

## **Modification in the Functional Properties of Sodium Caseinate-based Imitation Cheese through Use of Whey Protein and Stabilizer**

M. Hosseini<sup>1\*</sup>, M. B. Habibi Najafi<sup>1</sup>, and M. Mohebbi<sup>1</sup>

### **ABSTRACT**

Guar gum and whey proteins concentrate (WPC-35) were used as functional additives to improve the functional characteristics (hardness and meltability) of the Na-caseinate-based imitation cheese. Also, the alterations in the composition, sensory acceptance, color, and texture caused by these ingredients were evaluated. Imitation cheeses were formulated with three levels each of WPC (0, 1.5, and 3%) and guar gum (0, 0.3 and 0.6%) w/w in cheese formulation. Cheeses with higher guar and lower WPC were softer and melted to a greater degree. Use of the two food additives at increasing levels reduced the amount of protein significantly ( $P < 0.05$ ). Unlike guar, WPC increased pH values. Color of the cheese was affected positively by guar and negatively by WPC ( $P < 0.05$ ). The overall acceptability of the cheese was not affected by the levels of WPC, but it did improve with increasing level of guar used in the formulation. Taking into consideration the adverse impact of WPC on color and meltability and slight adverse effect of guar on the hardness of imitation cheese, use of WPC at 1.5% and guar at 0.3% level in the formulation of imitation cheese is recommended.

**Keywords:** Guar gum, Physico-chemical properties, Sensory characteristics, Whey protein concentrate.

### **INTRODUCTION**

Imitation cheeses or cheese analogues are defined as products made by blending food ingredients, involving sources of fat and protein along with water, acid, emulsifying salts (ES), and cheese flavoring, and employing thermal and mechanical energy to produce a homogeneous product, simulating a specific cheese variety (Noronha *et al.*, 2008). The protein in cheese analogue stabilize oil-in-water emulsion through reducing the interfacial tension and increasing the viscosity of the aqueous phase, thereby decreasing the frequency of collisions between oil droplets (Ennis and Mulvihill, 1999). Imitation cheese is mostly used as an ingredient of ready-to-eat foods

and is used in both unmelted and melted states. In the unmelted state, cheese is exposed to operations involving a combination of shear and compressive stresses (e.g. cutting) that results in large deformation and fracture into smaller pieces. As an ingredient, analogue cheese is also used extensively in cooking applications, e.g. on pizzas and cheeseburgers. A key aspect of cooking performance of cheese is its heat-induced functionality, including melting or flowability (O’Riordan *et al.*, 2011). Thus, the functional attributes of importance for imitation cheese include the hardness and exhibiting the desired degrees of meltability.

The main protein source in dairy-based imitation cheese products is rennet casein or

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<sup>1</sup> Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran.

\* Corresponding author; e-mail: marziehossini@yahoo.com



sodium (Na) and/or calcium (Ca)-caseinates. Rennet casein, due to its functional properties and desirable flavor, is preferred over alternative protein sources. For rennet casein which has a high calcium-to-casein ratio (~36 mg g<sup>-1</sup> casein), the degree of calcium sequestration and *para*-casein aggregation is easily controlled by using the correct blend of ES to give the desired degree of fat emulsification in imitation cheese. This, in turn, gives the desired degree of flow and firmness on cooking the imitation cheese (Guinee *et al.*, 2004). With Na-caseinate, the phenomenon of over-creaming is reported that when the oil-phase in analogue cheeses was over-emulsified, the cheese product had poor meltability and firmness characteristics. Thus, cheese analogues produced only on the base of Na-caseinate is undesirable as a pizza topping and some other applications (O’Riordan *et al.*, 2011). On the other hand, the high cost of rennet casein have forced processors to search for readily available low-cost substitutes to partially or totally replace this type of milk protein in analogue cheeses. In the present study, Na-caseinate was employed due to its availability and reasonable cost in Iran. On the basis of literature review, it was presumed that optimal levels of guar gum and WPC in combination may confer the desired firmness and meltability to the Na-caseinate based imitation cheese.

