

Optimization of textural characteristics of analogue UF-Feta cheese made from dairy and non-dairy ingredients

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Abstract

In this study, a mixture of milk protein concentrate, whey protein concentrate, skim milk powder, soymilk, margarine, butter and water was used for production of recombined UF-Feta cheese analogue. Variables were milk protein concentrate (8%, 9%, 10%), whey protein concentrate (0%, 1.5%, 3%), soymilk (5%, 10%, 15%) and margarine (0%, 5%, 10%). Textural properties of Samples were analyzed 3 days post-manufacture. The central composite design was employed and the results were modeled and analyzed using response surface methodology. Coefficients of determination, R^2 , of fitted regression models for different variables were varied in the range of 89.59-97.80 and the lack-of-fit was not significant for all responses at 95%. Hence, the models for all the response variables were highly adequate. The results showed that the optimum processing conditions for producing cheese with suitable hardness and cohesiveness and lowest adhesiveness were: 9.13% milk protein concentrate, 3% whey protein concentrate, 15% soymilk and 7.65% margarine.

Keywords: Milk protein concentrate; Whey protein concentrate; Soymilk; Margarine; Analogue cheese; Texture profile.

Introduction

Cheese is the generic name for a group of fermented dairy products, produced throughout the world in a wide range of flavours, textures and forms (Fox *et al.* 2000). It is commonly believed that cheese evolved in the 'Fertile Crescent' between the Tigris and Euphrates rivers, that run through modern-day Iraq, about 8000 years ago (Fox 2011). It is estimated that more than 2000 varieties exist and the list may still be growing (Gunasekaran and Mehmet 2003). The broad range of different cheeses available is based mainly on regional conditions and production technology, which has been repeatedly adapted and optimized (Isam *et al.* 2010).

UF-Feta cheese is a cheese with soft and spreadable texture that is produced from milk which has been concentrated by ultrafiltration,

to achieve total solids of 35 %, and then enzymatically coagulation of retentate. This type of cheese contains 45-60% fat (on dry basis), 28% protein (on dry basis) and max. 3% salt and its final pH after 72 hours is 4.8. UF-Feta cheese is a fresh cheese that can be consumed 3 days after production. The shelf life of UF-Feta cheese is max. 2 months (Iran standards 12736 and 6629).

Analogue cheese is generally manufactured from dairy and non-dairy proteins, various edible fat or oil sources, types of starches, other ingredients and water (Fox *et al.* 2000; McSweeney 2007). The main advantages of Analogue cheeses over natural cheeses are lower cost and relatively high functional stability during storage (Fox *et al.* 2000).

According to Abou El Nour *et al.* (1998), the replacement of rennet casein by milk protein concentrate (MPC, 85% protein) powders increase the firmness but decrease the meltability of spread-type cheese analogue. Shakeel-Ur-Rehman *et al.* (2003) found that using a mixture of cream and liquid MPC in low fat Cheddar cheese formulation increase the yield.

Whey protein concentrate (WPC) and skim milk powder (SMP) are used in variety of

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process cheeses and analogues (Guinee *et al.* 2004). El-Neshawy *et al.* (1988) showed that increase in WPC caused an improvement in consistency and spreadability of the resultant processed cheese analogue. The addition of WPC increases the moisture content of cheese (Guinee *et al.* 2004). Gelation and water binding properties for WPC has also been implied (Harper 1991).

In spite of technological problems, Soymilk is one of soybean products that used in cheesemaking industry as a low cost substitute for milk protein. Ahmed *et al.* (1995) studied the feasibility of using soy protein as a partial replacement for casein in manufacturing of imitation cheeses and showed that increase of soy protein caused a decrease in firmness of cheeses. According to Metwalli *et al.* (1982), 20% of soymilk would be the maximum proportion of mixing with milk for cheese making. They observed that the higher concentration of soymilk (25-30%), resulted in the formation of a very weak curd. They also showed that autoclaving soymilk at 120°C for 15 min before mixing with milk, greatly improved curd firmness.

