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پست الکترونیکی: ifstrj@um.ac.ir

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Optimization of mucilage extraction conditions from *Plantago major L.* seed using response surface methodology

Younes Zahedi[1], Hadi Mahdavian Mehr[2], Seyed .M. A. Razavi[3*]

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Abstract

Identification of a new source of hydrocolloids is of interest due to their important effects on the textural attributes of food products. The objective of this study was to investigate the extraction conditions of *Plantago major L.* seed mucilage using a central composite rotatable design of response surface methodology. Temperature (25–85°C), pH (3–9) and water to seed ratio (50:1-50:4) were the factors investigated. Results showed that temperature was major factor in the extraction yield, whereas water to seed ratio and pH had minor effects on the yield. The maximum and minimum yields were 18.95% (conditions: temperature= 85 °C, water to seed ratio = 31.3 and pH= 6) and 6.35% (conditions: temperature = 25 °C, water to seed ratio= 31.3 and pH= 6), respectively. The optimal conditions were obtained at the temperature of 60 °C, water to seed ratio of 48.9 and pH of 3 in which predicted value for the extraction yield was 11.84%. The rheological properties of the mucilage, extracted at the optimal conditions, were investigated as a function of concentration at three levels of 3, 4 and 5% w/v, and shear rate ranged from 14 to 300s^{-1}. Mucilage dispersions showed non-Newtonian shear-thinning behavior at all studied concentrations. The Power law model well described the rheological behavior of the mucilage solutions with high determination coefficients (R^2>0.99). The flow behavior index (n) varied in the range of 0.30 to 0.36. The consistency coefficient (k) was in the range 6.13-17.81 Pa.s^n. Overall, *Plantago major L.* seed mucilage could be attended as a new beneficial source for use as a food thickening agent.

Keywords: *Plantago major L.*, Response surface methodology, Mucilage.

Introduction

Hydrocolloids are a wide range of polysaccharides and proteins that are widely used in food processing to provide thickening and gelling aqueous solutions, stabilizing foams, emulsions and dispersions, inhibiting ice and sugar crystal formation, the controlled release of flavors, etc. They can perform a significant influence on the textural and organoleptic properties of food products at concentrations of less than 1% (Phillips and Williams, 2009). Humans have traditionally used the gum and mucilage obtained from different plants for food preparation (Koocheki *et al.*, 2009b). Starch and its derivatives, galactomannans, carrageenans, pectin, agars, alginates, gum arabic and cellulose are mostly used as hydrocolloid in food systems (Karazhiyan *et al.*, 2009). Beside these commercial hydrocolloids, new sources of gum and mucilage from different seeds such as flaxseed, white mustard, fenugreek, prosopis flexuosa, mesquite, durian, *Lallemantia royleana, Salvia macrosiphon* and *Gleditsia triacanthos* have been introduced by researchers in the last decades (Koocheki *et al.*, 2009b). Each of them has individual composition which possibly confers particular functional properties.

Plantago major L. (PM) is a perennial plant that belongs to the *Plantaginaceae* family. Some of its common names are Soldier's Herb, Broad-leaved Plantain, Hen Plant, Lambs Foot, Road weed and White Man's Foot. PM produces large amounts of seeds (up to 20000 per plant). The seeds are quite small with an ovate shape and a slightly bitter taste. The seed endosperm has highly thickened cellulosic walls with the cell lumen filled with oil and

1. Assistant Professor, Department of Food Science and Technology, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran.
2 & 3. Former Ph.D Student and Professor, Department of Food and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad.
(*- Corresponding Author Email: s.razavi@um.ac.ir)
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protein. It forms the major part of the seeds and surrounds the embryo completely. The seeds are located in capsules (8–16 per capsule) and become sticky in humid weather due to the swelling of the polysaccharides present mainly in the seed coat. The seeds contain up to 30% mucilage including the monosaccharides glucose, fructose, xylose and rhamnose as well as the disaccharide sucrose and the trisaccharide planteose. The outer seed coat contains polysaccharides that swell in contact with water and forms mucilage with high viscosity (Samuelsen, 2000). The leaves and the seeds possess anti-bacterial, haemostatic, anti-complementary, anti-inflammatory, anti-septic, laxative, anti-nociceptive, anti-leukaemia, anti-carcinoma, anti-viral, ophthalmic and diuretic properties, and are also used as a remedy for dysentery and diarrhea, and treatment of parasitic worms (Samuelsen *et al.,* 1999; Türel *et al.,* 2009).

Aqueous extraction is the common method for the extraction of mucilaginous components from different seeds. Previous studies have been showed that different factors may influence the extraction parameters such as yield, protein content and rheological properties of the extracted gum (Cui *et al.,* 1994; Wu *et al.,* 2007; Koocheki *et al.,* 2009b; Razavi *et al.,* 2009; Bostan *et al.,* 2010; Karazhiyan *et al.,* 2011). The most important of these factors are the ratio of water to seed, pH and temperature, which could be different for various seeds and must be determined in a laboratory. Although the effects of some extraction conditions may be predictable, most of them are not known to researchers; for example an increase in water to seed ratio might result in increase of the extraction efficiency, but the influence of some extraction factors such as pH might be complex and sometimes minor (Cui *et al.,* 1994; Wu *et al.,* 2007; Koocheki *et al.,* 2009b; Bostan *et al.,* 2010), or sometimes noteworthy (Razavi *et al.,* 2009; Karazhiyan *et al.,* 2011). For this reasons, it is necessary to evaluate different extraction conditions for each of the gum or mucilage resources and select the best conditions from the aspects of the extraction yield, impurity, energy consumption, rheological characteristics and so on.

Response surface methodology (RSM) is a useful technique for the investigation of several input variables which influence the performance and quality characteristics of the product or process under investigation. The technique provides mathematical and statistical procedures to study relationships between one or more responses (dependent variables) and a number of factors (independent variables) (Karazhiyan *et al.,* 2011). RSM has been used to study the effect of the different extraction conditions on the hydrocolloids obtained from different sources and optimize the extraction process (Cui *et al.,* 1994; Wu *et al.,* 2007; Koocheki *et al.,* 2009b & 2010; Razavi *et al.,* 2009; Bostan *et al.,* 2010; Karazhiyan *et al.,* 2011). There is no published data for optimizing the mucilage extraction from PM seeds. Therefore, the objectives of this research were 1) to investigate the effect of the extraction temperature, pH and water to seed ratio on the extraction yield of mucilage from PM seeds, 2) to find out the optimum conditions for mucilage extraction from PM seeds using RSM. Furthermore, rheological parameters of the mucilage, extracted under optimum conditions, were measured.

Materials and methods

Materials

PM seeds were prepared from the local medical plant market, Mashhad, Iran. The seeds were manually cleaned to remove all foreign matter such as dust, capsules, stones and chaffs. All chemicals used in the assay were purchased from Dr Mojallali chemical laboratories (Iran) unless otherwise noted.

Extraction procedure

PM seeds mucilage was extracted from the whole seeds using of the distilled water (25 to 85°C) at pH 3-9. The seeds dispersed in the water and the slurry was mixed over 10 minutes and extraction was carried out using a centrifugal basket extractor after 2.5 hours hydration. The extract was vacuum filtered,

dried at 70°C, milled and then screened to achieve the fine powder. The extraction yield was calculated as percentage of hydrocolloid powder to the seed weight.

Preparation of mucilage dispersions

The mucilage powder, extracted under optimum conditions, was slowly added to the distilled water for approximately 15 min under constant stirring rate at room temperature. Then, it was stored at the room temperature for 24 h to complete hydration prior to rheological assessment.

Rheological measurement

Apparent viscosity of mucilage dispersions were measured at constant temperature of 25°C and different shear rates ranged from 14 to 300 s^{-1} at three concentration levels (3, 4, and 5% w/v) using a rotational viscometer (Visco 88, Bohlin instruments, UK) equipped with C30 measuring spindles (based on viscosity of dispersion) and a heating circulator (Julabo, Model F12- MC, Julabo Labortechnik, Germany). For each test, about 25 ml sample was transferred to sample compartment (bob and cup) following by 9, 10, 12 min pre-shearing at 50 s^{-1} to obtain uniform solution and time independent conditions for concentrations 3, 4 and 5% w/v, respectively. The shear rate was increased linearly from 14 to 300 s^{-1} in 4 min. The flow behavior index (n) and consistency index (k) values were computed by fitting the power law model (Eq. 1) using Slide Write software version 2.0.

$$\tau = k\dot{\gamma}^{n} \qquad (1)$$

Where, τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), k is the consistency coefficient ($Pa.s^{n}$) and n is the flow behavior index (dimensionless). The measurements were performed at least two replications.

Experiment design

The optimization experiments were carried out according to a central composite rotatable design (CCRD) with three variables including extraction temperature (25-85°C), pH (3-9) and water to seed ratio (50:1-50:4). As presented in Table 1, the coded values of the independent variable are arranged as -1.68 (lowest level), -1, 0 (medium level), +1 and +1.68 (highest level) which have the same predictive power in all directions from the centre point with the best precision. The complete design consisted of 20 experimental points including 6 replications of the centre point and the experiment was carried out in the random order. Parameter in mucilage extraction that was measured as dependent variable was the extraction yield. The data were analyzed using the Design Expert software (version 6.0.2) to fit the following quadratic polynomial equation:

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^{2} + \sum_{i=1}^{3} \beta_{ij} x_i x_j \qquad (2)$$

Where, Y is the dependent variable (extraction yield) and β_0 is a constant, β_i, β_{ii} and β_{ij} are regression coefficients of the model, while x_i and x_j are the code of the independent variables.

Results and discussion

Statistical analysis and modeling

The experimental data for extraction yield under different conditions are presented in Table 1. The maximum yield (18.95%) was obtained at the condition of temperature 85°C; water to seed ratio 31.3 and pH 6. The minimum yield was 6.35% that acquired at the temperature 25°C, water to seed ratio 31.3 and pH 6. The average yield of six middle points was 7.95% and standard deviation of ±0.245 that represent high reproducibility among those points. The second-order polynomial response surface model (Eq. 3) was fitted to the response variable i.e. yield (Y). For the corresponding fitting of the explanatory models and the variation of the extraction yield, the sum of squares of the sequential model was analyzed (Table 2). The value of lack-of-fit for regression equation of quadratic model was small in comparison with other model indicating that this model has a good fitness.

Regression analysis and ANOVA were used for fitting the model and to examine the

statistical significance of the terms. The estimated regression coefficients of the quadratic polynomial models for the response variables, along with the corresponding coefficients of determination (R^2) are given in Table 3. After elimination of non-significant terms, final equation (Eq. 3) for response variable fitted to empirical data:

$$Y = 7.93+3.65X_1 + 0.26X_3 +1.8X_1^2 + 0.55X_2^2 +0.25X_3^2 \quad (3)$$

Where, X_1 , X_2 and X_3 are temperature, pH and water to seed ratio, respectively.

Table 1. Process variables and experimental data for the three factors at five levels of response surface design of *Plantago major L.* seed mucilage.

Run order	Actual levels			Code levels			Yield (%)
	T (^{o}C)	pH	Water to seed ratio				
1	55	9.0	31.3	0	1.68	0	9.1
2	37	4.2	20.1	-1	-1	-1	6.73
3	73	7.8	42.4	1	1	1	14.4
4	55	6.0	31.3	0	0	0	8.021
5	73	7.8	20.1	1	1	-1	13.82
6	37	4.2	42.4	-1	-1	1	7.2
7	55	6.0	31.3	0	0	0	7.604
8	37	7.8	20.1	-1	1	-1	7.133
9	73	4.2	20.1	1	-1	-1	14.33
10	55	3.0	31.3	0	-1.68	0	10.19
11	55	6.0	31.3	0	0	0	8.229
12	55	6.0	31.3	0	0	0	8.125
13	55	6.0	50.0	0	0	1.68	8.666
14	55	6.0	31.3	0	0	0	8.021
15	85	6.0	31.3	1.68	0	0	18.95
16	55	6.0	31.3	0	0	0	7.708
17	73	4.2	42.4	1	-1	1	14.97
18	25	6.0	31.3	-1.68	0	0	6.79
19	55	6.0	12.5	0	0	-1.68	7.9
20	37	7.8	42.4	-1	1	1	7.76

Table 2. Analyze of sequential model sum of squares for the extraction yield.

Source	Sum of squares	DF	Prob > F	Lack of fit tests
				Prob > F
Mean	1885.35	1		
Linear	183.19	3	< 0.0001	< 0.0001
2FI	0.52	3	0.98	< 0.0001
Quadratic	48.63	3	< 0.0001	0.054
Cubic	0.10	4	0.98	0.005
Residual	1.63	6		
Total	2119.43	20		

Extraction yield

From the model of extraction yield, linear effect of extraction temperature and water to seed ratio and quadratic effect of all independent factors were significant ($P<0.05$), whereas no interaction terms were significant (Table 3). The results also showed that variables with the largest effect were the linear and quadratic terms of extraction temperature. This is because of the water temperature had

great effects on the mass transfer rate of the water- soluble polysaccharides in the cell wall (Shi *et al.,* 1996). Based on the sum of squares, the importance of the independent variables on the yield could be ranked in the following order: extraction temperature > water to seed ratio > pH.

The relationship between independent and dependent variables is illustrated in three-dimensional representations of the response surface and contour plots generated by the model (Figs 1a, b; 2a, b; 3a, b).

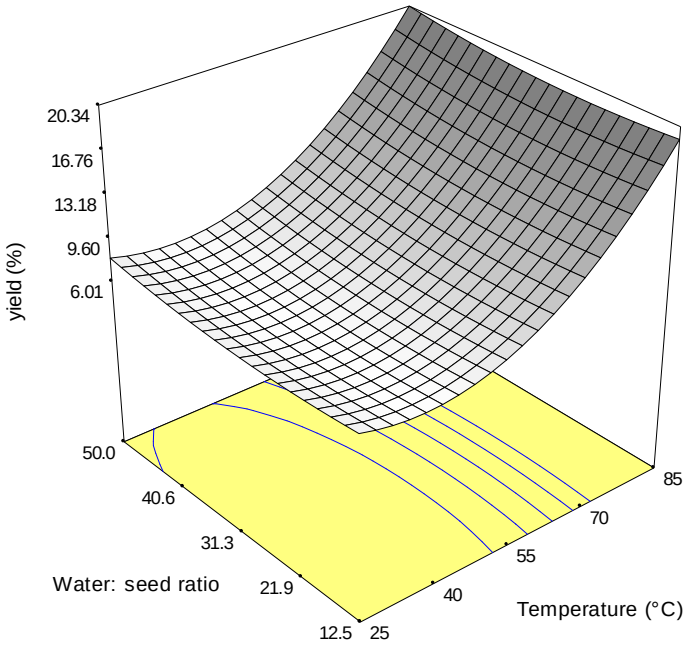
Table 3. ANOVA and regression coefficients of the second-order polynomial model for the extraction yield.

Source	DF	Coefficient	Sum of squares	P-Value
Model	9	7.930	232.35	< 0.0001
Linear				
β_1	1	3.650	182.24	< 0.0001
β_2	1	-0.017	3.806E-003	0.88
β_3	1	0.260	0.95	0.04
Quadratic				
β_{11}	1	1.800	46.56	< 0.0001
β_{22}	1	0.550	4.42	0.0005
β_{33}	1	0.25	0.93	0.04
Interaction				
β_{12}	1	-0.260	0.52	0.11
β_{13}	1	0.015	1.891E-003	0.92
β_{23}	1	0.012	1.176E-003	0.93
Residual	10		0.17	
Pure error	5		0.059	
Total	19			
R^2		0.993		
Adj- R^2	0.985			
C.V.		4.282		

The variation of yield with extraction temperature and water to seed ratio at constant pH 6 is presented in Figs. 1a and b. As it shows, the yield increased exponentially with temperature. It can be explained by the fact that the viscosity of the seeds mucilage reduces with increasing temperature and adhesiveness of seeds might be less than low temperatures. As a result, the mucilage can be easily released from the seeds and the extraction yield raises (Koocheki *et al.,* 2009b). Similar plots were drawn for the temperature and pH at water to seed ratio of 31.3 (Figs. 2a and b). In the plots demonstrated for pH and water to seed ratio (Figs. 3a and b), extraction yield at initial and terminal pH was maximum and as the water to seed ratio increased, it enhanced exponentially. This is because more water would dissolve more mucilaginous substances of the seeds.

Effect of temperature on the extraction yield

Temperature was the major factor affecting the extraction yield of PM seed mucilage. As the temperature increased from 25°C to 85°C, the yield increased from 6.79 to 18.95%, irrespective of the changes in pH or water to seed ratio. This may be due to the fact that high temperature led to solubility increase of mucilaginous components and weakening of adhesive force between this components and seed hull. Other researchers have reported the same results for flaxseed gum, boat-fruited sterculia, wild sage, basil and *Lepidium perfoliatum* seeds (Cui *et al.,* 1994; Wu *et al.,* 2007; Koocheki *et al.,* 2009b; Razavi *et al.,* 2009; Bostan *et al.,* 2010; Karazhiyan *et al.,* 2011). Influence of temperature on the yield has not always been noteworthy at high degree. For instance, research of Sepulveda *et al.* (2007) showed that the temperature had no remarkable influence on the mucilage yield of *Opuntia ficus indica*. Also in other study, the highest yield of polysaccharides was obtained at 80 °C from *Opuntia milpa alta* seed, while using high temperature decreased the yield because of increasing the hydrolysis of polysaccharides (Cai *et al.,* 2008).



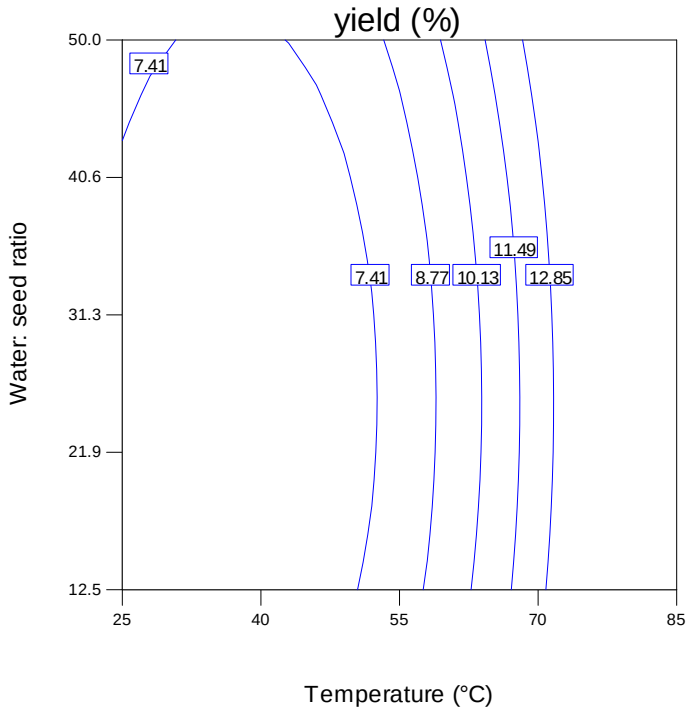


Figure 1. Response surface (a) and contour (b) plots for the effect of temperature and water to seed ratio on the yield extraction.

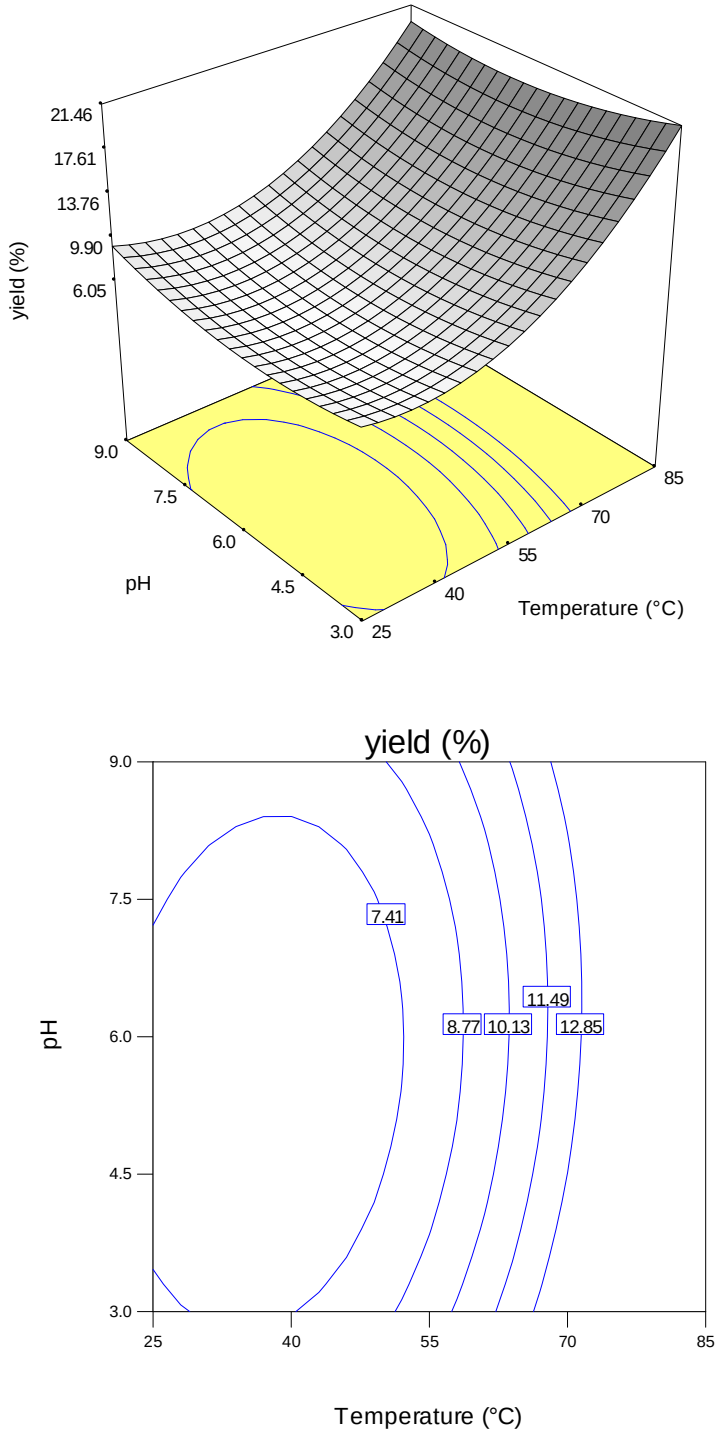


Figure 2. Response surface (a) and contour (b) plots for the effect of pH and temperature on the yield extraction.

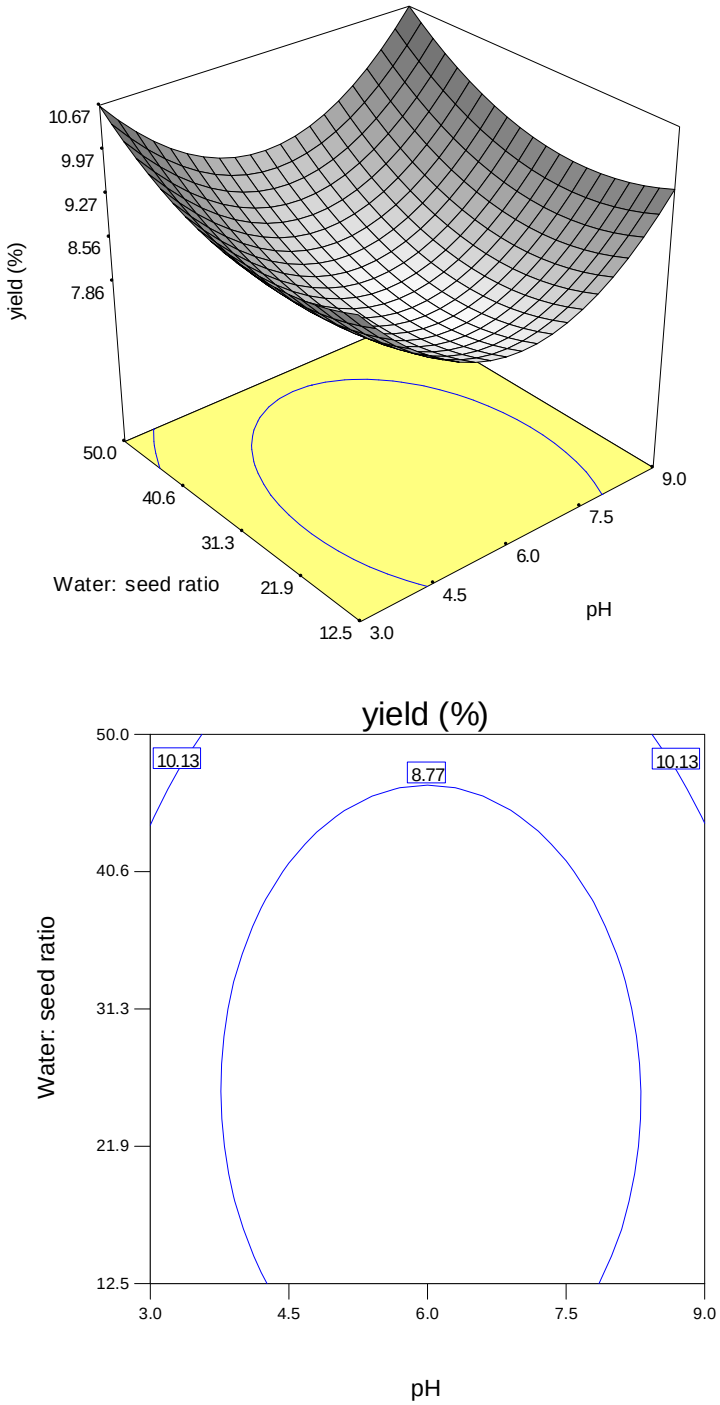


Figure 3. Response surface (a) and contour (b) plots for the effect of pH and water to seed ratio on yield extraction.

Effect of water to seed ratio on the extraction yield

Water to seed ratio had significant effect on the extraction yield of PM seed mucilage. As water to seed ratio increased, the extraction yield continuously increased too. This is due to the availability of more liquid which increases the driving force of mucilage out of the seeds

(Koocheki *et al.,* 2009b). Similar effects were reported for boat-fruited sterculia, wild sage and *Lepidium perfoliatum* seeds (Wu *et al.,* 2007; Koocheki *et al.,* 2009b; Bostan *et al.,* 2010). However, water to seed ratio showed non-significant effect on the extraction yield of flaxseed gum (Cui *et al.,* 1994). Also, Sepulveda *et al.* (2007) reported that mucilage yield of *Opuntia ficus indica* indicated a small tendency to increase when the amount of water used for the extraction was increased. Cai *et al.* (2008) observed that the yield of the extracted polysaccharides from *Opuntia milpa alta* seeds initially increased as the ratio of water to seed increased to 3-4 folds, but more increasing of this ratio caused to the yield reduction.

Effect of pH on the extraction yield

The yield of mucilage extraction from PM seed was not influenced by the pH. This result is in agreement with findings of Koocheki *et al.* (2009b) and Bostan *et al.* (2010) for *Lepidium perfoliatum* and wild sage seeds, respectively. Cui et al. (1994) reported the impact of pH on the mucilage separation is minor and only very acidic pH was relatively suitable for the extraction. The pH had significant effect on the yield of polysaccharide of boat-fruited sterculia seeds and the neutral pH provided the maximum extraction yield (Wu *et al.,* 2007).