When WPC is heated over the denaturation temperature, the tertiary structure of the protein molecule (especially  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin) is unfolded. This phenomenon allows both the exhibition of hydrophobic sites and the sulfhydryl/disulfide interchange reactions that induce the formation of aggregates (Meza *et al.*, 2010). Thus, substitution of casein with WPC produces cheese with the highest firmness values. Firmness of processed cheese analogues obtained from acid casein and partially replaced by whey products was much higher than standard analogues with an increase in pH (5.0-7.0). Such phenomena have been ascribed to the

interactions between casein and whey proteins (Solowiej 2007). Mleko and Foegeding (2000) reported that whey proteins interact with casein network as filler. The most probably disulphide bonds between  $\kappa$ -casein and  $\beta$ -lactoglobulin was formed, but also  $\alpha$ -lactalbumin could participate in the interaction with casein. It was recommended to replace casein by whey protein up to 2% only.

WPC has been implicated in reducing the meltability of imitation cheese (Mleko and Foegeding, 2000; Guinee *et al.*, 2004; Solowiej, 2007). According to Cavalier-Salou and Cheftel (1991), the melting ability correlated with pH, texture, degree of casein dissociation, and degree of fat emulsification. Since the viscosity of fully hydrated solution of guar gum decreased with increasing temperature, it can improve heat-induced flowability of cheese (Jackson *et al.*, 2001). The properties of gels brought about by guar gum largely varies depending upon the amount of gum used; it creates rubbery mass which exhibits flow properties, coalesce readily after being subjected to shear, and shows no syneresis. Moreover, guar is capable of forming hydrogen bonds. Therefore, even when used in small quantities, it can bring about intense competition in absorption of water with various hydrocolloids and water-soluble proteins (Chudzikowski, 1971). In general, stabilizers (such as guar) are one such ingredient, which, in spite of their low consumption level in the formulation, impart specific and important functions to the finished product (Bahram Parvar *et al.*, 2013). Various gums i.e. guar, locust bean, xanthan, arabic gum, and their admixtures have been used to create a heat reversible gel for use in preparation of Soybean mozzarella cheese analogue (MCA). In order to obtain MCA having superior flowability; guar is required in very little amounts than other gums. Guar gum led to MCAs having greater hardness when compared to use of other gums (Yang, 1983).

The upper and lower limits of the levels of WPC and guar gum to be used in the product that could give a cheese-like structure were determined in the preliminary studies. For example, when there was only guar, the structure was fluid and in level above 0.6%, the structure was too fluid. WPC at the levels between 1-3% was required to harden the structure.

The objective of this study was to investigate the influence of different levels of WPC and guar gum on the composition, sensory acceptance, colour, and especially functionality (texture and melting properties) of the Na-caseinate based imitation cheese.

## MATERIALS AND METHODS

### Materials

Sodium caseinate (90% protein) was purchased from Iran Caseinate Company (Tehran, Iran). Hydrogenated soybean oil (Ghoncheh Vegetable Oil Company, Tehran, Iran) and rapeseed oil (Aliagolestan Food Company, Tehran, Iran) were used for preparation of imitation cheese. Trisodium citrate, citric acid, potassium sorbate, and guar gum were all obtained from Merck Company (Darmstadt, Germany). NPNZ company (Auckland, New Zealand) provided WPC (35% protein) and sodium chloride was obtained from SepidDanesh

Trading Company (Shiraz, Iran).

### Manufacture of Imitation Cheese

A basic formula and manufacturing method similar to Noronha *et al.* (2008) were used with some modifications for the preparation of imitation cheese samples. Imitation cheese, with an average moisture content of 52% (w/w), was manufactured in an experimental twin-screw cooker (dimensions: 10×25×15 cm, capacity: 1,800 cm<sup>3</sup>, mixer speed: 50 rpm, setting temperature by circulating, electromotor power: 180 watt) that had been prepared for this purpose (Tamesh Company, Mashhad, Iran). The ingredients for preparing imitation cheese were 24.5% Na-caseinate, 52% water, 14% hydrogenated soybean oil, 7% rapeseed oil, 0.5% trisodium citrate, 1.3% NaCl, 0.2% potassium sorbate, and 1% citric acid. It should be noted that sodium caseinate was replaced with WPC and guar gum. Indeed, 0, 0.3 and 0.6% guar and 0, 1.5, and 3% WPC were used instead of the same contents of Na-caseinate (Table 1). Imitation cheese (800 g per batch) was manufactured by blending the vegetable oil with water at 50°C for 2 minutes. Then, this mixture was fed to the cooker; mixing speed was 50 rpm and temperature of cheese attained was 85°C. Sodium chloride, potassium sorbate and trisodium citrate were added and mixed for a further minute. Subsequently, the required amounts of WPC