Attempts to reduce cheese costs have led to the use of vegetable fat and oil to replace the more costly milk fat (Mounsey and O'Riordan 2001; Chavan and Jana 2007). Fat and oil, apart from their nutritional significance in cheeses, are two of the most important ingredients affecting the sensory and textural properties of cheese (Miočinović *et al.* 2011). Metzger and Mistry (1995) studied the effect of fat on cheese structure and reported that the weakness of protein matrix was affected by the fat globule distribution. The use of vegetable fats in cheese formulations resulted in analogues that were harder or similar to cheeses manufactured with butter (Cunha *et al.* 2013). Soybean fat conferred hardness and adhesiveness to the cheese analogues, but decreased their cohesiveness and springiness (Lobato-Calleros *et al.* 1997).

The objective of the current study was to produce a mixture similar to retentate to produce a recombined cheese like UF-Feta cheese after addition of Enzyme, starter and

salt and evaluation of its textural properties.

Material and Methods

Calcium chloride (food-grade) was obtained from Kemira Agro Ltd. (Helsinki, Finland). Milk protein concentrate containing 75% protein (MPC-75) was supplied by Milei GmbH (Stuttgart, Germany). Whey protein concentrate (WPC-35), skim milk powder (SMP) and butter were prepared from the Khorasan Pegah Dairy Co. (Mashhad, Iran). Margarine was obtained from BehinehWazin Co. (MahgolTM) (Tehran, Iran). Full fat soy flour was purchased from Soyan Toos Co. (Mashhad, Iran).

The chemical composition of the MPC (proteins: 75%, water: 5%, ash: 7.6%, lactose: 10.9%, fat: 1.5%), WPC (proteins: 35%, water: 4.6%, ash: 7.2%, lactose: 50.2%, fat: 3%), SMP (proteins: 36%, water: 4%, ash: 7.85%, lactose: 50.8%, fat: 1.35%), Butter (proteins: 0.49%, water: 16%, fat: 82%), Margarine (proteins: 0.5%, water: 18%, fat: 80%) and soy flour (proteins: 38%, water: 13.7%, soluble carbohydrate: 15%, non-soluble carbohydrate: 15, fat: 18%) were declared by their producers.

Safelt 2, as a Blend of mesophilic and thermophilic bacteria, was obtained from Chr. Hansen A/S (Hørsholm, Denmark). This culture contains specially selected strains chosen for their phage resistance and ability to produce lactic acid quickly. This culture does not produce CO₂. As coagulant, Fromase[®] 2200 TL granulate (microbial rennet from *Rhizomucor miehei* obtained from DSM Co., Netherland) was used.

Preparation of soymilk, enzyme and starter

Full fat soy flour was used to make soymilk. For this purpose, soy flour and boiling water were poured into a blender (Model A707A, Kenwood MFG, Surrey, UK) in the ratio of one part soy flour to six parts boiling water and then mixed for 10 minutes. This mixture, was then poured into sealed glass containers and heated to 100°C and kept at this temperature for 15 minutes before

mixing with other components. After conditioning, soymilk was not filtered and wholly used in cheese formulation.

Rennet was added at a concentration of 30 mg per kg of recombined retentate. Rennet was diluted in sterile water. The dilution was standardized so that 5 mL of solution would deliver 30 mg of chymosin to 1 kg of recombined retentate.

0.1 mg of freeze-dried culture was dispersed in 50 mL sterile water and 5 mL was then added directly into 1 kg of recombined retentate.

Cheese manufacture

To achieve homogenous mixture, initially, MPC, WPC and SMP powders were mixed with warm water (60°C) in a laboratory blender for 5 minutes. Then, the previously prepared soymilk was added and mixed thoroughly. After cooling to 40°C, melted (40°C) margarine and butter were added and mixed completely. To prevent separation of fat globules during process, the mixture was homogenized using an Ultra-Turrax® T25 homogenizer (IKA® Werke, Janke & Kunkel GmbH & Co KG, Staufen, Germany) at a speed of 20000 rpm for 1.5 min (Day *et al.* 2007). After adding salt (1.4%), the resulting mixture was pasteurized and cooled to approximately 34°C. Rennet, starter culture and calcium chloride (0.02%) were then mixed with the mixture. Rennet includes the enzyme chymosin, which makes the casein in mixture coagulate (Bylund 1995). After addition of mentioned materials, the mixture was thoroughly stirred and poured into 100-ml sterile cups and covered with aluminum foils and then incubated at 34°C for 25 min to coagulate. After completion of coagulation, cheese samples were incubated at 27-28°C for 19 h to achieve pH 4.8 and finally stored at 5°C for 3 days. Textural analyses were performed on 3-day-old cheeses.