Optimization

The optimum conditions for extraction of PM seed mucilage were determined to achieve the extract with appropriate quality and yield. For this purpose, the temperature was set in 60 °C, pH and water to seed ratio was selected in range of 3-9 and 12.5-50, respectively and the extraction yield was set on maximum. After optimization, 10 points was suggested that prime point (temperature= 60°C, pH= 3 and water to seed ratio= 48.9) with desirability of 0.66 was selected (Table 4). The predicted extraction yield under these conditions was 11.84%.

Table 4. Ten optimized conditions to achieve to the maximum extraction yield.

Number	Temperature (°C)	pH	water to seed ratio	yield (%)	Desirability
1	60	3	48.9	11.84	0.660
2	60	9	50	11.71	0.652
3	60	8.8	50	11.56	0.643
4	60	3	43.8	11.41	0.634
5	60	9	43.9	11.15	0.616
6	60	9	12.5	10.75	0.591
7	60	9	13.1	10.72	0.589
8	60	9	28.6	10.47	0.571
9	60	8.6	12.5	10.39	0.566
10	60	3.8	12.5	10.36	0.564

Rheological properties

The PM seed mucilage dispersions showed a non-Newtonian behavior and shear-thinning (Pseudoplastic) flow. When the flow behavior index is less than 1, it means that the dispersion macromolecules have oriented in network and they have aligned in the direction of the shearing force (Cancela *et al.,* 2005). The power law was suitable model to describe the flow behavior of the PM seed mucilage dispersions with high determination coefficients (R^2>0.99) for each concentration level. As the concentration of the mucilage solution increased, the consistency coefficient (k) increased, while flow behavior index values (n) decreased (Table 5). Similar results have been observed for some other hydrocolloids such as *Alyssum homolocarpum* mucilage (Koocheki *et al.,* 2009a), salep (Farhoosh and Riazi, 2007) and carboxy methylcellulose (Cancela *et al.,* 2005).

Table 5. The power law equation parameters for *Plantago major L.* seed mucilage dispersions at different concentrations*.

Parameter	3%	4%	5%
n (dimensionless)	0.36± 0.02	0.32± 0.03	0.30± 0.007
k (Pa.s^n)	6.13± 0.33	10.36± 0.54	17.81± 0.22
R^2	0.998	0.998	0.996

*The values are means of at least two replications.

Values of flow behavior index of PM seed mucilage were lower than those of the salep, cashew, starch and pectin dispersions (Mothe and Rao, 1999; Marcotte *et al.*, 2001) indicating great tendency of this mucilage to shear-thinning behavior. Whatever the mucilage solution possess high pseudoplasticity, the mouth feel characteristic will be favorite. The effect of shear rate on the apparent viscosity of the PM seed mucilage is shown in Fig. 4. It can be seen that the apparent viscosity of mucilage dispersions were shear rate dependent at all concentrations and it was maximum in low shear rates, but the apparent viscosity decreased sharply at shear rate around 50 s^{-1}. The apparent viscosity was governed of the mucilage concentration, and as the concentration increased, the apparent viscosity enhanced. This can be explained by the fact the higher solid contents generally lead to an increase in the viscosity resulting from mainly molecular movements and interfacial film formation (Maskan and Gogus, 2000).

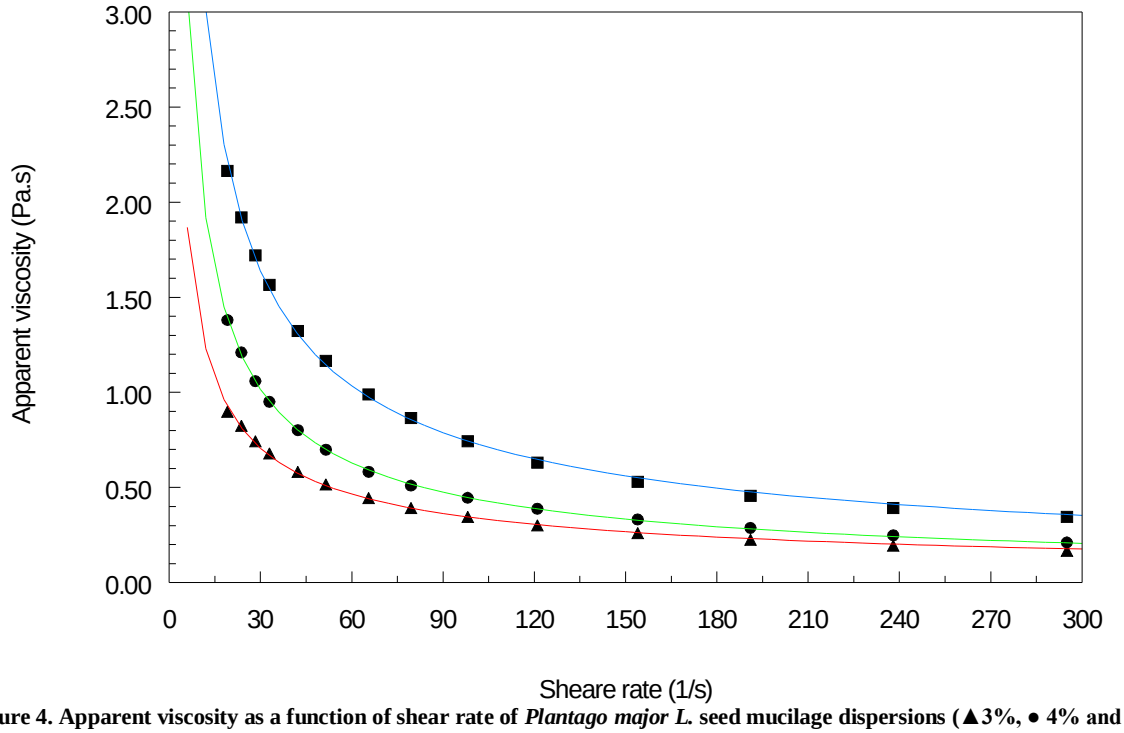


Figure 4. Apparent viscosity as a function of shear rate of *Plantago major L.* seed mucilage dispersions (▲3%, ● 4% and ■ 5%) at temperature 25°C.

Conclusion

This study investigated of the optimal conditions for the mucilage extraction from PM seeds. Results indicated the temperature was most important factor in the extraction process and as the temperature increased, the extraction yield increased. A pseudoplastic behavior observed for the mucilage dispersions at all concentrations. The power law model well described the rheological behavior of the mucilage dispersions with high determination coefficients. Increasing the solution concentration increased consistency

coefficient, while the flow behavior index decreased.

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بهینه‌یابی شرایط استخراج موسیلاژ از دانه بارهنگ توسط روش سطح پاسخ

یونس زاهدی[1]- هادی مهدویان مهر[2]- سید محمدعلی رضوی[3*]

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چکیده

به دلیل نقش و جایگاه هیدروکلوئیدها در ویژگی‌های بافتی فراورده‌های غذایی، شناسایی منابع جدید آنها حائز اهمیت و مطلوب است. هدف از این پژوهش بررسی شرایط استخراج موسیلاژ از دانه بارهنگ توسط طرح مرکب مرکزی چرخش‌پذیر روش سطح پاسخ بود. درجه حرارت (°C 85-25)، pH (9- 3) و نسبت آب به دانه (50 به 1 الی 50 به 4) فاکتورهای مورد بررسی بودند. عامل اصلی تاثیرگذار بر میزان بازدهی استخراج، درجه حرارت بود، در حالی‌که نسبت آب به دانه و pH تاثیر بسیار کمی داشتند. بیشترین میزان بازدهی 95/18% در شرایط دمایی 85°C، نسبت آب به دانه 3/31 و pH برابر6 و کمترین راندمان 35/6% در دمای 25°C، نسبت آب به دانه 3/31 و pH برابر 6 به‌دست آمد. شرایط بهینه به‌دست آمده دمای 60°C، نسبت آب به دانه 9/48 و pH برابر 3 بود. در شرایط بهینه مقدار پیش‌بینی شده برای بازدهی 84/11% بود. رفتار رئولوژیکی موسیلاژ استحصال شده در شرایط بهینه در سه غلظت 3، 4 و 5 درصد وزنی- حجمی و سرعت برش بین 14 تا 300 بر ثانیه مورد بررسی قرار گرفت. محلول‌های موسیلاژی رفتار شل شوندگی با برش (غیرنیوتنی) را در تمامی غلظت‌ها از خود نشان دادند. مدل قانون توان توانست به خوبی رفتار رئولوژیکی محلول‌های موسیلاژی بارهنگ را با ضریب تبیین بالا (R^2>0/99) توصیف نماید. شاخص رفتار جریان (n) در محدوده 0/3 تا 0/36 تغییر یافت. ضریب قوام (k) نیز در محدوده $Pa.s^n$ 13/6 تا 81/17 متغیر بود. نتایج نشان داد موسیلاژ به‌دست آمده از دانه بارهنگ می‌تواند به‌عنوان قوام‌دهنده در غذاها مورد استفاده قرار گیرد.

واژه‌های کلیدی: بارهنگ، روش سطح پاسخ، موسیلاژ.

1- استادیار، گروه علوم و صنایع غذایی، دانشکده کشاورزی و منابع طبیعی، دانشگاه محقق اردبیلی.
2 و 3- دانش‌آموخته دکتری و استاد، گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه فردوسی مشهد
(*- نویسنده مسئول : Email: s.razavi@um.ac.ir)

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Microencapsulation of anthocyanin pigments obtained from seedless barberry (*berberis vulgaris* L.) fruit using freeze drying

Hassan Mirhojati[1], Parvin Sharayei[2*], Reihaneh Ahmadzadeh Ghavidel[3]

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Abstract

The acidified ethanol extracts of dried barberry which have a relatively high anthocyanin content (376.28± 1.45 mg c3g/Kg dmp) were freeze dried using maltodextrin (MDX), polyvinyl-pyrrolidone (PVP) and mixture of MDX and calcium alginate (MDX-CaAlg) as a carrier and coating agents. The qualitative attributes of the powders were characterized by their productively encapsulation efficiency, moisture content, bulk density, colour values (L*, a*, b*, C and H°), particle size, total phenolic compounds (TPC), free radical scavenging activity of DPPH (RSA), ferric reducing-antioxidant power (FRAP) and minimized 50% of radical- scavenging activity (IC_{50}). Scanning electron microscope was used for monitoring the structures of the powders. To determine the stability and half- life period of microencapsulated pigments, samples were stored under different storage temperatures (4°C and 25°C) at relative humidity 75%. Results showed that the encapsulated powder containing PVP 8% as wall material represented the best powder quality (p<0.05). The total anthocyanin content of microcapsules decreased during storage at different temperatures, but encapsulated powder containing PVP 8% had the lowest rate of their decrease. Finally, the obtained results showed that microencapsulation by freeze drying could be recommended as a suitable method for stabilizing anthocyanins of barberries' extract.

Keywords: Anthocyanins, Antioxidant activity, Barberry, Carrier agents, Encapsulation, Freeze drier, Stability.

Introduction

The colour is one of the most important qualitative properties of food that has a significant impact on the popular market. Therefore, the application of colour-producing agents in the food industry has a specific position. Since consumers' desire for red foods is more than any others, therefore more attention has been paid to this colour and its sources. Most of the synthetic red colours which are used in the food industry, including Azorubine (also called Carmoisine), Ponceau 4R (also called Brilliant Scarlet 4R or Cochineal Red A) and Allura Red AC have chemical sources with harmful health effects (Anon, 1995). Today, there are more tendencies to use natural colorants instead of synthetic ones, because of their anticancer and antioxidant properties (Andersen *et al*., 2010). Fruits, vegetables, herbs, nuts, spices are known as potential sources of natural pigments.

Seedless barberry (*Berberis vulgaris* L.) is one of the economical sources of anthocyanin pigments (Fallahi et al., 2010). Barberry is a dicotyledonous, perennial species and well-known medicinal shrub in Iran (Shamsa *et al.*, 1999; Rezvani Moghaddam *et al.*, 2007; Ebadi *et al.*, 2010). It is cultivated as a domestic plant in Southern Khorasan province in the eastern part of Iran since 200 years ago (Kafi and Balandari, 2004; Rezvani Moghaddam *et al*., 2007; Aghbashlo *et al.*, 2008; Fallahi *et al.*, 2010). Currently, barberry is cultivated in more than 11000 hectares with annual production about 9200 tons dry fruit in this region (Radmehr, 2010).

Anthocyanins, a large group of water - soluble pigments, are responsible for red to

1. Former MSc Student, Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran.
2. Assistant Professor, Agricultural Engineering Research Department, Khorasan Razavi Agricultural and Natural Resources Research and Education Center, AREEO Mashhad, Iran.
3. Assistant Professor, Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran
(*Corresponding Author: parvin_sharayei@yahoo.com)
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blue colours of fruits and vegetables, are commonly used in acidic solutions as a red pigment in soft drinks, jams, and confectionary and bakery products. Attractive colour and functional properties such as antimicrobial properties, antiemetic, antipyretic, anti-itch, anti-inflammatory, hypertensive, antiarrhythmic (irregular heartbeats), sedative, analgesic and antioxidant properties of anthocyanins make them a good substitute for synthetic pigments in the food industry (Castaoveda- Ovando *et al*., 2009;Khosrokh-avar *et al*., 2010). The stability is an important aspect to consider for use of anthocyanins as antioxidants and colourants in foods. The stability of anthocyanins has been studied by several researchers and it has been shown that they are affected by pH, temperature, light, oxygen, ascorbic acid, enzymes, sugars, degradation products and metallicions (Patras *et al*., 2010; Bakowska *et al*., 2003).

Encapsulation of anthocyanin extracts may enhance their stability for the use as food colorants (Ersus and Yurdagel, 2007). Microencapsulation is a technique by which the sensitive ingredients are packed within a coating or wall material. The wall material protects the sensitive ingredients against adverse reaction and controls release of the ingredients. In addition, microencapsulation can convert liquids into powders, which are easy to handle. Numerous wall materials or encapsulating agents are available for food application (Shahidi and Han, 1993; Desai and Park, 2005; Pu, 2010). Different kinds of carbohydrates (starch, maltodextrin, dextrin, sucrose and annular dextrin) cellulose (carboxymethyl cellulose, methyl cellulose, ethyl cellulose, nitrocellulose and acetate cellulose), gums (acacia, sodium alginate and carrageenan), fats (wax, paraffin, beeswax, diglyceride, monoglyceride and oils), proteins (gluten, casein, gelatine, albumin, haemoglobin and peptides) and polymers (polypropylene and polyvinyl acetate) are commonly used as wall materials or carrier substances (Kaushik and Roos, 2007). Maltodextrins with different dextrose equivalent (DE) are used as wall material mainly due to their high solubility in water, low viscosity, bland flavor, and colorless solutions (Gibbs *et al*., 1999; Ersus and Yurdagel, 2007; Saénz *et al*., 2009; Avaltroni *et al*., 2004). Polyvinylpyrrolidone (PVP) is a light flaky hygroscopic powder, readily absorbing up to 40% of its weight, has excellent wetting properties and readily forms film. These make it good as a coating, or an additive, or a wall material in encapsulation processes. Furthermore, PVP has amorphous properties and does not form crystals in encapsulated particles (Selim *et al*., 2000). Its safety and biocompatibility have been reported in general biological studies (Xu *et al*., 2010).

In various experiments on encapsulation of anthocyanins, different materials and compounds have been used in spray drying (Cai and Corke, 2000; Robert *et al*., 2010, Mahdavi *et al*., 2016 a and b) or freeze drying techniques such as glucan gel (Xiong *et al*., 2006), maltodextrins with different DEs (Ersus and Yurdagel, 2007; Fang and Bhandari, 2011; Saenz *et al*., 2009, Laine *et al*., 2008; Coralia *et al*., 2010; Nayak *et al*., 2010, Robert *et al*., 2010), maltodextrin and inulin (Bakowska-Barczak and Kolodziejczyk, 2010), maltodextrin, ascorbic acid and mesquite gum (Kandansamy and Somasundaram, 2012), maltodextrin, Arabic gum, and tapioca starch (Tonon *et al*., 2010) and PVP (Xiaoyi *et al*., 2010; Selim *et al*., 2000). But, encapsulation of barberry anthocyanins by freeze drying has not been investigated yet. Therefore, encapsulation of barberry anthocyanins with freeze drying technique through wall materials of maltodextrins and Polyvinylpyrrolidone and then, evaluating the stability of anthocyanin and colour of encapsulated powders during storage could be an initial step to produce natural and highly stable food colorants.

Material and methods

Raw materials

Barberry fruit (dried with moisture content of 12.37 ± 0.85%) were purchased from a local market in Mashhad. After cleaning, samples were packed in low-density polyethylene film with a thickness of 140 microns. Samples were

kept in a freezer at -18°C for further analyses. Polyvinylpyrrolidone 40 (molecular weight of 40,000 Daltons), maltodextrin (dextrose equivalent 16.5-19.5), Calcium alginate salt and other materials were purchased from Merck, Sigma- Aldrich, and Caledon companies.

Extraction and concentration of anthocyanins

Ethanolic extracts of anthocyanins were prepared as follows: frozen fruits were ground with Armfield ball grinder without thawing. Four volume of 96% ethanol 1:1.5 N HCl (85:15 v/v) blend was added to barberries to extract anthocyanins and then were subjected to ultrasonic waves (Hielscher, Germany, UP400S, 24 KHz) for 10 min with 20% intensity. After 24 h stiring at ambient temperature (25± 1◦C), samples were filtered through a filter paper Whatman grade 1. Extraction solvent was evaporated at 45°C under vacuum by the rotary evaporator (Laborota 4000 efficient, Germany) until a level of 8± 0.3% soluble solids was reached (Ersus & Yurdagel, 2007).

Preparation of microencapsulated powders

Wall materials maltodextrin (MDX) 20% (w/v), polyvinylpyrrolidone (PVP) 8 and 15% (w/v) and mixture of maltodextrin 10% and calcium alginate (MDX-CaAlg) 0.1% (w/v) dissolved in distilled water at ambient temperature (25± 1°C). The solution was kept in the refrigerator for complete hydration in 24 h. Then, extract of anthocyanins from barberry and wall materials were mixed in a weight ratio (w/w) of 1:5 (extract: wall material), then mixed with a rotor-stator (120 rpm, 30 min), (Wang *et al.,* 2013). Total anthocyanins of samples were measured through differential pH method (Giusti and Wrolstad, 2001) before drying. The solutions were dried in a freeze dryer (Operon- Korea) for 48 h (−55°C, 0.15mm Hg pressure). Dried materials were ground using a pestle and mortar and passed through a 0.71mm mesh and stored in brown glass bottles with screwed caps in a freezer (−18◦C) until usage. For the preparation of the blank sample, the concentrated extract (8± 0.3% soluble solids) without wall materials freeze- dried in similar conditions with other samples.

Physical and chemical analyses

Moisture content

Moisture of the samples was determined using appropriate device working by infrared (MX-50, Japan) heating at 105± 1°C until a constant weight (Kaushik and Roos, 2007).

Bulk density

The bulk density of powders was measured by weighing 20g of samples and pouring them into a 20ml graduated cylinder. The bulk density was calculated by dividing the powder mass by the volume occupied in the cylinder (g/cm^3), (Tonon *et al*., 2010).

Colour measurement

The color of encapsulated powders was measured using computer vision method adapted from Koocheki *et al.,* (2009) with some modifications. The indices of Hue angle ($H^o= \tan^{-1}(b/a)$), Chroma ($C= (a^2+ b^2)^{1/2}$) was calculated and the mean of three replicates were reported.

Total anthocyanin content

Total anthocyanin content (TAC) was evaluated by pH differential method (Giusti and Wrolstad, 2001). 0.2g of each sample was dissolved in 10 ml of distilled water in a volume flask far from the light. Absorbance was measured at 510 nm and 700nm. Total anthocyanin pigment concentration in the powder (TAC) was calculated according to the following equations:

$$TAC(mg/kg): DA \times Mw \times D_f \times 1000/(M_a \times L) \quad (1)$$

$$DA=(A_{510}\_A_{700})\,pH_{1.0}\,\_(A_{510}\_A_{700})\,H_{4.0} \quad (2)$$

With DA: difference of absorbance; Mw: molecular weight for cyaniding-3-glucoside (449.2 g mol^{-1}); D_f: the dilution factor of the samples; Ma: molar absorptive of cyaniding-3-glucoside (26,900 $Lcm^{-1}mg^{-1}$); and L: Diameter of spectrophotometer cell (cm). Results were expressed as mg of Cyaniding-3-

glucoside Equivalents per Kg of dry matter of powder (mg c3g/Kg dmp).

Encapsulation efficiency

Encapsulation efficiency was calculated using following equation (Najaf Najafi, 2010).

$$\text{Encapsulation efficiency}(\%) = \frac{\text{Total mass of the capsules obtained before microencapsulation}}{\text{Total mass of solids obtained after microencapsulation}} \times 100 \quad (3)$$

Particles size

Laser diffraction particle size analyser (SAL, D-2101, Shimadzu, Japan) was used to measure particle size in terms of diameter. The samples were dispersed in hexane using ultrasonic waves (24 kHz and 20% intensity) for 2 min and then particles size was directly determined (Parrarud and Pranee, 2010).

Scanning electron microscopy

Particle structures of the encapsulated powders were evaluated by scanning electron microscope (LEO-1450, Germany). Powders were attached to SEM stubs using a 2-sided adhesive tape and left in desiccators containing phosphorous pentoxide for 48h. Samples were coated with 200˚A gold under vacuum before the examination. SEM was operated at an accelerating voltage of 10 kV.

Glass transition

Samples of freeze-dried pigment powders were equilibrated at 75% RH (NaCl saturated solution) for 1 week. Glass transition temperature (Tg) was determined using a differential scanning calorimeter (DSC1 Mettler Toledo, Switzerland). The temperature range was set from -40°C to 200°C with a heating rate of 10°C/min (Cai and Corke, 2000). Five milligrams powder was weighed directly into a DSC sample pan and sealed. An empty pan was used as a reference.

Total phenolic compounds

The total phenolic content (TPC) of the extracts was determined using the Folin–Ciocalteau method (Singleton *et al.*, 1999). 100 µl of the sample solutions (100 mg in 10 mL of Methanol), 6 ml of twice distilled water and 500 µl of Folin-Ciocalteau reagent were added; after waiting between 8 s and 8 min at room temperature, 1.5 ml of sodium carbonate (20% w/v) were added. The extracts were mixed and allowed to stand for 30 min at room temperature before measuring the absorbance at 765 nm. A mixture of water and reagents was used as blank. Results were reported as mg Gallic acid equivalents per kg, (mg GA/kg). A calibration curve of Gallic acid in methanol was performed in the concentration range of 0.04–0.40 mg per mL.

Determination of antioxidant activity

Total antioxidant activity was estimated by two standard procedures, DPPH and FRAPS assays. In DPPH radical- scavenging assay, various concentrations of the methanolic sample solutions (1 ml) were mixed with 1 ml of methanolic solution containing DPPH radicals (0.006 % w/w). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm (Ramadan *et al.*, 2003). The radical-scavenging activity (%RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\%RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100 \quad (4)$$

Where A_S is the absorbance of the solution when the sample has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. α- Tocopherol was used as the positive reference while methanol was used as negative one.

Ferric reducing-antioxidant power (FRAP) was measured using 2, 4, 6-Tripyridyl-S-triazine (TPTZ) according to Benzie & Strain, (1996). Acetate buffer (0.3 M, pH 3.6) was prepared by dissolving 3.1 g $C_2H_3O_2Na$ -$3H_2O$

and 16 mL of acetic acid in 1 L of distilled water. TPTZ solution was prepared by dissolving 23.4 mg of TPTZ in 7.5 mL of 40 mM HCl solution. Ferric solution (20 mM) was prepared using $FeCl_3$-$6H_2O$. The final working FRAP reagent was prepared freshly by mixing acetate buffer, TPTZ, and ferric solutions at a ratio of 10:1:1. In brief, 900 mL FRAP working reagent was mixed with 90 mL distilled water and was warmed to 37°C in a water bath. The reagent control reading was recorded at 595 nm, followed by adding 30 mL of sample solutions (100 mg in 10 mL of methanol). The absorbance was taken at 595 nm, against the control solution. A standard curve was prepared using different concentrations of $FeSO_4$-$7H_2O$ (200– 2000 µmol per L). All solutions were freshly prepared. The results were expressed in µmol Fe^{2+} per liter.

Storage stability evaluation

Encapsulated powders were stored at controlled temperature and humidity and in the absence of light for 42 days. Samples of 3 g of each powder were transferred to closed low-density polyethylene bags (5cm× 5cm). The samples were placed on sealed desiccator containing saturated NaCl solution to obtain humidity values of 76% and placed at 4 and 25°C temperature (Gradinarua *et al*., 2003).

Statistical analysis

All experiments and measurements were carried out in triplicate, and data were subjected to analysis of variance (ANOVA).

ANOVA and regression analyses were performed according to the MStatC and Excel software. Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

Results and discussion

Physical properties of encapsulated powders

Table 1 summarizes the results for physical properties of samples with different wall materials.

Table 1. Physical properties of microencapsulated powders

Parameters	MDX	MDX -CaAlg	8% PVP	15% PVP
Efficiency (%)	69.26± 1.23d	71.26±0.98c	92.54±0.15a	80.64±1.68b
Moisture (%)	4.77 ±0.12c	7.84±0.16b	8.34±0.59a	4.66±0.72d
Density (kg/m^3)	513 ±1.34c	607±1.87b	654±2.70a	469±1.90d
*L**	54.22 ±0.78b	46.07±1.03c	51.81±1.44b	78.45±0.51d
*a**	29.81 ±0.56a	30.26±1.12a	14.23±0.26b	5.06±0.41c
*b**	23.14± 0.48a	22.10±1.85a	14.60±0.25b	10.21±0.31c
C	37.73± 0.12	37.47±0.18	20.38±0.05	11.40±0.14
H°	37.82 ±0.23	36.14±0.10	45.73±0.28	63.64±0.03
Particles size (µm)	19.71± 0.36d	49.20±0.50a	36.91±1.01c	46.53±0.19b
Glass transition temperature (°C)	23.17±0.69b	10.66±0.78d	19.49±0.82c	46.70±0.92a

Means ± SD (standard deviation) with the same lowercase letters are not significantly different at $P < 0.05$.

The type and concentration of the wall had significant effects on physical characteristics of encapsulated powders. It has been reported that type and concentration of polymers as wall material affect the encapsulation efficiency (Jalil and Nixon, 1990). As can be seen from table 1, unexpectedly, encapsulation efficiency decreased with increasing concentration of PVP from 8 to 15%. It has been proven that increase in encapsulation efficiency (more mass microencapsulation), depends on moisture content. The final moisture content of microencapsulated powders is affected by different factors such as the number of groups linking wall material to water, sealing methods of containers and storage temperature (Najaf Najafi, 2010). This might be due to low moisture content in microencapsulated powder prepared with PVP 15% compared with PVP 8%. In addition, low encapsulation efficiency in microencapsulated powders prepared with MDX compared with

MDX- CaAlg might be due to gelatinous texture of the wall on account of calcium alginate (Belscak *et al*., 2011). Cai and Corke, (2000) in their work on encapsulated anthocyanin extract from Amaranthus plant obtained similar results in the effect of moisture content (1.25 to5.67%) on microencapsulation efficiency.

