

## Research Article

# Application of an optimum level of acidic extracted grass carp (*Ctenopharyngodon idellus*) gelatin as a fat replacer in low-fat milk cream development

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### Abstract

The main objective of this study was to determine the optimum application level of acidic extracted grass carp (*Ctenopharyngodon idellus*) gelatin as a protein-based fat replacer in the development of low-fat milk cream. A cream mixture consisting of fish gelatin at different concentrations (0-8%), milk cream, and cow milk was used to find the best formula for developing a low-fat milk cream. The optimization of the formula was based on the physicochemical and sensory properties of the prototypes using a mixture design model. Results showed that the crude protein content of the prototypes was increased by increasing fish gelatin concentration. However, the fat content was significantly reduced from 30% in the control to 12.5% in the low-fat sample with 8% fish gelatin. The highest values of freeze-thaw stability and water holding capacity were seen in the low-fat samples with 4% and 8% fish gelatin. The microstructural characteristics revealed that fish gelatin could cause smaller particles of oil globules compared to the full-fat cream. The lightness value was decreased significantly in the prototypes by increasing fish gelatin concentration, while the highest yellowness index was observed in the prototype with 8% fish gelatin. This study revealed the successful application of fish gelatin in low-fat milk cream at the level of 8%. At this level, the sensory attributes and texture properties were improved. This study noted that it is possible to develop a low-fat milk cream enriched fish gelatin as a novel functional ingredient with high nutritional values and potential health benefits.

**Keywords:** Grass carp, Fish gelatin, Fat replacer, Low-fat milk cream, Acidic extraction

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## Introduction

Today, the global demand for food products with fat low and calories is rising dramatically due to consumers' awareness of the effects of these products on their health. Therefore, the food industry is encountered with the ever-increasing shift of producing reduced-fat products based on consumer purchase behavior for healthy foods (Akbari *et al.*, 2019). Milk cream (an oil-in-water emulsion) is one of the most famous dairy products that are widely used in the production of desserts, cakes, cookies, and ice creams (Sajedi *et al.*, 2014). Milk cream has a high level of fat and may increase the risk of heart disease, hypertension, and weight gain in consumers. However, fat plays a major role in emulsified food products by creating proper texture and flavor (Akbari *et al.*, 2019). Therefore, fat substitute materials should have similar sensory properties (Cheng *et al.*, 2008). In this regard, hydrocolloids such as gelatins have many functional properties including viscosity improver, emulsifiers, gel former, and they may create lipid sensory taste in the mouth. These functionalities make them an excellent choice as a fat replacer (Nguyen *et al.*, 2017).

Gelatin is derived from collagen hydrolysis and is currently used in a variety of food and pharmaceutical applications (Ataie *et al.*, 2020). Gelatin is used as a stabilizing agent in dairy products and it can also reduce the amount of fat and energy without

adversely affecting the functional properties of reduced-fat products (Yousefi and Jafari, 2019). It has been reported that fish gelatin has been formulated for producing low-fat spread (Cheng *et al.*, 2008; Jamili *et al.*, 2019), low-fat yogurt (Pang *et al.*, 2017), and low-fat mayonnaise (Ataie *et al.*, 2020). Moreover, one of the advantages of replacing fat with fish gelatin is increasing the final product's shelf life due to the high antioxidant and antibacterial properties of fish gelatin peptides (da Trindade Alfaro *et al.*, 2015; Sae-Leaw *et al.*, 2017). Therefore, the objective of this study was to develop a low-fat milk cream using the optimum level of acidic extracted grass carp gelatin and to investigate the physicochemical and sensory characteristics of the products. The mixture design model and principal components analysis (PCA) were applied to reach this goal. The result could be useful for the commercial application of fish gelatin in low-fat dairy products.

## Materials and methods

### *Fish gelatin extraction*

The fresh grass carp (*Ctenopharyngodon idellus*) skins were obtained from a local market (Tehran, Iran), kept in a polystyrene iced box with ratio skin to the ice of 1:2 (w/w), transported to the processing lab at Science and Research Branch University (Tehran, Iran), and stored at -20°C until the further use. Acidic extraction was used for gelatin

production (Giménez *et al.*, 2005). The oven-dried gelatin was ground using a kitchen grinder (Pars Khazar, Iran). The gelatin powder was packed in polyethylene zip lock bags and was stored in the refrigerator until use.