Texture profile analysis (TPA)

Texture profile analysis (being the most

commonly used method for assessment of cheese texture) has been found to be effective for evaluating cheese texture (International Dairy Federation 1991). TPA parameters were determined by using Universal Testing Machine, Model QTS-25 (CNS Farnell, Ltd., Hertfordshire, England) equipped with a 5-25 kg load cell. A flat plate probe with 36 mm of diameter was attached to moving crosshead. Six Cubic samples (20×20×20 mm) were prepared from each cheese block using a metal borer at 4–6°C. Samples were compressed to 70% of their original height at a speed of 60 mm min⁻¹. For reducing friction at the sample – compression plate interface, plates were lubricated with mineral oil. The results of hardness (N), cohesiveness, adhesiveness (Ns), springiness (mm) and gumminess (N) were presented as an average of six analyses.

Experimental design and statistical analysis

A four-factor, three-level Central Composite Design (CCD) was used for obtaining optimum levels of MPC, WPC, Soymilk and margarine. The central composite design with a quadratic model was employed. Four independent variables namely MPC (X₁), WPC (X₂), Soymilk (X₃) and margarine (X₄) were chosen. Each independent variable had 3 levels which were -1, 0 and +1. A total of 30 different combinations (including six replicates of center point) were chosen in random order according to a CCD configuration for four factors. The coded values of independent variables are shown in Table 1. Analysis was performed by using Design-Expert, Version 8.0.7.1 program. A significance level of 0.05 was used in the statistical evaluations. Results of the optimization analysis were validated by producing a trial cheese with the optimized formula. The Kolmogorov–Smirnov's test was applied to verify if the experimental data had a normal distribution (p>0.05). Mean values of experimental and predicted data were then compared using parametric one sample t-test.

Table 1. Uncoded and coded levels of the independent variables

Independent variables	Index	Coded levels		
		+1	0	-1
MPC (%)	X ₁	10	9	8
WPC (%)	X ₂	3	1.5	0
Soymilk (%)	X ₃	15	10	5
Margarine (%)	X ₄	10	5	0

The study was carried out according to the central composite design and the experimental points used according to the design are shown in Table 2. A second-order polynomial

equation was used to express Y (dependent variable or response) as a function of independent variables.

$$Y = K + AX_1 + BX_2 + CX_3 + DX_4 + ABX_1X_2 + ACX_1X_3 + ADX_1X_4 + BCX_2X_3 + BDX_2X_4 + CDX_3X_4 + A^2X_1^2 + B^2X_2^2 + C^2X_3^2 + D^2X_4^2 \quad (1)$$

Table 2. Central composite design used for preparation of UF-Feta cheese analogues

Sample run	Coded level				Real levels (%)			
	1	2	3	4	MPC	WPC	Soymilk	Margarine
1	-1	-1	1	-1	8	0	15	0
2	-1	1	-1	-1	8	3	5	0
3	-1	-1	-1	1	8	0	5	10
4	-1	0	0	0	8	1.5	10	5
5	-1	-1	1	1	8	0	15	10
6	-1	1	1	-1	8	3	15	0
7	-1	-1	-1	-1	8	0	5	0
8	-1	1	-1	1	8	3	5	10
9	-1	1	1	1	8	3	15	10
10	0	1	0	0	9	3	10	5
11	0	0	0	0	9	1.5	10	5
12	0	0	0	0	9	1.5	10	5
13	0	0	0	1	9	1.5	10	10
14	0	0	-1	0	9	1.5	5	5
15	0	0	0	0	9	1.5	10	5
16	0	0	0	0	9	1.5	10	5
17	0	-1	0	0	9	0	10	5
18	0	0	0	-1	9	1.5	10	0
19	0	0	0	0	9	1.5	10	5
20	0	0	0	0	9	1.5	10	5
21	0	0	1	0	9	1.5	15	5
22	1	1	-1	1	10	3	5	10
23	1	-1	-1	1	10	0	5	10
24	1	1	1	-1	10	3	15	0
25	1	-1	1	-1	10	0	15	0
26	1	-1	-1	-1	10	0	5	0
27	1	-1	1	1	10	0	15	10
28	1	1	-1	-1	10	3	5	0
29	1	1	1	1	10	3	15	10
30	1	0	0	0	10	1.5	10	5

Samples contained MPC in the range of 8-10%, 0-3% WPC, 6% SMP, 0-15% soymilk, 0-10% margarine, 1.4% NaCl, 0.02% calcium chloride and water. The proportion of fat was held constant (16%) and the amount of butter was adjusted according to the proportion of fat in the formula.