The bulk density of encapsulated powders had significant differences ($P<0.05$), and varied between 469 (related to the samples with 8% PVP) to 654 g/cm^3 (related to the samples with 15% PVP). Size of the crashed particles, frangibility, and moisture content and flow properties may influence the bulk density of produced powders. The heavier the material, more easily it accommodates into the spaces between the particles, thus occupying less space and resulting in a higher bulk density (Tonon *et al.*, 2010).

Changes in the color indices (a*, b*, L*, C, and H◦) in microencapsulated powders with different wall materials are shown in table 1. Differences in wall materials had significant effects on *L** values ($p<0.05$). The lightness increased with increasing PVP concentration from 8 to 15% (from 51.81 to 78.45 units). a* and C values of samples with 15% PVP were found to be significantly lower than the samples produced with MDX, MDX-CaAlg, and 8% PVP. The chroma value was proportional to the strength of the colour and indicates its degree of saturation (Maskan, 2001). H° values of PVP (8 and15%) samples were found to be significantly higher than other samples. Higher a* and lower H° indicates bright and purple shade of red color. It is concluded that increasing of PVP, the color of anthocyanins becomes paler.

Table 2 shows the particle size distribution for the powders produced with the different carrier agents. The diameters of encapsulated powders varied from 19 to 49µm. This increase in particle sizes is related to the molecule size of each carrier agent and also longitudinal breakage and sublimation of ice crystals. Our results are in accordance with Zuidam and Shimoni, (2010) and Heinzelmanna *et al*. (2000) findings. These results coincided with the changes in bulk density. The same behavior was observed by. According to Keogh *et al*. (2003) and Shrestha *et al*. (2007), the particles with higher median diameter occupied higher volume because of increasing interstitial air content between particles.

Glass transition temperature (T_g) of PVP15% was the highest. Lower T_g of carrier agents caused higher hygroscopicity of the encapsulated powders. Maltodextrins are the components with low molecular weight and contain shorter chains and more hydrophilic groups. Therefore, powders prepared with carrier agent MDX, MDX- CaAlg had rubbery and soft texture in ambient temperature. In rubbery and soft polymers, core materials tend to release or transfer from wall materials. Also, Glass transition temperature decreased with increasing moisture content.

Chemical properties of encapsulated powders

The Chemical properties of encapsulated powders are given in Table 2.

Table 2. Chemical properties of microencapsulated powders

Parameters	MDX	MDX-CaAlg	8% PVP	15% PVP
Total Anthocyanin content (mg c3g/Kg dmp)	160.85±2.35d	178.30±0.67c	303.50±0.54a	214.40±0.62b
TPC (mg GA/kg)	13.78±0.56b	16.78±0.83a	15.19±0.38ab	13.69±0.11b
RSA (%)	49.83±0.32a	61.98±0.459b	79.96±0.16d	59.75±0.17c
FRAP (Fe^{2+}, µmol/l)	1055.30±3.56d	1727.05±2.45c	3949.20±2.71a	1942.08±1.98b

Means ± SD (standard deviation) with the same lowercase letters are not significantly different at $P < 0.05$.

The highest total anthocyanin content (303.50 mg c3g/Kg dmp) and DPPH radical-scavenging activity (RSA, 79.96%) belonged to the encapsulated powders with 8% PVP carrier. The highest total phenolic compounds with no statistical differences belonged to the encapsulated powders with MDX- CaAlg (61.98 mg GA/kg) and 8% PVP (15.19 mg

GA/kg) as a carrier agents. The direct correlation between antioxidant activity and anthocyanin or phenolic compounds content was observed. Although phenolic compounds have several critical biological activities such as free radical scavenging, are often considered from the antioxidant activity point of view (Aparicio *et al*., 1999; Morello *et al*., 2009). Previous researchers reported antioxidant activity has high correlation with anthocyanin content and total phenolic composition of food materials (Brand-Williams *et al*., 1995; Sanchez *et al*., 2006; Belscak *et al*., 2011; Parejo *et al*., 2000; Camire *et al*., 2002; Moyer *et al*., 2002; Wanget al., 1997).

The FRAP test is a simple, reproducible, rapid, and inexpensive procedure that measures the ability of anti- oxidative compounds to convert the ferric ion Fe^{3+} to ferrous Fe^{2+}, as a measure of total antioxidant capacity (Prior and Cao, 1999). Many similarities were found to exist between the results of the FRAP test and those of the DPPH radical-scavenging activity assay. The encapsulated powders with wall material 8% PVP had a FRAP quantity (3949.20 µmol Fe^{2+} per liter) significantly higher than that of the others.

Microstructure of encapsulated powders

The outer topography of the freeze-dried capsules was affected by wall composition (Fig 1, A-D). All encapsulated powders except for MDX- CaAlg showed amorphous glassy shapes which were formed during dehydration, grounding, and sieving of freeze-dried solid materials. These glassy structures can protect entrapped anthocyanins from exposure to heat and oxygen (Roos, 1995). According to the figure 1, encapsulated powders with MDX are more spherical and have the smooth surface and fewer wrinkles compared with powders with PVP. These differences might be due to the difference between covering power and spatial structure of MDX and PVP. Furthermore, wrinkles might be due to mechanical stresses induced by atomization or drying conditions. Wall composition and drying speed, especially at early stages, affect surface characteristics of encapsulated powders (Lee and Rosenberg, 2000). Encapsulated powders with MDX- CaAlg as a wall material had globular shape, because of gelatinous properties of alginate. Similar results were obtained by Semyonov *et al*. (2010).

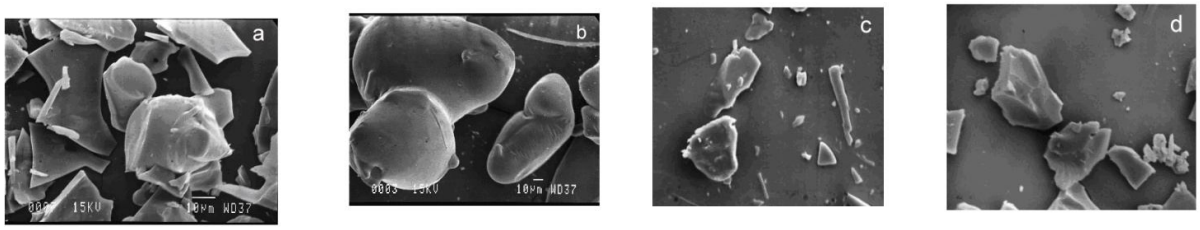

Fig.1. Scanning electron microscopy (5000×), a: MDX, b: MDX-CaAlg c: 8% PVP, d: 15% PVP.

Storage stability evaluation of barberry microencapsulated anthocyanins

Stability of anthocyanins in freeze-dried microencapsulated powders was evaluated under various conditions of storage temperature (Figs 2, 3). The increase of temperature led to faster anthocyanin degradation, at the end of 6 weeks storage period, the pink colour of the samples was not changed at 4˚C where it was turned to brown colour at 25˚C, which was expected since these pigments are highly thermosensitive. This negative influence of temperature on anthocyanin stability has been observed by many researchers (Pacheco-Palencia *et al*., 2007; Ersus and Yurdagel, 2007; Tnon *et al*., 2010). The faster anthocyanin degradation at the higher temperature may also be related to the presence of sugars, together with proteins, which can result in the Maillard reaction (non-enzymatic browning), which generally occurs during food processing at high temperatures or during food storage for a long time. According

to Von Elbe and Schwartz (1996), the presence of sugars or products resulting from their degradation can accelerate the anthocyanin degradation, since this reaction rate follows the rate of conversion of sugars to furfural. Furfural, which is a derivative from aldopentoses, as well as hydroxyl methyl furfural, which is a derivative from keto-hexoses, are products resulting from the Maillard reaction that condenses together with the anthocyanins, leading to the formation of compounds with brown coloration. This reaction is highly dependent on temperature, being accelerated by the presence of oxygen and occurring more frequently in fruit juices.

The kinetics of degradation of anthocyanins was monitored over the storage period, rate constants and half- life values of reactions were determined (Table 3). Previous works on anthocyanin degradation showed that reaction followed in first- order degradation kinetics (Calvi and Francis, 1978; Cemerog *et al.*, 1994; Kırca *et al.*, 2003; Markasi, 1974). An increase in storage temperature led to an increase in rate constants.

Rate constants were predicted with the use of equations as:

$$\text{Log}(C_o/C_t) = k \times t \text{ and } t_{1/2} = {}^{-}\ln 0.5/k \quad (5)$$

Where k is the slope, C_o is the initial anthocyanin content, C_t is the anthocyanin content at a specific time and t is time. Half-life ($t_{1/2}$) values were then determined and are given in Table 3.

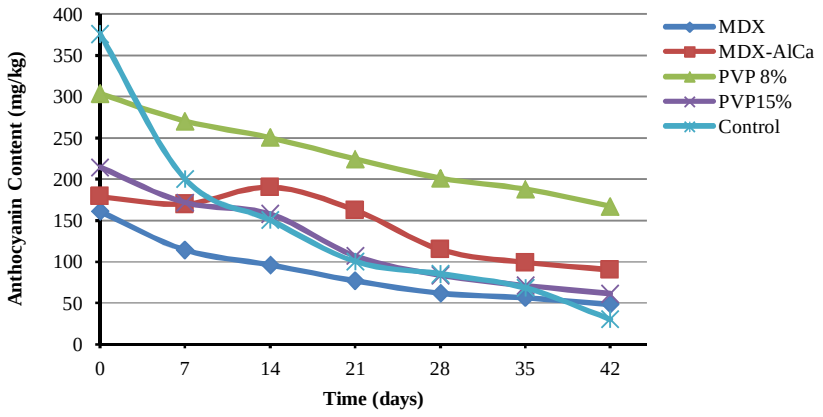

Fig.2 Anthocyanin content of freeze dried microencapsulated powders with different wall materials during storage at 25°C

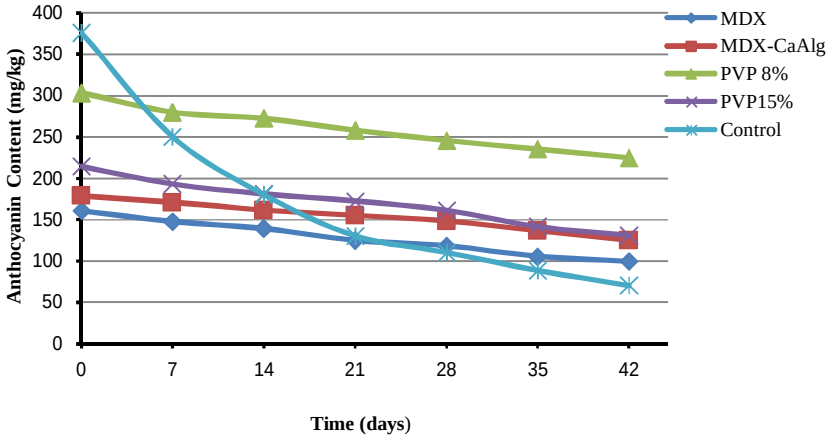

Fig.3 Anthocyanin content of freeze dried microencapsulated powders with different wall materials during storage at 4°C.

Table 3. Kinetic degradation data for freeze - dried microencapsulated powders with different wall materials under different storage conditions

Storage condition	Microencapsulated powders	K (1/days)	Half live(days)	R^2
4°C	MDX	0.011	63	0.99
	MDX-CaAlg	0.008	87	0.97
	PVP8%	0.006	116	0.99
	PVP15%	0.011	63	0.98
	Control	0.039	18	0.98
25°C	MDX	0.027	26	0.97
	MDX-CaAlg	0.018	39	0.83
	PVP8%	0.014	50	0.99
	PVP15%	0.031	22	0.98
	Control	0.052	13	0.97

The half- life of anthocyanins at storage temperature (4°C) was found 63- 115 days where it is 29- 50 days for 25°C storage temperature. Concerning to the different carrier agents used, the particles produced with PVP 8% had the highest half-life, in the conditions studied, followed by those produced with MDX-CaAl. The powders prepared with MDX showed higher degradation rates and, consequently, lower half-lives, with respect to the others

Conclusion

In conclusion, encapsulation of barberries' extract with freeze-drying technique and wall materials MDX, MDX-CaAlg and PVP (with different concentration) could protect anthocyanins during storage. PVP with 8% concentration as a wall material gave the highest anthocyanin content powder at the end of the drying process. Storage at 4°C increased half -life of freeze dried anthocyanin pigments 2.3- 2.8 times according to 25°C storage temperature.

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حسن میرحجتی[1]- پروین شرایعی*[2]- ریحانه احمدزاده[3]

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چکیده

در این پژوهش، عصارهٔ الکلی اسیدی حاصل از زرشک که حاوی مقدار نسبتا بالایی آنتوسیانین بود (376/26±1/45 بر حسب سیانیدین -3-گلیکوزید بر وزن خشک) با استفاده از دیواره‌های مالتودکسترین، پل‌وینیل‌پیرولیدون و مخلوط مالتو دکسترین و آلژینات‌کلسیم و با روش خشک‌کردن انجمادی ریزپوشانی شدند. خصوصیات ریزکپسول‌های حاصل نظیر راندمان ریزپوشانی، رطوبت، دانسیته‌توده، مولفه‌های‌رنگی، اندازه قطرذرات، مقدارترکیبات‌آنتوسیانینی، مقدار ترکیبات فنلی، مقدار ترکیبات گیرنده رادیکال آزاد، مقدار ترکیبات احیاءکننده‌آهن III و دمای انتقال شیشه‌ای مورد ارزیابی قرار گرفتند. از میکروسکوپ الکترونی نیز برای بررسی ریزساختار ریزکپسول‌ها استفاده شد. همچنین میزان رهایش ترکیبات آنتوسیانینی طی 42 روز نگهداری در دمای 4 و 25 درجه سلسیوس و رطوبت نسبی 75درصد مورد پایش قرار گرفت. نتایج نشان داد که ریزکپسول‌های تهیه شده با مادهٔ دیوارهٔ پلی‌وینیل‌پیرولیدون 8 درصد، دارای خصوصیات کمی و کیفی بهتری نسبت به سایر ریزکپسول‌ها بودند ($p<0/05$). میزان آنتوسیانین کل ریزکپسول‌ها طی مدت نگهداری کاهش یافت، اما ریزکپسول‌های حاوی مادهٔ دیوارهٔ پلی‌وینیل‌پیرولیدون 8 درصد، کمترین میزان کاهش ترکیبات آنتوسیانینی را داشتند. به‌طور کلی نتایج نشان داد ریزپوشانی ترکیبات آنتوسیانینی زرشک با استفاده از خشک‌کن انجمادی، روشی مناسب و قابل توصیه برای حفظ و پایداری این ترکیبات می‌باشد.

واژه‌های کلیدی: ترکیبات دیواره، پایداری، خشک¬کن انجمادی، ریزپوشانی، زرشک، فعالیت آنتی¬اکسیدانی.

1- دانش‌آموخته کارشناسی ارشد، دانشگاه آزاد اسلامی، واحد قوچان، گروه صنایع غذایی.
2- استادیار، پژوهش بخش تحقیقات فنی و مهندسی کشاورزی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان خراسان رضوی، سازمان تحقیقات، آموزش و ترویج کشاورزی، مشهد، ایران.
3- استادیار، گروه صنایع غذایی، دانشگاه آزاد اسلامی، واحد قوچان، قوچان، ایران.
(*- نویسنده مسئول : Email: parvin_sharayei@yahoo.com)

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Antioxidant Properties of Various Solvent Extracts of Indian Frankincense *(Boswellia serrata)* Oleogum Resin

Adeleh Mohammadi[1], Saeedeh Arabshahi-Delouee*[2], Kyriaki G. Zinoviadou[3], Charis M. Galanakis[4]

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Abstract

Methanol, ethanol, acetone and water extracts of Indian Frankincense *(Boswellia serrata)* were evaluated for their total phenolic contents and antioxidant properties using various methods including 2,2-diphenyl-1-picrylhydrazyl, iron (III) reducing power, total antioxidant capacity and oxidative stability index (Rancimat). The four extracts showed varying degrees of antioxidant activity in a dose - dependent manner in each assay. Methanol extract containing the highest amount of phenolic compounds exhibited the strongest antioxidant capacity in all the assays used. Moreover, all the extracts were able to improve the oxidative stability of soybean oil as evaluated by the Rancimat test. On the basis of the results obtained, *B. serrata* oleo-gum resin was found to serve as a potential source of natural antioxidants due to their considerable antioxidant activity.

Keywords: *Boswellia serrata*, Phenolic compounds, Antioxidant properties, Rancimat, Oleogum Resin.

Introduction

Lipid oxidation is one of the most important processes of food deterioration because it can affect food safety, color, flavor and texture (Wasowicz *et al.*, 2004). In addition, oxidation leads to health disorders such as atherosclerosis and carcinogenesis among others. Hence, the presence of antioxidants in foods is recommended for controlling rancidity with its deleterious consequences (Pizzale *et al*., 2002; Koleva *et al*., 2003). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ) are widely used to prevent the oxidation of oils and fats and extend the shelf-life of lipid-containing foods (Mohdali, 2010). However, the use of synthetic antioxidants have been recently restricted due to their possible toxic and carcinogenic effects (Padmashree *et al*., 2007; Valentao *et al*., 2002). It has been also suggested that there is an inverse relationship between dietary intake of foods rich in natural antioxidants and human diseases (Yildirim *et al*., 2001). Consequently, there is an increasing interest in finding naturally occurring alternatives from plants for use in food and pharmaceutical industry. The important of plant-based antioxidants in foods is appreciated for preserving foods against oxidative deterioration as well as supplying the essential antioxidants in vivo (Shahidi, 1977). A large number of plants have been screened as viable sources of natural antioxidants including tocopherols, vitamin C, carotenoides and phenolic compounds, which are responsible for maintenance of health and protection from coronary heart disease and cancer (Castenmiller *et al.*, 2002).

The name frankincense is derived from the ancient French term “franc enens” meaning “pure incense”, it is a natural oleogum resin acquired from Boswellia tree that belongs to the family *Burseraceae* comes from three distinct regions India, North Africa and the Middle East. Generally, the frankincense tree

1. PhD Student, Department of Food Science and Technology, Gorgan University of Agriculture and Natural Resources, Gorgan,Iran
2. Assistant Professor, Department of Food Science and Technology, Azadshahr Branch, Islamic Azad University, Azadshahr, Iran.
3. Assistant Professor, Department of Food Science and Technology, Perrotis College, American Farm School, GR-551 02, Thessaloniki, Greece
4. Assistant Professor, Department of Research and Innovation, Galanakis Laboratories, Skalidi 34, GR-73131 Chania, Greece
*Corresponding author:saeedeh_arabshahi@yahoo.com
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is a small, 3 – 6m high and scrubby tree which grows in rough, wild and inhospitable arid mountainous regions. This genus contains 23 species and the major species used for frankincense production are *Boswellia carteri, Boswellia frereana, Boswellia serrata* and *Boswellia papyrifera* (Khan & Farooqi, 1991; Dharmananda, 2003). The resin is harvested by scraping shallow incisions in the bark, this compounds convert into globular, pear or club shaped tears when exposed to the air and sun (Mathe *et al*., 2004). For at least 3000 years frankincense had been a significant trade material for the civilizations located in the North Africa and Arabian Peninsula, In addition to ceremonial and religious purposes, It has also been used in medicine and perfumery (Al-Dubai & Al-khulaidi, 1996; Mothana *et al*., 2011).

The active components of frankincense are various and can be divided in three groups; essential oils (5-9%), alcohol-soluble resins (65-85%) and the remaining water soluble gums (Tucher, 1986). The oleo-gum-resin of frankincense is mainly consisting of volatile oil, monoterpenes, diterpenes and lipophilic pentacyclic triterpene acids of the oleanane (α-boswellic acids), ursane (β- boswellic acids), polysaccharides, and lupine type (Sharma *et al*., 2009).

Frankincense therapeutic effect significantly depends on the amount of oleoresin. These effects include anti-inflammatory, hepatoprotective, anticancerous, anti-HIV, anti-microbial, antifungal, anti ulcerous, gastroprotective, hypoglycemic and antihyperlipidemic properties (Hussein *et al*., 2000; Basar *et al*., 2001; Al-Harrasi & Al-Saidi, 2008; Shen & Lou, 2008; Singh *et al*., 2008; Aman & Balu, 2009; Shah *et al*., 2009).

The objective of this study was to determine the total phenolic contents and the antioxidant properties of various solvent (methanol, ethanol, acetone and water) extracts of *B. serrata* oleogum resin by different methods including DPPH radical scavenging activity, reducing power, total antioxidant capacity and oxidative stability index in vitro.

Materials and Methods

Chemicals and Materials

Tert-Butylhydroquinone (TBHQ) was purchased from Nova international (India), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the Folin–Ciocalteau reagent were purchased from Sigma–Aldrich (St. Louis, MO, USA) while all solvents and reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). *Boswellia serrata* oleogum resin was supplied from a local factory (Gorgan, Iran). The oleo-gum resin was ground to a fine powder, passed through a 40- mesh sieve and kept in an air-tight container at 4°C until further use. Refined soybean oil pure of antioxidant additives was purchased from a local oil refining factory (Alia Golestan Co., Iran).

Extraction of antioxidants from *B.serrata*

The dried powder of *B.serrata* (25g) was extracted overnight in 250 ml each of methanol, ethanol, acetone and water, respectively, in a mechanical shaker (IKA, MTS 2/4) at room temperature and each extract was filtered with Whatman No. 1 filter paper. The filtrates obtained from methanol, ethanol and acetone extractions were evaporated to dryness at 40°C in a rotary evaporator (Buchi,V800, Switzerland) and the water extract was freeze-dried (-40°C) (Operon, FDB-5503, Korea). The dried sample of each extract was weighed to determine the yield of soluble constituents and stored at 4 °C until use.

Estimation of total phenolics

Total phenolic content of each extract was determined by the Folin–Ciocalteu method (Slinkard and Singleton, 1977). Briefly, 20 µl of extract solution were mixed with 1.16 ml distilled water and 100 µl of Folin–Ciocalteu reagent, followed by addition of 300 µl of Na_2CO_3 solution (20%) after 8 minutes. Subsequently, the mixture was incubated at oven (Memmert, WB14, Germany) at 40 °C for 30 minutes and its absorbance was measured at 760 nm (Cecil, Aquarius, England). Gallic acid was used as a standard

for the calibration curve. The phenolic content expressed as gallic acid equivalent was calculated using equation obtained by performing linear regression on calibration curve:

$Y=0.0011X-0.0001$ (1) $R^2=0.9949$

Where Y is the absorbance at 720 nm and X is concentration of phenolic compounds as gallic acid equivalents (µg/ml).

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the method of Blois (1958). Briefly, 1 ml of a 0.1 mM methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 100–1000 µg/ml). The mixture was then vortexed and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and antioxidant activity was expressed as percentage DPPH scavenging relative to the control using the following equation:

DPPH scavenging activity (% =[(Absorbance of control− Absorbance of sample)/ Absorbance of control] ×100 (2)

Also IC_{50} was determined by linear correlation analysis of different concentrations of the samples. The methanol is used as experimental control. The control sample was prepared by mixing methanol and DPPH radical solution.

Reducing power assay

The ability of extracts to reduce iron (III) was assessed according to Yildirim *et al*, (2001). The extracts (100– 1000 µg/ml) in 1 ml of the corresponding solvent were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide $[K_3Fe(CN)_6]$ (1%) and then the mixture was incubated at 50 °C for 30 min. After incubation, 2.5 ml of tri-chloro-acetic acid (100 g/ l) were added and the mixture was centrifuged at 1650g for 10 min. Finally, 2.5 ml of the supernatant were mixed with 2.5 ml of distilled water and 0.5 ml of Ferric Chloride ($FeCl_3$, 1g/l) and the absorbance was measured at 700 nm. Higher absorbance indicates better reducing power under the reaction conditions.

Total antioxidant capacity

Total antioxidant activity of the extracts was determined according to Prieto *et al*, (1999). Briefly, a 0.1 ml aliquot of sample solution (containing 100– 1000 µg/ml of different extracts in corresponding solvent) was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in Eppendorf tube. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank solution contained 1 ml of the reagent solution and the appropriate volume of the same solvent used for the sample and was incubated under the same conditions as the rest of the samples.

Oxidative stability index (Rancimat test)

The effectiveness of the *B.serrata* extracts against oxidation of soybean oil was determined by the Rancimat (Metrohm, 743, Switzerland) (AOCS, 2007). That is an accelerated technique most commonly used for the assessment of the oxidative stability of edible fats, oils and fat-containing foods (Farhoosh, 2007). The concentration of the antioxidant agents varied from 100-1000ppm, the air flow rate was set at 20 l/h and the temperature at 110°C The oxidative stability was expressed as induction time while antioxidant index (AI) was calculated from the measured induction times according to Forster *et al*, (2001) using the following formula:

AI= Induction time of soybean oil oxidation with antioxidant/ Induction time of soybean oil oxidation without antioxidant. (3)

Statistical analysis

All the experiments were carried out in triplicate and the collected data were analyzed by ANOVA; the means were compared by the

Duncan's multiple range tests at the 5% level using SPSS version 21 (IBM, USA).

Results and discussion

Extract yield and total phenolics

Solvent extraction has become one of the most popular methods for the preparation of extracts from plant materials (Dai and Mumper, 2010). In general, solvent extraction of polyphenols depends on two events; dissolution of each compound in the plant material matrix followed by their diffusion in the external solvent medium (Shi *et al.*, 2005). Consequently, the extraction efficiency depends on a great number of factors such as polarity of solvent, extraction conditions like time and temperature, chemical and physical properties of the samples as well the solvent/ sample ratio (Dai and Mumper, 2010). The effect of different solvents on the extraction yield and the total phenolic content of *B.serrata* oleo-gum resin are presented in Table 1. The amount of extractable components expressed as percentage by weight of dried material ranged from 11.25% (acetone extraction) to 20.58% (water extraction). According to the results, the water solvent showed the highest yield of extraction because of the highest polarity index. The solvent polarity is an important parameter for extraction of natural antioxidants and the higher polarity leads to better solubility of phenolic compounds (Tomsone *et al.*, 2012).

Table 1. Effect of solvent type on the extraction yield and total phenolics content of *Boswellia Serrata.*

Sample	Yield[1]	Total phenolic content[2]
Methanol extract	11.53 ± 0.19^{c}	254.27 ± 1.22^{a}
Ethanol extract	12.42 ± 0.14^{b}	172.47 ± 0.98^{b}
Acetone extract	11.25 ± 0.09^{c}	158.11 ± 1.31^{c}
Water extract	20.58 ± 0.23^{a}	112.13 ± 1.05^{d}

[1]Grams of extract per 100 g of dried powder.
[2]mg of gallic acid per 100 g dry weight of extract.
Different letters within the same column indicate significant differences ($p<0.05$)

The amount of total phenolics (gallic acid equivalents) expressed as percentage by weight of dried extract ranged from 112.13 in the water extract to 254.27 in the methanol extract. Methanol was found to be the most effective solvent in extraction of phenolic compounds from *B.serrata* oleo gum resin. This is in agreement with previous studies which reported that methanol can be an effective solvent for the extraction of antioxidants from different plants (Siddhuraju *et al.*, 2003; Arabshahi-Delouee & Urooj, 2007; Chirinos *et al.*, 2007). It has been shown that methanol is more efficient in extracting lower molecular weight phenols while aqueous acetone is more suitable for the extraction of higher molecular weight compounds. Consequently, there is no universal extraction procedure that can be applied for the extraction of all phenolics (Dai & Mumper, 2010).