#### *Product design and development*

Homogenized and pasteurized full-fat milk cream (30% fat and protein 2.5%) and semi-skimmed cow milk with 2.5% fat were purchased from a local market (Tehran, Iran). A mixture design was used to optimize the levels of fish gelatin in the formula of cow milk cream (Shaviklo *et al.*, 2020). Statistical software package Design-Expert (Version 6.0.2, State-Ease, Minneapolis, MN) was applied to make and analyze the designs. Characteristics like spreadability, brightness, aromatic from fish gelatin, cream flavor, fishy flavor, bitterness, mouth-coating, texture, surface shine, and overall acceptance were also determined as the responses. Therefore, 5 representative formulations were suggested by the software.

The fat content of the low-fat cream treatment was reduced from 30% to 15% by calculating the proportions of semi-skimmed cow milk (2.5% fat) and full-fat milk cream (30% fat) using Pearson's Square method. Then, the concentration of the cow milk in the reduced-fat formula (45%) was replaced with the fish gelatin at 2, 4, 6, and 8 %. In summary, a certain amount of cow milk was poured into a sterile beaker, different levels of fish gelatin were

added gradually, and the mixture was heated to 45-50°C in a bain-marie until a homogeneous mixture was prepared. After blending the mixture, the temperature of the solution was increased to 75°C for 1 min. The beaker was removed from the bain-marie, then milk cream was added using a kitchen blender (Pars Khazar, Iran), for 1 min. The concentration of the milk cream in the reduced-fat formula was 55% in all prototypes. The prototypes were kept in a refrigerator for 24 h and they were used for sensory evaluation tests to find the optimum mixture. The optimized prototypes were developed using the same aforementioned process. The prototypes were kept in the refrigerator at 4°C±1 for further physicochemical and sensory measurements.

#### *Analytical techniques*

Proximate compositions and pH were determined according to the AOAC method (AOAC, 1996) and national standard method (INSO, 2019) respectively. The freeze-thaw stability of the emulsions was measured for one freeze-thaw cycling. Initially, 10 g of each sample was transferred to 50 mL centrifuge tubes and allowed to freeze at -18°C in a freezer for one-day. All tubes were removed from the freezer and thawed at 40°C for 30 min using a water bath (Memmert, WNB 14, Germany). Then, the heated tubes were centrifuged at for 7500 rpm for 15 min and the supernatants were decanted, and the residues were

weighed to measure freeze-thaw stability as follow:

$$\text{Freeze - thaw stability (\%)} = \frac{\text{Weight of decanted liquid after centrifuge}}{\text{Weight of sample before centrifuge}} \times 100 \quad (1)$$

The water holding capacity (WHC) of the milk cream samples was measured using Balti *et al.* (2011) method. Briefly, 0.5 g milk cream was diluted with 50 ml distilled water and kept for 1 h at room

temperature. The solution was centrifuged at 4500 rpm for 20 min. After separation of the higher part, the residue was filtered and WHC was reported using the following equation:

$$\text{WHC(\%)} = \left(1 - \frac{\text{Weight of sample after centrifugation}}{\text{Weight of sample}}\right) \times 100 \quad (2)$$

Color characteristics of milk cream samples were determined by placing them in a test tube (25 mm in diameter) which was read in a Hunter lab digital colorimeter (Vis UltraScan, Virginia, USA) in L\*a\*b\* measuring mode with a CIE standard Illuminant C and 10° viewing angle. Results were presented as lightness (L\*), redness (a\*), and yellowness (b\*). Accordingly, whiteness and yellowness indexes were measured using the following equations (Rhim *et al.*, 1999).

$$\text{Whiteness Index} = \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (3)$$

$$\text{Yellowness Index} = \frac{142.86 \times b^*}{L^*} \quad (4)$$

To study microscopic structure, milk cream samples were diluted by cold distilled water and placed on a glass plate of a light microscope (Olympus, Japan) equipped with a digital camera (Sony, Japan). The microscopic structure of a milk cream drop was evaluated at 100 X (total magnification).

Textural characteristics of milk cream samples were analyzed using a texture analyzer (Brookfield CT3; Brookfield Engineering Labs, Middleboro, MA) equipped with a cylindrical probe (15 mm diameter) as described by Bonezar *et al.* (2002). The measured parameters were firmness (g), cohesiveness (g), resilience, and adhesiveness (g/s). The apparent viscosity (Pa.s) of the samples was measured according to the method of Wang *et al.* (2013) using a rheometer (Physica MCR 301, Anton Paar, Graz, Austria) with the ability to control the shear stress.