Results and Discussion

Effects of MPC, WPC, Soymilk and margarine on textural properties of UF-Feta cheese analogues

UF-Feta cheese analogues were prepared by using a basic formula and manufacturing method which was developed in the preliminary studies. Four ingredients, MPC, WPC, Soymilk and margarine, were found to be the major components effective on physical properties of the cheeses. The upper and lower levels of mentioned ingredients that could give a cheese-like structure were determined in the preliminary studies.

The amount of fat was kept constant at a value of 16% and the reduction or addition in the amount of margarine was compensated by increasing or decreasing the level of butter in the formula, respectively.

Hardness

Hardness is the force required to penetrate the sample with the molar teeth or force necessary to attain a given deformation (Gunasekaran and Mehmet 2003). The linear

effects of MPC, WPC and Soymilk levels on hardness were found significant ($P < 0.05$). A significant interaction effect between MPC and WPC was also observed ($P < 0.05$). According to Fig. 1, hardness increased with increasing levels of MPC and WPC significantly because of the increase in dry solids content. Hennelly *et al.* (2005) also observed that an increase in the hardness of imitation cheese was associated with an increase in dry solids. The increase in hardness was more obvious at higher MPC and WPC concentrations so that the highest hardness was related to 10% MPC and 3% WPC. Addition of soymilk to mixture followed by heat treatment at approximately 100°C for 15-20 min caused an increase in hardness. This is probably due to the more denaturation of the soy proteins at higher temperatures as compared to the lower temperatures. Hardness was also significantly influenced by margarine content ($P < 0.05$; result not shown). Cunha *et al.* 2010 also found that the substitution of 25% and 50% of the dairy fat by vegetable fat resulted in increased hardness.

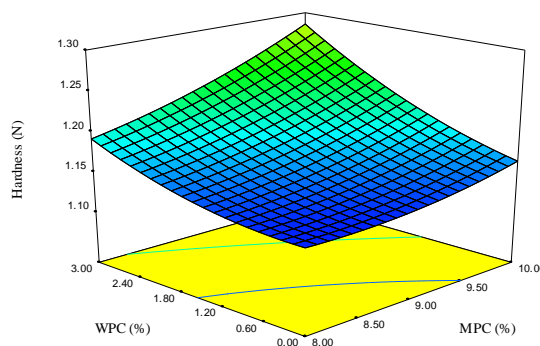


Fig.1. The response surface for hardness of cheese as function of MPC and WPC concentrations in TPA test.

Cohesiveness

Cohesiveness is the amount of deformation undergone by a material before rupture when biting completely through the sample using molars or strength of the internal bonds making up the body of the product (Gunasekaran and Mehmet 2003). The amount of cohesiveness significantly increased with increasing MPC, WPC and soymilk concentrations ($P < 0.05$) (Fig. 2). Furthermore, the effect of MPC on increasing cohesiveness

was higher as compared with WPC. By increasing MPC, WPC and soymilk, the amount of protein was increased and resulted in reinforced the gel structure and cohesiveness. In comparison, the cohesiveness values of the cheese made from UF milk were higher than those made from un-concentrated milk (Toufeili and Özer 2006). Scanning electromicrograms of traditional and UF Urfa cheese (a white-brined Turkish cheese) also showed that the cheese made from UF

concentrated milk had a more compact structure than the cheese manufactured from

un-concentrated milk (Özeret *et al.* 2003).