DPPH radical scavenging activity

The DPPH essay has been widely used in order to evaluate the ability of various compounds to scavenge free radicals (Musa *et al.*, 2013). This method is simple and requires mild experimental conditions, which is advantageous in comparison with other methods that require preliminary sample treatment (Musa *et al.*, 2013). However, it can be affected by factors such as the type and amount of the solvent used, the water content, pH and the presence of metal ions (Dawidowicz *et al.*, 2012). The scavenging ability of the extracts in comparison with the synthetic antioxidant TBHQ are presented in Figure 1. As it can be seen, the scavenging activity of the four extracts against DPPH was concentration-dependent.

The methanolic extract was found to be the most active radical scavenger followed by ethanol, acetone and water extracts and this can be attributed to its higher content of total

phenolic compounds. However, it was not as effective as the reference control, TBHQ, since the amount of extract required to scavenge 50% of DPPH radicals present in the reaction mixture (IC_{50}) was significantly ($p<0.05$) higher than that of TBHQ (Table 2). IC_{50} values of the extracts ranged from 515.46± 0.76 in methanol extract to 982.12± 0.87µg/ml in water extract. A high correlation between total phenolic content and free radical scavenging activity of different plant extracts has been reported by many researchers (Peterson *et al*., 2001; Jimenez-Escrig *et al*., 2001; Arabshahi-Delouee & Urooj, 2007). In general, increasing the concentration of phenolic compounds increases the ability of the extracts to inhibit free radicals but the type of phenolic compounds is crucial as wel.

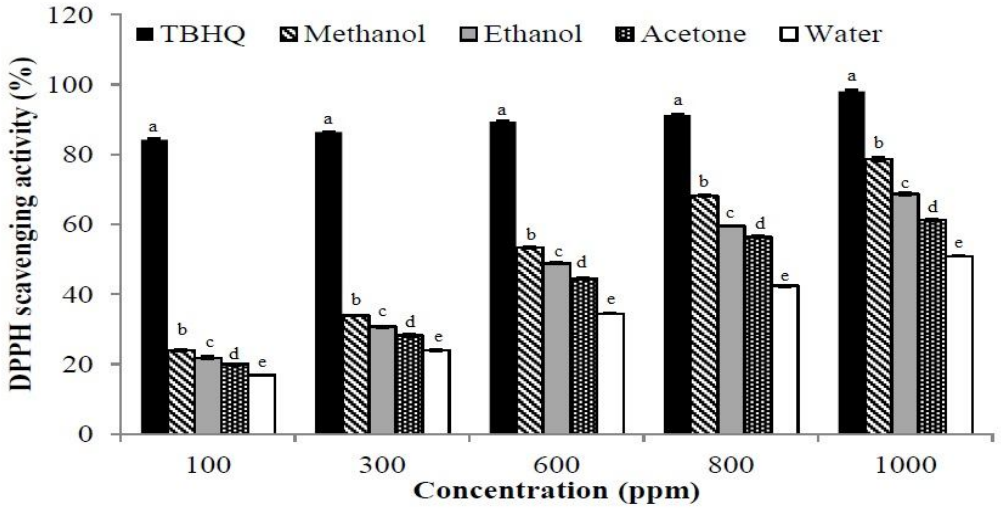

Fig. 1. DPPH radical scavenging activities of methanol, ethanol, acetone and water extracts of *Boswellia serrata* oleogum resin and TBHQ.

Table 2. Ic_{50}[1] (µg /ml) of different solvent extracts from *Boswellia serrata* oleogum resin and TBHQ.

Sample	DPPH	Reducing power	Total antioxidant capacity
TBHQ	59.32±0.87[a]	40.8±0.79[a]	159.36±0.38[a]
Methanol extract	515.46±0.76[b]	96.15±0.43[b]	359.71±0.61[b]
Ethanol extract	636.76±0.54[c]	185.18±0.62[c]	692.38±0.59[d]
Acetone extract	694.44±0.92[d]	200±0.19[d]	437.6±0.72[c]
Water extract	982.12±0.87[e]	232.55±0.66[e]	ND[2]

[1] The effective concentration in which the absorbance was 0.5 for reducing power and total antioxidant capacity; DPPH radicals were scavenged by 50%.

[2] Not detectable.

Values denoted by different letters within each column are significantly different ($p< 0.05$).

Reducing power

It has been previously reported that the reducing power was associated with the antioxidant activity (Siddhuraju *et al*., 2002). In this assay, the ability of the extracts to reduce iron (III) to iron (II) was determined. All the extracts showed some degrees of electron donation capacity in a concentration-dependent manner, but their capacities were inferior to that of TBHQ (Figure 2). Methanolic extract containing the highest amount of total phenolics, was the most potent reducing agent, whereas the water extract with the lowest phenolic content, was the weakest one.

Regarding the calculated IC50 values for different extracts, they followed the order of water> acetone> ethanol> methanol, which corrected well with their total phenolic contents (Table 2). Similar relations between

iron reducing activity and total phenolics content of plant extracts have been reported previously (Gao et al., 2000; Tsao et al., 2005; Arabshahi-Delouee & Urooj, 2007).

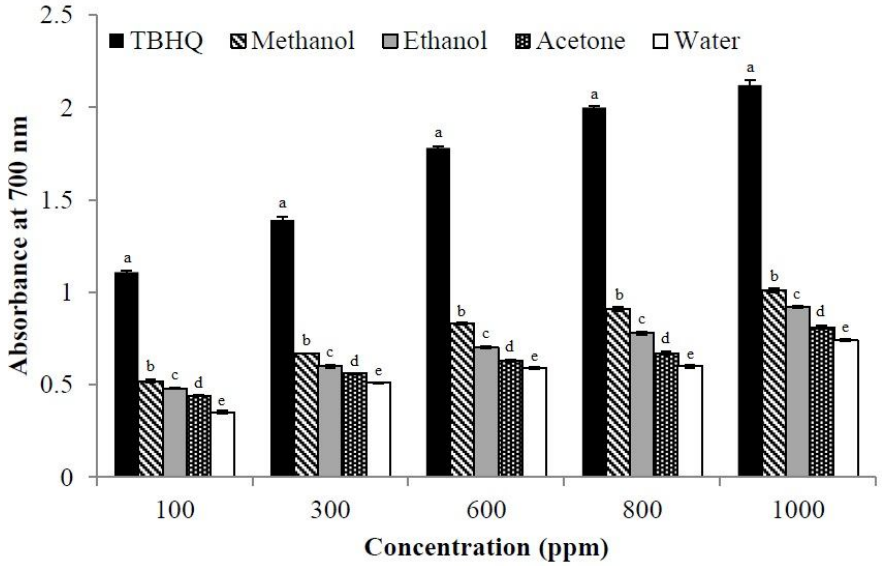

Fig.2. Reducing powers of methanol, ethanol, acetone and water extracts of *Boswellia serrata* oleogum resin and TBHQ.

Total antioxidant capacity

The phosphomolybdenum assay which is a quantitative method for evaluation of water-soluble and fat soluble antioxidants (Prieto *et al*.,1999) has been widely used to determine the total antioxidant capacity of many plant extracts (Kumaran & Karunakaran, 2007; Arabshahi-Delouee and Urooj, 2007; Jiang *et al*., 2014). As shown in Figure 3, the antioxidant capacity of the extracts was concentration-dependent; however, their capacities were inferior to that of TBHQ. In this assay, methanol extract showed the highest activity followed by acetone, ethanol, and water extracts. The IC_{50} values calculated for various extracts and TBHQ are presented in Table 2. Among the organic solvent extracts, the lowest (359.71 ± 0.61 µg/ml) and the highest (692.38± 0.59 µg/mL) IC_{50} values belonged to the methanol and ethanol extracts, respectively, while the IC_{50} of the water extract could not be determined. Results of this assay demonstrated electron-donating capacity of different extracts of *B. serrata* oleogum resin.

In this study, the DPPH radical scavenging and the reducing power activities assays of the extracts correlated to the amount of their phenolic contents, but in the total antioxidant capacity assay the order was different. Unlike previous assays (DPPH radica scavenging and reducing power), the total antioxidant capacity of the extracts did not correlate well with their total phenolic contents. Singh *et al*, (2008) has reported that apart from the phenols present in the *B. serrata resin*, boswellic acid which is a mixture consisting of four major pentacyclic triterpene acids can be found. The presence of such antioxidant compounds may explain the differences observed in the results of different assays.

Oxidative stability index

The rancimat method is based on the conductivity changes incurred by deionised water after collecting the volatile organic acids produced in the final steps of the accelerated oil oxidation process (García-Moreno *et al*., 2012). The method can provide a fast assessment of the efficiency and the thermal stability of different antioxidants under more challenging conditions than using the Scall oven test (Aladedunye *et al*., 2014). A high

correlation between sensory properties or analytical data and the results of rancimat analysis has been reported (Coppin & Pike, 2001; Anwar *et al*., 2003). Table 3 shows the oxidative stability of soybean oil containing different concentrations of various extracts of *B.serrata* compared to synthetic antioxidant, TBHQ. All the extracts of *B.serrata* were able to improve the oxidative stability of soybean oil, as indicated by longer induction periods compared to that of control without any antioxidant, however the activities were inferior to that of TBHQ. Methanolic extract containing the highest amount of total phenolics, exhibited the greater protective effect against the oxidation of soybean oil, whereas the water extract was the least effective one.

The amount and type of antioxidant components, the solubility in soybean oil, and the stability of the components during thermal processing, are the factors that affect the effectiveness of the antioxidant agents when evaluated with the rancimat test.

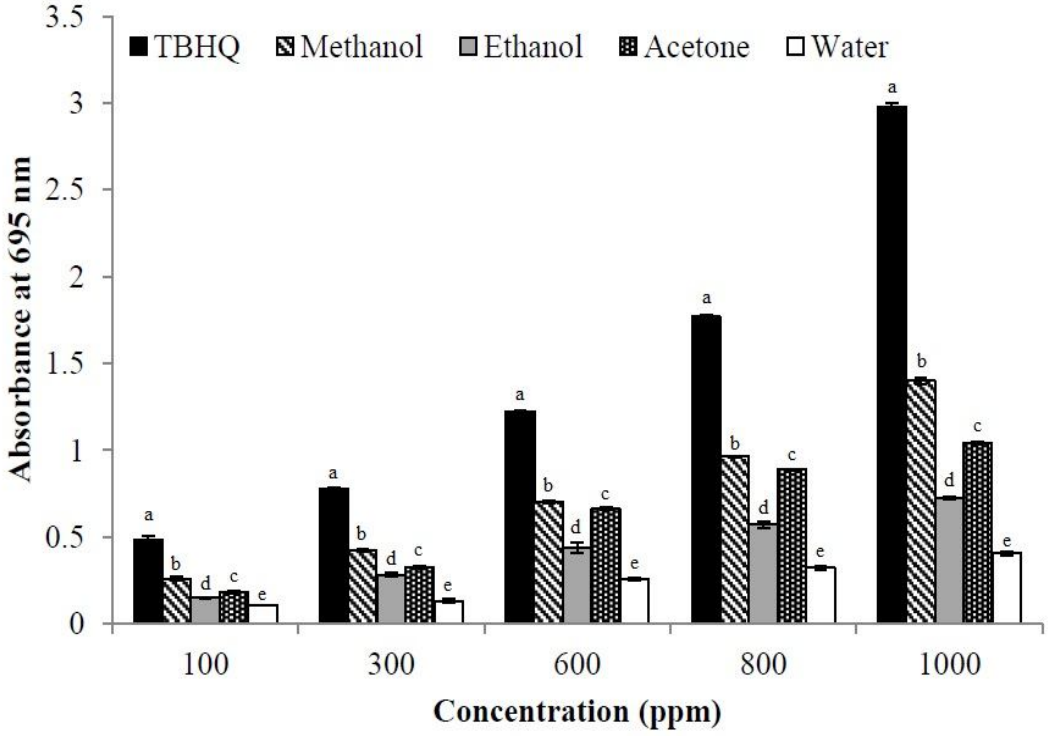

Fig. 3. Total antioxidant activities of methanol, ethanol, acetone and water extracts of *Boswellia serrata* oleogum resin and TBHQ.

Conclusions

Various solvent extracts of *B.serrata* oleogum resin showed varying degrees of antioxidant activity in different test systems in a concentration dependent manner. The type of extraction solvent and the concentration of the extract were assigned as important parameters in the overall activity of the extracts. Methanol proved to be the most efficient solvent for extraction of antioxidants from *B.serrata* as the related extract contained the highest amount of phenolic compounds and also exhibited the strongest antioxidant capacity in all the assays used. Overall, *B.serrata* was found to serve as a potential source of natural antioxidants for utilization in food and biological systems. In addition, potential exploitable beneficial effects and safety in humans need to be proved in clinical trials and the effect of the applied extracts on the sensory attributes of fats and oils should be further studied.

Table 3. Rancimat analysis of soybean oil samples containing different concentrations of *Boswellia serrata* extracts, or TBHQ at 110 °C. Values denoted by different letters within concentrations of the same extract are significantly different (p< 0.05).

Samples	Concentration(ppm)	Induction time (h)	AI[1]
Blank	-	6.14 ± 0.09	-
TBHQ	100	12.25 ± 0.11	1.99
Methanol extract	200	6.98 ± 0.09[d]	1.13
	500	7.30 ± 0.20[c]	1.18
	800	7.67 ± 0.05[b]	1.24
	1000	8.07 ± 0.09[a]	1.31
Ethanol extract	200	6.93 ± 0.10[d]	1.12
	500	7.16 ± 0.06[c]	1.16
	800	7.42 ± 0.04[b]	1.20
	1000	7.64 ± 0.05[a]	1.24
Acetone extract	200	6.71 ± 0.07[d]	1.09
	500	6.92 ± 0.05[c]	1.12
	800	7.28 ± 0.12[b]	1.18
	1000	7.41 ± 0.07[a]	1.20
Water extract	200	6.60 ± 0.06[d]	1.07
	500	6.79 ± 0.01[c]	1.10
	800	7.02 ± 0.05[b]	1.14
	1000	7.25 ± 0.08[a]	1.18

[1]Antioxidant activity index for Rancimat method

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ویژگی‌های آنتی اکسیدانی عصاره‌های مختلف اولئوگم رزین کندر *(Boswellia serrata)*

عادله محمدی[1]- سعیده عربشاهی دلویی[2*]- کریاکی زینوویادو[3]- کریس گالاناکیس[4]

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چکیده

ترکیبات فنولی کل و خصوصیات آنتی‌اکسیدانی عصاره متانولی، اتانولی، استونی و آبی اولئوگم رزین کندر توسط روش‌های مختلف مهار رادیکال‌های آزاد DPPH، قدرت احیاءکنندگی، ظرفیت آنتی‌اکسیدانی کل و آزمون رنسیمت مورد ارزیابی قرار گرفت. عصاره‌های مختلف فعالیت آنتی‌اکسیدانی متفاوتی را بروز دادند. عصاره متانولی حاوی بیشترین ترکیبات فنولی بود و ظرفیت آنتی‌اکسیدانی بالاتری را در تمامی آزمون‌های انجام گرفته نشان داد. علاوه بر این، همه عصاره‌ها توانستند پایداری اکسایشی روغن سویا را در آزمون رنسیمت بهبود بخشند. براساس نتایج به‌دست آمده، می‌توان از اولئوگم رزین کندر به‌عنوان منبعی بالقوه از آنتی‌اکسیدان‌های طبیعی استفاده نمود.

واژه‌های کلیدی: *Boswellia serrata*، ترکیبات فنولی، خصوصیات آنتی‌اکسیدانی، رنسیمت، زرشک، اولئوگم رزین

1- دانشجوی دکتری علوم و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، گرگان، ایران
2- استادیار گروه علوم و صنایع غذایی، دانشگاه آزاد اسلامی، واحد آزادشهر، آزادشهر، ایران
3- استادیار، گروه علوم و صنایع غذایی، دانشکده پروتیس، تسالونیکی، یونان
4- استادیار، گروه تحقیقات و نوآوری، آزمایشگاه‌های گالاناکیس، خانیا، یونان
(*- نویسنده مسئول : Email: saeedeh_arabshahi@yahoo.com)

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Effect of mixed edible coatings containing gum tragacanth and Aloe vera on postharvest quality of strawberries during storage

Aryou Emamifar[1*], Sudabeh Bavaisi[2]

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Abstract

The effects of mixed coating based on aloe vera (AG) and gum tragacanth (GT) on the microbial, physicochemical and sensorial properties of fresh strawberries were evaluated during 20 days of storage (1 °C, 95 % RH) compared to uncoated fruits. The coating solutions were prepared by mixing solution of AG diluted 1:3 with distilled water and GT solution (0.6 % w/v in distilled water) at different concentrations (25 % AG +75 % GT, 50 % AG +50 % GT and 75 % AG +25 % GT). Microbial stability (fungi (yeasts and molds) and total aerobic bacteria), physiochemical characteristics (ascorbic acid (AA), weight loss, firmness, titratable acidity, soluble solid content (SSC), anthocyanin content, total phenolic and antioxidant activity) and sensory attributes (color, taste, odor and overall) of the samples were evaluated after 0, 4, 8, 12, 16 and 20 days of storage compared to uncoated fruits. Comparing with untreated fruits, 50 % AG +50 % GT treatment significantly ($p<0.05$) decreased the microbial growth, weight loss and AA degradation and maintained firmness, anthocyanin and phenol contents and antioxidant activity. A greater visual acceptance was observed in fruits treated with 50 % AG + 50 % GT. The combination of AG/GT solution as a proper coating formulation in addition to have high antimicrobial activity; have great potential to extend shelf life of fresh strawberries.

Keywords: Strawberry; Aloe vera; Gum tragacanth; Shelf life.

Introduction

Strawberry fruits have a short shelf life, so after harvest, the marketability of them will reach about 5 d at temperature between 0 and 4 °C with 90-95 % relative humidity (Jiang *et al*. 2001). The loss of fresh strawberries can reach 40 % from harvesting to consumption (Park *et al*. 2005). There have been many studies on reducing postharvest losses of strawberry fruits using pre or post-harvest treatments including: polyamines (Ponappa *et al*. 1993), methyl jasmonate (Ayala-Zavala *et al*. 2005), UV (Erkan *et al.* 2008), nitric oxide (Wills *et al*. 2000), 1-methylcyclopropene (Bower *et al*. 2003) ultrasound (Aday *et al*. 2013), salicylic acid (Zhang *et al*. 2010) as well as application of edible coating (Tanada-Palmu and Grosso 2005; Del-Vallea *et al*. 2005). Application of edible coating on perishable fruits such as strawberries or fresh cut fruits is one of the most common methods to increase their shelf life through reducing the respiration rate, water loss, improving the sensory attributes and retarding the microbial growth (Bifani *et al*. 2007). Alginate, cellulose, chitosan, chitin, lipids, mucilage, milk protein, starch, wax, and zein have been widely used as an edible coating in the food industry (Valverde *et al*. 2005). Gum tragacanth is one of the most popular herbal gum that exudates from stem of *Astragalus gummifer*. Safety assessment and approving their use in foods (emulsifying agent, stabilizer, filing and thickening agent) have been confirmed (E-number E413) by the Scientific Committee for Food of the European Community (Gavlighi *et al*. 2013; Samanta *et al.* 2010; Fayazzadeh *et al*. 2014). Gum tragacanth is an acidic hetero-polysaccharide that contained some protein-bounded polysaccharides (Saffari *et al*. 2013). It consists of two different fractions including tragacanthin (water-soluble) and bassorin (water insoluble) with different rheological

1. Assistant Professor, Department of Food Science and Technology, Buali-Sina University, Hamedan, Iran
2. Master in Horticultural Science, Department of Horticultural Science, University of Kurdistan, Sanandaj, Iran.
(*Corresponding author email: a.emamifar@basu.ac.ir)
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properties (Balaghi *et al*. 2010). Aloe vera (*Aloe barbadensis Miller*) is a natural plant with functional properties such as antibacterial, antifungal and antioxidant activity (Nejatzadeh-Barandozi 2013). The transparent gel of aloe vera that contains nutritional components has also edible and medicinal values (Zafari *et al*. 2015). Two main components obtained from the fresh harvested aloe vera leaves including liquid fraction containing bitter excaudate and semisolid fraction containing parenchyma tissue. Moreover, it has improved postharvest life quality of fruits such as grape, nectarine, papaya and pomegranate arils (Valverde *et al*. 2005; Navarro *et al*. 2011). Many researches have been done on the applications of gum tragacanth in food formulations. Recently, it has been used in food packaging (Mostafavi *et al*. 2016). There are few reports on the use of tragacanth as an edible coating. The main mechanism of the film formation in polysaccharide films is the breaking apart polymer segments and reforming of the polymer chain into film matrix or gel that achieved by several film-forming mechanisms including solvent evaporation, creating hydrophilic and hydrogen binding or electrolytic and ionic crosslinking (Park *et al.* 2014). The safety of biopolymer materials, solutions and additive that used as fruit coatings is very important for its application on the food industry (Šuput *et al.* 2015). The main purpose of this research is to evaluate the effects of gum tragacanth combined with aloe vera gel on the microbial contamination, physicochemical and sensory properties of fresh strawberries during cold storage.

Materials and Methods

In the first step, the optimal concentration of diluted gum tragacanth in distilled water as an edible coating solution for fresh strawberries was determined and in the next step, the effects of the mixed edible coating containing aloe vera (AG) and gum tragacanth (GT) at different GT/ AG ratio on the postharvest quality of strawberries were evaluated.

Plant material

Under the guidance of expert botanists, at the University of Kurdistan, fresh strawberries (Fragaria X anannasa Duch.), cv. 'Parous', were harvested at maturity stage (based on 80 % red color on the surface) from a commercial farm in Sanandaj, Iran. They were immediately precooled (at 1^{o}C for 1 hour) after harvest and were directly transported to the laboratory. Then, fresh fruits were sorted for uniform size, color, and removed unripe and damaged and then were stored at 1 ^{o}C and 95 % RH.

Preparation of coating formulation (Gum tragacanth solution)

The ribbon tragacanth gum (slight-yellowish clear appearance) was purchased from the local market in Sanandaj, Iran. They were grinded (National, K039131, Cixi City, China) and sieved (Retch sieve, 0.425 mm, Lincoln, U.K.) to produce fine powder. The powdered gum tragacanth at different concentrations (0, 0.3, 0.6 and 0.9% w/v; of total solid) was added gently to the distilled water, mixed for 1 h and let stand overnight at ambient temperature to fully hydrate. Gum tragacanth solutions were pasteurized in a water bath at 70°C for 45 min and were cooled to 25^{o}C (Marpudi *et al*. 2011).

Coating and storage

Strawberry fruits were immersed and the coating treatments were performed at 25°C by immersing the strawberries for 5 min in gum tragacanth coating solutions and dried on a sterile stainless-steel screen under fans for about 1 h. Gum tragacanth solutions were pasteurized in a water bath at 70°C for 45 min and were cooled to 25^{o}C (Marpudi *et al.* 2011). Sterile distilled water was used as the control solution. The coated and uncoated (Control) fruits were then packaged in polyethylene packages (72 packages, each containing 15 pieces of strawberries) and stored at 1°C with 95 % RH. The samples were evaluated at three replications (Each replicate consisted of one packed with 15 plants) for their microbiological, physicochemical, and sensory characteristics

at 0, 4, 8, 12, 16 and 20 d of storage. After the selection of the best concentration of gum tragacanth solution, it was applied for preparing the mixed coating solution with aloe vera gel solution.

Preparation of coating formulation (Aloe vera gel)

Aloe vera leaves were cut from greenhouse-grown plants (University of Kurdistan, Sanadaj, Iran). They were washed with water, disinfected (in a 2% sodium hypochlorite solution for 30 min) and rinsed with distilled water. Aloe vera gel matrix was then separated from the outer cortex of leave, ground, and filtered to produce a fresh aloe vera gel. After pasteurization (at 70ºC for 45 min) the gel was cooled to 25ºC (Marpudi *et al*. 2011). The coating solution was prepared by aloe vera gel diluted 1:3 with sterile distilled water (Valverde *et al*. 2005; Hassanpour 2015).

Preparation of coating formulation (Aloe vera and Gum tragacanth solutions)

Mixtures of pasteurized (GT) and aloe vera (AG) solutions at different concentrations (25% AG+ 75 % GT, 50% AG+ 50% GT and 75% AG+ 25% GT) were prepared as an edible coating. Fruits were dipped in coating solutions or sterile distilled water as the control solution, at 25°C for 5 min. They dried on a sterile stainless-steel screen under fans for about 1 h to ensure surface dryness. The coated and uncoated (control) fruits were packaged (72 packages, each containing 15 pieces of strawberries) and stored (under the same conditions as mentioned above). Samples were evaluated exactly according to section 2.3. (Coating and storage).

Microbiological evaluations

At each sampling time, for each replicate a mixed sample with quarter of each fruit was transferred aseptically to a stomacher bag and homogenized. Decimal dilutions of the suspension of strawberries in sterile peptone water (0.1%) were prepared. With the spread plate and pour plate methods, enumeration of total aerobic bacteria (at 30°C for 2 d), yeasts and molds (at 25°C for 2d) were carried out on the plate count agar (PCA, Scharlau Chemie, S.A., Barcelona, Spain) and the potato dextrose agar (PDA, Scharlau Chemie, SA., Barcelona, Spain), respectively. Serial dilutions (10^{-2} to 10^{-3}) were performed in triplicate and dilutions were plated in duplicate. The results were expressed as colony-forming units (CFU) per gram (Sogvar *et al*. 2016).

Physicochemical properties

Weight loss (WL) and Firmness

The weight of each packaged (replicate) was recorded immediately after packaging and after each 4d of storage. Weight loss was expressed as the percentage loss of the initial fresh weight. The firmness of strawberries in each box replicate was evaluated by texture analyzer (Santam, STM-1) with an 8 mm probe with constant speed of 20 mm min^{-1}. Two different measurements were done on opposite sides of the central zone of each fruit (Sogvar *et al.*, 2016). Force values were expressed as newton (N).

Titratable acidity (TA), soluble solid content (SSC) and Ascorbic acid

A mixed sample of all fruit with one-eighth segments of each fruit per replicate juiced together and used for measuring the titratable acidity (TA), soluble solid content (SSC) and ascorbic acid. TA was measured based on titrimetric method by using 0.1 M NaOH (to pH 8.1) and was expressed as percentage of citric acid per 100 mL fruit juice (AOAC, 2002). SSC was detected by digital refractometer (AOAC, 2002). Ascorbic acid was determined based on titrimetric method by 2,6-dichlorophenolindophenol as a titrant (AOAC 967.21) and expressed as mg kg^{-1} fresh weight basis of fruits (AOAC, 2002).

