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Potential of β-d- glucan to enhance physicochemical quality of frozen soy yogurt at different aging conditions

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Abstract

β-d- glucan as a soluble dietary fiber, has many desirable physical and physiological characteristic. In this research the effect of β-d- glucan and aging conditions (Time and Temperature) on some physicochemical and textural properties of frozen soy yogurt was investigated. Three variables including concentration of oat β-d-glucan (0, 1 and 2%), aging time (2, 13 and 24 h) and aging temperature (2, 4 and 6°C) were studied. The results showed that the addition of β-d- glucan to frozen yogurt increased viscosity, overrun, hardness and fat destabilization but the melting resistance and L*value were decreased. In terms of aging conditions, it was revealed that increasing aging time could improve the quality of product whereas higher temperature had an undesirable effect on the quality of frozen soy yogurt. Longer aging time caused an increase in viscosity, hardness, fat destabilization and melting resistance. By increasing aging temperature, fat destabilization, overrun and viscosity were decreased and melting rate was increased. It was concluded that addition of β-d-glucan as a dietary fiber and prolonged aging time at low temperature could adjust textural properties of frozen soy yogurt and improve quality of this frozen dessert.

Key words: Frozen soy yogurt, β -d- glucan, Aging time, Aging temperature

Introduction

Food producers attempt to develop new food formulations towards functional foods and ingredients according to the customer demands for healthy nutrition (Havrlentova *et al*., 2011). Addition of soluble dietary fiber to foods is one of the ways to produce functional food and to improve health. Dietary fibers are a class of storage and cell wall polysaccharides that resist to human digestive enzymes (Bangari, 2011).

β -d- glucan is a soluble fiber that found in some cereals such as oat and barely. It is a polymer of glucose with β (1→3) and β (1→6) glycosidic linkage and is extracted from cereal, mushroom, seaweed and yeasts (Kobayashi *et al*. 2005; Rhee *et al*., 2008). Addition of this dietary fiber to food is beneficial physiologically because it can reduce the serum LDL and total cholesterol level. It also adjust glycemic index due to its fermentability effect (Nikoofar *et al*., 2013). Furthermore, it could affect textural characteristic of products and therefore sensory perception (Vasiljevic *et al*., 2007). There are some researches about application of β-d- glucan in dairy. Nikoofar *et al*. (2013) studied the effect of oat β-d- glucan as a fat replacer on rheological properties of nonfat set yogurt. They stated that yogurt containing β-d- glucan had firm and creamy texture with darker color. Vasiljevic *et al*. (2007) investigated the effect of β-d- glucan on a probiotic yogurt. They reported that addition of β-d- glucan caused a weaker gel incapable of retaining water. Bangari (2011) studied the effects of oat β-d- glucan on the stability and textural properties of milk beverage fortified with β-d- glucan. The author reported that samples containing different levels of β-d-glucan had higher viscosity than control

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sample. However there is no report about applying β-d- glucan in frozen soy yogurt.

Frozen yogurt or yogurt ice cream is a functional dairy dessert that combines the physical characteristics of ice cream with the sensory properties of fermented milk product (Marshall *et al*. 2003). It is prepared by freezing a pasteurized mix containing milk fat, SNF, sweetener, stabilizer and yogurt (Guner *et al*., 2007). The use of soy products in the manufacturing of foodstuff has some positive effects related to human health. It has capability to reduce the risk of cancers such as breast and prostate cancers. Incorporating soy to dairy dessert may potentially serve as carriers of valuable soy ingredients (Mahdian *et al*., 2012). Dervisoglou *et al*. (2005) investigated the effect of soy protein concentrate (SPC) on physical, chemical and sensory properties of ice cream. Their results showed that SPC positively influenced viscosity, melting rate and appearance.

Aging is an important step of production dairy dessert such as ice cream and frozen yogurt. Aging condition promotes some significant effect in ice cream mix (Botega, 2012). In this process, the fat crystallizes, protein adsorbs on the fat globules and hydration of the protein occurs (Issariyachaikul, 2008). These events need to continue for a few hours. Aging conditions could affect the events that occured in this period. Thus, the objective of this study was to investigate the effects of β-d- glucan addition and aging conditions on some physicochemical and qualitative properties of frozen soy yogurt.

Materials and Methods

Soymilk was prepared according to the method of Mahdian *et al*. (2012) with some modifications. Soymilk blend contains 8% soy flour (Toos soyan Co. Iran), 4% skim milk powder (Pegah Co. Iran) and 1% food grade sodium citrate (Merck, Germany) was prepared by dissolving the mentioned components in hot water (90-95°C). β-d- glucan from oat (Imam Co. China) was added to the mixture at different levels (0, 1 and 2%). The mixture (13% dry matter) was stirred completely by a hand blender (Gosonic, Turkey) so that homogenous and free of sandy mouth feel soy milk was obtained. This mixture was heated at 85 °C for 30 minutes. After cooling to 42°C, yogurt starter (0.2 %) (YF-L812, CR-Hansen, Denmark) was added and followed by incubation at 42°C. When acidity reached to 80° Dornic, fermentation process was stopped and the yogurt was cooled to 10°C.

To prepare frozen yogurt mix, sugar (16%), stabilizer- emulsifier mix (0.2%, lux6700, Cargill, France) and skim milk powder (to adjust total solid to 35%) were dissolved in water and heated at 85°C for 30 minutes. After cooling to 10°C, the mixture was blended with yogurt and aged at different temperatures (2, 4 and 6°C) for certain times (2, 13 and 24 hr). The aged frozen soy yogurt mix was frozen using a laboratory batch ice cream maker (Musso, Italy) for 30 minutes. After freezing, samples were distributed among polyethylene cups (50 ml) and hardened at -18°C until analysis.

Measurement of overrun

Overrun is an important property of ice cream and frozen yogurt that affects the physical condition and storage stability. The overrun was calculated using the following equation (Rossa *et al*., 2012):

(Weight of unit volume of mix before freezing– weight of unit volume of ice cream)/ Weight of unit volume of ice cream)×100 (1)

Measurement of viscosity

Viscosity measurement was done for frozen soy yogurt mix immediately after aging. Apparent viscosity of samples (250 ml) was measured according to the method of Akalin and Erisir (2008) with some modifications. Viscosity was determined using Brookfield DVII RV (USA) viscometer equipped with spindle 4 at 100 rpm and at 4°C after 20s. Viscosity reported in Centipoises.

Measurement of melting rate

Melting resistance of frozen yogurt samples was determined according to the method of Mahdian *et al*. (2013) with some modifications. Frozen yogurt (30 ± 2 gr) was placed on a wire

screen (o.5 mm hole diameter) on top of a funnel that was attached to a flask. The samples were placed in a controlled temperature chamber at 25°C. The weight of melted frozen yogurt was recorded after 45 min and expressed as percentage of melted weight.

Measurement of fat destabilization

Fat destabilization was determined by spectrophotometery method. Briefly, an aliquot of frozen yogurt mix and final frozen yogurt was diluted to 1: 500 with distilled water and absorbance was measured at 540nm (Issariyachaikul, 2008).The smaller the fat destabilization value, the greater is the fat destabilization in the ice cream (Adapa *et al*., 2000). The percent of fat destabilization was determined using the following formula:

(Turbidity of the final frozen yogurt/ turbidity of the frozen yogurt mix)*100 (2)

Hardness determination

Hardness of frozen yogurt samples was measured using Texture Analyzer (TA-TX, Stable Microsystem Ltd, Uk). Samples were rapidly transported to the texture analyzer and analysis completed within 30s to minimize variability due to sample warming (Muse & Hartel, 2004). Measurements were carried out with a cylindrical probe (2 mm diameter). The penetration depth in the samples was 10mm and penetration speed was set at 2 mm/s. hardness of sample was determined as the maximum compression force during penetration.

Measurement of foam stability

To evaluate the foam stability, 200 ml of the frozen yogurt mix after aging was blended for 2 min in a domestic blender (Gosonic). Then the mix was poured into 250 ml graduated cylinder and the volume of produced foam was recorded. After 15 min, the final volume of foam was measured. Percent of foam stability was expressed as percent of final volume of foam to initial volume of foam (Alakali *et al.*, 2009).

Color measurement

The color of samples was evaluated by image processing method (Hashemi-Shahraki *et al.* 2014). A digital camera was fitted on top of sample in a box (with 60 × 60 cm length and width). Interior walls of the box were covered with dark cloth for evade light scattering. The obtained pictures were transferred to a computer and saved in JPEG format. Image processing analysis was conducted using Image J software (Version 1.42, Wayne Rasband, National Institue of Health, USA). The color space of picture was converted from RGB to CIElab by using of converter space color. Mean of each color parameter was expressed as L* (lightness), a* (redness) and b* (yellowness).

Statistical analysis

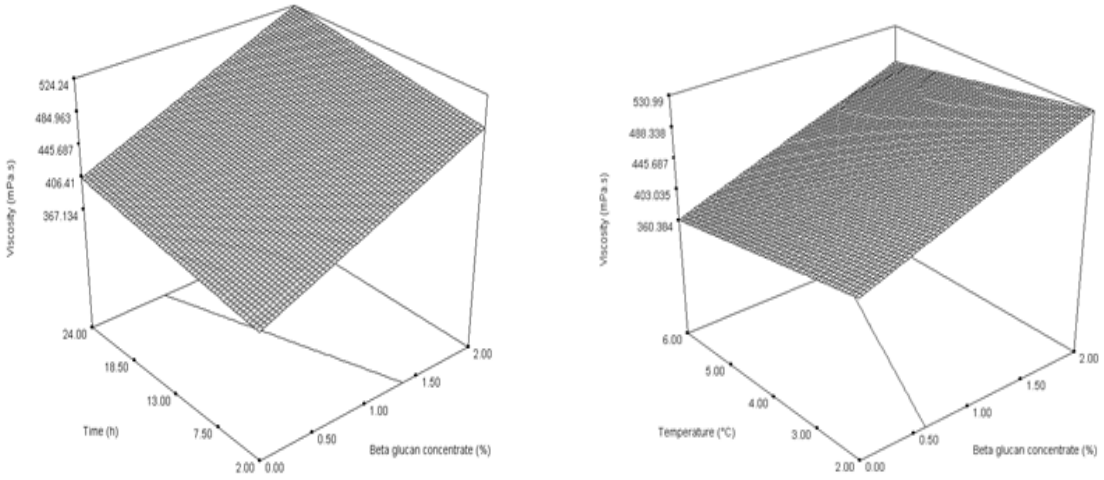
Response surface methodology with a central composite design was used to design and analyze the experiments. B-d- glucan concentration, aging temperature and aging time were chosen as independent variables. Design Expert software (Version 6.0.2, Stat-Ease, Inc) was used to design, arrange and analyze of experiments.

Results and Discussion

Viscosity

Viscosity as one of the most important rheological properties of ice cream mix is affected by mix composition, type and quality of the ingredients, processing of the mix and temperature (Bahram Parvar and Mazaheri-Tehrani, 2011). Fig.1 shows the effect of β-d-glucan and aging condition on the viscosity of soy frozen mix. The results showed that β-d-glucan addition and extended aging time caused an increase in viscosity of soy frozen mix (Fig. 1-a) but aging temperature had inverse effect (Fig. 1-b). Higher apparent viscosity of samples containing higher concentration of β-d- glucan could be explained by potential ability of β-d-glucan to interact with soy milk protein. Maximum viscosity was observed when β-d-glucan concentration was 2% and after 24 hours aging. In terms of aging temperature, the highest viscosity was observed at 2°C. Previous studies also reported that addition of some fibers such as inulin to ice cream (Issaryachakul 2008) and frozen yogurt (Rezaei *et al.*, 2014) caused a significant increase in viscosity. They stated that interaction of this dietary fiber and

the liquid part of ice cream mix is the main reason for viscosity improvement. Hydration of β-d- glucan and other hydrocolloids occur during aging time so it was expectable an increase in viscosity.



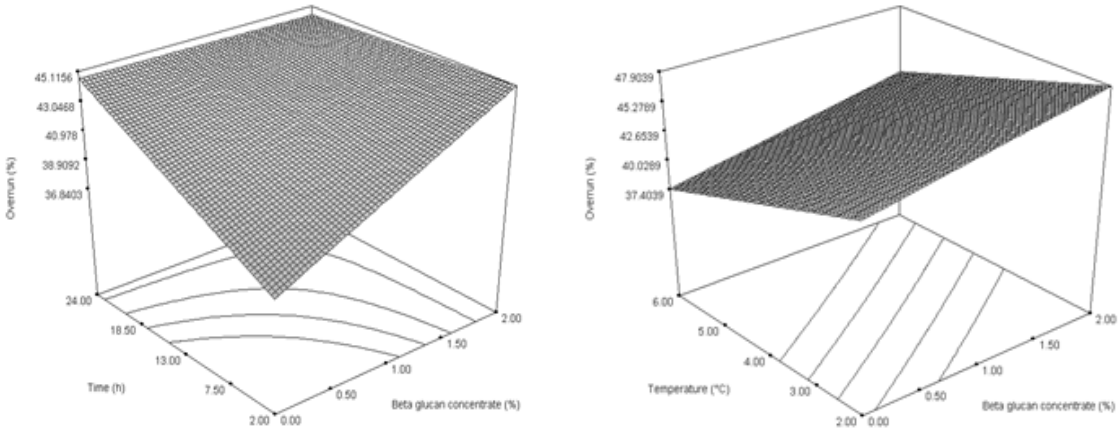
(a) **(b)**

Fig. 1. Effect of β-d- glucan and aging condition (a: time and b: temperature) on the viscosity of frozen soy yogurt mix

Overrun

Overrun is a numerical indicator of the amount of air that is whipped into a product. The amount of air in frozen dessert is important because it affects the quality and profit (Bahram Parvar and Mazaheri-Tehrani, 2011). This factor also influences melting rate and sensory properties of product. It has been shown that hydrocolloids affect the ability of product to incorporate air and hold it for sufficient period of time (Lammann, 2011).

Results of this study showed that addition of β-d- glucan increased overrun of frozen soy yogurt and maximum overrun was attained by addition of 2% β-d- glucan (Fig. 2-a).



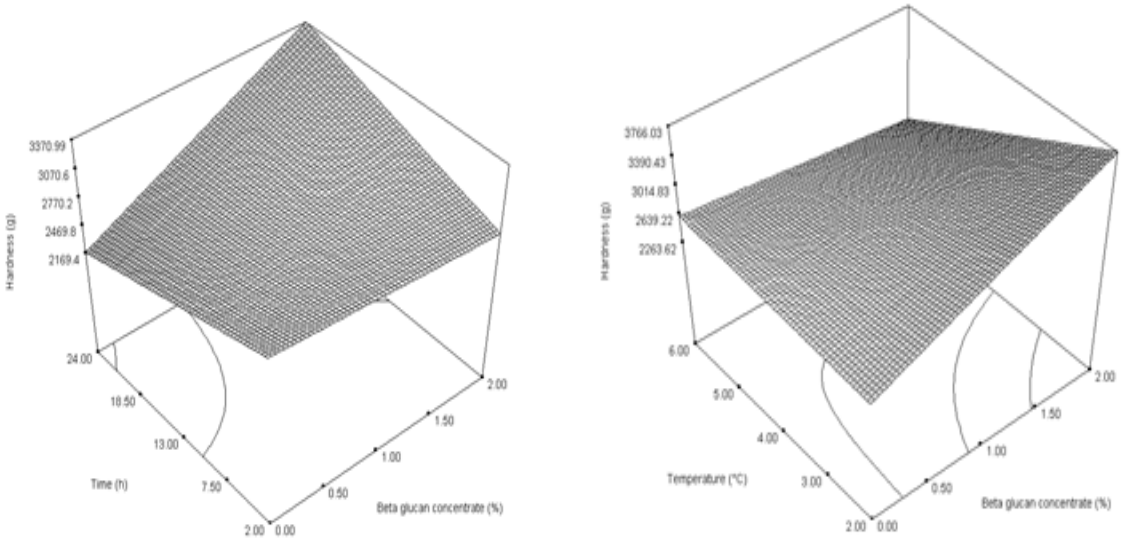
(a) **(b)**

Fig. 2. Effect of β-d- glucan and aging condition (a: time and b: temperature) on the overrun of frozen soy yogurt

Ability of a hydrocolloid to increase overrun could be attributed to their capability to form a polymer entanglement and control the air cells unstability (Soukoulis *et al*., 2008). Akin *et al*. (2007) reported that addition of inulin increase overrun values of the ice cream sample but it was insignificant. A similar result was obtained by Rezaei *et al.* (2011). They also stated inulin increased the overrun of frozen yogurt. Aging time also had a positive effect on the overrun and highest overrun was observed after 24h of aging period (Fig. 2-a). Non-aged ice cream mix shows a loose stand up and unpredictable whipping. The whipping ability of the mix usually improves with aging (Goff, 1997). Temperature of aging had negative effect on overrun and decreased it. Lowest overrun was belonged to sample without β-d- glucan that aged at 6°C and it was seen that overrun increased at lower temperature (Fig. 2-b).

Hardness

Hardness of ice cream is an index of resistance of ice cream to deformation when an exterior force is applied (Muse and Hartel, 2004). Conventionally, hardness of frozen dairy products is determined by a penetrometer test (Adapa *et al*., 2000). This test shows the required force to penetrate the probe into samples. The higher the force, the higher is the hardness. Soukoulis *et al*. (2008) reported that hardness measurments reveal the impact of ingredients such as hydrocolloids and processing conditions (such as aging) on the product. Our results showed that hardness enhanced with increasing β-d- glucan level and aging time. The maximum hardness was observed in sample containing 2% β-d- glucan and aged for 24 hours (Fig. 3-a). Actually, addition of β-d- glucan and increasing aging time caused an increase in viscosity that resulted in rising the hardness. Rheological property of the mix is a factor that affects the hardness. The ice cream was harder when the apparent viscosity was greater (Muse and Hartel, 2004).



(a) **(b)**

Fig. 3. Effect of β-d- glucan and aging condition (a: time and b: temperature) on the hardness of frozen soy yogurt

According to Akalin and Erisir (2008) ability of some ingredients to bind water and form a gel network can improve the firmness of products. Gustaw *et al*. (2011) studied the influence of selected prebiotic on the some properties of bio-yogurt. They reported that addition of 1% FOS increased viscosity and hardness of yogurt. They explained it was a part of the structural network being formed during fermentation. Another factor that affects

the hardness is fat destabilization. Hardness of ice cream increased as level of destabilized fat increased. Results of fat destabilization measurement of this study confirmed this claim. Lower temperature had a positive impact on the hardness and maximum hardness was detected at 2°C and 2% β-d- glucan (Fig. 3-b)

Fat destabilization

Destabilized fat in the form of partially coalesced fat globules coats and stabilizes air cells (Mous and Hartel, 2004). Fat destabilization in ice cream affects some properties such as melting rate and overrun. Segall and Goff (2002) reported that positive characteristics of ice cream that associated with the destabilized fat are the high overrun and slow melt down rate. Our results showed that maximum fat destabilization occurred when β-d- glucan concentration and aging time were in the highest level (Fig. 4-a).

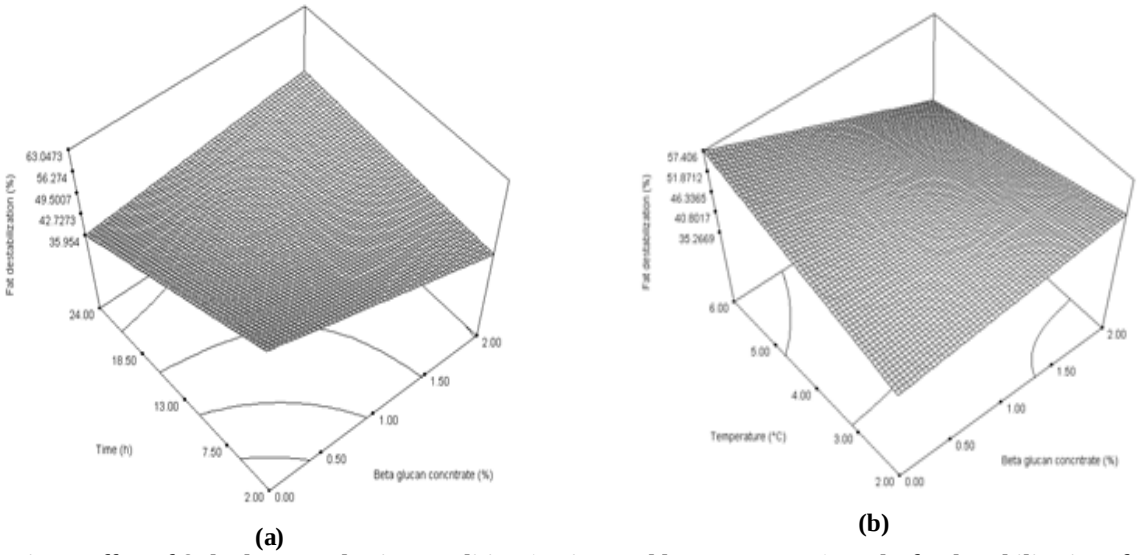


Fig. 4. Effect of β-d- glucan and aging condition (a: time and b: temperature) on the fat destabilization of frozen soy yogurt

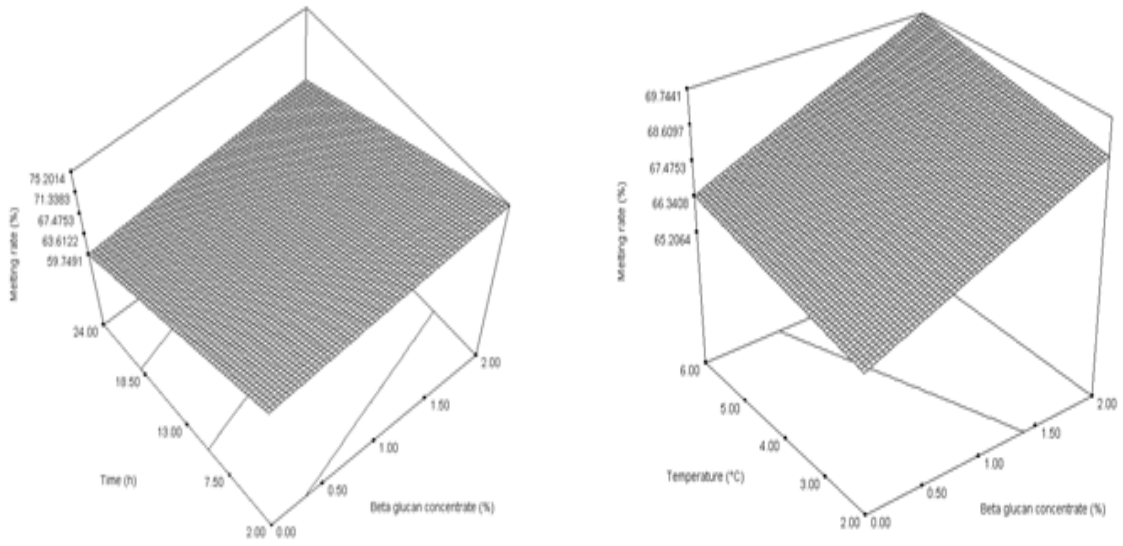
By increasing β-d- glucan, the level of destabilized fat increased. This phenomenon could be attributed to high viscosity of mix as a result of β-d- glucan addition. Muse and Hartel (2004) also reported that ice cream made with corn syrup (DE=20) had higher viscosity that led to higher level of fat destabilization. Higher aging temperature had an inverse effect on fat destabilization (Fig. 4-b). Lower temperature of aging led to the formation of partially crystalline fat droplet. Partial crystallization of fat droplet in ice cream mix promoted partial coalescence which caused to formation of a stable ice cream structure (Botega, 2012).

Melting rate

The melt down rate of ice cream is affected by many factors including the amount of incorporated air, the nature of ice crystals and the network of fat globules formed during freezing (Muse and Hartel, 2004). A slow, uniform melting of ice cream is desirable (Baer *et al.*, 1999).Our results showed that addition of β-d- glucan increased melt down rate of soy frozen yogurt (Fig. 5-a).

Our findings were in contrast with some researches that stated dietary fibers such as inulin reduced melting rate (Aykan *et al*. 2008; Rezaei *et al*. 2011). This event may be related to incompatibility between casein and β-d-glucan as a non-interacting polysaccharide that led to form a weak network (Nikoofar *et al.*, 2013). Vasiljevic *et al.* (2007) also verified this behavior of β-d- glucan in network containing casein. Unlike β-d- glucan concentration, prolongation of aging time caused a decrease in melt down (Fig. 5-a). This effect may be explained with higher consistency of mixes when aged for long time. Higher viscosity could retard the melting of product (Akalin and Erisir,

2008). Actually, when serum viscosity increased, more time is required for the water to be diffused into the concentrated serum before it flows to the ice cream exterior (Sokoulis *et al*., 2008). Moeen Fard and Mazaheri-Tehrani (2008) showed that in all samples containing different stabilizers (Panisol ex, salab and a mix of stabilizers and emulsifiers include sodium alginate, guar, carageenan and polysorbate 80) melting resistance increased significantly. Temperature of aging caused an increase in melt down rate of soy frozen yogurt and maximum melt down rate was observed at 6°C. This event could be explained with lower viscosity and lower overrun of samples that aged at higher temperature. Lower overrun is correlated to higher heat transfer rate and therefore fast melting rate of these samples.