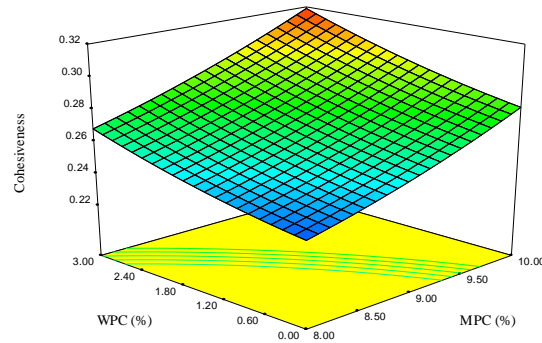


Fig.2. The response surface for cohesiveness of cheese as function of MPC and WPC concentrations in TPA test.

Adhesiveness

Adhesiveness is described as the force required to remove the food from the palate during eating or work necessary to overcome the attractive forces between the surface of the food and surface of other materials with which the food comes in contact (Gunasekaran and Mehmet 2003). Adhesiveness values were significantly decreased by increasing MPC and WPC concentrations ($P < 0.05$) because of increasing total solids. The highest value of

adhesiveness was found for 8% MPC and 0% WPC (Fig. 3). Adhesiveness decreased with decreasing moisture content (Bryant *et al.* 1995). Watkinson *et al.* (2002) reported that an increase in the moisture content of model Cheddar-like cheeses, from 40 to 48%, w/w, resulted in a large increases in adhesiveness. Addition of soymilk also significantly reduced adhesiveness ($P < 0.05$) and this may be due to the increased protein and dry solids contents.

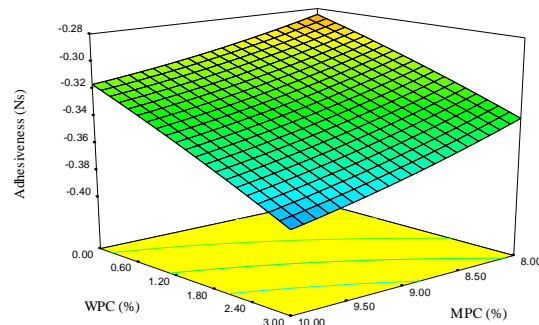


Fig.3. The response surface for adhesiveness of cheese as function of MPC and WPC concentrations in TPA test.

Springiness

Springiness is degree or rate at which the sample returns to its original size/shape after partial compression between the tongue and palate or the distance recovered by the sample during the time between end of first bite and start of second bite (Gunasekaran and Mehmet 2003). WPC and Soymilk had significant linear effects on springiness ($P < 0.05$). Significant interaction effects between MPC

and WPC and between soymilk and WPC were also observed ($P < 0.05$). As seen from Fig. 4, springiness increased significantly with increasing soymilk concentration. This may be due to the lower moisture content of cheeses and larger amounts of proteins (Gunasekaran and Mehmet 2003; Zisu *et al.* 2005; Hassan 2008). Increasing the concentration of WPC reduced springiness significantly, while the addition of MPC had no significant effect on

it. By increasing casein concentration in the cheese matrix, the number of intra- and inter-strand linkages are increased and finally the matrix become more elastic(Guinee and Kilcawley2004). Increasing of WPC reduced casein concentration and caused loss of elasticity of cheeses.

Gumminess

The results showed that the linear effects of MPC, WPC and Soymilk levels on gumminess were significant (P <0.05). In addition, an interaction effect between MPC and WPC was found significant (P <0.05). Fig. 5. shows the effect of MPC and WPC on gumminess (when fixed soymilk at 10% and margarine at 5%).

According to this figure, the gumminess of the analogue cheese was significantly increased by increasing of MPC and WPC contents so that the highest gumminess was found at levels of 10% MPC and 3% WPC. This is due to the increase in protein concentration and the decrease of water content in cheese matrix. Romeih *et al.* (2002) also observed that with increasing protein to fat and water ratios in low-fat cheeses, the gumminess of cheese increased. The increase in soymilk concentration also increased the gumminess of analogue cheese which may have attributed to an increase in the content of protein (result not shown).

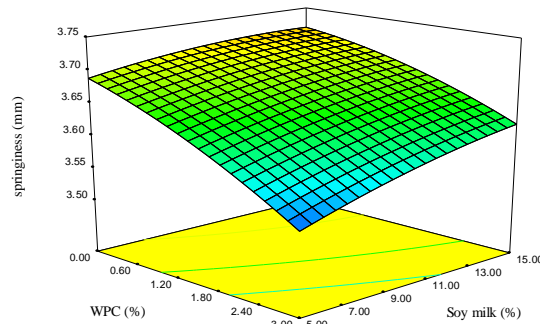


Fig.4. The response surface for springiness of cheese as function of Soy milk and WPC concentrations in TPA test.