Sensory descriptive analysis was carried out to evaluate milk cream samples by an expert panel (Meilgaard *et al.*, 2007) consisted of 6 trained experts (3 females) with an average age of 35 years old. The panel evaluated different milk cream prototypes and discussed their specification and provided 11 attributes and definitions to have a common language when the assessment of the prototypes (Table 1). The evaluation and selection of the

prototypes were based upon the highest scores of sensory attributes except for fishy odor, bitterness, and mouth coating, which had to be minimum. Low-fat milk cream prototypes were first prepared and then kept in a refrigerator until 30 min before serving

when samples were placed at room temperature (25°C.) The samples were presented in plastic cups, labeled with 3-digit random numbers. The panelists were requested to initially evaluate each sample by sniffing alone and then by tasting. They rinsed their mouths with water after evaluating each sample (Meilgaard *et al.*, 2007).

**Table 1: Lexicon for the sensory attributes of full-and low-fat milk cream samples (adapted from Shaviklo *et al.*, 2020).**

Sensory attribute	Scale (0-100)	Definitions
Spreadability	Difficult-easy	Ease of moving product over the skin
Brightness	Dull-bright	The chroma/ purity of the color/ ranging from dull to bright color
Odor	None-much	Those volatiles related to milk and butter
Aromatics from fish gelatin	None-much	The aromatics associated with fish gelatin/ mild fish odor
Cream flavor	None-much	The flavor of high fat fresh cream or fresh butter
Fishy flavor	None-much	The flavor associated with fish gelatin/ mild fish flavor
Bitterness	None-much	Basic taste associated with various compounds
Mouth coating	None-much	The amount of film left on the mouth surface after swallow/ eating product
Texture	Smooth-rough	The overall amount of small and large particles in the surface
Surface shine	Dull-shiny	Amount of light reflected from the products surface
Overall acceptance	None-much	The overall liking of milk cream

### Statistical analysis

Multivariate comparison of sensory attributes of low-fat milk cream prototypes was also done with Principal Component Analysis (PCA) using the statistical program Unscrambler® V 9.7 (CAMO Software AS, OSLO, Norway). PCA provides a way to visualize the relationship between the product (low-fat milk cream) and its attributes and characteristics. In fact, PCA uses a multidimensional dataset to create a simple view, which can allow us to easily identify differences and similarities in the samples. Analysis of variance (ANOVA) was performed using the statistical program NCSS 2007

(NCSS, Statistical Software, Kaysville, UT) for the statistical analysis of physicochemical results. The results were shown as a mean  $\pm$  standard deviation. The significance of difference was defined at the 5% level.

### Results

The extracted gelatin from grass carp skin contained  $0.93 \pm 0.01\%$  lipid,  $97.12 \pm 0.15\%$  crude protein, and  $1.83 \pm 0.02\%$  ash contents. The yield of gelatin of grass carp skin was 8.1%. From the results (Table 2), the fat content of low-fat milk cream samples was reduced from 30% in full-fat cream to 12.51% in the low-fat ones with 8%

fish gelatin. The protein content of the prototypes was increased by increasing fish gelatin incorporation and the highest protein content was observed in the treatment with 8% fish gelatin (7.1%). However, the lowest crude protein level

was observed in the full-fat cream sample (3.2%). As shown in Table 2, there was no significant difference among the treatments for the pH value (6.75-6.88).

**Table 2: Ingredients, chemical composition, and pH of full-and low-fat milk cream samples.**

Parameters	Milk cream prototypes				
	T0	T2	T4	T6	T8
<b>Ingredients (%)</b>					
Fish gelatin	0	2	4	6	8
Semi-skimmed cow milk	0	43	41	39	37
Full-fat Milk cream	100	55	55	55	55
Total	100	100	100	100	100
<b>Proximate compositions (% in wet weight) and pH</b>					
Fat	30.00±0.00 <sup>a</sup>	14.59±0.09 <sup>b</sup>	13.76±0.05 <sup>c</sup>	13.11±0.16 <sup>d</sup>	12.51±0.06 <sup>e</sup>
Protein	3.19±0.07 <sup>c</sup>	3.41±0.11 <sup>c</sup>	5.23±0.04 <sup>b</sup>	6.03±0.04 <sup>b</sup>	7.09±0.09 <sup>a</sup>
Ash	0.73±0.02 <sup>c</sup>	0.75±0.05 <sup>c</sup>	0.84±0.02 <sup>b</sup>	0.95±0.03 <sup>ab</sup>	1.24±0.09 <sup>a</sup>
Moisture	65.26±0.21 <sup>e</sup>	79.67±0.08 <sup>a</sup>	78.73±0.12 <sup>b</sup>	77.64±0.10 <sup>c</sup>	76.27±0.16 <sup>d</sup>
pH	6.57±0.08 <sup>a</sup>	6.65±0.03 <sup>a</sup>	6.70±0.06 <sup>a</sup>	6.78±0.03 <sup>a</sup>	6.88±0.10 <sup>a</sup>

Different superscripts denote significant differences within a column ( $p < 0.05$ ). T0: Full-fat milk cream with 0% fish gelatin as control; T2: Low-fat milk cream with 2% fish gelatin; T4: Low-fat milk cream with 4% fish gelatin; T6: Low-fat milk cream with 6% fish gelatin; T8: Low-fat milk cream with 8% fish gelatin.

