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Color and weight changes of fresh-cut banana slices coated by quince seed gum: Effect of concentration, storage temperature and duration

R. Farahmandfar[1*], M. Asnaashari[2], M. Amraie[3], M. Salehi[3]

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Abstract

The consumer's acceptance significantly reduces during the storage of fresh cut fruits. Edible coating is one of the most innovative ways to maintain quality and improve shelf life of fresh fruits and vegetables. The objective of this study was to assess the suitability of quince seed gum (QSG) at different concentrations (0.5, 1 and 1.5%) as edible coatings for banana slices and to determine their influence on changes in physicochemical properties during storage at 4°C and 40°C. Data on shrinkage, weight loss and color were collected and subjected to statistical analysis. Banana slices which coated with 1% of QSG and stored at 4°C showed significantly better physicochemical characteristics. Higher temperatures result in more rapid changes of quality parameters. On the other hand, samples coated with gum reduced the weight loss and shrinkage during storage. It is recommended that 1% quince seed gum can be used to reduce the surface fresh-cut banana browning.

Keywords: Banana, Coating, Fresh fruits cut, Quince seed gum

Introduction

Fresh fruits and vegetable are susceptible to decay, and loss of their important beneficial components, if they are not preserved properly (Asnaashari, Tajik, & Khodaparast, 2015). Fruit decay is caused by several agents like loss of quality and shorten the shelf life, loss of water and moisture, process of Millard, texture damage and microbial contamination (Rojas-Graü, Tapia, & Martín-Belloso, 2008). Several techniques, like low temperature, controlled atmosphere packaging (CAP), and modified atmosphere packaging (MAP) were applied to maintain the quality of fresh food. However, each preservative technique has positive and negative effects on freshness and quality of fruits.

Edible coating exerts to maintain the quality and freshness of fruit slices and vegetables, hinder the microbial activity and decreasing metabolic processes like respiration in the fresh fruit. This technique used for increase the storage time of the fresh cut fruit (Fisk, Silver, Strik, & Zhao, 2008; Gonzalez-Aguilar et al., 2008; Vargas, Pastor, Chiralt, McClements, & Gonzalez-Martinez, 2008).

Edible coatings have demonstrated the capability of improving food quality and prolonging shelf life of fresh fruits and vegetables. Edible coatings can be used to help in the preservation of minimally processed fruits, providing a partial barrier to moisture, oxygen and carbon dioxide, improving mechanical handling properties, carrying additives, avoiding volatiles loss, and even contributing to the production of aroma volatiles (Olivas & Barbosa-Cánovas, 2005).

Gums are polysaccharides, which have high molecular weight and easily disperse in water, thus they can increase the viscosity of solutions (Asnaashari, Motamedzadegan, Farahmandfar, & Rad, 2016). Gums obtained from different sources (plant, epiphyte and animal extracts) are widely used in the food systems for various purposes, such as thickeners, stabilizers, gelling agents and texture modifiers. Hydrocolloids from plants have also the advantage over those from

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animals due to more acceptability by consumer. Plants seeds are a traditional and ancient source of gums (Koocheki, Taherian, Razavi, & Bostan, 2009).

Postharvest processes such as respiration can be reduced by coating with protective compound, by changing the atmosphere around the fruits, thereby delay ripening. Some researchers showed that edible coatings preserve quality and extended shelf life of fruits (Ali, Muhammad, Sijam, & Siddiqui, 2011; Brasil, Gomes, Puerta-Gomez, Castell-Perez, & Moreira, 2012), apples (Chiumarelli & Hubinger, 2014), strawberries (Gol, Patel, & Rao, 2013), red bell peppers (Poverenov et al., 2014), and guavas (Hong, Xie, Zhang, Sun, & Gong, 2012).

Quince seeds contain hydrocolloid, which is native of middle east which include Caucasus region, Iran, Dagestan, Syria, Afghanistan and Antalya (Trigueros, Pérez-Alvarez, Viuda-Martos, & Sendra, 2011). Experiment of quince seeds gum shows that its seeds have antioxidant compounds and have medicinal use, for diarrhea and stomach ulcers (Šarić-Kundalić, Dobeš, Klatte-Asselmeyer, & Saukel, 2011). Qum extracted from quince seed can apply for encapsulation of essential oil (Jouki, Mortazavi, Yazdi, & Koocheki, 2014), and have positive effect for removal of T-2 toxin in dermal of rabbit (Hemmati, Kalantari, Jalali, Rezai, & Zadeh, 2012). Study of quince seed gum shows this seed have several polysaccharide such as cellulose (Vignon & Gey, 1998).

Banana is a fruit usually growth in tropical, and popular among people. It includes mineral and micronutrient such as vitamin which are positive for health human. While harvested banana, they are unripe and placed at room temperature. Banana become decay quickly, especially to be ripe, during storage (Golding, Shearer, Wyllie, & McGlasson, 1998; Marriott & Palmer, 1980). Purpose of this work was to determine the effect of quince seed edible coating on quality and shelf life extension of cut slice banana and also maintain of moisture and its possible effect on discoloration and shrinkage of cut slice during storage.

Materials and Methods

Extraction and preparation of solution gum

Quince seed purchased from the local market at Mahmudabad in Iran. QSG prepared according to the method of (Jouki, Yazdi, Mortazavi, & Koocheki, 2013). About 10 g of quince seeds sieved and washed with its triple weight of ethanol (96%, w/v) for 5 min under constant stirring. Then ethanol was removed and the seeds dried in an oven at 45˚C. Aqueous quince seed mucilage was extracted from whole seeds using distilled water (water to seed ratio of 30:1). Then, the swelled seeds were stirred with a rod paddle blender (Rondo-2500, KA702, France) at 6000 rpm and 50˚C for 20 min to scrape the mucilage layer off the seed surface. The solutions were then filtered with cheese cloth and the obtained mucilage was dried by an oven at 45˚C. Solution of gum (w/v) was prepared by slowly dissolving 0.5, 1, and 1.5 g gum powder in 100 ml distilled water and constant stirring at 45°C for 15 min. Then solution gum prepared placed in 4°C temperature.

Preparation of banana slices

Banana was purchase from local market (Mahmoudabad in Iran). Banana fruit for this study were selected based on uniformity in size and color and lack of spot. Banana slices was similar in size and shape. Then they were immersed into quince seed coating solution with different concentrations for one minute. Weight and diameter of each of samples is 3.5 to 5.5 g and 0.5 ± 0.05 cm.

Weight loss determination

Each slice banana was weighted on each inspector. Weight loss calculated by comparison the weight of coat sample at its initial condition and its final condition (Farahmandfar, Mohseni, & Asnaashari, 2017).

Shrinkage

Change of size and diameter toward smaller defined as shrinkage (Farahmandfar et al., 2017). Reason of this change was egress of banana moisture during storage, which cause

change pressure balance between inside and outside banana. This stress leads shrinkage in material. Apparent shrinkage (Sapp) is defined as the ratio of the apparent volume at a given moisture content (V_{app}, m^3) to the initial apparent volume of materials before processing (V_{0app}, m^3):

$$S_{app} = \left(\frac{V_{app}}{V_{0app}} \right) \times 100 \qquad (1)$$

Analysis of color

The changes in color of banana slice was calculated by measuring the L*, a* and b* parameters (Eshghi, Asnaashari, Haddad Khodaparast, & Hosseini, 2014). Sample illumination was achieved with two fluorescent lights (10 W, 40 cm in length), were placed in a wooden box (0.5×0.5×0.5 m^3). The interior walls of the box were painted black to minimize background light. A color digital camera was located vertically at a distance of 20 cm from the sample (Panasonic, Model DMC-FS42, Japan). Since the computer vision system perceived color as RGB signals, which is device-dependent, the images taken were converted into L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness) units to ensure color reproducibility. The measure color parameters for estimate changes in total color and whiteness index (No, Meyers, Prinyawiwatkul, & Xu), based on following equation:

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \qquad (2)$$

While L*, a* and b* are the color parameter values of the white standard backgrounds (L*= 93.49, a*= -0.25 and b*= -0.09) and L*, a* and b* are the color parameters of sample.

Statistical Analysis

For statistical analysis, a completely randomized factorial design with at least three replications for each treatment was applied. Analysis of variance (ANOVA) was performed to study the differences between the effects of coating, storage temperature and storage time on quality parameters of banana slice using (SPSS). Between groups means were analyzed by Duncan's multiple-range test. Statistical significance was set at 0.05 probability level.

Results and Discussion

Shrinkage

Shriveling and withering can cause loss of weight, lead to decrease consumer acceptance. The means of shrinkage percentage during storage at two temperatures of 4°C and 40°C are shown in Table 1 and 2. for coated banana slices sample and the control.

Table 1. Effect of coating treatment and storage time on shrinkage (%) at 4°C

	Storage time (hours)									
Treatments	**0**	**12**	**24**	**36**	**48**	**60**	**72**	**84**	**96**	**108**
Control	88±0.98^{a}	04±0.33^{a}	66±0.36^{a}	.07±0.39^{a}	.99±0.09^{b}	.93±0.12^{d}	.66±0.64^{b}	67±1.64^{a}	.48±0.96^{b}	72±0.44^{a}
QSMF 0.5%	02±3.93^{a}	34±3.79^{a}	89±5.37^{a}	.92±3.76^{a}	.12±0.83^{a}	.57±1.83^{a}	.99±1.28^{a}	12±1.43^{a}	.91±1.06^{a}	06±3.37^{a}
QSMF 1%	65±0.47^{a}	92±0.39^{a}	46±0.87^{a}	.78±0.93^{a}	.48±0.72^{a}	.15±0.64^{b}	.74±2.67^{b}	70±4.98^{a}	.09±0.17^{a}	70±1.03^{a}
QSMF 1.5%	78±0.38^{a}	30±0.20^{a}	82±0.20^{a}	.47±0.37^{a}	.99±1.06^{a}	.74±0.99^{c}	66±1.66^{ab}	39±1.73^{a}	.68±0.55^{a}	31±3.35^{a}

Different letters in each row show significant difference at P < 0.05. (C, Control/ Quince seed mucilage fraction)

The result of experimental showed with increasing the storage time, shrinkage value enhanced while the volume of banana slices became smaller. Moreover, as the temperature of the storage time increased, the shrinkage was also enhanced. So that, the rate of increasing shrinkage values of control samples and sample with 0.5% were higher than the

other samples. However, the percentage of shrinkage at samples with higher concentrations of gum was lower, remarkably. The reason was that gum with higher concentrations could easily inhibit water loss. In this study, at temperatures 4°C and 40°C, samples with 1.5% of the gum showed the lowest shrinkage value. These results showed quince seed gum could be a marvelous barrier in banana slices.

Weight loss

Table 3 and 4 showed the effect of different coating and storage temperatures on weight loss. Results showed that it was a significant difference between control sample and coated samples at both temperatures 4 and 40°C, which explains that quince gum prevents the loss of water and moisture in coated samples. Although weight loss in control sample higher than all coated sample, in higher temperatures more changes of weight loss were happened. However, when fruit stored, its moisture was removed and caused weight loss. Coating the samples with quince seed gum in all concentrations was not change the weight loss significantly. However, at 1.5% of the quince seed gum, the weight loss was slightly lower. As it was shown Table 2, at high temperature (40°C), the rate of aspiration and metabolic process in banana was higher, that caused diminish substrates resulting into more decrease weight loss of banana. Indeed, one of the agent's effects to the weight loss is respiration. The principle mechanism of weight loss from fruit is exit of water from inside fruit to environment by vapor pressure at different locations in banana.

Color changes evaluation

Color change in fresh fruits is one of the reasons for the reduction of consumer acceptability during storage (Farahmandfar *et al*., 2017). Effect of edible coating and storage conditions on color parameter of fresh-cut banana was demonstrated by ANOVA Duncan test. The experimental results for the color changes in fresh-cut banana coated with the different concentrations of gum and uncoated samples by lightness L *, redness a*, yellowness b* and total color difference (ΔE) in the storage temperatures 4 and 40°C are shown in Figure 1 and 2. The results showed that the fresh-cut banana using coating and store at low temperature cause reduce of color changes. L* and b* changes increased with increasing time at both temperatures studied. Also, it was observed that with an increase in storage time, a* and ΔE increased. In this research, all of the coating samples reduced L* changes during storage.

Table 2. Effect of coating treatment and storage time on shrinkage (%) at 40°C.

	Storage time (hours)						
Treatments	**0**	**16**	**24**	**32**	**40**	**48**	**56**
Control	18.90 ± 0.00^{Fa}	19.47 ± 0.19^{Eab}	19.69 ± 0.09^{Eab}	20.28 ± 0.29^{Da}	21.89 ± 0.00^{Cab}	26.28 ± 0.60^{Bb}	31.49 ± 0.13^{Aa}
QSMF 0.5%	19.85 ± 3.62^{Ea}	21.49 ± 2.81^{Eab}	22.66 ± 3.45^{Eab}	25.65 ± 4.93^{Da}	28.78 ± 3.99^{Ca}	31.42 ± 2.33^{Ba}	34.68 ± 3.14^{Aa}
QSMF 1%	16.09 ± 3.57^{Ea}	16.21 ± 3.59^{Eb}	16.41 ± 3.71^{Eb}	17.95 ± 2.77^{Da}	19.62 ± 1.43^{Cb}	27.65 ± 0.19^{Bab}	33.19 ± 3.29^{Aa}
QSMF 1.5%	21.78 ± 0.89^{Ea}	22.94 ± 1.00^{Ea}	24.66 ± 2.47^{Da}	25.09 ± 2.84^{Da}	27.71 ± 3.74^{Ca}	32.95 ± 2.62^{Ba}	36.61 ± 2.07^{Aa}

* Means followed by the same capital letter is in line and the same lower case letter in the columns, do not differ statistically at P>0.05.

The data showed that with an increase of storage time, sample treated with concentration 1% of gum quince seed had the lowest value of L* changes. For example, L* changes of control, QSMF 0.5, 1 and 1.5% at the end of storage at 4°C were -16.21, -13.38, -12.28 and -12.7, respectively. The lowest value of b* changes was observed in 1% QSMF during time. The lowest and the highest changes of a* and ΔE have seen for sample treated with concentration 1% of gum and control, respectively. Usually, changes in L* value are appertained to tissue surface modifications caused by polyphenol oxidases activity, so this reduction can be relative to the obstacle formed by the coating on samples (Soliva-Fortuny, Lluch, Quiles, Grigelmo-Miguel, & Martín-Belloso, 2003).

Regarding L* changes, several researchers have indicated that a decrease in this parameter associated to an increase in pigment concentration and the using of a coating, since this act could restrict the action of polyphenol oxidase as an oxygen barrier (Rojas-Graü et al., 2008). L* values decreased for coated and uncoated sample during the storage time, possibly because of surface moisture loss, which could be caused seen darker color (Perdones, Sánchez-González, Chiralt, & Vargas, 2012).

Table 3. Effect of coating treatment and storage time on weight loss (%) at 4°C.

	Storage time (hours)									
Treatments	**0**	**12**	**24**	**36**	**48**	**60**	**72**	**84**	**96**	
Control	10.80±0.24^{a}	24.61±0.98^{a}	36.94±0.10^{a}	46.03±0.49^{a}	51.88±0.64^{a}	55.81±1.10^{a}	62.66±3.15^{a}	63.57±3.67^{a}	64.26±3.89^{a}	64.85±3.95^{a}
QSMF 0.5%	7.00±1.23^{b}	17.64±0.20^{b}	30.20±0.16^{b}	38.39±0.32^{b}	44.94±0.00^{b}	48.57±1.23^{b}	56.22±0.12^{b}	57.13±0.62^{b}	58.04±0.04^{a}	58.77±0.34^{a}
QSMF 1%	2.55±1.03^{c}	18.68±0.19^{b}	30.94±0.31^{b}	39.83±0.35^{b}	45.55±0.26^{b}	50.25±0.70^{b}	57.30±0.88^{b}	58.12±1.47^{ab}	58.74±1.78^{a}	59.04±1.63^{a}
QSMF 1.5%	3.98±0.02^{c}	14.46±0.50^{c}	30.92±2.11^{b}	40.56±1.57^{b}	47.07±1.90^{b}	51.99±2.08^{b}	58.17±1.37^{ab}	59.63±0.38^{ab}	59.75±1.83^{a}	59.96±1.54^{a}

Different letters in each row show significant difference at P < 0.05. (C, Control/ Quince seed mucilage fraction)

Table 4. Effect of coating treatment and storage time on weight loss (%) at 40°C.

	Storage time (hours)							
Treatments	**0**	**16**	**24**	**32**	**40**	**48**	**56**	**64**
Control	48.47±0.42^{Da}	64.59±0.5^{Ca}	69.63±0.5^{Ba}	70.15±0.18^{Ba}	71.14±0.25^{Aa}	71.73±0.48^{Aa}	72.03±0.59^{Aa}	72.12±0.72^{Aa}
QSMF 0.5%	41.89±1.88^{Eab}	57.55±1.35^{Dab}	65.96±1.50^{Ca}	70.15±0.21^{Ba}	70.56±0.47^{Aa}	70.98±0.45^{Aa}	71.19±0.45^{Aa}	71.29±0.60^{Aa}
QSMF 1%	42.58±2.63^{Dab}	59.55±1.40^{Cab}	67.45±1.39^{Ba}	68.73±0.48^{Ba}	69.32±0.56^{Aa}	69.61±0.44^{Aa}	69.81±0.45^{Aa}	69.90±0.33^{Aa}
QSMF 1.5%	35.79±6.22^{Db}	51.88±7.97^{Cb}	62.12±8.64^{Ba}	64.03±9.74^{Ba}	67.26±6.34^{Aa}	67.64±6.08^{Aa}	67.84±6.10^{Aa}	68.03±5.84^{Aa}

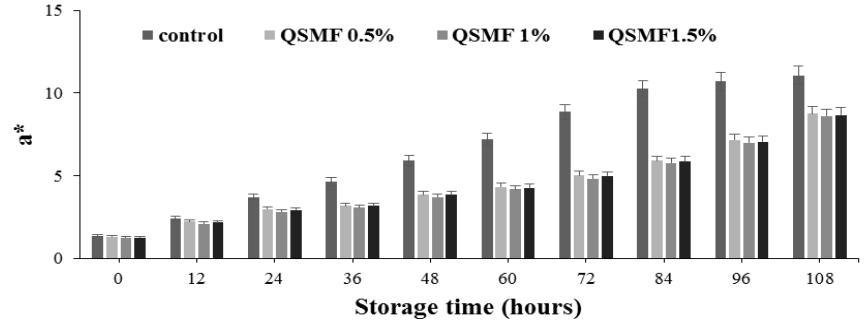
* Means followed by the same capital letter is in line and the same lower case letter in the columns, do not differ statistically at P>0.05.

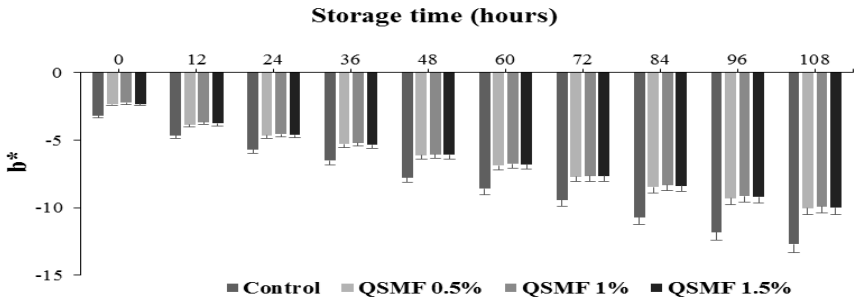
In similar research de Aquino, Blank, and de Aquino Santana (2015) exhibited that an edible chitosan coating blending guavas coated with edible coating containing 2.0% cassava starch, 2.0% chitosan and 3.0% Lippia gracilis Schauer genotype mixtures had more effect in preventing the browning of during storage than no coating. As a* value changes was shown, an increase of a* means a higher browning index (Rojas-Graü, Sobrino-López, Soledad Tapia, & Martín-Belloso, 2006). Control samples exhibit forceful increase in a* values that associate to changes in the browning index, whiles for the treated samples with quince seed gum showed significant difference ($p > 0.05$). So, coating have the best effective property against changes a* increase for 1% QSMF with 8.67 at 4°C after 120 hours of storage time and 13.02 in 40°C after 84 hours of storage time. Rojas-Graü et al. (2008) express that higher a* value is pointing to increasing browning index in apples during storage. As a consequence, this study is recommended that coating with quince seed gum (1%) could be used to reduce the surface fresh-cut banana browning. This affection is most probably ascribed to a decrease in oxygen accessible (Zambrano-Zaragoza, Mercado-Silva, Gutiérrez-Cortez, Castaño-Tostado, & Quintanar-Guerrero, 2011).

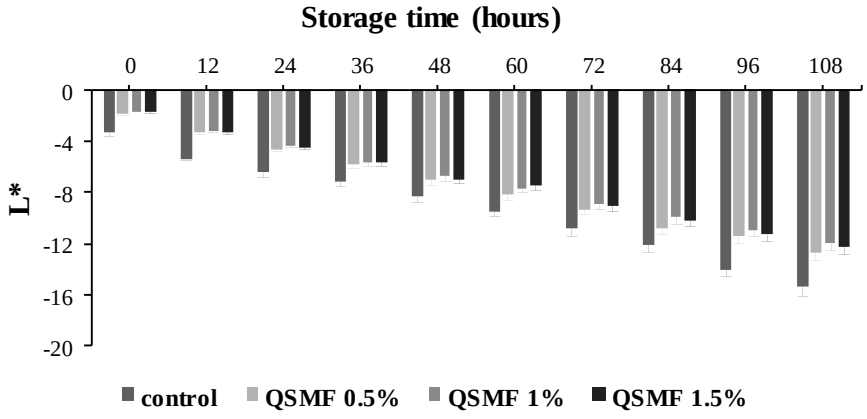
Conclusion

This study showed that several physicochemical quality changes of stored banana slice were dependent on the storage conditions (time and temperature) and used concentration of coating. Most changes were accelerated at higher temperatures, and hence, lower temperatures suggested better choices

for storage. Quince seed gum has beneficial effects on maintain quality and extend the shelf life. Samples of coated with gum reduced the weight loss and shrinkage during storage. In addition, the results of this study is recommend that coating with quince seed gum (1%) could be used to reduce the surface fresh-cut banana browning.