(a) **(b)**

Fig. 5. Effect of β-d- glucan and aging condition (a: time and b: temperature) on the melting rate of frozen soy yogurt

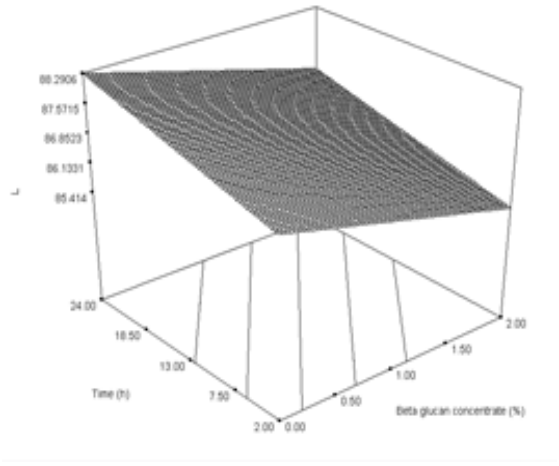
Color

Color is a key attribute in foods because it is usually the first property that observed by the customer (Sanabria, 2007). Different factors such as ingredients and manufacturing parameters influence the color of products. Results of this study showed that addition of β-d- glucan had considerable effect on the color of soy frozen yogurt. It lowered the L*(lightness) and increased b* (yellowness) values that could be related to pale yellow color of β-d- glucan powder that was used for frozen soy yogurt formulation. Nikoofar *et al*. (2013) investigated yogurt containing β-d- glucan and explained L* value was decreased with increase in amount of β-d- glucan and b* values in yogurt containing β-d- glucan were decreased. Aging conditions also affected on color indexes. Whereas aging time increased L* and b* values, aging temperature decreased L* and increased b*. Effects of β-d- glucan, aging time and aging temperature on a* was observed in Fig. 6-a and 6-b.

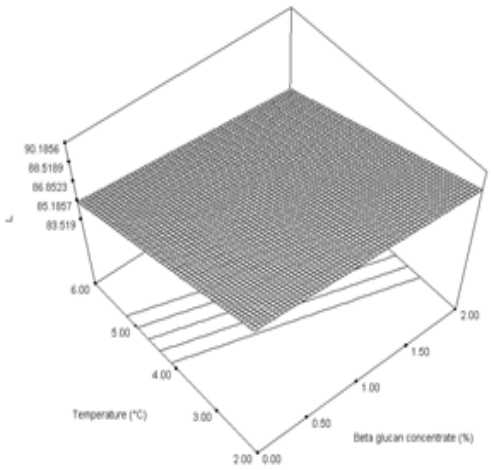
Foam stability

Fig. 7 shows the effect of β-d- glucan and aging conditions on the foam stability of soy frozen yogurt mix.

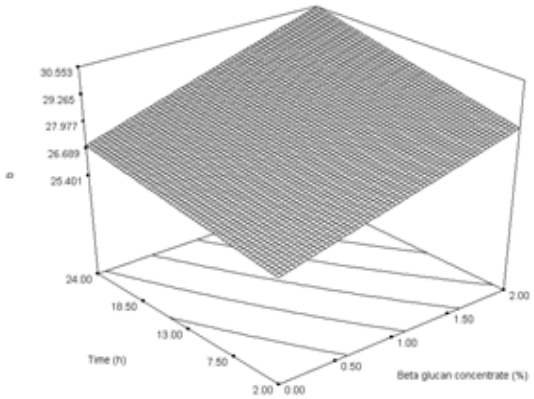
According to our data, it is found that maximum foam stability was obtained in central point of aging time and highest concentration of β-d- glucan. Temperature had no considerable effect on the foam stability. Amount of foam formation of soy frozen yogurt mix inversely correlated with foam stability (data not shown).



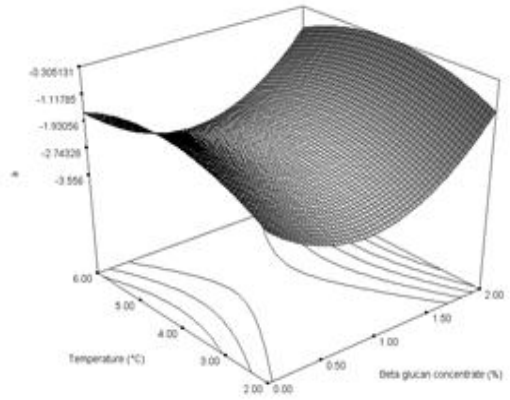
(1-a)



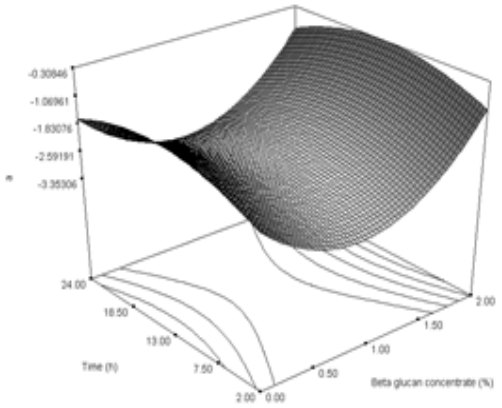
(1-b)



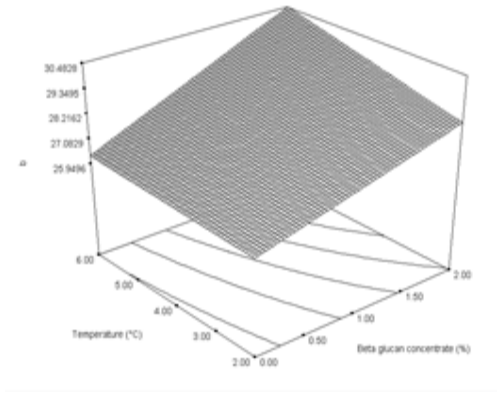
(2-a)



(2-b)



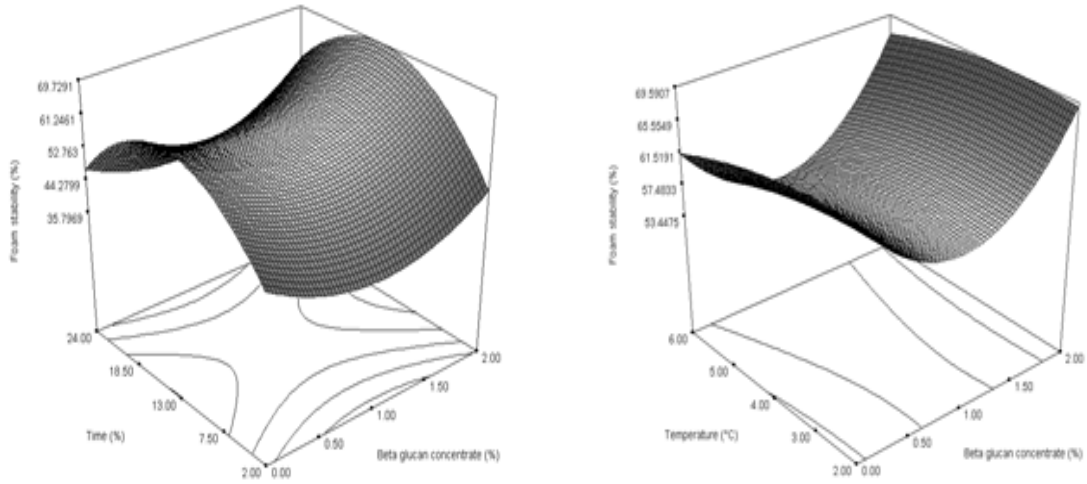
(3-a)



(3-b)

Fig. 6. Effect of β-d- glucan and aging condition (a: time and b: temperature) on the color (1: L*, 2: a* and 3: b*) of frozen soy yogurt

It seems that viscosity plays an important role for foam formation and its stability. By increasing viscosity, foam stability was improved. Higher viscosity and heavy body of ice cream mix caused that air cells remain invariable, consequently foam stability increased (Alakali *et al.*, 2009).



(a) **(b)**

Fig. 7. Effect of β-d- glucan and aging condition (a: time and b: temperature) on the foam stability of frozen soy yogurt

Conclusion

This study investigated some physicochemical properties of frozen soy yogurt containing β-d- glucan manufactured at different aging conditions. Results of this study showed that addition of β-d- glucan could improve some characteristics of frozen soy yogurt. Overrun viscosity, hardness and fat destabilization of product increased by adding β-d- glucan. Prolongation aging time to 24 hour had positive effect on viscosity and hardness but decreased overrun. Our results also revealed that aging at lower temperature (2°C) is preferable to make this dessert. So, we can introduce β-d-glucan as a proper option to modify frozen soy yogurt texture and sensory properties

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پتانسیل بتاگلوکان جهت بهبود کیفیت ماست سویا منجمد تحت شرایط مختلف دوره رساندن

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چکیده

در این مطالعه، تاثیر بتاگلوکان و شـرایط رساندن (دما و زمان) بر برخی ویژگی‌های فیزیکوشیمیایی و بافتی ماست سویا منجمد مورد بررسی قرار گرفته است. سه متغیر شامل مقدار بتاگلوکان (صفر، 1و 2 درصد)، زمان رساندن (2، 13 و 24 ساعت) و دمای رساندن (2، 4 و 6 درجه سانتی‌گراد) مورد مطالعه قرار گرفت. نتایج نشان داد که افزودن بتاگلوکان به ماست منجمد باعث افزایش ویسکوزیته، اوران، سختی و ناپایداری چربی گردید اما مقاومت به ذوب و شـاخص L* را کاهش داد. از نظر شرایط رساندن، مشخص شد که افزایش زمان رساندن کیفیت محصول را افزایش داده در حالی که دمای بالاتر تاثیر نامطلوبی بر کیفیت ماست سویا منجمد بر جای گذاشت. زمان رساندن طولانی ترسبب افزایش ویسکوزیته، سختی، ناپایداری چربی و مقاومت به ذوب شـد. با افزایش دمای رسـاندن ناپایداری چربی، اوران و ویسکوزیته کاهش یافت و سرعت ذوب افزایش یافت. بر اساس این یافته‌ها می‌توان نتیجه گرفت که افزودن بتاگلوکان به‌عنوان فیبر رژیمی و زمان رساندن طولانی در دمای کمتر می‌تواند ویژگی‌های بافتی ماست سویا منجمد را تعدیل و کیفیت این دسر منجمد را بهبود دهد.

واژه‌های کلیدی: ماست سویا منجمد، بتاگلوکان، دمای رساندن، زمان رساندن

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Preparation of anti-Pseudomonas bioactive films by embedding *Lactobacillus casei* ATCC 39392 in Sodium caseinate and Methyl cellulose matrices

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Abstract

Biodegradable films containing lactic acid bacteria (LAB) are considered as new tools for advanced methods of food storage. In this study, *Lactobacillus casei* ATCC 39392 (*L. casei* 39392**)** was directly incorporated into a film formation solution of sodium caseinate (NaCas) and methyl cellulose (MC). The bioactive films were prepared in a manner to contain 10^6CFU/cm^2 *L. casei* 39392. The moisture content, solubility in water, water vapor permeability (WVP), color, opacity, tensile strength, percentage of elongation at break, and the elastic modulus of the films were studied. The survival rate of *L. casei* 39392 was examined during 30 days of storage (5 °C, RH 75%) and the films inhibitory effect on the growth of *Pseudomonas aeruginosa* PTCC 10832 was also studied at 5 °C for 12 days. The presence of *L. casei* 39392 increased the film's opacity and its WVP compared to the control ($p < 0.05$). The survival rate of *L. casei* 39392 was higher in NaCas films than in methylcellulose films ($p < 0.05$). A higher inhibitory effect on the growth of *P. aeruginosa* 10832 (85.3%) was observed in the MC bioactive film, and this inhibitory effect became noticeable from the fourth day of storage onwards ($p < 0.05$). Our results showed that the bioactive films containing *L. casei* 39392 could be used and recognized as biofilms containing natural preservatives.

Keywords: Anti-Pseudomonas, Edible film, *Lactobacillus casei*, Methyl cellulose, Sodium caseinate

Introduction

The development of food storage methods is given an ongoing attention by researchers. So far, various methods have been proposed such as freezing, drying, vacuum packaging and the use of antimicrobial preservatives for food storage; however, some of those methods cannot be used in certain groups of food such as ready-made meals (Quintavalla & Vicini, 2002). Spoilage by microorganisms is a major concern in the realm of food preservation. Bacteria such as *Listeria monocytogenes*, *Pseudomonas spp.* and *Escherichia coli* can cause food spoilage, and their presence in food is often a critical issue when considering the storage of food products (Angiolillo et al., 2014). *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic pathogen which mainly targets patients with hematological malignancies (Neves *et al.*, 2014). *P. aeruginosa* is also reported to be the major cause of contamination in poultry, fish, beef and dairy products (Van Tassell *et al.*, 2012). Previous research has shown that the direct use of free antimicrobial compounds in foods result in an immediate but short-term reduction of bacterial populations (Quantavalla & Vicini, 2002). Therefore, a new method was considered, known as active packaging. In this packaging technique, antimicrobial agents are placed inside the film matrices. As a result, antimicrobial effects are maintained for a longer period of time. (Sozer & Kokini, 2009). The duration of antimicrobial activity in the film depends on how antimicrobial compounds are released from the film and how they react with the film's matrix (Leonard *et al.*, 2014). Biodegradable and edible films generally receive greater attention than synthetic films, because they do not end up as waste material, and therefore they exist as environmental friendly (Sozer & Kokini, 2009). Recently, the use of lactic acid bacteria (LAB) in edible films has been considered (Lopez de Lacey *et al.*, 2014). Several reports confirm the effect of metabolites produced by lactic acid bacteria,

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such as the production of bacteriocin-like inhibitory substances (BLIS) (Galvez, Abriouel, & Ben Omar, 2007); organic acids and hydrogen peroxide (Tahiri *et al*., 2009) which inhibit the growth of bacterial pathogens. A well known probiotic LAB that can produce antimicrobial compounds is *Lactobacillus casei* (*L. casei*). Pervious studies have shown that *L. casei* significantly reduce the growth of pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *P. aeruginosa* (Sunaryanto *et al.*, 2014; Erddougrul & Erbilir 2006)

There are numerous reports regarding the use of methyl cellulose (MC) and sodium caseinate (NaCas) biopolymers in the production of edible films (Jimenez et al., 2013; Noronha *et al.*, 2014). Methyl cellulose and NaCas films are biodegradable, inexpensive and easily available. Their ability in preventing the permeation of naturally occurring gases in the atmosphere makes them more applicable (Matsakidou *et al.*, 2013).

Previous studies have also shown that the chemical composition of the matrix can affect the antimicrobial and physical properties of bioactive films (Kanmani and Lim, 2013; Lopez de Lacey *et al*., 2014).

Odila Pereira *et al.* (2016) stated that the viable number of *LAB* decreased only three logarithmic cycles in 60 days in edible films containing whey protein. In a study, *Lactobacillus plantarum* GG ATCC 53103, *Lactobacillus reuteri* ATCC 55730 and *Lactobacillus acidophilus* DSM 20079 were embedded in matrices made of pullulan and various starches. LAB caused slight changes to the mechanical and physical properties of the bioactive films. Also, pullulan and pullulan/potato starch were found to be effective in shaping the aforementioned properties of the films (Kanmani &Lim, 2013).

The first aim of this study was to prepare a new edible film with anti-pseudomonas properties by embedding *Lactobacillus casei* ATCC 39392 (*L. casei* 39392) in a carbohydrate based matrix (methyl cellulose, MC) and in a protein based matrix (NaCas). The second aim was to understand how such embedding can affect the films qualities and structures, including their physicochemical and mechanical properties, and the survivability of the *L. casei* 39392 in the films. Measurements spanned the 30 days of storage (at 5°C and 75% relative humidity). The antimicrobial activity of films against *Pseudomonas aeruginosa* PTCC 10832 (*P. aeruginosa* 10832) was investigated invitro.

Materials and Methods

Propagation of bacterial cells

L. casei ATCC 39392 and *Pseudomonas aeruginosa* PTCC 10832 (*P. aeruginosa* 10832) were purchased in lyophilized form from credible institutions. Lyophilized *L. casei* 39392 was inoculated in 20 ml de Man Rogosa Sharpe Broth (MRS Broth, Liofilchem, Italy) in sterile conditions, and then it was incubated (at 30°C for 24 hours) (Vescovo *et al*., 2006). The cells of *P. aeruginosa* 10832 were transferred to the culture medium of Tryptic Soy Broth (TSB) for 48 hours at 37°C which was then incubated under sterile conditions (Tyagi and Malik, 2011). Microbial suspensions were centrifuged (Model Eppendorf 5810, Germany) for 15 minutes at 3500 g, and the plate was obtained after being washed twice with phosphate-buffered saline (PBS, Merck, Germany) pH=7 to obtain microbial suspensions. Thereafter, the population growth curve for each group of cells was plotted using the serial dilution and pour plate method. The absorbance value of each aliquot was measured using the UV-VIS spectrophotometer device (Cecil model, England) at 600 nm.

Preparation of films

Four grams of the relevant powder (CAS 9004-67-5, Sigma-Aldrich, Madrid, Spain) was dissolved in 100 ml of sterile distilled water at 25 °C in order to prepare the methyl cellulose film, according to Sanchez-Gonzalez et al. (2013). Glycerol was used at 25% w/w, based on its polymer weight (Merk, Germany). The glycerol was added to the film formation solution (FFS) as a plasticizer. It was homogenized in a rotor-stator ultraturrax (DI25, Germany) at 13500 rpm for 4 min. In

order to remove the unwanted cells, the FFS was heated to 80°C for 30 minutes and then quickly cooled to 5°C. By the assistance of a vacuum pump (Jencons, England), degassing was carried out at room temperature.

The NaCas film-forming solution was prepared according to the procedure of Broumand *et al*. (2011) with slight modifications. Five grams of NaCas powder (CAS 9005-46-3, Sigma-Aldrich, Madrid, Spain) was added to 100 mL of sterilized distilled water containing glycerol (30% w/w, based on its polymer weight) at a temperature of 65 ± 5°C in order to prepare the FFS. After complete dissolution, FFS was stirred for 1 hour at 85 ± 2 °C and a vacuum pump was used for its degasification. By transferring 10 µl of *L. casei* 39392 broth culture into 10 mL of MRS broth, bioactive films were became ready to be incubated at 30°C for 24 h. LAB were processed and obtained after being centrifugation at 3500 rpm for 20 min. Reassurance was made to wash the pellets twice with PBS at pH= 7.0 accordingly. Aliqoutes of 240 and 300 µl per 98 ml, respectively of *L. casei* 39392 was then added into the FFS of MC and NaCas-based films. The solution was then mixed on a magnetic stirrer for 5 minutes, and it was endeavored to bring the concentration of *L. casei* 39392 in dry films to 10^6 CFU/cm^2. The FFS of bioactive MC (bio-MC) and bioactive NaCas (bio-NaCas) were transferred to plastic dishes and were dried in an oven at 25 °C for 24 hours. All steps were carried out by using the technique of aseptic and sterile conditions. The average thickness and the total density of the film samples were 0.071 ± 0.05 and 52.2 g/m^2, respectively. In order to adjust moisture (to constant weight), all films were positioned in the desiccator for seven days at 5 °C and at a relative humidity (RH) of 75% before carrying out the mechanical and physical examinations. The 75% RH was reached with the help of NaCl solution that was ultra-saturated.

Survival rate of *L. casei* 39392

The survival rate of *L. casei* 39392 was quantified according to the method used by Gialamas *et al.* (2010) with slight modifications. Bio-NaCas and bio-MC films were placed inside plates and were then collectively placed in the desiccator which had 75% relative humidity at 5°C. This system was maintained for 30 days. A container having saturated salt (NaCl) was used inside the desiccator which caused the moisture content reach 75%. This RH was monitored by a digital hygrometer device (Samwon, South Korea). Every 10 days, the bioactive films were transferred to the Stomacher blender bag (Interscience French model) containing 100 mL of phosphate buffered saline which was then homogenized for 2 minutes. Thereafter, 1000 µl of the sample were removed and, after preparing serial dilutions, it was cultured on MRS agar. The plates were incubated at 30°C for 48 h. Lactobacillus Survival rate was calculated according to the following below formula:

$$\text{Survival rate } (\%) = (N_t / N_0) \times 100 \quad (1)$$

Where N_0 represents the initial number of *L. casei* 39392 and N_t represents the number of alive *L. casei* 39392 in every 10 days of storage**.**

Evaluation of antimicrobial characteristics of bioactive films

From the microbial suspension, aliqout of 50 µl *P. aeruginosa* 10832 was transferred and cultured on the TSA medium. Infusion was particularized in a manner that set the final concentration of *P. aeruginosa* at 10^2 CFU/cm^2 on the medium. Then, films of the control group and the bioactive films were separately placed on the TSA which had been inoculated with the target pathogen. Collectively, this system was incubated at 5°C for 12 days. Under aseptic conditions, samples were transferred to Stomach bags with 100 ml of PBS. Samples were shaked in Stomacher device (interscience, France) for two minutes and serial dilutions were then prepared. Aliqout of 100 µl of the sample was transferred to the TSB and MRS media in order to count the *P. aeruginosa* 10832 and *L. casei* 39392, respectively, in accordance with the procedure described in

section 2.1. The inhibitory effect on the cells' growth rate was calculated in relation to the initial population of cells (Zheng and Zhu, 2003).

$$\text{Inhibition of growth rate } (\%)=((N_0-N_T)/N_0) \times 100 \quad (2)$$

Where: N_0, is the initial number of target bacteria colonies and N_T is the final number of pathogenic bacteria colonies in every 4 days of storage.

Characterization of films

Moisture content

Determination of moisture content of films was performed based on the method introduced by Kurek *et al.* (2014) with some modifications. Fifty mg of the film sample was placed inside the oven at 60°C for 24 hours. Then, the sample was weighed again and, finally, the moisture content of the film was calculated.

Measurement of film solubility in water

Film samples (with the dimensions of 2 × 3 cm) were dried at 60°C for 24 hours and were subsequently weighed. The procedure was followed according to the method proposed by Pinottia *et al.* (2007). Film samples were soaked in 80 ml of water and were then stirred for 1 hour. Finally, the film's insoluble pieces were separated and dried at 60 C overnight. The film's percentage of solubility was calculated according to the following equation.

$$\text{Solubility } (\%)=((W_I-W_F)/W_I) \times 100 \quad (3)$$

Where: W_I is the initial weight of the film and W_F is its weight after the solubility test. Measurements were performed in triplicate.

Water vapor permeability (WVP)

The WVP of the film was evaluated based on the ASTM method E96-95 with some modifications described by Vargas *et al.* (2011) whereby 10 ml of distilled water was added into the glass vial (with an outer diameter of 2 cm), and the film samples were attached firmly to the vials with the help of parafilm. Then, the prepared vials were placed inside the desiccator with a relative humidity of 75% at 5°C. Water vapor transmission was measured according to the vials' weight loss which was measured every 12 hours by using a balance with 0.0001 precision (AND GF-300 model, Japan).

The gradient curve of the weight loss was calculated in relation to time and was plotted over the area (A, m^2) of the film to calculate the water vapor transmission rate (WVTR). WVP of the film was calculated by the following formula.

$$WVP= WVTR \times d/ \Delta P \quad (4)$$

Where d is thickness (mm) and Δp is water vapor pressure difference (Kpa) (Beristain-Bauza *et al.*, 2016).

Color

The colorimetric features of films were evaluated by using the Hunter lab system (Colorimeter, Minolta CR-400, Japan) and values of L (white– black+), a (red– green+) and b (yellow– blue+) were determined. Color differences of each film with the standard sample (Films made of pure biopolymer) were calculated according to the following formula.

$$\Delta E= [(L-L^*)^2+ (b-b^*)^2 + (a-a^*)^2]^{1/2} \quad (5)$$

Where L, a, b are the color parameters of the films formulated with *L. casei* 39392 and L*, a*, b* are the color parameters of the pure film (without lactic acid bacteria) (Beristain-Bauza et al., 2016).

Opacity measurement

The opacity of films was measured according to Núnez-Flores *et al.* (2012) with slight modifications. Firstly, the film was cut into a rectangular shape (0.7×1.5 cm^2) and placed carefully within the spectrophotometer quartz cell. Then, the absorbance value of the film was measured by using a UV-VIS spectrophotometer device (Cecil model, England) at 600 nm, and the air was used as blank for calibration, so that all films were measured in triplicates. The opacity of various films was calculated according to the following formula.

Opacity= A/X (6)

Where: A is absorption at 600λ and X is film thickness.

Mechanical properties

Mechanical tests included tensile strength (TS), percentage of elongation at break (E%), and elastic modulus (EM) which were evaluated by using a texture analyzer (Testometric, M350-10CT, England). Before the test, moisture of film samples was adjusted to 75% relative humidity at 5°C. Then, they were cut into a rectangular shape with the dimensions of 25.4 × 100 mm according to the Standard ASTM D-882 (ASTM, 2001). Grip separation was set at 50 mm and the cross-head speed was 50 mm per minute (Vargas *et al.* 2011).