The results of freeze-thaw stability showed that the lowest values were recorded in the full-fat sample ( $96.88 \pm 0.08\%$ ) followed by low-fat milk cream with 2% fish gelatin ( $99.35 \pm 0.02\%$ ), while it was 100% in the samples incorporated with 4, 6, and 8% fish gelatin. The same trend was observed in WHC. Samples incorporated with 4, 6, and 8% fish gelatin had the highest WHC (100%) and the lowest values were found in the full-fat sample ( $97.86 \pm 0.10\%$ ) followed by low-fat milk cream with 2% fish gelatin ( $98.37 \pm 0.05\%$ ). Lightness ( $L^*$ ), redness ( $a^*$ ), and whiteness indexes showed the highest values in the full-fat sample, and

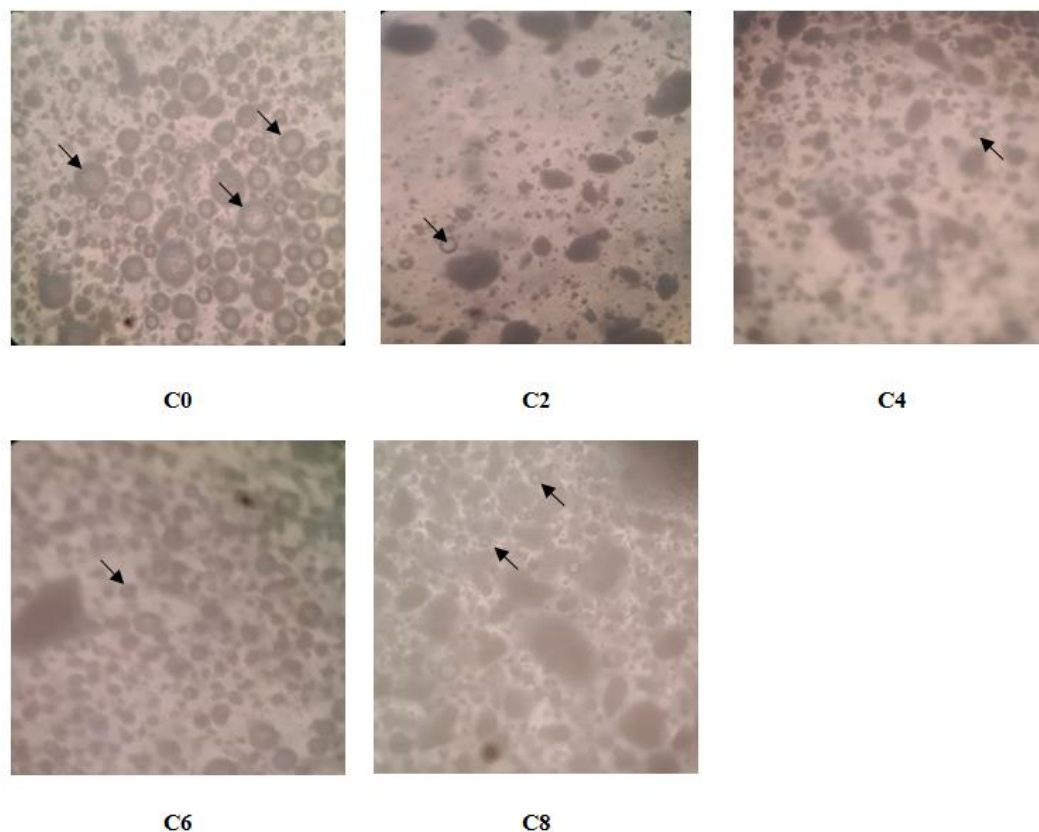
the lowest value was observed in the low-fat samples containing fish gelatin. There were no significant differences among treatments in the  $b^*$  parameter, while the highest yellowness index was obtained in the low-fat creams incorporated with fish gelatin (Table 3).