Total anthocyanin content (TAC), total phenolic concentrations (TPC) and Total antioxidant activity (TAA)

At the sampling time, a bulked sample of all fruits with 1/8 th of each fruit per replicate was cut, frozen and stored at -80ºC and they were used for determination of total anthocyanin content (TAC), total phenolic

(TP) concentrations and total antioxidant activity. Total anthocyanin content was measured by a pH differential method (Cheng and Breen 1991). The absorbance of the combined sample extract with quarter of each fruit was read at 510 nm and 700 nm in buffers at pH 1.0 and 4.5. Results were expressed as milligrams of pelargonidin 3-glucoside (P 3-G) equivalents per kilogram of fresh weight. Total phenolic concentrations were determined according to Folin Ciocalteu method using gallic acid as the standard curve and the results were presented as milligram of gallic acid equivalent per kilogram of fruit fresh weight (Singleton *et al.,* 1999). Measurement of the total antioxidant activity of frozen samples was carried out by 2, 2-diphenyl-1-picryl-hidrazil (DPPH) radical-scavenging method (Sanchez Moreno *et al*. 1999). The absorbance was measured at 517 nm, using a spectrophotometer (UNICO UV-2100, Shanghai, China) and the results presented as the percentage inhibition of DPPH radicals.

Sensory analysis

Sensory characteristics (color, odor, taste, and overall) of uncoated and coated strawberries were evaluated based on a 5-point scale (extremely like= 5, moderately like= 4, neither liked nor disliked= 3, moderately dislike= 2 and extremely dislike= 1) by 10 expert panelists. Fruits were sampled at 0, 4, 8, 12, 16 and 20 days of storage (Emamifar *et al.* 2010).

Statistical analysis

Analysis of variance was carried out using the SAS statistical software release 6.12 (SAS Institute, Cray, NC) based on completely randomized designs. Significant differences among the data were represented as $p< 0.05$.

Results and Discussion

Experimental section 1

Table 1, shows the variations of the mean microbial populations, weight loss and ascorbic acid content in the tragacanth coated fruits compared to control (uncoated) during storage (at 1^{o}C and 95% RH). In all samples, the microbial populations and weight loss increase and ascorbic acid content decreases at different rates as the storage time increases, significantly ($p<0.05$). The mean initial populations of yeasts and molds and total aerobic bacteria in freshly harvested strawberries immediately after packaging (control samples) were found to be 1.47 log CFU $g^{-1}$and 1.38 log CFUg^{-1}, respectively. For the coated strawberry fruits with 0.6% gum tragacanth solution, significance decreases were observed in yeasts and molds and total aerobic bacteria populations compared to all other coated and uncoated samples, after 20 d. The weight loss of uncoated fruits was increased significantly ($p<0.05$) up to 34.15% as compared to 26.58, 18.85 and 22.38 % for strawberries coated with 0.3, 0.6 and 0.9% gum tragacanth solutions, respectively. Ascorbic acid degradation in control samples was higher than in other coated samples after 20 d of storage. At the end of storage period, strawberries coated with 0.6% gum tragacanthe solution maintained higher ascorbic acid content (64%) compared to other that coated with 0.3% gum tragacanthe solution (52%), 0.9% gum tragacanthe solution (58%) and control (46%), significantly ($p<0.05$). However, the 0.6% w/v solution of gum tragacanth treatment shows a significant ($p<0.05$) effect on reducing the microbial populations and weight loss and maintaining the ascorbic acid content in strawberry fruits as compared to the other treatments and control during storage. These results are in agreement with the findings of Mohebbi *et al*. (2012). Treviño-Garza *et al*. (2015) found that edible active coatings based on polysaccharides reduced the microbial growth in coated strawberry fruits and increased their shelf life up to 15 d. Gol *et al*. (2013) showed that the combination of Hydroxypropyl methyl cellulose (HPMC) and chitosan as an edible coating can maintain the ascorbic acid content in fresh strawberries during cold storage. Significant reductions of weight loss were observed in strawberries coated with cassava starch (Garcia *et al*. 2012) and chitosan-based edible coatings (Han *et al*.

2004) during cold storage. Strawberry fruits coated with 0.6% w/v gum tragacanth solution showed significantly ($p<0.05$) higher values of firmness, total anthocyanin, total phenol, antioxidant activity and sensory attributes and lower values of SSC and TA compared to other treatments and control (data not shown).

Table 1. Effect of tragacanth (mean ± sd) [a] on the yeasts and molds, total aerobic bacteria populations, weight loss and ascorbic acid of strawberries stored at 1 °c for 20 d.

	Tragacanth concentration (%)	Storage time(days)					
		0	4	8	12	16	20
Molds and Yeasts (log CFU g^{-1})	0	1.47 ^{k} ± 0.05	2.25 ^{g} ± 0.04	2.61 ^{e} ± 0.01	2.81^{cd} ± 0.04	3.04 ^{b} ± 0.02	3.37 ^{a} ± 0.05
	0.3	1.47 ^{k} ± 0.04	1.88 ^{j} ± 0.03	2.29 ^{g} ± 0.05	2.57 ^{e} ± 0.05	2.78 ^{d} ± 0.02	2.89 ^{c} ± 0.07
	0.6	1.47 ^{k} ± 0.04	1.61 ^{j} ± 0.03	1.93 ^{i} ± 0.02	2.08 ^{h} ± 0.05	2.39 ^{f} ± 0.05	2.57 ^{e} ± 0.02
	0.9	1.47 ^{k} ± 0.01	1.62 ^{j} ± 0.07	2.06 ^{h} ± 0.01	2.39 ^{f} ± 0.05	2.61 ^{e} ± 0.07	2.74 ^{d} ± 0.02
Total aerobic bacteria (log CFU g^{-1})	0	1.38 ^{m} ± 0.03	2.13 ^{gh} ± 0.01	2.45 ^{d} ± 0.04	2.70 ^{c} ± 0.01	2.91 ^{b} ± 0.03	3.29 ^{a} ± 0.05
	0.3	1.38 ^{m} ± 0.07	1.84 ^{ij} ± 0.03	2.04 ^{h} ± 0.03	2.26^{ef} ± 0.06	2.54 ^{d} ± 0.01	2.78 ^{c} ± 0.08
	0.6	1.38 ^{m} ± 0.06	1.54 ^{l} ± 0.04	1.76 ^{j} ± 0.02	1.91^{l} ± 0.05	2.19 ^{fg} ± 0.08	2.48 ^{d} ± 0.07
	0.9	1.38 ^{m} ± 0.02	1.65 ^{k} ± 0.06	1.91 ^{l} ± 0.01	2.16 ^{g} ± 0.04	2.34 ^{e} ± 0.02	2.70 ^{c} ± 0.02
Weight loss (%)	0	0.00 ^{j} ± 0.00	6.75 ^{i} ± 0.70	15.76 ^{e} ± 0.36	21.10 ^{c} ± 0.40	26.64 ^{b} ± 0.45	34.15 ^{a} ± 1.01
	0.3	0.00 ^{j} ± 0.00	6.10 ^{i} ± 0.28	12.90 ^{f} ± 0.65	18.09 ^{d} ± 0.60	21.57 ^{c} ± 0.22	26.58 ^{b} ± 0.17
	0.6	0.00 ^{j} ± 0.00	5.20 ^{i} ± 0.69	9.12 ^{h} ± 0.44	12.51 ^{fg} ± 0.48	15.69 ^{e} ± 0.18	18.85 ^{d} ± 0.19
	0.9	0.00 ^{j} ± 0.00	5.95 ^{i} ± 0.09	10.85 ^{gh} ±0.13	15.04 ^{e} ± 0.34	17.78 ^{d} ± 0.24	22.38 ^{c} ± 0.22
Ascorbic acid (mg kg-1)	0	555.50 a ± 3.34	435.50 e ± 11.1	382.25 fg ± 7.79	347.75 ji ± 1.12	307.75 l ± 3.36	255.50 m± 11.12
	0.3	555.50 a ± 5.57	487.70 c ± 8.86	425.50 e ± 2.22	371.15 gh± 1.16	335.50 jk ± 1.19	294.40 l ± 3.39
	0.6	555.50 a ± 1.12	532.25 b ± 2.23	488.87 c ± 3.34	422.25 e ± 7.72	385.50 fg ± 8.88	355.50 hi ± 1.16
	0.9	555.50 a ± 7.78	592.20 b ± 1.14	455.50 d ± 3.32	395.50 f ± 6.63	355.51 hi ± 3.34	326.65 k ± 1.09

Values followed by the same letter in the same row are not significantly different ($p < 0.05$).

Experimental section 2

Microbiological evaluation

Strawberry fruits can become contaminated with spoilage microorganisms during growth in the field, harvesting and postharvest handling, or during storage (Barth *et al.* 2009). Moreover, the fruit surface has long been considered a suitable environment for the growth of microorganisms. More generally, fruits surface microbes have impact on the rates of food spoilage (Akhtar *et al.* 2016).Freshly harvested strawberries contain more nutrients and high water content. Therefore, they are highly susceptible to microbial decay. There are multiple factors that considerably affect microbial decay during storage including temperature, relative humidity and O_2 concentration (Kader 1998). Edible coating act as barriers to moisture and oxygen during storage and delayed decay development in coated fruits as compared to uncoated (Valverde *et al.* 2005). The variations of the mean microbial populations in coated fruits compared to control (uncoated) during cold storage are shown in Fig. 1. For all samples, the microbial population increased significantly ($p<0.05$) at different rates as the storage time increased. The populations of yeasts and molds and total aerobic bacteria in control samples reached 3.81 log $CFUg^{-1}$and 3.82 log $CFUg^{-1}$, respectively, after 20 d. According to Fig. 1. The mixed gum based treatments have significant ($p<0.05$) effects on retarding microbial growth in all strawberry samples during cold storage. In comparing all treated and untreated samples, 50 % GT + 50 % AG treatment showed the most antifungal (2.72 log CFU g^{-1}) and antibacterial (2.78 log CFU g^{-1}) activity on fresh strawberries during 20 d of storage at 1 ^{o}C. The effect of the volume ratio of AG to GT on antimicrobial activity of mixed coating material is

pronounced significantly ($p<0.05$). As shown in Fig. 1, increased AG: GT ratio is believed to be related to the decreased antimicrobial activity of coating materials. However, coating formulation containing lower concentrations of aloe vera (50%) have significantly more antimicrobial activity than coating formulation containing 75% of aloe vera ($p<0.05$). Aloe vera contains several special compounds with antimicrobial activity, including anthraquinones (Garcia-Sosa *et al*. 2006), dihydroxy- anthraquinones (Wu *et al*. 2006) and saponins (Reynolds and Dweck 1999). Ferro *et al*. (2003) showed that aloe vera gel exhibited an antimicrobial activity against gram positive and gram-negative bacteria as well as yeasts and molds. Moreover, the antifungal activity of the aloe vera gel against fungi spores such as *Penicillium*, *Botrytis* and *Alternaria* and against mycelium growth of fungi such as *Rhizoctonia*, *Fusarium and Colleotrichum* (Saks and Barkai-Golan 1995; Jasso de Rodriguez *et al*. 2005) have reported. Shelf life extension and microbial load reduction of pomegranate arils, table grape and sweet cherry using aloe vera gel as an antimicrobial coating have been reported (Martínez-Romero *et al*. 2013; Valverde *et al*. 2005; Martinez-Romero *et al*. 2006). Recently, antimicrobial activity of gum tragacanth against some gram negative and gram-positive bacteria and molds and yeasts has been reported (Ghayempour *et al*. 2015; Singh *et al*. 2015). Muthulakshmi (2013) showed that the interaction between gum tragacanth amino groups with gram-negative bacterial cell wall as well as the binding of gum tragacanth carboxylate groups with the gram-positive bacterial cell and the fungal cell wall are the most important reasons for antimicrobial activity of this gum. Moreover, the complex structure and high molecular weight of gum tragacanth have more positive effects on antimicrobial activity of gum tragacanth (Muthulakshmi 2013).

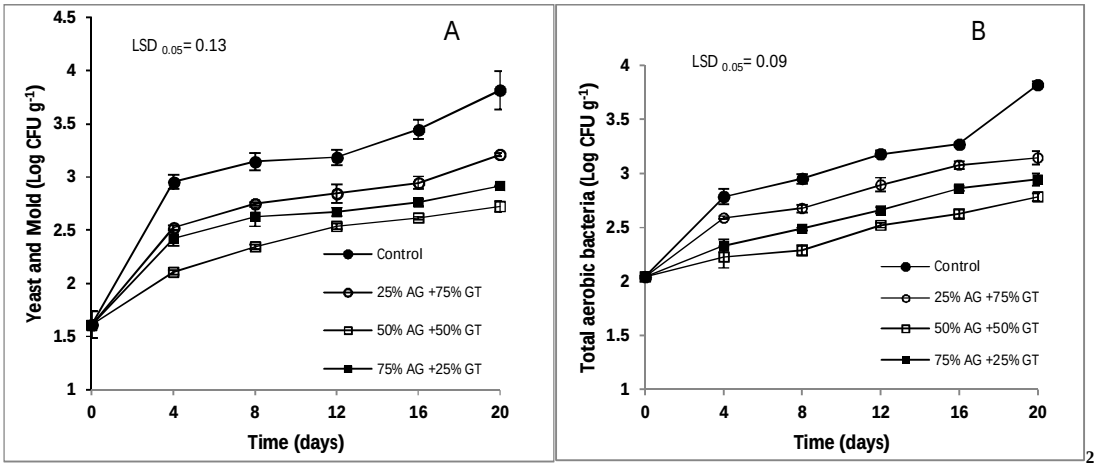

Fig. 1. Effect of mixed coating based on aloe vera (AG) and gum tragacanth (GT) on the yeasts and molds populations (a) and total aerobic bacteria populations (b) of strawberries stored at 1 °C for 20 d. Vertical bars represent standard error (n = 3).

Weight loss

According to Fig. 2.A., in all samples, the weight loss increases at different rates as the storage time increases, significantly ($p<0.05$). Strawberries coated with 50% GT+ 50% AG showed the lower rate of weight loss compared to the others, so that after 20 d of storage, the weight loss of uncoated fruits reached to 26.6% as compared to 21.1% (25% AG+ 75% GT), 18.7 %(50% AG+ 50% GT) and 19.3% (75% AG+ 25% GT), respectively (Fig. 2.A.). No significant differences were observed in weight loss between two-treatment coating including 50% AG+ 50% GT and 75% AG+ 25% GT, except on the 8th d ($p<0.05$). Weight loss often occurred due to water evaporation in

fruits and vegetables during respiration process (Yaman and Bayoindirli 2002). However, the main impact of coatings application are based on their hygroscopic properties, which form a water barrier layer between fruits and environments and reduce the fruit surface evaporation (Mohebbi *et al.* 2012). Aloe vera coating has also been reported to reduce the weight loss of table grapes, sweet cheery, apple and papaya stored at cold storage (Valverde *et al.* 2005; Martinez-Romero *et al.* 2006; Marpudi *et al.* 2011; Ergun and Satici 2012). Mohebbi *et al.* (2012) reported that the combination of aloe vera and gum tragacanth are more effective on reducing the weight loss of button mushroom. It can be concluded that the combination of aloe vera and gum tragacanth in the specified concentration ranges have a synergetic effects on reducing the weight loss of the strawberries during cold storage.

Firmness

The firmness is one of the most important physical quality characteristics of fruit during storage (Pasquariello *et al.* 2013). As shown in Fig. 2.B., with increasing the storage time the rate of softening increased in all of strawberries, but it was faster in uncoated than those of coated samples (Fig. 2.B.). After 20 d, firmness value decreased from 3.05 N to 1.41, 1.73, 2.26 and 1.93 N in uncoated and coating treatments including 25% AG+ 75% GT, 50% AG+ 50% GT and 75% AG+ 25% GT, respectively (Fig. 2.B.). 50% AG+ 50% GT coating was more effective in firmness retention of the strawberries compared to others ($p<0.05$). These results are in agreement with the results reported by many studies which stated that the firmness of strawberry fruits treated with edible coating including cactus mucilage (Del-Valle *et al.* 2005), starch (García *et al.* 2012) and gluten (Tanada-Palmu and Grosso 2005) were maintained during storage. Degradation of the middle lamella of the cortical parenchyma cells and cell separation and subsequent microbial infection are the main reasons for the softening of strawberry fruits during storage (Rahman *et al.* 2016).

Titrable acidity (TA) and soluble solids content (SSC)

For all samples, SSC increased and TA decreased during storage (Fig. 2.C and D). The variations of TA and SSC values in uncoated fruits during storage were significantly ($p<0.05$) higher than those of coated fruits. According to Fig. 2C. and D, after 20 d of storage; the highest TA (0.705%) and the lowest SSC (6.9%) were recorded in strawberries treated with 50% AG+ 50% GT, compared to the other treatments and control samples, indicating uncoated strawberries presented a more pronounced ripening development than coated strawberries, similarly to that found in starch-coated strawberry (Mali and Grossmann, 2003). These results agreed with those found by EL Gaouth *et al.* (1991), Pelayo *et al.* (2002) and Koyuncu (2004). Zheng *et al.* (2007) showed that decreasing in titratable acidity of strawberry fruits during cold storage caused by increasing their respiration rate. The increase in SSC of strawberry fruits during storage could be related to degradation of cell-wall polymers or other polysaccharides such as hemicelluloses and water loss (Hernandez-Munoz *et al.* 2008; Tanada-Palmu and Grosso 2005).

Ascorbic acid content

Ascorbic acid content of strawberries can be influenced by pre-harvest and postharvest factors including cultivar, growing season, environmental conditions, harvest time and storage conditions (Skrovankova *et al.* 2015; Cordenunsi *et al.* 2005). The average ascorbic acid content of strawberry fruits is ranged from 190 mg kg^{-1} to 715 mg kg^{-1} (Lee and Kader 2000). According to Fig. 3.A., ascorbic acid in all coated and uncoated samples reduced over 20 d of storage. Coating containing 50% AG+ 50% GT was the most effective treatment to maintain ascorbic acid (29.26%) in strawberries during storage up to 20 d while this value was decreased to 17.29 % for uncoated and 23.31 and 28.35% for coating containing 75% AG+ 25% GT and

25% AG+ 75% GT, respectively. Autoxidation is one of the main reasons for ascorbic acid depletion during storage of fruits (Plaza *et al*. 2006). Coating materials provide a protective thin layer between the fresh fruit and its surrounding atmosphere that decrease the moisture transfer, O_2, and CO_2 exchange (Tapia *et al*. 2008; Atress *et al*. 2010). Adetunji *et al*. (2012) have found that pineapple coated with aloe vera gel showed the shelf life extension by decreasing the oxygen permeability.

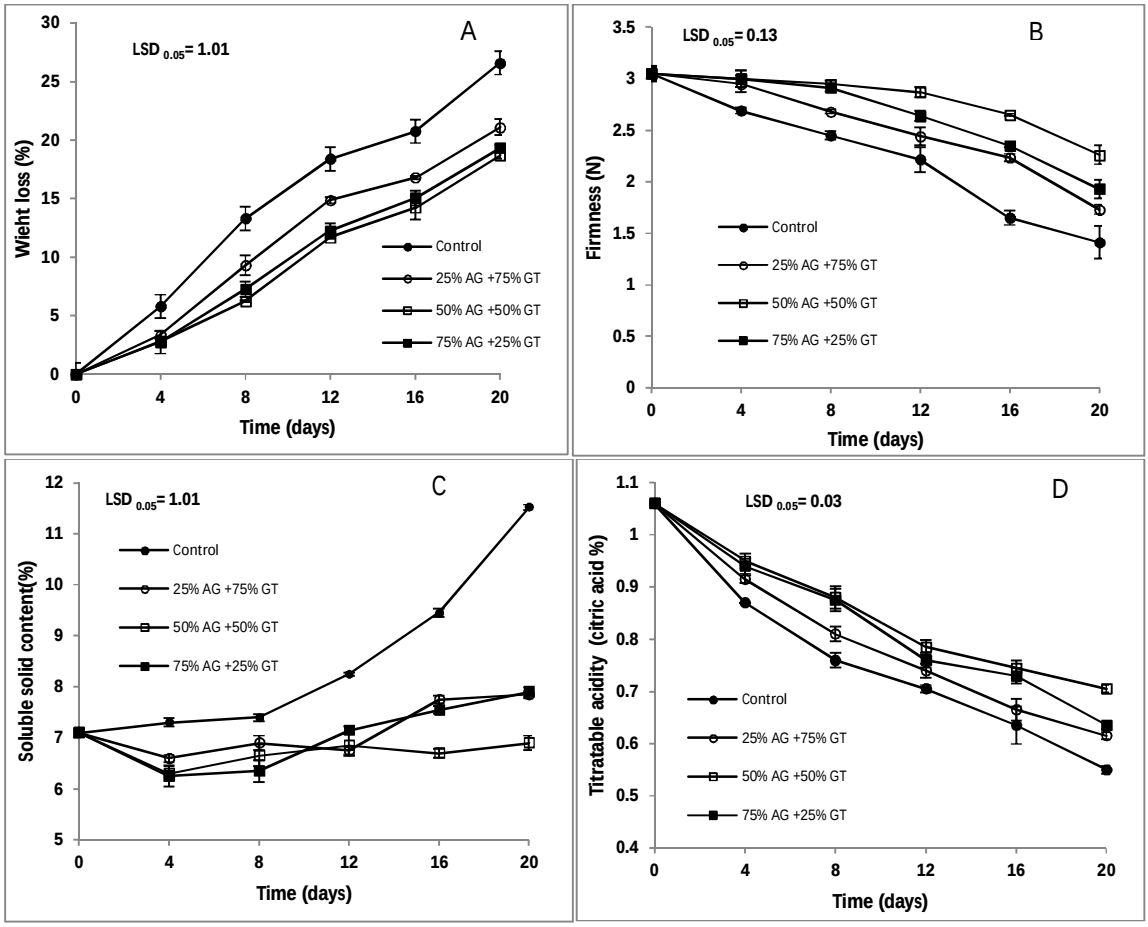

Fig. 2. Effect of mixed coating based on aloe vera (AG) and gum tragacanth (GT) on the weight loss (A), Firmness (B), Soluble solid content (C) and Titratable acidity (D) of strawberries stored at 1°C for 20 d. Vertical bars represent standard error (n = 3).

Total anthocyanin content (TAC), total phenolic concentrations (TPC)

Anthocyanin and phenolic compounds are beneficial phytochemicals that affect the functional properties of strawberry fruits (Giampieri *et al*. 2012). There is an increase in consumers' demand of fresh strawberry fruits due to beneficial properties (Flores-Félix *et al*. 2015). It is therefore critical to consider their changes during storage. Coating treatments and storage time significantly ($p<0.05$) affected the initial total anthocyanin content (50.5 mg kg^{-1} of pelargonidin-3-glucoside) and total phenol content (355 mg kg^{-1} of garlic acid on fresh weight basis) of fresh strawberries during storage (Fig. 3.B. and C.). After 4 d of storage, a sharp increase in anthocyanin was observed in all coated fruits compared to uncoated (Fig. 3. B.). Fruits treated with 50%

AG+ 50% GT had the greatest anthocyanin content than those of other treatments and untreated fruits after 20 d of storage ($p<0.05$). The amount of anthocyanin content of strawberry is dependent on the cultivar, storage temperature and O_2 concentration (Cordenunsi *et al*. 2005). Several studies have shown that the biosynthesis of the strawberry fruits have continued during storage (Kalt *et al*. 1999; Cordenunsi *et al*. 2005; Ayala-Zavala *et al*. 2005). However, a slight increase in anthocyanin content for all samples was observed up to 20 d of storage (Fig. 3.B). According to Fig 3.C., the total phenolic contents in all uncoated samples decreased following storage for 20 d (223 mg kg^{-1}) but they decreased in coated samples up to 16 d in 25% AG+ 75% GT (252 mg kg^{-1}), 50% AG+ 50% GT (278.5 mg kg^{-1}) and 75% AG+ 25% GT (271 mg kg^{-1}) and then increased up to 20 d. The total phenolic contents decrease at different rates as the storage time increases, significantly ($p<0.05$). However, phenol degradation in control was higher than other coated samples after 20 d (Fig.3.C.). Strawberries coated with 50% AG+ 50% GT maintained higher total phenols (295 mg kg^{-1}) compared with other coated treatments at the end of storage. Coating materials stabilize the TSS/ TA ratio in fruits and cause the pH remain at a low level. It is leading to a decrease in enzyme activity of polyphenol oxidase and increase total phenol concentrations (Singha *et al*. 2009). Gol *et al*. (2013) showed that total phenolic contents increased in strawberries during cold storage.

Antioxidant activity

Antioxidant activity in coated and uncoated strawberry fruits decreased during cold storage at 1°C (Fig.3.D.). Reducing the rate of antioxidant activity in coated fruits was faster than that of uncoated. The high antioxidant activity was observed in strawberry fruits that were coated with 25% AG+ 75% GT (45.5%), 50% AG+ 50% GT (55.5%) and 75% AG+ 25% GT (50.6%) compared to control (36.5%) after 20 d. As shown in Fig.3.D, coating containing 50% AG+ 50% GT is the most effective treatment to maintain antioxidant activity in strawberry fruits during cold storage. However, aloe vera gel has inherent antioxidant capacity resulted in aloe gel coated fruits retained their antioxidant activity. Previous studies have shown that antioxidant capacity of aloe vera at different stages of development, is due to many active compounds with different degrees of antioxidant capacity (Vieira *et al.* 2016; Wu *et al.* 2006). Ascorbic acid and the total phenolic contents in strawberry fruits could have a significant impact on the antioxidant activity (Kelebek *et al.* 2009; Wang and Lewers 2007). Studies have shown that grapes (Serrano *et al.* 2006) and raspberry (Hassanpour 2015) coated with aloe vera gel had higher antioxidant capacity than uncoated.

Sensory evaluation

According to Fig.4. Significant differences ($p<0.05$) were determined in the sensory characteristics of coated strawberries compared to uncoated except for the color score. Scores for all sensorial attributes fall down as the storage time increased, significantly ($p<0.05$). After 20 d, the panelists assigned the highest color, odor, taste and overall scores to strawberry fruits coated with 50% AG+ 50% GT, 75% AG+ 25% GT and 25% AG+ 75% GT, respectively and the lowest score being associated with uncoated samples (Fig. 4.). The odor and texture scores correlated well with the microbial load and firmness values presented in Fig. 1. and Fig. 2.B, respectively. It can be concluded that, 50% GT+ 50% AG treatment as the best coating combination, could keep the sensory attributes of strawberries over the storage period. Garcia *et al.* (2012) reported that sensory characteristics of the strawberries coated with cassava starch had been accepted by consumers up to 12 d compared to uncoated fruits. Benitez *et al.* (2013) showed that the coating of kiwifruit slices with 5% aloe vera gel improved their marketability up to 8 d.

Conclusion

The results obtained in the present research showed that application of combined edible coating containing aloe vera and gum trgacanth is a new preservation approach for fresh strawberry fruits at 1ºC. Using solution coating containing 50 % diluted aloe vera (1: 3) and 50% gum tragacanth solution (0.6 % w/v), extended the shelf life of fresh strawberries up to 20 d without any side effects on their physicochemical characteristics and sensorial attributes. However, application of combined coating containing aloe vera and gum tragacanth for storage of fresh strawberries is not sufficient for long time storage. This study revealed that for extension shelf life of fresh strawberries, it is necessary to apply another treatment as a new hurdle in addition to coating treatments.