Morphological characterization using scanning electron microscopy

First, the samples in the liquid nitrogen were broken and, then, they were mounted onto aluminum stubs via tapes that were double-sided. After coating the films with a thin layer of gold, cross-section images were obtained by an electron microscope (EM3200, KYKY, USA) with an acceleration voltage of 26 kV.

Statistical analyses

Results were analyzed by SAS software version 9.1 using the factorial experiment in a completely randomized design (CRD) with three replications and the comparisons of mean values of the physical, mechanical and biological data were performed with the Duncan and Tukey's tests, respectively. All tests were performed in triplicates.

Results and discussion

Moisture, water solubility and WVP

Moisture, water solubility and WVP of the films are three of the most important factors in edible films that determine the duration of shelf life and the quality of foods. Moisture, water solubility and WVP of the films are shown in Fig. 1 (a, b and c). The NaCas film (10.83±0.81%) had more moisture content in comparison with the MC film (9.67± 0.60%), which indicates that the NaCas film exhibits more hydrophilicity compared to the MC ($p<0.05$).

There was no significant difference in the moisture content (10.83±0.8- 10.90±0.82%) and water solubility (85.17±0.4- 85.62±0.5) when comparing NaCas films and bio-NaCas ($p<0.05$). But the bio-MC film showed the highest moisture content (12.83±0.40%) and the highest water solubility (94.07 ±0.7%) ($p<0.05$).

Under lactose starvation, *L. casei* can change its metabolic pathways and produce catabolic enzymes that enable *L. casei* to use beta-glucosidase, maltose, trihalose and ribose, and this results in structural changes to the polysaccharide polymers; however, the metabolism of proteins and amino acids remained largely unchanged or slightly suppressed during starvation (Naseri *et al.*, 2013).

Piermaria *et al.* (2015) reported that the presence of *Lactobacillus plantarum* and *Saccharomyces marxianus* did not change the moisture content of the Kefiran films. This observation could be due to the difference in the chemical structure of the films and the type of embedded microorganisms. Beristain-Bauza *et al.* (2016) stated that the addition of microbial metabolites to biofilms would increase solubility. There are differences in the ionization of hydroxyl and carboxyl groups and the decomposition of hydrogen and ionic bands which is probably due to the high polarity of film composition or water penetration (Soradech *et al*., 2012). The results showed that the WVP of NaCas and MC films were significantly different which can be related to the hydrophilicity of the NaCas polymer ($P<0.05$). Bio-films (16.85- 19.73 g× mm KPa^{-1} h^{-1} m^{-2}) produced significantly higher WVP compared to pure films (14.09- 17.33 g× mm KPa^{-1} h^{-1} m^{-2}) with similar biopolymers. This parameter is influenced by some factors such as thickness, biopolymer structure, intermolecular reactions, type of softener used and conditions of storage (including temperature and relative humidity) (Bertuzzi *et al*., 2007). The images of bio-films obtained by the SEM (2B and 2D)

show that the presence of *L. casei* 39392 causes discontinuities in the matrices of films, thereby increasing the movability of polymer chains in the transfer of water molecules (Sanchez-Gonzalez *et al*., 2013). This result is not in line with those reported by Gialamas *et al*. (2010), and the previous researcher did not observe significant differences in terms of the barrier properties of the sodium caseinate films when the bioactive medium was added. Kurek *et al*. (2014) stated that the high moisture content of films could cause an increase in WVP. This is probably due to the fact that water molecules react with polar groups of the polymer chains, causing the film's WVP to increase (Slavutsky & Bertuzzi, 2012).

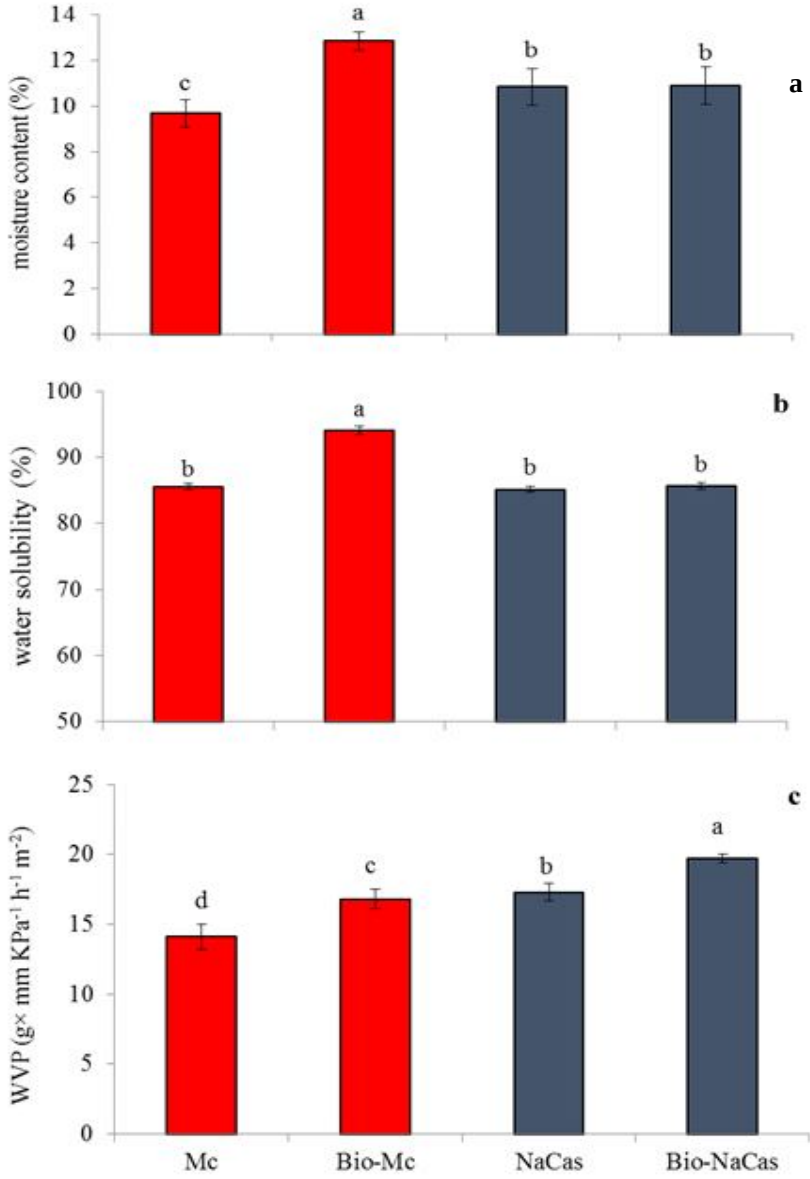

Fig 1. Changes in moisture (a), water solubilty (b) and water vapor permability (WVP, c) of films.
The values are the means± SD of triplicate expriments. [a-d] Difference letters in each column indicate significant differences ($p<0.05$). MC, Methylcellulose; Bio-MC, MC film containing *Lactobacillus casei* ATCC 39392; NaCas, Sodium caseinate; Bio-NaCas, NaCas film containing *Lactobacillus casei* ATCC 39392.

Optical properties

Table 1 shows the mean values of the color and opacity parameters of the films. The results showed that the highest amount of *L* was observed in the Bio-MC. Incorporating *L. casei* 39392 into the film matrix increased the clarity (*L*) of the film. The highest *a* value was observed in the MC film. There was no significant difference among the amount of *a* parameter in the NaCas, Bio-NaCas and Bio-MC films (P>0.05).

Table 1- Changes in optical properties of films

Film	Optical properties				
	L	*a*	*b*	*ΔE*	opacity (A650/X)
NaCas	88.85±0.05^{d}	1.72±0.03 ^{b}	-2.32±0.10 ^{ab}	----	2.50±0.11^{a}
Bio-NaCas	89.05±0.01^{c}	1.74±0.06^{b}	-2.16±0.10^{a}	0.30±0.10^{b}	1.11±0.10 ^{b}
Mc	89.28±0.13^{b}	1.82±0.03 ^{a}	-2.64 ±0.10^{bc}	----	1.21±0.09^{b}
Bio-MC	89.54±0.10 ^{a}	1.69±0.01^{b}	-2.93±0.30^{c}	0.60±0.15^{a}	0.50±0.02^{c}

The values are the means ± SD of triplicate expriments. $^{a-d}$Difference letters in each column indicate significant differences (p<0.05). NaCas, Sodium caseinate; MC, Methylcellulose; Bio-MC, MC film containing *Lactobacillus casei* ATCC 39392; Bio-NaCas, NaCas film containing *Lactobacillus casei* ATCC 39392

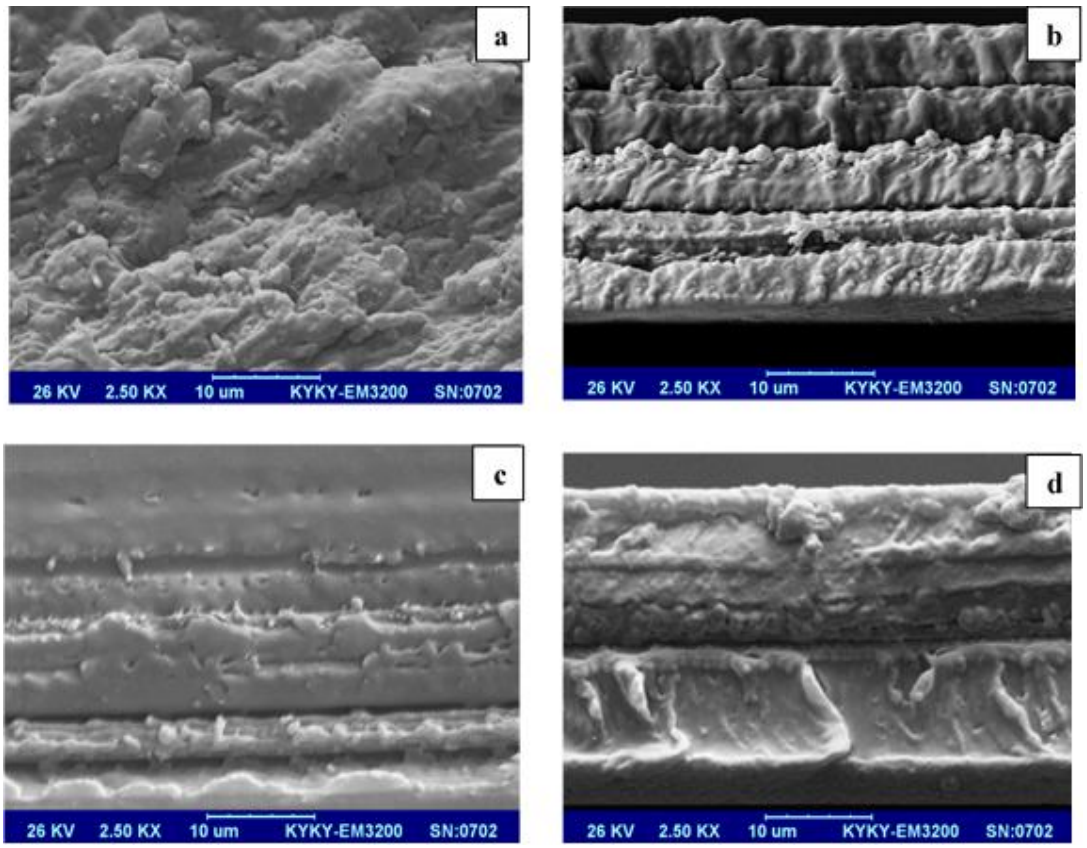

Fig. 2. Scaning electron microscop micrographs of the cross-section of films. a: Methylcellulose; b: Methylcellulose + *Lactobacillus casei* ATCC 39392; c: Sodium caseinate; d: Sodium caseinate *Lactobacillus casei* ATCC 39392

Furthermore, the results showed that there was no significant difference between the *b* values of NaCas and Bio-NaCas films, and also no significant difference between the MC and Bio-MC in that respect, which indicates that *L. casei* 39392 had no significant effect on the parameter *b* (blue-yellow) ($P>0.05$). The results also showed that the ΔE value for Bio-MC was significantly higher than the value for Bio-NaCas. Given that the calculated ΔE is less than 2 units, the resultant color change would not be visible. Beristain-Bauza *et al.* (2016) stated that applying 18 mg/ ml of a cell-free supernatant of *Lactobacillus rhamnosus* in a NaCas matrix and a whey protein isolate can decrease the parameter *L* and increase the parameter *b* and color variation (ΔE), in both matrices. The different results might be due to the difference in the biopolymer and the additive to the film matrix. As Table 2 shows, the MC

Film was more opaque than NaCas ($P<0.05$) which can be due to the more uniform structure of the NaCas film (fig.2c) compared to the MC (fig.2a). The highest value of opacity was observed in the NaCas. Matsakidou *et al.* (2013) stated that the increase in the moisture content of the film could reduce the opacity, while our results indicated that the moisture content of the NaCas and Bio-NaCas films did not differ significantly. However, there was a significant difference in opacity among the films ($P<0.05$). The results showed that the biofilms are more transparent than the pure films with the same matrix, which indicates the significant effect of *L. casei* 39392 on film opacity. Kanmani and Lim (2013) reported that embedding *L. plantarum*, *L. reuteri* and *L. acidophilus* in the matrix of pullulan/starch, tapioca and potato starch could reduce opacity. However, those results do not match the results obtained in this study due to the variation in chemical structures of the films as well as in the microorganisms embedded.

Mechanical properties

The mechanical properties of films were investigated with parameters of TS (MPa), EM (MPa) and E (%). Fig.1 shows that the TS (Fig. 1a), EM (Fig. 1b), and E values (Fig. 1c), in the MC film are higher than in the NaCas film ($p<0.05$). This is probably due to the difference in the chemical composition and the homogeneous structure of the MC film (Fig. 2a) ($p<0.05$). The results show that the mechanical properties of bio-NaCas have no significant difference compared to pure NaCas films, which indicates that *L. casei* 39392 had no effect on the mechanical properties of NaCas-based films ($p>0.05$). Adding *L. casei* 39392 to the FFS of MC served to reduce the TS and EM, but increased the E values compared to pure MC films ($p<0.05$). It has been reported that *Lactobacillus casei* and *Bifidobacterium animalis* Bb-12 were embedded in whey protein isolate matrices which resulted in the reduction of TS but did not change the EM and E% values of the film (Pereira *et al*., 2016). The mechanical properties of films are belived to be influenced by parameters such as the moisture content and the interconnected film matrix. Moisture content acts as a plasticizer, thereby reducing the TS and EM but increasing the E% values (Pittia & Sacchetti, 2008; Aguirre-Loredo *et al.*, 2016). This is consistent with the results obtained in this study.

Survival rate of *L. casei* 39392 in the biofilm

The survival rate (SR) of *L. casei* 39392 in Bio-MC and Bio-NaCas is shown in Fig. 4 for 30 days of storage (5°C and relative humidity of 75%). The results showed that during the storage of *L. casei* 39392, the SR decreased significantly and the lowest SR in Bio-MC ($71.4 \pm 0.6\%$) was observed on day 30 of storage. Romano *et al.* (2014) reported that the SR value of LAB decreased with time in MC films. It was also observed that the viability of *Bifidobacterium animalis* and *L. casei* in whey protein-based films decreased by 1 and 2 logarithmic cycles during storage at 4°C. (Odila Pereira *et al*., 2016). Furthermore, it has been reported that embedding LAB, as a softening compound, in the film accelerates enzymatic and chemical reactions such as the oxidation of the cytoplasmic membrane lipids, and reduces the survival rate (Soukoulis *et al*., 2016).

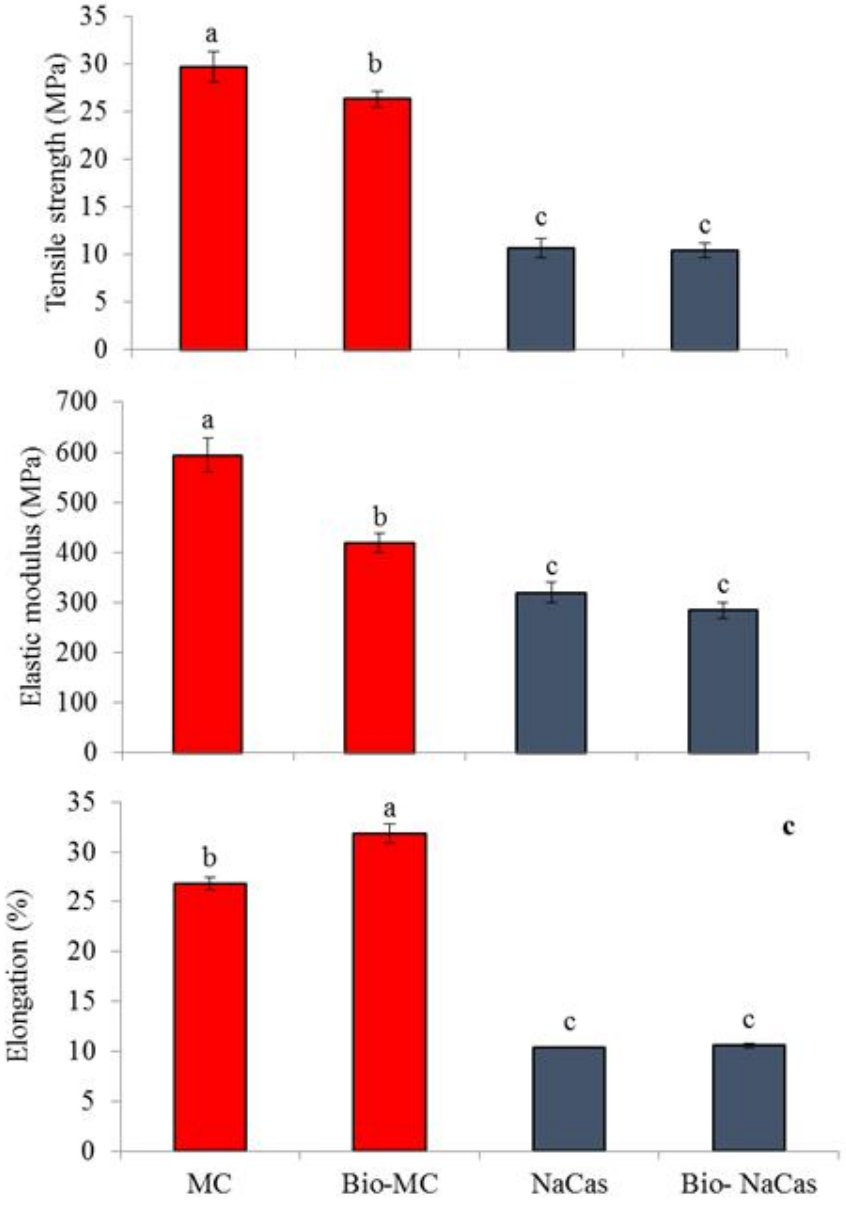

Fig. 3. Changes in tensile strength (a), elastic modulus (b) and elongation at break (c), of films.
The values are the means ± SD of triplicate expriments. [a-c] Difference letters in each column indicate significant differences ($p<0.05$). MC, Methylcellulose; Bio-MC, MC film containing *Lactobacillus casei* ATCC 39392; NaCas, Sodium caseinate; Bio-NaCas, NaCas film containing *Lactobacillus casei* ATCC 39392 .

The Bio-NaCas in this research appeared to have the highest SR compared to the Bio- MC, and this can be confirmed by studying the SR of *L. casei* 39392 embedded in the biofilm matrix throughout the 30 days of storage ($p<0.05$). Previous studies have shown that *Lactobacillus paracasei* and *Lactococcus lactis* have higher survival rates in the alginate-NaCas matrix than in alginate (Leonard *et al*., 2015) which can be due to a higher buffering capacity,

better bacterial placement, nutrient supply (peptide and amino acid) and better control over free radicals by caseinates (Leonard *et al*., 2014). Fu and Chen (2011) stated that the viability rate of LAB depends on the strain of microorganisms, the type of matrix, storage temperature, oxidative stress and film structure.

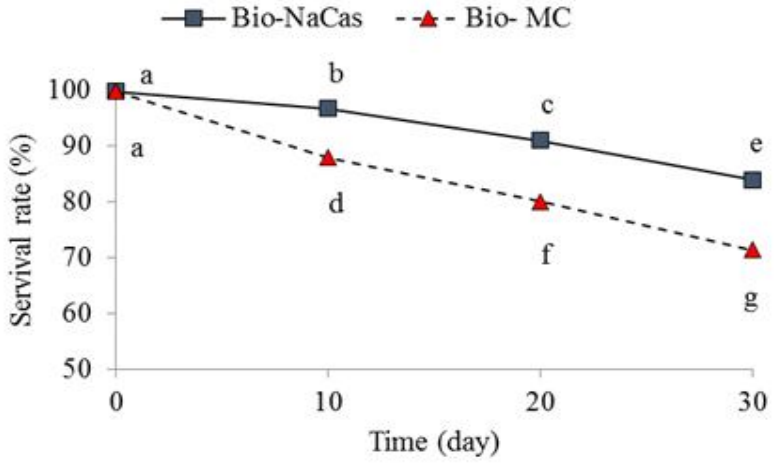

Fig. 4. Survival rate *Lactobacillus casei* ATCC 39392 embedded in films, on the tryptone soya agar medium contaminated with *Pseudomonas aeruginosa* PTCC 10832.

The values are the means of triplicate expriments. [a-g] Difference letters indicate significant differences among films with different formulation at the storage time ($p < 0.05$). Methylcellulose film containing *Lactobacillus casei* ATCC 39392; Bio-NaCas, Sodium caseinate film containing *Lactobacillus casei* ATCC 39392 .

Anti-pathogenic effect of films

The results varied among the Bio-MC and Bio-NaCas films regarding their effects on the growth inhibition of *P. aeruginosa* 10832 (at 5℃ for 12 days of storage) (Fig. 5).

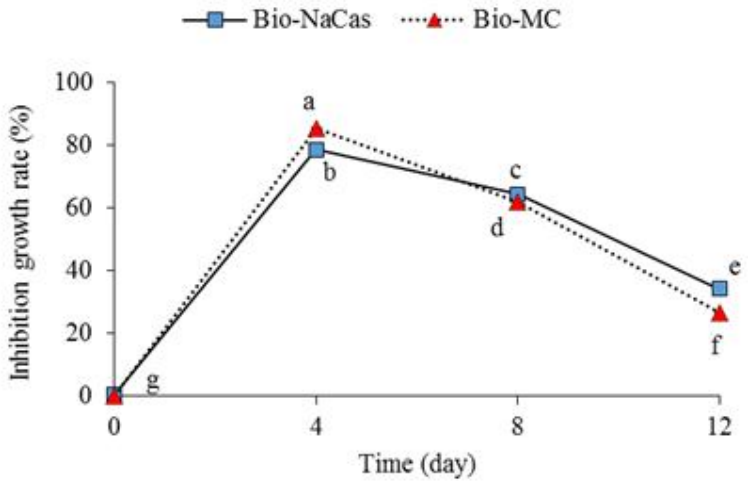

Fig 5. Effects of biofilms containing *Lactobacillus.casei* ATCC 39392 on growth inhibitory and *Pseudomonas aeruginosa* PTCC10832 in the tryptone soya agar medium.

[a-g] Difference letters indicate significant differences among films with different formulation at the storage time (p 0.05).

The results showed that Bio-MC and Bio-NaCas had inhibitory effects on the growth of *P. aeruginosa* 10832. A similar inhibitory effect was observed in the whey protein isolate

and in the calcium caseinate films which contained a cell-free supernatant of *Lactobacillus rhamnosus* (Beristain-Bauza *et al*., 2016).

Bio-MC showed the highest growth inhibition rate against *P. aeruginosa* 10832 (85.3%) on the fourth day. It has been reported that the presence of polysaccharides in an environment can have impacts on the metabolism of the LAB and can increase the production of antimicrobial compounds such as bacteriocin, organic acids and hydrogen peroxide (Galvez *et al*., 2007).

Pure NaCas and MC films showed no inhibitory effects on the pathogens' growth throughout the storage period. Furthermore, the high solubility of the Bio-MC can accelerate the diffusion of antimicrobial compounds. On the eighth and twelfth day of storage, the Bio-NaCas showed a stronger inhibitory effect on the growth of P. aeruginosa 10832, whereas the Bio-MC had a weaker inhibitory effect ($p<0.05$).

Furthermore, with respect to the higher survival rate of *L. casei* 39392 in Bio-NaCas, the inhibitory effect on pathogenic growth was more sustainable in Bio-NaCas up to the end of storage period.

Conclusion

The results showed that the chemical structure of the film affects the viability of the lactic acid bacteria, the intensity of antimicrobial effects, and the changes in the physical and mechanical properties of the film. *L. casei 39392* did not change the mechanical properties of the NaCas film but improved its elongation and reduced the elastic modeulus and Tensile strenght of the MC film by changing the structural of a backbone polymer. It also increased the opacity (41-44%) and the *L* parameter of the films (%0.22-0.26). *L. casei* 39392 was able to survive more when embedded in the NaCas matrix, compared to MC matrix. Ultimately, it can be claimed that the bio-MC film had a stronger anti-pseudomonas effect for up to 6 days of chilled storage. The results of this study demonstrate the anti-pathogenic properties of these bio-films *in vitro*. Our findings engender a new approach to the natural preservation, and may be able to increase the shelf life of some food products such as meat and cheese.