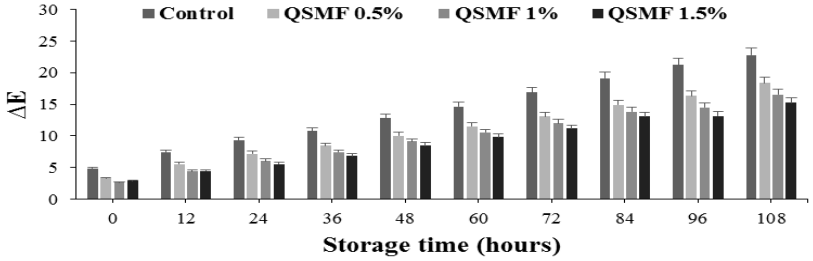
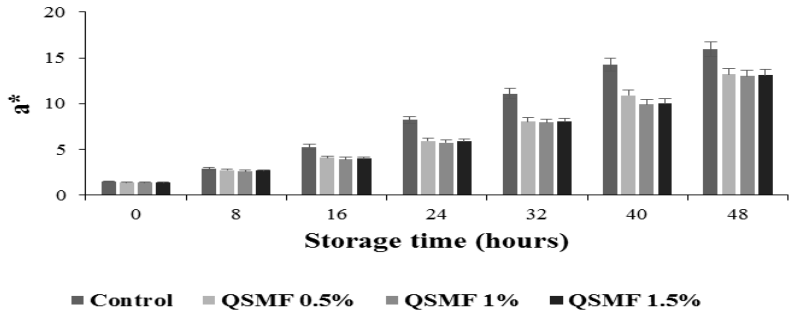
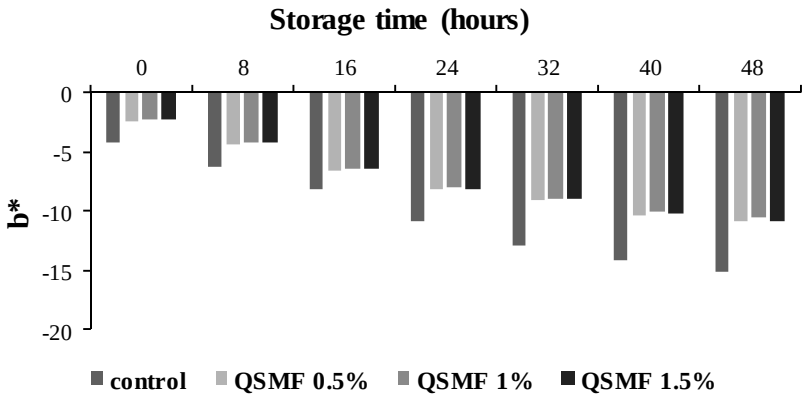
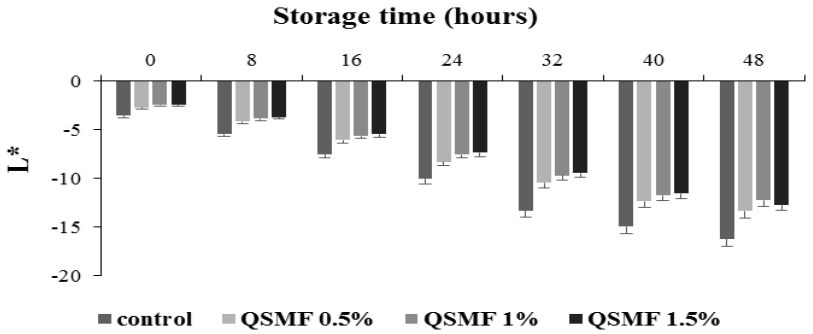


Fig. 1. Effect of coating treatment and storage time on changes of color parameters at 4°C.







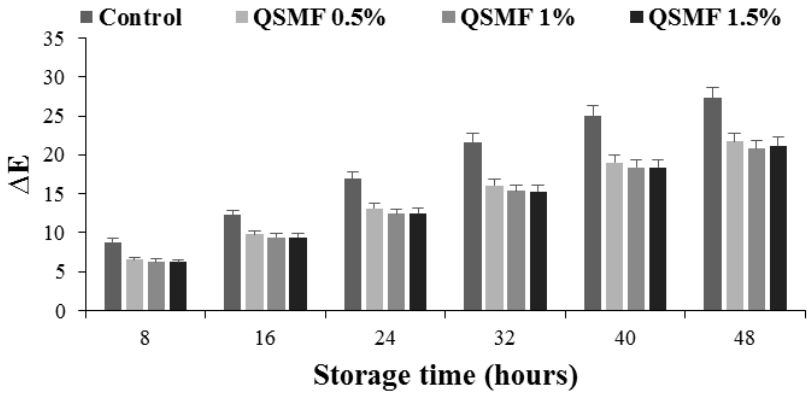


Fig. 2. Effect of coating treatment and storage time on changes of color parameters at 40°C.

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کیفیت برش‌های موز پوشش داده شده با صمغ به: اثر غلظت، دما و مدت انبارداری

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چکیده

پذیرش مصرف‌کننده در طی انبارداری میوه‌های برش یافته به شدت کاهش می‌یابد. پوشش‌های خوراکی یکی از جدیدترین روش‌ها بـرای حفـظ کیفیت و افزایش دوره ماندگاری میوه‌ها و سبزی‌های تازه است. هدف از این مطالعه ارزیابی میزان مناسب بودن صمغ دانه "به" در غلظت‌های مختلف (0/5، 1 و 1/5 درصد) به‌عنوان پوشش‌های خوراکی برای برش‌های موز و تعیین تاثیرات آنها بر تغییرات خصوصیات فیزیکوشیمیایی طی انبارداری در 4 و 40 درجه سانتی‌گراد بود. داده‌های چروکیدگی، افت وزن و رنگ جمع‌آوری شد و مورد آنالیز آماری قرار گرفت. برگه‌های موز پوشش داده شده با 1% صمغ "به" و نگهداری شده در دمای 4 ^{o}C خصوصیات فیزیکوشیمیایی بهتری نشان دادند. افزایش دما منجر به تغییرات سریع‌تر در خصوصیات کیفی شد. از طرف دیگر، نمونه‌های پوشش داده شده با صمغ میزان افت وزن و چروکیدگی را طی انبار داری کاهش دادند. توصیه می‌شود که غلظت 1% صمغ "به" می‌تواند برای کاهش قهوه‌ای شدن سطح برش‌های موز مورد استفاده قرار گیرد.

واژه‌های کلیدی: موز، پوشش، کیفیت، صمغ دانه به

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Optimization of low-calorie sweet cream formulation via response surface methodology

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Abstract

In this study, the effect of stevia (0-0.04 g/100g) as a sucrose replacer, milk protein concentrate (mpc) (0-4 g/100g), and modified waxy corn starch (0-3 g/100g) as fat replacers on the physico-chemical and sensory characteristics of 15% fat cream were analyzed using a central composite rotatable design. Response surface methodology was used for optimization of low calorie cream formulation. Results showed that an increase in sucrose substitution with stevia and mpc concentration was followed by an increase in cream acidity, while pH decreased. Increasing sucrose substitution with stevia in cream decreased firmness, apparent viscosity and consistency, whereas increasing concentration of milk protein concentrate and modified starch increased the cream firmness, apparent viscosity and consistency. However, according to multiple response optimization, the optimum levels of 0.034 g/100g stevia, 1.64 g/100g mpc and 2.30 g/100g modified starch predicted acidity 0.15% acid lactic, pH 6.5, firmness 1.4 N, apparent viscosity 28730.3 mPa.s and consistency 0.52 cm/30 s. The calorie value of formulated cream was 46.44% less than the control sample (cream with 30% fat and 12% sucrose), and no significant difference in total acceptance between them was found, while formulated cream had higher score for taste and creamy state.

Keywords: Low calorie cream, Stevia, Milk protein concentrate, Modified starch, Response surface methodology, Optimization

Introduction

Sweet Cream is a milk product comparatively rich in fat separated from milk which takes the form of an emulsion of fat-in-skimmed milk (FAO, 2000). Sweet cream due to the high level of sucrose and fat content produces high calories. The relationship between dietary fat and calorie and the development of cardiovascular disease, hypertension, diabetes and obesity has created the increased demand for consumption of low calorie products. Therefore, food manufacturers' response to consumer demands has led to rapid market growth for low calorie products (Thaiudom *et al*. 2011). However, in addition to nutrition, fat influences rheological properties, sensory characteristics (flavor, mouthfeel and texture) and stability of fat-based product such as emulsion (Drake *et al.* 1999). Sucrose is not consumed only for its sweetness. It also has many functional properties in foods that make it useful as a bulking agent, texture modifier, mouthfeel modifier and preservative (Salminen *et al.* 2002). By increasing total solids Sucrose increases firmness and also provides sweetness and calories of product. These properties are very hard to reachieve in low/without fat and sucrose formulations. Modification of these products by using appropriate fat and sucrose replacers is often viewed as an effective way to overcome such problems due to the reduction in fat and sucrose content (Drake *et al.* 1999; Worrasinchai *et al.* 2006) but those properties might be changed (Thaiudom *et al.* 2011).

Stevia is a natural sweetener that is used as sucrose substitute (Cardello *et al.* 1999). The leaves of *Stevia Rebaudiana Bertoni* accumulate diterpenoids with sweet taste which is known as Steviol glycosides (Brandle *et al*. 2007). The major sweet compounds are rebaudioside A and stevioside. Rebaudioside

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A is 200-300 times sweeter than sucrose on a weight basis (Lindley, 2012). Rebaudioside A (purity>97% by HPLC) is also known as rebiana. Rebiana provides zero calories and has a clean sweet taste with no significant undesirable taste characteristics (Prakash *et al*, 2008). Milk protein concentrates (MPC_s) are dairy protein powders with protein content in the range of 42-85%. They are manufactured by removing the lactose and minerals from skim milk using membrane technology. The retentate obtained from this process is further concentrated by evaporation, and spray dried. MPCs are used as protein-based fat substitutes for their nutritional and functional properties. Higher-protein MPCs provide protein enhancement and a clean dairy flavor without adding significant levels of lactose to food and cream formulations. MPCs are multifunctional ingredients and provide benefits such as emulsification (Banach, 2012; Patel *et al.* 2006). Sodium octenyl succinate starch (E1450) is made by substituting hydroxyl groups in the polysaccharide chains by anhydrous octenyl succinic under alkaline conditions (Tesch *et al.* 2002). This polysaccharide exhibits amphiphilic character which enhances its emulsifying property. E1450 is also used as thickening agent by forming network with other polymers in aqueous solution through hydrophobic interaction. This increases the viscosity of the system and can stabilize droplet particles (Ortega-Ojeda *et al.* 2005). Various researches have been carried out about the use of sucrose and fat replacers in dairy products. Bagheri *et al.* (2014) investigated the possibility of substituting sugar with stevioside in breakfast cream and reported that if the bitter taste of stevioside can be covered, it could be used as an appropriate sugar replacer.

Response surface methodology (RSM) is a collection of statistical and mathematical technique useful for developing, improving and optimizing the formulation (Myers *et al.* 2009).

The objective of this study was to investigate the effects of fat replacers (MPC, and modified starch (MS)) and sucrose substitute (stevia) at different levels on the physico-chemical and sensory properties of low calorie sweet cream and optimize its formulation ingredient using RSM to obtain low calorie sweet cream with acceptable textural and sensory properties.

Materials and Methods

Pasteurized and homogenized milk with 2% fat content and sterilized and homogenized cream with 30% fat content were purchased from Damdaran Company, Tehran. MPC (with 70% protein content) and MS (sodium octenyl succinate waxy corn starch (E1450)) were purchased as fat replacers from Westland Company, New Zealand, and Pars Khoosheh Pardaz Company, Shiraz, Iran, respectively. Rebaudioside A 97% was obtained from Techfa Industrial Services Co., Tehran, Iran, as sucrose replacer and sucrose was procured from a local market.

Experimental design

Response surface methodology (RSM) which involves design of experiments, selection of levels of variables in experimental runs, fitting mathematical models and, finally, selecting variables' levels by optimizing the response was employed in the study. A central composite rotatable design (CCRD) was used to design the experiments comprising three independent variables at five levels each (Myers *et al.* 2009). One sucrose replacer (stevia) and two fat replacers (MPC and MS) were selected as independent variables. Moreover, six dependent variables (responses)-namely acidity, pH, firmness, syneresis, apparent viscosity, and consistency-were determined by the model to evaluate the optimum levels of the independent variables. The complete design included 20 experiments with six replications of the center point. The independent variables, their levels, and experimental results from this study are presented in Table 1.

Table 1. Central composite rotatable design for the independent variables and responses of dependent variables

Run no.	Independent variables						Response variables					
	X_1		X_2		X_3		Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
	Coded	Actual	Coded	Actual	Coded	Actual						
1	-1	0.008	-1	0.81	-1	0.61	0.12	6.61	0.417	14.24	5100	5.6
2	1	0.032	-1	0.81	-1	0.61	0.13	6.57	0.451	11.5	4600	6.3
3	-1	0.008	1	3.19	-1	0.61	0.17	6.46	0.515	3.04	10600	5
4	1	0.032	1	3.19	-1	0.61	0.18	6.41	0.446	0	5600	5.3
5	-1	0.008	-1	0.81	1	2.39	0.12	6.62	1.908	0	34800	0.3
6	1	0.032	-1	0.81	1	2.39	0.13	6.55	1.315	0	26200	0.6
7	-1	0.008	1	3.19	1	2.39	0.17	6.42	1.947	0	37100	0
8	1	0.032	1	3.19	1	2.39	0.18	6.38	1.908	0	36300	0
9	-α	0	0	2	0	1.5	0.15	6.53	1.187	0.34	21700	0.8
10	+α	0.04	0	2	0	1.5	0.16	6.49	0.741	0	12200	2.3
11	0	0.02	-α	0	0	1.5	0.11	6.64	0.716	0.4	17900	3.1
12	0	0.02	+α	4	0	1.5	0.19	6.35	1.045	0	21600	1.8
13	0	0.02	0	2	-α	0	0.16	6.52	0.314	15.88	3100	8.3
14	0	0.02	0	2	+α	3	0.15	6.51	2.477	0	46000	0
15	0	0.02	0	2	0	1.5	0.15	6.54	0.868	0.2	18900	2.4
16	0	0.02	0	2	0	1.5	0.15	6.55	0.878	0.1	16600	1.8
17	0	0.02	0	2	0	1.5	0.15	6.51	0.829	0.2	17900	2
18	0	0.02	0	2	0	1.5	0.15	6.50	0.849	0.1	20000	2.4
19	0	0.02	0	2	0	1.5	0.15	6.48	1.104	0	23200	2.1
20	0	0.02	0	2	0	1.5	0.16	6.44	1.01	0	23200	2.4

X_1 (concentration of Stevia, g/100g), X_2 (concentration of MPC, g/100g), X_3 (concentration of MS, g/100g)
Y_1 (Acidity, % acid lactic), Y_2 (pH), Y_3 (Firmness, N), Y_4 (Syneresis, %), Y_5 (Apparent viscosity, mPa.s), Y_6 (Consistency, cm/30 s)

Cream preparation

Control cream was prepared by addition of sucrose (12 g/100g) to UHT cream with 30% fat. Experimental creams were prepared based on the 15% fat in the formulated cream. The amount of stevia, MPC and MS were followed at certain level suggested by RSM (Table 1). As per the experimental design (Table 1), stevia and sugar powder composition were added to cream and mixed using mixer (Black & Decker.250w, England) for 20 s. MPC and MS were added to milk respectively and mixed well at room temperature. Milk mix and cream were mixed for 30 s and then the mix temperature reached 90ºC using a hot water bath in 20 min and was kept at this temperature for 20 s. Then, formulated cream was cooled in an ice bath to 60ºC and immediately stored at 6ºC until the day of analysis.

Analytical methods

Acidity

Titratable acidity was measured using the method of AOAC (1990), by titration of samples with 0.1 N NaOH solution containing 1% phenolphthalein as an indicator. Titratable acidity was calculated as a lactic acid percentage (%) as Eq. (1):

$$Acidity(\%) = \frac{0.1N\ NaoH\,(ml) \times 0.009}{sample(g)} \times 100 \quad (1)$$

pH

The pH was determined by using a digital pH meter (model Jenway, 3505, VK) at 20ºC. Buffer solutions of pH 7 and 4 were used to

standardize the pH meter (Gonzalez-Martinez *et al.* 2002).

Firmness

The firmness of cream samples was determined at 7ºC by texture analyzer (STM-5, Santam, Iran). The firmness is defined as the force (N) necessary to puncture the cylindrical probe (diameter 22 mm) into the depth of 10 mm of the cream sample at a constant speed of 1 mm/s (Kovacova *et al.* 2010).

Syneresis

The cream samples were kept at room temperature of 25ºC in order to reach uniform temperature in the samples, and then 10 g of each cream sample was centrifuged in a centrifuge Z200A HERMLE (4000 rpm, at 20ºC for 15 min). After centrifugation, the mass of the separated water was determined. The percentage of syneresis was calculated as follows in Eq. (2) (Gonzalez-Martinez *et al.* 2002):

$$Syneresis(\%) = \frac{Separated\ water(g)}{Cream(g)} \times 100 \qquad (2)$$

Apparent viscosity

Viscosity of the cream samples was measured using a digital rotational viscometer (Myr, model V2-R, VISCOTEC Co., Spain) at 7ºC with spindle TR11. All measurements were recorded after 5 s at 20 rpm (shear rate =5 s^{-1}) and reported as the apparent viscosity (Emam-Djome *et al.* 2008).

Bostwick consistency

Consistency was determined by measuring the distance (cm) over which the sample flowed in a Bostwick consistometer at 6ºC for 30 s. Consistency was related to distance inversely (Gonzalez-Martinez *et al.* 2002).

Calorie values

Moisture, ash, fat, and protein contents were determined according to AOAC (1990) official methods. Carbohydrates were determined by subtracting the sum of moisture, protein, fat, and ash percentages from 100%. Calorie values of control and optimum samples were calculated as follows in Eq. (3) (Worrasinchai *et al.* 2006):

$$Calorie Values = (4 \times protein) + (9 \times fat) + (4 \times carbohydrate) \qquad (3)$$

Sensory evaluation

A semi trained consumer panel, consisted of ten students (8 female and 2 male), of Food Science and Technology Department of Sari Agricultural Sciences and Natural Resources University, rated the sensory quality of control and optimum samples on the following attributes: appearance (color, separation of whey, foaming), odour, flavour, mouthfeel, consistency, spreadability, creamy texture and overall acceptance. The samples from each test were placed in glass containers and presented to each panelist at once. The samples were coded without a name, with a form that was pre-designed for this test, along with a meal presented to the panelists. A 5-point hedonic scale (1-1.99=dislike, 2-2.99=neither like nor dislike, 2-3.99=like moderately, 4-4.99=like very much and 5=like extremely) was used to evaluate the samples (Barzegari, 2012).

Statistical analysis

The obtained experimental data were fitted to a backward quadratic polynomial equation, and the 1% and 5% levels of significance were selected as the significance threshold. The CCRD test results were analyzed using Design-Expert software (version 7.0.0) to define a regression model and produce analysis of variance (ANOVA) tables and surface profile plots for all six responses. The results of sensory evaluation were analyzed in a randomized complete block design using SPSS 16.0 software to determine the difference between panelists, so that panelists were considered as blocks and samples (control and optimum) as treatments, but due to not significant difference between blocks, difference between treatments was analyzed in a complete randomized design.

Optimization

Numerical optimization technique of the Design-Expert (7.0.0) software was used for

simultaneous optimization of the multiple responses. The desired goal for each independent factor and response was chosen. All the independent factors were kept within the range of the experimental study (Table 1). The responses, acidity and pH were kept within the range of standards of Iran, firmness was kept within the range of control cream and apparent viscosity and consistency were maximized.

Results and Discussion

Response models

The experimental results of the optimization study are given in Table 1. Also, the results obtained from the ANOVA are shown in Table 2. *P*-values of <0.01 indicate that all predicted response surface models were statistically significant at 99% confidence interval. Meanwhile, it was observed that the lack-of-fit test (Table 2) for all the models except syneresis were insignificant, implying that the models were accurate enough to predict the responses, while syneresis model due to the significant lack of fit was not appropriate. The variability explained by all the models was more than 80 percent ($R^2 > 0.80$). Ergo, all the models exept syneresis exhibited statistical adequacy and were hence used to study the effect of independent variables on the various responses. The results of calculating the coefficients of regression to predict the regression model obtained by using the Design-Expert statistic software are shown in Table 2. The coefficients of the terms along with their *p*-values show which terms contributed significantly to the responses ($p<0.01$ and $p<0.05$).

Table 2 Regression coefficients (β) and ANOVA for the response surface models in terms of coded units

particulars	Acidity	pH	Firmness	Syneresis	Apparent viscosity	Consistency
Intercept	0.15	6.50	0.93	0.33	19846.89	2.28
X_1	0.00416^{**}	-0.020^{*}	-0.10^{**}	-	-2260.92^{**}	0.28^{**}
X_2	0.024^{**}	-0.086^{**}	0.094^{*}	-1.71^{**}	1839.56^{*}	-0.34^{**}
X_3	-	-	0.65^{**}	-4.06^{**}	13227.71^{**}	-2.58^{**}
X_1^2	-	-	-	-	-1136.20	-0.21
X_2^2	-	-	-	-	-	-
X_3^2	-	-	0.17^{**}	2.88^{**}	1550.80^{*}	0.71^{**}
X_1X_2	-	-	-	-	-	-
X_1X_3	-	-	-0.075	-	-	-
X_2X_3	-	-	-	2.84^{**}	-	-
Model F-value	320.64^{**}	82.67^{**}	94.39^{**}	43.07^{**}	94.98^{**}	206.97^{**}
Lack of fit	0.7165^{NS}	0.9977^{NS}	0.4250^{NS}	$<0.0001^{**}$	0.7882^{NS}	0.2730^{NS}
R^2	0.9742	0.9068	0.9712	0.9199	0.9714	0.9867
C.V. %	2.39	0.39	11.24	70.40	11.54	11.86
Adequate precision	58.672	29.456	33.987	22.063	34.957	52.504

** Highly Significant ($p < 0.01$)

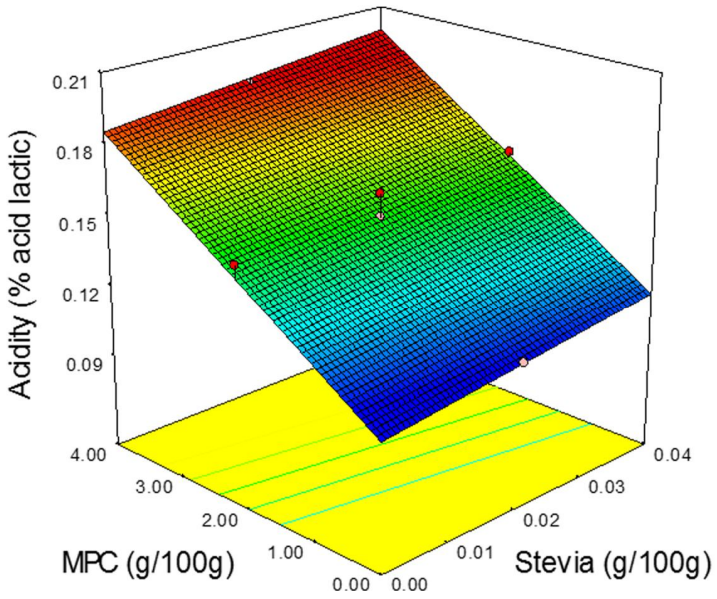
* Significant ($p < 0.05$)

^{NS} Non- Significant ($p > 0.05$)

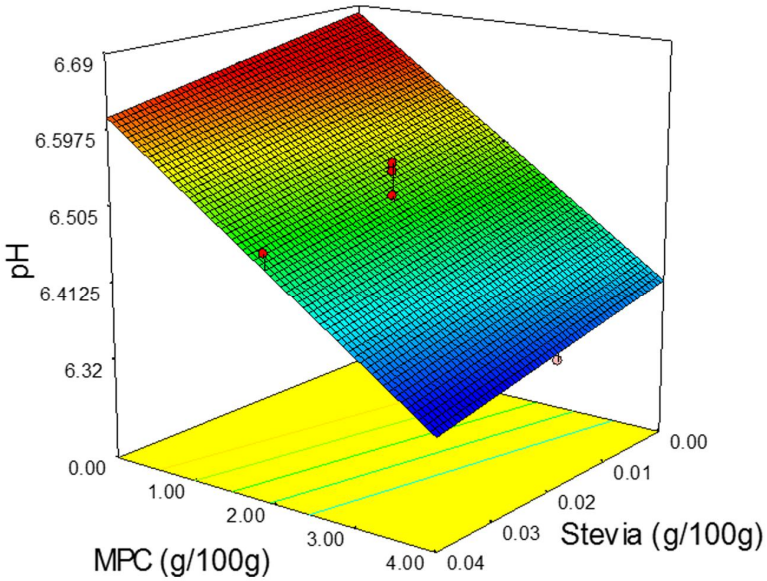
X_1 (concentration of Stevia, g/100g), X_2 (concentration of MPC, g/100g), X_3 (concentration of MS, g/100g)

Acidity

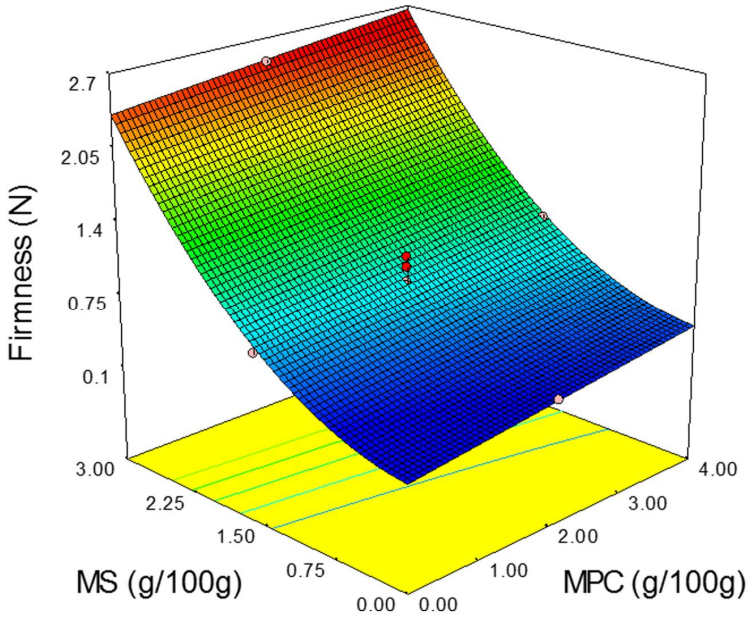
ANOVA of the independent variables impact on cream acidity (Table 2) indicated that the effects of stevia (X_1) and MPC (X_2) were significant ($p<0.01$) and the general regression model could be described as a linear equation. The effect of MPC was more pronounced (β =0.024) and pursued. Positive coefficients of X_1 and X_2 indicated linear effect to increase acidity. No significant effect was observed for MS (X_3) on acidity. The three-dimensional Fig. 1(a) shows two independent variables of the predictive model for acidity.