**Table 1.** Actual values of Na-caseinate, WPC and guar for 9 treatment of imitation cheese.<sup>a</sup>

Sample run	Na-caseinate (%)	WPC (%)	Guar gum (%)
1	24.5	0	0
2	24.2	0	0.3
3	23.9	0	0.6
4	23	1.5	0
5	22.7	1.5	0.3
6	22.4	1.5	0.6
7	21.5	3	0
8	21.2	3	0.3
9	20.9	3	0.6

<sup>a</sup> The other ingredients in the formulation were stable.



along with sodium caseinate were added and the process continued until a uniform mass was obtained (approximately 15 minutes). Guar gum, hydrated in a part of formulation water, was added and mixing continued for 2 more minutes. Later on, citric acid was added at the end of the production process for one final minute of mixing at 85°C. The imitation cheese was discharged into a plastic lined cardboard box with dimensions 6×10×6 cm and was then transferred to a refrigerator and cooled to 4°C for 24 hours. Then, the cheese was divided into sections of 200 g, vacuum-packed (Model A200 H, Webomatic, Bochum, Germany) and stored at 4°C till it was analyzed.

### Compositional Analysis

The fat content of imitation cheese was determined by Gerber method, moisture by oven drying method (ISIRI, 2002), the protein content was determined by micro-Kjeldhal. The ash content was determined by AOAC official method (AOAC, 2002). A glass/Ag/AgCl pH electrode, attached to a pH meter (Model H18314, Unicam Ltd., Cambridge, UK), was used to measure the pH value. The salt content of samples was determined by using potentiometric method of Fox (1963). All the measurements were performed in triplicate.

### Texture Profile Analysis

Textural properties of the imitation cheeses were examined using a texture analyser (QTS Texture Analyser; CNS, Farnell, UK) according to the texture profile analysis method at cheese temperature of 20°C (Van Hekken *et al.*, 2007). Samples with 15 mm height and 15 mm diameter were taken from the cheeses with a cork borer and a slicer. A load cell of 5 kg with a speed of 100 mm min<sup>-1</sup> applying 50% compression and a cylindrical probe with a diameter of 3.5 cm was used. Five samples from each cheese block were analysed.

### Melt Test

A modification of Schreiber method and image processing technique were used to assess imitation cheese meltability (Wang and Sun, 2002; Hajimohamadi Farimani, 2008). At first, disc of cheeses with 5 mm diameter and 22 mm thickness were cut and placed in the center of a glass plate. The discs were heated in an air draft forced oven (Model SH2006, Paat-Ariya Company, Mashhad, Iran) at 90°C for 15 minutes. Photographs of the samples were taken before and after heating in oven using camera (Power shot EOS 1000D) connected to the computer equipped with Zoom Browser EX software (version 5.0) and saved in JPEG format.

Area measurement was performed using Clemex software (version 4.0.021). The melting property of cheese was determined using the equation:

$$MD_t = (A_t/A_0) \times 100 \quad (1)$$

Where,  $MD_t$  and  $A_t$  are melting degree (%) and area (mm<sup>2</sup>) of cheese disc after heating and melting, respectively.  $A_0$  was considered as the initial cheese area (mm<sup>2</sup>). Three samples from each batch of imitation cheese were examined.

### Color Test

The RGB images (in JPEG format) of samples with 2×2 cm sizes were captured using an image processing system consisting of a digital camera (Cannon, power shot EOS 1000D) fastened vertically at a distance of 20 cm from the sample, connected to a Pentium IV computer with Cannon remote capture software (Version 5.00). Images were taken under two fluorescent lamps each with 11 W inside a wooden box with black walls to avoid the light and reflection from the box. Images were taken at their maximum resolution (2272×1704). For extracting imitation cheese from its black background in the obtained images, thresholding-based method was used. Adobe-RGB-to- $L^*a^*b$  transformation was

carried out using image J software (Version 1.42e). In food industry, the most used color space system is the  $L^*$ ,  $a^*$  and  $b^*$ , which is also referred to CIELAB system. According to the CIE system,  $L^*$  is defined as the lightness of the color of the sample;  $a^*$  shows the red and green characteristics and  $b^*$  shows the yellow and blue characteristics (Pillonel *et al.*, 2007). The color test was performed in triplicate for each batch.

