0.01$. Observing Table 1, the highest mean pH value was found in AIC, although the lowest mean value was determined in PIC sample. These obtained results showed compliance with the organic acid profiles of nuts and ice cream samples.

According to Table 1, the highest mean viscosity value was found in WIC (11975 cP) and it was followed by PIC (11863 cP), AIC (3880 cP), HIC (3320 cP) and control (2773 cP), respectively. As seen in Table 1, WIC and PIC samples showed similar values in terms of viscosity changes, while other ice cream samples were completely different ($p<0.01$) from each other, and WIC and PIC samples statistically.Although all of the nuts were minced in equal size and added to the mixes in equal amounts, their different structural properties and hardness prevented forming homogenous mixture in the ice cream mixes.AIC and HIC remain hard and grain-structured in the ice cream mixes so, they are not effective in increasing the viscosity of mixes.This situation might occur as a result of the different structural characteristics, fibre, oil, mucilaginous compound contents of using nuts for ice cream production (El–Samahy et al., 2009).

Overrun (OR) is an increase in the volume of ice cream mix and this value shows the amount of air in the ice cream (Sofjan and Hartel, 2004). In the ice cream samples, the highest mean value of overrun (34.62%) was found in WIC sample and the lowest value (25.10%) was determined in the HIC sample. This situation might occur due to the fibre content, structural properties, oil contents and water binding capacities of using nuts. As seen in Table 1, control, AIC, WIC and PIC samples showed a similar trend with respect to statistical evaluations, although HIC sample was completely different ($p<0.01$) from them statistically.

The longest mean first dripping time and complete melting time values were found in HIC and followed by control, AIC, PIC and WIC samples, respectively (Table 1). The statistical evaluations showed that first dripping time values of C and HIC were similar to each other; and WIC and PIC samples showed a similar trend with respect to statistical evaluations. Contrary to this situation, this sample groups showed statistically significant differences ($p<0.01$) from in each other and AIC sample. On the other hand, AIC and PIC samples were similar in terms of complete melting time values, while control, HIC and WIC samples were found different from each other and AIC and PIC samples at the level of $p<0.01$. The differences in the first dripping and complete melting time values of the samples might be due to the different properties of nuts, the amount of air found in the mixes and treatments applied to the mixes (mixing

and hardening process) (Sakurai et al., 1995; Sofjan and Hartel, 2004).

3.2.Colour values of the experimental ice creams

Visual and sensory parameters of foods affect the consumer demands on them. Accordingly, determination of colour and sensory parameters of food products carries more importance. As seen in Table 2, the highest mean values of *L** (87.42) was found in control sample, and the lowest mean value (71.32) was determined in the WIC sample. The *L** values of the ice creams were found completely different ($p<0.01$) from each other statistically. The highest mean value (-0.37) of *a** was determined in HIC and it was followed by WIC (-0.80), AIC (-0.86), control (-3.72) and PIC (-4.97) samples, respectively. According to the statistical evaluations HIC, AIC and WIC samples were similar in terms of *a**colour values. Conversely, control and PIC showed statistically significant differences ($p<0.01$) from each other and this sample group. The highest *b** value was determined in the PIC (23.56) sample and this sample was followed by samples of AIC (15.30), WIC (14.77), HIC (14.47) and control (13.21), respectively. The *b** values of the AIC and WIC samples showed a similar trend with respect to statistical evaluations, although the control, HIC and WIC samples differed from each other and the other samples ($p<0.01$) statistically (Table 2).