Acidity (% acid lactic)
0.21
0.18
0.15
0.12
0.09
4.00
3.00
2.00
1.00
0.00
MPC (g/100g)
0.00
0.01
0.02
0.03
0.04
Stevia (g/100g)

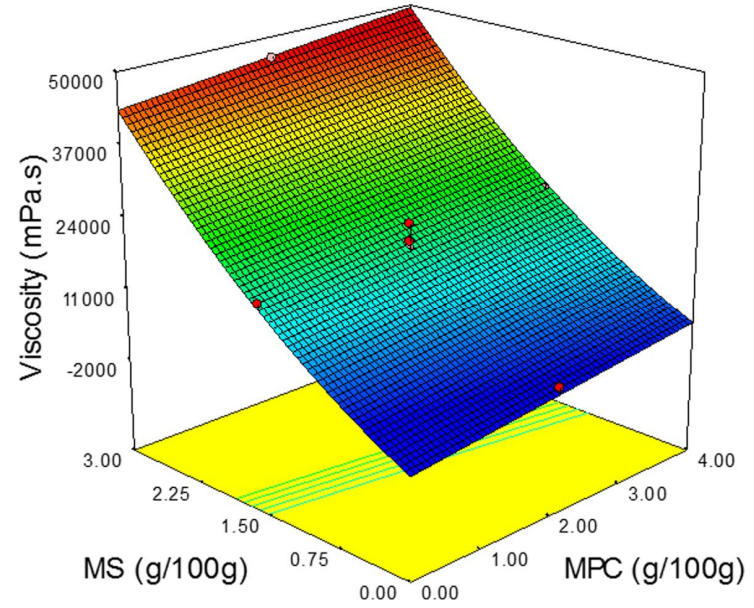
(a)


pH
6.69
6.5975
6.505
6.4125
6.32
0.00
1.00
2.00
3.00
4.00
MPC (g/100g)
0.00
0.01
0.02
0.03
0.04
Stevia (g/100g)

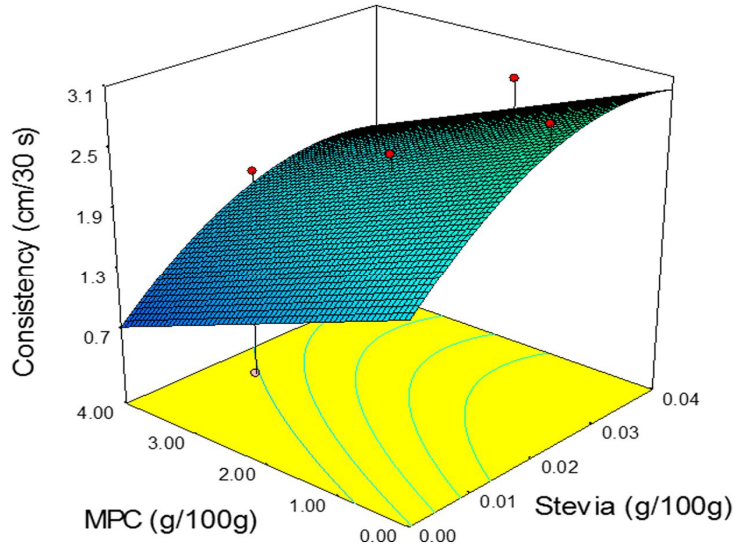
(b)


Firmness (N)
2.7
2.05
1.4
0.75
0.1
3.00
2.25
1.50
0.75
0.00
MS (g/100g)
0.00
1.00
2.00
3.00
4.00
MPC (g/100g)

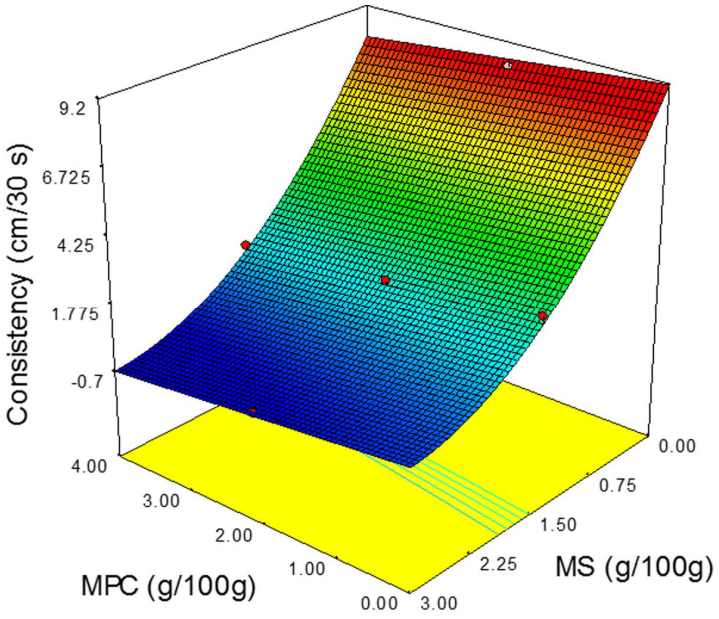
(c)


Viscosity (mPa.s)
50000
37000
24000
11000
-2000
3.00
2.25
1.50
0.75
0.00
MS (g/100g)
0.00
1.00
2.00
3.00
4.00
MPC (g/100g)

(d)



(e)



(f)

Fig. 1 Response surface plots showing the effects of stevia and MPC on acidity (a), stevia and MPC on pH (b), MPC and MS on firmness (c), MPC and MS on apparent viscosity (d), stevia and MPC on consistency (e), and MPC and MS on consistency (f) of creams

These provide geometrical representation of the behavior of acidity within the experimental design. In this study, acidity was in the range of 0.11 to 0.19% acid lactic for formulated creams. Based on the results, with increasing sucrose substitution with stevia at different MPC concentrations, acidity increased (Fig. 1(a)). It seems that the presence of amino acids

and fatty acids in the extract of the leaves of Stevia plant is effective. Tadhani *et al.* (2006) identified six fatty acids in the leaf extract of Stevia that contains palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. Furthermore, increasing MPC concentration increased acidity so that the maximum acidity was observed in the sample with the maximum concentration of MPC, thus this can be attributed to higher acidity of MPC to cream. Increase in acidity with an increase of stevia substitution and MPC concentration has been reported by Bagheri *et al.* (2014).

pH

It is evident from ANOVA (Table 2) that pH was dependent on stevia (X_1) and MPC (X_2). However, no significant effect was observed for MS (X_3) or any quadratic and interaction terms on pH. In this study, pH was in the range of 6.35 to 6.64 for formulated creams. The pH was affected more significantly ($p<0.01$) by MPC than by stevia ($p<0.05$). Negative coefficients of stevia and MPC indicated linear effect to decrease pH. As seen in Fig. 1(b) pH linearly decreased as sucrose substitution with stevia increased due to increase of acidity. Moreover, decrease in pH was followed by an increase in MPC. So that the minimum pH was observed in the sample with the maximum concentration of MPC. It seems that the MPC has affected water absorption and mobility of hydrogen ions and thus has reduced the cream pH. Decrease in pH with an increase of MPC concentration has been reported by Patel *et al.* (2006).

Firmness

The firmness of cream texture is defined as its resistance to deformation by external forces and processes such as homogenization of cream fat and protein contents can affect the texture of the final product. The force necessary for the probe to penetrate into the cream samples, which is indicative of texture firmness, was in the range of 0.314 to 2.477 Newton for formulated creams in this research. Review of Table 2 shows significant effect of all the independent variables on firmness. The effect of stevia (X_1), MS (X_3) and its quadratic term (X_3^2) upon firmness was significant at $p<0.01$, whereas firmness was significantly affected at $p<0.05$ by MPC (X_2). The MS had the largest coefficient ($\beta= 0.65$) (Table 2) and maximum effect. No significant effect was observed for any interaction terms on firmness. The interaction effect of stevia and MS (X_1X_3) on firmness was not significant, however, it was not removed from the model due to its impact on the regression coefficients. As seen in Table 2 firmness linearly decreased as sucrose substitution with stevia increased due to reduction of total solid. According to Fig. 1(c) there was an increase in the firmness as the MPC and MS concentration were increased. The firmness of cream is highly dependent on total solids content, on the protein content of the product and also on the type of protein (Oliveira *et al.* 2001). Thus, in the present study, firmness increased with increase of MPC concentration along with increase of total solids and casein content. Moreover, increase in firmness was followed by an increase in MS concentration. So that the sample with the maximum concentration of MS had the maximum firmness. MS may cause increased firmness due to water absorption ability and strong network formation (Woo *et al.* 2002).

Syneresis

The experimental results showed that syneresis decreased with increase of MPC concentration despite increased acidity (Table 1), which is due to the high water absorption of MPC. Also, according to Table 1 syneresis decreased with increase of MS concentration in the constant concentration of Stevia and MPC, so that in the maximum concentration of MS no syneresis was observed but the maximum syneresis (15.88%) was observed in the sample without MS. On the other hand, according to the ANOVA (Table 2), the model of syneresis was statistically significant ($p<0.01$) but the lack-of-fit test was significant ($p<0.01$). The significant lack of fit for a model does not endorse the accuracy of the

model to fit the data and indicates that the points are not well-located around the model. Therefore, the model cannot be used to predict the values of function variables. Emam-Djome *et al.* (2008) also concluded that with increase of whey protein concentrate, syneresis of cream decreased.

Apparent viscosity

Apparent viscosity of formulated creams varied from 3100 to 46000 mPa.s. According to the ANOVA (Table 2), stevia (X_1), MPC (X_2) and MS (X3) significantly ($p<0.01$ and $p<0.05$) influenced the viscosity. As seen in Table 2, by increasing sucrose substitution with stevia, viscosity decreased due to reduction of total solid followed by decrease of sucrose concentration. Disaccharides such as sucrose produce high osmolality solutions due to their solubility and hydrophilic characteristic and have the capacity to make hydrogen bonds with water molecules by a hydroxyl group, which in turn augments viscosity of the creams (Alizadeh *et al.* 2014). Guggisberg *et al.* (2011) also reported that the apparent viscosity of low-fat set yoghurt decreased as the substitution of sucrose with stevia increased. Figure 1(d) shows the 3D response surface plot at varying MPC and MS concentrations. From Fig. 1(d) it can be concluded that the viscosity of formulas increases with increase in both variables. It is obvious that the increase made by MS addition was more evere. So that the maximum viscosity was observed in the sample with the high content of MS. It is also evident from the results shown in Table 2 that apparent viscosity was severely dependent on MS. By increase in protein level of creams followed by increase of MPC concentration, increased water absorption ability and viscosity improved (Aminigo *et al.* 2009). The increase of MS concentration enhances total solids and firmness of creams and increases creams viscosity followed by reduction of molecules mobility and increase of emulsion stability.

Bostwick consistency

In Bostwick consistometer, less traveled distance by sample over time (30 s) is indicative of its higher consistency. The consistency of formulated creams varied from 0 to 8.3 cm/30 s. Analysis of variance of the independent variables (Table 2) showed that the effect of all the independent variables upon consistency was significant at $p<0.01$. The MS had the largest coefficient (β= -2.58) and maximum effect on cream consistency (Table 2). Positive coefficient of stevia (X_1) indicated linear effect to increase traveled distance by sample and decreased consistency. It can be concluded from the Fig. 1(e) that with increasing the level of sucrose substitution with stevia at different MPC concentrations, traveled distance by sample increased; thus, cream consistency decreased due to reduction of total solids as a result of decrease of sucrose concentration. However, the regression coefficient of cream consistency (Table 2) showed that with increase of MPC and MS concentration traveled distance by sample decreased and consistency increased. It can be inferred from Fig. 1(f) that the traveled distance by sample would be minimized as the MPC and MS reached their maximum values. Protein matrix of MPC is formed from the casein micelles; and casein is the key factor to obtain a firm consistency. By increase in MS concentration, traveled distance by formulated creams decreased; thus, cream consistency increased due to increase of viscosity and firmness, and also reduce of molecules mobility.

Numerical optimization of formulations

Design Expert statistics software (version 7.0.0) was used for simultaneous numerical optimization of the processing variables. The optimum values of the independent variables were achieved after assigning certain constraints upon the processing conditions and the responses (Table 3). The value of importance was as per the default setting of the software (importance= 3) for all the variables. The optimum values of the independent variables and their corresponding responses are reported in Table 3. The best conditions for meeting the maximum desirability (0.978)

were obtained at 0.034 g/100g stevia (equivalent to 10.2 g/100g of sucrose), 1.64 g/100g MPC, and 2.30 g/100g MS. The corresponding predictions for the dependent variables under these conditions were 0.15% acid lactic for acidity, pH 6.5, firmness 1.4 N, apparent viscosity 28730.3 mPa.s and consistency 0.52 cm/30 s. The experiments were also conducted under the predicted optimum conditions to verify the efficacy of the models. Review of Table 3 shows that the predicted values had non-significant difference from experimental values.

Table 3. Goals set for constraints to optimize the formulation of low calorie sweet cream and verification of the response models by comparing the experimental values with the predicted values

Independent variable	Goal	Lower limit	Upper limit	Optimum value [a]		
Stevia (g/100g)	in range	0	0.04	0.034		
MPC (g/100g)	in range	0	4	1.64		
MS (g/100g)	in range	0	3	2.30		
Responses				Predicted value	Actual value [b] ± SD	p-value
Acidity (% acid lactic)	in range	0.09	0.15	0.15	0.155±0.007	0.500
pH	in range	6.5	6.8	6.5	6.56±0.01	0.105
Firmness (N)	in range	1	1.4	1.4	0.95±0.07	0.070
Apparent viscosity (mPa.s)	maximize	3100	46000	28730.3	26550±353.55	0.073
Bostwick distance (cm/30 s)	minimize	0	8.3	0.52	0.15±0.07	0.086

[a] the desirability for this result was 0.978
[b] means from triplicate experiments

Calorie values

The proximate analysis and calorie values of the optimized and control cream are listed in Table 4. The moisture content of optimized cream decreased with increasing level of sucrose substitution with stevia and decreasing fat content due to reduction of total solids. The calorie value of optimized cream was 46.44% less than the control sample. This calorie reduction was due more to reduction of fat content. Although by addition of MPC as a fat replacer protein content of optimized cream increased and also carbohydrate content decreased due to reduction of sucrose amount in cream formulation. Besides, the energy amount produced by carbohydrate and protein is less than the one produced by fat.

Table 4. Chemical compositions (g/100g) and calorie values of cream samples

Sample	Moisture	Fat	Protein	Ash	Carbohydrate	Calorie values (Kcal/100g)
Control	52	30	2.02	0.70	15.28	338
Optimized	72.7	15	3.36	0.795	8.14	181.02

Sensory evaluations

As seen in Fig. 2, no significant difference between the optimum and control creams in appearance, odour, mouthfeel, consistency, spreadability and overall acceptance was found and both of them earned high scores, while in terms of flavour and creamy texture there was a significant difference ($p<0.01$) between them. The flavour score of formulated cream was significantly higher than control. This can be attributed to more favorable sweetness and flavour as a result of sucrose substitution with rebaudioside A and adding MPC as a fat replacer. MS had no effect on flavour. Also, in terms of creamy texture the formulated cream earned a higher score than the control which shows that simultaneous use of MS and MPC can cover qualitative defects caused by reduction of fat in low fat cream.

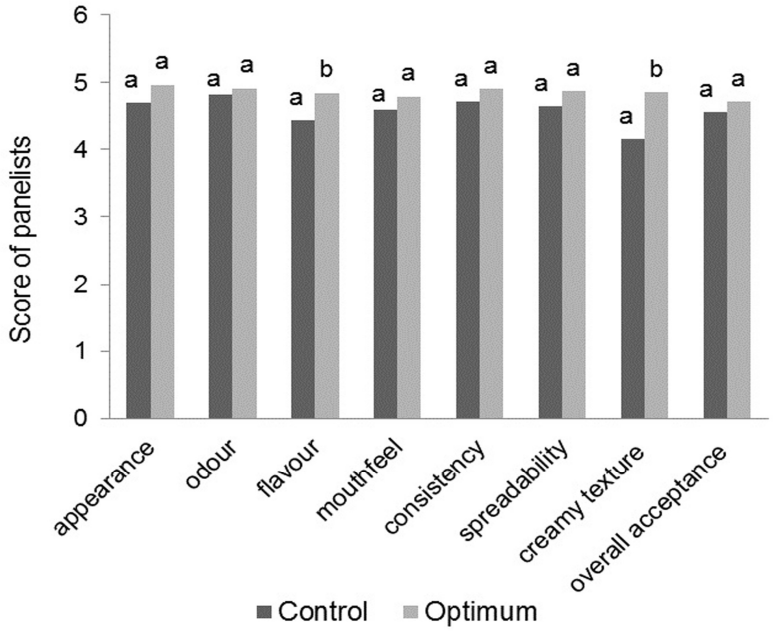


Fig. 2. The results of sensory evaluation based on obtained scores

Conclusion

Response surface methodology was effective in optimizing formulation for the manufacture of low calorie sweet cream form different blends of stevia, MPC and MS. The regression analysis yielded models that were used for obtaining optimum formulation for desired responses within the range of conditions applied in this study. The formulation with 0.034 g/100g of stevia, 1.64 g/100g of MPC and 2.30 g/100g of MS was found optimum for low calorie sweet cream preparation. Price per kilogram of optimized and control creams was estimated 38500 and 40000 Rials respectively, that shows new formulation is more affordable for the consumer economically.

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بهینه‌سازی فرمولاسیون خامه شیرین کم‌کالری با استفاده از روش سطح پاسخ

سیده فرشته حسینی[1]، زینب رفتنی امیری[2*]

تاریخ دریافت: 1396/10/18
تاریخ پذیرش: 1397/08/02

چکیده

در این تحقیق، تأثیر استویا (0/04-0 درصد وزنی) به‌عنوان جایگزین ساکارز، کنسانتره پروتئینی شیر (4-0 درصد وزنی) و نشاسته اصلاح‌شده ذرت مومی (3-0 درصد وزنی) به‌عنوان جایگزین‌های چربی بر روی خصوصیات فیزیکوشیمیایی و حسی خامه 15 درصد چربی با استفاده از طرح مرکب مرکزی چرخش‌پذیر بررسی شد. برای بهینه‌سازی فرمولاسیون خامه کم‌کالری از روش سطح پاسخ استفاده شد. نتایج نشان داد که افزایش جایگزینی ساکارز با استویا و غلظت کنسانتره پروتئینی شیر موجب افزایش اسیدیته خامه شد، در حالی‌که pH کاهش یافت. با افزایش جایگزینی ساکارز با استویا در خامه، سفتی بافت، ویسکوزیته ظاهری و قوام کاهش یافت، در حالی‌که افزایش غلظت کنسانتره پروتئینی شیر و نشاسته اصلاح‌شده منجر به افزایش فاکتورهای ذکر شده گردید. بر اساس بهینه‌سازی چند پاسخ، سطوح بهینه برای استویا 0/034 درصد، کنسانتره پروتئینی شیر 1/64 درصد و نشاسته اصلاح‌شده 2/30 درصد تعیین و مقادیر اسیدیته 0/15درصد بر مبنای اسیدلاکتیک، pH 6/5، سفتی بافت N 1/4، ویسکوزیته ظاهری mPa.s 28730/3 و قوام cm/30se 0/52 پیش‌بینی شدند. ارزش کالری خامه فرموله شده 44/46 درصد کمتر از نمونه شاهد (حاوی 30 درصد چربی و 12 درصد ساکاروز) بود. خامه فرموله شده از نظر پذیرش کلی اختلاف معنی داری با شاهد نداشته و در عین حال امتیاز بالاتری از نظر مزه و حالت خامه ای داشت.

واژه‌های کلیدی: خامه کم‌کالری، استویا، کنسانتره پروتئینی شیر، نشاسته اصلاح‌شده، روش سطح پاسخ، بهینه‌سازی

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Proteolysis of sodium caseinate using *Withania coagulans* extract: An optimization study

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Abstract

In this study, sodium caseinate was hydrolyzed with *Withania coagulans* extract and the response surface methodology (RSM) was applied to optimize the effects of hydrolysis conditions including hydrolysis temperature, enzyme concentration and hydrolysis time on the degree of hydrolysis, solubility, and foaming properties. The analysis of variance in RSM showed that the linear effects of enzyme level and hydrolysis time and quadratic effects of hydrolysis temperature were important factors affecting the hydrolysis process remarkably ($P<0.0001$). Results were indicative of the fact that the increase in responses was obtained by an increase in hydrolysis time and enzyme level. The generated quadratic model showed that the optimum conditions for maximizing the responses were when enzyme concentration of 1.75 (%w/w), temperature of 55.43°C and hydrolysis time of 490 min.