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جلد 15، شماره 3، مرداد - شهریور 1398، ص. 27-13

تهیه پوشش زیستی ضدسودوموناس با قرار دادن *لاکتوباسیلوس کازیی* ATCC 39392 در ماتریکس‌های سدیم کازئینات و متیل سلولز

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تاریخ دریافت: 1396/10/03
تاریخ پذیرش: 1397/08/28

چکیده

لفاف‌های زیست تخریب‌پذیر حاوی باکتری‌های اسید لاکتیک از روش‌های جدید نگهداری مواد غذایی است. در این مطالعه باکتری *Lactobacillus casei* ATCC 39392 (*L. casei* 39392) مستقیما به محلول سازنده لفاف‌های کازئینات سدیم و متیل سلولز به‌صورت جداگانه افزوده گردید، به‌طوریکه لفاف زیستی تهیه شده حاوی 10^6CFU/cm^{2}بود. خصوصیات مقدار رطوبت، حلالیت در آب، قابلیت نفوذ به بخار آب، رنگ، شفافیت، مقاومت در برابر کشش‌پذیری، ازدیاد طول فیلم تا نقطه پاره شدن و مدول الاستیک فیلم‌ها بررسی گردید. همچنین نرخ زنده‌مانی *L. casei* 39392 در طی 30 روز نگهداری (دمای 5 درجه سانتی‌گراد، رطوبت نسبی 75%) و اثر مهارکنندگی لفاف‌ها بر رشد باکتری *Pseudomonas aeruginosa* PTCC 10832 (*P. aeruginosa* 10832) طی 12 روز در 5 درجه سانتی‌گراد بررسی گردید. نتایج نشان داد افزودن *L. casei* 39392 به لفاف‌های تهیه شده شفافیت و نفوذپذیری به بخار آب بالاتری را در مقایسه با لفاف خالص نشان داد ($p<0/05$)، نرخ زنده‌مانی *L.casei* 39392 در لفاف سدیم کازئینات نسبت به لفاف متیل سلولز بالاتر بود ($p<0/05$)، بیشترین نرخ بازدارندگی رشد در برابر *P. aeruginosa* 10832 (85/3%) توسط لفاف زیستی متیل سلولز در روز چهارم نگهداری مشاهده گردید ($p<0/05$). نتایج ما نشان داد لفاف‌های زیستی حاوی *L.casei* 39392، می‌تواند تهیه لفاف‌های حاوی نگهدارنده‌های طبیعی را توسعه دهد.

واژه‌های کلیدی: آنتی-سودوموناس، فیلم خوراکی، *Lactobacillus casei*، متیل سلولز، سدیم کازئینات

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Effect of pH, ionic strength, temperature and sugar concentration on orange peel essential oil/ T60: propanol and water microemulsion zone using Response Surface Methodology

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Abstract

In this study, microemulsification of orange peel oil (OPO) using Tween 60:propanol with the ratio of 1:1 was studied under different conditions of pH, ionic strength, and sugar concentration.. Results showed that critical temperature (the temperature in which one- phase microemulsion system was still stable) for the microemulsions with higher sucrose concentrations (in the range between 0 to 30%) was lower while by decreasing in sugar concentration, critical temperature shifted to higher temperatures, as it reached to 90°C for the samples without sugar. The prepared microemulsions were stable at 5 and 25°C for seven days, but samples with higher concentrations of sugar (25 and 30%) became turbid at 45°C, whereas all other samples exhibited a one-phase microemulsion system at this temperature. Microemulsions were not stable at -3°C (freezing temperatures). In sensory evaluation, it was observed that the microemulsified OPO was dissolved in water as soon as it was added into the medium, in contrast to free essential oil as it was spreading on the surface of the solution. Encapsulation of OPO caused lower release of aroma, resulting a milder odor and taste (lower intensity) in samples which were preferred by the panelists. The overall acceptability of all samples containing OPO microemulsion was significantly higher than samples with free essential oil.

Keywords: Microemulsion, Orange peel oil, Response surface methodology, Modeling

Introduction

Orange peel essential oil is a by-product of sweet orange that is extracted mostly by cold press from the peel of the fruit (Ashurst, 1999). As the most compounds of orange peel oil are more lipophillic, therefore they have normally lower solubility in aqueous and sugar-containing aqueous beverages due to lower hydrogen bonding. Orange peel oil is a popular flavoring agent in food, pharmaceutical or cosmetic formulations due to its specific aroma and low cost. In addition, it has other beneficial effects such as anti-inflammatory, anti-depressant, anti- spasmodic, anti-septic, aphrodisiac, carminative, diuretic, tonic, sedative and cholagogue effects (Duke *et al.*, 2002). Apart from these advantages, its low solubility in water and aqueous media, high volatility and degradation during processing and storage, which leads to changes in the sensory properties of the product, are some of the major concerns which has limited its applications. In order to overcome the abovementioned limitations, over the past few decades many efforts have been done (Ponce Cevallos *et al.*, 2010; Haroldo *et al.*, 2010), to entrap the essential oil within a protective layer of coating material known as "encapsulation" by different techniques. Emulsification is a process which can be considered as a technique of encapsulation; because, an emulsion normally consists of two immiscible liquids (usually oil and water), and that one liquid is dispersed as small spherical droplets in the other one (McClements, 1999). In this regard, microemulsions have several advantages over macroemulsions, such as enhanced solubility, better thermodynamic stability, ease of manufacturing, stability against oxidation and controlled release (Zhong *et al.*, 2009). In the last years microemulsions has attracted interests for being used in cosmetic, pharmaceutical and food industries to encapsulate and deliver compounds or allow solubility of high polar molecules (Polizelli *et al.*, 2006). Where stability and homogeneity of the finished product is desired, the stability and small droplet size of

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microemulsions can be helpful (Bidyut and Satya, 2001). Microemulsions are homogeneous, clear, and thermodynamically stable solutions, which consist of different ratios of oil, surfactant, co-surfactant and water (McClements, 1999; Radomska *et al.*, 2000; Feng *et al.*, 2009). They usually have spherical droplets with a radius less than 50 nm (Feng *et al.*, 2009). Microemulsions can be used for coating of volatile compounds such as essential oils to increase their stability during storage or processing (Zhong *et al.*, 2009). In terms of food systems, their applications are restricted mostly due to several factors such as the surfactant types which are allowed as additive in foods (Flanagan, 2006) as well as the high amount of surfactant needed for formation of microemulsions (Zhong *et al.*, 2009). An important factor which limits the application of microemulsions in food industry is its sensitivity to formulation. In other words, after formulation of a stable microemulsion and its addition into a food medium, the microemulsion might be broken due to the high or low pH, sugar or salt concentration and temperature. Few studies have been performed concerning the stability of microemulsions in media with different concentrations of electrolyte, sugar as well as pH. In this regard, Abbasi & Radi (2016) showed that the presence of salts (NaCl and $CaCl_2$) slightly increased the W/O areas of canola oil/ lecithin:*n*-propanol/ water microemulsions, whereas pH variation was not effective on the microemulsion formation. Ahmad *et al.* (2008) studied the effect of electrolytes on the ζ potential of vegetable oil microemulsions stabilized by a non-ionic surfactant where its value was negative for some tested electrolytes (Na^+, Ca^{2+} and Mg^{2+}). Hsu and Nacu (2003) also showed that ζ potential was dependent on pH and the higher concentrations of Na^+ and K^+ was able to separate the oil phase from soybean oil-in-water emulsion. Furthermore, Rajib and Bidyut (2005) showed that addition of NaCl (up to 0.2 mol dm^{-3}) increased the microemulsion area while its further addition (0.5 mol dm^{-3}) decreased the microemulsion formation area in Brij-35 and AOT [Sodium bis-2 (ethylhexyl) sulfosuccinate]/ eucalyptus oil/ butanol microemulsion. Similar observations were also recorded for glucose addition in the same system. Radi and Abbasi (2018) showed that the single phase microemulsion of canola oil/ lecithin:propanol/ water areas were increased by increasing the temperature. A good freeze-thaw, pasteurization and short UV exposure stability was recognized for lycopene microemulsion (Amiri-Rigi & Abbasi, 2017).

As it was mentioned, to the best of our knowledge, the effects of ionic strength, pH, temperature and sucrose concentration on the microemulsion area of edible flavors had not been studied yet. Therefore, the main objectives of this study were to evaluate the influence of these variables on the formation of orange peel oil microemulsion stabilized with Tween 60/propanol using response surface methodology (RSM).

Materials and methods

Orange peel oil (OPO) (100%, w/w) was obtained from Giah Esanse Company (Gorgan, Iran). Propanol, $CaCl_2$, sucrose, NaOH, HCl, and Tween 60 (T60) [polyoxyethylene (20) sorbitan monostearate] purchased from Merck Chemical Co. (Darmstadt, Germany). All other chemicals were analytical grade and commercially available. Distilled water was used for microemulsion formation.

Preparation of microemulsion using RSM

The phase diagrams were constructed to evaluate the effect of pH, ionic strength and sugar concentration on the microemulsion zones of T60: Propanol (1:1). Response surface methodology (RSM) was used to design experiments as well as to model the above mentioned variables. Each independent variable was coded at three levels between −1 and +1. Therefore, the pH over the range 3 (-1) to 7 (+1), ionic strength 0 (-1) to 70 (+1), and sugar concentration 0 (-1) to 60 (+1), were given to the Design Expert 7.0 software (Stat-Ease Inc., Minneapolis, USA) to model different combinations of three variables (Table 1). Twenty experiments were planned and their responses [microemulsion area (%)] were measured. The statistical software package (Design- Expert, Stat-Ease Inc., Minneapolis, USA) was also used to plot response surface. Therefore, pH, ionic strength and sugar concentration of water phase was adjusted according to Table 1. The pH of formulating water was adjusted using HCl and NaOH 0.5 M. Sucrose, as the most applicable sugar in food systems, and calcium chloride were used for tuning the sugar concentration and ionic strength, respectively. It is noteworthy that the ionic strength, *I*, of a solution is a function of the concentration of all ions present in that solution (Skoog *et al.*, 2004).

$$I = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2 \quad (1)$$

Where c_i is the molar concentration of ion (mol dm^{-3}), z_i is the valence of that ion, and the sum is taken over all ions in the solution.

Afterwards, the microemulsions were prepared using formulated water, T60: propanol (1:1) and OPO. For OPO/ formulated water/ T60:propanol (1:1) microemulsion preparation, mixtures of formulated water (or OPO) with T60:propanol were made with the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1, respectively. The prepared mixtures were diluted by adding aliquots (10 µl) of OPO (or formulated water) stepwise, until the transparency disappeared. Then, the microemulsions were kept for 24 h at room temperature to ensure equilibrium (Flanagan *et al.*, 2006; Zhong *et al.*, 2009). Therefore, the microemulsion regions were determined based on the visual transparency of the mixtures. In the next step, the phase diagram was constructed to find the points that represent transparent, one-phase systems (microemulsions).

The particle size distribution of a microemulsion (containing T60: propanol (1:1) 28, water 71 and OPO 1% wt.) was also measured using dynamic light scattering technique (Zetasizer, Nano ZS, 4mW He–Ne laser, Malvern Instrument Ltd., UK) at ambient temperature (wavelength of 633 nm, detection angles 70 and 90◦, dynamic viscosity of sample 8.76 mPa.s).

Validation experiment

The mathematical model created by RSM implementation was validated by conducting some experiments on some selected conditions.

The effect of temperature and sugar concentration on orange peel oil/ T60: propanol and water microemulsion zone

For evaluating the combined effect of sugar concentration and temperature, different levels of sugar concentration (0, 5, 10, 15, 20, 25 and 30% w/w) were prepared and their microemulsions were constructed with OPO/T60: propanol under 5, 25 and 45 °C. For this purpose, microemulsions were prepared using water formulated with different concentrations of sugar, OPO and T60: propanol (1:1), then their phase diagrams were constructed. Microemulsifications were operated at constant temperatures of 5, 25 and 45°C.

Heat stability measurement

For evaluating the heat stability of microemulsions prepared at different sugar concentration, a point from the stable systems [containing T60: propanol (1:1) 34, sugar containing water 65 and OPO 1% w/w] was selected using their phase diagrams and the heat stability of the prepared microemulsions were evaluated. The temperature, in which the transparency of emulsions changed to turbid, was considered as the point of instability.

In another experiment, the same microemulsion systems were kept at 5, 25 and 45°C for two months and their stability (visual appearance, phase separation) was assessed.

Sensory analysis

A non- flavored carbonated water was purchased from a local supermarket, in which OPO (free and in the microemulsion form) was added to give a final concentration of 10 ppm and the sensory properties of samples were evaluated. Samples were evaluated for color, viscosity, taste and odor by 12 experienced panelists selected from members and students of Azad University of Yasooj. Assessors evaluated the intensity of odor and taste in each sample and rated their liking score (0 to 10) for odor intensity, taste intensity (0 to 10) and overall acceptability of each sample.

Results and Discussion

The influence of different parameters on the microemulsion area

The phase diagrams of OPO microemulsions are shown in Fig.1a. The transparent, one phase regions were considered as the microemulsion regions (38.66%) whereas the OPO, T60, propanol and water were soluble (Liu *et al.*, 1998; Zhong *et al.*, 2009). The boundaries between three regions of O/W, bicontinuous and W/O for OPO, water and T60 with propanol microemulsion area were determined (Fig. 2), measuring the electrical conductivity and the viscosity of the system along with three dilution lines of 10:90, 20:80 and 30:70 OPO/ surfactant: cosurfactant (data not shown) (Kim *et al.*, 2009). As it can be seen, the W/ O region of T60 in combination with propanol was significantly wider than those of others. Moreover, the O/ W region in T60 was almost evident. Therefore, it can be concluded that T60 was likely efficient in terms of OPO entrapment.

According to the Dynamic light scattering (DLS) results (Fig. 3), the mean particle size of over 90% of particles was 2.495 nm and only about 10% had greater size (18.44 nm) for a sample from the O/W region of T60:propanol microemulsion. The polydispersity index was 0.394, indicating the homogenicity of the microemulsion. The

polydispersity index is the indicator of particle homogenicity and varies from 0 to 1. When the value is closer to zero, the system is more homogenous (Amiri *et al.*, 2013). The small sizes of the micelles obtained in this study can be related to high ratio of surfactant to OPO, as demonstrated by Polizelli *et al.* (2009).

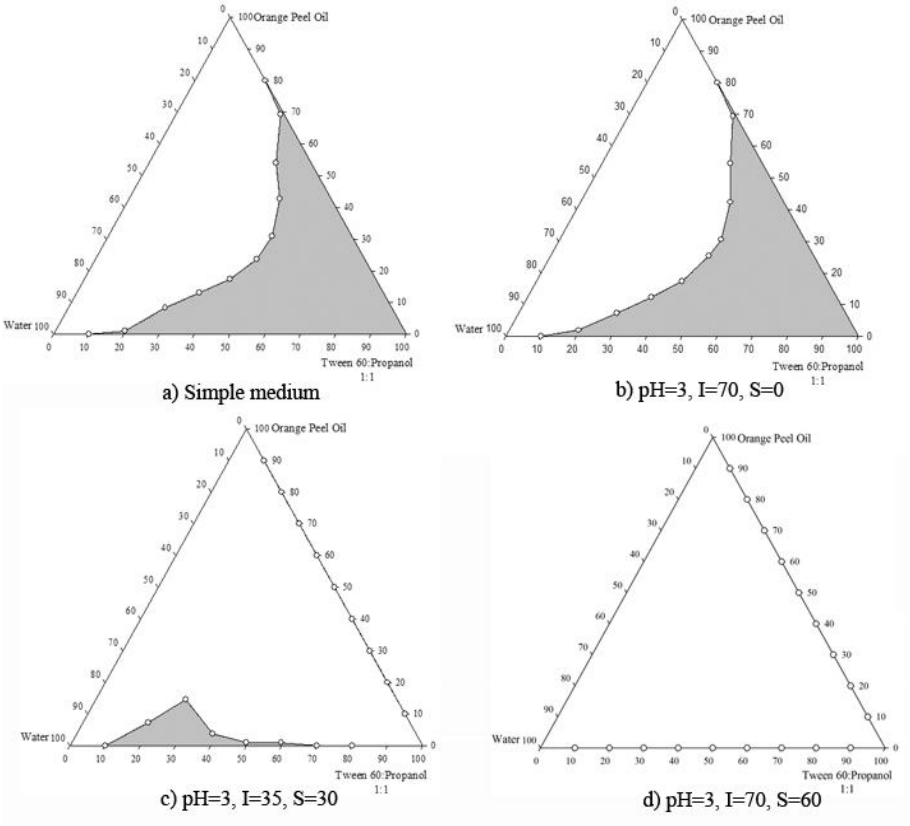

Fig. 1. Phase diagrams of OPO microemulsions with the surfactant:cosurfactant ratio of 1:1 at ambient temperature under different conditions of ionic strength (mM) (I) and sugar concentration (%)(S). The grey zones show the microemulsion areas. All ratios were by weight.

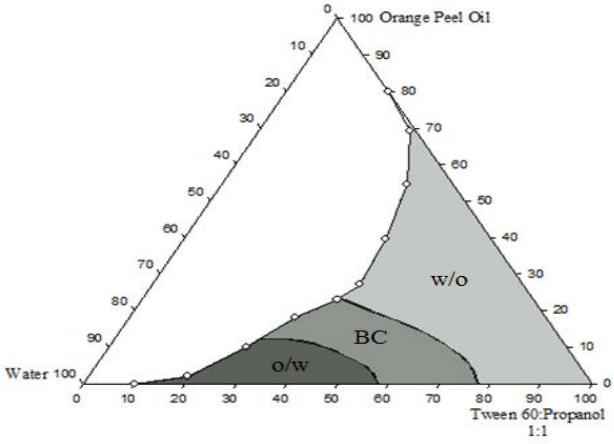

Fig. 2. Phase diagram of OPO microemulsions with the surfactant:cosurfactant ratio of 1:1 at ambient temperature. The grey, dark and light colors show the W/O, bicontinuous and O/W microemulsion areas. All ratios were by weight

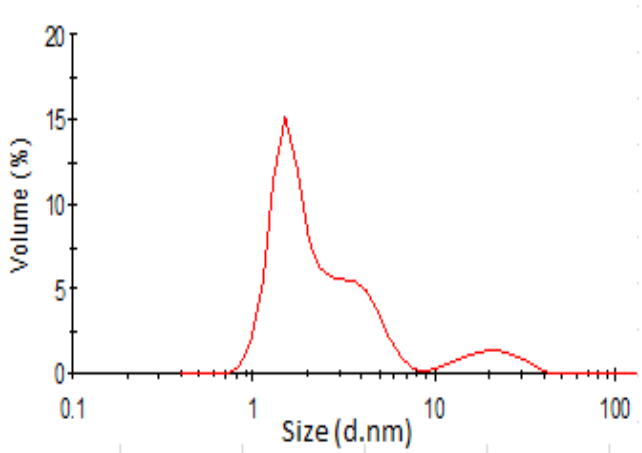

Fig. 3. Size distribution of OPO microemulsion (71 % water/1% OPO/28% surfactan:cosurfactant).

Apart from the above mentioned debate, the response surface methodology was used to evaluate the influence of pH, ionic strength, sugar concentration as well as their interactions on the microemulsion area of OPO/ T60:propanol. Central composite design (CCD) was used to design the experiment. The phase diagrams of OPO microemulsion under different conditions of pH, ionic strength, sugar concentration and their interactions are depicted in Figs.1b, and c (all data not shown) and their microemulsion areas (%) in CCD experimental design were calculated (Table 1). The software predicted a Quadratic Model for three examined factors.

Table 1. Effect of pH, ionic strength and sugar concentration onon mono-phase microemulsion area using central composite design.

Runs	Variables			Response
	pH	Ionic Strength (mM)	Sugar concentration (%)	Microemulsion area (%)
1	5	35	0	37.89
2	3	70	0	39.26
3	3	0	0	38.66
4	5	35	30	5.35
5	5	35	30	4.89
6	5	35	30	5.23
7	5	35	30	5.18
8	5	35	30	5.07
9	5	35	30	5.75
10	5	70	30	5.30
11	5	0	30	5.66
12	7	35	30	5.66
13	7	70	0	38.13
14	7	0	0	38.56
15	3	35	30	5.40
16	3	70	60	0.00
17	3	0	60	0.00
18	5	35	60	0.00
19	7	70	60	0.00
20	7	0	60	0.00

The equation given by the software was:

Microemulsion Area (%) = +40.12 - 1.28E - 003 Ionic Strength - 0.647 pH -1.56 Sugar Conc. - 1.84E-003 Ionic Strength × pH - 2.02E-005 Ionic Strength × Sugar Conc. + 2.56E-003 pH × Sugar Conc. + 1.51E-004 Ionic Strength^2 + 0.06 pH^2 + 0.02 Sugar Conc.^{2}

The statistical significance of the model equation was evaluated by the F-test for analysis of variance (ANOVA). The F-value (5221.30) of obtained model shows the significance of the quadratic model. In this model, sugar concentration and sugar concentration^2 were found as the most significant parameters. In addition, the obtained Lack of Fit F-value of 1.33 implies the Lack of Fit was not significant relative to pure error. Furthermore, Fig. 4 depicts combined effect of pH and ionic strength on microemulsion area (%) at a constant sugar concentration as a response surface plot. The microemulsion area of T60:propanol system was not affected by pH, ionic strength or their interactions. Since these parameters and their interactions were detected to be not significant in the model, therefore their results appeared in a flat form (Fig. 4). Consequently, the three-dimensional response surface curves for microemulsion area (%) had two stationary parts along with two dimensions of pH and ionic strength, which means that microemulsion area was not altered when pH or ionic strength were changed. The Brij-35 stabilized eucalyptus oil/butanol system was also indifferent toward salinity of the system with NaCl, but inorganic salts had strong effects on the behavior of microemulsions which stabilized by ionic surfactants as it was observed for NaCl in Rajib and Bidyut studies (Rajib & Bidyut, 2005). It is reported that the shielding effect of NaCl on polar head group of an O/W microemulsion droplet increases the cohesive interaction among the droplets and subsequently could result in the coagulation of droplets and decreasing the water solubility of AOT and cause the phase separation of the microemulsion (Rajib & Bidyut, 2005). T60, as a powerful steric stabilizer with no effect on electrostatic stabilization due to its non-ionic nature, is a valuable polysorbate emulsifier for preparing emulsions or concentrates that must be diluted by water with unknown hardness (Whitehurst, 2004; Polizelli *et al.*, 2006).

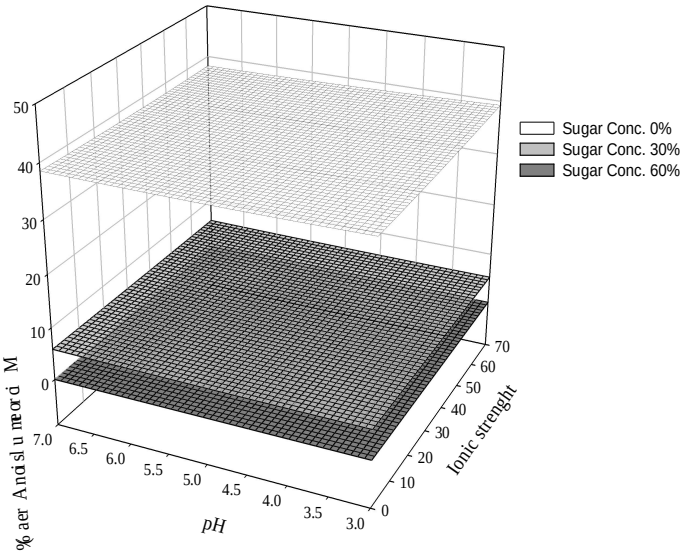

Fig. 4. Response surface for microemulsion area (%) as a function of ionic strength (mM) and pH at constant sugar concentration.

The three-dimensional response surface curve for microemulsion area (%) had a decreasing part in one dimension with a stationary part in another dimension (Fig. 5). The stationary point is the point at which the slope of the response surface is zero when taken in all directions. The response surface decreased as sugar concentration was increased as at sugar concentration of 60%, no

microemulsion zone was observed. Therefore, sugar concentration played an important role in the microemulsion formation and area. The response surface had a stationary region in different pH (Fig. 5) or ionic strenght (not shown) variations, indicating that pH or ionic strength had no effect on microemulsion area.

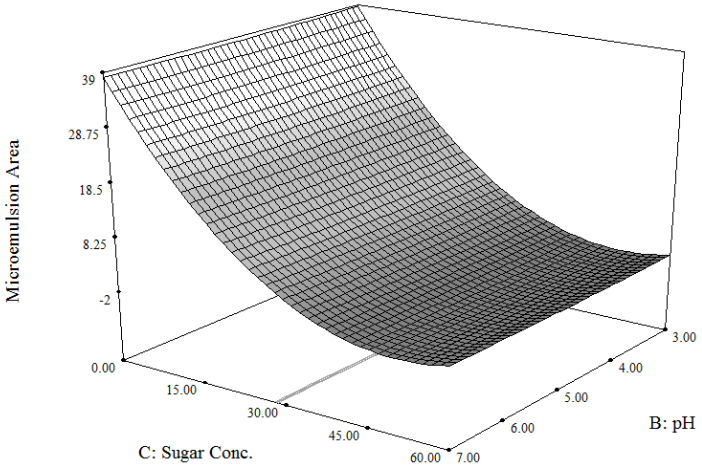

Fig. 5. Response surface for microemulsion area (%) as a function of sugar concentration (%) and pH at constant ionic strength of 35%.