### Sensory Analysis

An untrained 12-member panel conducted the sensory evaluation of the imitation cheeses. The panel of judges comprised of 6 males and 6 females aged between 24 and 37 years, selected from students of Ferdowsi University of Mashhad. All panelists were seated in separate booths, and samples were presented under a red/green light to avoid visual bias. Prior to assessment, each sample was cut into 10 g cubes and was placed in small plastic containers coded with 3-digit random numbers; then, allowed to stand for 0.5h in room temperature to equilibration of volatiles in the headspace. Seven sessions took place, with 8 cheeses tasted each time from the tenth day after production. Cheeses were randomly assigned in each day. The samples were assessed for aroma, flavor, texture, appearance, and overall acceptability on a general taste panel using a 5-point hedonic scale where 1= Extremely dislike, 2= Dislike, 3= Neither like nor dislike, 4= Like; 5= Extremely like.

### Statistical Analysis

Two separate batches of imitation cheeses containing three levels of WPC (0, 1.5, and 3%) and guar gum (0, 0.3, and 0.6%) were manufactured. A factorial completely randomized design was used for all the treatments. Analysis of variance (ANOVA) was conducted using Minitab 16.1 software (Minitab Inc., State College, PA, USA). In

case of significant differences, (at  $P < 0.05$ ), Duncan's multiple range test was performed.

## RESULTS AND DISCUSSION

### Compositional Analysis

The compositional analysis of the imitation cheeses formulated with substitution of Na-casein with WPC and guar gum at different levels are shown in Table 2. Protein content of imitation cheeses significantly decreased ( $P < 0.05$ ) as the extent of WPC used to substitute Na-caseinate was increased, as a result of considerably lower protein content of WPC (35%) compared to the protein content of Na-caseinate (90%). The protein content of the cheeses decreased with increasing level of guar, but the trend of this decrease was not so clear.

There was a significant decrease in pH value with increasing level of guar gum used. This decrease might be due to lack of buffering power of Na-caseinate. High pH value of WPC (6.8) resulted in an increase in pH of imitation cheese samples, when it was used to substitute part of Na-caseinate.

Increasing guar gum did not lead to significant difference in the ash content of cheeses. WPC, due to the higher ash content (7.8%) compared to sodium caseinate (3.6%), increased the ash content of imitation cheese samples.

No significant differences were obtained between imitation cheeses containing guar gum and WPC for moisture, salt, and fat values.

### Texture Profile Analysis

Table 3 shows the results obtained in the instrumental texture evaluation. As expected, WPC increased the hardness of the cheese. This effect was ascribed to the interaction between casein and denatured WPC formed during high-temperature

**Table 2.** Proximate composition of imitation cheese containing WPC and guar gum.<sup>a</sup>

Formula	WPC (%)	Guar (%)	Protein (%)	Fat (%)	pH	Moisture (%)	Salt (%)	Ash (%)
1	0	0	22.24±0.21 <sup>a</sup>	21.60±0.38 <sup>a</sup>	5.91±0.01 <sup>b</sup>	51.92±0.17 <sup>a</sup>	1.42±0.13 <sup>a</sup>	4.22±0.03 <sup>c</sup>
2	0	0.3	21.92±0.12 <sup>ab</sup>	21.68±0.44 <sup>a</sup>	5.86±0.04 <sup>c</sup>	51.74±0.23 <sup>a</sup>	1.47±0.09 <sup>a</sup>	4.23±0.14 <sup>c</sup>
3	0	0.6	21.73±0.07 <sup>ab</sup>	21.58±0.29 <sup>a</sup>	5.80±0.05 <sup>c</sup>	51.66±0.41 <sup>a</sup>	1.29±0.12 <sup>ab</sup>	4.21±0.09 <sup>c</sup>
4	1.5	0	21.42±0.14 <sup>bc</sup>	21.38±0.36 <sup>a</sup>	5.92±0.05 <sup>b</sup>	51.46±0.36 <sup>a</sup>	1.21±0.19 <sup>b</sup>	4.34±0.10 <sup>b</sup>
5	1.5	0.3	21.09±0.05 <sup>cd</sup>	21.90±0.17 <sup>a</sup>	5.88±0.05 <sup>c</sup>	51.78±0.24 <sup>a</sup>	1.35±0.10 <sup>ab</sup>	4.36±0.09 <sup>b</sup>
6	1.5	0.6	20.88±0.17 <sup>d</sup>	21.17±0.40 <sup>a</sup>	5.83±0.03 <sup>d</sup>	51.82±0.29 <sup>a</sup>	1.35±0.09 <sup>ab</sup>	4.36±0.08 <sup>b</sup>
7	3	0	20.68±0.11 <sup>d</sup>	21.76±0.17 <sup>a</sup>	5.95±0.07 <sup>a</sup>	51.86±0.41 <sup>a</sup>	1.32±0.16 <sup>ab</sup>	4.47±0.05 <sup>a</sup>
8	3	0.3	20.78±0.12 <sup>d</sup>	21.44±0.41 <sup>a</sup>	5.91±0.01 <sup>b</sup>	51.95±0.22 <sup>a</sup>	1.39±0.19 <sup>ab</sup>	4.49±0.07 <sup>a</sup>
9	3	0.6	20.11±0.23 <sup>e</sup>	22.07±0.36 <sup>a</sup>	5.88±0.04 <sup>c</sup>	51.78±0.20 <sup>a</sup>	1.35±0.11 <sup>ab</sup>	4.48±0.07 <sup>a</sup>