Keywords: Sodium caseinate, Hydrolysis, *Withania coagulans* protease, Solubility, Foaming properties.

Introduction

Enzymatic proteolysis and the effect of hydrolysis parameters on the functional properties of proteins such as emulsifying, foaming, viscosity and solubility have been extensively investigated and reported by several researchers (Banach *et al*. 2013; de Castro *et al.* 2015; Guan *et al*. 2007; Miedzianka *et al*. 2014; Pralea *et al*. 2011). Process conditions including pH, time, enzyme to substrate ratio and temperature are influential on the hydrolysate composition and thereby the functional properties (Ovissipour *et al*. 2012).

Caseins are the main proteinaceous component of milk (approximately 80% of the total nitrogen in milk consists of three proteins, α-, β- and γ-caseins). Sodium caseinate is a dairy ingredient that its solubility is poor at acidic conditions near its isoelectric point (Luo *et al.* 2014). Different proteases such as a bacterial protease (Hidalgo *et al.* 2015), papain, pancreatin, trypsin (Luo *et al*. 2014) and chymotrypsin (Pralea *et al.* 2011) have been implemented for the hydrolysis of sodium caseinate and some physical, chemical and biochemical properties of the obtained hydrolysates have been characterized. Slaterry and Fitzgerald (1998) employed commercial Bacillus proteinase complex to hydrolyze sodium caseinate. These hydrolysates have improved the emulsion activity and foam expansion at low degrees of hydrolysis (0.5 and 1.0% DH).

Response surface methodology has been used successfully to model and optimizes the enzymatic hydrolysis of proteins from different sources (Nilsang *et al*. 2005; Ovissipour *et al.* 2012; Surówka *et al*. 2004). It is a valuable tool for investigating complex processes which determine the effects of the multiple variables and their interactions on response variables (Majd *et al*. 2014).

Withania coagulans is belonging to the *Solanaceae* family that is grown commonly in Pakistan, Afghanistan, India and the south of Iran (Sarani *et al.* 2014). Fruits from this plant have been widely used as the main source of milk coagulant (Beigomi *et al*. 2014) for the preparation of traditional cheese in Sistan and Baluchistan province of Iran. Also, the

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purification and characterization of *W. coagulans* enzyme (Beigomi *et al*. 2014; Naz *et al*. 2009) and the production of mozzarella cheese (Nawaz *et al*. 2011), Feta cheese (Pezeshki *et al*. 2011)and tofu (Sarani *et al*. 2014) using *W. coagulans* as a new coagulant has been reported. However, it is noteworthy that no study has been carried out on the other applications of the *W. coagulans* protease.

Withania coagulans is available in natural habitats of Iran. Considering the fact that in the available literature, there has been no report on the use of *Withania coagulans* protease for modifying functional properties of proteins. Hence, the aim of the present research was to optimize the effects of reaction conditions (i.e., enzyme activity, hydrolysis temperature, and hydrolysis time) of *Withania coagulans* protease on the degree of hydrolysis, foaming properties and solubility of sodium caseinate.

Materials and Methods

Dried fruits of *W. coagulans* were collected from wild plants which grow in Nikshahr city (Sistan and Baluchistan, Iran). Fruits were washed with distilled water and dried at ambient temperature. Casein sodium salt from bovine milk, bovine serum albumin (BSA) and O-phthaldialdehyde (OPA) were purchased from Sigma-Aldrich Co. (Steinheim, Germany). Other chemicals and solvents were of analytical grade.

Enzyme extraction

According to the method optimized by Shavandi *et al*. (2015), the dried fruits powder was subjected to the extraction by immersing 10 g of powder in 62 mL sodium phosphate buffer, 0.1 mol L^{-1} (pH 3) for 3 hours at 44.2ºC with agitation. Samples were filtered and then centrifuged (Gallenkamp centrifuge 200, Gallenkamp, UK) at 4,000×rpm for 20 minutes. Ammonium sulfate was added to supernatants (85% saturation) and after 24 h of storage at 4ºC, the sediment was separated by centrifugation (10,000×g for 20 min). The precipitate was re-suspended in phosphate buffer (25mM, pH 7.5) and dialyzed (D9527; Sigma-Aldrich) against the same buffer for 24 h at 4ºC. The resulting extracts have been freeze-dried (freeze dryer Christ Alpha 1-4/LD Plus, Christ, Germany), and stored at -20ºC for further use.

Protease activity

The protease activity of *Withania coagulans* extract was determined according to the previously published protocol of Anson (1938), with some modifications. Aqueous enzyme extracts were prepared by dissolving 0.15 g of freeze-dried extract in 5 mL of extraction buffer (pH=7.5, 10 mM sodium acetate and 5 mM acetate calcium). 1 mL of enzyme extract was added to 5 mL of 0.65% casein as a substrate and incubated at 37 ºC for exactly 10 min. After this time, 5 mL of trichloroacetic acid (110 mM) was added to stop the reaction, which was in turn incubated at 37 ºC for 30 min. Following this, each of the test solutions has been filtered and then 5 mL of sodium carbonate (500 mM) and 1 mL of Folin & Ciocalteu's Phenol Reagent were added to 1 mL of solution and the mixture was incubated at 37 ºC for 30 min. The absorbance of the solution was measured using a spectrophotometer at 660 nm. The protease activity was calculated using a standard curve of L-tyrosine.

Hydrolysis of sodium caseinate

Samples of 0.05 g mL^{-1} of sodium caseinate were dissolved in 25 mM phosphate buffer, pH 7. *Withania coagulans* extract was added according to the experimental runs shown in Tables 1. All reactions have been performed in a shaking incubator (Heidolph incubator 1000, Heidolph, Germany) with constant agitation (300 rpm). After inactivating the enzyme by boiling it for 20 min, the samples were cooled on ice and centrifuged at 5000 rpm for 20 min to remove any insoluble contents. Finally, supernatants were freeze-dried and stored at 20ºC for further use (Luo *et al.* 2014).

The degree of hydrolysis (DH)

The DH of hydrolysates was determined by reacting free amino acids with o-

phthaldialdehyde (OPA), according to the method modified by Luo *et al*. (2014). The fresh preparation procedure of the reagent was carried out by mixing 25 ml of 0.1 M Borate buffer (pH 9.5), 2.5 ml of 20% sodium dodecyl sulphate aqueous solution, 40 mg of OPA in 1 ml ethanol, 100 µl of b-mercaptoethanol and the remainder being distilled water to a total volume of 50 ml (Church et al. 1983). Samples (50 µl) were mixed with 3 ml of OPA reagent and the mixture was allowed to stand for 2 min before measuring the absorbance at 340 nm. To achieve the maximum DH, sodium caseinate was hydrolysed with 6 N HCl for 24 h at 120°C. The DH of the samples was calculated by the following equation:

$$DH(\%)= \frac{A_{sample}-A_{blank}}{A_{total}-A_{blank}} \times 100 \qquad (1)$$

Where A_{total} was the absorbance of the sample with maximum DH and A_{blank} was the absorbance of sample replaced with distilled water to the same procedure of the hydrolyzed samples.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

The SDS-PAGE was carried out by the method of Luo et al (2014) on 15% polyacrylamide gel. The hydrolysates were diluted in an SDS–PAGE sample buffer containing b-mercaptoethanol and heated at 95 °C for 5 min. Samples with 4 mg/ml protein were loaded onto the gel for electrophoresis at 200 V. Protein marker (Fermentas, Biotechnology company), ranging from 20 kDa to 120 kDa, was also loaded to estimate peptide molecular weight. After staining using Coomassie brilliant blue G-250, the gel was scanned.

Solubility measurement

Hydrolysates were dispersed in acetate buffer (pH 5, 25 mM) at 10 mg/ml. After incubation for 30 min at room temperature, samples were centrifuged at 10,000g for 10 min (Refrigerate Centrifuge SIGMA 3-30K). The protein content in the supernatant was measured by the biuret method (Layne 1957). The solubility was calculated as (Banach et al. 2013):

Solubility (%)= (protein in supernatant/protein in dispersion) × 100 (2)

Foaming capacity and stability determination

Foaming properties were measured according to the method described by Guan *et al* (2007) with minor modifications. Portions of 20 ml sample solutions (2 mg/ml) at pH 5 were homogenized for 1.5 min at the speed of 10,000 rpm. Foaming capacity (FC) was determined as the percentage increase in the volume of the sample solutions upon mixing. Foam stability (FS) was evaluated as the percent of the foam remaining after 30 min.

Experimental design for optimization

One of the main experimental designs which have been widely used in the second order response surface models is the central composite design (CCD) (Chabeaud et al. 2009; de Castro *et al*. 2015). In this investigation, the effects of three process variables, namely the enzyme loading, hydrolysis time and hydrolysis temperature as well as optimization of the hydrolysis conditions were studied using a full-factorial rotatable CCD. The dependent variables (responses) were the degree of hydrolysis, foaming properties, and solubility. The factors and their levels have been determined based on the preliminary experiments and previously published studies (Shavandi *et al*. 2015). The levels of three variables were: hydrolysis time (30, 145, 260, 375 and 490), hydrolysis temperature (30, 40, 50, 60 and 70 °C), and enzyme loading (1%, 2%, 3%, 4% and 5%). The level of alpha was 2.

A three-factor CCD design with a total of 20 runs, including 8 factorial points, 6 axial points and 6 replicates at the center points was employed to determine the response pattern and then to establish a model. Design-Expert 7 software was used to analyze the observed data. The design of the experiments and response variable values are given in Table 1. The responses could be related to the

independent variables by a second-order polynomial as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{1 \le i \le j} \beta_{ij} X_i X_j \quad (2)$$

Where Y is the predicted response, k is the number of the factors, x_i and x_j are the independent variables, β_0 is a constant term, β_i, β_{ii}, β_{ij} are the regression coefficients for the linear, squared and cross-product terms, respectively. The optimum extraction conditions of different independent variables were determined based on the highest value of responses including DH, solubility, FC, and FS by using 'Numerical Optimization' of the Design Expert 7 software.

Table 1. Central Composite Design (CCD) with the experimental values for the response variables (degree of hydrolysis, solubility and foaming capacity, and stability) applied for hydrolyzing sodium caseinate by *Withania coagulans* extract

Run	X_1	X_2	X_3	DH	S	FC	FS
1	5	50	260	18.15	62.20	128.57	35.55
2	2	40	375	13.89	39.55	142.86	66.49
3	4	40	375	17.32	61.34	150	38.22
4	2	60	145	10.42	35.02	110.91	71.22
5	3	70	260	9.74	27.03	92.86	68
6	2	60	375	14.91	48.9	142.86	72.59
7	4	60	375	15	46.45	92.86	48.48
8	3	50	260	15.28	52.28	117.86	62.59
9	3	50	260	14.03	56.16	135.7	55.16
10	1	50	260	12.66	24.88	121.43	83.22
11	4	60	145	12.04	38.47	103.57	66.67
12	4	40	145	14.23	39.98	142.86	51.72
13	3	50	260	14.20	60.91	125	53.54
14	2	40	145	9.45	24.66	117.24	67.55
15	3	50	260	13.43	57.89	125	47.22
16	3	50	490	16.83	68.14	132.14	37.5
17	3	50	30	11.87	22.72	114.29	73.72
18	3	30	260	8.04	34.58	135.71	52.02
19	3	50	260	15.33	57.67	128.57	51.75
20	3	50	260	14.05	57.02	114.29	58.02

X1: Enzyme level (%w/w); X_2: Hydrolysis temperature (°C); X_3: Hydrolysis time (min); DH, the degree of hydrolysis; S, Solubility; FC, Foam capacity; FS, Foam stability

Results and discussion

Enzyme activity and SDS-PAGE

Withania coagulans extract displayed a proteolytic activity of 0.323 UmL^{-1}(0.141 U mg $protein^{-1}$) according to the casein method (Anson 1938). The resulting extract was run in SDS-PAGE in combination with protein marker for correct size identification of the partially purified protease. Proteins obtained from the extract were observed in an electrophoretogram with the protein band of approximately 60 kDa with a range of Mw values from 20 to 35 kDa (Fig. 1). These bands had Mw values which were similar to the reported Mw for *W. coagulans* protease by Beigomi *et al*. (2014) and Naz *et al*. (2009).

The degree of hydrolysis (DH) and SDS-PAGE characterization

To evaluate the influences of the three contributing factors, namely the enzyme loading, hydrolysis temperature and hydrolysis time on DH, the design matrix of experimental conditions with the corresponding response values in Table 1 was fitted to a polynomial model. A quadratic model was proposed, based on the results of the sequential model, sum of squares and the calculated statistics for all model terms. The mathematical equation suggested for this response in terms of coded values is shown in Table 3. The coefficient of determination (R^2) of the model could be applied for checking the experimental data variability. R^2 for DH indicated that the model could explain 94.15% of the variability in the response and only 5.85% of the total variation could not be attributed to the variables. In addition, lack of fit test was insignificant (Table 1) that indicates the model is careful to predict the degree of hydrolysis.

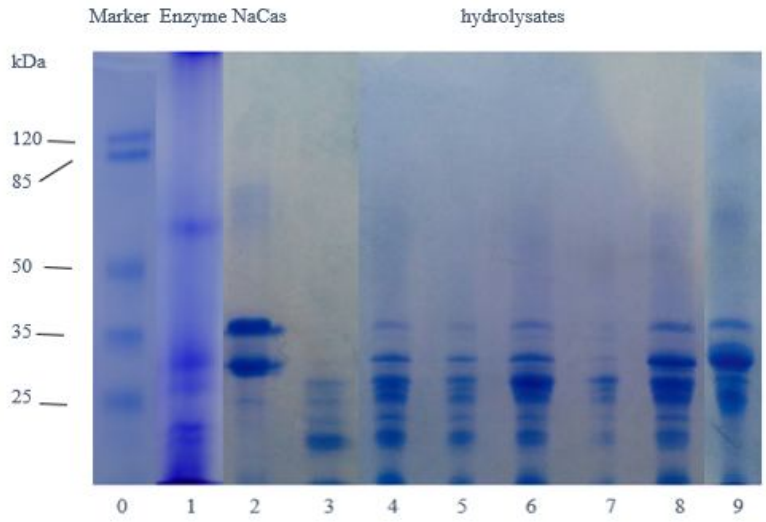

Fig. 1. SDS-PAGE of the enzyme (lane 1), sodium caseinate (NaCas, lane 2) and its hydrolysates. Lanes 3, 4, 5, 6, 7, 8 and 9 represent samples 3, 12, 2, 4, 11, 14 and 5, respectively. Lane 0 shows protein markers.

Among the independent variables, linear effects of the enzyme level and hydrolysis time and quadratic effects of hydrolysis temperature had a very prominent effect ($p<0.0001$). Furthermore, the effect of the interaction between the enzyme loading and hydrolysis temperature on the DH was notable ($p<0.01$).

Figure 2(A) depicts the response surface plots of the effects of the three variables, the enzyme loading, hydrolysis time and hydrolysis temperature on DH. The temperature demonstrated quadratic effects on the response; hence DH increased up to approximately 50 ^{o}C followed by a decline with its further increase. However, hydrolysis time and enzyme loading caused a linear increase in the response. Considering that the DH is defined as the percentage of peptide bonds cleaved by protease (Adler-Nissen 1979), the hydrolysate with high DH is believed to contain more low-molecular-weight peptides in comparison with the hydrolysate with low DH. Similar trends of DH growth with the enhancement of hydrolysis time has been reported by Nilsang *et al.* (2005), and with the rise of enzyme loading by Ovissipour et al. (2012). The reduction of the degree of hydrolysis with an increase in the temperature (above 50^{o}C) is probably due to the decreased enzyme activity at high temperatures.

Results of SDS-PAGE presented in Fig. 1 generally agreed with DH. The hydrolysis of sodium caseinate resulted in hydrolysates with smaller molecular weights, as observed in SDS-PAGE (Fig. 1). Among the SDS- PAGE samples, lower molecular weights were observed in sample 3 (Lane 3) with the highest DH.

Solubility

A second-order polynomial regression model was selected to predict the solubility response. The simplified model is shown in Table 3. The model was found significant for solubility and the lack-of-fit test indicated no significance with p-values <0.0001 for the model (Table 2).

This indicates that the fitted model is quite appropriate for predicting and determining the values for solubility within the design space (Jain & Anal. 2016). Regression coefficients have indicated that the solubility of the hydrolysate was firstly dependent on hydrolysis time, secondly on enzyme level, and thirdly on hydrolysis temperature in their linear and quadratic forms.

Table 2. ANOVA table for the degree of hydrolysis, solubility and foaming properties of sodium caseinate hydrolyzed by *Withania coagulans* extract

Regression	Sum of squares	df [a]	Mean square	F-value	p-value
Degree of hydrolysis					
Model	127.27	5	25.45	45.04	< 0.0001
X_1	27.30	1	27.30	48.31	< 0.0001
X_3	38.78	1	38.78	68.63	< 0.0001
$X_2 X_1$	5.27	1	5.27	9.33	0.0086
X_2^2	48.92	1	48.92	86.56	< 0.0001
X_1^2	1.88	1	1.88	3.32	0.0899
Lack of Fit	5.03	9	0.56	0.97	0.5451
Solubility					
Model	3658.48	6	609.75	27.35	< 0.0001
X_1	794.69	1	794.69	35.64	< 0.0001
X_3	1386.74	1	1386.74	62.19	< 0.0001
$X_2 X_1$	162.96	1	162.96	7.31	0.0181
X_2^2	1149.73	1	1149.73	51.56	< 0.0001
X_1^2	322.23	1	322.23	14.45	0.0022
X_3^2	242.74	1	242.74	10.89	0.0058
Lack of Fit	250.35	8	31.29	3.96	0.0731
Foam capacity					
Model	4347.36	5	869.47	20.78	< 0.0001
X_2	2220.17	1	2220.17	53.07	< 0.0001
X_3	502.96	1	502.96	12.02	0.0038
$X_2 X_1$	1014.68	1	1014.68	24.25	0.0002
$X_1 X_3$	467.20	1	467.20	11.17	0.0048
X_2^2	142.36	1	142.36	3.40	0.0863
Lack of Fit	294.47	9	32.72	0.56	0.7870
Foam stability					
Model	2847.66	4	711.91	23.89	< 0.0001
X_2	280.11	1	280.11	9.40	0.0078
X_1	1765.96	1	1765.96	59.25	< 0.0001
X_3	673.61	1	673.61	22.60	0.0003
$X_1 X_3$	127.99	1	127.99	4.29	0.0559
Lack of Fit	307.57	10	30.76	1.10	0.4866

[a] df = degree of freedom; X1: Enzyme level (%w/w); X_2: Hydrolysis temperature (°C); X_3: Hydrolysis time (min)

Response surface plots were created from the polynomial model to demonstrate the effect of each pair of independent variables on the solubility (Fig. 2(B)). An increase in solubility was achieved by an increase in hydrolysis time and enzyme level. Results presented in Fig. 2(B) depict that the solubility with increasing temperature up to an average value increased and then began to fall.

A remarkable rise in protein solubility during the enzymatic hydrolysis has been reported by Luo *et al* (2014). The low molecular weight of hydrolysates and the corresponding increase in the number of the exposed ionizable amino and carboxyl groups accounted for the high solubility of hydrolysates (Panyam & Kilara. 1996).

Table 3. Simplified second-order polynomial equations of the four responses studied

Responses	Simplified polynomial model (coded factors)	CV (%)	R^2	Adj R^2	PRESS
DH	$Y_1 = 14.42 + 1.31X_1 + 1.56X_3 - 0.81X_1X_2 - 1.36X_2^2 + 0.27X_1^2$	5.55	0.9415	0.9206	16.10
S	$Y_2 = 56.55 + 7.05X_1 + 9.31X_3 - 4.51X_1X_2 - 6.76X_2^2 - 3.58X_1^2 - 3.11X_3^2$	10.31	0.9266	0.8927	1248.11
FC	$Y_3 = 125.56 - 0.64\times X_1 - 11.78\times X_2 + 5.61\times X_3 - 11.26\times X_1X_2 - 7.64\times X_1X_3 - 2.29\times X_2^2$	5.23	0.8813	0.8389	1270.02
FS	$Y_4 = 58.06 + 4.18X_2 - 10.51X_1 - 6.49X_3 - 4X_1X_3$	9.4	0.8643	0.8281	781.69

CV, Coefficient of variation; R^2, Coefficient of multiple determination; Adj R^2, Adjust R^2; PRESS, Predicted Residual Sum of Squares; X1: Enzyme level (%w/w); X_2: Hydrolysis temperature (°C); X_3: Hydrolysis time (min); DH, degree of hydrolysis; S, Solubility; FC, Foam capacity; FS, Foam stability

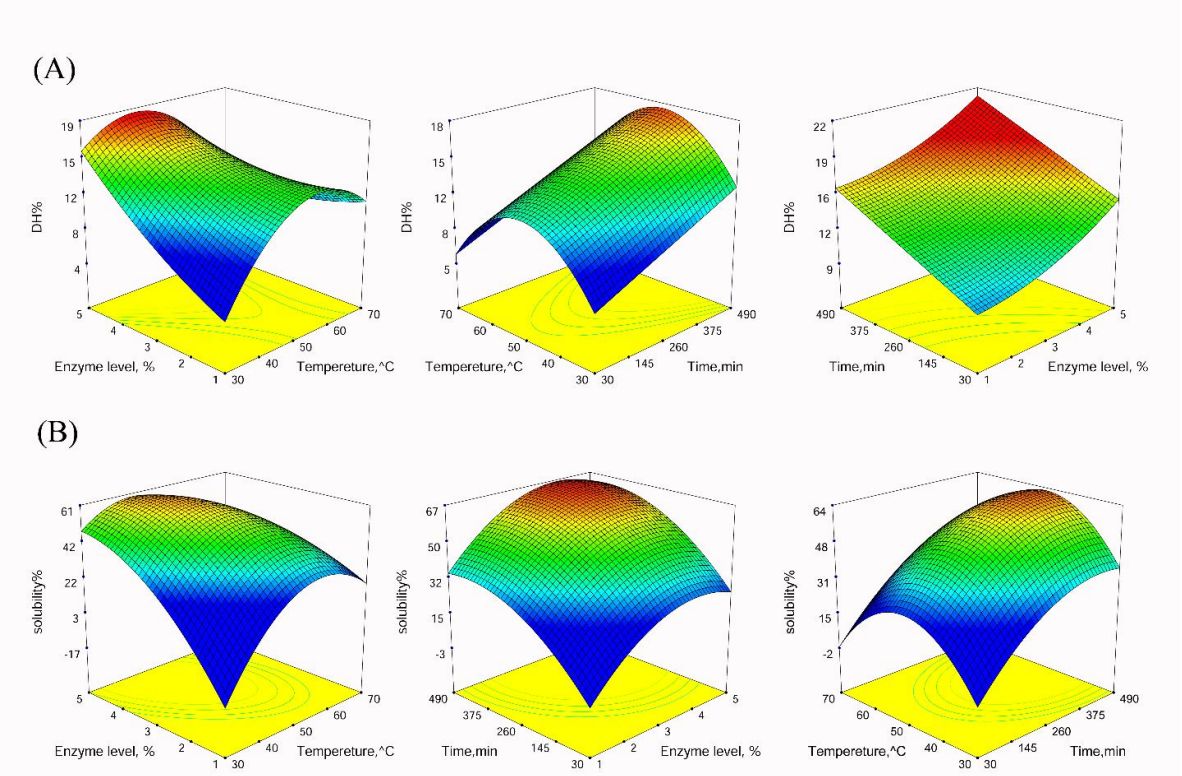

Fig. 2. Response surfaces for the degree of hydrolysis (DH) (A) and solubility (B) as a function of hydrolysis time, hydrolysis temperature, and enzyme level. The missing independent variable in each plot was kept at the center of levels.