Validation of optimized results

In order to verify the model, several experiments (5%, 10% and 20% sugar concentration) were performed under the predicted conditions. The experimental values did not agree with the results obtained from the model, showing the unsuitability of the model for prediction of microemulsion area (%) due to weak prediction on sugar concentration. As a result, in the next step for strict modeling of sugar concentration effect, the pH and ionic strength parameters were omitted and the sugar concentration was given to Design expert software between two levels of -1 (0) and +1 (40%). Therefore, one factor design was applied by the software. The variety of values chosen by the software for sugar concentration was much more than the previous one (Table 2). Ten experiments were planned and the response, microemulsion area (%), was measured.

Table 2- Effect of sugar concentration on microemulsion area.

Runs	Sugar concentration (%)	Microemulsion area (%)
1	0.00	38.66
2	25.00	8.15
3	30.00	4.38
4	5.00	38.99
5	40.00	0.27
6	0.00	38.13
7	15.00	32.19
8	40.00	0.26
9	20.00	23.53
10	20.00	23.04

Software suggaested a cubic model as folllows:

Microemulsion Area (%) =+37.83 + 1.47 Sugar Conc. - 0.17 Sugar Conc.^{2} + 2.64E-003 Sugar Conc.^{3}

The ANOVA results of cubic model for the one dependent variable (sugar concentration) are presented in Table 3. The F-value (152.62) of model for sugar concentration implied that the cubic model was significant. There is only a 0.01% chance that a "Model F-Value" could occur due to noise. Values of "Prob>F" less than 0.05 indicate that the model terms are significant and hence the cubic model can be used for prediction of microemulsion area when sugar concentration is changing from 0 to 40%. In this case, *A* and A^3 (Table 3) were significant model terms and values greater than 0.1000 indicate that the model terms were not significant. The "Lack of Fit F-value" of 116.33 also implies the significance for sugar concentration. Moreover, there was only a tiny chance (0.13%) that a "Lack of Fit F-value" could occur due to noise. The normal % probability versus studentized residuals graphs for responses of microemulsion area yielded fairly straight line (Fig. 6), showing normal distribution of the data. Hence, the residual plots indicated a normal distribution and there was no apparent problem with normality in all cases.

Table 3- ANOVA analysis for response "mono-phasic microemulsion area" with one factor at a time (sugar concentration).

Source	Sum of squares	DF	Mean square	*F* value	Prob > *F*	
Model	2321.09	3	773.70	152.62	< 0.0001	significant
A^a	544.81	1	544.81	107.47	< 0.0001	
A^2	17.48	1	17.48	3.45	0.1127	
A^3	129.67	1	129.67	25.58	0.0023	
Residual	30.42	6	5.07			
Lack of Fit	30.16	3	10.05	116.33	0.0013	significant
Pure Error	0.26	3	0.086			
Cor Total	2351.51	9				

[a]*A*=Sugar concentration

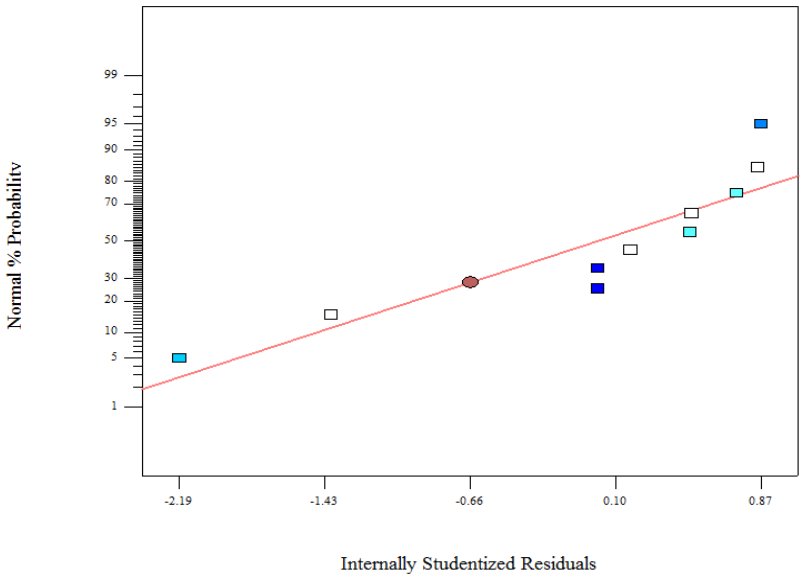

Fig. 6. Normalized % probability versus studentized residuals for microemulsion area (%) with one factor.

Fig. 7 depicts actual microemulsion areas, the measured response data obtained by experiments and the predicted values obtained by cubic models. High correlation coefficient of 0.9865 was indicating good

microemulsion area prediction of model with variation in sugar concentration.

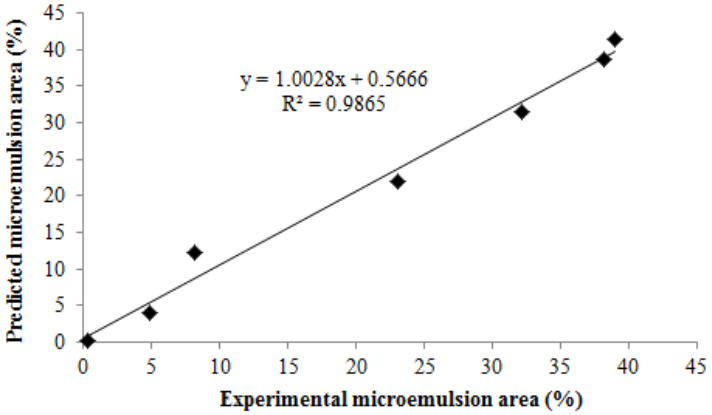

Fig. 7. Correlation between experimental and predicted microemulsion areas in the presence of different sugar concentration.

The model given by RSM for predicting the combined effect of sugar concentration and temperature on the microemulsion area (%) (the following model), was recognized unsuitable, as the experimental data did not confirm the predicted model (data not shown). It was concluded that RSM is not suitable for predicting the effect of factors on the response, where response (Table 4) doesn't have a constant trend (a decreasing or increasing ternd). Consequently, the combined effect of sugar concentrations (0, 5, 10, 15, 20, 25 and 30%) and temperatue (5, 25 and 45°C) on the microemulsion area were evaluated in which its 3D surface plot is shown in Fig. 8.

Microemulsion Area (%) =+44.56599-1.16820× Sugar Conc.-4.65090E-003× Tem

Table 4- Microemulsion areas (%) of OPO microemulsions with the surfactant:cosurfactant ratio of 1:1 under different sugar concentrations (%)(S) and temperatures (C°)(T).

Microemulsion (Sugar conc.)	Microemulsion area (%)		
	5°C	25°C	45°C
0 %	35.76	38.66	39.98
5%	38.05	38.99	44.64
10%	36.18	38.15	38.82
15%	33.87	32.19	23.40
20%	27.71	23.04	11.52
25%	12.18	8.15	1.23
30%	5.11	4.89	0.53

Based on our findings, low concentration of sugar (<5%) had no effect on the microemulsion area, whereas at higher concentrations (>5%) microemulsion area decreased. However, this decrease was very low at 10% sucrose. Regarding the justification of these behaviors it can be said that sucrose most likely affects the hydration capacity of the surfactant head group as high sugar concentrations attract the water and takes water away from the access of surfactant. That is why with increasing sucrose concentration, the W/O type microemulsion was impeded till completely disappeared (at 25% sucrose). Indeed, the water of high sugar aqueous solutions is highly bonded with sucrose molecules. Such phenomenon is true for both O/W and W/O microemulsion particularly for W/O microemulsion due to its lower amount of water which results in sugar precipitation. Similar results were obtained in Brij-35 stabilized eucalyptus oil/butanol

system in the presence of glucose (Rajib & Bidyut, 2005).

Evaluating the combined effect of temperature and sugar concentration showed that both parameters are effective on the microemulsion area (Fig. 9). With no sugar addition or at low concentrations of sugar (up to 5%), the microemulsion area was increased slightly with increasing temperature from 5 to 45°C. This was due to decrease in the surface tension of water with temperature (Shahin & Servet, 2006), which results in wider microemulsion zones.

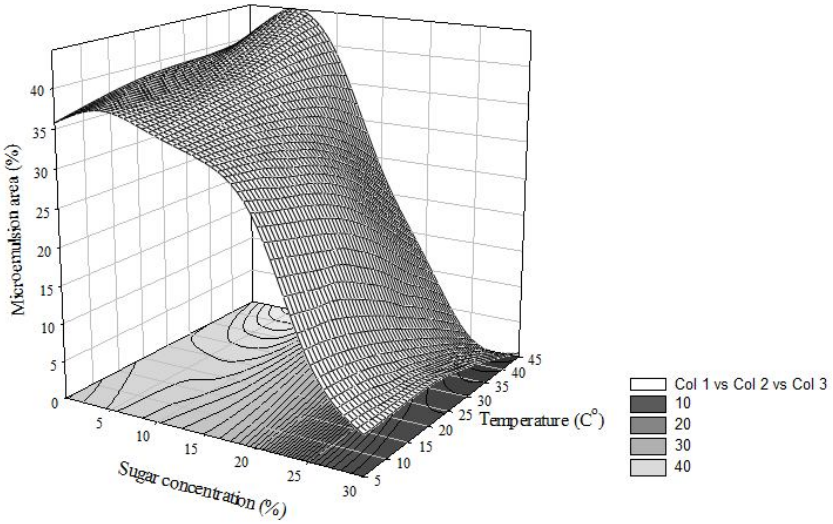

Fig. 8. Response surface for microemulsion area (%) as a function of temperature (C°) and sugar concentration (%).

At 10% sugar concentration, less microemulsion zone was constructed at 5°C due to higher surface tension of sucrose solutions (Shahin & Servet, 2006), but a slight increase was observed in the microemulsion area when temperature was increased from 25 to 45°C (Fig. 8). It seems that in 10% sugar concentration, the concentration is not such high for immobilizing the water, but can form hydrogen bonds with polyoxyethylene chains of T60, and hence increased the hydrophilicity of the surfactant (Rajib & Bidyut, 2005). Increasing in the hydrophilicity of the surfactant results in W/O microemulsion region reduction, as simultaneously increases the O/W microemulsion zone. Therefore, increasing the extent of a region in the microemulsion area and decreasing another part of the microemulsion area, causes no significant increase in the microemulsion area from 25 to 45°C.

At higher sugar concentrations (>10%), a significant decrease in the microemulsion area was observed with increasing temperature. As it was described before, at higher sugar concentrations, the amount of water in which forms hydrogen bonds with polyoxyethylene chain of T60, decreases as it was bonded with sugar molecules. The amount of free water which is available for microemulsion formation diminished more with increasing temperature due to breakage of hydrogen bonds between the water molecules and the hydrophilic head of the surfactant molecules. This results in decreasing the microemulsion area. Such reduction is more pronounced in W/O microemulsion regions as the low amount of their water becomes inaccessible. Consequently, the O/W region is less affected than W/O or bicontinuous zones. In such systems (with high sugar concentrations), precipitation of sugar was observed when breakage of microemulsion occurred.

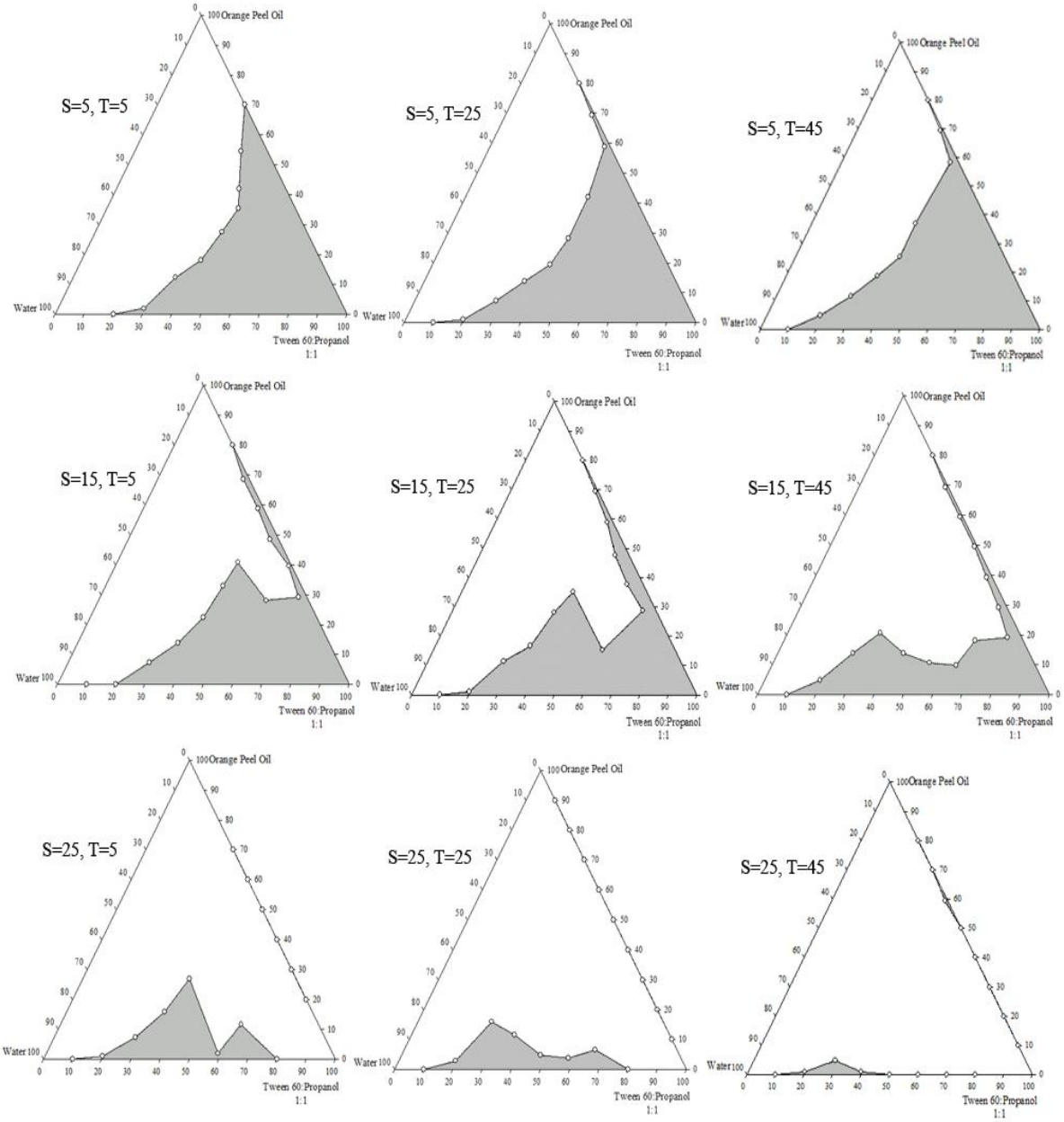

Fig. 9. Phase diagrams of OPO microemulsions with the surfactant:cosurfactant ratio of 1:1 under different sugar concentrations (%)(S) and temperatures (C°)(T). All ratios were by weight.

Heat stability

The effect of sucrose concentration on the critical temperature, above which microemulsion becomes turbid, was examined (Fig. 10). This critical temperature is defined as the cloud point of the surfactant at a particular concentration. In the formulated microemulsion systems, the concentration of sugar was changed from 0 to 30% while the other formulation components such as OPO (1%), T60: propanol (34%) and water (65% wt.) were fixed. Systems with higher sucrose concentrations became turbid at lower temperatures while by decreasing in sugar

concentration, the maximum temperature in which one-phase microemulsion system was still stable, shifted to higher temperatures as reached to 90°C for the samples without sugar. By further heating the solutions above the critical temperature, the emulsions became turbid. Cloudiness is associated with a rapid increase in the micelle aggregation, with the formation of long cylinders (Tadros, 2005). It is also worthy to note that the temperature ranges in which microemulsion formation occurred on heating overlap well with the temperature ranges in which microemulsion formation occurred on cooling. Temperature increase, significantly changes the hydrophile-lipophile balance (HLB) of nonionic surfactant, due to the dehydration of the oxyethylene group which results in more lipophilicity of the surfactant and phase separation of the microemulsion. On the other hand, the effect of temperature is less evident for ionic surfactants (Rajib & Bidyut, 2005). Rajib and Bidyut (2005) found the Brij-35 stabilized eucalyptus oil/butanol system highly dependent on temperature, whereas the AOT and mixed (AOT+ Brij-35) stabilized systems were insensitive to temperature.

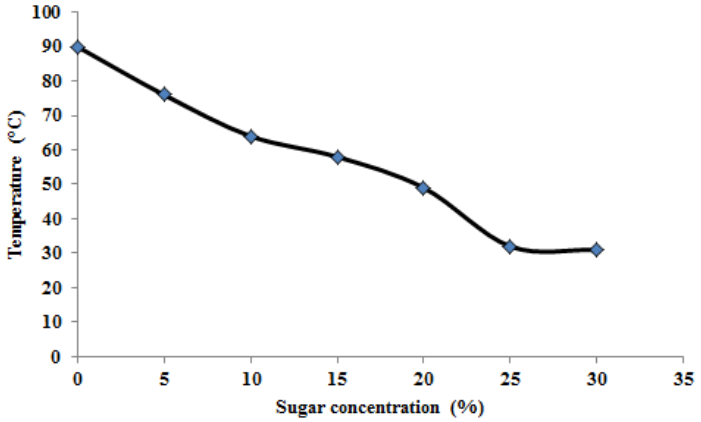

Fig. 10. Effect of sugar concentration on the critical temperature of OPO microemulsion formation.

The stability of OPO microemulsions at 5, 25 and 45°C

The stability of the prepared microemulsions was also examined at three temperatures of 5, 25 and 45°C for seven days (Table 5). All samples were stable at 5 and 25°C.

Table 5- Influence of storage temperature and sugar concentration on the stability of OPO (1%)/ T60: propanol (1:1) (34%) and water (65% wt.) microemulsion for seven days.

Sugar conc. (%)	Storage temperature (°C)			
	-3	5	25	45
0	unstable	stable	stable	stable
5	unstable	stable	stable	stable
10	unstable	stable	stable	stable
15	unstable	stable	stable	stable
20	unstable	stable	stable	stable
25	unstable	stable	stable	unstable
30	unstable	stable	stable	unstable

The samples which contained high concentrations of sugar (25 and 30%) were turbid at 45°C, whereas all other samples exhibited a one-phase microemulsion system at this temperature. On the basis of our findings, microemulsions were not stable at freezing temperatures (-3°C). At subzero temperatures, the water is frozen and the essential oil is solidified. These factors result in separation of the aqueous and oily phases

from the microemulsion system and therefore the breakage of microemulsion.

Sensory profile

The microemulsified OPO were dissolved in the solutions as fast as adding to the medium. It was completely in contrast with what happened for the free essential oil as it was spreading on the surface of the solutions. Presence of CO_2 did not influence the solubility of the microemulsion. Statistical analysis revealed significant differences between samples containing free and encapsulated OPO. No significant differences were recognized in color and viscosity of all samples for the free and encapsulated essential oil in comparison with control (without OPO).

Although the odor and taste intensity of free oil in all samples were higher than the capsulated ones, samples containing OPO microemulsions were recognized as preferred samples in comparison with samples containing free OPO ($p<0.05$). Encapsulation of OPO caused lower release of aroma, resulting a milder odor and taste (lower intensity) in samples which were preferred by the panelists. The lower intensity of odor and taste in samples containing microemulsified OPO were verified for all samples by the assessors. The overall acceptability of all samples containing OPO microemulsion was significantly higher than the samples with free oil ($p<0.05$).

Conclusions

Results obtained from this study showed that sugar concentration and temperature affect the microemulsion area of OPO/ T60: propanol system, significantly. Moreover, microemulsion area and the heat stability of the OPO microemulsions were also affected by sucrose addition as the heat stability was decreased with increasing sucrose concentration. The effectiveness of microemulsions in encapsulating of OPO was proved by panelists who confirmed the lower odor and taste intensity of carbonated water containing microemulsified OPO in comparison with sample containing free essential oil.

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تاثیر pH، قدرت یونی، دما و غلظت شکر بر شکل‌گیری میکروامولسیون اسانس پوست پرتقال/ توئین 60: پروپانل و آب با استفاده از روش سطح پاسخ

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چکیده

میکروامولسیون‌ها سامانه‌هایی هموژن هستند که قطر ذرات فاز پراکنده در آن‌ها کمتر از 100 نانومتر می‌باشد. از آن‌جا که شکل‌گیری این سامانه‌ها، تحت تاثیر پارامترهای مختلفی قرار می‌گیرد، در این تحقیق تشکیل میکروامولسیون اسانس پوست پرتقال با استفاده از توئین 60 و پروپانل (با نسبت 1:1) تحت شرایط مختلف pH، قدرت یونی، غلظت شکر و دما مورد مطالعه قرار گرفت. برای این منظور، از روش سطح پاسخ برای تعریف تعداد آزمون‌ها و ترکیب فاکتورهای مورد بررسی استفاده گردید. نتایج نشان داد که pH و قدرت یونی و تعامل آن‌ها در شکل‌گیری میکروامولسیون موثر نیستند و سطح میکروامولسیون تحت تاثیر پارامترهای مذکور قرار نمی‌گیرد. غلظت شکر به‌طور معنی‌داری بر شکل‌گیری میکروامولسیون موثر بود و با افزایش غلظت شکر از صفر به 30 درصد، منطقه میکروامولسیون از 38% به حدود 4/5% کاهش یافت. مدل کیوبیک تاثیر غلظت شکر بر سطح میکروامولسیون را به خوبی پیش‌بینی کرد. علاوه بر این، منطقه میکروامولسیون با افزایش دما در غلظت‌های پایین شکر (تا 10%) افزایش یافت اما در غلظت‌های بالاتر شکر کاهش معنی‌داری را نشان داد. همچنین پایداری حرارتی میکروامولسیون اسانس پوست پرتقال/ توئین 60: پروپانل/ آب با افزایش غلظت شکر به شدت کاهش یافت. نتایج آنالیز حسی نشان داد که استفاده از میکروامولسیون اسانس پوست پرتقال در آب گازدار در مقایسه با اسانس آزاد، انحلال و پذیرش محصول را خصوصا از نظر فاکتورهای طعم و آروما به‌طور معنی‌داری بهبود بخشید.

واژه‌های کلیدی: میکروامولسیون، اسانس پوست پرتقال، روش سطح پاسخ، مدل کردن

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Study on the physicochemical/ microbial properties and gas chromatography profile of synbiotic yogurt

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Abstract

A synbiotic yogurt was provided by adding aerobic (*Lactobacillus acidophilus*) and anaerobic (*Bifidobacterium bifidum*) probiotics and inulin to the yogurt. The effects of aerobic and anaerobic probiotics, storage time, and inulin on the physicochemical/microbial properties of synbiotic yogurt in terms of acidity, viscosity, syneresis, and microbial count were studied. A head-space solid phase microextraction-gas chromatography (HS-SPME-GC) method was used to extract and detect of VOCs profile (total peak area and total peak height) of yogurt sample by nano-sized polyaniline fiber. The D-Optimal Combined Design (DOCD) was used to analyze the effect of probiotics type (aerobic and anaerobic), inulin percent (W/W %), and storage time of yogurt (day) on the physicochemical/microbial properties of synbiotic yogurt. Results showed that the aerobic and anaerobic probiotics, inulin, and storage time of yogurt affect the yogurt physicochemical/microbial property and there were relations between the physicochemical/microbial properties of yogurt and VOCs gas chromatography profile (total peak area and total peak height). The current research also enables us to obtain microbial count by total peak area and total peak height of the VOCs GC-profile of yogurt sample.

Keywords: Synbiotic Yogurt, Physicochemical property, Microbial property, Nano-sized, Gas chromatography.

Introduction

Yogurt is a type of fermented milk by which lactose converts to lactic acid by the starter microorganisms consist of a mixture of *Lactobacillus delbrueckii ssp. bulgaricus* (*Lactobacillus bulgaricus*) and Streptococcus salivarius ssp. thermophilus (*Streptococcus thermophilus*) [1 and 2]. Some other starters such as *Lactobacillus delbrueckii ssp. Lactis* and *Lactobacillus helveticus* have also been used [1 and 2]. Probiotic yogurts due to their advantages like health benefits gained high popularity during the last two decades. Probiotic bacteria improve intestinal microbial balance in the host animal and were used as live microbial feed supplements. Two famous probiotics that are used in dairy industry include *Lactobacillus acidophilus* and *Bifidobacterium spp.* These probiotics produce acetic acid and lactic acid that decrease the pH of the colonic content so the lower pH inhibits the development of *E. coli.* The probiotics are believed to decrease the uptake of nutrients and space for putrefactive and pathogenic bacteria in the gut (Ziemer *et al*., 1998)

The high molecular-weight polymers composed of saccharide subunits are called polysaccharides. Several monosaccharide residues by chemical reactions are joined together by glycosidic linkages and polysaccharides are formed (Degeest *et al*., 2001). The extracellular polysaccharides and intracellular polysaccharides are two types of polysaccharides that synthesized by organisms. Inulin as an intracellular polysaccharide is produced by plant and glycogen is produced by microorganisms and animals. Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/ or activity of one or a limited number of bacteria in the colon (Degeest *et al*., 2001). This selectivity has been demonstrated for Bifidobacterium, whose growth may be promoted by the uptake of substances such as fructo- oligosaccharrides, transgalactosylated oligosaccharides and soybean oligosaccharides (Degeest *et al*., 2001). Beside their prebiotic properties, certain oligosaccharides have shown a number of functional effects on the GIT physiology

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including reduced fat and cholesterol absorption, modulation of microbial proliferation, thus subsequently reducing intestinal disturbances, cardiovascular diseases and intestinal cancer (Degeest *et al.*, 2001).