<sup>a</sup> Values followed by the different letter in each column are significantly different at  $P < 0.05$ .

(85°C) processing. Pinto *et al.* (2007) also observed that application of WPC in production of processed cheese spread was responsible for increasing the hardness of the product. In contrast to our finding, Dees (2002) reported that cheese analogue containing 2% native whey protein did not differ significantly with regard to hardness compared to the one devoid of whey proteins. According to Dees (2002), the susceptibility of the proteins to thermal denaturation tends to be lower in presence of high lactose concentration, as was the case in WPC. The hardness of analogues made using guar gum combination tends to decline. Guar gum, as a partial replacement of casein in imitation cheese, increased the ratio of free water to bound water within the product matrix, possibly due to its higher water-holding capacity. The unbound water facilitates cheese particles to flow, thereby resulting in a cheese product with lower hardness. Similarly, YuetHee *et al.* (2008) reported that guar gum decreased the strength of gels formed by the gelatin in simulating soft cheeses.

WPC was found to reduce adhesiveness in imitation cheese. This is a minor effect as the only significant differences were seen between 0 WPC/0 guar and the 3 WPC/0, 0.3%, +0.6% guar treatments. It has been suggested that inter- and intra-molecular bonds in the protein network of imitation cheese containing WPC was raised and protein-protein interactions occurred to the detriment of protein-water interactions. Therefore, this poorly hydrated protein matrix possibly contributed to low adhesiveness (O’Riordan *et al.*, 2011). Kaminarides and Stachtiaris (2000) also reported a marginal reduction in the adhesiveness of process cheese with increasing use of WPC levels. In the current experiment, increasing the level of guar up to 0.6% did not significantly affect the adhesiveness of imitation cheeses.

Increasing the concentration of WPC increased the cohesiveness of cheeses. This is a minor effect as the only significant differences were seen between 3 WPC/0

**Table 3.** Textural properties of imitation cheese as influenced by WPC and guar gum.<sup>a</sup>

Formul a	WPC (%)	Guar (%)	Hardness (N)	Adhesiveness (N/S)	Cohesiveness s (-)	Springiness (mm)
1	0	0	18.5±0.10 <sup>f</sup>	0.34 ±0.01 <sup>a</sup>	0.55 ±0.02 <sup>c</sup>	4.41±0.02 <sup>cd</sup>
2	0	0.3	16.6 ±0.12 <sup>g</sup>	0.33 ± 0.01 <sup>ab</sup>	0.56 ± 0.00 <sup>bc</sup>	4.44 ± 0.06 <sup>bc</sup>
3	0	0.6	14.7 ±0.14 <sup>h</sup>	0.33 ±0.02 <sup>ab</sup>	0.56± 0.01 <sup>bc</sup>	4.50 ±0.05 <sup>a</sup>
4	1.5	0	28.0 ± 0.17 <sup>c</sup>	0.32 ±0.01 <sup>abc</sup>	0.58± 0.01 <sup>ab</sup>	4.37±0.05 <sup>f</sup>
5	1.5	0.3	26.2 ± 0.17 <sup>d</sup>	0.32 ±0.00 <sup>abc</sup>	0.58 ±0.01 <sup>ab</sup>	4.40±0.01 <sup>de</sup>
6	1.5	0.6	24.1 ± 0.14 <sup>e</sup>	0.32±0.01 <sup>abc</sup>	0.58 ±0.02 <sup>ab</sup>	4.46 ±0.03 <sup>b</sup>
7	3	0	32.9 ±0.08 <sup>a</sup>	0.31±0.03 <sup>bc</sup>	0.60±0.01 <sup>a</sup>	4.33 ± 0.04 <sup>g</sup>
8	3	0.3	31.0 ± 0.22 <sup>b</sup>	0.30 ± 0.00 <sup>c</sup>	0.58±0.00 <sup>ab</sup>	4.38±0.06 <sup>ef</sup>
9	3	0.6	28.2 ± 0.15 <sup>c</sup>	0.30 ±0.01 <sup>c</sup>	0.59±0.02 <sup>a</sup>	4.41±0.03 <sup>cd</sup>