Foaming properties

The analysis of variance (ANOVA) indicated that the P-values for foaming properties (FC and FS) were less than 0.01, suggesting that the proposed models showed high significance at a 99.99% confidence level (Table 2). In addition, the R^2 values indicated the models were able to explain 88.13% (FC) and 86.43% (FS) of the experimental data variability. Moreover, the models show statistically insignificant lack of fit, as is evident from the P value of 0.787 and 0.4866 for FC and FS, respectively (Tables 2 & 3). This shows that the models are sufficiently accurate for predicting the foaming properties for any combination of experimental independent variables.

As shown in Fig. 3, the foaming capacity increased with the rise in the enzyme concentration, hydrolysis time and hydrolysis temperature and decreased with higher levels of these factors. It should be mentioned that the foam stability decreased with the increase in the enzyme concentration and hydrolysis time. The aforementioned results demonstrate that a limited amount of hydrolysis is favorable to increase foam capacity, but foam stability is highly decreased as a result of such hydrolysis. Hydrolysis produces smaller peptides which are probably owing to an initial growth in the polypeptide content and allows more air to be incorporated. However, the polypeptides do not have the ability to stabilize the air cells. Foaming capacity has been reported to experience improvements in enzyme-modified food proteins (Ma 1985; Puski 1975). Some larger protein components were required to make a stable foam (Turner 1969). In this study, the breakdown of larger protein components due to enzyme treatment could cause the loss of the foam stability. In addition, an increase in charge density because

of the hydrolysis leads to the reduction of the foam stability since foam stability is developed when the electrostatic repulsion of proteins is at its minimum amount (İbanoğlu & İbanoğlu. 1999).

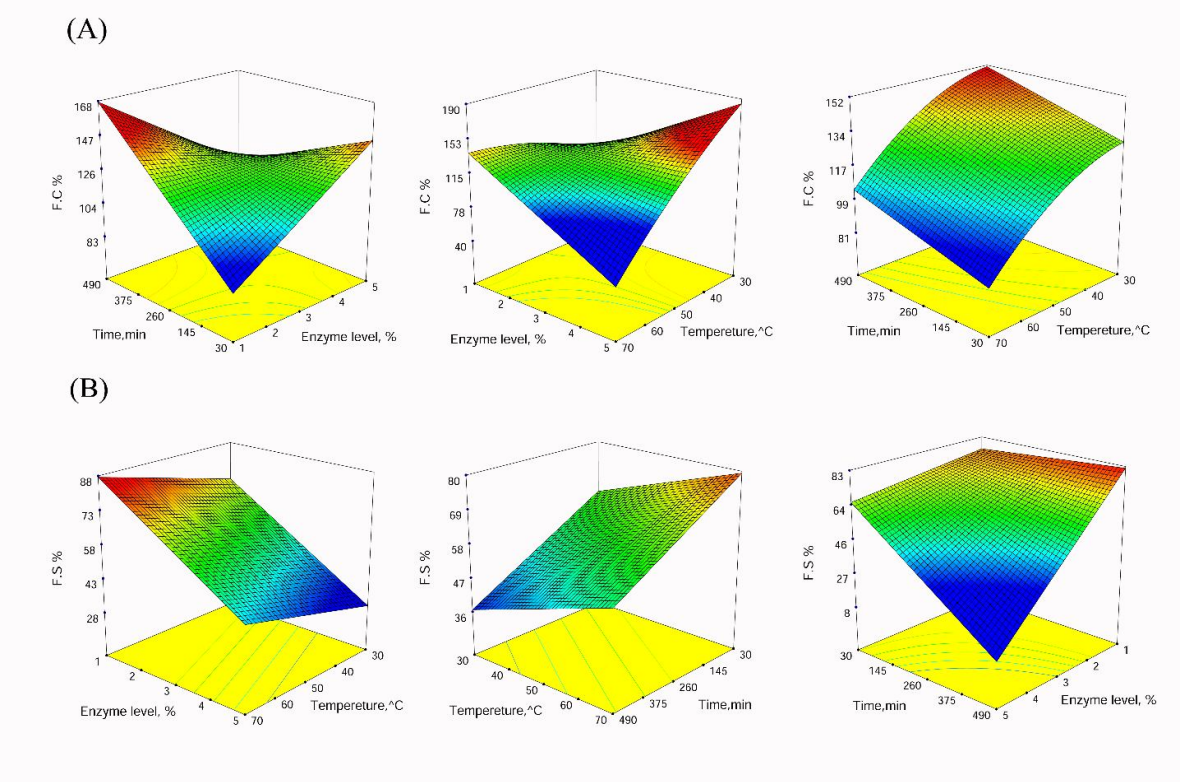

Fig. 3. Response surfaces for foam capacity (A) and foam stability (B) as a function of hydrolysis time, hydrolysis temperature, and enzyme level. The missing independent variable in each plot was kept at the center of levels.

Optimization

Numerical optimization was used to determine the optimal extraction condition. Optimization was done based on the highest values of DH, solubility, FC, and FS as responses. The suitability of the models for producing peptides with the highest functional properties was tested under the conditions: enzyme concentration of 1.75 (%w/w), temperature of 55.43 °C and hydrolysis time of 490 min. Under these conditions, the predicted. DH, solubility, FC, and FS of sodium caseinate hydrolysates were 16.47%, 49.42%, 156.43% and 70.47%, respectively.

A controlled hydrolysis is desirable to increase functional properties of the sodium caseinate. Therefore, the application of *W. coagulans* in the food industry to produce the hydrolyzed proteins with desired properties is highly recommended. Considering the fact that the *W. coagulans* has been employed for the local and medicinal purposes in the human diet, the safety of this plant for industrial applications is approved. The results of the current research present new perspectives on the use of this medicinal plant as an alternative to commercial enzymes for the modification of proteins. Further researches regarding the determination of the other functional properties of sodium caseinate and the use of different proteins are necessary to be done.

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پروتئولیز کازئینات سدیم با استفاده از عصاره *ویتانیا کوآگولانس*: یک مطالعه بهینه‌یابی

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چکیده

میوه ویتانیا کوآگولانس یک منبع پروتئاز گیاهی جدید برای استفاده در صنایع غذایی به‌عنوان جایگزین آنزیم‌های تجاری است. بنـابراین مطالعـه کاربردهای متفاوت این پروتئاز گیاهی در دسترس ضروری است. هدف این مطالعه تولید هیدرولیزات کازئینات سدیم بر اساس خصوصیات کف‌کنندگی، حلالیت و درجه هیدرولیز بهینه، از طریق ترکیب عوامل هیدرولیز مانند زمان، درجه حرارت و میزان آنزیم است. یک طرح مرکب مرکزی برای آزمون‌ها و یک چند جمله‌ای درجه دو برای مدل‌سازی اثرات زمان، درجه حرارت و آنزیم بر روی خصوصیات عملکردی استفاده شد. نتایج نشان داد که افـزایش زمان هیدرولیز و میزان آنزیم سبب افزایش پاسخ‌ها شد. مدل درجه دو ایجاد شده نشان داد که شرایط بهینه برای ماکزیمم پاسخ‌ها غلظت آنزیم 1/75% (وزنی/ وزنی)، درجه حرارت 55/43 درجه سانتی‌گراد و زمان هیدرولیز 490 دقیقه بود. خصوصیات عملکردی هیدرولیزات کازئینات سدیم به‌وسیله روش سطح پاسخ بهینه‌یابی شد و نتایج نشان داد که هیدرولیز کنترل شده برای افزایش خصوصیات عملکردی کازئینات سدیم مطلوب است.

واژه‌های کلیدی: کازئینات سدیم، هیدرولیز، پروتئاز ویتانیا کوآگولانس، خصوصیات عملکردی، بهینه‌یابی

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Numerical Calculation F-value and Lethality of Non-Newtonian Food Fluid during Sterilization based on Can Geometry

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Abstract:

Amount of heat transfer temperature was stimulated in the slowest heating zone of 3.5% starch dispersion during canning sterilization with 10% headspace. The computational fluid dynamics software COMSOL 4.1 was used and governing equations for energy, momentum, and continuity were computed using a finite volume method. The effect of container geometry (cylinders with 6*10cm and 10*6cm dimensions, and cones with 10 cm height and 5 cm radius on 0 and 180° position) on heat penetration parameter (j) and microbial lethality (L) in slowest heating point were investigated. The temperature of the slowest heating zone was monitored by a thermocouple and then compared with the predicted temperature by software. It was determined that cone-shaped container had the fastest heat transfer during sterilization. Also, container geometry has a significant effect on slowest heating zone shape, position, final temperature, j, L, and F-value.

Keywords: Microbial lethality, Numerical calculation, Sterilization, Container geometry, Heat penetration parameter

Introduction

Canning is one of the economical sterilization methods for food preservation (Karaduman, Uyar *et al.* 2012). In canning industries, in order to prevent the overcooking and preserving the quality of the product, the estimation of good sterilization method is necessary (Karaduman, Uyar *et al*. 2012). The industrial sterilization process is based on a temperature-time profile which ensures the product shelf life and quality by deactivating the special microorganism or enzyme and reducing the loss of nutritional factors in the product. Heating rate and heat transfer mechanism in canned foods (conduction and convection) depends on several factors like difference between retort and product temperature, the ratio of surface to volume, product viscosity (Tattiyakul, Rao *et al*. 2002) package shape, and rotation rates. Several studies have numerically investigated the factors affecting on slowest heating zone (SHZ) shape, position and temperature profile during sterilization of canned foods. Datta and Teixeira (1988) numerically predicted the velocity and temperature of canned water in static retort and suggested that cold area is donate-like and is near one-tenth length from the bottom (Datta and Teixeira 1988). Kumar *et al*. (1990) also used finite element method to simulate non-Newtonian food heating in vertical metal cans which were heated from the top at 121˚C (KUMAR, Bhattacharya *et al*. 1990). They realized that in natural convection, the cold area is near the bottom of the can. Wiese and Wiese (1992) used different numerical methods for determining the break point (WIESE and WIESE 1992). Ghani *et al.* (1999) numerically simulated the natural convection of canned water and CMC with PHOENICS and predicted the fluid flow and internal temperature of these model fluids. They found cold area moves to the bottom during the heating process and its size and shape is different in water and CMC (Ghani, Farid *et al*. 1999). Ghani *et al*. (2002) also studied the sterilization of canned orange soup. They found the temperature of cold area reaches a maximum temperature of 107˚C and SHZ moves to the bottom due to natural convection and stay in 20-25% (Ghani, Farid *et al.* 2002). Farid and Ghani (2004) used a

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new computational technique to estimate the sterilization time of canned foods. In order to predict the temperature profile of the cold area and natural convection in the can, they used computational fluid dynamics with PHOENIX. They suggested that due to little vertical dimension the effect of natural convection in a horizontal can is lower than vertical can (Farid and Ghani 2004). Dimou and Yanniotis (2011) predicted temperature and velocity profile of 7.6*10.9 cm cans containing 4% brine and 0, 1, 8 and 19 asparaguses. They found the cold area is near the %13.5 from the bottom and is not dependent on asparagus numbers (Dimou and Yanniotis 2011). Dimou *et al.* (2011) studied the effect of process variables on temperature profile, flow patterns, and location of the cold area in static cans with computational fluid dynamics. They used asparagus, olive in %4 brine, and peach in %20 sucrose in 76*109 mm cans and have found the flow and temperature are affected by shape, type and arrangement of particles inside the can, and properties of the filling fluid (Dimou and Yanniotis 2011). Srghini and Erdogdu (2016) investigated the effect of rotation rate (end-over-end) and viscosity (water and 1.5% CMC) on heat transfer characteristics of a canned food with headspace (Sarghini and Erdogdu 2016).

The mechanism of unsteady convection is very complex and a valid modeling is essential to determine heat process requirements to predict temperature during heating. In order to make more realistic data from CFD modeling, more accurate conditions are required. The shape, situation, and temperature of SHZ is strongly depended on package geometry, heating time, and product viscosity (Abdul Ghani and Farid 2006, Kannan and Sandaka 2008, Kumar, Wee *et al.* 2012). Since most of the food fluids are non-Newtonian and they have a complex behavior during heating, different studies were done to simulate heating rate in such model liquids such starch dispersions. During the heating, process starch makes a viscose paste (Lagarrigue and Alvarez 2001) that the heat transfer mechanism changes from convection to conduction. This phenomenon leads to a slope change in time-temperature curve at a determined time known as a breakpoint (X_{bh}) (Yang and Rao 1998) (Fig. 1). Due to break in the heating curve, these fluids are known as broken heating fluids. There is limited information about the effect of container geometry on heat penetration parameters of SHZ in starch distribution. However, no study has addressed the numerical calculation of heat penetration parameters and microbial lethality during starch sterilization in its real canning state with headspace. The present study is an attempt to evaluate different container geometry on heat penetration parameters and microbial lethality of starch dispersion SHZ during static sterilization.

Materials and methods

Experimental Procedure

Starch dispersion (3.5% w/w) was filled in two cylinders (6*10 cm and 10*6 cm) and one cone (10 cm height and 5 cm radios) with equal volumes. In real canning procedure there is a headspace above the product and according to Ranjbar *et al.* (2016) the SHZ is near the air-product interface, the T- type thermocouple was placed in 2 cm below the air-product interface (Ranjbar, Ziaiifar *et al.* 2015, Ranjbar, Aman Mohammad *et al.* 2016). All measurements were performed in triplicates. The 8-port data logger (Pico-TC08, England) and related software (PicoLog) were used to record the temperature data with 10s intervals. The steam was assumed to maintain a constant temperature of 394.14 K at all boundaries. The initial temperature of starch dispersion was 323.14 K.

Governing Equations

Since starch dispersion is a breaking heating fluid, the equations (1-6) were used to represent the apparent viscosity of 3.5% starch dispersion as a function of temperature (Yang and Rao 1998):

Viscosity increase section in 78-89.5˚C:

$$\eta \times \left(\frac{\omega}{\omega_r}\right) = 7.4 \times 10^{-6.0} \left(\frac{T}{100-T}\right)^{6.108} \quad (1)$$

The end of viscosity increase (pick

viscosity) and the beginning of viscosity reducing in 89.5-92.5˚C (2):

Reducing viscosity in 92.5-121˚C (3):

Since ω and η^* are dynamic shear data, in order to relating them to static shear data ($\dot{\gamma}$ and η_a), modified Cox-Mers rule is used (4) (Barnes, Hutton *et al*. 1989, Yang and Rao 1998).

C and α shift factor at 25˚C are equal to 2.07 and 1.01, respectively. Thus we have Eq. (5): η^* (T) is as Eq. (6):

T is temperature (°C) and if $T\text{-}x \geq 0$ then $H(T-x)$=1 and if $T\text{-}x \leq 0$, then $H(T-x)$=0

$$\eta*\left(\frac{\omega}{\omega_r}\right) = -69122.86 + 2244.36T - 24.28T^2 + 0.088T^3 \quad (2)$$

$$\eta*\left(\frac{\omega}{\omega_r}\right) = 4.11 + exp\left[23298.3\frac{1}{T} - \frac{1}{366.1}\right] \quad (3)$$

$$\eta*(\omega) = C[\eta_a(\dot{\gamma})]^{\alpha} \quad (4)$$

$$\eta_a = \left[\frac{1}{2.07}\eta*(T)\left(\frac{\dot{\gamma}_r}{\gamma}\right)\right]^{\frac{1}{1.01}} \quad (5)$$

$$\begin{aligned}\eta*(T) = {} & \left(7.4\times 10^{-69}\left(\frac{T}{100-T}\right)^{6.203}\right)\times[H(T-50)-H(T-89.5)] \\ & + (-69122.86 + 2244.36T - 24.28T^2 + 0.088T^3)\times[(H(T-89.5)-H(T-95)] \\ & + \left(4.11 + exp\left[23298.3\frac{1}{T} - \frac{1}{366.1}\right]\right)\times[(H(T-95)-H(T-121)]\end{aligned} \quad (6)$$

Other physical and thermal characteristics of 3.5% starch dispersion is shown in table 1:

Table 1- Starch dispersion heat characteristics (Yang and Rao 1998)

Quantity	Parameter	Equation	Unit
Density	rho	1000*(1-0.00053*(T-IT))	[kg/m^3]
Conduction transfer coefficient	k	0.66	[W/(m*K)]
Special heat coefficient	Cp	4180	[J/(kg*K)]
Convection transfer coefficient	h	Nu_starch*k_starch/(h)	

Numerical simulation was includes pairing two physical phenomena: heat transfer and fluid flow. Since the system was cylindrical and cone shaped cans containing food with natural convection, non-isothermal laminar flow equations were used. For each problem, one 3-D geometry was defined and meshing was done according table 2.

Table2- Different cans meshes for cone 0°, cone 180°, cylinder1, and cylinder2 geometries

Can Characteristics	Number of element	Maximum element size
Cone 0 °	61,549	0.0335
Cone 180 °	61,549	0.0335
Cylinder1 (6*10cm)	54,680	0.039
Cylinder2 (10*6cm)	107,356	0.0201

A BDF[1] method for time stepping and Backward Euler to time discretization were used. The system used to run the test and solve the equation was IntelVR CoreTM i5CPU M 460 @ 1.70 GHz and 6GB RAM.

The governing equations for non-isothermal laminar flow for domains were defined. The equations of continuity (7), energy (8), and momentum (9) are as below:

Continuity equation:

$$\frac{\partial \rho}{\partial t} + \nabla.(\rho u) = 0 \qquad (7)$$

Energy equation:

$$\rho c_p \frac{\partial T}{\partial t} + \rho c_p u \cdot \nabla T = \nabla \cdot (k \nabla T) + Q + Q_{vh} + W_p \qquad (8)$$

Momentum equation (9).

$$\rho \frac{\partial u}{\partial t} + \rho(u \cdot \nabla)u = \nabla \cdot \left[-pI + \mu(\nabla u + (\nabla u)^T) - \frac{2}{3}\mu(\nabla \cdot u)I\right] + F \qquad (9)$$

Where *P* is the static pressure, *U* is velocity field, *T* is Temperature, μ is viscosity, ρ is density and *F* is the external body force including gravitational effects. To simplify the problem, following assumptions was used (Farid and Abdul Ghani 2004, Abdul Ghani and Farid 2006, Erdoğdu 2008, Kannan and Sandaka 2008, Dimou, Stoforos *et al.* 2011, Dimou and Yanniotis 2011, Erdogdu and Tutar 2012, Kumar, Wee *et al.* 2012):

Data related to cooling phase were ignored.

Effect of gravity (9.8 m/s) was expressed as a body force.

The estimation of food density as a function of temperature for Bostwick calculation for starch was used.

Fluid was considered homogeneous.

Heat Penetration and Lethality Analysis

Since in thermal diffusion analysis, the formula methods are correct than empirical methods (Yang and Rao 1998), formula methods were used in this study for calculating *j* parameter. The smallest *j* indicates there is less time required to reaching the uniform heating. The accuracy of these calculations was evaluated using CFD (Wang and Sun 2003). The parameter of J_h index, as a dimensionless correction factor, is calculated by the Eq. (10):

$$j_h = \frac{T_\infty - T_m}{T_\infty - T_i} \qquad (10)$$

Where T_∞ is steam temperature (121°C), T_m is temperature at time=t (s), and T_i is initial temperature of product. Since pH of starch dispersion is 6, the goal of sterilization is based on reduction or deactivation of *C. Botulinum* spores. Lethality (L) in coldest point was according to Eq. (11):

$$L=10^{(T-121.1)/10} \qquad (11)$$

Where, 121.1°C is the reference temperature and T is the predicted or measured temperature in the coldest point of the can and z is the temperature required to change thermal death time by a factor of 10 that is 10 °C.

The F value for a process is the number of minutes required to kill a known population of microorganisms in a given food under specified conditions. This F value is usually set at 12 D values to give a theoretical 12 log cycle reduction of the most heat-resistant species of mesophilic spores in a can of food. For example, if there were 10,000 spores of a species of spore in a can of food and a 12 D process was given, the initial 10,000 spores (10^4 spores) would be reduced to a theoretical 10^{-8} living spores per can, or again, in theory, one living spore per 10^8 cans of product (one spore per one hundred million cans). F-value is calculated according to Eq. (12):

$$F=\Delta t \times \sum L \qquad (12)$$

Where t is time (s). All these parameters were calculated for two different holding time at 121.1°C at 15 min (900s) and 20 min (1200s) after heating by 121.1°C assumed vapor (tables 3 and 4, respectively).

Results and Discussion

Model validation

The predicted and experimental temperature of SHZ was shown in Fig. 2. Differences was not significant using T student test ($P<0.05$).

[1] Backward Differentiation Formula

The (r) of experimental and simulated data was 0.9484 and the root mean square of errors (RMSE) was 0.124°C. It shows the good accuracy between experimental and model temperature data during CFD modeling.

Heat transfer analysis

Calculation of heat penetration parameters and microbial efficiency is critical to study the uniformity of heat transferring in canning industry (Smout, Ávila *et al.* 2000, Smout, Loey *et al.* 2000). But these calculations are a little complex in non-Newtonian fluids. Starch dispersion is a non-Newtonian solution that has a viscosity dependent on the temperature. By increasing the time and temperature of the heating process, the starch granules start to swelling and then gelatinization (Lagarrigue and Alvarez 2001). Before gelatinization, the heat transfers convectively and after gelatinization, due to conductive heating, the rate of heat transferring and temperature profile is change (Fig. 1).

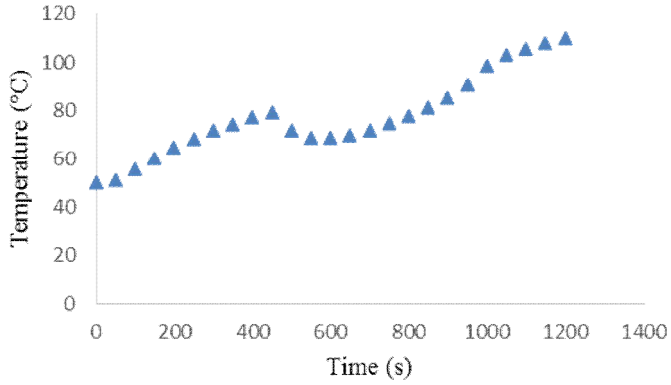

Fig. 1. Temperature-time profile of 3.5% starch dispersion during sterilization (the situation of X_{bh} is determined as 450s or 7.5 min)

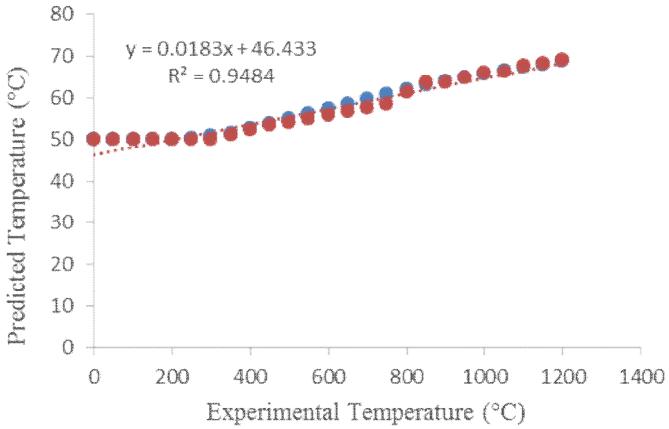

Fig. 2. Comparison of experimental and predicted temperature of starch dispersion SHZ

As can be seen in Fig, 1, the conduction heating leads to a delay in temperature increase. These changes in viscosity significantly affect the heat transfer mechanism. In order to simulation, the temperature profile of such fluids, the simulation of the effect of viscosity on SHZ position, shape, and temperature is necessary (Sarghini and Erdogdu 2016). Calculation of heat transfer parameters in combination with

viscosity changes and container geometry is a complex phenomenon (Varma and Kannan 2005, Buckow, Baumann et al. 2011). Fig. 3 shows the significant effect of geometry on the slowest heating point of different studied geometry.