3.3.Organic acid profiles of the nuts and experimental ice creams

The organic acid profiles of different types of nuts and ice cream samples are presented in Table 3 and Table 4. Citric acid is a weak organic acid and is found naturally in several fruits. It can be found in small quantities in milk and dairy products (Fernandez-Garcia and McGregor, 1994). As seen in Table 3, the highest mean concentration of citric acid was found in pistachio and it was followed by walnut, hazelnut and almond, statistically. All of the nuts showed statistically significant differences ($p<0.01$) from each other. Otherwise, the lowest mean concentration of citric acid was found in the AIC (35.69±0.91), while the highest mean value was determined in PIC (40.72±0.71) (Table 4). The obtained results showed parallelism with the organic acid profiles of nuts. According to statistical evaluations, HIC and AIC showed a similar trend, but control, WIC and PIC samples were completely different ($p<0.01$) from each other and HIC and AIC samples statistically. Orotic acid occurs during the biosynthesis of nucleic acids. It can be found in milk and dairy products in important quantities (Okonkwo and Kinsella, 1969; Fernandez-Garcia and McGregor, 1994; Güzel-Seydim et al., 2000; Kavaz and Bakirci, 2014). Observing Table 3, the highest mean concentration of orotic acid was found in walnut, while it was not detected in hazelnut and almond. Statistical evaluations showed that hazelnut, almond and pistachio were similar to each other, while walnut were found completely different ($p<0.01$) from them statistically (Table 3). As seen in the orotic acid concentrations of ice cream samples, the highest mean value was found in WIC and followed by PIC, control, AIC and HIC samples, respectively. Observing Table 4, control, WIC and PIC were similar and HIC and AIC showed similarity in terms of statistical evaluations. Contrary to this, sample groups showed differences from each other at the level of $p<0.01$. Malic acid is known as dicarboxylic acid and is generally used as flavouring agent and food additive in foods (Sniffen et al., 2006). The highest mean value of malic acid was determined in hazelnut, while it was not detected in walnut. Almond and pistachio were found similar in terms of malic acid concentration, while hazelnut and walnut

showed significant differences ($p<0.01$) from each other and other nuts statistically (Table 3). As seen in Table 4, the highest mean malic acid value was determined in HIC and it was followed by PIC, respectively. Conversely, malic acid was not found in control, AIC and WIC samples. Statistical evaluations showed that control, AIC and WIC samples were similar, but HIC and WIC samples showed statistically significant differences ($p<0.01$) in between and from other samples (Table 4). Lactic acid is the most important organic acid. It is generally found in significant amounts in milk, and fermented dairy products (Tormo and Izco, 2004; Kavaz and Bakirci, 2014). Observing Table 3, the highest mean value of lactic acid was determined in pistachio and it was followed by hazelnut, walnut and almond, respectively. According to the statistical evaluations, hazelnut, almond and walnut showed a similar trend, but pistachio was completely different ($p<0.01$) from them statistically (Table 3). The mean lactic acid values of ice cream samples ranged between 2.49 µg/g (control) and 254.4 µg/g (PIC) (Table 4). As seen in Table 4, AIC and WIC were similar with respect to statistical evaluations. Contrary to this, control, HIC and PIC samples showed differences from each other and AIC and WIC samples at the $p<0.01$ level. Acetic acid is a natural organic acid and occurs as an intermediate substance in the metabolism of plants and animals (Sholberg and Gaunce, 1995; Kavaz and Bakirci, 2014). As seen in Table 3, the highest mean acetic acid value was found in hazelnut and followed by walnut, almond and pistachio, respectively. Statistical evaluations showed that all of the nuts were statistically different ($p<0.01$) from each other. Observing Table 4, the highest mean acetic acid value was in WIC (1.91 µg/g), while it was not determined in other ice cream samples. According to the statistical evaluations all ice cream samples except for WIC showed similarity, while the differences of WIC from other samples was at the level of $p<0.01$.