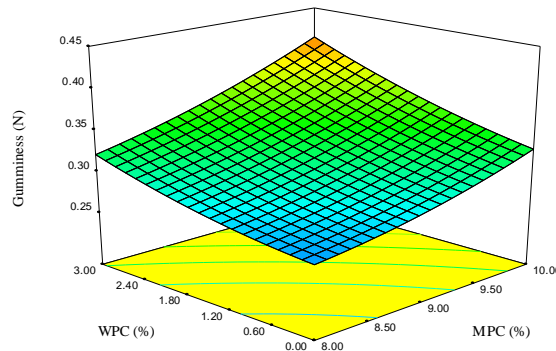


Fig.5. The response surface for gumminess of cheese as function of MPC and WPC concentrations in TPA test.

Optimization

The final objective of this study was to optimize the experimental variables in such a way that the sum of responses could be received the highest scores. The results showed that the optimum ingredient

combination for producing cheese with suitable hardness and cohesiveness and lowest adhesiveness was: 9.13% MPC, 3% WPC, 15% soymilk and 7.65% margarine. Hardness, cohesiveness, adhesiveness, springiness and gumminess of an analogue cheese based on the

optimized formula were estimated to be approximately 1.26 N, 0.3, -0.38 Ns, 3.62 mm and 0.38 N, respectively. A trial cheese was manufactured by using the optimized formula to validate these estimations. Hardness, cohesiveness, adhesiveness, springiness and gumminess of trial cheese were 1.09 N, 0.28, -

0.44 Ns, 3.42 mm and 0.30 N, respectively. According to the results obtained, no significant differences between adhesiveness and springiness of optimized and trial cheeses were observed, however, hardness, cohesiveness, and gumminess of cheeses were significantly different ($P < 0.05$) (Fig. 6.).

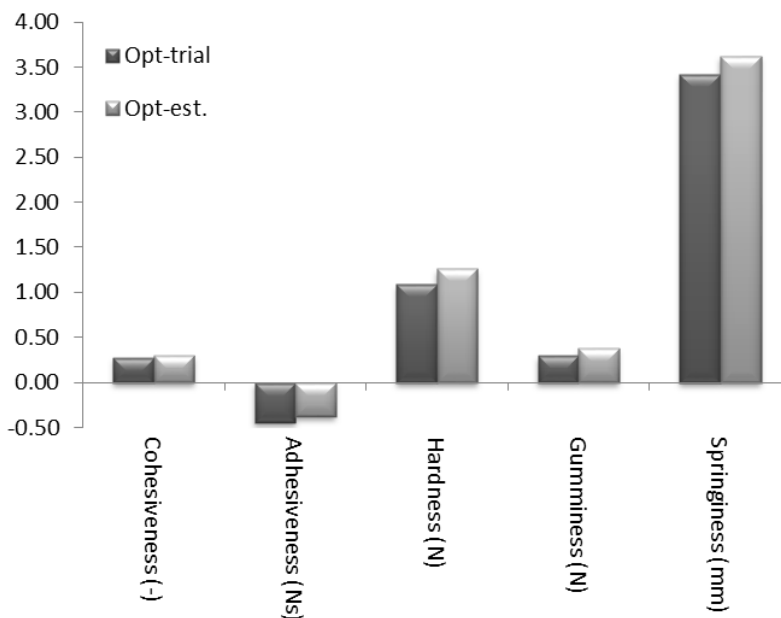


Fig.6. Comparison of textural properties estimated in the optimization and obtained in a trial cheese that was produced according to the optimized formula (Opt-est.: Estimated value in the optimization, Opt-trial: Trial cheese).

One approach to evaluate responses simultaneously is the study of the overlaid contour plot of responses. The contour plot for hardness, cohesiveness, adhesiveness and springiness based on corresponding models were plotted and overlaid into a single diagram (Fig. 7.). The best conditions were obtained at 9.13% MPC, 3% WPC, 15% soymilk and 7.65% margarine.