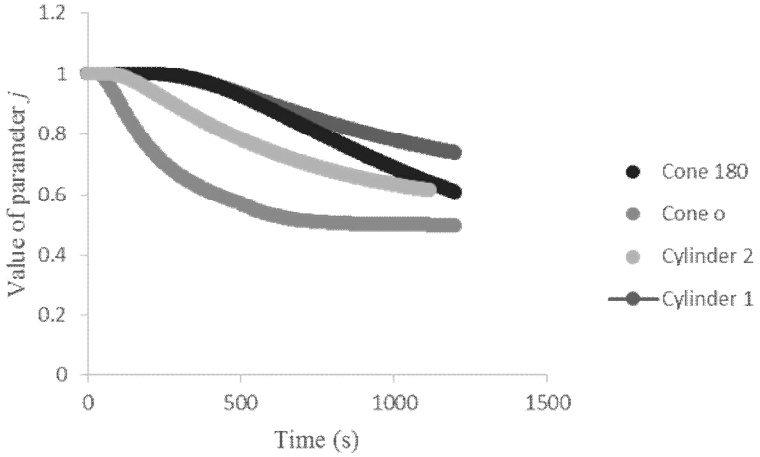

Fig. 3. Values of parameter *j* for different container geometries

The cone 0° shows a good reduction in j by heating time. It indicates the time required for uniform heating in cold point is low in cone 0° (Tutar and Erdogdu 2012). But the cylinder 1 has a relatively higher j parameter than other. It may be due to the higher height of container that fluid should rise doing convection heat transferring. It is however because of the low velocity of starch dispersion during heating (about 0- 65×10^{-8} (m/s)) that increase the time of starch movement before its gelatinization begins to start (Yang and Rao 1998, Lagarrigue and Alvarez 2001). It also has an effect on L and F. As can be seen from tables 3 and 4, the L, F, and temperature of SHZ (0, 0, 7,5) of cone 0° is higher than other geometries; both at the end of 15 min or 20 min holding time.

Table 3- The heat penetration parameter *j* and Lethality factor at 15 min (900s) after reaching the container wall to 121.°C for different container geometry filled with IT=50°C of starch dispersions

Can Characteristics	j_h	Temperature of SHZ (°C)	L	F (min)
Cone 0°	0.4999	85.5	0.0195	0.293
Cone 180°	0.731	69.07	0.00017	0.0025
Cylinder1 (6*10cm)	0.803	63.9	0.00008	0.001
Cylinder2 (10*6cm)	0.652	74.7	0.00096	0.014

Table 4- The heat penetration parameter *j* and Lethality factor at 20 min (1200s) after reaching the container wall to 121.°C for different container geometry filled with IT=50°C of starch dispersions

Can Characteristics	j_h	Temperature of SHZ (°C)	L	F (min)
Cone 0°	0.496	85.72	0.0323	0.646
Cone 180°	0.608	77.78	0.00086	0.0172
Cylinder1 (6*10cm)	0.735	68.8	0.0003	0.006
Cylinder2 (10*6cm)	0.613	77.45	0.0024	0.048

When the starch temperature is 75°C (viscosity increasing section is at 78-89.5°C (gelatinization phase) (Yang and Rao 1998)), its viscosity increase with temperature increasing. The layers near the wall are slowly gelatinized and heat transfers gently from gelatinized layers to other (Yang and Rao 1998).

Figures 4-7 shows the temperature- time profiles of 4 studied geometries at 15 and 20 min holding times. It is interesting that the shape and situation of SHZ are absolutely different in each geometry. The shape of SHZ in cone 0° at time=360s is like an Erlenmeyer flask and with increasing the heating time, two different SHZ with shapes and different temperatures appeared. But finally the SHZ near the air-fluid interface remains and one near the bottom disappears rapidly. This is due to a short height near the cone wall that starch dispersion should rise during convection heating (Ranjbar, Ziaiifar *et al*. 2015, Ranjbar, Aman Mohammad *et al*. 2016).

The holding time during sterilization is critical to destruction the goal microorganism or enzyme and retaining the nutritional materials in foods. At the end of two selected holding time (15 and 20 min), the position of the slowest heating point was determined. The cold point position for cone 0°, cone 180°, cylinder1, and cylinder2 at the end of 900s (15 min) was respectively (0, 0, 8.5 and 0, 0, 1.4), (0, 0, 7), (0, 0, 7.2), and (0, 0, 1.5). And after 1200s (20 min) holding time, was at (0, 0, 8), (0, 0, 8), (0, 0, 8), and (0, 0, 1.8). In cone 0° after 900s, the bottom part of SHZ disappeared and the part near the air-product interface remains. Varma and Kannan (2006) studied the effect of geometry and position of metal container on SHZ. They assumed fulfilled containers. In their study because of the connection of upper wall with the product, the SHZ in cone 180° is at the bottom of the container (Varma and Kannan 2006). But when the air phase is used in a container, the effect of air as a preventing area for direct heat transferring from the wall to the product should be considered.

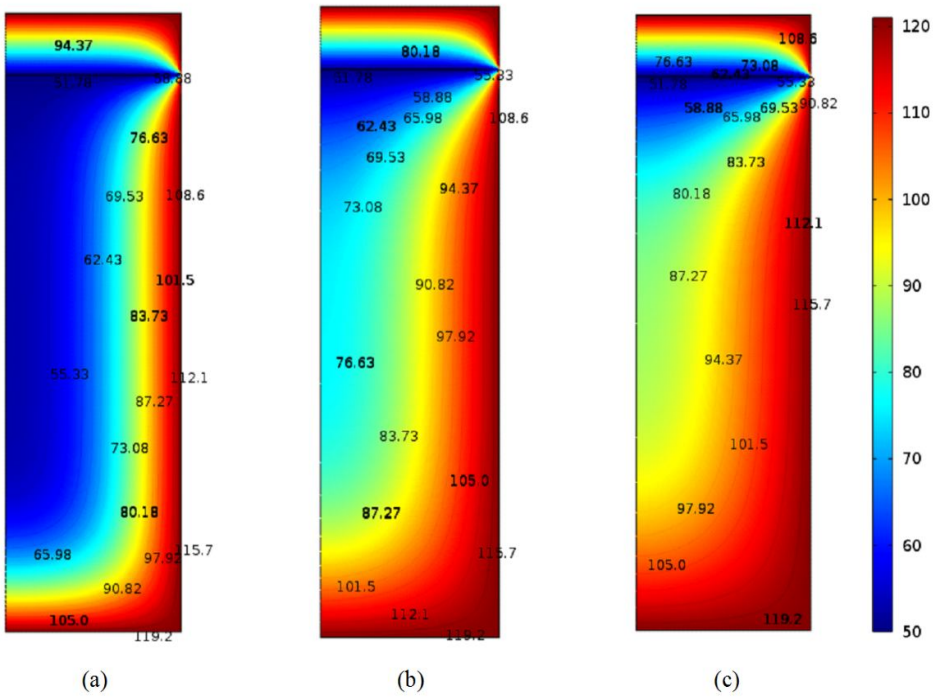

Fig. 4. Temperature counters of cylinder 1 at (a) t=360s, (b) t= 900s, and (c) t=1200s

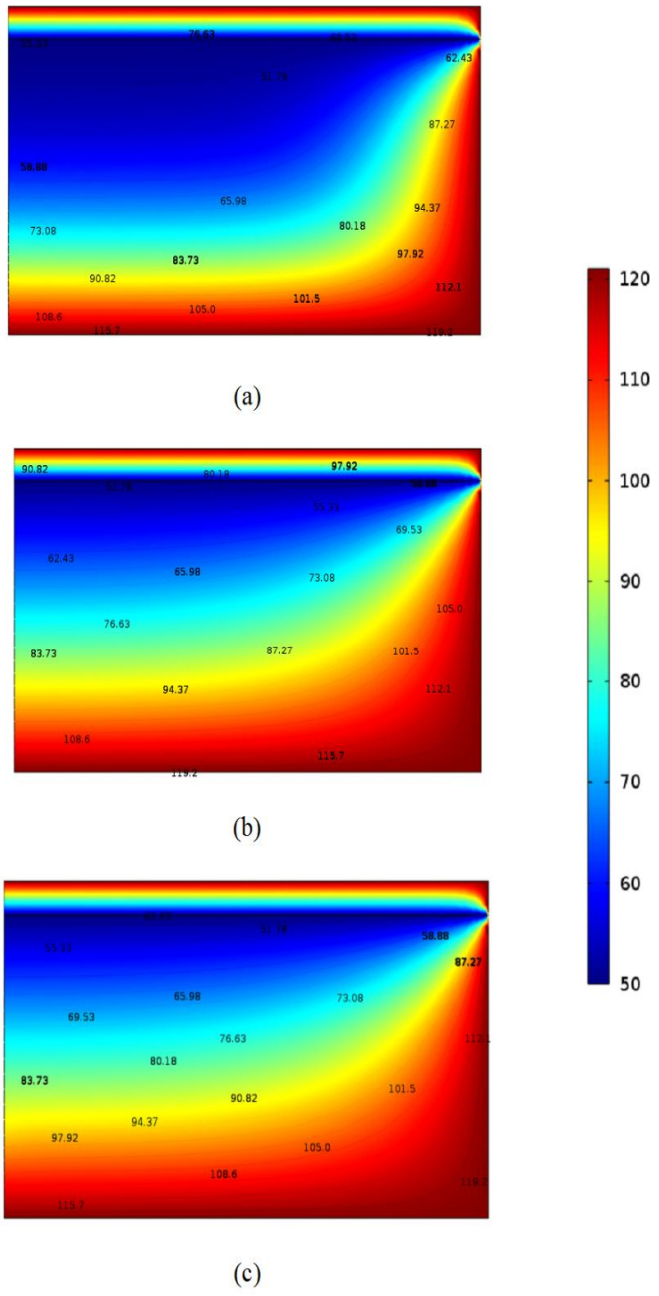

Fig. 5. Temperature counters of cylinder 2 at (a) t=360s, (b) t= 900s, and (c) t=1200s

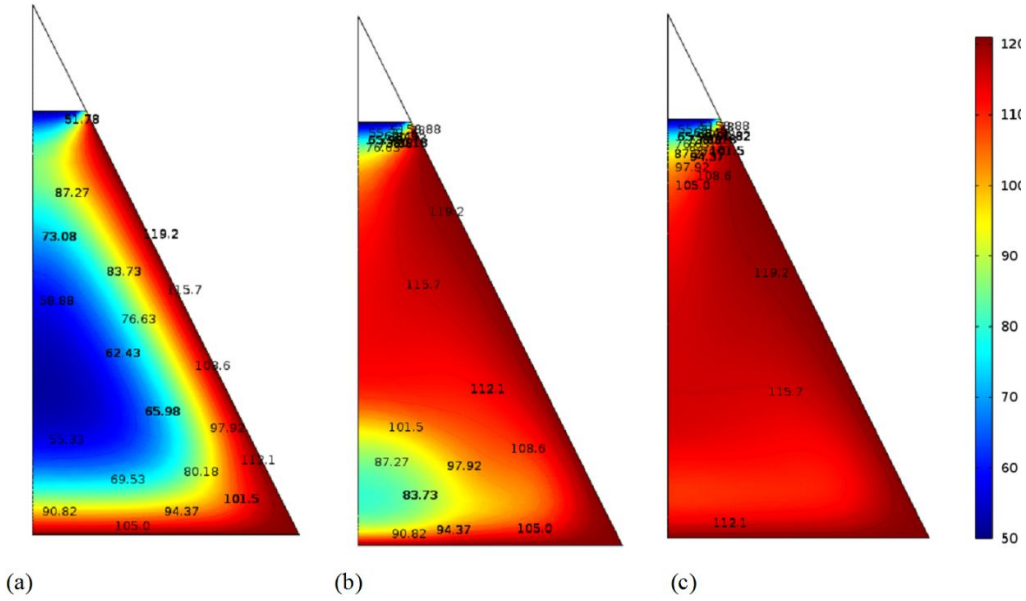

Figure 6- Temperature counters of cone 0° at (a) t=360s, (b) t= 900s, and (c) t=1200s

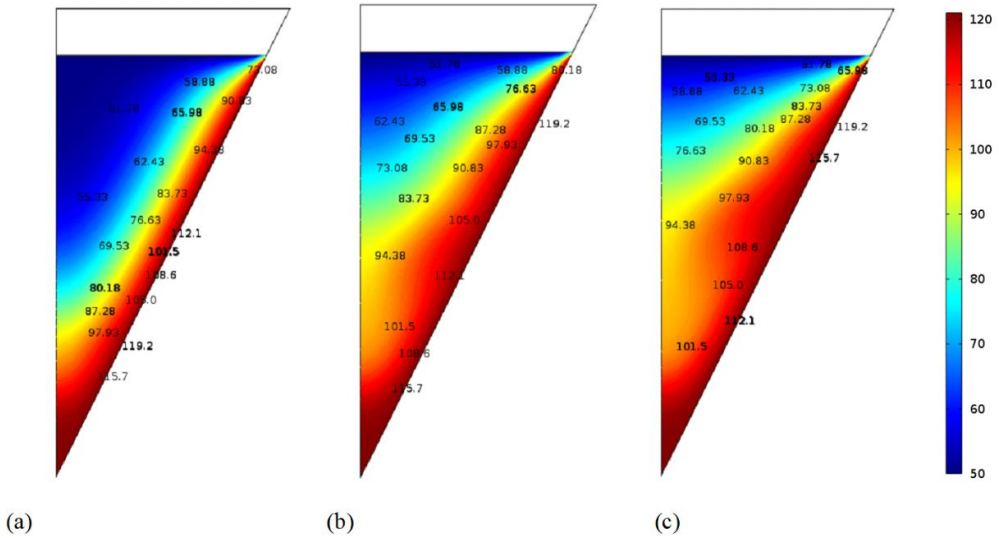

Fig. 7. Temperature counters of cone 180° at (a) t=360s, (b) t= 900s, and (c) t=1200s

In this study, it was demonstrated that at 121.1°C, the 20 min holding time is critical to reaching a good L and F value (tables 3 and 4). The 5 min additional holding time leads to about 1.6, 5, 3.75, and 2 fold on Lethality effect of temperature in cone 0°, cone 180°, cylinder1, and cylinder2, respectively. Also, F-value arises 2.2, 6.8, 6, and 4 fold, respectively. It shows the importance of the selection of a good temperature-time profile during sterilization. But before all, the data showed that the selection of an accurate cold area in heat transfer monitoring is a key step in food sterilization. Because of microbiological and technical considerations, there is a headspace on top of the food product that in stationary sterilizations (such fluids as starch dispersions that high shear rate breaks their

structure (Lagarrigue and Alvarez 2001)), has a great effect on place and shape of SHZ and should be considered in heat transfer studies (Varma and Kannan 2005). Ranjbar et al. (2015) studied the role of the headspace on the processing time of 3.5% starch dispersion. They found heating rate was higher in the samples which were fulfilled while when the headspace is used in a container, a larger volume of headspace leads to a faster healing process. The cold area in 100% fill samples is near the 10% length from the bottom of the can. SHZ in samples with 80 and 90% fill levels, is near the air-product interface. In static sterilization of the food metal containers, heat transfer rate in 100% filled cans are more and if there is headspace in the can, the heating rate decrease with decreasing the air volume but the final temperature is higher (Ranjbar, Ziaiifar *et al.* 2015).

Conclusion

A CFD model was developed to study the time-temperature distribution of a 3.5% starch dispersion in different can shapes. The experimental and predicted temperature at SHZ was in a good agreement. CFD modeling showed the container geometry has a significant effect on SHZ shape, position, final temperature, j, L, and F-value. Also holding time at 121.1°C for 20 min leads to a significant decrease in j and increase in F compared with holding time of 15 min. Also numerically demonstrated that in a static metal container which contain fluids with natural convection behavior during the heating process, the SHZ is close to the interface of air-product.

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محاسبه عددی اندیس F و L سیال خوراکی غیرنیوتنی طی استرلیزاسیون بر حسب هندسه قوطی

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چکیده

مقدار درجه انتقال حرارت در ناحیه سرد دیسپرسیون نشاسته 3/5 % طی استرلیزاسیون در قوطی دارای 10% سرفضا شبیه سازی شد. از نـرم‌افـزار محاسبات عددی COMSOL ورژن 4/1 استفاده شد و معادلات مربوط به انرژی، جابه‌جایی، و پایستگی با استفاده از روش حجم محدود حل شدند. اثر هندسه قوطی (استوانه با ابعاد 10× 6/6 × 10 سانتی‌متر و مخروط با ارتفاع 10 سانتی‌متر و قطر 5 سانتی‌متر به شکل عمودی رو به بالا و رو به پایین) بر پارامتر نفوذ حرارتی (j) و کشندگی میکروبی (L) در ناحیه سرد بررسی شد. درجه حرارت ناحیه سرد توسط یک ترموکوپل پایش و با نتایج پیش‌بینی شده نرم‌افزار مقایسه شد. مشخص شد که قوطی مخروطی شکل سریعترین انتقال حرارت طی استرلیزاسیون را داراست. همچنین هندسـه قـوطی اثـر معنی‌داری بر شکل، موقعیت، دمای نهایی، پارامتر j، L و F ناحیه سرد دارد.

واژه‌های کلیدی: کشندگی میکروبی، محاسبه عددی، استریلیزاسیون، هندسه قوطی، پارامتر نفوذ حرارتی

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Chemical and antimicrobial properties of silver carp surimi enriched by Thyme leaves extract

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Abstract

In this study, effect of Thyme (*Thymus vulgaris*) ultrasonic extract on the quality of *Hypophthalmichthys molitrix* surimi was evaluated. For this purpose, the leaves of *Thymus vulgaris* were dried, grinded and soaked in methanol (1:10 w/v) for 30 min at 45˚C and sonicated at 30 kHz for 15 min at 40˚C. The extract was suspended by Dimethyl sulphoxide and mixed with surimi (0.4 and 0.8% w/w). Then, the analysis of chemical (free fatty acid, peroxide value, Thiobarbituric acid and total volatile base nitrogen) and microbial (mesophilic and psychrotrophic viable count) properties of the samples were done at specific intervals after zero, 4, 8, 12 and 16 days of storage at 2 ºC. Results of chemical and microbial analysis showed that 0.8% concentration of *T. vulgaris* could increase the shelf life of *Hypophthalmichthys molitrix* surimi and there is significant difference between control and treated samples. Moreover, the results could be claimed that the *T. vulgaris* due to marvelous antioxidant and antimicrobial component such as thymol (52.17%), ρ-cymene (14.42%), carvacrol (9.11%) and γ- terpinene (4.45%) has significant effect on preventing the *Hypophthalmichthys molitrix* surimi oxidation and microbial growth. The results also showed ultrasound was the effective way to extract the *Thymus vulgaris* beneficial compounds.

Keywords: Antioxidant; Fish; Shelf life; Surimi, Thyme

Introduction

Rancidity caused by oxidation of fish lipids is one of the major problems encountered in fish processing, producing off-flavor and reducing their nutritional value (M. Asnaashari, Farhoosh, & Sharif, 2014). Many efforts have been carried out for supplying fresh fish according to consumer's demand (Farhoosh *et a*l,. 2016).

The use of natural antioxidants is one of interest to processing industry (Asnaashari *et al*,. 2015; Eshghi *et al.*, 2014). Many natural compounds have been extracted and used as antimicrobial agents in food preservation like essential oils and extracts from herbal plants acting against food spoilage and suppressing a wide variety of pathogens (Asnaashari *et al*., 2015; Asnaashari *et al*., 2016; Farahmandfar, *et al*., 2015). Thyme (*Thymus vulgaris*), belongs to *Lamiaceae* family (Gandomi *et al*., 2009) is extensively used as a flavor ingredient in a wide variety of food in Iran. This plant possesses carvacrol, thymol as main phenolic compounds and p-cymene as main non-phenolic compounds (Sharififar *et al*., 2007). Its antioxidant property is due to free radical production chain with one hydrogen atom to break up fat oxide and subsequent delay that able to inhibit linoleic acid oxidation (Asnaashari *et al*., 2015). Thyme has also antibacterial effect and could be used as an antibacterial preservative in hamburger or other meat products (Sharafati Chaleshtori, *et al*., 2013).

Production of fish protein ingredients such as surimi, minced fish and fish protein isolate is common (Farahmandfar *et al*., 2016; Ruusunen & Puolanne, 2005; Shaviklo & Johannsson, 2006). Surimi contains about 76% water, 15% protein, 6.85% carbohydrate, 0.9% fat and 0.03% cholesterol (Chaijan *et al*., 2004; Jin *et al*., 2007). Suitable properties of surimi such as light color, bland odor, low fat

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content and high in myofibrillar proteins as well as considerable functional characteristics due to the unique gelling property of its concentrated proteins, make surimi as a perfect functional ingredient to make up new food products (Lanier *et al*., 2013).

In recent years, ultrasound-assisted maceration (UAM) has received considerable attention for the recovery of different compounds from various sources. This technique is attractive because of its simplicity and low equipment cost and temperature (avoid thermal damage), high efficiency and saving time of extraction compared with solvent extraction. It has been suggested that improvement of solvent extraction from material by ultrasound is due to mainly the mechanical effects of acoustic cavitation, which enhances mass transfer and solvent penetration into the plant material by disrupting the cell walls (Farahmandfar *et al*., 2015)

The aim of this study was to use of ultrasound-assisted maceration for extraction of *T. vulgaris* and its effect on quality properties of *Hypophthalmichthys molitrix* surimi after 0, 4, 8, 12 and 16 days of storage at 2°C.

Materials and Methods

Materials

T. vulgaris wild leaves were collected from Mazandaran (north of Iran), and dried in oven (Behdad, Iran) then leaves were powdered by mixer (Panasonic, MK-G20NR). 5 Silver carp (*Hypophthalmichthys molitrix*) were purchased from warm water fish farm in Mazandaran province (north of Iran). Average characteristics of fish were male, 1 year, 40 cm length, 10 cm width and 1.25 kilograms. They were transferred to laboratory under cool chain and stored at 2˚C for 4h for further processing. All chemical reagents used for experiments were analytical grade.

Ultrasound-assisted extraction

Crushed dried *T. vulgaris* leaves were added to appropriate methanol (1:10 w/v) in a sample bottle. The leaves were extracted in a shaking water bath for 30 min at 45˚C. The mixture was sonicated with an ultrasonic probe system (Branson, 8510R-Mt, Canada) at 30 kHz for 15 min at 40˚C. The extracts were filtered through Whatman No.1. The organic solvents were concentrated to near dryness using rotary evaporator bath (Buchi EL 141, Switzerland) under reduced pressure (Albu, Joyce, Paniwnyk, Lorimer, & Mason, 2004).