<sup>a</sup> Values followed by the different letter in each column are significantly different at  $P < 0.05$ .

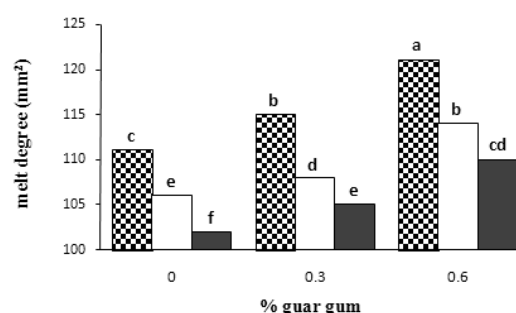
guar and the 0 WPC/0.3%+0.6% guar treatments. This was attributed to interaction between casein and whey proteins that possibly strengthened the network formed by Na-caseinate. In fact, WPC increased the strength of internal bonds through disulfide interaction with casein. Kaminarides and Stachtariis (2000), reported that WPC improved the cohesiveness of product insignificantly. The cohesiveness of the imitation cheeses was not significantly affected by inclusion of guar in the cheese formulation.

Incorporation of WPC reduced the springiness of the imitation cheese samples. Casein gives resilience to cheese structure when external forces are applied. Thus, replacing it with whey proteins decreases springiness of the product. Researchers have reported that the replacement of 0 to 39.25% of Kasar cheese by WPC reduced the elasticity of processed cheese. The effect of casein on the elasticity of cheese was greater than that exerted by WPC (Kaminarides and Stachtariis, 2000). According to Solowiej (2007), there was an increase in the elasticity of the process cheese analogue containing WPC as part substitute of casein. Springiness of the structure increased significantly with increasing guar gum level. Yang (1983) also noticed slight increase in the springiness of soybean mozzarella cheese analogue with inclusion of guar gum in its formulation. The increase in springiness of cheese containing guar may

be due to a decrease in the protein matrix compactness because the addition of guar gum increases water binding capacity of protein matrix in the product.

### Melt Test

The meltability of imitation cheese is another important characteristic, because one of the main applications of the product is as an ingredient in cooked foods (Noronha *et al.*, 2008). Guar gum and WPC significantly affected the meltability of the imitation cheese. The results presented in Figure 1 indicate that increasing the amount of WPC reduced the meltability of imitation cheeses. Similar to our findings, Mleko and Foegeding (2000, 2001) and Solowiej



**Figure 1.** Meltability of imitation cheese containing 0 (▨), 1.5 (□), or 3% (■) WPC and 0 to 0.6% of guar gum. Values with different letter(s) are significantly different at  $P < 0.05$ .



(2007) reported that the meltability of process cheese analogue decreased with increasing levels of whey protein. This could have affected the strength of bond created between casein and whey proteins. Contrary to our findings, Abd El-Salam *et al.* (1996) found that the meltability of process cheese spread increased as the amount of WPC increased from 0 to 40%. Perhaps because the processed cheese spread contains less quantum of intact casein (Relative casein content) than does imitation cheese or process cheese. Guar gum helped in improving the meltability of imitation cheese. The softening and better flow property of imitation cheese, when heated, was evident when using increasing guar gum level in the formulation. Such effect may be attributed to the significant decrease in the protein content of cheeses containing higher level of guar gum that might have resulted in weaker emulsion and increased melting. Also, since the viscosity caused by guar gum is reduced at high temperature (90°C), the melting of the product improves.