The micrographs of the low-fat milk cream are shown in Figure 1. The graphs showed that by decreasing the concentration of fish gelatin, a larger number of fatty granules were observed.

**Table 3: Color analysis of full-and low-fat milk cream samples.**

Parameters	Milk cream prototypes				
	T0	T2	T4	T6	T8
L*	90.43±0.00 <sup>a</sup>	83.30±0.11 <sup>b</sup>	84.47±0.04 <sup>b</sup>	83.50±0.04 <sup>b</sup>	83.24±0.00 <sup>b</sup>
a*	0.39±0.00 <sup>a</sup>	0.01±0.00 <sup>c</sup>	0.05±0.00 <sup>c</sup>	0.10±0.00 <sup>b</sup>	0.15±0.00 <sup>b</sup>
b*	9.36±0.00 <sup>a</sup>	9.15±0.00 <sup>a</sup>	9.83±0.00 <sup>a</sup>	9.70±0.00 <sup>a</sup>	9.58±0.01 <sup>a</sup>
Whiteness (%)	86.61±0.05 <sup>a</sup>	81.87±0.01 <sup>b</sup>	81.62±0.01 <sup>c</sup>	81.65±0.01 <sup>c</sup>	80.69±0.00 <sup>d</sup>
Yellowness	12.08±0.01 <sup>c</sup>	13.78±0.05 <sup>b</sup>	14.63±0.70 <sup>a</sup>	16.53±0.70 <sup>a</sup>	16.44±0.00 <sup>a</sup>

Same lower script shows no significant in a row ( $n=3$ ,  $p<0.05$ ). T0: Full-fat milk cream with 0% fish gelatin as control; T2: Low-fat milk cream with 2% fish gelatin; T4: Low-fat milk cream with 4% fish gelatin; T6: Low-fat milk cream with 6% fish gelatin; T8: Low-fat milk cream with 8% fish gelatin.



**Figure 1: Microscopic structure of various milk cream samples (×100 total magnification). C0: milk cream with 0% fish gelatin; C2: milk cream with 2% fish gelatin; C4: milk cream with 4% fish gelatin; C8: milk cream with 8% fish gelatin. Arrows show a number of the fat droplets.**

Texture profile analysis showed that the levels of firmness, cohesiveness, resilience, and adhesiveness were increased significantly with increasing fish gelatin levels and the highest values were measured in the low-fat sample containing 8% fish gelatin (Table 4).

As Figure 2 illustrated, the viscosity of low-fat creams was significantly

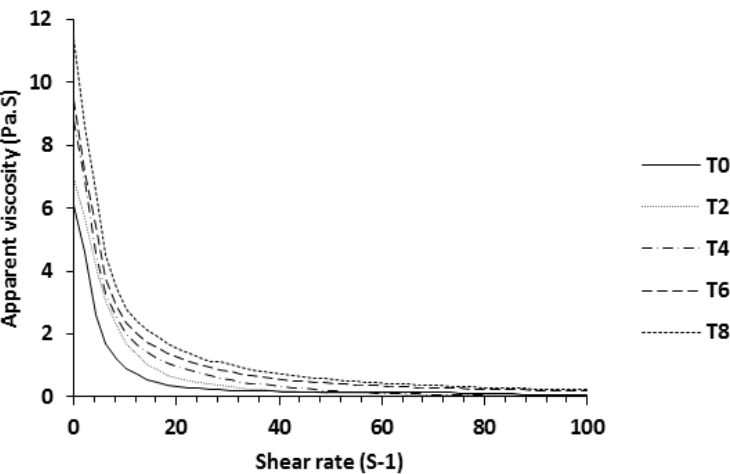
increased by increasing fish gelatin levels compared to the full-fat sample. Moreover, the variation in sensory attributes of the prototypes as given by the three-component design is shown in Figure 3. As the level of fish gelatin increased, the acceptance of the product decreased. The design-expert plot presents that incorporating fish gelatin in

low-fat cream decreased lightness and whiteness and increased yellowness in the prototypes. The addition of fish gelatin also improved the texture properties of low-fat cream samples.