Propionic acid can be formed naturally in the metabolism of human, animal and some plants. Propionic acid is widely used as a fungicide and bactericide for controlling the microbial activity in foods, feeds and pharmaceuticals (Anonymous, 1991). The highest mean value of propionic acid was in pistachio and followed by almond. Contrary to this, propionic acid was not found in other nuts. Pistachio was found statistically different ($p<0.01$) from hazelnut, almond and walnut (Table 3). As seen in Table 4, the highest mean value of propionic acid was in PIC, while it was not determined in control and HIC samples. The control and HIC were statistically similar and WIC and PIC showeda similar trend with respect to propionic acid values. These sample groups were statistically significantly different ($p<0.01$) form each other and from sample AIC (Table 4). Butyric acid is formed as a result of microbial activity, hydrolysis of milk fat, deamination of amino acids and during the anaerobic fermentation (Molkentin, 1998).

Table 1. The effect of different types of nuts on pysico-chemical properties of ice creams (mean±sd)

Icecream samples	Total solids (%)	Fat (%)	Protein (%)	pH	Viscosity (cP) 50 rpm	Over run (%)	First dripping times (s)	Complete melting times (s)
Control	41.23±0.12c**	5.30±0.12c**	5.31±0.21c**	6.68±0.01c**	2773±133.03d**	33.47±1.71a**	533±33.01a**	3039±838.94b**
HIC	41.62±0.47c**	6.20±0.23bc**	5.88±0.16b**	6.73±0.02b**	3320±456.36c**	25.10±0.61b**	545±71.94a**	6226±15.37a**
AIC	43.31±0.13b**	7.60±1.08a**	6.67±0.56a**	6.79±0.03a**	3880±543.98b**	32.40±2.01a**	443±28.87b**	2808±66.48bc**
WIC	43.19±0.12b**	7.30±1.05ab**	6.02±0.02b**	6.69±0.02c**	11975±95.45a**	34.62±2.98a**	372±22.25c**	2349±37.74c**
PIC	44.93±0.55a**	5.85±0.60c**	5.82±0.38b**	6.64±0.01d**	11863±313.13a**	31.80±0.23a**	406±26.36bc**	2603±20.35bc**

Table 2. The effect of different types of nuts on the colour parameters of ice creams (mean±sd)

Icecreamsamples	L*	a*	b*
Control	87.42±1.39a**	-3.72±0.18b**	13.21±0.29c**
HIC	77.42±0.63c**	-0.37±0.22a**	14.47±0.22bc**
AIC	82.40±0.85b**	-0.86±0.34a**	15.30±0.90b**
WIC	71.32±1.46e**	-0.80±0.16a**	14.77±0.39b**
PIC	74.20±1.33d**	-4.97±0.70c**	23.56±1.65a**

Table 3. Organic acid profiles of different types of nuts that used for the ice cream production (mean±sd)

Nuts	Citric acid (µg/g)	Orotic acid (µg/g)	Malic acid (µg/g)	Lactic acid (µg/g)	Acetic acid (µg/g)	Propionic acid (µg/g)	Butyric acid (µg/g)
Hazelnut	58.74±8.90b**	0.00±0.00b**	481.48±6.08a**	5.04±1.10b**	55.62±33.66c**	0.00±0.00b**	0.00±0.00
Almond	39.50±0.54c**	0.00±0.00b**	42.78±0.44b**	0.61±0.14b**	4.22±1.67b**	0.56±0.07b**	0.00±0.00
Walnut	72.00±1.50d**	0.24±0.10a**	0.00±0.00c**	3.24±1.27b**	144.79±2.59d**	0.00±0.00b**	0.00±0.00
Pistachio	84.89±1.97a**	0.02±0.01b**	40.30±1.26b**	3275.50±155.43a**	0.00±0.00a**	5.40±1.11a**	0.00±0.00

Table 4. The effect of different types of nuts on the organic acid profiles of the ice creams (mean±sd)