### Measuring Color

When the color attribute of imitation cheese with varying WPC levels were compared (Table 4), it was observed that the lightness value ( $L^*$ ) diminished ( $P < 0.05$ ), indicating that the cheeses became duller. Darker color of WPC compared to Na-caseinate is one of the reasons for such effect. In addition, reduction in the lightness

value of imitation cheese containing WPC can be attributed to the lactose available in the WPC (lactose content of WPC was 46.5%) which might have undergone Maillard's reaction during processing of cheese. Lightness of the product was increased by increasing levels of guar. Esparan *et al.* (2010) reported that in tofu cheese, carrageenan at the level of 0.1% increased  $L$ -value of the product, but use of 0.2% carrageenan had less impact on  $L$ -value than did 0.1% level.

For all samples, the parameter  $b^*$  is presented by positive values (Table 4), indicating a tendency of the samples toward yellow colour; Na-caseinate being a yellow powder must have contributed to such colour unit. The results indicated an increase in the value of yellowness with increasing levels of WPC. On the other hand, the  $b^*$  values were significantly ( $P < 0.05$ ) reduced when utilizing guar at increasing levels. Thus, the use of guar had opposite effect on the colour of cheese compared to that exerted by WPC. According to Noronha *et al.* (2008) a clean white color is considered as a highly desirable attribute in unmelted imitation cheese. Yoder *et al.* (1996) also reported that the gums (pectin and carrageenan) in combination with gelatin in the formulation of imitation cheese reduced the yellow tint imparted by gelatin.

The variations in the parameter  $a^*$  of samples containing WPC and guar is shown in Table 4. The greenness of cheese was decreased as a result of increase in the levels of WPC used. The parameter  $a^*$  increased

**Table 4.** Color properties of imitation cheese as affected by WPC and guar gum.<sup>a</sup>

Formula	WPC (%)	Guar (%)	$L^*$	$b^*$	$a^*$
1	0	0	81.25±0.19 <sup>d</sup>	27.30 ± 0.27 <sup>bc</sup>	-7.34 ± 0.14 <sup>ab</sup>
2	0	0.3	82.61 ± 0.32 <sup>c</sup>	26.31 ± 0.21 <sup>bc</sup>	-7.35 ± 0.10 <sup>bc</sup>
3	0	0.6	85.45 ± 0.24 <sup>a</sup>	25.08 ± 0.12 <sup>a</sup>	-7.59 ± 0.21 <sup>c</sup>
4	1.5	0	79.76 ± 0.47 <sup>ef</sup>	28.15 ± 0.32 <sup>de</sup>	-7.10 ± 0.22 <sup>a</sup>
5	1.5	0.3	82.49 ± 0.37 <sup>c</sup>	27.05 ± 0.20 <sup>cde</sup>	-7.21 ± 0.16 <sup>ab</sup>
6	1.5	0.6	84.08 ± 0.14 <sup>b</sup>	25.93 ± 0.17 <sup>ab</sup>	-7.43 ± 0.12 <sup>bc</sup>
7	3	0	78.96 ± 0.28 <sup>f</sup>	28.49 ± 0.17 <sup>f</sup>	-6.80 ± 0.18 <sup>a</sup>
8	3	0.3	80.39 ± 0.42 <sup>c</sup>	28.36 ± 0.23 <sup>c</sup>	-7.01 ± 0.11 <sup>a</sup>
9	3	0.6	82.77 ± 0.35 <sup>c</sup>	28.20 ± 0.19 <sup>bcd</sup>	-7.23 ± 0.28 <sup>a</sup>

<sup>a</sup> Values followed by the different letter in each column are significantly different at  $P < 0.05$ .



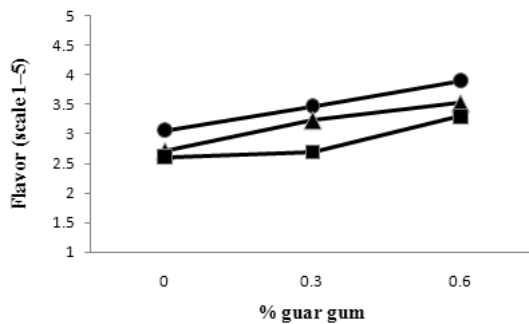
with increasing levels of guar used in the product formulation. Esparan *et al.* (2010) observed decrease in the redness of tofu when utilizing carrageenan.