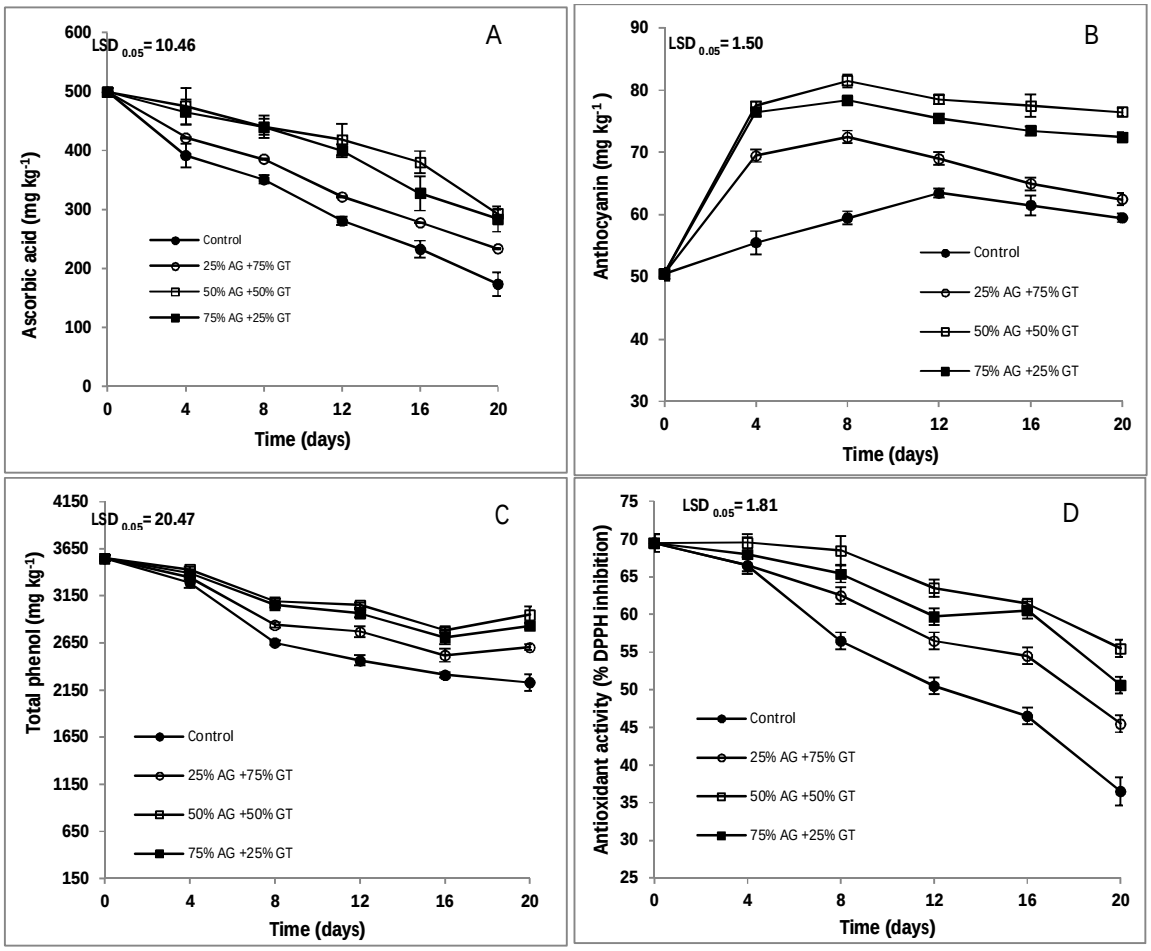

Fig.3. Effect of mixed coating based on aloe vera (AG) and gum tragacanth (GT) on Ascorbic acid (A), Anthocyanin (B), Total phenols (C) and Antioxidant activity (D) of strawberries stored at 1 °C for 20 d. Vertical bars represent standard error (n = 3).

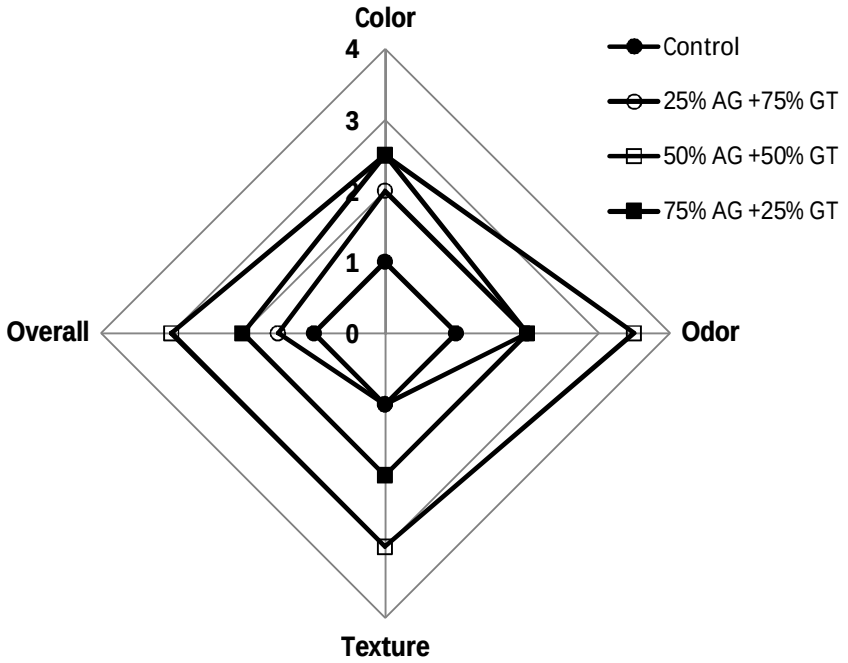

FIG. 4. Effect of mixed coating based on aloe vera (AG) and gum tragacanth (GT) on the sensory attributes of strawberries after 20 d at 1 ºC.

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تاثیر پوشش خوراکی ترکیبی بر پایه صمغ کتیرا و آلوئه‌ورا بر کیفیت پس از برداشت توت‌فرنگی طی انبارداری

آریو امامی‌فر[1*] - سودابه باویسی[2]

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چکیده

اثرپوشش ترکیبی بر پایه ژل آلوئه ورا و ژل کتیرا بر ویژگی های میکروبی، فیزیکوشیمیایی و حسی توت فرنگی تازه طی 20 روز انبارداری (دمای یک درجه سانتی گراد و رطوبت نسبی 95 درصد) در مقایسه با نمونه بدون پوشش ارزیابی گردید. پوشش ها با مخلوط کردن محلول ژل آلوئه ورا رقیق شده (به نسبت 1:3 وزنی حجمی در آب مقطر) و ژل کتیرا (با غلظت 0/6 درصد وزنی حجمی در آب مقطر) در غلظت های مختلف (25% ژل آلوئه ورا + 75% ژل کتیرا، 50% ژل آلوئه ورا + 50% ژل کتیرا و 75% ژل آلوئه ورا + 25% ژل آلوئه ورا + 75% ژل کتیرا) تهیه شدند. پایداری میکروبی (تعداد کپک و مخمر و کل باکتری های مزوفیل هوازی)، خصوصیات فیزیکوشیمیایی(اسید آسکوربیک، کاهش وزن، سفتی، اسیدیته، مواد جامد محلول، محتوی آنتوسیانین، فنل کل و فعالیت ضد اکسایشی) و ویژگی های حسی (رنگ، طعم، بو و پذیرش کلی) نمونه ها پس از 0، 4، 8، 12، 16 و 20 روز از شروع انبار داری در مقایسه با نمونه بدون پوشش (شاهد) ارزیابی گردید. پوشش های حاوی50% ژل آلوئه ورا + 50% ژل کتیرا در مقایسه با نمونه های بدون پوشش به صورت معنی داری ($p< 0/05$) رشد میکروبی، کاهش وزن و تخریب اسید آسکوربیک را در نمونه های توت فرنگی کاهش داده و سفتی بافت، محتوی آنتوسیانین، فنل کل و ظرفیت ضد اکسایشی آن ها را حفظ کردند. همچنین بیشترین امتیاز ویژگی های حسی به توت فرنگی های پوشش داده با 50% ژل آلوئه ورا + 50% ژل کتیرا اختصاص یافت. به هر ترتیب محلول ترکیبی از دو ژل کتیرا و آلوئه ورا به عنوان یک فرمول پوششی مطلوب، علاوه بر خاصیت ضد میکروبی زیاد، از توانایی بالایی در افزایش ماندگاری توت فرنگی تازه برخوردار بود.

واژه‌های کلیدی: توت‌فرنگی، آلوئه ورا، صمغ کتیرا، عمر نگهداری

1- استادیار، گروه صنایع غذایی دانشگاه بوعلی سینا، همدان.
2- دانشجوی کارشناسی‌ارشد، گروه صنایع غذایی دانشکده کشاورزی دانشگاه کردستان.
(*- نویسنده مسئول : Email: a.emamifar@basu.ac.ir)

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Canola seeds losses during harvest using grain combine harvester as a function of thermal properties of canola unbroken pod

Ehsan Ghajarjazi[1], Mohsen Azadbakht[1*], Farshid Ghaderifar[2]

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Abstract

The present study investigated the thermal properties of canola pods (thermal conductivity, specific heat, and thermal diffusivity), canola losses (natural losses, platform losses, and total combine harvester losses), and unbroken pods in three common canola varieties cultivated in the North of Iran (Hyola 420, Hyola 401, and Hyola 50) at three times of pre-harvest, harvest, and post-harvest. Furthermore, the relation between the thermal properties of canola pods and the amounts of losses during harvest was studied. Thermal conductivity coefficient, specific heat, and thermal diffusivity were determined using line heat source, mixture method, and calculation methods, respectively. Seed losses were calculated, using a built grain collector. The results revealed that adjustments, variety, and sampling time had significant effects on thermal conductivity and specific heat of canola varieties at the probability level of 1%. The effect of the interaction between variety and time on thermal conductivity, specific heat, and thermal diffusivity was considerable at the probability levels of 1% and 5%, respectively. Furthermore, the effects of canola varieties and harvest time on natural losses, total combine harvester losses, as well as unbroken pods were substantial at 1% probability. In addition, a notable relation was observed between thermal conductivity coefficient and platform losses at 5% and unbroken pods at 1%. However, unbroken pods indicated a substantial relation with specific heat and thermal diffusivity at 1%.

Keywords: Canola pod, Harvest losses, Thermal conductivity coefficient, Thermal diffusivity coefficient, Specific heat

Introduction

Since 1970s, canola has not been comparable to any other plants in terms of cultivation due to its high oil content. Therefore, the ensuing significant increase in canola production has been observed mostly in European countries and Canada. In Iran, the lack of raw materials drew the attentions to this plant which is a good source of edible oils (Soleimani and Kasraie, 2012). Management efforts such as the two-stage harvest (which includes harvesting in higher moisture content and then cutting and stripping) and crushing can lead to faster harvest at proper moisture content and reduced losses. In the two-stage harvest, the stems are cut when 30 cm high at the first step, and the pods are possibly untouched so that they would be harvested by the remover head at the due time. The two-stage harvest approach, i.e. striping and harvesting at 35% and 10% moisture, respectively, would significantly decrease the losses, since the pods are stripped off when they look like rubbers. Furthermore, it is worth notifying that as soon as canola seed moisture is reduced to 40%, the weight of the dry matter may still remain unchanged (Afzali and Sheikh-Davoodi, 2008). Thus, finding proper solutions to reduce harvest losses requires knowing the thermal properties of canola, which is the main objective of the present research. In what follows, we review a number of studies conducted on the thermal properties of various crops as well as canola harvest losses.

Researchers have studied the thermal properties of soybean pods in terms of yield moisture content and temperature. It was

1- Department of Bio-system Mechanical Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
2- Department of Agronomy, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
(*Corresponding author: azadbakht@gau.ac.ir)
DOI: 10.22067/ifstrj.v1396i3.62905

found that a rise in temperature and moisture results in an increase in specific heat from 1.856 to 4.39 (kJ/kg.˚K) as well as in thermal conductivity coefficient from 0.038 to 0.338 (W/m.˚c). In addition, at all temperature levels, higher moisture causes lower thermal diffusivity (Azadbakht *et al*., 2013). Other scholars have studied the dependence of moisture on thermal properties of peanut pod, shelled peanut, and the skin. They have observed that increasing the moisture may increase specific heat and thermal conductivity coefficient and decrease thermal diffusivity. In this research, specific heat was determined through a vacuum calorimeter through mixing with hot water; thermal conductivity coefficient was measured by line heat source method, and thermal diffusivity coefficient was calculated, using an equation from literature (Bitra *et a*l., 2010). Gharibzahedi *et al*. (2012) analyzed the thermal characteristics of the Iranian black seed and observed that thermal conductivity, specific heat, and thermal diffusivity varied from 0.17 to 0.22 ($W\ m^{-1}K^{-1}$), 1642 to 2035 ($J.kg^{-1}K^{-1}$), and 9.3 to 10.4 × 10^{-8} (m^2s^{-1}), respectively. Scholars have investigated the effect of moisture, temperature, and variety on the specific heat of Pistachio, using a mixed approach. They have found that higher moisture and temperature result in increased specific heat in all varieties within the range of 0.419-2.93 (KJ/Kg.˚c). However, the effect of moisture content was more substantial than those of temperature and variety (Razavi and Taghizadeh, 2007). Researchers have also studied the effect of harvest time on yield and losses of canola seeds and have observed that more seed losses occur over time (Madani *et al.*, 2008). By determining the best harvest time of spring canola as the second cultivation, Rabiei *et al.* (2014) studied the varieties of RGS 003, Hyola 401, Hyola 420, and Hyola 308 at four times. Their findings indicated that the variety of Hyola 401 at the moisture level of 35% and the variety of Hyola 308 at moisture level of 15% had the highest and the lowest yields, respectively.

The present research was aimed to determine the thermal conductivity, specific heat, and thermal diffusivity of canola pods, identify various losses at canola harvest, and investigate the relation between these two factors. Moreover, it aimed to study the effect of moisture and harvest time on the amount of seed losses by platform, seed losses in the whole combine harvester, and unbroken pods and to see the effect of thermal properties on canola losses. The results are relevant and applicable to drying before crushing, which is employed in the farm dryer unit, and reducing seed losses at canola harvest.

Material and Methods

Sampling

Initially, the three canola varieties of Hyola 420, Hyola 401, and Hyola 50 were selected from the farms of Aliabad-e Katul, Golestan Province, Iran. The sampling was performed at three times of pre-harvest, harvest, and post-harvest. There were four-day intervals between the harvest periods. When 85 percent of canola seeds were brown, was considered as harvest time. The moisture content of canola pods at pre-harvest, harvest, and post-harvest were found to be 28%, 15%, 8%, respectively. Thermal conductivity, specific heat, and thermal diffusivity of the three varieties of canola pods were determined at the three sampling times. Subsequently, seed normal losses, seed platform losses, seed losses in the whole combine harvester, and unbroken pods were measured; moreover, the relation between the losses and the thermal properties of canola pod was investigated.

Thermal conductivity

The thermal conductivity of canola pods was determined using the line heat source method (Mohsenin, 1980; Bitra *et al.*, 2010; Azadbakht *et al*., 2013; Yang *et al.*, 2002; Bart–Plange *et al*., 2012). This method is the most common transient method employed in food and agricultural products, which is proper for measuring the thermal conductivity of the masses of agricultural products (Salarikia, 2012). Measuring thermal conductivity, either by non-isolated wire or thermal conductivity

probe, is based on a line heat source with infinitesimal diameter, infinite length, and constant longitude heat located in a homogenous cylinder. Equation (1) presents the increase in temperature as follows:

$$\Delta T = \frac{Q}{4\pi K}\left[ln(t) + ln\left(\frac{4\alpha}{r^2 e^{0.5772}}\right)\right] \quad (1)$$

It is the increased temperature at the distance of r from the probe of line heat source (˚C). t is the time for (s), and Q is the heating power per probe length (W.m^{-1}); K denotes the thermal conductivity (W.m^{-1}.˚C^{-1}), α is the thermal diffusivity (m^{2}s^{-1}), and r is the distance from line (m) central vector.

Equation (2) demonstrates temperature difference (ΔT) against time normal logarithm (lnt):

$$S = Q.(4\pi K)^{-1} \quad (2)$$

The thermal conductivity is:

$$k = \frac{Q}{4\pi}\frac{\Delta ln(t)}{\Delta T} \quad (3)$$

As $Q=IR^2$, the relation (3) can be written as

$$k = \frac{I^2 R}{4\pi s} \quad (4)$$

R represents the thermal element electrical resistance per length (Ω/m), and I is the input current to heat source.

The test transient heat transfer device (Figure 1) is constructed by a line heat source in PVC cylinder (the height is 300 nm, and the diameter is 110 mm). The cylinder is enclosed at the top and the bottom by a 10-mm fiberglass. A nickel-chromium line heater, 0.127 mm in diameter, is placed along the cylinder main vector which is connected to an adjustable D.C power source (500 mA, 1.5-12 V) (Bitra *et al.*, 2010).

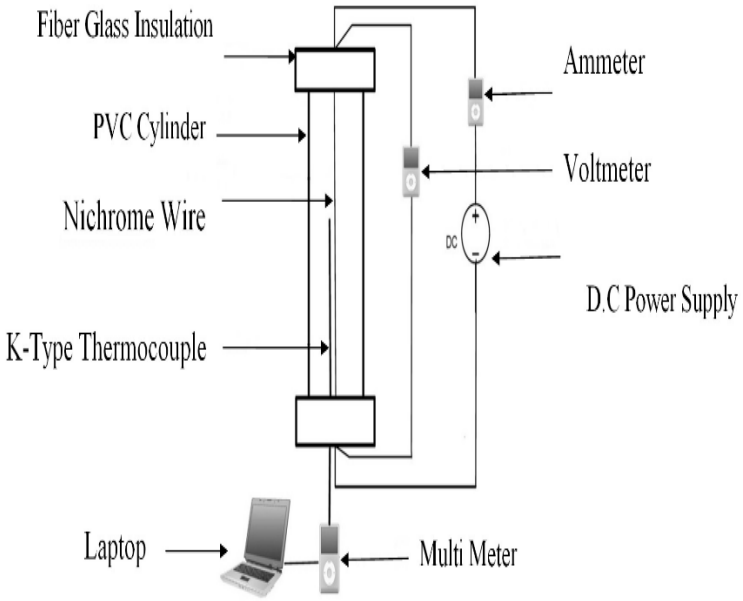

Fig.1. Line heat source device

In order to measure the core line, a K-type thermocouple of STANDARD ST-941 with the accuracy of 1˚C (made in China) was applied. The thermocouple was mounted on a base at a distance of 12 mm from the heat line source. During the test, it was assumed that the temperature of the container was fixed (constant); therefore, a K-type thermocouple was embedded in the outer surface of the container to monitor the temperature. Considering the recorded temperature per second of the data logger output, the schematic chart of the temperature value was drawn in the time natural logarithm within 600 seconds of the test. The slope and coefficient of determination (R^2) were measured for each

sample. The thermal conductivity was determined, using the charts in which R^2 value was larger than 0.990 (Azadbakht *et al*., 2013).

Specific heat

Specific heat was determined using mixture method (Mohsenin, 1980; Bitra *et al*., 2010; Razavi and Taghizadeh, 2007; Azadbakht *et al*., 2013; Bart–Plange *et al*., 2012). In order to measure the specific heat of the canola pods at a constant pressure, the calorimeter was first put in the refrigerator to cool down, as shown in Figure 2. Therefore, the low lost heat was negligible. Two hundred g of distilled water was boiled and then added to the calorimeter. Afterwards, the temperature was measured and recorded. Ten g of the sample was then added to the calorimeter at a given temperature (25˚C). The mixture was allowed to balance thermally. Finally, the specific heat of the pod was calculated, using balance Eq. (5).Between the heat acquired or lost by water and calorimeter and the heat acquired or lost by the sample (Azadbakht *et al.*, 2013).

$$C_s = \frac{C_w W_w (t_a - t_w) - C_c W_c (t_i - t_a)}{W_s (t_i - t_a)} \quad (5)$$

C_s is the specific heat of the sample (kJ/kg.˚C), C_w is the specific heat of water (kJ/kg.˚C), W_w is the added mass of water (g), t_a is the balance temperature (˚C), t_w is the initial temperature of water (˚C), C_c is the specific heat of the calorimeter (kJ/kg.˚C), W_c is the bucket mass of the calorimeter (g), t_i is the initial temperature of the sample (˚C), and W_s is the mass of the sample (g).

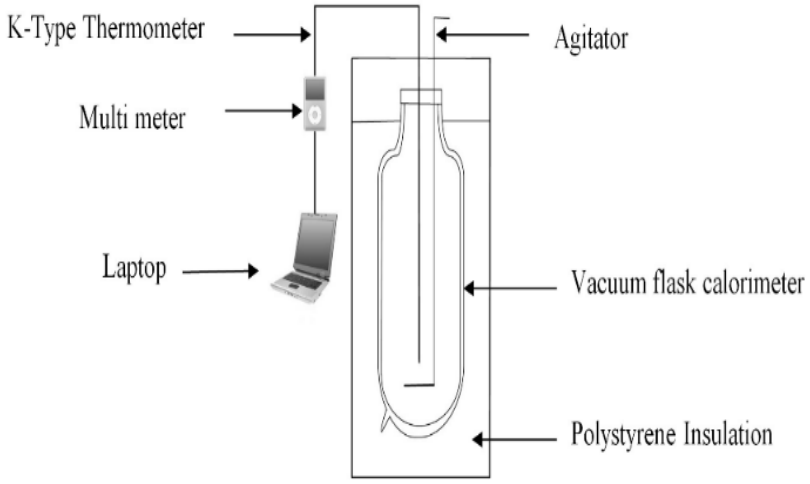

Fig. 2. Vacuum calorimeter

Thermal diffusivity

The thermal diffusivity of the pods was obtained by Eq. (6) (Ariara and Haque, 2001; Azadbakht *et al.*, 2013; Yang *et al.*, 2002; Bart–Plange et al., 2012).

$$\alpha = \frac{K}{\rho C_p} \quad (6)$$

α represents the thermal diffusivity (m^2s^{-1}), K is the thermal conductivity ($W.m^{-1}{}^{\circ}C^{-1}$), ρ is the bulk density (kg m^{-3}), and C_p is the specific heat (J $kg^{-1}{}^{\circ}c^{-1}$).

To measure the density of cumulus, a cylinder with known mass and volume was filled with pods without a gap and then was weighed. With knowing the volume of a cylinder (diameter of 26.44 mm and height of 71.04 mm), the bulk density was obtained.

Canola losses

In order to determinethe canola losses, natural losses, seed losses by platform, seed

losses in the whole combine harvester, and unopened pods were studied. Thus, a wooden frame with the dimensions of 50cm×50cm was provided. In each harvest time, the natural losses were initially estimated. Following that, in order to determine the combine harvester platform losses, the frame was placed at point 1 in Figure 3 to determine the combine harvester losses. In addition, the frame was placed at point 2 in Figure 3 to determine the losses of the whole combine harvester.

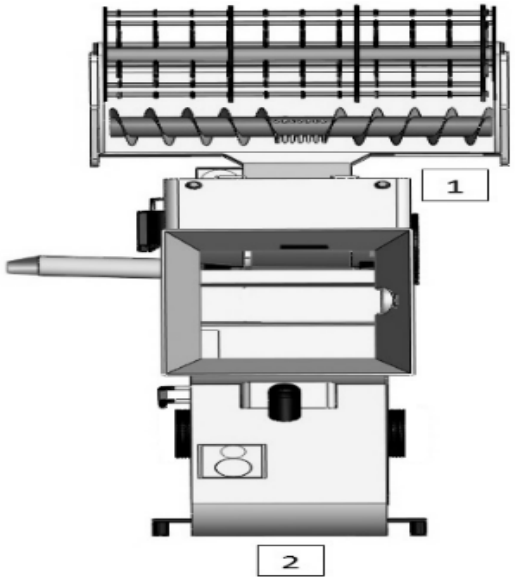

Fig. 3. The position of the wooden frame to the combine harvester in the field

The seeds in the frame were then collected by a built grain collector (Figure 4) and weighed. The collector was equipped with a silicon filter, which kept the seeds inside, and an air filter, which prevented the collector from being damaged by dust.

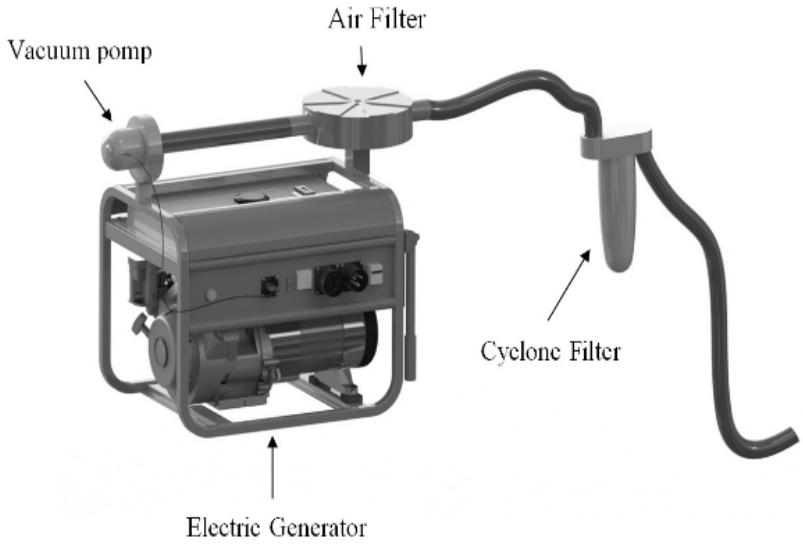

Fig. 4. Field grain collector

Results and Discussion

Natural losses

Changing the variety and the sampling time significantly influenced the canola natural losses at the probability level of 1%. Moreover, it was observed that the interaction between the variety and the sampling time at 1% affected the natural losses. The maximum and minimum values of natural losses were 0.56 and 0.04 g m^{-2}, respectively; post-harvest and pre-harvest in term of time; and, Hyola 420 and Hyola 50 in term of varieties.

Platform seed losses

Changes in the sampling time at 1% influenced the platform losses. The minimum losses were observed at the pre-harvest time due to the high moisture content.

Seed losses in the whole combine harvester

Changes in variety and harvest time affected seed losses at the probability level of 1%. The highest seed losses were assigned to the variety of Hyola 420; however, the lowest losses were observed in the variety of Hyola 50. The minimum seed losses were noticed in the pre-harvest period, which significantly increased over time to such an extent that post-harvest losses were 4 times more than the pre-harvest losses.

Unbroken pods

Changes in variety and harvest time influenced the value of canola unbroken pods at the probability level of 1%. The variety of Hyola 50 had the minimum unbroken pods, and the maximum amount of unbroken pods was observed at the pre-harvest time.

The relation between canola harvest losses and thermal properties of canola pods

Losses and thermal conductivity

The variety and the sampling time influenced the thermal conductivity coefficient at the probability level of 1%. Moreover, the interaction between the variety and the sampling time had a substantial effect on the thermal conductivity at this level. The maximum and minimum values of the thermal conductivity were 0.47 ($Wm^{-1}{}^{\circ}c^{-1}$) and 0.16 ($Wm^{-1}{}^{\circ}c^{-1}$) in the varieties of Hyola 50 and Hyola 420 in pre-harvest and post-harvest periods, respectively.

According to Table 1, the thermal conductivity is correlated with platform seed losses and unbroken pods at the probability levels of 5% at 1%, respectively. Moreover, it was observed that the thermal conductivity with the coefficient of 4.16 had a negative, inverse relation with platform seed losses. In other words, the higher thermal conductivity at a given composition the lower canola losses. It was also found that the thermal conductivity with the coefficient of 67.74 was directly correlated with unshelled pods.