**Table 4: Texture analysis of full-and low-fat milk cream samples.**

Texture properties	Milk cream prototypes				
	T0	T2	T4	T6	T8
Firmness (g)	122.98±7.06 <sup>d</sup>	206.49±43.83 <sup>c</sup>	1405.95±144.87 <sup>b</sup>	2333.75±144.87 <sup>b</sup>	3261.00±388.14 <sup>a</sup>
Cohesiveness (g)	17.00±0.02 <sup>d</sup>	19.61±0.57 <sup>c</sup>	23.80±0.95 <sup>b</sup>	27.86±0.75 <sup>b</sup>	31.92±0.610 <sup>a</sup>
Resilience	0.27±0.03 <sup>c</sup>	0.50±0.04 <sup>b</sup>	0.63±0.03 <sup>ab</sup>	0.67±0.04 <sup>ab</sup>	0.75±0.07 <sup>a</sup>
Adhesiveness (g/s)	0.15±0.00 <sup>d</sup>	0.71±0.10 <sup>c</sup>	2.28±0.25 <sup>b</sup>	3.01±0.35 <sup>b</sup>	3.75±0.21 <sup>a</sup>

Same lower script shows no significant in a raw (n=3,  $p<0.05$ ). T0: Full-fat milk cream with 0% fish gelatin as control; T2: Low-fat milk cream with 2% fish gelatin; T4: Low-fat milk cream with 4% fish gelatin; T6: Low-fat milk cream with 6% fish gelatin; T8: Low-fat milk cream with 8% fish gelatin.



**Figure 2: Apparent viscosity of full- and low-fat milk cream samples. T0: Full-fat milk cream with 0% fish gelatin as control; T2: Low-fat milk cream with 2% fish gelatin; T4: Low-fat milk cream with 4% fish gelatin; T6: Low-fat milk cream with 6% fish gelatin; T8: Low-fat milk cream with 8% fish gelatin.**

The reconstitution of the mixtures was done and no significant differences were found between the predicted responses and actual obtained response values. In this study, the addition of fish gelatin as a protein-based fat replacer could improve the sensory properties of the prototypes.

Multivariate analysis of the sensory data presented that 100% of variations between the samples were explained by the first two principal components (Fig. 4). All products were grouped on the left side of the plot. The control sample scored for fat content, lightness, and whiteness. Fish gelatin incorporated samples had similarities like yellowness, lightness, and fat content. The arrow shown in the diagram indicates the trend of improving the quality and sensory attributes of low-fat milk cream samples based on the increase in the amount of fish gelatin in the formulation. This means that by increasing the value of fish gelatin, these attributes are improved.