A synbiotic product is the combination of probiotics and prebiotics. A synbiotic enhances survival and activity of the organism, for example FOS in conjunction with a Bifidobacterium strain or lactitol in conjunction with *Lactobacillus* (Degeest *et al.*, 2001). The synbiotic has synergistic effects due to promotion the growth of existing strains of beneficial bacteria in the colon. Synbiotics also act to improve the survival, implantation and growth of newly added probiotic organisms. The synbiotic concept has been widely used by yogurt manufacturers in the world.

D-optimal designs are one form of design provided by algorithm. These types of computer-aided designs are particularly useful when classical designs do not apply. Unlike standard classical designs such as factorials and fractional factorials, D-optimal design matrices are usually not orthogonal and effect estimates are correlated (Gladitz *et al.*, 1982).These types of designs are always an option regardless of the type of model the experimenter wishes to fit (for example, first order, first order plus some interactions, full quadratic, cubic, etc.) or the objective specified for the experiment (for example, screening, response surface, etc.). D-optimal designs are straight optimizations based on a chosen optimal criterion and the model that will be fit. The optimality criterion used in generating D-optimal designs is one of maximizing |X'X|, the determinant of the information matrix X'X (Gladitz *et al.*, 1982).

Some polymers like polyaniline, polypyrrole, polythiophene as conducting polymers have some interesting properties like conductivity, nano-size, high temperature resistance and etc. These polymers have an increasing number of applications in various fields like solid phase extraction agent, electronic devices, chemical sensors, and separation filters. Among these polymers, polyaniline (PANI) has been studied most extensively in recent years since it can be synthesized easily, is comparatively stable in air, is relatively cheap and exhibits a number of interesting properties such as chemical sensitivity (Stejskal *et al.*, 2002). Conducting polymer composites have attracted considerable interest in the recent years because of their numerous applications in a variety of chemical sensors, chemical filters and electric and electronic devices. It has been found that such composites can exhibit some novel properties such as high surface area, positive temperature coefficient of resistance (PTC), and chemical separation ability (Stejskal *et al.*, 2002). A recent and very successful application of conducting polymers is the application of these polymers in the solid-phase microextraction (SPME). Pawliszyn and co-workers in 1989 invent the SPME as a type of SPE method that uses micro area solids compounds extraction from different phases. The SPME method by a single step dose all extraction process, including extraction, separation, concentration and sample introduction. In the SPME by using a fiber, analytes are extracted and concentrated from sample (Pawliszyn, 2003). The SPME method has some advantage compared to the SPE method like lower detection limit, lower cost and higher speed. Some techniques like gas chromatography (GC), HPLC, GC/ mass spectrometry (GC/ MS), LC/ MS and super critical fluid chromatography (SFC) are combined with SPME method and are used successfully for the extraction, detection and analysis of VOCs and semi-VOCs in the different samples like biological, environmental, industrial and food samples (Pawliszyn, 2003).

In this work, the effects of aerobic and anaerobic probiotics, storage time, and inulin on the physicochemical/microbial property of synbiotic yogurt were studied. The PANI fiber was used to extract and detect VOCs of yogurt by HS-SPME-GC method.

Material and methods

Low-fat milk (1.5% fat) was prepared from the Pegah factory from Urmia, Iran. The yogurt starter (set 1 type) containing *S. thermophilus*

and *Lactobacillus spp.*, probiotic microorganism starter namely *lactobacillus acidophilus* (L-10) were purchased from industrial enzymes company, Tehran, Iran. The probiotic starter (BB-12) *Bifidobacterium bifidum* was purchased from Chr. Hansen Denmark. Frutafit HD inulin with an average chain length was purchased from SENSUS Netherlands. The culture medium, including: MRS-Agar (Biolife), RCA (Merk), Oxgall-bile (Sigma), and Peptone Water (Merk) were used. Aniline purchased from Fluka, Switzerland and stored in a refrigerator in the dark prior to use. Potassium dichromate ($K_2Cr_2O_7$) was used as oxidant from Aldrich. All analytical reagents were purchased from Merck.

Apparatus

The PANI fiber was prepared by a chemical polymerization. An SPME fiber holder for manual sampling was designed and fabricated by Pirsa *et al.* (2016). The GC apparatus used in this study was from Agilent 7890 A, Wilmington, DE, USA. The scanning electron micrographs (SEM) using an SEM instrument (Philips XL30, Holland) was used to evaluate the morphology of PANI fiber.

Brookfield viscometer (Brookfield DVII+, USA), Incubator (Heraeus D6450 Hanau-type SI 6120), Mixer (NO.HA Model 3020, Japan), Autoclave (WEBO GmbH-bad Schwartau, Germany), Anaerobic Jar (Anaerocult, Merk, Darmstadt, Germany), Gas pack (Anaerocult A, Merk, Darmstadt, Germany) were used to test physicochemical and microbial analysis.

Yogurt preparation

Preparation of yogurt samples was done as follow: 10 Kg milk (pasteurized, homogenized and 1.5% fat) was heated to 45°C. Inulin hydrocolloid (0, 2, 3, and 4%Wt) was added to the milk according to the experimental design (Table 1). The mix was cooled (to 37°C) and yogurt starter, as well as probiotics *lactobacillus acidophilus*, and probiotic *Bifidobacterium bifidum* (BB-12) were added to the mix. Then the samples were packed and transported to the incubator (40-37°C). The sample pH was controlled during incubation. The samples in the pH=4.6 were transferred to the fridge (5°C). At 1, 11 and 21 days of storage, the physicochemical/ microbial properties of yogurt and VOCs gas chromatography profile (total peak area and total peak height) were analyzed (Ozer *et al.*, 2005)

Apparent viscosity measurement

The apparent viscosity was measured by using a Brookfield viscometer (Brookfield DVII+, America, LV2) after 30 seconds rotation. Yogurt samples were stirred for 1 minute before measurment (Ghasempour *et al.*, 2012)

Acidity determination

Acidity was determined by titration of 10 g yogurt sample (5 g sample and 5 g of distilled water) by NaOH solution (0.1 N) in the presence of phenolphthalein.

Syneresis measurement

25g of sample was poured onto the filter paper. The sample was placed in the refrigerator for 2 hours. Finally the syneresis was calculated as a percentage of the volume of separated clear liquid phase to the initial weight of the yogurt (Sahan *et al.*, 2008)

Survival of probiotics

To investigate changes in the *Lactobacillus acidophilus* and *Bifidobacterium bifidum* count, 1 gram of yogurt sample was well mixed with 9 ml peptone water (0.1%). The diluted suspended samples were cultured. For this purpose, 1 ml of diluted samples was transferred to the plates. Then, 15 ml of the appropriate culture medium was added to each plate (Kailasapathy, 2006).

Fiber preparation, chromatographic conditions and extraction procedure

The PANI nano-fiber was fabricated by chemical polymerization method at room temperature under atmospheric condition, according to our previous work (Pirsa *et al.*, 2013). HS-SPME extraction procedure was done as previously reported (Pirsa *et al.*, 2016). A gas chromatography instrument (Agilent7 890 A, Wilmington, DE, USA) with flame

ionization detector (GC-FID) at our previous research work condition was used (Pirsa *et al*., 2016). The gas chromatographic profile of yogurt VOCs at the optimum condition is shown in Fig. 1.

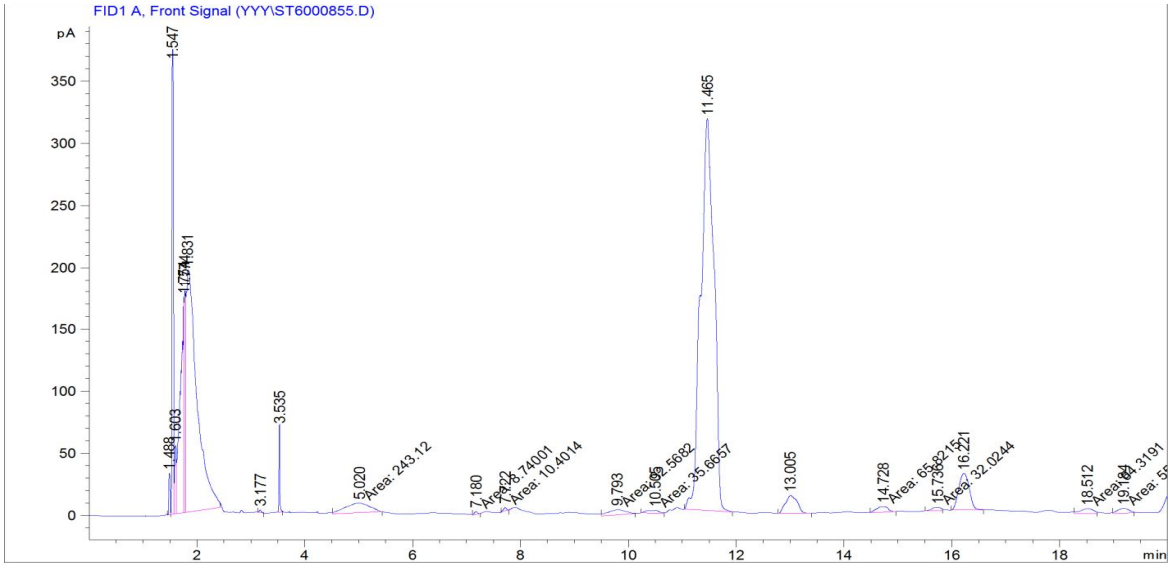

Fig.1. GC-FID chromatogram of synbiotic yogurt VOCs extracted by PANI fiber

Results and Discussion

Morphology

The morphology, size and porosity of the synthesized PANI film were studied by scanning electron microscopy (SEM). Fig. 2 shows the SEM micrographs of the PANI fiber. Results show that PANI particles are seed like and in the nano-size between 40- 100 nanometers.

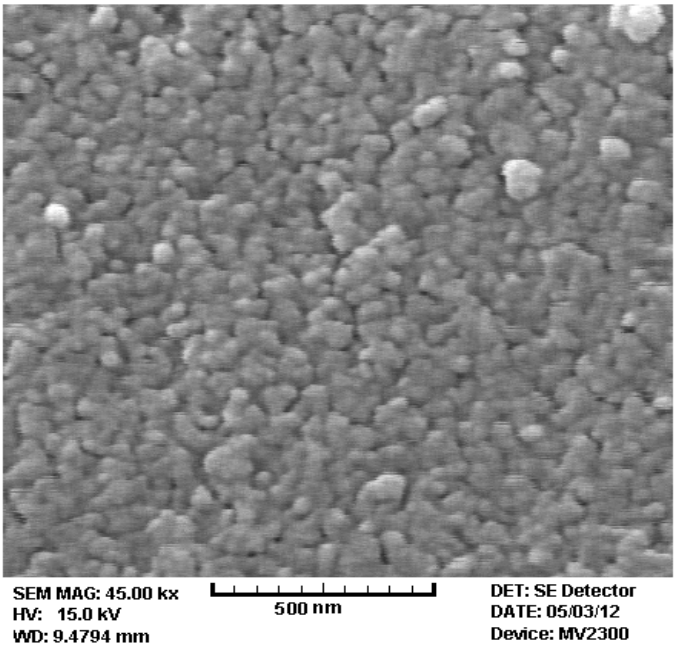

Fig.2. SEM images of PANI fiber

Experimental design

Effects of variables on the physicochemical/ microbial properties

The probiotic type (aerobic and anaerobic), storage time (day), and inulin level (% Wt) were three variableas that affect physicochemical and microbial properties of synbiotic yogurt including acidity, viscosity, syneresis, and microbial count. To study the effect of these variables on the responses, a D-Optimal Combined Design (DOCD) was used. Three variables, inulin percent (Wt %) (F1) (In four levels), storage time (day) (F2) (in 3 levels) and probiotic bacteria type (aerobic and anaerobic) as a categorical factor (F3) were investigated. In Table 1, the 3 processing variables as factors, levels and experimental design are given. Table 1 also presents the evaluated responses including, acidity, syneresis, viscosity, microbial count, total peak area, and total peak height.

Table 1- List of experiments in the DOCD and the responses of each run

Run	Factors			Physicochemical/microbial properties				GC chromatogram	
	Inulin %	Storage (day)	Probiotic type	Acidity	Syneresis (%)	Viscosity (mPas)	Microbial count(cfu/g)	TPA*	TPH**
1	2	1	anaerobic	0.75	40.8	1632	4.70E+08	1127.8	314.8
2	0	1	aerobic	0.829	35.2	1896	2.00E+05	705.7	22.3
3	4	1	anaerobic	0.72	38.4	1724	5.60E+07	1134.2	102.8
4	4	21	anaerobic	0.98	29.8	1996	1.24E+09	656.9	28.5
5	4	1	anaerobic	0.72	39.2	1900	4.90E+07	1060.4	175.6
6	4	21	aerobic	0.98	31.48	1908	1.00E+07	768.6	79.1
7	4	1	aerobic	0.756	36.4	2192	1.00E+07	861	160.5
8	3	11	anaerobic	0.81	44	1164	2.00E+08	945.5	98.5
9	0	11	aerobic	0.972	43.2	1488	2.00E+05	1276	158.9
10	0	21	anaerobic	1.031	32.4	1732	1.00E+09	246	23.1
11	0	21	anaerobic	1.001	34	1396	2.00E+09	803.9	91.6
12	2	1	aerobic	0.738	39.2	2128	1.00E+05	918	153.9
13	0	21	aerobic	1.067	31.2	1376	2.00E+05	870.7	78
14	2	11	anaerobic	0.954	42	1716	5.00E+08	1170.3	122.4
15	0	1	anaerobic	0.828	32.8	1984	3.60E+08	1394	61
16	0	21	aerobic	1.026	32	1532	4.20E+06	490.4	70.4
17	4	21	anaerobic	1.04	29.12	1600	5.00E+08	3212	359.9
18	0	11	anaerobic	0.9	44	1472	7.00E+08	1501.4	185.6
19	4	21	aerobic	1.074	30.16	1568	1.00E+07	790.5	64.8
20	3	11	aerobic	0.846	40	1448	1.00E+05	2012.8	145.7

*total peak area **total peak height

The Design-Expert software (version 7) and Minitab version 17 were used to perform statistical analysis. Initially, the full term second order polynomial response surface models were fitted to each of the response variables, according to the following equation:

$$Y = b0 + b1\times F1 + b2\times F2 + b3\times F3 + b4\times F1\times F1 + b5\times F2\times F2 + b6\times F3\times F3 + b7\times F1\times F2 + b8\times F1\times F3 + b9\times F2\times F3 \quad (1)$$

Where Y is the responses (acidity, syneresis, viscosity, and microbial count); F1, F2 and F3 are inulin percent (% Wt), storage time (day) and probiotic type respectively. For inulin four levels (0, 2, 3, and 4 %), storage time three levels (1, 11, and 21 days), and probiotic type (aerobic and anaerobic) were chosen to construct a design. b0 to b9 are the coefficient values obtained through multiple linear regressions.

Table 2- Some characteristics of the constructed models and analysis of variance for responses

Acidity						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value**	
Model	0.26786	2	0.13393	77.077	< 0.0001	Significant
A-inulin	0.011709	1	0.011709	6.7388	0.0188	
B-Time	0.247916	1	0.247916	142.67	< 0.0001	
Residual	0.029539	17	0.001738			
Lack of Fit	0.022031	12	0.001836	1.22	0.4404	Not Significant
Pure Error	0.007509	5	0.001502			
Cor Total	0.2974	19				
R^2=0.900675, Adj R2=0.888989, Pred R2=0.866264						
Syneresis						
Model	436.5174	4	109.1294	32.40407	< 0.0001	Significant
A-inulin	0.113234	1	0.113234	0.033623	0.8570	
B-Time(days)	130.2021	1	130.2021	38.66125	< 0.0001	
AB	23.63183	1	23.63183	7.017063	0.0182	
B^2	259.182	1	259.182	76.95961	< 0.0001	
Residual	50.51651	15	3.367767			
Lack of Fit	47.49411	10	4.749411	7.857019	0.0173	Significant
Pure Error	3.0224	5	0.60448			
Cor Total	487.0339	19				
R^2=0.896277, Adj R2=0.868618, Pred R2=0.814185						
Viscosity						
Model	668828.2	2	334414.1	7.554622	0.0045	Significant
B-Time(days)	300661.5	1	300661.5	6.792131	0.0184	
B^2	390296.5	1	390296.5	8.817041	0.0086	
Residual	752524.6	17	44266.15			
Lack of Fit	532212.6	12	44351.05	1.006551	0.5385	not Significant
Pure Error	220312	5	44062.4			
Cor Total	1421353	19				
R^2=0.470557, Adj R2=0.40827, Pred R2=0.399						
Microbial count						
Model	37.34429	2	18.67214	45.65753	<0.0001	Significant
B-Time(days)	2.501241	1	2.501241	6.116089	<0.0242	
C-probiotic type	35.90213	1	35.90213	87.78867	0.0001	
Residual	6.952334	17	0.408961			
Lack of Fit	5.953415	12	0.496118	2.483274	0.1622	Not significant
Pure Error	0.998919	5	0.199784			
Cor Total	44.29662	19				
R^2=0.84305, Adj R2=0.824586, Pred R2=0.774708						

Where possible, stepwise deletion of terms was applied to remove the statistically non-significant terms in order to simplifying the model. However, when the exclusion of such terms from the model decreases R^2 (adjusted) increases the estimator of the variance S, the term was included in the model. The statistically non- significant linear terms also remained in the model when the respective quadratic or interactive effects were statistically

significant. The quadratic polynomial models for all response functions accompanied by F values and corresponding R^2 were used. The analysis of variance and estimated regression coefficients are summarized in Table 2.

Response Surface and three-dimensional plot to study variables effect on the physicochemical/microbial properties

The three-dimensional (3D) plots, linear regression interaction curve and contour plots based on the model function were used to predict responses to survey influence of each variable on the analyzed physicochemical/ microbial properties.

Effects of variables on the syneresis

Syneresis is one of the important factors in determining the quality of yogurt. Syneresis in yogurt is due to the shrinkage of a three-dimensional network of protein structures, which leads to a reduction in the binding of whey proteins and leads to the outflow of water from yogurt (Lucey, 2005).

Fig. 3 shows the three-dimensional plot of syneresis versus inulin level and storage time. Results show that inulin level and storage time have affected the yogurt syneresis, but probiotic type doesn't have significant effect on the syneresis. At the initial sample storage time (1 day) yogurt syneresis is increased by increasing of the inulin percent, but at the end of sample storage time (21 days) yogurt syneresis is decreased by increasing the inulin percent.

The reason for this behavior can be due to the acidity of yogurt. By increasing the concentration of hydrogen ions, the forces of repulsion are reduced and in casein micelles, aggregation occurs, and a stronger protein network is created and syneresis is decreased.

Production of exopolysaccharides is another reason for reduction of the syneresis. Researchers such as Kailasapathy (2006), Souza and Saad (2009) have reported in probiotic yogurt a decrease in syneresis with increasing acidity.

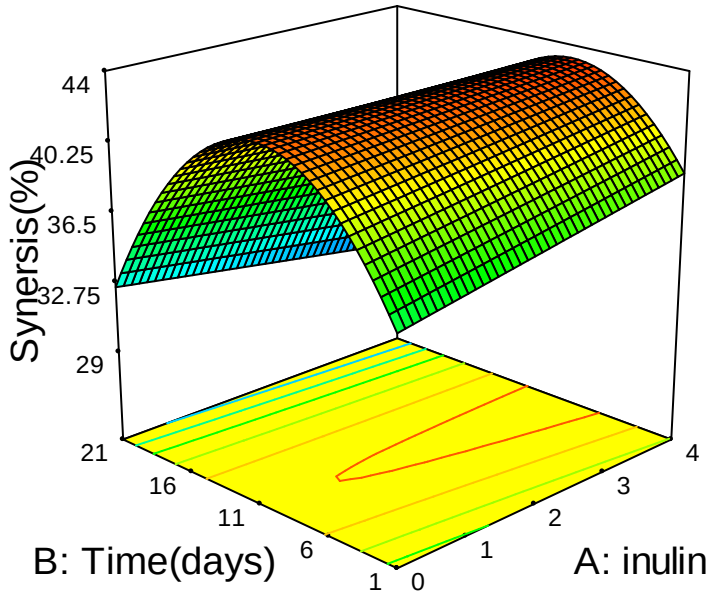

Fig.3. Three-dimensional plot of syneresis versus inulin percent and storage time

Linear regression curve of acidity based on variables

Fig. 4 (A) shows the linear regression curve of acidity versus storage time and fig. 4 (B) shows the linear regression curve of acidity versus inulin levels. Results show that probiotic type doesn't have a significant effect on the

acidity and storage time and inulin levels have an effect on the acidity, but there is not any interaction between storage time and inulin percent. According to the linear curves, yogurt acidity is increased by storage time and decreased by inulin level increasing.

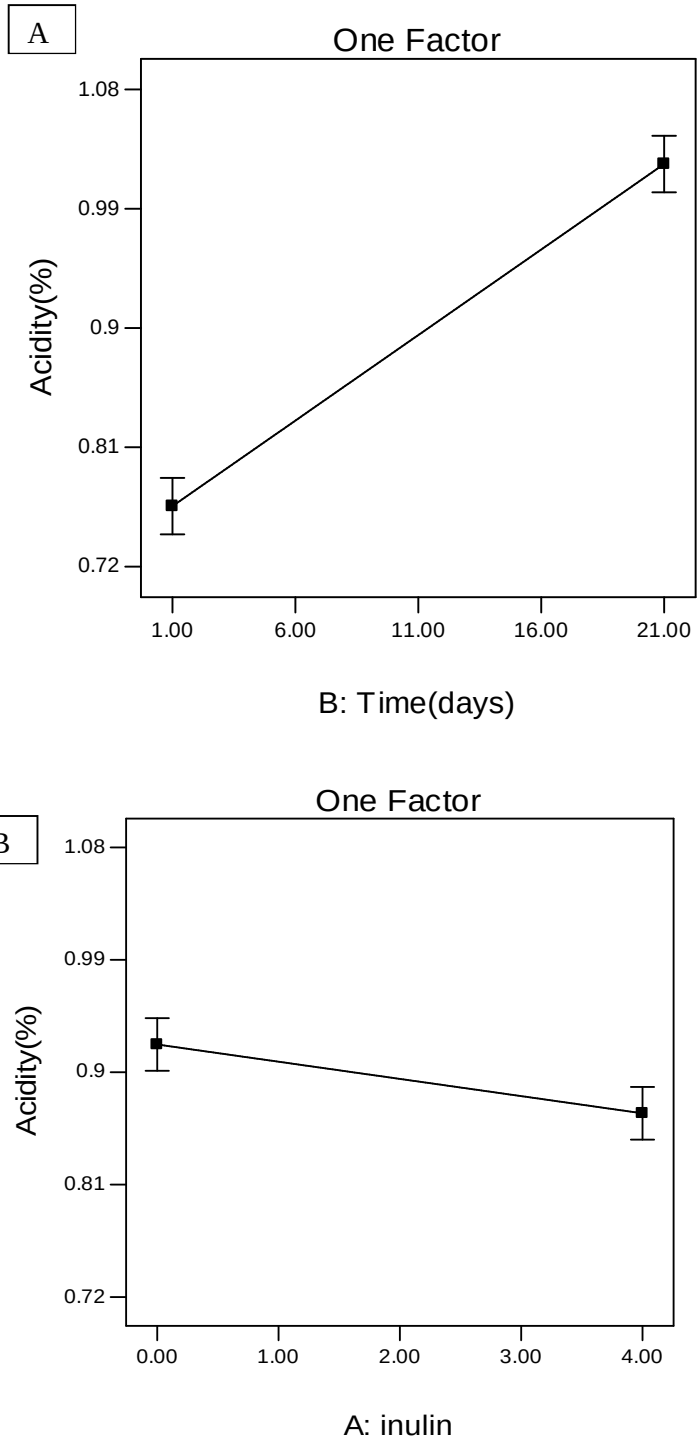

Fig.4. Linear regression curve of acidity versus (A) storage time and (B) inulin percent

Increasing acidity by increasing storage time can be due to the acidification phenomenon that results from the lactose fermentation process. Lactose fermentation is affected by traditional starter of yogurt and probiotics. Lactose fermentation produces lactic acid and increases the acidity of yogurt.