GC-MS analysis

The GC-MS was carried out on an Agilent model 5975C USA mass spectrometry operating in the ionizing energy mode at 70 eV, combined with the GC. The separation was done on a 30 m × 0.25 mm column coated with 0.25 μm HP5-MS. The analytical conditions were as follows: carrier gas, He, 1.3 mL/min in the constant flow mode; injector temperature, 250 ℃; injection volume, 1 μL; split ratio, 15:1; temperature program, 2 min at 40 ℃, raised at 3 ℃/min to 180 ℃, raised at 10 ℃/min to 280 ℃; transfer line to MSD, 280 ℃; MSD, 170 ℃. The ionization energy was 70 eV. The range *m/z* 40-300 was scanned at a rate of 0.52 scans/s. A mixture of the *n*-alkanes (C_9-C_{30}) was analyzed under the same conditions to calculate the retention indices. The compounds were identified according to their mass spectra and their retention indices (Sparkman, 2005).

Sample preparation

The fish were eviscerated, deboned and washed prior to be minced by a meat grinder (Panasonic, MK-G20NR), then the minced fish fillets were processed to surimi. The process involved three washing steps by cold water (10±1°C) for removing water soluble proteins and then dewatered using cloth filtration. In each step, the ratio of water to the minced meat was 3:1 (v/w). During each washing step, the mixture was stirred for 5 min. For more dewatering, at the third step, 0.2% NaCl was added to the mixture (Moosavi-Nasab *et al*., 2005). The extract was suspended by Dimethyl sulphoxide (DMSO) and two concentrations of ultrasound-assisted extraction of *T. vulgaris* (0.4 and 0.8%) were

added to surimi. The surimi was packaged in 100 g containers without open air exposure and evaluated in triplicate for microbial and chemical properties after zero, 4, 8, 12 and 16 days of storage at 2±1ºC. Control experiment was surimi untreated without any *T. vulgaris* extract.

Chemical analyses

Determination of free fatty acids (FFA)

The lipid extraction was performed using the ratio of 20 parts of 2:1 dichloromethane/methanol to 1 part of tissue. A weak salt solution (e.g., 0.66% NaCl) was then added to achieve a final ratio of 8:4:3 dichloromethane/methanol/water including the water contained within the tissue. The dichloromethane phase was concentrated in a rotary evaporator to 20 mL at ambient temperature. The flask was totally covered with a black material to avoid light influence. The lipid extract was deposited in tubes. All of the solvent was evaporated with nitrogen, and 3 mL of cyclohexane was added, followed by 1.0 mL of cupric acetate-pyridine reagent with agitation of the biphasic system for 30 s. After centrifugation at 2000*g* for 10 min, the upper layer was read at 710 nm (Bernárdez *et al*., 2005).

Measurement of peroxide value (PV)

Peroxide value (PV) was measured according to the AOAC method (Willian, 2000). Three samples (each sample 5 g) were weighed in a 250-mL glass and heated in a water bath at 60 °C for 3 min, then thoroughly agitated for 3 min with 30 mL acetic acid–chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman No. 1. Saturated potassium iodide solution (0.5 mL) was added to the filtrate, which was transferred into titrator equipped with stirrer and pH electrode. The titration was allowed to run against standard solution of sodium thiosulfate (25 g/L). PV was calculated and expressed as milliequivalent of oxygen per kg of sample:

$$PV = \left(\frac{S \times N}{W}\right) \times 1000 \qquad (1)$$

Where: S is the volume of titration (ml), N the normality of sodium thiosulfate solution (N=0.01), and W the sample weight (kg) (Namulema *et al*.,1999).

Determination of thiobarbituric acid reactive substances (TBARS)

The thiobarbituric acid value was determined colorimetrically (Pezeshk *et al*., 2011). A portion (200 mg) of sample was weighed into a 25 ml volumetric flask. An aliquot (1 ml) of 1-butanol was added to dissolve the sample. A portion (5.0 ml) of the mixture was added into 5 ml of TBA reagent. The test tubes were vortexed and placed in a water bath at 95°C for 120 min, then cooled. Absorbance (As) was measured at 530 nm against water blank. A reagent blank was run and absorbance (Ab) recorded. TBA value (mg of malonaldehyde equivalents/kg of tissue) was obtained by the formula.

$$TBA = \frac{50 \times (As - Ab)}{200} \qquad (2)$$

Determination of total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) was determined by distillation after the addition of magnesium oxide to fillet sample (Willian, 2000). The distillate was collected in an Erlenmeyer containing 3% aqueous solution of boric acid and mixed indicator from dissolution of 0.1 g of methyl red and 0.05 g of methylene blue to 100 ml of ethanol 96%. Finally, the boric acid solution was titrated with a 0.1 N hydrochloric acid solution (Namulema *et al.*, 1999).

Microbiological analyses

Ten grams of fish fillet samples (3 samples) were aseptically removed from the trays and homogenized for 1 min in a stomacher (VRN-200, Taiwan R.O.C) containing 90 ml of physiological saline solution (0.85% NaCl) (Merck, Darmstadt, Germany) in triplicate. After resuscitation (for 30 min at 25°C) further decimal serial dilutions were prepared from this homogenate in the same sterile diluent. The appropriate dilutions were subsequently used for enumeration in the samples, at each of

the pre-determined time intervals, during refrigerated storage. Mesophilic and psychrotrophic viable count (MVC and PVC) were determined by inoculating 0.1 mL of the sample homogenate onto triplicate sterile plates of dried Tryptic Soy Agar (Liofilchem, Italy) using the surface spread technique, then the plates were incubated for 48 h at 35 °C and for 10 days at 2°C for MVC and PVC, respectively. All counts were expressed as log CFU/g (Amin, 2012).

Statistical analysis

The obtained data were subjected to one-way analysis of variance using SPSS statistical software, release 18.0. Duncan's new multiple range test was performed to determine the significant differences of the means at the 5% probability level ($P<0.05$).

Results and Discussion

GC-MS analysis of thyme extract

According to GC-MS analysis, the main phenolic and non-phenolic active compounds in Thyme UAM extract were as: thymol (52.17%), ρ-cymene (14.42%), carvacrol (9.11%), γ- terpinene (4.45%), α- terpineol (1.25%), sabinene hydrate (1.56%), linalool (7.32%) and geraniol (1.78%). Thymol, ρ-cymene, carvacrol, γ- terpinene, α- terpineol, sabinene hydrate, linalool and geraniol were the most important compounds in Thymus. In comparison with other studies, recovery of different compounds in conventional method (solvent extraction) were more than UAE technique, however there was not any significant difference (Chizzola *et al*., 2008). In the present experiments, quantitative changes observed in the contents of γ-terpinene, *p*-cymene, carvacrol and thymol can be attributed to their localization in the biosynthetic pathway (Kowalski & Wawrzykowski, 2009). The increase of γ-terpinene concentration may result from the UAM influences on the shift of chemical balance through the conversion of thymol, carvacrol, and *p*-cymene in the biosynthetic pathway in the direction of γ-terpinene synthesis. Researchers found a reduction of the content of important components in tea extract after ultrasonically assisted extraction (Xia, *et al*., 2006). The quantity of these compounds can be also varied due to harvesting season, plant age, soil, climate, geographical sources and herb drying method (Bagamboula *et al*., 2004; Shafiee & Javidnia, 1997).

Chemical analyses of surimi

Free Fatty Acids (FFA)

Free Fatty Acids in fish muscle develop undesirable flavors and tissue damage by a combination of muscle protein. They also accelerate the degeneration and loss of product quality and increase of fat oxidation (Sayyari & Farahmandfar, 2016). The results of FFA in different treatments of *Hypophthalmichthys molitrix* surimi were shown in Table 1. The initial FFA value in all samples at first was 0.41 - 0.51 oleic acid. According to the results, the amount of FFA in all treatments was increased with the passage of time and reached 4.17, 2.43, and 2.11% oleic acid for control, 0.4 and 0.8% thyme, respectively. Similar results were reported by (Zolfaghari *et al*., 2011) study. Although the amount of FFA was increased during storage, but this increase was not regular. The time and concentration had a significant effect on the formation of FFA during storage. The results showed that in the higher concentrations of thyme, formation of FFA was less compared to the other treatments.

Table 1. FFA formation in *Hypophthalmichthys molitrix* surimi samples including 0.4% and 0.8% ultrasound thyme extracted during storage

Storage time (days)	Control	0.4% Extract	0.8% Extract
zero	0.51 ± 0.02^{aE}	0.41 ± 0.02^{aE}	0.45 ± 0.05^{aE}
4	1.21 ± 0.02^{aD}	0.64 ± 0.04^{bD}	0.52 ± 0.07^{bD}
8	2.35 ± 0.04^{aC}	1.29 ± 0.04^{bC}	0.95 ± 0.03^{cC}
12	3.12 ± 0.03^{aB}	1.85 ± 0.07^{cB}	1.43 ± 0.08^{dB}
16	4.17 ± 0.12^{aA}	2.43 ± 0.05^{cA}	2.11 ± 0.08^{dA}

Means with the same small letters in a raw and capital letters in a column were not significantly different ($P < 0.05$).

Peroxide value (PV)

PV values in treated and control samples of *Hypophthalmichthys molitrix* surimi are shown in Figure 1. The initial PV value in all samples at the beginning of storage period was 0.83-0.86 meqO_2/kg. PV values after 16 days of storage period were 5.13, 4.24 and 4.13 meqO_2/kg for control, 0.4 and 0.8% of thyme UAM extracts, respectively. Significant lower PV value was observed for treated samples during the storage period at 4ºC ($P<0.05$).

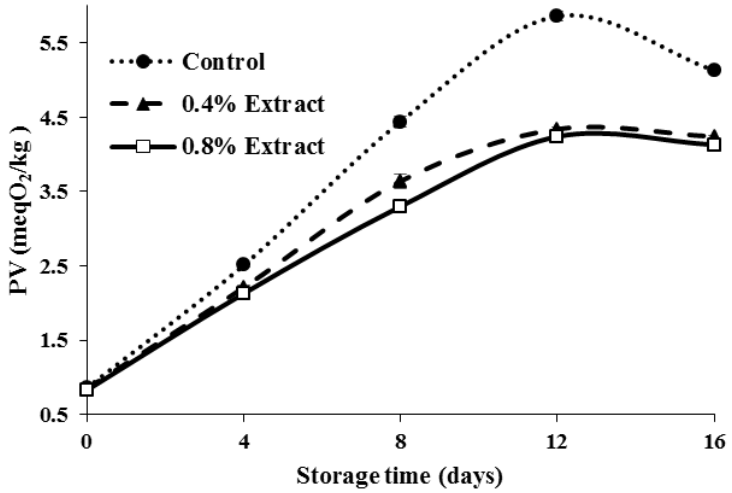

Fig. 1. Hydroperoxide formation (PV) in Hypophthalmichthys molitrix surimi samples including 0.4 and 0.8% ultrasound thyme extracted during storage

The PV value is an index of lipid oxidation measuring primary oxidation products (Farhoosh *et al*., 2016). Fish are very susceptible to both microbiological and chemical deteriorations, due to their chemical composition (Goulas & Kontominas, 2007). Storage of food products is accompanied by oxidation of unsaturated fatty acids. This process is important in seafood due to higher poly-unsaturated fatty acid content. Degradation of products formed during oxidation of unsaturated fatty acids is followed by the formation of low-molecular-weight volatile compounds, which accounts for foreign shades of odor and flavor (Misharina & Polshkov, 2005).

The primary value of PV in *Hypophthalmichthys molitrix* surimi was lower than raw and unwashed compared samples of *Hypophthalmichthys molitrix* reported by Asgharzadeh, Shabanpour, Aubourg, and Hosseini (2010). The results of present study indicate that the UAM is effective in retarding the production of primary lipid oxidation. Similar results, conventional maceration, were obtained by Mexis, Chouliara, and Kontominas (2009) and Ojagh, Rezaei, Razavi, and Hosseini (2010). The major protective effect of UAM thyme extract is owed to its carvacrol, thymol content (Mexis *et al*., 2009). Similar consequences were expressed by Ojagh *et al*. (2010).

Thiobarbituric acid reactive substances (TBARS)

TBA value in treated and control samples of *Hypophthalmichthys molitrix* surimi are shown in Table 2. The initial TBA value in all samples at first was 0.55-0.65 mg of malonaldehyde/kg fat. TBA value increased with time of storage at 2˚C for all treatments and reached 3.65, 3.22, and 2.87 for control, 0.4 and 0.8% thyme, respectively. Significant lower of TBA value was observed for treated samples and 0.8% of thyme during the storage period at 4˚C ($P<0.05$). TBA values of control and 0.4% samples reached the maximal

recommended limit (Lakshmanan, 2000) for TBA values of fish (2 mg of malonaldehyde/kg of tissue) at 8th of storage, while for 0.8% treatment, TBA content was still lower than upper acceptability limit.

Table 2- TBA value changes in *Hypophthalmichthys molitrix* surimi samples including 0.4% and 0.8% ultrasound thyme extracted during storage

Storage time (days)	Control	0.4% Extract	0.8% Extract
zero	0.55 ± 0.01^{aE}	0.65 ± 0.05^{aE}	0.61 ± 0.03^{aE}
4	1.85 ± 0.05^{aD}	1.32 ± 0.02^{bD}	1.25 ± 0.04^{bD}
8	2.65 ± 0.12^{aC}	2.25 ± 0.11^{bC}	1.84 ± 0.01^{cC}
12	3.25 ± 0.17^{aB}	2.56 ± 0.02^{bB}	2.29 ± 0.04^{cB}
16	3.65 ± 0.23^{aA}	3.22 ± 0.06^{bA}	2.87 ± 0.03^{cA}

Means with the same small letters in a raw and capital letters in a column were not significantly different ($P < 0.05$).

Thiobarbituric acid (TBA) is a common indicator for the assessment of degree of lipid oxidation (Manju *et al*., 2007). Results of Pezeshk *et al*. (2011) indicated that the effect of the turmeric extract and shallot extract on the rainbow trout samples decreased the TBA value and increased the shelf life of fish sample during the refrigerated storage. Similar results have been reported by Mexis *et al*. (2009) during storage of oregano essential oil-treated rainbow trout fillets at 2°C.

Total volatile basic nitrogen (TVB-N)

TVB-N values in treated and control samples of *Hypophthalmichthys molitrix* surimi are presented in Fig. 2. The initial TVB-N value in all samples at first was 11.22-11.27 mg N/100g. TVB-N values increased progressively with time of storage at 2˚C for all treatments and reached to 65.7, 53.98 and 38.13 for control, 0.4 and 0.8% thyme UAM extracts, respectively. However, significant lower TVB-N value was observed for treated samples during the storage period at 2˚C ($P<0.05$). TVB-N values of control and 0.4% samples reached the upper acceptability limit set by the EU (European-Union, 1995) for TVB-N values of fish (30 mg/ 100 g of fish flesh) at day 12 of storage, while for 0.8% treatment, TVB-N content was still lower than upper acceptability limit.

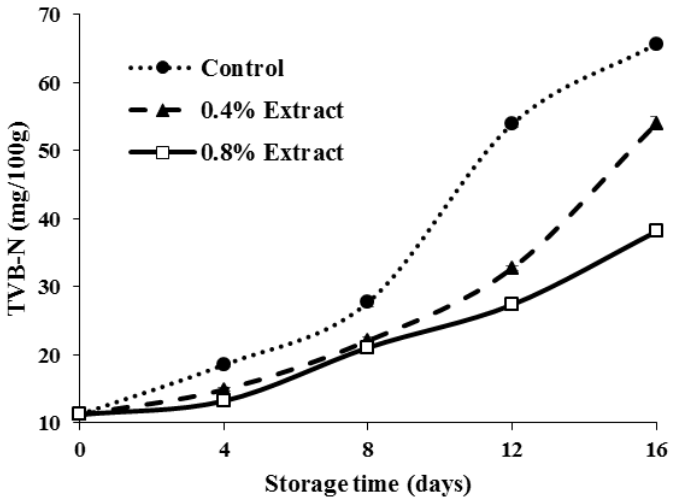

Fig. 2. TVB-N value changes in Hypophthalmichthys molitrix surimi samples including 0.4 and 0.8% ultrasound thyme extracted during storage

TVB-N content in fish muscle is not only different among species but also is variable in same species due to age, sex, season and environment (Razavi Shirazi, 2007). Some researchers concluded that TVB-N was not a good quality index for fish (Mexis *et al.*, 2009), but it could be used as a quality index. Because increasing TVB-N content of fish samples during storage is directly related to the activity of spoilage bacteria and endogenous enzymes (Goulas & Kontominas, 2007; Özogul, Polat, & Özogul, 2004). TVB-N is composed of different compounds including ammonia, methylamine, dimethylamine as well as trimethylamine (Razavi Shirazi, 2007) which are produced by spoilage bacteria and endogenous enzymes.

The lower TVB-N content in samples treated by thyme UAM extract may be attributed to the antibacterial properties of phenolic compounds such as carvacrol, thymol and linalool. Low levels of TVB-N in treated samples can be attributed to either decreased bacterial population or reduced capacity of bacteria for oxidative deamination of nonprotein nitrogen compounds or both (Manju *et al.*, 2007), which was due to the effect of thyme UAM extract. Similar results have been reported by Fahimdezhban, *et al.*, (2014) regarding to *Zataria multiflora* and *Rosemarinus officinalis* extracts on quality of minced frozen *Hypophthalmichthys molitrix*.

Bacteriological analysis of surimi

Changes in MVC and PVC of control and two concentrations of thyme UAM in *Hypophthalmichthys molitrix* surimi, during the refrigerated storage, are shown in Table 3. The initial MVC and PVC (log CFU/g) of samples were from 3.28 to 3.48 and from 3.24 to 3.34 ranges, respectively. By the day 12 of storage, however, MVC and PVC in 0.8% of thyme UAM was still below 7 log CFU/g, while that of controls and 0.4% of thyme UAM attained a count of 8.45 and 7.44 for MVC and 8.20 and 6.47 for PVC. There was a significant difference between control, 0.4 and 0.8% of thyme UAM ($P<0.05$).

Table 3- MVC and PVC changes in *Hypophthalmichthys molitrix* surimi samples including 0.4 and 0.8% ultrasound thyme extracted during storage

Storage time (days)	MVC			PVC		
	Control	0.4%Extract	0.8% Extract	Control	0.4%Extract	0.8% Extract
zero	3.48 ± 0.28^{aE}	3.47 ± 0.19^{aE}	3.28 ± 0.14^{aE}	3.25 ± 0.09^{aE}	3.34 ± 0.06^{aE}	3.24 ± 0.07^{aE}
4	5.44 ± 0.19^{aD}	4.59 ± 0.2^{bD}	4.26 ± 0.1^{bD}	5.31 ± 0.10^{aD}	4.35 ± 0.09^{bD}	4.19 ± 0.03^{bD}
8	6.72 ± 0.37^{aC}	6.07 ± 0.22^{bC}	5.49 ± 0.16^{cC}	6.37 ± 0.04^{aC}	5.51 ± 0.09^{bC}	5.23 ± 0.10^{cC}
12	8.45 ± 0.19^{aB}	7.44 ± 0.2^{bB}	6.36 ± 0.1^{cB}	8.20 ± 0.04^{aB}	6.47 ± 0.09^{bB}	6.24 ± 0.08^{cB}
16	9.36 ± 0.26^{aA}	8.4 ± 0.17^{bA}	7.4 ± 0.12^{cA}	3.25 ± 0.09^{aE}	3.34 ± 0.06^{aE}	3.24 ± 0.07^{aE}

Means with the same small letters in a raw and capital letters in a column were not significantly different ($P < 0.05$).

In the present study, initial MVC and PVC counts indicate good surimi quality (Mexis *et al.*, 2009). Most of the available literature on fresh fish total viable count (TVC) reports bacterial counts of 2-4 (log CFU/g) (Gelman *et al.*, 2001). Fan *et al.*, (2008) have demonstrated the increase of TVC in fish meat during storage. By the day 12 of storage, TVC counts in controls and 0.4% treatments were higher than the maximal recommended limit of 7 log CFU/g for TVC in raw fish (Sallam, 2007).

Most of plant extracts have been shown to inhibit growth of pathogenic bacteria. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal (Zaehner & Fiedler, 1995).

The gram-negative psychrotrophic bacterial count is major group of microorganisms responsible for spoilage of aerobically stored fish at chilled temperatures (Sallam, 2007). In the current study, the growth pattern of PVC was similar to MVC in different times. A significant increase in PVC with time has also been reported for salmon stored at 1˚C for 15 days (Sallam, 2007). The active compound for the inhibition of *E. coli* and *Salmonella*

enteritidis was identified as Gallic acid. It has been reported that gram-negative bacteria have low susceptibility to plant extracts when compared to gram-positive bacteria. The resistance of gram-negative bacteria to antibacterial substances is related to presence of lipopolysaccharides in their outer membrane. Generally, the extent of the inhibitory effects of the extracts could be attributed to their phenolic composition (Hasani & Hasani, 2014; Tesaki *et al*., 1999).

Conclusion

The shelf life of silver carp surimi is due to the chemical and bacteriological activities in fish leading to loss of quality and succeeding spoilage. Current study showed that silver carp surimi treated with Thyme leaves extract had lower microbiological and chemical indices, during refrigerated storage, because of the antimicrobial and antioxidant properties of the extract. It can be concluded that natural extract from Thyme leaves can be used by the food industry to extent the shelf life because they exhibited promising antioxidant and antimicrobial effect.

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خصوصیات شیمیایی و میکروبی سوریمی ماهی کپور نقره‌ای غنی شده با عصاره برگ آویشن

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چکیده

در این تحقیق، تاثیر عصاره فراصوت آویشن بر خصوصیات ماهی کپور نقره‌ای مورد ارزیابی قرار گرفت. به این منظور، برگ‌های آویشن خشک، آسیاب و در متانول (10:1 وزنی/ حجمی) به مدت 30 دقیقه در دمای 45°C خیسانده و در فرکانس 30 kHz به مدت 15 دقیقه در 40°C فراصوت‌دهی شد. عصاره در دی‌متیل سولفوکسید حل و با سوریمی (0/4 و 0/8 درصد وزنی/ وزنی) مخلوط شد. سپس آنالیز شیمیایی (اسید چرب آزاد، عدد پراکسید، اسید تیوباربیتوریک و ترکیبات فرار بر پایه نیتروژن) و میکروبی (میزان باکتری‌های مزوفیل و سرمادوست) نمونه‌ها در فاصله‌های زمانی معین صفر، 4، 8، 12 و 16 روز در دمای 2 °C انجام شد. نتایج آنالیز شیمیایی و میکروبی نشان داد که غلظت 0/8% آویشن می‌تواند عمر ماندگاری سوریمی کپور نقره‌ای را افزایش دهد و تفاوت معنی‌داری بین نمونه شاهد و تیمارها وجود دارد. علاوه بر این، نتایج نشان دادند که به دلیل مقادیر زیاد ترکیبات آنتی‌اکسیدانی و ضد میکروبی همچون تیمول (52/17%)، p- سیمن (14/42%)، کارواکرول (9/11%) و γ-ترپینن (4/45%) تاثیر معنی‌داری بر جلوگیری از اکسیداسیون و رشد ضد میکروبی سوریمی دارد. نتایج نشان داد که فراصوت روش مفید برای استخراج ترکیبات مؤثره گیاهی می‌باشد.