Table 1- Analysis of regression of grain losses and thermal conductivity

Variable	DF	Natural losses	Platform grain losses	Whole combine harvester grain losses	Unbroken pod
Thermal conductivity	1	-0.062^{ns}	-4.16^{*}	-7.84^{ns}	67.74^{**}
Intercept	1	0.021^{ns}	2.14^{**}	4.83^{**}	-2.84^{ns}

According to Table 1 and considering the coefficients, Eq. (7) demonstrates the relation between thermal conductivity and platform seed downfall. In addition, Eq. (8) indicates the relation between thermal conductivity and unbroken pods as follows:

$$Loss\ of\ platform = 2.14 - 4.16\,K \quad (7)$$

$$Unbroken\ pods = -2.84 + 67.74\,K \quad (8)$$

According to Figure 5, the increased thermal conductivity reduces platform losses. When the thermal conductivity is high, this means that the moisture is high as well. Therefore, the losses are reduced through increasing the thermal conductivity and the moisture. Moreover, increased thermal conductivity and higher moisture result in an increase in the number of unbroken pods.

Losses and specific heat

The variety and the sampling time at the

probability level of 1% and their interaction at the probability level of 5% were highly influential for the specific heat of canola pods. The maximum and minimum specific heat was 2.47 (kJ $kg^{-1}{}^{\circ}c^{-1}$) and 0.69 (kJ $kg^{-1}{}^{\circ}c^{-1}$) for the varieties of Hyola 420 and Hyola 50 at pre- and post-harvest periods, respectively.

According to Table 2, the regression coefficient of specific heat showed no significant relations with natural losses, platform seed losses, and combine harvester losses; nevertheless, this regression coefficient played a significant role in the relation between specific heat and unbroken pods at the probability level of 1%. According to Table 2, the specific heat with the coefficient of 11.45 was directly correlated with unshelled pods.

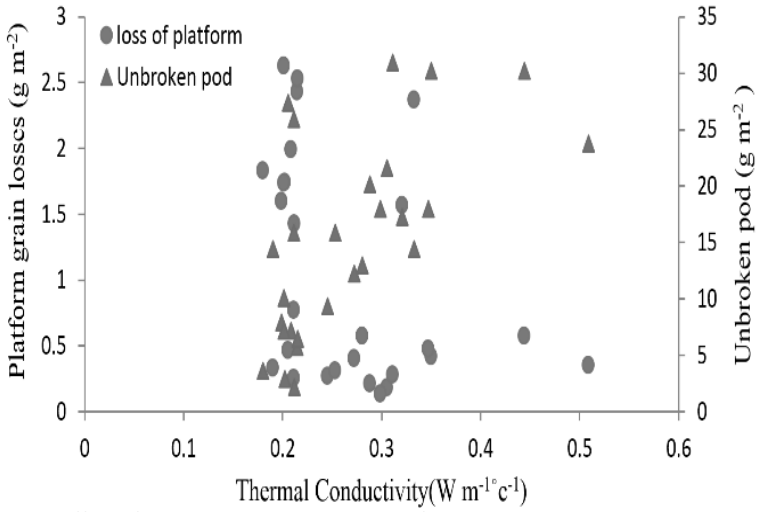

Fig. 5. Effect of thermal conductivity on platform grain losses and unbroken pods

Table 2. Analysis of regression of grain losses and specific heat

Variable	DF	Natural losses	Platform grain losses	Whole combine harvester grain losses	Unbroken pod
Specific heat	1	0.027^{ns}	-0.52^{ns}	-0.75^{ns}	11.45^{**}
Intercept	1	0.16^{ns}	1.72^{**}	3.74^{**}	-0.12^{ns}

By considering Table 2 and the coefficients, we can obtain the relation between the specific heat of canola pods and that of the unbroken pods by Eq. (9):

$$Unbroken\ pods = -0.12 + 11.45\,C \quad (9)$$

According to Figure 6, the amount of the unbroken pods increases by higher specific heat and moisture.

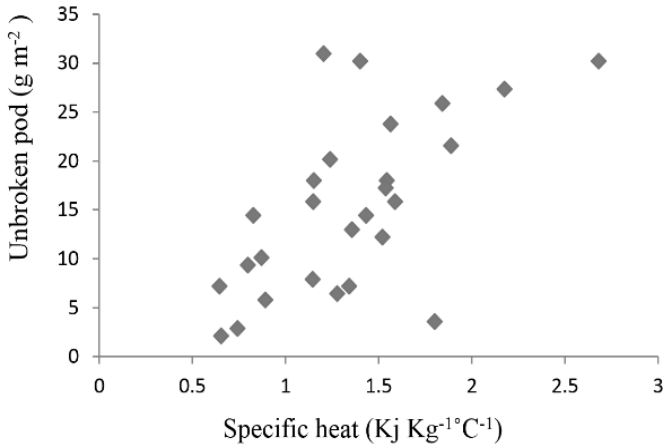

Figure 6- Effect of specific heat on unbroken pods

Losses and thermal diffusivity

The interaction between the varieties and the sampling time had a significant effect on the thermal diffusivity at the probability of 5%. The maximum and minimum values of thermal diffusivity were 1.87×10^{-6} (m^2s^{-1}) and 6.59×10^{-7} (m^2s^{-1}) in pre- and post-harvest periods, respectively, for the variety of Hyola 401.

As Table 3 depicts, the regression coefficient of thermal diffusivity and unbroken pods was effective at the probability level of 1%. Additionally, a negative, inverse relation was noticed between thermal diffusivity with the coefficient of 6183351 and the number of unshelled pods.

Table 3. Analysis of regression of grain losses and thermal diffusivity

Variable	DF	Natural losses	Platform grain losses	Whole combine harvester grain losses	Unbroken pod
Thermal Diffusivity	1	53110^{ns}	475242^{ns}	668464^{ns}	-6183351^{**}
Intercept	1	0.13^{ns}	0.4^{ns}	1.86^{*}	23.37^{**}

Equation (10) presents the relation between the thermal diffusivity of canola pods and unbroken pods with respect to Table 3 and the coefficients:

$$Unbroken\ pods = 23.37 - 6.18 \times 10^{6}\, C \quad (9)$$

As Figure 7 shows, the unbroken pods decrease by increasing the thermal diffusivity of canola pods.

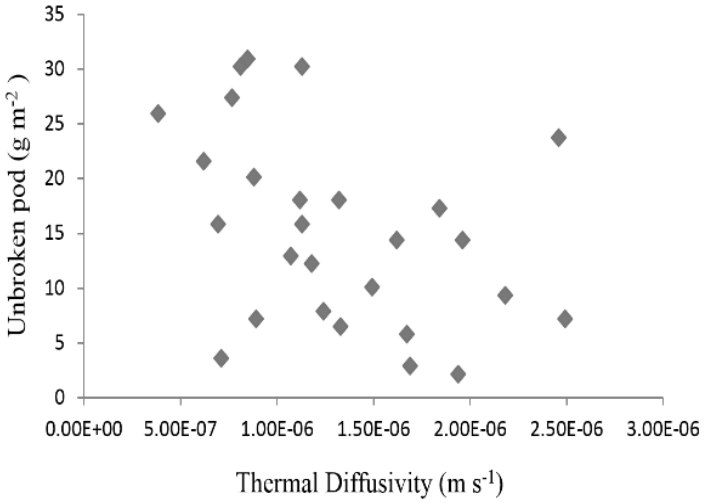

Fig. 7. Effect of thermal diffusivity on unbroken pods

Conclusion

The analysis of seed losses demonstrated that the minimum natural losses, platform losses, and combine harvester seed losses were observed in the pre-harvest period. Therefore, early harvesting may remarkably reduce the amounts of losses. Moreover, studying the canola varieties also revealed that the varieties of Hyola 420 and Hyola 50 had the maximum and minimum seed losses, respectively. Investigating the unbroken pods showed that the highest amount of unbroken pods was observed in the pre-harvest period. In addition, the minimum amount of unbroken pods was attributed to the variety of Hyola 50. In point of fact, the best variety, in terms of losses, was Hyola 50, and the best time was the pre-harvest time due to its lowest amount of losses. The results obtained revealed that the whole combine harvester losses are larger than the platform losses, indicating the high losses in the other parts of the combine harvester. Canola seeds are so tiny that require an accurate adjustment of combine harvester at harvesting in order to minimize the losses of this strategic product. Increased thermal conductivity reduces platform seed losses, while it increases the losses of unbroken pods. Increased specific heat also increases the unbroken pods. However, the higher thermal diffusivity of canola pods reduces the amount of the unbroken pods.

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تلفات دانه کلزا هنگام برداشت با کمباین برداشت غلات تحت تاثیر خواص حرارتی غلاف نشکسته کلزا

احسان قجرجزی[1]- محسن آزادبخت[1*]- فرشید قادری فر[2]

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چکیده

در تحقیق حاضر،خواص حرارتی غلاف کلزا شامل ضریب رسانندگی حرارتی، گرمای ویژه و ضریب انتشار حرارتی و میزان تلفات کلزا شامل تلفات طبیعی، تلفات پلاتفرم، تلفات کل کمباین و غلاف کوبیده نشده در سه سطح رقم مرسوم کلزای کشت شده در شمال ایران (هایولا 50، هایولا 401 و هایولا 420) و در سه زمان قبل از برداشت، حین برداشت و پس از برداشت اندازه‌گیری شدند. سپس ارتباط بین خواص حرارتی غلاف کلزا با میزان تلفات هنگام برداشت کلزا بررسی شد. ضریب رسانندگی با روش منبع حرارت خطی، گرمای ویژه از روش مخلوط و ضریب انتشار از طریق فرمول محاسبه شدند. برای اندازه‌گیری مقدار تلفات دانه از جاروبرقی صحرایی ساخته شده استفاده گردید. نتایج نشان داد که تغییرات، رقم و زمان نمونه‌برداری بر ضریب رسانندگی حرارتی و گرمای ویژه در سطح احتمال یک درصد معنی‌دار بوده است. و نیز اثر متقابل رقم و زمان بر ضریب‌رسانندگی در سطح یک درصد و بر گرمای ویژه و ضریب انتشار حرارتی در سطح 5 درصد موثر بوده است. همچنین اثرات رقم و زمان برداشت بر تلفات طبیعی، تلفات کل کمباین و غلاف کوبیده نشده در سطح یک درصد موثر بود. همچنین مشاهده شد بین ضریب رسانندگی حرارتی و تلفات پلتفرم در سطح 5 درصد و باغلاف کوبیده نشده در سطح یک درصد ارتباط معنی‌دار بود. و همچنین غلاف کوبیده نشده با گرمای ویژه و ضریب انتشار حرارتی در سطح یک درصد ارتباط معنی‌دار داشت.

واژه‌های کلیدی: غلاف کلزا، تلفات برداشت، ضریب رسانندگی حرارتی، ضریب انتشار حرارتی، گرمای ویژه.

1-گروه مهندسی مکانیک بیوسیستم، دانشگاه علوم کشاورزی و منابع طبیعی گرگان
2-گروه زراعت، دانشگاه علوم کشاورزی و منابع طبیعی گرگان
(*- نویسنده مسئول : Email: azadbakht@gau.ac.ir)

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Fatty acid composition, rheological and thermal properties of butter from sheep's and omega-3 cow's milks

Morteza Kashaninejad[1], Seyed M. A. Razavi[2*], Mostafa Mazaheri Tehrani[2], Mahdi Kashaninejad[3]

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Abstract

In this study, the compositional, rheological, thermal and textural properties of omega-3 cow's butter (OCB), conventional cow's butter (CCB) and sheep's butter (SB) were evaluated. The fatty acid composition of SB showed a relatively high content of the short chain fatty acids (SCFA) compared with that of cow's butters and higher levels of CLA and omega 3 fatty acids in OCB were observed. Regarding to the firmness, at refrigeration temperature (5 °C), SB was much firmer than CCB and OCB, but as a function of temperature, it was softened much quicker. However, at temperatures around 18°C it was already softer than the latter. From dynamic rheological data, it was found that butter samples display solid-like viscoelastic behavior since the values of G′ were much higher than those of G″ with a low dependence on frequency. The values of G′ and G″ also decreased in butters containing more percentage of unsaturated fatty acids. The temperature effect on the viscosity followed an Arrhenius-type relationship and OCB had a less activation energy than others, indicating that the butter containing high SCFA was more sensitive to temperature changes. Through differential scanning calorimetery, the thermal behavior of the butters during melting was analyzed.

Keywords: Butter, DSC, Firmness, GC, Omega-3, Rheology

Introduction

Milk and dairy products contribute considerably the consumption of essential nutrients in human populations (Drewnowski 2011). Health and nutrition professionals advise consumers to limit consumption of saturated fatty acids and increase the consumption of foods rich in polyunsaturated (PUFA) and n-3 fatty acids. An increase in the ratio of saturated to unsaturated fatty acids in milk fat is associated with an increased risk for cardiovascular disease and concentrations of total and low-density lipoprotein (LDL) cholesterol (Sacks and Katan 2002). A higher ratio of saturated fatty acids contributes to the hardness and poor spreadability of butter at refrigeration temperature (Edmondson *et al.* 1974; Taylor and Norris 1977; Ashes *et al.* 1997).

Omega-3 butter produced from milk with elevated PUFA and n-3 fatty acids levels caused by dietary manipulation appears to be improved in texture and nutritional value. Gonzalez *et al.* (2001) observed that Firmness values were lower for the high oleic and high linoleic butter when compared to the control. Oeffner *et al.* (2013) showed that the less saturated fatty acid profile was associated with decreased hardness and adhesiveness of refrigerated butter. Therefore, there has been a great deal of interest in omega-3 milk production containing more SCFA and PUFA in milk fat.

Dairy sheep has an important role in dairy industry of Mediterranean and Middle East region countries (FAO 2003). So far the value of sheep's milk in human nutrition has received very little academic attention and few facts are available (Haenlein 1987; Park 1991). In addition, little literature is available on the thermal, rheological and textural properties of butter from sheep's as well as omega-3 cow's milks.

Rheological measurements are useful in

1 and 2. Former MSc Student and Professor, Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, PoBox 91775-1163, Mashhad, Iran.
3. Professor, Faculty of Food Science & Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, 49138-15739, Iran
(*- Corresponding Author Email: s.razavi@um.ac.ir)
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objectively measuring properties related to texture, functionality, and also providing data for process modeling and quality (Fox and McSweeney 2003). Small amplitude oscillatory shear (SAOS) has been recently considered to predict correct rheological characteristics of semi-solid foods (Ahmed and Ramaswamy 2006) and could be implemented for butter samples. Rheological properties of butter are also influenced by temperature; commonly Arrhenius equation is used to describe temperature dependency of butter samples. Moreover, the viscosity and the melting properties are valuable as quality, process and storage parameters. Although it has long been recognized that the melting properties of butter is a key factor in determining its spreading quality, there have been only a few reports of the liquid fat content of' commercial butter.

Thus, the objective of this study was to compare the properties of omega-3 cow's butter and sheep's butter with conventional cow's butter. An instrumental texture analysis, a strain sweep test, a frequency sweep test and a temperature sweep test were carried out together with the fatty acid composition and melting behavior analysis. All of the tests were performed over a range of temperatures to gain insight into the performance of the materials in storage and consumer usage environments.

Materials and methods

Butter manufacture

For this study, conventional and organic dairy farms (Golshid-Mashhad Co and Shafashir Toos industrial dairy farmer's production and distribution cooperative) located in Northeast of Iran were selected (Mesgaran and Jafarpoor 2012). The sampling took place in January, at the winter for both the cow and sheep.

After pre-pasteurization (78°C for 30 s), the milk was skimmed at 40°C by a centrifugal cream separator (120 L/h, Alfa Pak, Iran). The cream collected was then standardized at 40% fat before being pasteurized (88°C for 1 min 30s). It was then cooled to 6°C using a plate heat exchanger. The cream was physically matured (18°C for 3 h) before being re-cooled and held at 6°C. The cream was then churned at 40 rpm in a 200L industrial churn (Alfa Pak, Iran) until the butter was granulated. After separating the buttermilk, the butter was washed in water at 8°C before being pre-worked in the churn, first at low speed for 15 min then at high speed for 1 min. Butter samples were stored in plastic containers at 5°C until further analysis.

Gas chromatography

Milk fat extraction and fatty acid separation were performed according to a procedure described by Chouinard *et al*. (1999). Fatty acid methyl esters were prepared by trans-methylation, then were quantified using a gas chromatograph (GC ystem 6890 Hewlett-Packard, Wilmington, DE) equipped with a flame-ionization detector and a CP-7489 fused-silica capillary column (100m× 0.25mm with 0.2µm film thickness; Varian, Walnut Creek, CA). Initial oven temperatures (50°C) was held for 1 min then ramped at 5 °C/min to 160°C where it was held for 42 min, and then ramped at 5°C/min to 190°C and held for 22min. Inlet and detector temperatures were maintained at 250°C, and the split ratio was 100:1. Hydrogen carrier gas flow rate through the column was 1 mL/min. Hydrogen flow to the detector was 30 mL/min, airflow was 400mL/min, and nitrogen make-up gas flow was 25mL/min. Peaks in the chromatogram were identified and quantified using pure methyl ester standards. Samples were analyzed in duplicate, and peak identification was accomplished through the analysis of authentic standards.

Differential scanning calorimetry (DSC)

A Perkin-Elmer Pyris-1 (LTI03-USA) differential scanning calorimeter was used to investigate the melting behavior of the samples. Nitrogen gas was used to prevent condensation in the cell and an empty pan was used as the reference. Samples (approximately 10 mg) were weighed in aluminum pans and tightly sealed with an aluminum lid. Measurements were carried out in the

temperature range of -40 to 90°C with heating rates of 10°C/min. The endothermic curves of heat flow as a function of temperature were recorded to analyze the non-isothermal melting kinetics. The temperature of each peak (T_{m1}, T_{m2} and T_{m3}) (°C) and enthalpy of melting (ΔH_{m1}, ΔH_{m2} and ΔH_{m3}) (J g^{-1}) were obtained from heating curves, as shown in Fig. 1. All measurements were performed in triplicate for each sample.

Instrumental texture analysis

To determine firmness, the penetration value (P) of the samples were measured at 5, 10 and 20°C, respectively; using a TA-XT2 texture analyzer (Stable Micro Systems, London, UK) with a 45° conical probe was lowered at 0.02 mm/s at a load of 102.5 g. The penetration value (P) is the depth of penetration of the cone into butter sample. Each test was repeated 3 times using fresh samples. The thousand fold value of the reciprocal of the penetration value (F=1000/P) was used for the characterization of the firmness of butter samples. The firmness was characterized by a linear relationship between temperature and firmness.

Dynamic rheological analysis

Small amplitude oscillatory shear (SAOS) measurements were performed using a controlled stress/strain rheometer (Physica MCR 301, Anton Paar GmbH, Stuttgart, Germany) equipped with parallel-plate geometry and a 50mm diameter set to a gap of 2 mm. Data were recorded with the Rheoplus software, version 2.65 (Anton Paar Germany GmbH). For each measurement, a new sample was used. Oscillatory measurements were performed at least in duplicate.

The linear viscoelastic region (LVR) for butter samples was determined by performing an amplitude sweep measurements (0.01-100%) at constant frequency (1 Hz) and two temperatures of 5 and 20°C. Then, frequency sweep tests at a constant strain in the LVE region were carried out to determine the viscoelastic properties of butters. The mechanical spectra were characterized by values of storage modulus (G´, Pa), loss modulus (G″, Pa) and complex viscosity (η*, Pa.s) as a function of frequency in the range of 0.01–100 Hz and two temperatures (5°C and 20°C). Finally, the temperature sweep measurements were performed at the constant strain of 0.01%, which was well within the linear viscoelastic region, while the frequency was fixed at 1 Hz. The experiments were carried out by heating the samples from 5 to 60°C at 5°C/ min^{-1}.

Results and discussion

Fatty acid profile

Milk fatty acid composition has been considered to be the main factor influencing nutrition (Palmquist 1991; Jensen 2002) and butter texture (Brunner 1974). Fatty acid compositions of the samples are summarized in Table 1. The SB showed a relatively high content of the SCFA, C4:0 to C10:0, 16.97% by weight, compared with that of cow's butters. Capric acid (C10) was the major SCFA present in SB (9.52% by weight).

Short-chain and medium-chain fatty acids (MCFA) in milk fat have certain interesting characteristics, and they are primarily absorbed through the portal vein during lipid digestion (Christophe and Devriese 2000). In addition, SCFA and, to a lesser extent, MCFA lower the melting point of triacylglycerols and, thus, their presence helps keep milk fat liquid at physiological temperatures.

The degree of saturation ranged between 69.88% by weight in OCB, and 74.17% in SB, in the CCB being relatively higher than that of OCB. The unsaturated fatty acids play an important role in the physical as well as nutritional properties of the milk fat (Marangoni *et al*. 2012). A decreasing degree of saturation of the fatty acid and a decreasing chain length results in a lower melting point compared to saturated fat with a long chain. Such changes in the chemical composition of milk fat are likely to change the rheological properties of produced butter. Of the saturated fatty acids, Stearic (C18) was predominant in both sheep's and cow's butters, while oleic acid was the major unsaturated fatty acid. The

oleic acid level was lowest (17.98% by weight) in the CCB and highest (20.14% by weight) in the SB.

26.68% of the fatty acids in OCB are mono-unsaturated (MUFA) with oleic acid (18:1) accounting for 19.19% by weight of the total fatty acids. Poly-unsaturated fatty acids (PUFA) in OCB constituted of 3.44% by weight of the total fatty acids and the main poly-unsaturated fatty acids were linoleic acid (18:2) and α-linolenic acid (18:3) accounting for 1.85 and 1.59% by weight of the total fatty acids. Therefore, it is remarkable that higher levels of CLA and omega 3 fatty acids in OCB were found in this study, which they have been reported before in organic butter (Bergamo *et al*. 2003).

Table 1. Fatty acid profile of different butters from gas chromatographic analysis

Fatty acids (wt %)	SB[1]	CCB[2]	OCB[3]
4:00	2.22	2.01	1.46
6:00	2.4	1.8	1.4
8:00	2.83	1.26	1.04
10:00	9.52	2.94	2.71
12:00	5.52	3.47	3.55
14:00	13.61	12.82	12.77
14:1,cis-9	0.89	1.51	2.19
15:1,cis-9	1.2	1.09	1.49
16:00	26.14	33.56	37.67
16:1,cis-9	1.65	2.19	3.81
17:00	0.67	0.86	0.85
18:00	10.6	15.65	8.32
18:1 Trans	17.42	16.97	18.07
18:1 Trans	2.72	1.01	1.12
18:2cis	1.48	1.75	1.85
18:2 Trans	0	0	0
18: 3	0.47	0.92	1.59
20:00	0.6	0.12	0.07
oleic	20.14	17.98	19.19
MUFA[4]	23.88	22.77	26.68
PUFA[5]	1.95	2.67	3.44
SCFA[6]	16.97	8.08	6.61
MCFA[7]	45.94	50.71	54.84
LCFA[8]	11.2	15.77	8.39
Saturated FA	74.17	74.56	69.88
Unsaturated FA	25.83	25.44	30.12

1. SB: sheep's butter.
2. CCB: conventional cow's butter.
3. OCB: omega-3 cow's butter.
4. MUFA (monounsaturated FA): C10:1, C14:1, C16:1, C17:1, C18:1 trans, C18:1cis, C20:1, C22:1, C24:1.
5. PUFA (polyunsaturated FA): C18:2 Trans, C18:2 cis, C18:3n-3.
6. SCFA (short-chain FA): C4:0 to C10:0.
7. MCFA (medium-chain FA): C11:0 to C17:0.
9. LCFA (long-chain FA): > C18:0.

Today, health authorities normally recommend a higher intake of these fatty acids as they are considered important for normal growth and development and for prevention of a number of diseases like hypertension, diabetes, cancer, and coronary heart disease.

Comparison of fatty acids composition of cow's and sheep's milk has been reviewed by Ramos and Juarez (1986). Their researches involve comparing the ratios of fatty acids. For example, the 12:0/10:0 fatty acid ratio is consistently higher in cow's milk (0.9–1.3) than in sheep's milk (0.4–0.8). Other ratios that have been considered include 14:0/12:0 and 14:0/8:0, and more complex combinations, such as 10:0/ (12:0+ 16:0+ 18:1).

Melting properties

Central to butter sensory character is a continuous-phase lipid composition, which influences melting properties and mouthfeel character. The melting properties of different milk fats give insight into the degree of fluidity at different temperatures as affected by the fatty acids (Osthoff, 2011).

The melting thermograms of SB, CCB and OCB determined by differential scanning calorimetry (DSC) are shown in Fig. 1. It could be seen that the melting profile ranged from −40°C to 90°C. On all melting curves, three endothermic peaks (T_{m1}, T_{m2} and T_{m3}) in accordance with literature data (TenGrotenhuis *et al*. 1999) could be observed. Vasic and DeMan (1968), Shukla and Rizvi (1995) and Chandra *et al*. (2009) also reported three melting zones in melting thermograms of butter. Although, in this study the second peak for OCB was broken down into two peaks.

The shape of the curve as well as height of melting peaks of butter change depending on fatty acid composition (KaisersBerger 1989). The values of peak temperature and melting enthalpy of butters are summarized in Table 2. Between 0°C and 0.7°C, an endothermic peak appeared due to the melting of water. The magnitude of these temperature peaks is related to the quantity of water present in the butter (Watson 1975). The lipids in butter samples occur in two groups; one group with

high amounts of PUFA and the other more of saturated ones. The results showed that these two groups of molecules melt between 11.93°C to 13.05°C, and 30.23°C to 32.97°C, respectively. Regarding enthalpies, the SB with the lowest level of PUFA showed the highest melting enthalpy of peak 2 (-4.21 Jg_{-1}), while OCB with the lowest saturated fatty acids exhibited the highest melting enthalpy of peak 3 (-3.77 Jg_{-1}). In addition, the melting enthalpy of peaks exhibited the opposite trend with the values of peak temperature.