Acidity reducing in the presence of inulin can be due to the ability of inulin to control the acidification process. Balthazar *et al*. (2015) examined the effect of different inulin concentrations on sheep's yogurt acidity, and concluded that yogurt containing inulin had less acidity than the control yogurt. Therefore it can be said that inulin can be used as a control agent for acidity in yogurt.

Linear interaction curve of microbial count based on variables

Fig. 5 shows the linear regression interaction curve of microbial count versus storage time and probiotic type. Results show that the inulin level doesn't have an effect on the microbial count. The microbial count is increased by increasing storage time in the presence of both aerobic and anaerobic probiotics. Also result showed that the probiotic type had significant effect on the microbial count, anaerobic probiotic showed more microbial count than aerobic probiotic. Inulin as a prebiotic promotes the growth and metabolic activity of probiotics. Ozer *et al*. (2005) successfully used inulin as an agent for the growth of *Lactobacillus acidophilus* and anaerobic *Bifidobacterium bifidum*. Sadek *et al*. (2004) demonstrated the increasing of viability of probiotics in the presence of inulin as a prebiotic.

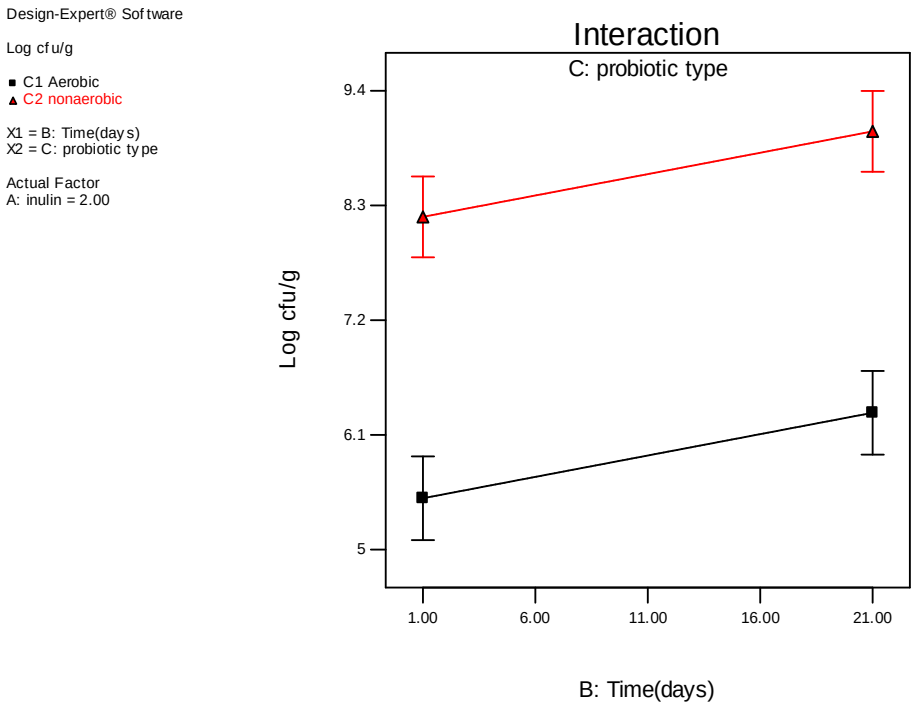

Fig. 5. Linear regression interaction curve of microbial count versus storage time

Regression plot of viscosity based on variables

Viscosity is resistance to flow and one of the important factors on the quality of food products. Viscosity is affected by factors such as particle size, dry matter, and compression of the protein network.

Fig. 6 shows the regression plot of viscosity versus storage time. Results show that only the

yogurt storage time affects the viscosity. The probiotic type and inulin level don't have a significant effect on the viscosity. The viscosity is decreased by increasing storage time to 11 days and then is increased.

In general, during storage period, the yogurt viscosity may be reduced due to changes in the compression of the protein network and the formation of pores in yogurt. The increase in viscosity after the eleventh day may be due to an increase in the hydration of inulin colloid, or creation of protein-protein interaction.

Toneli *et al.* (2007) reported a positive effect of inulin on the viscosity of low-fat or high-fat foods such as salads, chocolate and yogurt.

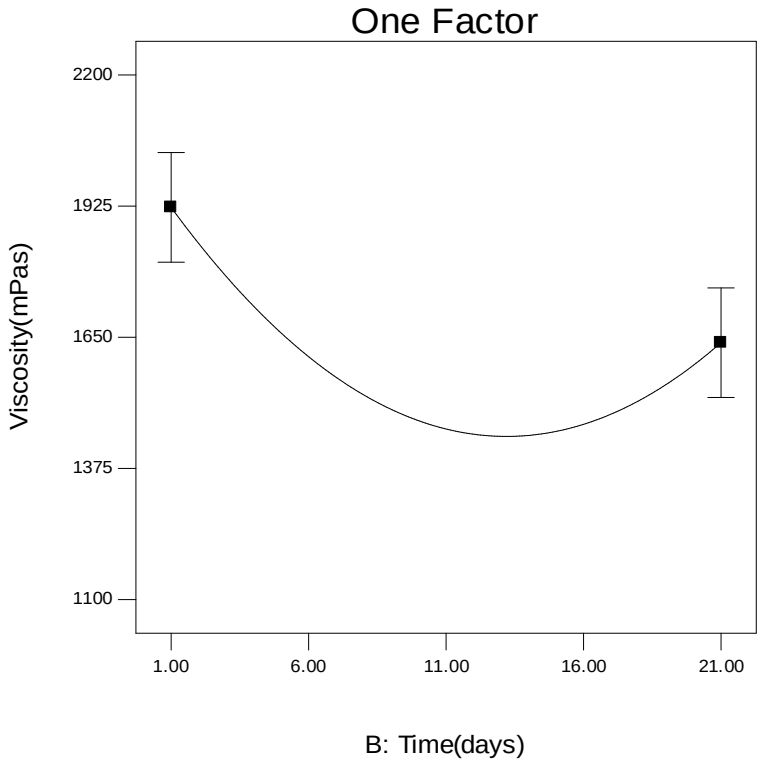

Fig. 6. Regression plot of viscosity versus storage time

Relation between physichochemical/ microbial properties of yogurt and volatile compounds

For evaluation of relation between physichochemical/ microbial properties of yogurt and volatile compounds, GC chromatograms, the acidity, viscosity, syneresis, and microbial count of yogurt were considered as variables and total peak area and total peak height are considered as responses. So the full term second order polynomial response surface models were fitted to each of the response variables, according to equation (1). Where Y is the responses (total peak area and total peak height); factors (F) are acidity, syneresis (%), viscosity (mPas), and microbial count (cfu/g), and b values are the coefficient values obtained through multiple linear regressions. The quadratic polynomial models for two response functions accompanied by F values and corresponding R^2 was used, the estimated regression coefficients summarized in Table 3.

Fig. 7 and Fig. 8 show the surface plot and counter plot of the total peak area and total peak height based on variables (acidity, syneresis (%), viscosity (mPas), and microbial count (cfu/g)) respectively.

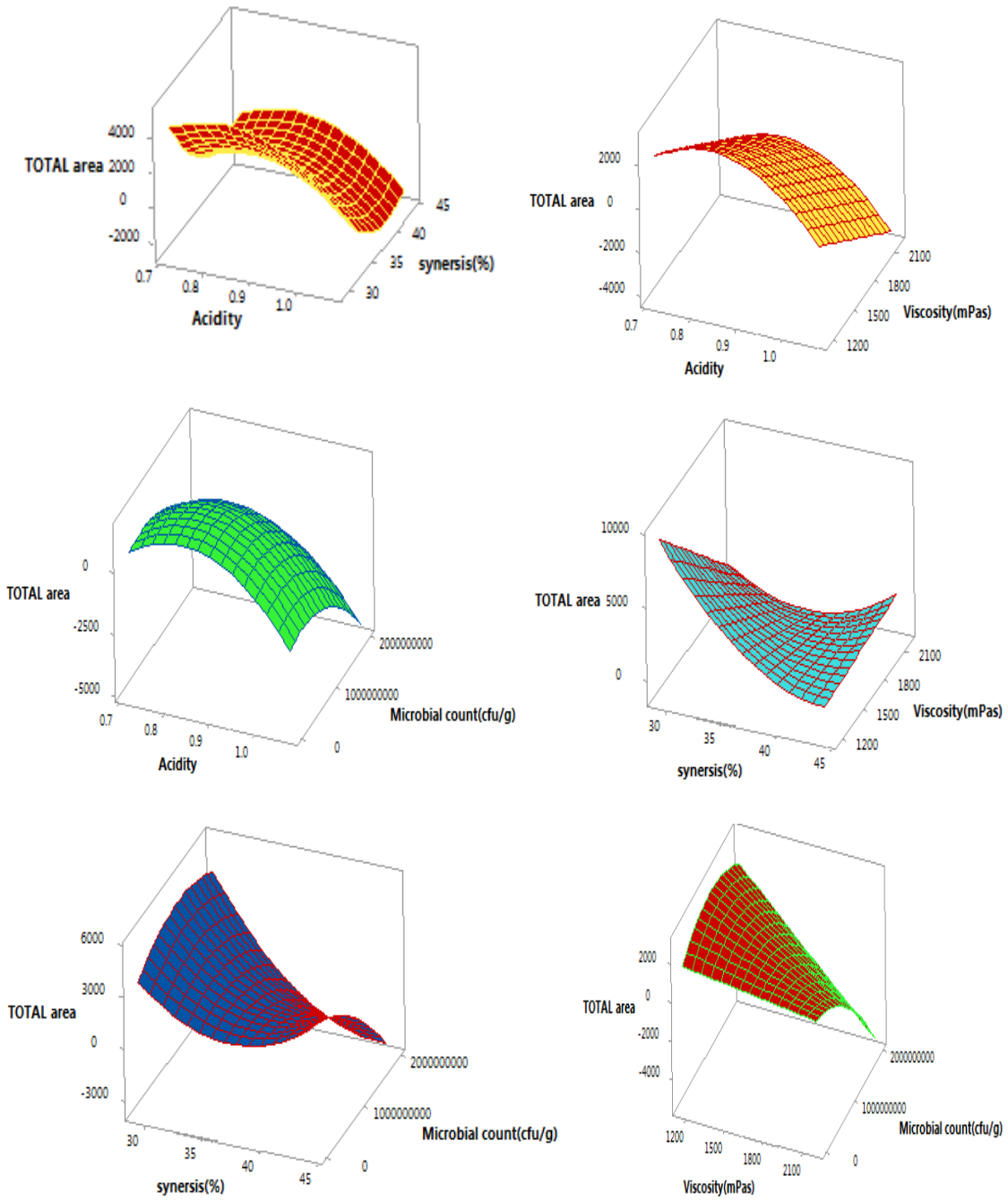

Fig. 7. Surface plots of total peak area based on variables

Results show that the total peak area and total peak height of yogurt VOCs are affected by physicochemical/ microbial properties of yogurt and there are good relation between physicochemical/ microbial properties of yogurt and volatile compound peaks in GC that can help to suggest and determine microbial count, viscosity, acidity and synersis of yogurt.

Table 3- Some characteristics of the constructed models for responses (total peak are and total peak height)

Regression equation	Model Summary
Total peak area = 52683 + 95755 Acidity - 3827 synersis(%) - 23.67 Viscosity(mPas) + 0.000015 Microbial count(cfu/g) - 57231 Acidity*Acidity + 37.5 synersis(%)*synersis(%) - 0.000000 Microbial count(cfu/g)*Microbial count(cfu/g) + 0.624 synersis(%)*Viscosity(mPas) - 0.000000 synersis(%) *Microbial count(cfu/g) - 0.000000 Viscosity(mPas)*Microbial count(cfu/g)	R-sq= 79.33% R-sq(adj)= 56.37%
Total peak height = 5990 + 10 Acidity - 272 synersis(%) - 1.583 Viscosity(mPas) + 0.000005 Microbial count(cfu/g) + 2.82 synersis(%)*synersis(%) - 0.000000 Microbial count(cfu/g)*Microbial count(cfu/g) - 0.000002 Acidity*Microbial count(cfu/g) + 0.0467 synersis(%)*Viscosity(mPas) - 0.000000 synersis(%)*Microbial count(cfu/g) - 0.000000 Viscosity(mPas) *Microbial count(cfu/g)	R-sq= 78.18% R-sq(adj)= 53.93%

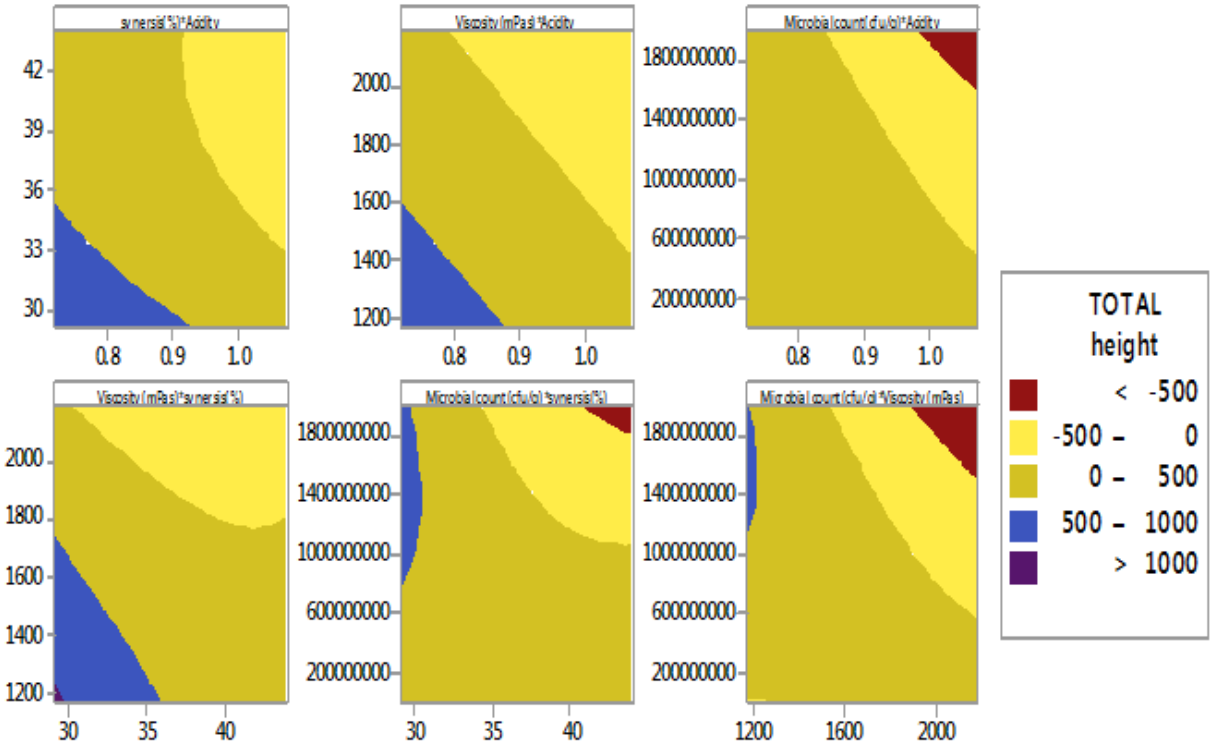

Fig. 8. Counter plots of total peak height based on variables

Conclusion

The physicochemical and microbial properties of yogurt are affected by probiotics type (aerobic and anaerobic), inulin level and storage time of yogurt. Physicochemical and microbial properties of yogurt have a strong effect on the VOCs of yogurt. The chemically synthesized nano- size Polyaniline was used to extract and analyze yogurt VOCs by HS-SPME- GC method. The D- Optimal Combined Design (DOCD) was used to study the effect of probiotics type (aerobic and anaerobic), inulin level (W/W %), and storage time of yogurt (day) on the physicochemical/microbial properties of synbiotic yogurt and the relation between physicochemical/ microbial properties and VOCs GC- characters (total peak area and total peak height). According to the results: 1- Inulin level and storage time have affected the yogurt syneresis, but probiotic type doesn't have significant effect on the syneresis, 2- Inulin level and storage time have effect on the sample acidity, but there is not any interaction between storage time and inulin level, 3- The microbial

count is increased by increasing storage time in the presence of both aerobic and anaerobic probiotics, and 4- total peak area and total peak height of yogurt VOCs are affected by physicochemical/ microbial properties of yogurt and there are good relation between physicochemical/ microbial properties of yogurt and volatile compounds peaks in gas chromatography.

Acknowledgment

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جلد 15، شماره 3، مرداد - شهریور 1398، ص. 59-45

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مطالعه ویژگی‌های فیزیکوشیمیایی/ میکروبی و پروفیل کروماتوگرافی گازی ماست سین‌بیوتیک

نسیبه عبودی[1]- محمد علیزاده[2]- سجاد پیرسا[3*]

تاریخ دریافت: 1397/09/03
تاریخ پذیرش: 1398/01/25

چکیده

ماست سین‌بیوتیک با افزودن پروبیوتیک‌های هوازی (*لاکتوباسیلوس اسیدوفیلوس*) و بی‌هوازی (*بیفیدوباکتریوم بیفیدوم*) و اینولین به داخل ماست تهیه شد. اثرات پروبیوتیک‌های هوازی و بی‌هوازی، زمان نگهداری و اینولین بر خواص فیزیکوشیمیایی/ میکروبی ماست سین‌بیوتیک از نظر اسیدیته، ویسکوزیته، سینرزیس و میزان بار میکروبی مورد بررسی قرار گرفت. برای استخراج و شناسایی پروفیل مواد فرار (مساحت سطح کلی پیک‌ها و ارتفاع کلی پیک‌ها) نمونه‌های ماست از روش ریز استخراج از فضای فوقانی با فاز جامد- کروماتوگرافی گازی (HS-SPME-GC) به‌وسیله فیبر نانوساختار پلی‌آنیلین استفاده شد. برای بررسی اثر نوع پروبیوتیک (هوازی و بی‌هوازی)، درصد اینولین و زمان نگهداری ماست بر روی خواص فیزیکوشیمیایی/ میکروبی ماست سین‌بیوتیک از طرح آماری مرکب بهینه استفاده شد. نتایج نشان داد که پروبیوتیک‌های هوازی و بی‌هوازی، اینولین و زمان نگهداری ماست بر ویژگی‌های فیزیکوشیمیایی ماست تاثیر می‌گذارند و ارتباط معنی‌داری بین خواص فیزیکو شیمیایی ماست و پروفیل مواد فرار کروماتوگرافی گازی (مساحت سطح کلی پیک‌ها و ارتفاع کلی پیک‌ها) وجود دارد. تحقیق ارائه شده، محاسبه بار میکروبی از طریق بررسی مساحت سطح کلی پیک‌ها و ارتفاع کلی پیک‌های نمونه‌های ماست را امکانپذیر می‌سازد.

واژه‌های کلیدی: ماست سین‌بیوتیک، خواص فیزیکوشیمیایی، خصوصیات میکروبی، نانومواد و کروماتوگرافی گازی

1- دانش‌آموخته گروه علوم و صنایع غذایی دانشگاه ارومیه
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نشریه پژوهشهای علوم و صنایع غذایی ایران
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Improvement of Physicochemical and Nutritional quality of sponge cake fortified with microwave- air dried quince pomace

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Abstract

In the present study, the effects of addition of quince pomace powder (0- 15%) and water content (25- 35%) on the batter rheological properties, physicochemical characterizes and sensory properties of sponge cake were evaluated. The results showed that increasing substitution of quince pomace increased the viscosity and consistency batter and the dietary fiber, firmness, overall acceptability of cake and reduced the moisture content, and density of cake. Results of RSM based desirability function showed cakes formulated with 12.56% of quince pomace powder and 29.62% of water content had the most and desired physicochemical quality. Total phenol content (7.71 mg/g), iron (0.263 mg/Kg dry weight) and calcium (340 mg/Kg dry weight) of the control sponge cake was improved to 8.32 (mg/g), 0.361 (mg/Kg dry weight) and 1160 (mg/Kg dry weight) in the optimal sponge cake, respectively. SEM results showed the quince powder increased in the number of cavities in the cake's structure and the uniformity of these cavities.

Key words: Physicochemical properties, Quince pomace, RSM, Sponge cake.

Introduction

Bakery products are widely consumed all over the world and among them; cakes are particularly popular and associated in the consumer's mind with a delicious product with particular organoleptic characteristics (Hafez, 2012; Matsakidou *et al*., 2010). Cakes are favorite foods but of low fiber content and refined wheat flour lack the natural bioactive components found in dietary fiber and consequently may yield lowered health benefits compared with whole wheat, Therefore, increasing the fiber content of the cakes can enhance the nutritional quality of these products (Kim *et al*., 2012; Majzoobi *et al*., 2015).

Dietary fiber functions as a bulking agent and increases the intestinal mobility and moisture content of the feces and lack of fiber in the diet has been associated with constipation, diverticulitis, diabetes, obesity, cardiovascular disease, and cancer (Sudha *et al*., 2007).

Many reports have highlighted that the intake of dietary fiber is much lower than the recommended value, resulting in a number of serious diseases such as cancer, obesity, diabetes, blood pressure, and cardiovascular problems (Mellen *et al*., 2008; Nouri *et al*., 2017). Besides this, fibers can be used for technological purposes because of its functional properties. As functional additives, their usage can range from bulking agent to fat substitute (Fissore *et al*., 2007).

Different sources of dietary fiber have been added to the cakes to increase the dietary fiber content such as Opuntia ficus indica (Ayadi *et al.*, 2009), cross-linked resistant rice starch (Ren and Shin, 2013), Oat Fiber (Majzoobi *et al*., 2015) and Carrot(Salehi *et al*., 2016), have been added to increase dietary fiber content of bakery products.

Quinces (*Cydonia oblonga*) have received attention in the last ten years because of their high content in biologically active phytochemicals and antioxidant capacity. Several studies have recently described antioxidant properties (Wojdyło *et al.*, 2014), phenolics compounds (Carvalho *et al*., 2010),

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antimicrobial activity (Fattouch *et al*., 2007; Silva and Oliveira, 2013), antiallergic (Shinomiya *et al*., 2009), antihemolytic (Costa *et al*., 2009), and antiproliferative properties (Carvalho *et al*., 2010) of quince. Besides, quince has low fat content and it is an important source of organic acids, sugars, crude fiber and minerals, such as potassium, phosphorous and calcium (Carbonell- Barrachina *et al*., 2015). However, this pomace is quite susceptible to deterioration due to its relatively high moisture content and enzymes such as those accelerating non-enzymatic browning reactions (Noshad *et al*., 2011). Drying is a common preservation method, mainly because of water removal and consequently reduction in enzymatic deterioration. Using the microwave- assisted drying can improve the physicochemical quality of the dried powder (Hernández- Ortega *et al*., 2013).

To our knowledge the effect of quince pomace (QP) powder dried in microwave-assisted drying on the properties of sponge cake have not been studied yet. Therefore, the current work was to optimize and evaluate the efficacy of QP in production of a sponge cake in order to improve its quality and nutritional value.

Materials and Methods

Fresh quinces (Isfahan variety) with approximately same size and ripen were bought from a local market in Ahvaz, Iran**.** The ingredients used in the formula of sponge cakes were cake flour (Ard jonob Company, Iran), white fine sugar, sunflower oil (with commercial name of Oila, Iran), fresh whole eggs, baking powder (containing sodium bicarbonate and tartaric acid), vanilla (with commercial name of Zamen, Iran), water and nonfat dry milk powder (Pegah Company, Iran) were purchased from locally market.

Microwave drying of quince pomace

The quince pomace consisted of the peel and pulp remaining after juicing. The quince pomace was kept in air tight plastic bottles and stored at a temperature of 4°C until the drying process. microwave oven dryer (Rotosynth, Milestone s.r.l., Italy) were used for quince pomace drying (Anvar et al., 2017). Microwave drying conditions of quince pomace optimized with respect to quality attributes (moisture content, color change and consumer acceptance) using response surface methodology (RSM) (Anvar *et al.*, 2017). The dried quince pomace was ground to powder to pass through 35 mm sieve (AS 200 Basic model, Retsch, Haan, Germany).

Sponge cake preparation

First, sucrose and sunflower oil were mixed for 4 min. Whole egg was added to the bowl, and then mixed for 2 min. The quince pomace (QP) powder (0- 15 g/100 g samples of wheat flour), cake flour (100- 85 (g)), baking powder (2 g/100 g samples of wheat flour), vanilla (0.5 g/100 g samples of wheat flour) and nonfat dry milk powder (2 g/100 g samples of wheat flour) was gradually poured into a bowl, and mixed for 4 min. Water was added to the bowl, and then mixed for 1 min (Salehi *et al*., 2015). For each cake, 334 g of cake batter was poured into a cake pan and baked at 180°C (top) or 160°C (bottom) for 30 min in an oven toaster (Noble, Model: KT- 45XDRC) (Salehi *et al*., 2016). After baking, the cakes were removed from the pans and allowed to cool for 1 h at room temperature. The cooled cakes were packed in plastic zipper bags at room temperature before quality parameters analyses.

Determination of physicochemical properties of cakes

The physicochemical properties of sponge cakes, including moisture base on a wet weight, pH and ash, were determined by Movahed *et al* (2011) method (Movahed et al., 2011). The total dietary fiber(TDF) were also determined by Alharbi *et al* (2015) methods (Alharbi *et al*., 2015). The baking loss rate (%) of each type of sponge cake was calculated based on the percentage of cake weight lost after baking and the weight of the sponge cake batter (Turabi *et al*., 2008). The density of the sponge cake was determined by the rapeseed displacement method from four replications(Eriksson et al., 2014).