واژه‌های کلیدی: آنتی‌اکسیدان، ماهی، مدت ماندگاری، سوریمی، آویشن

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Using Principle Component Analysis for Evaluation of the Camel Burger quality

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Abstract

In the present study the cow meat was replaced with camel meat (0, 25, 50, 75 and 100%) in burger formulation. Principal component analysis (PCA) was performed to understand quality variables differences and similarities of thirty-five sample burgers. Score plot, represents Principal component analysis of datasets derived from evaluated variables of thirty-five samples (samples contain of 0, 25, 50, 75 and 100% camel meat). Overall, six principal component was obtained which 65.8% of the total variance was concentrated into three first PCs. Cooked L*, cooked b* shrinkage, springiness, flavor, texture, juiciness, color and overall acceptability were the variables which separated by the first PC. The PC2 is characterized by the rest of instrumental texture parameters and the third by cooked a* and fat. The evaluation of score plot shows burgers contain higher amount of camel meat (50, 75 and 100%) had the higher moisture and fat content after cooking, higher scores in flavor, texture, juiciness and overall acceptability.

Keywords: Camel meat burger, Principal component analysis, Overall acceptability

Introduction

Today industry and research are involved with interpretation of large data sets. Usually we face with such data sets which have numbers of columns and rows. In order to interpret such data, one needs statistical methods that can extract the most important information. Principal components analysis (PCA) originally introduced by Pearson (1901) and independently by Hotelling (1933), is a technique used to display patterns in multivariate data. It aims to graphically display the relative positions of data points in fewer dimensions while retaining as much information as possible, and explore relationships between dependent variables. In other word, we can use principal component analysis (PCA), as a useful multivariate statistical method to analyze the variations among physical, color, and sensory properties of meat. The procedure is based on the fact that when there are many measures on a particular object then some of these are likely to be correlated. Variables that are inter-correlated can 'represent' one another. For instance, if variables 1, 2, 3 and 4 are highly correlated with variable 5, then they will all change as variable 5 changes. A composite variable derived from these, could reduce these five variables to one. In PCA analysis, this could constitute the first component. A second component (uncorrelated with the first) can then be derived to examine more variation.

Camelus dromedaries which belong to *Camelus* genera are very important in the case of economy, health and food security in many countries. They have unique properties which help them to stand with the harsh environmental situation, produce milk and meat. Compare to other farm animal it can produce large quantity of meat which is comparable in taste and texture to beef. It characterized by low fat and high moisture content, low content of cholesterol and valuable source of vitamins and some important minerals. In spite of these advantages, public have negative perception and except that arid and semi- arid people, others avoid consumption of the camel meat. However, camel meat can be more acceptable by using in processed meat products such burger and sausage. The present study evaluated various chemical, physical and sensory variables of burgers by Principal Component Analysis (PCA) in order to

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determine the relationship between them and describe camel burger quality.

Material and methods

Burger preparation:

Five formulations of burger were produced and burgers were different in the level of camel meat 0% (beef only), 25%, 50%, 75%, 100% (camel only). 75% Meat was ground through a 5-mm plate in a grinder (Kenwood, MK-G20NR, Spain) and with the other ingredients consist of 12.5% flour, 10% onion, 1.1% sodium chloride and 1.4% spices (black pepper, red pepper, nut Meg, thyme, cinnamon, garlic powder) was thoroughly mixed to obtain a homogenous mixture. Thereafter mixture was shaped by using hamburger patty forming machine (Zophre Co., Ltd., Esfahan, Iran) to obtain patties of approximately 70 g and 1cm thickness. Finally, until analysis in designated times (once every two weeks during 3 months storage) the burgers were placed in plastic containers and were kept under frozen condition (–18 ºC).

Burger quality measurement:

Cooking properties:

Cooking characteristics was evaluated using a process of measure and remeasuring of thickness and diameter before and after cooking of burgers by contact grilling on a preheated electric grill (Delonghi, model 31100, Italy), then calculate as follow:

$$\%\text{Diameter reduction} = ((\text{raw diameter} - \text{cooked diameter})/(\text{raw diameter}))\times 100 \quad (1)$$

$$\%\text{Thickness increase} = \left(\frac{\text{cooked thickness} - \text{raw thickness}}{\text{raw thickness}}\right)\times 100 \quad (2)$$

$$\%\text{Fat retention} = \frac{(\text{cooked weight} \times \%\text{fat in cooked burger})}{\text{raw weight} \times \%\text{fat in raw burger}} \times 100 \quad (3)$$

$$\%\text{Moisture retention} = \frac{(\text{cooked weight} \times \%\text{moisture in cooked burger})}{\text{raw weight} \times \%\text{moisture in raw burger}} \times 100 \quad (4)$$

$$\%\text{Cocking yield} = \frac{\text{cooked weight}}{\text{raw weight}} \times 100 \quad (5)$$

$$\%\text{Shrinkage} = \frac{(\text{raw thickness} - \text{cooked thickness}) + (\text{raw diameter} - \text{cooked diameter})}{\text{raw thickness} + \text{raw diameter}} \times 100 \quad (6)$$

Color attributes:

Color was described by coordinates: lightness (*L**), redness (*a**, ±red-green) and yellowness (*b**, ±yellow-blue) using a colorimeter (Chroma Meter CR-410, Japan) equipped with a light source Illuminant *C* (2° observer).

Texture profile

Hardness (kg), *cohesiveness*, *springiness* (cm), *gumminess* (kg) and *chewiness* (kg× cm) were evaluated as texture profile parameters with a Texture Analyzer QTS following AMSA (1995) procedures. Cubic samples (1×1×1 cm) were cut from patties and subjected to a two-cycle compression test. Samples were compressed to 70% of their original height with a cylindrical probe of 3.5 cm diameter at a compression load of 25 kg, and a cross-head speed of 20 cm/min (modified method of Sánchez-Zapata *et al*, 2010).

Sensory properties:

The appraisal of color, texture, flavor,

juiciness and overall acceptability was done using a 5-point structured hedonic scale for sensory evaluation. Evaluation was performed by 30 trained panelists and each of them evaluated two replicates of all formulas.

Statistical analysis:

The data were analyzed with XLSTAT package (XLSTAT, 2013), after standardization of the variables to mean of zero and variance of one.

Results & Discuction:

Table 1 shows the correlation coefficients between the 17 variables of burger quality. Results show that these variables are significantly correlated in some cases. For instance, cooked L* showed high positive correlation with cooked b*($r=0.86$, $p<0.05$), also cooked L* had positive correlation with sensory color ($r=0.64$,$p<0.05$) that it can be reasonable on the basis of Valin, *et al* (1992) who reported that myoglobin content is important factor in meat redness and darkness. Meat color affected the Panelists judgment positively on sensory color and ultimately negatively on overall acceptability. This means that they recognize the degree of darkness or lightness, redness and yellowness and to give score samples based on them. Camel meat have lower L* than beef and it made a negative concept in terms of color acceptance on panelist. Otherwise cooked a* had a negative and positive correlation with cooked moisture and fat respectively. Other remarkable correlation coefficient is: positive correlation between instrumental texture that all of them changed in one direction, besides; among them just springiness was fairly impressive on the data derived from sensory evaluation such as texture, juiciness, overall acceptability and color, its negative correlation with the first three means sensorial evaluation scores increase with decreasing springiness and vice versa. Since shrinkage has the positive correlation with springiness and negative with juiciness, texture and overall acceptability, springiness is the other unpleasant effective variable on these sensory scores.

Table 1- Correlation coefficient between burgers quality variables

Variables	Cooked l	Cooked a	Cooked b	ΔE	Shrinkage	Cohesiveness	Chewiness	Hardness	Gumminess	Springiness	Cooked Moisture	Cooked Fat	Flavour	Texture	Juiciness	Color	Overal Acceptability
Coocked l																	
Coocked a	-0.299																
Coocked b	**0.864**	0.030															
ΔE	**-0.637**	0.004	**-0.678**														
Shrinkage	**0.449**	0.297	**0.586**	-0.300													
Cohesiveness	0.093	0.277	0.270	-0.074	0.225												
Chewness	0.092	0.244	0.071	0.032	0.101	**0.539**											
Hardness	-0.028	0.148	-0.098	0.096	-0.079	**0.404**	**0.963**										
Gumminess	-0.053	0.169	-0.078	0.063	-0.080	**0.529**	**0.945**	**0.977**									
Springiness	**0.532**	0.118	**0.537**	-0.222	**0.642**	0.185	0.333	0.160	0.075								
Cooked Moisture	0.179	**-0.389**	0.125	0.068	-0.113	0.037	-0.004	0.050	0.079	-0.104							
Cooked Fat	-0.269	**0.482**	-0.037	-0.120	0.169	0.305	-0.036	-0.069	0.056	-0.170	-0.314						
Flavour	**-0.437**	0.137	-0.285	-0.032	-0.112	-0.116	-0.087	-0.007	0.045	-0.293	0.167	0.254					
Texture	**-0.487**	0.002	**-0.566**	0.148	**-0.636**	0.008	0.000	0.146	0.194	**-0.680**	0.085	0.214	**0.376**				
Juiciness	**-0.523**	-0.075	**-0.579**	0.300	**-0.501**	-0.083	-0.022	0.134	0.186	**-0.718**	0.262	0.171	**0.549**	**0.826**			
Color	**0.642**	0.129	**0.715**	-0.307	**0.621**	0.278	0.075	-0.094	-0.079	**0.599**	0.005	-0.049	**-0.444**	**-0.608**	**-0.600**		
Overal Acceptability	**-0.502**	-0.023	**-0.474**	0.066	**-0.370**	-0.188	-0.026	0.128	0.154	**-0.390**	0.109	0.239	**0.790**	**0.547**	**0.543**	**-0.607**	

Values in bold are different from 0 with a significance level alpha=0.05

So springiness and shrinkage were not favorable for panelists. On the other hand sensory evaluation data are inter-dependent with each other. For example, color had significantly negative correlation with overall acceptability, juiciness had positive and negative correlation with color and overall acceptability respectively. Otherwise, texture had positive correlation with juiciness and overall acceptability, finally flavor had positive correlation with texture, juiciness and overall acceptability. In summary we can say flavor had the highest positive effect on overall acceptability and color highest negative effect on it.

The results of the principal component analysis are shown in Table 2 for these 17 principal components (PC). The analysis represents that near 33.6% of the total variation is expressed by the first principal component, 53.6% by the first two principal components and the 65.8% by the first three principal components. In other words, 65.8% of the total variance in the 17 variables can be more concentrated into three first PCs.

Table 2- Results from the principal component analysis for the first six principal components

	F1	F2	F3	F4	F5	F6
Eigenvalue	5.715	3.404	2.080	1.670	1.097	0.942
Variability (%)	33.618	20.023	12.233	9.822	6.454	5.539
Cumulative %	33.618	53.641	65.874	75.696	82.150	87.689

Table 3 shows that the most important variables for the first PC are cooked L*, cooked b* shrinkage, springiness, flavor, texture, juiciness, color and overall acceptability. So, the first PC is defined by the sensory parameters, shrinkage, two colored parameters and one instrumental texture parameter.

Table 3- Squared cosines of the variables

	F1	F2	F3	F4	F5	F6
coocked L	0.648	0.002	0.095	0.151	0.015	0.029
coocked a	0.002	0.128	0.565	0.030	0.001	0.009
coocked b	0.711	0.000	0.000	0.197	0.014	0.000
ΔE	0.206	0.002	0.024	0.465	0.012	0.226
shrinkage	0.506	0.006	0.149	0.013	0.064	0.071
cohesiveness	0.052	0.433	0.030	0.008	0.171	0.084
chewness	0.014	0.928	0.027	0.003	0.009	0.007
hardness	0.006	0.874	0.066	0.002	0.014	0.020
gumminess	0.011	0.924	0.033	0.002	0.000	0.005
springiness	0.600	0.045	0.001	0.007	0.184	0.000
cooked moisture	0.006	0.000	0.339	0.210	0.000	0.355
cooked fat	0.027	0.026	0.638	0.031	0.072	0.002
flavour	0.325	0.001	0.085	0.283	0.198	0.025
texture	0.651	0.018	0.000	0.041	0.130	0.013
juiciness	0.700	0.009	0.005	0.061	0.036	0.028
color	0.719	0.001	0.005	0.000	0.021	0.055
overal acceptability	0.530	0.007	0.018	0.167	0.158	0.010

Actually, these variables are placed far from the origin of the first PC in the loading plot (Fig. 1). The sensory parameters placed to the left in the loading plot are close together and, therefore, positively correlated and the other ones are in the right of the loading plot completely in contrast with the sensory parameters. The PC2 is characterized by the

rest of instrumental texture parameters. These variables are placed on the top in the loading plot, far from the origin of the second PCs and positively correlated with each other. The third PC is defined by cooked a* and fat content, the forth by ΔE and finally the sixth by moisture.

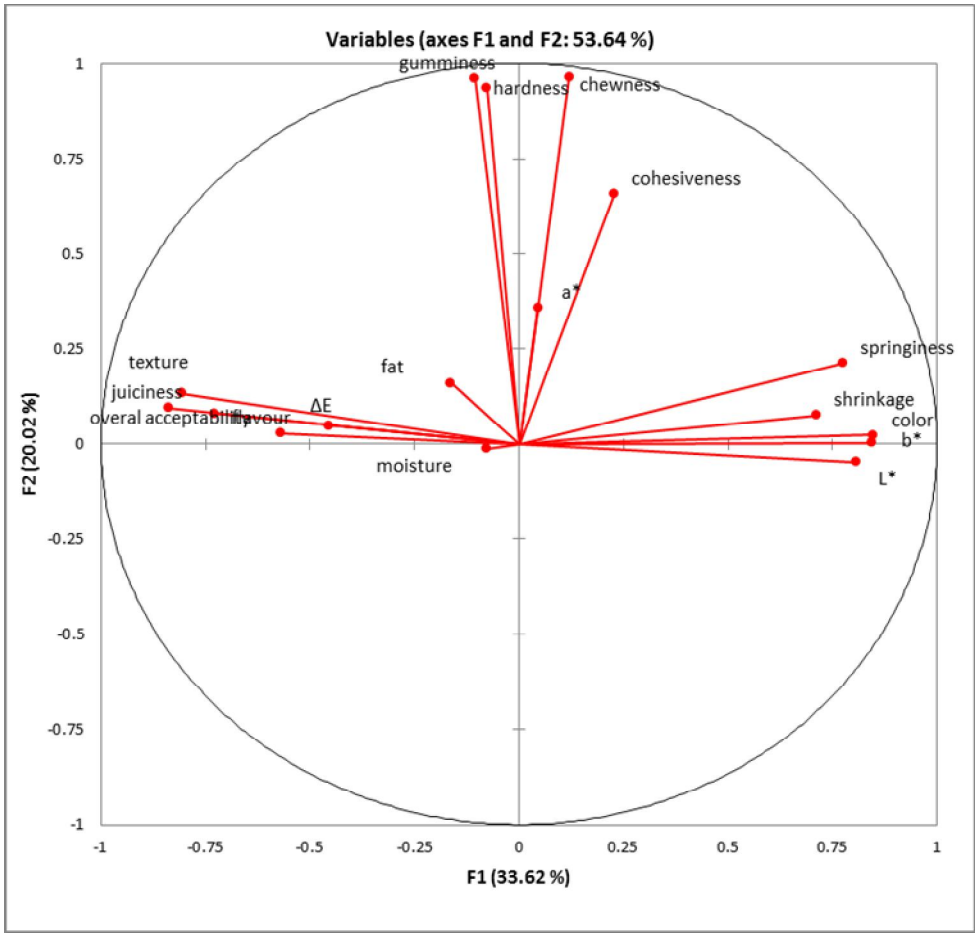

Fig 1: Loading plot

The score plot (Fig. 2) shows the location of the objects in the multivariate space of two first principal component score vectors. It can be seen that the scores are approximately divided in two groups. The first one includes burgers containing 50, 75 and 100% camel meat and the second one mostly includes burgers consist of 25% camel meat and 0 %(beef only). So the burgers containing 50, 75 and 100% camel meat in general show, higher moisture and fat content after cooking which verify the finding by Elsharif (2008) that reported camel meat sausages had higher water and fat retention during cooking compared to beef sausages. Besides, according to the score plot, in this study burgers contain higher amount of camel meat had higher scores in flavor, texture, juiciness and overall acceptability which are in the line with Elsharif (2008) about the increased sensorial scores of camel sausage. In the case of juiciness, McMillin, & Hoffman (2009) mentioned that the difference in juiciness is related primarily to the ability of muscle to retain fat and water during cooking. Listrat *et al* (2016) and Troutt *et al*, (1992) believed that fat is the effective factor in flavor and juiciness. Similarly, fat is important factor in texture (Ahmed *et al*. 1990; Serdaroglu & Sapancı-O zsumer. 2003), flavor and overall

acceptability (Serdaroglu *et al*, 2005). On the other side, the burgers consist of 25% camel meat and 0% (beef only) shows higher values of instrumental texture, instrumental color, shrinkage and sensory color. On the basis of Gregg *et al* (1993), Elsharif (2008) and Ahmed *et al* (1990) findings in different research cases, we can attribute the shrinkage and texture properties to the capacity of meat in moisture and fat retention therefore burgers containing higher amount of beef meat had higher value of shrinkage and texture properties. Higher value of both instrumental color and sensory color of these groups is also due to the highest values of lightness in beef meat. Among them, burgers containing 50% camel meat were evaluated in day 0, have poor sensory score and low moisture as the same as the burgers consisting of 25% camel meat and 0%.

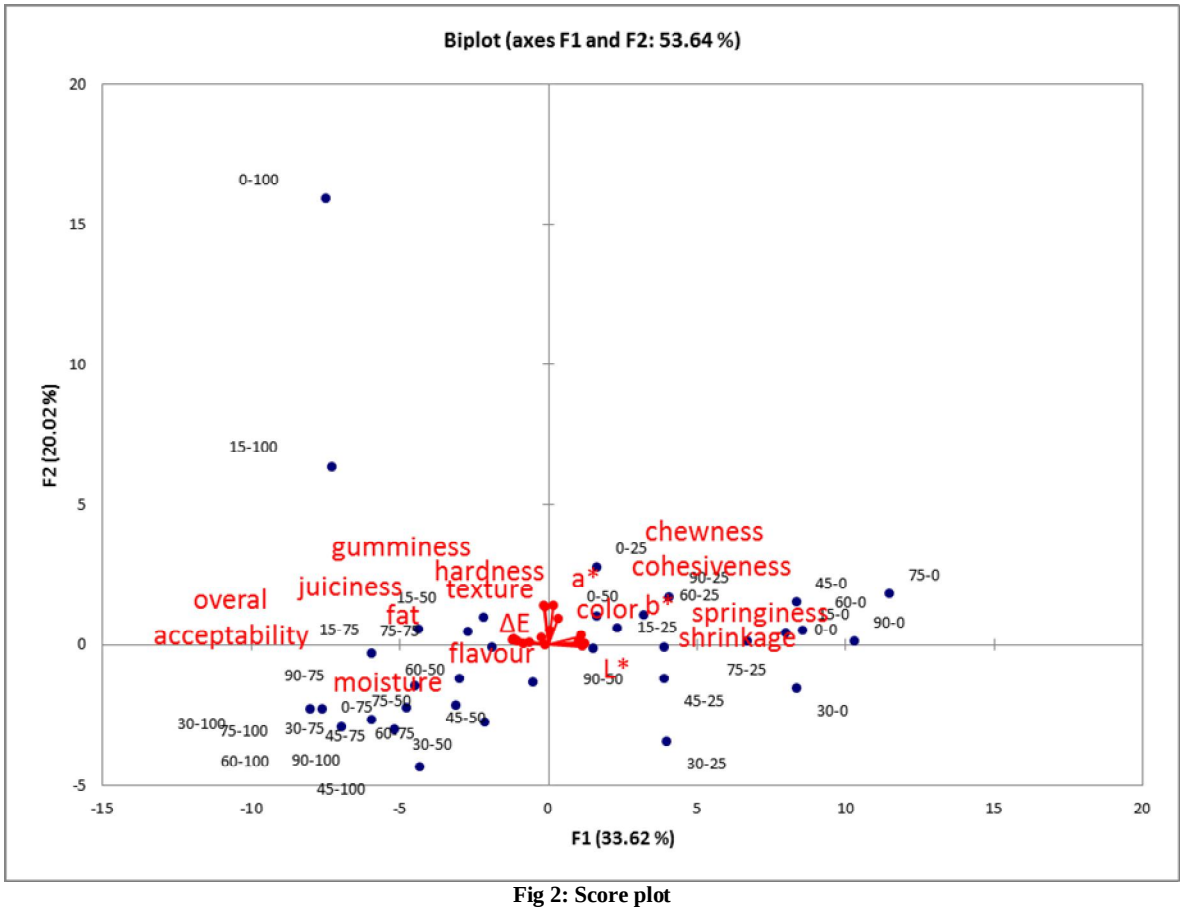

Fig 2: Score plot

Conclusion

The analysis on the basis of PCA showed that amount of meat had a more decisive contribution on the quality difference than the storage time. The results showed that springiness was the most important negative properties on the understanding and acceptance of sensory characteristics. On the basis of the score plot, the samples including the higher amount of camel meat were more acceptable in sensory qualification, so we can conclude the springiness is lower in this type of samples.

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ارزیابی کیفی برگر شتر با استفاده از تجزیه و تحلیل مولفه اصلی

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چکیده

در این تحقیق، گوشت شتر در نسبت‌های مختلف (صفر، 25، 50، 75 و 100) جایگزین گوشت گاو موجود در فرمولاسیون برگر شد. تجزیه و تحلیل مولفه اصلی (PCA) برای درک هر چه بیشتر شباهت‌ها و تفاوت‌ها میان متغیرهای کیفی ارزیابی شده در 35 نمونه برگر انجام شده است. منحنی امتیاز، تحلیل مولفه‌های اصلی را برای مجموعه داده‌های حاصل از اندازه‌گیری متغیرهای شیمیائی برای 35 نمونه (نمونه کنترل، نمونه‌های حاوی 25، 50، 75 و 100) نشان می‌دهد. در مجموع، 6 مولفه اصلی حاصل گردید که مولفه اصلی اول (PC1)، دوم (PC2) و سوم (PC3) در مجموع 65/8% از کل واریانس داده‌ها را پوشش دادند. مولفه اصلی اول امکان جداسازی درجه روشنایی و زردی نمونه پخته، چروکیدگی، خاصیت ارتجاعی و ویژگی‌های حسی (طعم، بافت، آبداری، رنگ و پذیرش کلی) را دارد. مولفه اصلی دوم توسط دیگر ویژگی‌های بافتی و مولفه اصلی سوم توسط درجه قرمزی نمونه پخته و چربی مشخص شده‌اند. امکان تشخیص چه متغیرهای شاخص و در جداسازی کدام گروه‌ها وجود داشت، اشاره شود. بررسی منحنی امتیاز نشان می‌دهد که برگرهای پخته حاوی میزان بالاتر گوشت شتر (50، 75 و 100)، میزان رطوبت و چربی بیشتر و امتیاز طعم، بافت، آبداری و پذیرش کلی بالاتر می‌باشند.

واژه‌های کلیدی: برگر شتر، تحلیل مولفه اصلی، پذیرش کلی

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