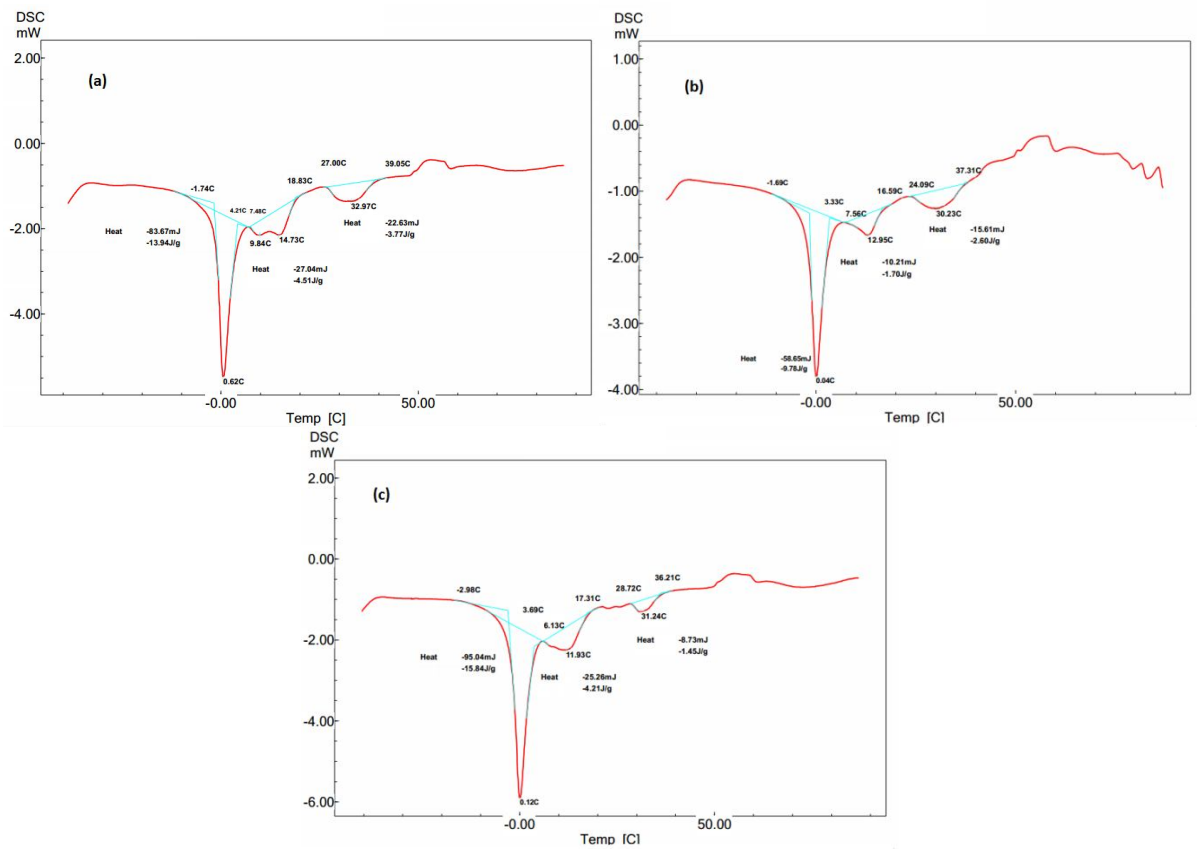

Fig. 1. DSC Curves of melting of omega-3 cow's butter (A), conventional cow's butter (B) and sheep's butter (C).

Table 2. Temperatures and enthalpies of butter melting process measured by DSC

Butter	Temperature (°C)			Enthalpy (Jg^{-1})		
	T_{m1}	T_{m2}	T_{m3}	ΔH_{m1}	ΔH_{m2}	ΔH_{m3}
CCB[1]	0.04	13.05	30.23	-9.78	-1.70	-2.60
OCB[2]	0.62	9.2/12.9[4]	32.97	-13.94	-1.51/-2.2[4]	-3.77
SB[3]	0.12	11.93	31.24	-15.84	-4.21	-1.45

1. CCB: conventional cow's butter.
2. OCB: omega-3 cow's butter.
3. SB: sheep's butter.
4. The peak is breaking down into two peaks, which indicates the decomposition of this fraction to another two ones.

The most important aspect to be noted is the initial and final melting points. Comparison of the results obtained for SB, showed that fat melting peaks started at a lower temperature than the other samples and also indicated the largest enthalpy of melting and values of peak temperatures. This result is probably because of being richer in SCFA

(Table 1), displayed greater melting in the low temperature range than cow's butters. A high initial, as well as final melting point of CCB is indicative of high amounts of saturated long-chain fatty acids. The second aspect is the different melting peaks observed for the butter samples, which is probably the result of triglycerides composed of different types of fatty acids.

As seen in Table 2, fat melting peaks for CCB were detected as endothermic peaks at 13.05 and 30.23°C, for OCB peaks appeared at 12.09 and 32.97 °C and for SB peaks appeared at 11.93 and 31.24 °C. This means that the butter samples are hard at 5°C and the majority of fat melts at room temperature (25°C).

Firmness

Fig. 2 shows the change of firmness of butters from omega-3 cow's milk, conventional cow's milk and sheep's milk as a function of temperature. It is obvious that at refrigeration temperature (5°C), SB is much firmer than others, but as a function of temperature it softens much quicker and near the 18°C it is already softer than the latter. In addition, the slope of the straight line in the SB is greater than the cow's butters, indicating less temperature stability because of its higher SCFA content. These findings are in agreement with Schaffer *et al*. (2001), who reported the enriched butter with low melting point triglycerides (LMP) versions of traditionally butters are firmer at a low temperature than the samples of butters, and also that the slopes of the straight line are greater.

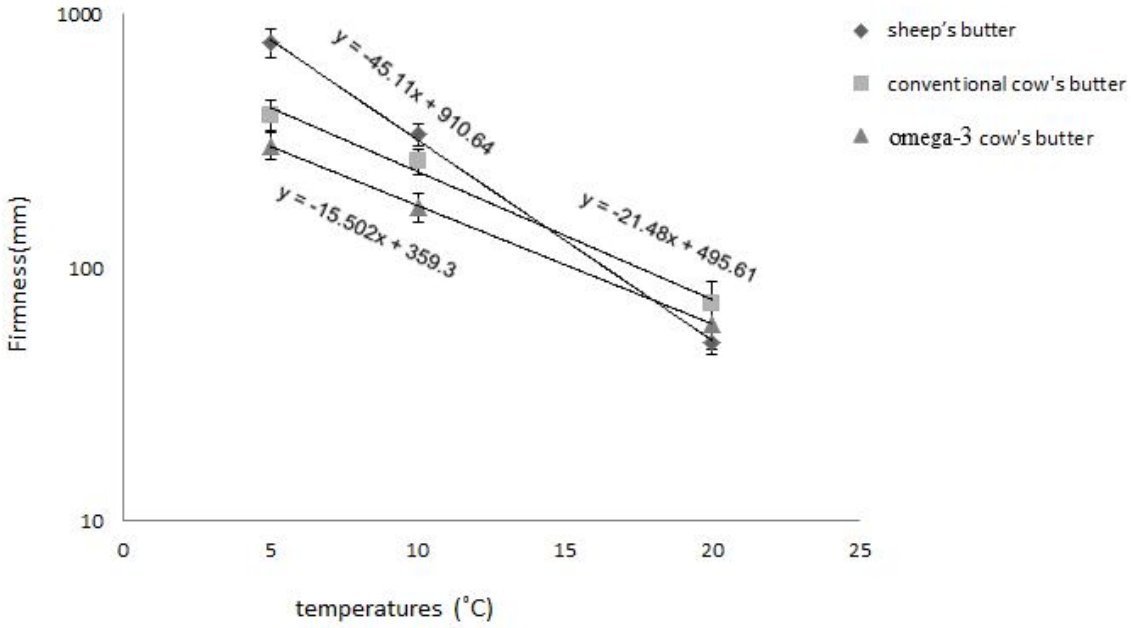

Fig. 2. Firmness of omega-3 cow's butter, conventional cow's butter and sheep's butter as a function of temperature

Generally, OCB with the highest percentage of unsaturated fatty acids had a more depth of penetration (at 5°C and 20°C) than others. Our results for CCB are also consistent with those of Middaugh *et al*. (1988); Lake *et al*. (1996); Lin *et al*. (1996); Baer *et al*. (2001) and Bobe *et al*. (2003).

Rheological properties

Linear viscoelastic domain

Prior to prepare the dynamic measurements of the sample's microstructure, the linear viscoelastic region (LVE) must be defined. Measurement of linear viscoelastic properties is a useful way of gaining information about a

foods' microstructure and how it influences the rheological properties (Narine and Marangoni 1999; Gunasekaran and Ak 2002). Fig. 3 shows the strain sweep (G′ and G″ vs strain) determined for SB at 20ºC. It could be seen that the G′ value was almost constant up to a certain strain, and then it began to decrease suddenly when the strain was further increased. The sudden decrease in the G′ may indicate the breaking of fat crystal network and a transition from a linear to a non-linear behavior.

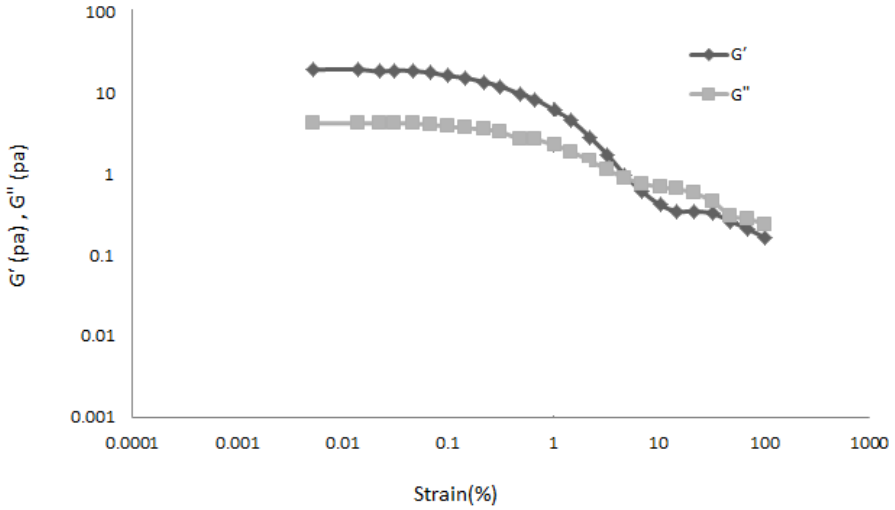

Fig. 3. Strain sweep dependency of storage modulus (G′) and loss modulus (G″) measured for sheep's butter (20 ºC & *f*=1 Hz).

The results indicated that the length of LVE ranged from 0.045% for SB at 20ºC to 0.067% for CCB, depending on the butter, this region lies at a strain of less than 1.0% or even less than 0.1% (Rohm and Weidinger 1993). Chandra *et al*. (2009) proposed that the LVR at 20ºC for butter samples was longer than that at 5ºC. As a result, the strain of 0.01% was selected for frequency sweep and temperature sweep tests since it was well fitted within the linear viscoelastic region for all experimental conditions, where the weak gel network was not damaged by the strain imposed during the measurements.

It was found from strain sweep that the values of G′ and G″ at LVE region increased for the butters with the highest percentage of saturated fatty acids. Furthermore, increasing temperature from 5 to 20ºC decreased the structural strength (G´ at LVE) of samples.

Viscoelastic properties

Fig. 4 shows the changes in storage modulus (G´), loss modulus (G″), and complex viscosity (η^*) as a functions of the frequency (f) for the CCB at 5ºC. It can be seen that the η^* values decreased steeply as the frequency increased, but G´ and G″ showed relatively less frequency dependence, which their G´ and G″ values increased as the frequency increased. The G´ values were greater than G″ values at any given frequency, indicating behavior essentially like that of solids.

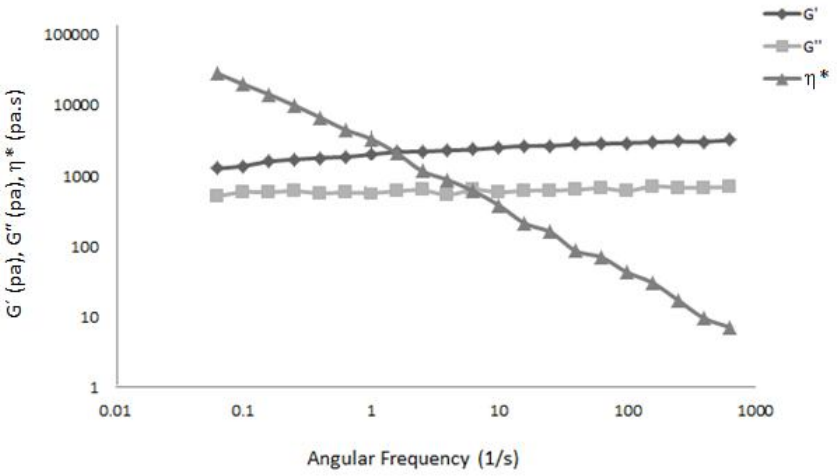

Fig. 4. Storage modulus (G′), loss modulus (G″) and complex viscosity (η*) versus angular frequency of conventional cow's butter (5 °C & γ=0.01%)

The dynamic rheological data of G´, G″ and η* versus angular frequency determined for butters at different temperatures (5–20˚C) were also subjected to power-law type equation, as suggested by Nolan et al. 1989. Table 3 contains the values of slopes (n', n" and n*), intercepts (k', k" and k*), and R^2 for the following equations:

$$G' = k'(\omega)^{n'} \quad (1)$$

$$G'' = k''(\omega)^{n''} \quad (2)$$

$$\eta^* = k^*(\omega)^{n^*} \quad (3)$$

From these dynamic rheological data, it was found that the butter displayed solid-like viscoelastic behavior because the values of k'(0.02– 2.81) are much higher than those of k" (0.002– 0.83) with a low dependence (n'=0.09– 0.12; n"=0.06– 0.17) on frequency.

According to Kokini and Plutchok (1987), for hydrocolloid gels (interwoven network of macromolecules), G' dominates over G" because the network bonding forces prevent translational movement. In a plastic fat, the intertwined crystal aggregates perform a similar function, leading to increased G' and G" values. At higher frequencies, a more solid-like response is observed (Drake *et al.* 1994; Rohm and Weidinger 1993).

Table 3. Storage modulus (G'), loss modulus (G") and complex viscosity (η^*) of butter samples at 5 and 20°C (frequency: 1 Hz).

Type of butters	Temperature (ºC)	G´			G″			η^*		
		n'	k'	R^2	n"	k"	R^2	n*	k*	R^2
CCB[1]	5	0.12	2.81	0.95	0.17	0.83	0.97	-0.80	1.32	0.99
	20	0.10	0.27	0.91	0.06	0.06	0.97	-0.89	0.28	0.99
OCB[2]	5	0.12	2.30	0.92	0.13	0.52	0.97	-0.86	1.25	0.99
	20	0.09	0.18	0.94	0.14	0.02	0.94	-0.91	0.19	0.99
SB[3]	5	0.10	1.16	0.99	0.16	0.22	0.99	-0.90	1.12	0.96
	20	0.09	0.02	0.96	0.13	0.002	0.97	-0.89	0.025	0.99

1. CCB: conventional cow's butter.
2. OCB: omega-3 cow's butter.
3. SB: sheep's butter.

The G' and G" values of butter samples at different temperatures (5–20 °C) showed little frequency dependence, indicative of the presence of a network with low possibility of rupture of junction zones within the low-frequency range used. Similar observations

were made by Doubler and Choplin (1989), Rohm and Weidinger (1993), Shukla and Rizvi (1995), and Narine and Marangoni (1999).

The k' values decreased as temperature increased, indicating greater elastic components at lower temperatures. Trends were similar for k" and k* values, indicating lower loss modulus and complex viscosity at higher temperatures. Diener and Heldman (1968), Rohm and Weidinger (1993), Shukla and Rizvi (1995) and Chandra *et al*. (2009) also reported similar behavior for butter.

These observations could be explained by the crystallization of fat at lower temperatures, which resulted in behavior more like that of solids. Narine and Marangoni (1999) proposed that higher values of the fractal dimension are associated with more ordered systems. At higher temperatures, crystallization proceeds more slowly than at lower temperatures. As a result, crystals will have more time to arrange themselves in more ordered networks.

Compared with CCB and SB, OCB had lower intercept values of k', k" and k* at similar temperatures, which associated with the highest percentage of unsaturated fatty acids (Table 3). It means that the values of k' and k" decreased in butters with more percentage of unsaturated fatty acids. The value of G", which is the viscous response, decreased with increasing the temperature. It is a well-known phenomenon that increasing temperature would decrease the viscosity of many fluid foods due to an increase in kinetic energy (Katsuta and Kinsella 1990).

Temperature dependency

Fig. 5 shows a typical dynamic shear profile of temperature sweep obtained with a CCB. In general, the values of G' and G" decreased steadily between 5 and 60°C. Below 40°C, the G' values were higher than G" values, indicating a solid-like behavior, but the two curves of G' and G" crossed at 38.51°C, indicating a liquid- like behavior above the crossover temperature. The crossing point for CCB and SB were different and detected at 40.50°C and 39.07°C, respectively. Increasing temperature from 5 to 40°C resulted in both G' and G" decrease, which expressed the melting of butter and fat liquefaction. While decreasing, G' remained over G", which can be explained by the mechanical resistance of the fat network while butter fat melts progressively. The lipid fraction with high amounts of PUFA has a lower melting point than the other more of saturated ones. Thus it can be assumed that there is a progressive melting of the different lipid fractions in fat up to 30 and 40°C, where the triglycerides of butter are liquids. The decrease in G' indicated a weakening of the butter structure, an effect which might be due in large part to liquefaction of the fat phase, fully liquid at about 40 °C.

The influence of temperature on the complex viscosity of the butters were demonstrated in Fig. 6. In general, for all the butters, the complex viscosity was decreased with increasing temperature from 5 to 60°C

The dependence of viscosity on the temperature can be interpreted by a free volume concept: an increase in temperature allows more thermal motion of macromolecules and greater free volume in the butter, which leads to a decrease in intermolecular or intra-molecular resistances associated with viscosity. The order of the viscosity levels of three butter types was the same as for loss modulus, a fact that confirms the dependence between the G″ and η*. The values of complex viscosity at 5°C ranged from 300004 Pa.s for OCB to 770296 Pa s for SB.

Because of the regular dependence of the viscosity of butter on temperature, a linear relationship between log complex viscosity and the reciprocal of absolute temperature can be obtained. Thus, it is possible to apply the Arrhenius equation;

$$\eta^{*} = \eta_0 \exp\left(E_a / RT\right) \quad (4)$$

Where η^{*} is the complex viscosity (Pa.s), η_0 a constant (Pa.s), E_a is the activation energy, R is the universal gas constant (1.987 kcal/mol.K) and T is the absolute temperature (K).

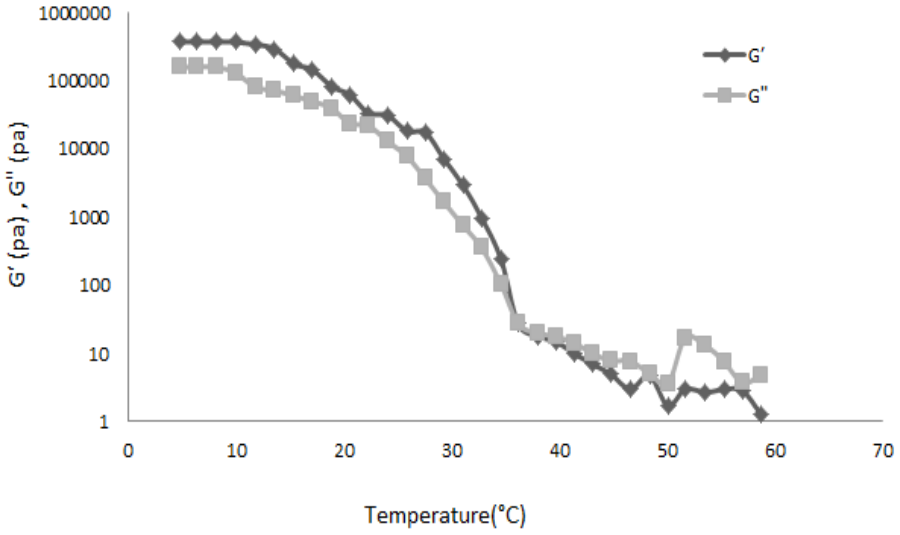

Fig. 5. Temperature dependence of the storage modulus (G′) and loss modulus (G″) of omega-3 cow's butter (*f*=1 Hz, γ=0.01%)

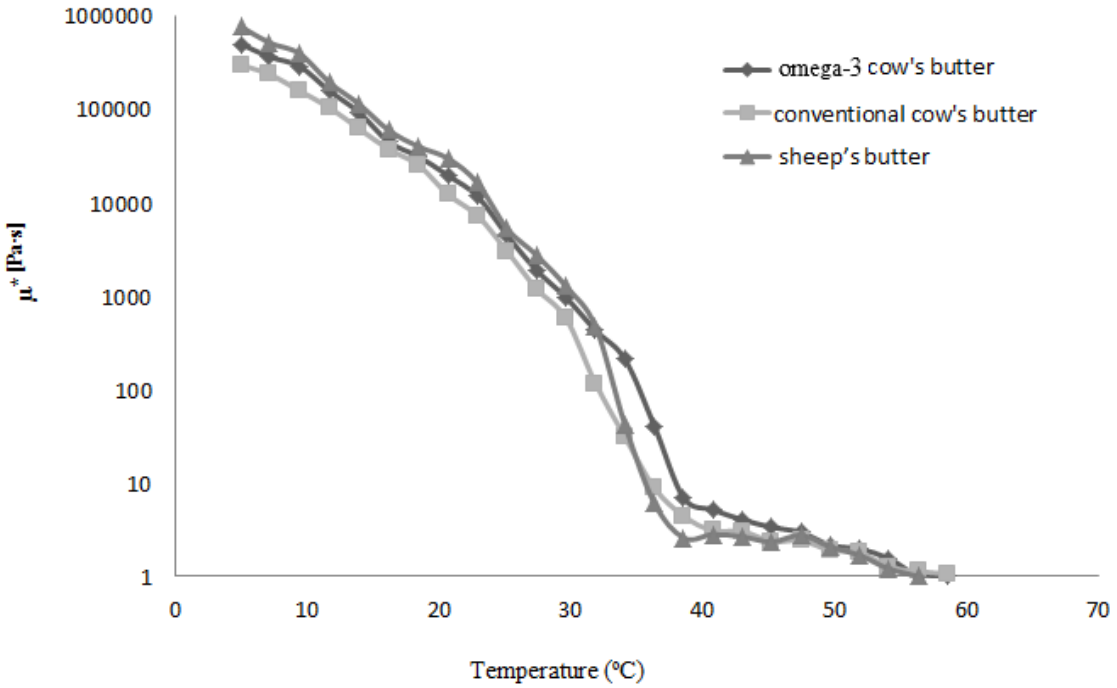

Fig. 6. Complex viscosity changes of butter samples as a function of temperatures (*f*=1 Hz, γ=0.01%)

Activation energy was determined as slope multiplied by R. A linear plot of ln (η^*) versus (1/T) was drawn to obtain the activation energy. The correlation coefficients (R^2) for each butter variety are presented in Table 4. It can be seen that the activation energy (E_a) ranged from 51.55 to 52.63 (kcal/mol). Shukla *et al*. (1994) found activation energy for fractionated high- melting triglyceride (HMT) and anhydrous milk fat (AMF) are 114.47 and 52.68 kcal/mol, respectively. Activation energy levels indicate the sensitivity of the viscosity to the temperature change. Higher E_a means that the butter viscosity is relatively more sensitive to temperature change. Data presented in Table 4 showed that OCB is the least sensitive (lowest E_a value), while SB is the most sensitive (highest E_a value) among the sample examined. The viscosity behavior is a function of composition components present in the butter. Generally, OCB with the lowest percentage of SCFA had a less activation energy than others, indicating that the viscosity is more sensitive to temperature changes at high SCFA contents.

Table 4. The parameters of Arrhenius model determined for different butters

Type of butter	η_0 (Pa.s)	E_a (kcal/mol)	R2
Omega-3 cow's butter	9.45×10^{-40}	51.55	0.95
Conventional cow's butter	6.17×10^{-36}	52.37	0.94
sheep's butter	2.27×10^{-34}	52.63	0.94

R^2 value for the linearied Arrhenius model (lnl vs.1/T)

Conclusions

based on the results obtained in this study, it can be concluded that the OCB with a more levels of CLA and omega 3 fatty acids composition probably have a more health-promoting fatty acid composition and is softer (at 5ºC and 20ºC) than the others. On the other hand, SB with high content of SCFA at the refrigeration temperature (5°C) is much firmer than others, but as a function of temperature, it softens much quicker and near the 18°C, it is already softer than the latter. The melting thermograms of SB displayed fat melting peaks started at a lower temperature than the others. Viscoelastic properties of butter samples were determined using dynamic mechanical analysis, which were dependent upon strain level, frequency, temperature, and chemical composition of samples. From dynamic rheological data, it was found that the butters displayed solid- like viscoelastic behavior, which a more solid- like response is observed at higher frequencies. Compared to CCB and SB; OCB had lower intercept values of k', k" and k* at similar temperatures, which associated with the highest percentage of unsaturated fatty acids. In addition, OCB with the lowest percentage of SCFA had a less activation energy than others, indicating that the complex viscosity is more sensitive to temperature changes at higher SCFA contents.

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خصوصیات حرارتی، بافتی و رئولوژیکی کره حاصل از شیر گاوی ارگانیک و شیر گوسفندی

مرتضی کاشانی‌نژاد[1]- سید محمد علی رضوی*[2]- مصطفی مظاهری طهرانی[2]- مهدی کاشانی نژاد[3]

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چکیده

دراین مقاله خصوصیات حرارتی، بافتی و رئولوژیکی کره حاوی آلفالینولنیک ناشی از تغذیه دام، کره گاوی تجاری وکره گوسفندی با پروفایل اسیدهای چرب مختلف مورد بررسی قرارگرفت. نتایج حاصل از اندازه‌گیری پروفایل اسید های چرب نشان داد که کره گوسفندی دارای مقادیر نسبتا بالاتری از اسیدهای چرب کوتاه زنجیر درمقایسه با کره‌های گاوی می باشد در حالی که کره گاو آلفالینولنیک دارای سطوح بالای CLA و اسید چرب آلفالینولنیک (امگا 3) است. همچنین با توجه به نتایج حاصل از آزمون نفوذ بالاترین میزان سختی در دمای 5 درجه سانتی‌گراد به نمونه کره گوسفندی و کمترین آن به نمونه کره آلفالینولنیک اختصاص داشت. در آزمون‌های رئولوژیکی دینامیک درآزمون کرنش متغیر برای نمونه‌های مختلف کره نشان داد که طول ناحیه ویسکوالاستیک خطی (LVE) برای کره با درصد بالاتر اسیدهای چرب اشباع شده بالاتر است و افزایش دما از 5 تا 20 درجه سانتی‌گراد نیز باعث کاهش LVE می‌شود. در آزمون فرکانس متغیر، وابستگی مدول‌های الاستیک و ویسکوز با فرکانس به‌صورت معادله قانون توان نشان داد نمونه‌های کره رفتار ویسکوالاستیک شبه جامدات با وابستگی اندک به فرکانس را دارند زیرا مقادیر (0/02-2/78) K' بالاتر از مقادیر (0/002-0/83) K'' بودند. در آزمون دما متغیر نیز مقادیر G' و G'' به‌طور پیوسته بین 5 تا 60 درجه سانتی‌گراد کاسته می‌شد و در کمتر از 40 درجه سانتی‌گراد، G' بالاتر از G'' بود که این امر رفتار شبه جامدات کره را در این بازه دمایی نشان می داد. همچنین در آزمون حرارتی DSC در منحنی ذوب چربی، سه پیک گرماگیر درنمونه های کره وجود داشت که پیک اول به میزان آب نمونه‌ها، پیک دوم به اسیدهای چرب غیراشباع و پیک سوم به اسیدهای چرب اشباع ارتباط داشت.

واژه‌های کلیدی: کره، DSC، GC، امگا 3، رئولوژیکی

1 و 2- دانش‌آموخته کارشناسی ارشد و استاد، گروه علوم و مهندسی صنایع غذایی، دانشکده کشاورزی، دانشگاه فردوسی مشهد
3- استاد، دانشکده علوم و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان
(*- نویسنده مسئول : Email: s.razavi@um.ac.ir)

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