Ice cream samples	Citricacid (µg/g)	Oroticacid (µg/g)	Malicacid (µg/g)	Lacticacid (µg/g)	Aceticacid (µg/g)	Propionicacid (µg/g)	Butyricacid (µg/g)
Control	32.92±0.72d**	6.96±0.23a**	0.00±0.00c**	2.49±1.53d**	0.00±0.00b**	0.00±0.00c**	4.68±0.50bc**
HIC	35.76±1.39c**	6.62±0.13b**	37.77±0.71a**	12.76±0.07b**	0.00±0.00b**	0.00±0.00c**	6.62±0.46a**
AIC	35.69±0.91c**	6.65±0.13b**	0.00±0.00c**	4.31±0.52c**	0.00±0.00b**	10.62±1.82b**	4.54±0.48c**
WIC	37.22±0.48b**	7.15±0.18a**	0.00±0.00c**	4.35±0.40c**	1.91±0.69a**	12.14±0.23a**	4.40±0.84c**
PIC	40.72±0.71a**	7.08±0.16a**	4.82±0.35b**	254.41±1.09a**	0.00±0.00b**	12.75±0.30a**	5.62±0.75b**

Table 5. Mineral composition of different types of nuts used for the ice cream production (mean±sd)

Nuts	Ca (ppm)	Al (ppm)	Cu (ppm)	Mg (ppm)	Fe (ppm)	K (ppm)	Zn (ppm)	Na (ppm)
Hazelnut	1559.35±149.84b**	0.00±0.00	12.95±1.34ab**	2056.00±85.70b**	0.00±0.00c**	4850.15±464.78b**	18.50±0.42c**	53.65±2.19a**
Almond	1614.65±68.24b**	0.00±0.00	8.35±0.78c**	1557.55±85.06c**	0.30±0.42c**	7312.10±328.24a**	21.60±0.28c**	36.20±4.38b**
Walnut	1296.10±110.73b**	0.00±0.00	14.30±0.57a**	1865.45±144.32b**	4.25±6.01b**	3476.95±562.79c**	36.20±2.83a**	33.00±1.84b**
Pistachio	2581.15±224.36a**	0.00±0.00	10.60±0.57bc**	3127.15±109.53a**	26.85±4.17a**	5868.85±293.80b**	31.50±0.00b**	33.50±1.56b**

Table 6. The effect of different types of nuts on the mineral composition of ice creams (mean±sd)

Icecreamsamples	Ca (ppm)	Al (ppm)	Cu (ppm)	Mg (ppm)	Fe (ppm)	K (ppm)	Zn (ppm)	Na (ppm)
Control	4674.05±514.00a**	0.00±0.00	9.15±10.20b*	455.40±1.41d**	0.00±0.00b*	3766.35±173.74b*	26.05±0.7a**	678.30±2.55d**
HIC	2236.80±304.34b**	0.00±0.00	9.00±0.28b*	614.15±28.21c**	0.00±0.00b*	2876.40±181.30c*	14.05±0.21d**	806.10±4.81a**
AIC	4065.60±33.23a**	0.00±0.00	4.95±0.64b*	658.50±39.17bc**	0.00±0.00b*	4571.15±290.13a*	14.20±0.14d**	794.85±7.00a**
WIC	2935.40±229.81b**	0.00±0.00	23.85±2.05ab*	736.40±72.69b**	0.95±1.34b*	3796.55±476.24b*	22.95±0.07b**	750.00±2.97c**
PIC	3962.40±325.41a**	0.00±0.00	5.60±0.42b*	974.55±8.13a**	6.05±8.56a*	3633.90±43.98b*	17.95±0.07c**	777.45±7.42b**

Table 7. The effect of different types of nuts on some sensory properties of ice creams (mean±sd)

Icecream Samples	Colour andappearance	Texture	Gummingstructureandmelting in mouth	Flavour	Sweetness	Overallacceptability
Control	7.50±0.00ab*	7.11±0.33	7.20±0.64	6.74±0.01b*	7.45±0.06	7.17±0.03b**
HIC	7.68±0.14ab*	7.65±0.23	7.70±0.29	7.92±0.42a*	7.83±0.43	7.77±0.15a**
AIC	7.21±0.70b*	7.00±0.69	7.20±0.46	7.00±0.58b*	7.24±0.62	7.26±0.18b**
WIC	7.39±0.44ab*	7.53±0.61	7.37±0.05	6.86±0.53b*	7.39±0.33	7.35±0.23b**
PIC	7.92±0.21a*	7.70±0.17	7.37±0.32	6.63±0.72b*	7.56±0.30	7.06±0.33b**