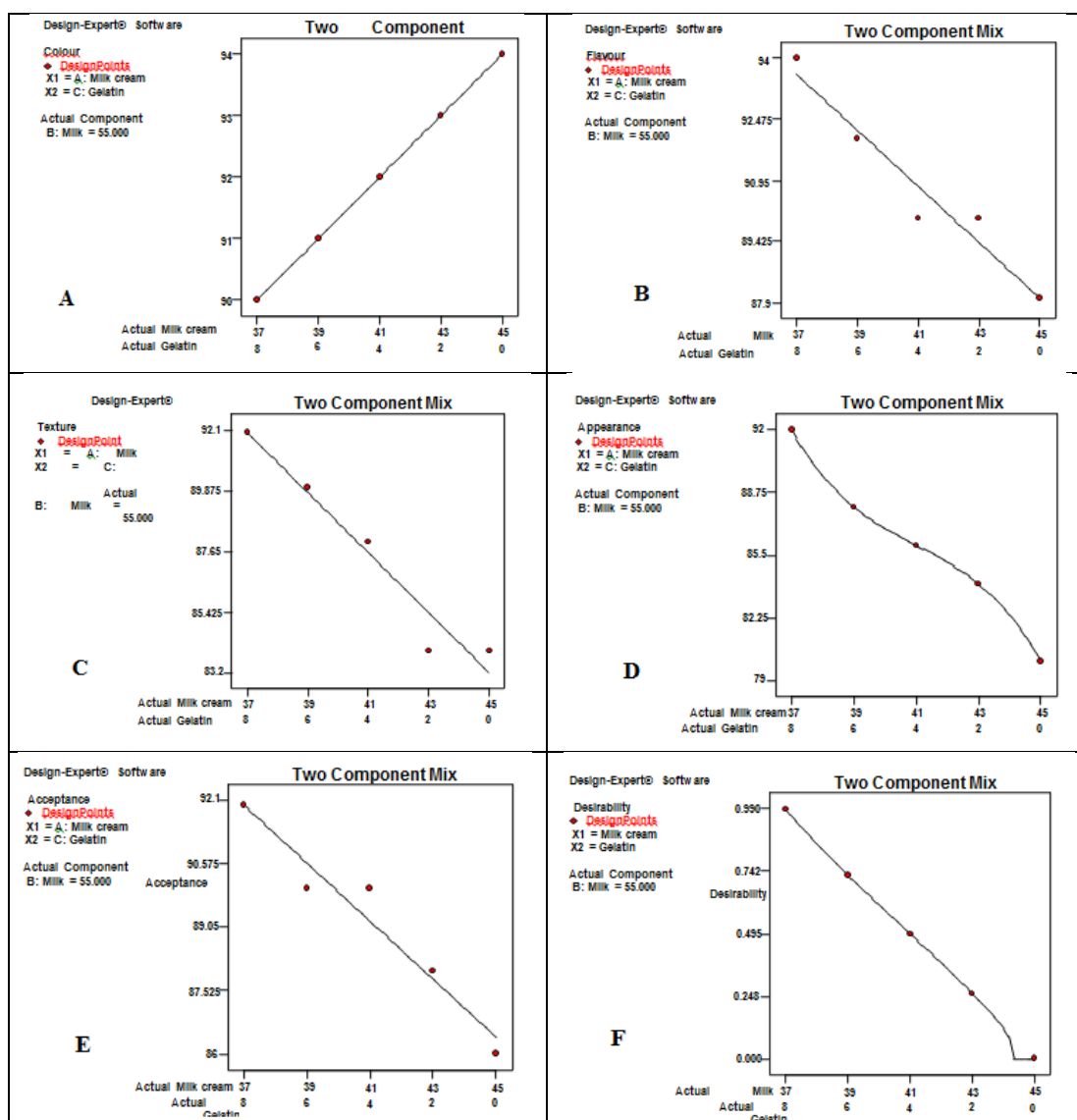


Figure 3: Variations in color (A), flavor (B), texture (C), appearance (D), overall acceptance (E), and desirability (F) of low-fat milk creams prototypes.

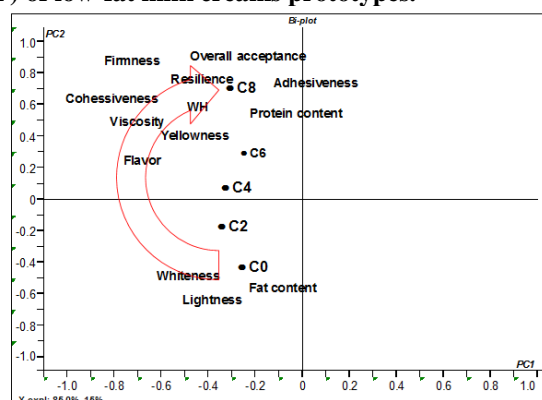


Figure 4: Principal component analysis (PCA) based on sensory data and some quality characteristics of low-fat milk creams prototypes. C0: Full-fat milk cream with 0% fish gelatin as control; C2: Low-fat milk cream with 2% fish gelatin; C4: Low-fat milk cream with 4% fish gelatin; C6: Low-fat milk cream with 6% fish gelatin; C8: Low-fat milk cream with 8% fish gelatin. The arrow located in the diagram indicates the trend of improving the quality and sensory attributes of low-fat milk cream samples based on the increase in the amount of fish gelatin in the formulation. This means that by increasing the value of fish gelatin, these attributes were improved.

## Discussion

The crude protein content of the low-fat emulsions was increased compared to the full-fat sample. It seems logical that an increase in the concentration of fish gelatin in the low-fat cream samples caused a significant increase in the protein content. Several studies indicated that by increasing the hydrocolloids such as gelatin, the content of moisture would be decreased in the final products, which play a key role in improving the textural properties of dairy products (Murtaza *et al.*, 2017). This was due to the creation of protein-protein and protein-water bounds between hydrocolloids and feedstuffs which can give an elastic property to the final product (Damodaran, 2007). The level of moisture in all treated cream samples containing fish gelatin was significantly higher than full-fat cream, while the lowest value was obtained in 8% fish gelatin among low-fat samples. The higher ash contents in the low-fat cream samples compared to full-fat ones showed a higher level of minerals which probably due to the relatively higher content of ash in the extracted fish gelatin (Ataie *et al.*, 2020). The addition of fish gelatin did not affect the pH value of the low-fat creams and it was in the range (6.5-6.8) recommended by the Iranian National Standard (INSO, 2019).