Conclusions

The production of analogue cheese encountered to some technological problems and use of non-dairy components, such as soy flours or its isolates, may also have negative effects on functional or sensory properties of analogue cheeses, but, it is possible to minimize these problems by modifications of

formulations and process conditions and to produce products, which have unique characteristics and meet consumers' expectations. In this study, the optimum ingredient combination for producing cheese was: 9.13% MPC, 3% WPC, 15% soymilk and 7.65% margarine. This combination resulted in the suitable hardness and cohesiveness and the lowest adhesiveness. However, hardness, cohesiveness and gumminess of optimized and trial cheeses had significant differences, but no significant differences were observed between their adhesiveness and springiness. It should be noted that, in the previous study (Gholamhosseinpour *et al.* 2014), the sensory and chemical parameters of optimized and trial cheeses had no significant differences.

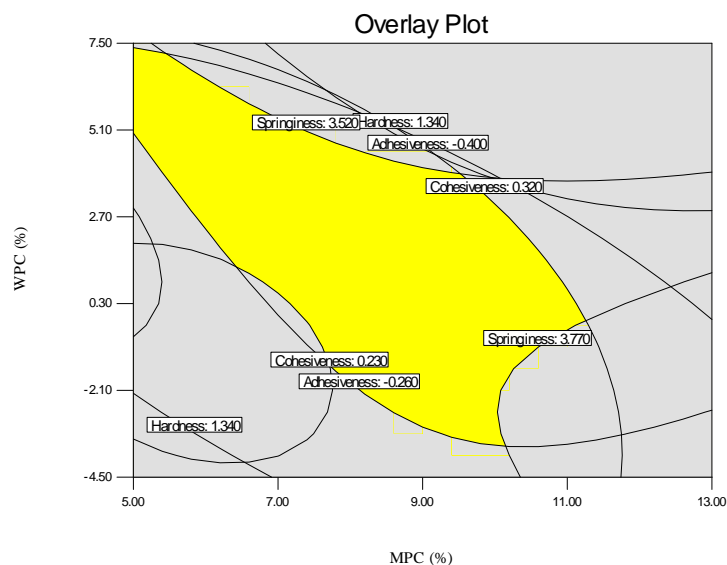


Fig.7. Overlaid contour plot for hardness, cohesiveness, adhesiveness and springiness at different levels of MPC and WPC and constant values of soymilk and margarine.

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بهینه‌سازی ویژگی‌های بافتی پنیر فتای فراپالایش آنالوگ تولید شده از ترکیبات لبنی و غیرلبنی

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چکیده

در این پژوهش از مخلوط کنسانتره پروتئینی شیر، کنسانتره پروتئینی آب‌پنیر، شیر خشک پس‌چرخ، شیرسویا، مارگارین، کره و آب برای تولید پنیر فتای فراپالایش بازساخته استفاده شد. متغیرها عبارت بودند از کنسانتره پروتئینی شیر در سه سطح 8، 9 و 10 درصد، کنسانتره پروتئینی آب‌پنیر در سه سطح صفر، 1/5 و 3 درصد، شیرسویا در سه سطح 5، 10 و 15 درصد و مارگارین در سه سطح صفر، 5 و 10 درصد. نمونه‌ها از نظر ویژگی‌های بافتی پس از روز سوم تولید، آنالیز شدند. نتایج در قالب طرح مرکب مرکزی بررسی و به روش سطح پاسخ مدل‌سازی و تجزیه و تحلیل شدند. ضریب تبیین مدل‌های رگرسیون برازش شده برای صفات مختلف بین 89/59 تا 97/80 متغیر بوده و فاکتور عدم برازش تمامی صفات در سطح اطمینان 95 درصد معنی‌دار نبود، از این رو صحت مدل برای برازش اطلاعات تایید گردید. با توجه به نتایج، شرایط بهینه به دست آمده برای تولید پنیری که سختی و پیوستگی مناسبی داشته و از کمترین مقدار چسبندگی برخوردار باشد عبارت بود از: 9/13 درصد کنسانتره پروتئینی شیر، 3 درصد کنسانتره پروتئینی آب‌پنیر، 15 درصد شیرسویا و 7/65 درصد مارگارین.

واژه‌های کلیدی: کنسانتره پروتئینی شیر، کنسانتره پروتئینی آب‌پنیر، شیرسویا، مارگارین، پنیر آنالوگ، پروفیل بافت.

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