Mean values ± standard deviations of ice creams manufacturing with duplicate samples. The letters a, b, c and d indicates means that significantly different atp<0.01 and p<0.05 levels; **: p<0.01, *: p < 0.05

According to Table 3, butyric acid was not determined in all nuts. As seen in Table 4, the highest mean value was determined in HIC and it was followed by PIC, control, AIC and WIC, respectively. According to the statistical evaluations, AIC and WIC were similar, while other samples were statistically different ($p<0.01$) from each other and this two ice cream samples.

Obtained results showed that organic acid profiles of ice cream samples showed compliance with the organic acid values of used nuts for ice cream production.

3.4. Mineral composition of the nuts and experimental ice creams

Mineral composition of the different types of nuts and ice cream samples were shown in Table 5 and Table 6, respectively.

In the nuts, the highest mean concentration of calcium (Ca) was found in pistachio and the lowest value was determined in the walnut. Pistachio was found statistically different ($p<0.01$) from other nuts (Table 5). As seen in Table 6, the highest mean Ca value was in control and followed by AIC, PIC, WIC and HIC, respectively. The Ca values of the control, AIC and PIC samples were similar to each other, likewise HIC and WIC samples showed a similar trend with respect to statistical evaluations, whereas these two sample groups were completely different ($p<0.01$) from each other statistically (Table 6). In this research, aluminum (Al) was not determined in any types of nuts and in any ice cream samples.

Observing Table 5, the highest mean value of copper (Cu) was determined in walnut and it was followed by hazelnut, pistachio and almond, respectively. Statistical evaluations showed that all of the nuts were different from each other at the level of $p<0.01$. As seen in Table 6, the highest mean values of Cu were found in WIC, and the lowest mean value was determined in the AIC sample. The Cu contents of control, HIC, AIC and PIC samples were similar to each other, although WIC showed differences ($p<0.05$) from them statistically.

As seen in the magnesium (Mg) content of the nuts, the highest mean value was in pistachio and followed by hazelnut, walnut and almond, respectively. Hazelnut and walnut were statistically similar in terms of Mg amounts, while almond and pistachio were different ($p<0.01$) in between and from hazelnut and walnut (Table 5). As seen in Table 6, the highest mean value of Mg was determined in PIC, whereas the lowest mean value was determined in control sample. According to statistical evaluations, all ice cream samples showed statistically significant ($p<0.01$) differences to each other (Table 6).

Iron (Fe) values of the nuts were determined as 26.85 ppm (pistachio), 4.25 ppm (walnut), 0.30 ppm (almond) and 0.00 ppm (hazelnut), respectively (Table 5). Hazelnut and almond showed a similar trend with respect to the statistical evaluations. Conversely, walnut and pistachio were different ($p<0.01$) in between and two other nuts statistically (Table 5). As seen in Table 6, the highest mean value was found in PIC, but Fe was not detected in three ice cream samples (control, HIC and AIC). All ice cream samples, except for PIC ($p<0.05$), showed similarity in terms of statistical evaluations (Table 6).

In the nuts, the highest mean concentration of potassium (K) was found in almond and followed by pistachio, hazelnut and walnut, respectively. Observing Table 5, hazelnut and pistachio were similar with respect to statistical evaluations. Contrary to this, almond and walnut were different from each other and from other nuts at the $P<0.01$ level. As seen in Table 6, the mean K values of ice cream samples ranged between 4571.15 ppm (AIC) and 2876.40 ppm

(HIC). According to statistical evaluations, control, WIC and PIC were similar and HIC and AIC were completely different ($p<0.05$) from each other and WIC and PIC samples statistically (Table 6).