From the results, adding fish gelatin as a fat replacer in low-fat milk cream samples led to a significant increase in freeze-thaw stability. In fact, the amount of water in the low-fat samples was decreased by increasing the level of fish gelatin. This reduction can be explained

by the creation of water syneresis between fish gelatin and milk cream binding to improve the functional properties of the low-fat product (Degner *et al.*, 2014). It has been reported that applying gelatin minimizes the growth of ice crystals in emulsified food systems to aggregate macromolecules and maximize their stability (Damodaran, 2007). In this vein, Li *et al.* (2017) examined the effects of fish gelatin on the stability of oil-in-water emulsions. Their results indicated that the incorporation of the fish gelatin in the formulation increased the stability of the final product due to the creation of bonds between the particles of the internal emulsion and gelatin functional groups. Their results are in line with the results obtained in this research.

The lowest amount of WHC was obtained in the full-fat sample (97.86%), while it reached 100% value in the samples containing 4, 6, and 8% fish gelatin. The application of hydrocolloids at high concentrations in oil-in-water food systems leads to more coagulation ratios in the emulsion and gives it more stability due to form a stronger structure protein network in the liquid phase stability. In addition, the presence of gelatin creates a stress-resistant network that increases WHC (Wang *et al.*, 2013).

The lightness parameter of the creams incorporated with fish gelatin was significantly decreased in comparison with the control sample. The increase of yellow color in low-fat samples incorporated with fish gelatin is probably due to the presence of high

natural carotenoids in fish gelatin (Ataie *et al.*, 2020; Jridi *et al.*, 2020). However, the source of raw materials and the extraction methods of fish gelatin can greatly affect the turbidity and color of the extracted fish gelatin (Ataie *et al.*, 2020).

The size of the oil particles was decreased by increasing the amount of gelatin replacement in low-fat milk cream samples and the lowest oil droplet size was observed in the sample with 8% fish gelatin (Fig. 1). One of the reasons for the smaller particles of oil globules during the replacement process can be the repulsion of fat droplets, which is due to the reduction of the moisture in the emulsion (Urgu *et al.*, 2018). In general, fish gelatin as an effective stabilizer can prevent the formation of larger oil droplets by covering the droplets during emulsification and prevent the coalescence during storage over time. Hydrocolloids increase the absorption of protein and surfactant in the lipid level, which can alter the surface tension and the number of fat droplets to air bubbles and causing smaller particles to be interconnected (Wang *et al.*, 2013).

The lowest textural parameters were observed in the full-fat cream compared to the low-fat samples. It can be concluded that the network of protein and fat was weakened in the full-fat cream samples and fish gelatin could form water-protein binds to create a strong scaffold in the reduced-fat samples (Murtaza *et al.*, 2017). Since there is a strong relationship between

hardness and WHC in different food systems, a higher WHC value in the low-fat samples incorporated with fish gelatin might lead to form a firmer structure.

Viscosity and stability are two important viscoelastic properties for milk cream, which affect the quality and acceptability of the product. The higher viscosity of low-fat creams is mainly due to the bonding ability of fish gelatin with free water and resulted in increasing the flow resistance by forming a strong casein network in the low-fat samples (Akalin *et al.*, 2008).

In general, a three-dimensional structure of water and other compounds is formed due to the presence of fish gelatin which can improve viscosity (Nguyen *et al.*, 2017). In addition, the apparent viscosity reduction was observed in the final shear rate in all samples. This reduction was more severe in the initial shear rate of the sample containing 8% fish gelatin compared to the other fish gelatin-containing samples. The apparent viscosity reduction in the sample was due to the discontinuity of the molecular network bands due to the forces imposed on the sample (Ataie *et al.*, 2020).

Mixture response surface contour plots display that the mixture of milk cream and fish gelatin affected sensory properties significantly. It reveals that fish gelatin played a major role in milk cream odor, flavor, texture, and overall acceptance. However, using such data for product design and development and prototype selection depends on the

specifications of the product and expert panel comments (Hoseini Shekarabi *et al.*, 2019). The desirable maximization of the low-fat milk cream was performed by numerical techniques using the mathematical optimization procedure of the Design-Expert Software Package. The optimization criterion was based on the highest level of sensory scores including acceptance, lightness, yellowness, and texture properties which are thought to be the most important parameters in product development studies (Shaviklo *et al.*, 2020). The solution was obtained using the software, which sought to maximize the desirability function by being at random starting points and proceeding on a path of the steepest slope to a maximum. The best among them was taken as optimum. The desirability model (Fig. 3-F) was obtained from the software (Design-Expert) calculation. It recommended a mixture containing 8% fish gelatin, 37% milk cream, and 55% cow milk. This study revealed the successful application of fish gelatin in low-fat milk cream at the level of 8%. At this level, the sensory attributes and texture properties were improved. This study noted that it is possible to develop a low-fat milk cream using fish gelatin as a novel functional ingredient with high nutritional values and potential health benefits.

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