Determination of cakes textural properties

The texture analyzer (TA-XT-PLUS, Micro stable system, made in English) at room temperature with a 36 mm diameter cylindrical probe, 25 % compressing and a test speed of 0.25 mm s^{-1}, time interval of 10 s, pre- test speed 5.0 mm s^{-1}, post-test speed 5.0 mm s^{-1} and trigger force 5 g was used to evaluate the texture of samples (Torbica *et al.*, 2010).

Sensory evaluation

Cakes were evaluated for their organoleptic characteristics (color, flavor, taste, texture, and overall acceptability) by performing a five-point hedonic test using trained panelists. The panelists were asked to evaluate the samples and score them between 1 (most disliked) to 5 (most liked) (Stone *et al.*, 2012).

Color determinations of sponge cake

Crumb and crust color of fresh cake was measured with a Konica Minolta (model CR-400, Japan) set for Hunter L (lightness), a (redness), b (yellowness) values. The results of the Hunter L, a, and b values were averaged from 10 replications.

Evaluation Antioxidant Activity
Determination of total phenol content

Total phenolic contents analysis was determined according to the method of (Sudha *et al.*, 2007) as follow: 1 g of defatted sample (after refluxing with chloroform and petroleum ether, (1:1 v/v) followed by drying) was mixed with 10 mL of water for aqueous extraction. Similarly, for methanol extraction, 1 g of defatted sample was mixed with 10 mL of methanol, stirred and centrifuged at 2000g for 15 min. The above supernatants were referred as WE and ME, respectively. Sample aliquot of 20–100 mL were added to 900 mL water, along with 1 mL of Folin– Ciocalteau reagent and 2 mL of 10% sodium carbonate solution were together mixed and incubated for 1 h at room temperature. The absorbance was measured at 765 nm with a Shimadzu UV– Visible Spectrophotometer (Shimadzu, Germany). The total phenolic content was expressed as milligram gallic acid equivalent (GAE) per gram sample (Sudha *et al.*, 2007).

Determination of DPPH radical-scavenging capacity

The antioxidant activities were determined on the quince pomace flour, optimized and control baked products by measuring their capacity to scavenge the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical. Antioxidant solution in methanol (0.1mL) was added to 3.9 mL of a 6×10^{-5}mol/L methanol DPPH solution (Brand- Williams *et al.*, 1995).

DPPH = (blank Absorption - sample Absorption/ blank Absorption)× 100 (1)

Scanning Electron Microscopy (SEM) of Sponge Cake

The method of Scanning Electron Microscopy (SEM) was based on the report of (Shyu and Sung, 2010). The sponge cake was frozen in liquid nitrogen. Once the samples were completely frozen, they were cut into small chunks with a size about 0.8 ×0.8 ×0.3 cm and then were freeze-dried for about 2 days. After drying, the surface of the sample was sputter coated with an electrically conductive layer of gold (LADD No. 30800 Sputter Coater, Vermont, USA). The microstructure of the sample was scanned with a Scanning Electron Microscopy (SEM) (Hitachi S-2500 Scanning Electron Microscopy 20 kV, Tokyo, Japan).

Determination of mineral

The mineral analysis was determined according to the AOAC (2007) method using atomic absorption device (Model ContorAA300, Germany). Wavelengths 248.3, 213.9 and 324.7 nm in order to read iron, zinc and copper was used (Ooi *et al.*, 2012).

Experimental Design

Response Surface Methodology (RSM) was used to find the best formulation of sponge cake. The independent factors were quince pomace (0- 15%) and water content (25- 35 %). The responses were moisture content (Y_1), color crust (Y_2), fiber (Y_3), hardness(Y_4), chewiness (Y_5), cohesiveness (Y_6), overall acceptability (Y_7). Minitab Version 16 was used for optimization; the models fitted in this study could also be utilized for optimization purposes using the desirability function.

Results and discussion

Proximate composition and physicochemical characteristics of sponge Cakes-

As shown in table 1, the linear effects of adding QP powder on the fiber content, moisture content and pH were statistically significant ($p \leq 0.05$) and linear effect of water content on the moisture content were statistically significant ($p \leq 0.05$). Measuring the physicochemical characterization of sponge cakes supplemented with different levels of QP powder showed that the pH ranged from 7.01 to 6.52 and was significantly decreased upon addition of QP powder ($P<0.0001$). The ash ranged from 1.05 to 1.66 and was not significantly increased upon addition of QP powder ($P>0.05$). The moisture contents of the sponge cakes significantly increased with increasing QP powder level ($P<0.05$). On the other hand, total dietary fiber content (TDF of cake containing QP powder was as high as 2.425% while it was 0.026% for control cake. This clearly indicates that QP powder can be an alternative source of dietary fiber in cake making.

The density of the cakes increased from 0.569 to 0.651 g/ml, which is also, reflected in the texture measurement values. (Sudha *et al.*, 2007) reported that addition of 0 to 30% apple pomace to the sponge cake reduced cake volume and increased cake density possibly due to the high ability of the apple pomace to preserve water. Uniformity index (UI) and symmetry of the cake, increased significantly ($p<0.0001$) with increasing pomace level.

Baking loss is of concern for the structural transformation of cake and decreases the shelf life of products (Kim *et al.*, 2012). In the present study, water capacity is believed that have increased as a result of the dietary fiber in the quince powder, and baking loss is believed that have decreased as the addition of quince powder increased, causing good effects in terms of texture and the unique quality of bakery products and affecting the initial water holding capacity for volume during the baking process.

Table 1- Regression models for the response variables: fiber, pH, moisture, ash, baking loss, uniformity index, and symmetry

	Fiber	Density	pH	Moisture	Ash	Baking loss	Uniformity index	Symmetry
p (Model)	0.002^{**}	0.000^{****}	0.01^{*}	0.346^{ns}	0.053^{*}	0.000^{****}	0.001^{****}	0.001^{**}
β_0	7.51	0.8	1.71	0.346	0.053	3.17	0.13	3.39
Quince pomce(β_1)	-0.031^{****}	0.008^{****}	-0.029^{ns}	-0.042^{*}	0.004^{**}	-0.13	-0.002^{****}	0.07^{****}
Water(β_2)	-0.033^{ns}	-0.01^{ns}	-0.048^{ns}	0.011^{ns}	0.287^{ns}	0.05^{ns}	-0.006^{ns}	0.13^{ns}
$\beta_1\beta_1$	-6.64^{ns}	-1.72^{ns}	0.004^{**}	0.297^{ns}	-0.808^{ns}	-0.004^{*}	0.0002^{****}	-9.43^{ns}
$\beta_2\beta_2$	0.0005^{ns}	0.00^{ns}	0.0009^{ns}	0.975^{ns}	2.701^{ns}	-0.001^{ns}	9.8^{ns}	-0.002^{ns}
$\beta_1\beta_2$	0.0004^{ns}	-4.28^{ns}	-0.001^{ns}	0.708^{ns}	0.904^{ns}	0.002^{ns}	2.66^{ns}	-5.73^{ns}
p (Lack of fit)	0.599^{ns}	0.12^{ns}	$0.0.579^{ns}$	0.636^{ns}	0.154^{ns}	0.99^{ns}	0.61^{ns}	14.25^{ns}
R^2	90.46	93.69	84.21	97.07	89.34	94.22	95.93	89.21
Adj-R^2	83.64	89.19	72.92	92.70	93.37	90.09	93.02	81.50
CV (%)	0.86	0.81	6.52	2.95	2.39	7.84	7.54	0.56
PRESS	0.071	0.002	0.07	10.317	6.61	0.32	0.0001	0.32

Texture profile analysis of sponge cake (TPA)

The magnitude of F values in table 2 indicates the linear effects of adding QP powder on Springiness and chewiness were statistically significant ($p \leq 0.05$. The linear effects of water content positive significant effect on the cohesiveness. As expected, table 2 shows that increase in quince powder level and water content decreased the cohesiveness, gumminess and chewiness whilst increased the hardness. the quadratic terms of quince powder positive effect on hardness, Springiness and cohesiveness, and the quadratic terms of water content positive effect on hardness, Springiness and chewiness, the interactions of ' quince powder and water content' has not significant effect on the all responses. Kim et al. (2012) reported that the firmness of cake was directly related to the density of tested materials (indirectly to its volume). Overall, as the percentage of quince powder increased,

hardness and gumminess increased whereas cohesiveness and chewiness decreased.

Table 2-Regression models for the response variables: gumminess, cohesiveness, chewiness, springiness, and hardness

	Gumminess	Cohesiveness	Chewiness	Springiness	Hardness
p (Model)	0.002^{**}	0.235^{ns}	0.927^{ns}	0.18^{ns}	0.99^{ns}
β_0	-0.127	59.74	4.89	1.00	8.77
Quince pomace(β_1)	0.023^{****}	0.66^{ns}	0.042^{*}	-2.20^{*}	0.86^{ns}
Water(β_2)	0.019^{ns}	0.039^{*}	0.792^{ns}	-9.90^{ns}	0.8^{ns}
$\beta_1\beta_1$	0.02^{**}	0.045^{*}	0.768^{ns}	-2.20^{*}	0.038^{*}
$\beta_2\beta_2$	-9.74^{ns}	0.45^{ns}	0.036^{*}	1.65^{**}	0.044^{*}
$\beta_1\beta_2$	-3.47^{ns}	1.00^{ns}	0.75^{ns}	1.43^{ns}	0.848^{ns}
p (Lack of fit)	0.133^{ns}	0.163^{ns}	0.944^{ns}	0.837^{ns}	0.817^{ns}
R^2	90.34	96.04	95.12	59.38	92.09
Adj-R^2	83.44	94.63	91.00	60.36	90.00
CV (%)	4.80	1.31	1.15	0.00	1.33
PRESS	0.012	0.04	0.36	2.90	2.03

Color measurement

The magnitude of F values in table 3 indicates the only just linear effects positive contribution of quince powder on the all responses. As shown in table 4 with the increase in quince powder and water content, for crumb color, the L* and b* value decreased but the a* values increased, indicating that a darker, redder, and more yellow crumb was obtained as a result of quince powder substitution. This is probably due to the QP powder contains some sugar and protein which was a synergistic agent for Millard reaction to produce dark red and reddish compounds and also due to present pigments in the QP powder. The crumb of the control sample was lighter and more yellow than any of the other cakes. Kim et al. (2012) also reported that for crumb color, the addition of green tea powder or chive powder caused L* and b* values to decrease while a* value increased. Browning degrees by amino carbonyl reactions and pyrolysis are reported to influence the chromaticity of prepared cake.

Table 3. Regression models for the response variables: colorimetric crust, crumb

	Crust			Crumb		
	L^*	**a^***	**b^***	**L^***	**a^***	**b^***
p (Model)	0.000^{****}	0.000^{****}	0.001^{****}	0.000^{****}	0.000^{****}	0.009^{****}
β_0	20.14	-3.04	38.68	89.83	-6.77	19.35
Quince pomce(β_1)	-2.88^{****}	0.81^{****}	-1.43^{****}	-1.39^{****}	1.37^{***}	-0.86^{****}
Water(β_2)	2.98^{ns}	0.11^{ns}	0.75^{ns}	-1.34^{ns}	-0.27^{ns}	1.15^{ns}
$\beta_1\beta_1$	0.05^{ns}	-0.04^{**}	0.002^{ns}	0.003^{ns}	-0.05^{**}	0.01^{ns}
$\beta_2\beta_2$	-0.05^{ns}	-0.003^{ns}	-0.01^{ns}	0.02^{ns}	0.002^{ns}	-0.02^{ns}
$\beta_1\beta_2$	0.02^{*}	0.004^{ns}	0.008^{ns}	0.01^{ns}	0.004^{ns}	0.004^{ns}
p (Lack of fit)	69.67^{ns}	25.91^{ns}	0.63^{ns}	18.79^{**}	1.99^{ns}	177.01^{****}
R^2	94.17	91.78	92.83	98.80	97.23	84.54
Adj-R^2	90.00	85.90	87.71	97.95	95.25	73.49
CV (%)	0.71	7.39	4.81	0.8	-13.19	0.5
PRESS	172.137	21.58	76.56	71.93	16.86	90.63

Sensory evaluation

Sensory evaluation and consumer acceptance is one of the most quality factors. As shown in table 4, the linear effects of quince powder were statistically significant ($p \leq 0.05$) effect on all responses and linear effect of water content has positive effect on overall acceptability ($p \leq 0.05$). As shown in table 4 with

the increase in quince powder and water content, the chewiness, flavor, texture, taste, and overall acceptability increased. This is probably due to the flavor of quinces's pomace. While, Salehi *et al.* (2016) reported that the addition of carrot pomace reduced the overall acceptance of cake.

Table 4- Regression models for the response variables: texture, hardness, flavor, chewiness, overall acceptability

	Texture	Hardness	Flavor	Chewiness	Overall Acceptability
p (Model)	0.000^{****}	0.002^{ns}	0.003^{**}	0.001^{**}	0.024^{*}
β_0	5.05	2.56	4.24	4.96	10.22
Quince pomace(β_1)	0.1^{****}	0.06^{****}	0.15^{****}	0.089^{****}	0.007^{**}
Water(β_2)	-0.14^{ns}	0.03^{ns}	-0.105^{ns}	-0.10ns	0.023^{*}
$\beta_1\beta_1$	-0.008^{**}	-8.26^{ns}	0.001^{ns}	-0.0038^{*}	0.327^{ns}
$\beta_2\beta_2$	0.002^{ns}	-8.60^{ns}	0.002^{ns}	0.0012^{ns}	0.399^{ns}
$\beta_1\beta_2$	0.004^{ns}	0.0006^{ns}	-0.002^{ns}	0.0013^{ns}	0.182^{ns}
p (Lack of fit)	0.045^{ns}	0.005^{ns}	0.244^{ns}	0.598^{ns}	0.33^{ns}
R^2	94.19	90.84	89.35	91.94	92.45
Adj-R^2	90.04	84.30	81.75	86.18	85.78
CV (%)	2.18	1.25	3.15	3.58	8.33
PRESS	1.09	0.93	0.63	0.38	3.64

Optimization procedure and verification of results

In this study, second order polynomial models were utilized for each response in order to determine the specified optimum conditions. These regression models are valid only in the selected experimental domain. By applying desirability function method, solution was obtained for the optimum covering the criteria. At this point, 12.56% for quince pomace and 29.62% for water content.

The amount of Total phenol content, DPPH radical-scavenging capacity, Scanning Electron Microscopy (SEM) and mineral elements including Iron, Copper, Zinc, Phosphorus and Calcium of optimum cake was evaluated.

Total phenol content of the optimized formulation

The results of evaluation of phenolic cake control, optimum cake, wheat flour and quince powder are shown in table 5. The results of the evaluation showed that both the aqueous and methanolic extracts of the quince powder had a higher phenol than wheat flour. The crust and crumb optimum cake extract also showed higher levels of phenol than the control cake. Although baking or drying at 50°C is considered as undesirable because of induction of oxidative condensation or thermal decomposition of compounds such as phenol (Sudha *et al.*, 2007). The existence and amount of phenol quince powder is consistent with the results (Khoubnasabjafari and Jouyban, 2011) (table7). The higher amount of phenolic content in the optimized cake can also be due to components derived from quince pomace and the formation of intermediates such as enediols and reductions during baking process, which interferes with the colorimetric assay *(Sudha et al.*, 2007).

Table 5. Phenol content of wheat flour, QP powder, control cake and optimized cake

Sample	Water extract (mg/g)	Methanol extract (mg/g)
Wheat flour	2.12 ± 0.01^{E}	3.12 ± 0.001^{F}
Quince pomace	6.14 ± 0.01^{A}	6.16 ± 0.001^{A}
Control cake crust	2.83 ± 0.01^{F}	3.42 ± 0.001^{B}
Control cake crumb	3.44 ± 0.01^{D}	4.26 ± 0.001^{C}
Optimized cake crust	3.48 ± 0.01^{C}	3.81 ± 0.001^{D}
Optimized cake crumb	3.89 ± 0.01^{B}	4.43 ± 0.06^{B}

The different letters in each column show a significant difference at $p<0.05$

DPPH radical- scavenging capacity of the optimized formulation

The results of the DPPH radical-scavenging capacity are shown in table 6. The results of the study showed that the optimized cake had more the percentage of radical- scavenging capacity (RCS) than control cake. This is due to the presence of several compounds with antioxidant properties in quince powder. (Alesiani *et al.*, 2010) examined the DPPH radical inhibitory capacity in a research into the antioxidant activity of the quince peel. The results of this study showed the strongest antioxidant capacity for quercetin flavone and 3-o-rutinoside. In particular, quercetin reduced the DPPH radical by 56.7%. Also, the quince acid derivatives, chromogenic acid and neochlorogenic Acid determined an average DPPH radical reduction of 35.0% respectively (Alesiani *et al*., 2010).

Scanning Electron Microscopy (SEM) of the optimized formulation

Figures. 1. Shows the structure of the pores of the optimized and control cake. The results of the study showed that the addition of QP powder caused the formation of fine, the uniformity cavities and increase in the number of cavities in the cake's structure. The reason for this difference, the presence of quince powder in the optimized cake structure and its effect on the wheat flour gluten network (Bhat and Anju, 2013).

Proximate composition of mineral elements characteristics of the optimized formulation

Table 7 shows the results of atomic absorption of the quince powder, the control and the optimized cake. Measurement of mineral elements by atomic absorption device showed that the mineral elements of quince powder and cake were better than the control cake sample. According to research results, (Carbonell- Barrachina *et al*., 2015), fruit is an important source of minerals such as iron, phosphorus and calcium, which is consistent with the results of this study (Carbonell-Barrachina *et al.*, 2015).

Table 6- DPPH radical-scavenging capacity

Sample	DPPH (%)
Quince pomace	67.62± 0.81 ^{A}
Control cake	0.07±0.01 ^{C}
Optimized cake	18.5±1.69 ^{B}

The different letters in each column show a significant difference at $p<0.05$

Table 7- The amount of minerals, quince powder, the control cake and the optimized cake (mg/l)

Mineral	Quince powder	Control cake	Optimized cake
Fe	0.386±0.04^{a}	0.263±0.03^{b}	0.361±0.07^{a}
Cu	0.072±0.01^{a}	0.002±0.001^{c}	0.010±0.004^{b}
Zn	0.129±0.05^{a}	0.066±0.006^{b}	0.128±0.003^{a}
P	0.145±0.02^{a}	0±0.00^{c}	0.01±0.001^{b}
Ca (mg/kg dry Weight)	1920±5.14^{a}	340±2.47^{c}	1160±4.98^{b}

The different letters in each row show a significant difference at $p<0.05$

Conclusion

Response surface methodology was an efficient statistical tool to model the influence of quince pomace and water contact of sponge cake on quality, chemical, sensory and image properties of sponge cake and fiber characteristics. Quince pomace having high amount of TDF can function as a valuable source of dietary fiber in cake making. Addition of quince pomace in cake making can avoid the addition of other flavoring ingredients as the cakes prepared with quince pomace had pleasant fruity flavor. Quince pomace also has the potential for use in cake making as a good source of polyphenols which has antioxidant properties. These results also suggested that by modifying the proportion of these components, a large range of variations may be obtained.

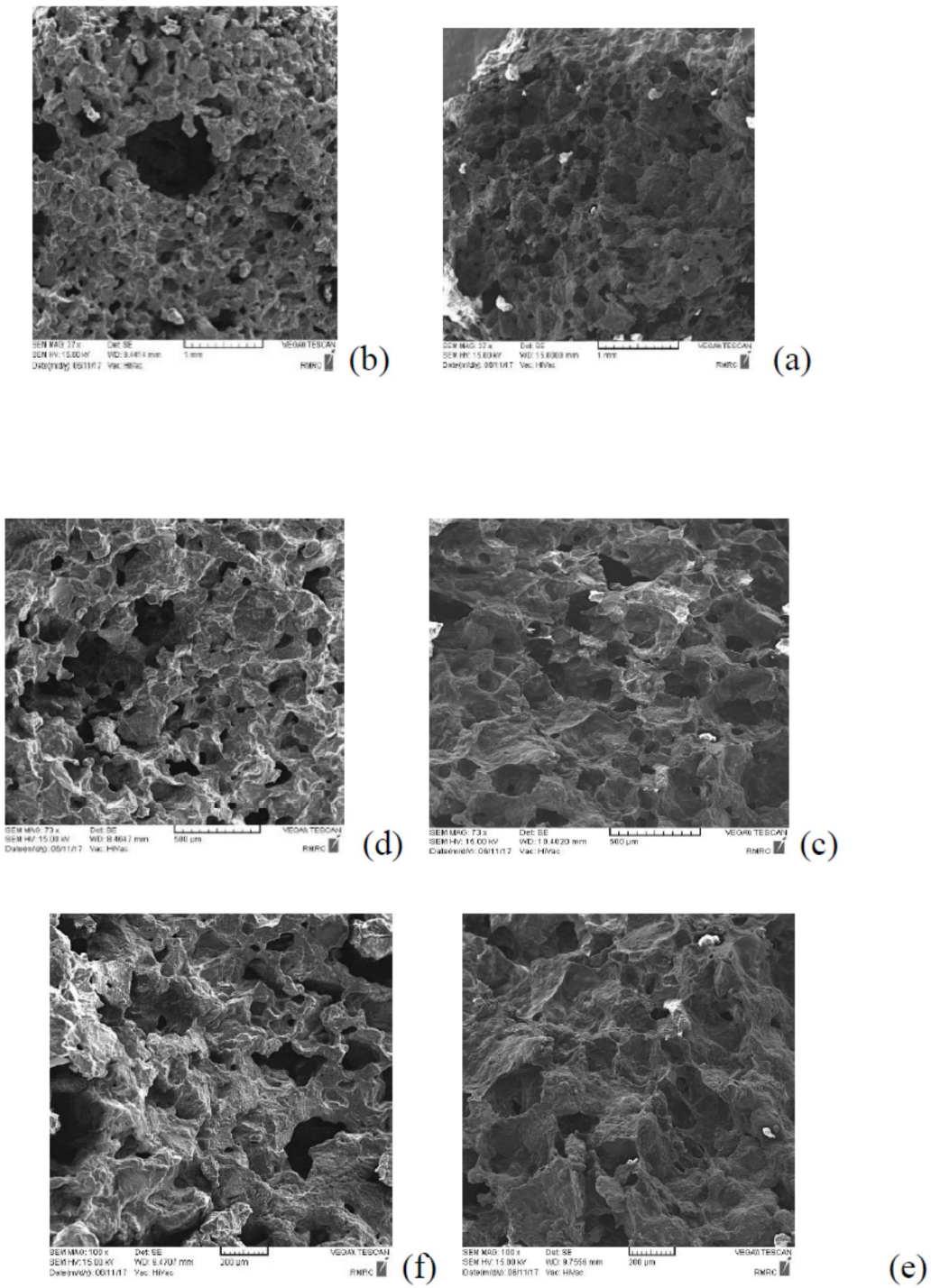

Fig. 1. Scanning electron microscopy ((a) (×37) of optimized cake; (b) (×37) Control cake; (c) (×73) of optimized cake; (d) (×73) Control cake; (e) (×100) of optimized cake (f) (×100) Control cake .

In addition to establishing best formulation, the image processing and texture analysis have been shown to be useful tools to investigate, approximate and predict a large number of cake properties. This study was preliminary and needs to be studied at molecular level in detail.

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نشریه پژوهشهای علوم و صنایع غذایی ایران
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بهبود ویژگی‌های فیزیکوشیمیایی و ارزش تغذیه‌ای کیک اسفنجی با استفاده از پسماند میوه به

عادیه انور[1]- بهزاد ناصحی[2*]- محمد نوشاد[3]- حسن برزگر[4]

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چکیده

در این پژوهش، تأثیر افزودن پودر پسماند به (15-صفر درصد) و مقدار آب (25 -35 درصد) بر خصوصیات فیزیکوشیمیایی و حسی کیک اسفنجی مورد مطالعه قرار گرفت. نتایج نشان داد که افزایش مقدار پسماند به باعث افزایش فیبر رژیمی، سفتی و پذیرش کلی کیک و کاهش مقدار رطوبت و دانسیته نمونه‌ها شد. همچنین افزایش پودر پسماند میوه به سبب افزایش ویسکوزیته و قوام خمیر شد. براساس روش تابع مطلوبیت، کیک تولید شده حاوی 12/56 درصد پودر پسماند میوه به و 29/62 درصد آب دارای بهترین و بیشترین کیفیت فیزیکوشیمیایی بود. کیک تولید شده با فرمول بهینه دارای (8/32 mg/g) ترکیبات فنلی، (0/361 mg/Kg dry weight) آهن و (1160 mg/kg dry weight) کلسیم بود. نتایج SEM نشان داد که افزودن پودر پسماند میوه به باعث افزایش تعداد حفرات و یکنواختی حفرات در ساختمان کیک شده است.

واژه‌های کلیدی: کیک اسفنجی، پسماند میوه به، ویژگی‌های فیزیکوشیمیایی، RSM

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مندرجات

نشریه پژوهش های علوم و صنایع غذایی ایران

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