The highest mean value (36.20 ppm) of zinc (Zn) was found in the walnut and the lowest mean value (18.50) was determined in hazelnut. Hazelnut and almond were similar statistically, while walnut and pistachio showed differences ($p<0.01$) from each other and other nuts statistically (Table 5). Observing Table 6, Zn concentrations of samples changed between the 26.05 ppm (control) and 14.05 ppm (HIC). HIC and AIC samples were found similar with respect to Zn concentrations, while other ice cream samples were completely different ($p<0.01$) from each other and HIC and AIC samples statistically.

Sodium (Na) values of the nuts were determined as 53.65 ppm (hazelnut), 36.20 ppm (almond), 33.50 ppm (pistachio) and 33.00 ppm (walnut), respectively. According to statistical evaluations, almond, walnut and pistachio were similar to each other, but these sample groups were statistically different ($p<0.01$) from hazelnut (Table 5). The highest mean value of Na was determined in HIC and it was followed by AIC, PIC, WIC and control samples, respectively. Observing the statistical evaluations, the HIC and AIC could be grouped together. Conversely, control, WIC and PIC samples were found statistically different ($p<0.01$) from each other and HIC and AIC samples.

From these results it might be said that K was the highest concentration mineral component that was found in the observed hazelnut, almond, walnut and pistachio, and it was followed by Mg, Ca, Na, Zn, Cu, Fe, respectively. As seen in the mineral amounts of the ice creams the most important one of the observed minerals was Ca and followed by K, Na, Mg, Zn, Cu, Fe, respectively. Generally, the mineral profiles of nuts and ice cream samples showed a compliance with each other. Obtained differences might stem from the milk, ingredients, used containers, storage and from other environmental factors.

3.5.Sensory properties of the experimental ice creams

The effect of different types of nuts on the sensory characteristics of experimental ice cream samples were presented in Table 7. PIC sample was appreciated in terms of colour and appearance, texture and gumming structure by the panellists, while the highest mean scores of gumming structure and melting in mouth, flavour, sweetness and overall acceptability scores were given to HIC sample. Statistical evaluations showed that the effects of different types nuts on the colour, appearance and flavour scores of the samples were at the level of $p<0.05$, while their effects on the overall acceptability scores were at $p<0.01$ level. However, other sensory parameters of the ice cream samples were not affected statistically by the addition of different types of nuts. As seen in Table 7, control, HIC, and PIC samples showed similarity in terms of colour and appearance scores, while AIC and PIC samples were found different ($p<0.05$) from each other and from other ice creams statistically. However, HIC sample showed statistically significant differences from control, AIC, WIC and PIC samples with respect to flavour ($p<0.05$) and overall acceptability ($p<0.01$) scores (Table 7).

4. Conclusions

Finally, the obtained results showed that using different types of nuts (Hazelnut, almond, walnut and pistachio) for ice cream production caused significant effects on the majority of observed quality characteristics of ice creams. Addition of nuts caused an

increase on the physicochemical properties and colour values of samples except for pH (PIC), overrun (HIC, AIC and PIC), first dripping (AIC, WIC and PIC) and complete melting time values (AIC, WIC and PIC), *L** (HIC, AIC, WIC and PIC) and *a** (PIC) values. From the obtained results, it might be said that the organic acid profiles and mineral composition of ice cream samples showed generally a compliance with the organic acid values and mineral amounts of nuts that were used for ice cream production. Generally, HIC and PIC samples came to fore with respect to observed sensory quality characteristics of ice creams. However, the highest overall acceptability score belonged to the hazelnut added ice cream sample. The obtained results also showed that each of the nuts gavedifferent values to the ice creams and they can be considered as suitable natural additives for ice cream production due to their nutritional values, flavour properties, organic acid profiles and mineral compositions.

5.References

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