### Sensory Profile of Cheese

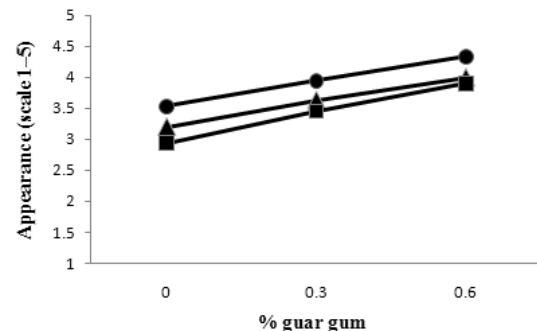
Figure 2-5 depicts the average sensory scores of imitation cheeses with different levels of WPC and guar gum. It can be seen from the Figure 2 that substitution of Nacaseinate with WPC decreased the flavor score. Similar results reported by Kaminarides and Stachtiaris (2000), who claimed that cheeses containing the highest amounts of WPC exhibited the lowest flavor score. Likewise processed cheese containing higher levels of WPC (3.0 and 4.5%) were found to be tasteless damaging the mild flavor (Pinto *et al.*, 2007). In our study,

increasing the level of guar in the cheese formulation led to better flavor scores. Increasing acceptance of taste could be due to the partial reduction of caseinate or counteracting the flavor of caseinate by guar.

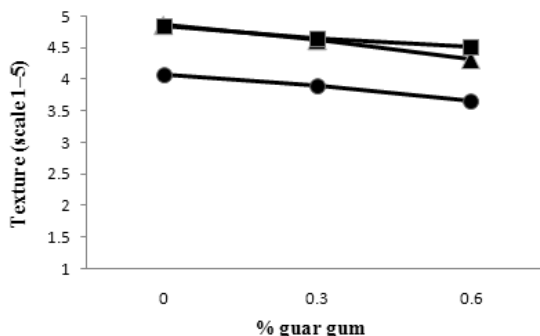
Appearance scores of samples decreased with increasing the levels of WPC (Figure 3). This result is in agreement with the results measured through image processing. Pinto *et al.* (2007) did not find any impact of using WPC on the appearance of processed cheese spread. In this study appearance scores increased with increasing levels of guar. Jana *et al.* (2010) also did not observe any significant difference in the color score of Mozzarella cheese analogue samples containing different hydrocolloid mixtures. However, when these cheese analogues were used as pizza topping, samples containing



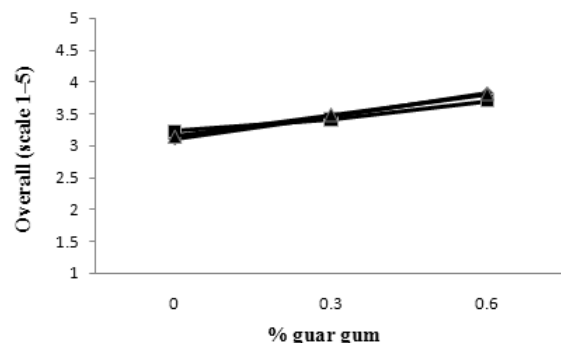
**Figure 2.** Flavor scores of imitation cheese containing 0 (●), 1.5 (▲), or 3% (■) WPC and 0 to 0.6% of guar gum.



**Figure 3.** Appearance scores of imitation cheese containing 0 (●), 1.5 (▲), or 3% (■) WPC and 0 to 0.6% of guar gum.



**Figure 4.** Texture scores of imitation cheese containing 0 (●), 1.5 (▲), or 3% (■) WPC and 0 to 0.6% of guar gum.



**Figure 5.** Overall acceptability scores of imitation cheese containing 0 (●), 1.5 (▲), or 3% (■) WPC and 0 to 0.6% of guar gum.



locust bean gum had superior appearance score than those containing xanthan or carrageenan.

It was observed (Figure 4) that presence of WPC in the imitation cheeses had a favorable influence on the sensory score for texture. These findings are in agreement with the data reported by Pinto *et al.* (2007) for the processed cheese spread. Contrary to this, the texture scores of cheeses decreased with increasing level of guar used in the formulation. Since results from TPA test indicated that samples containing more WPC and less guar were harder, it can be said that the panelists preferred cheeses with more hardness.

Statistical analysis for the imitation cheeses containing WPC and guar gum did not show any differences in the consumer preference for aroma scores, indicating that the WPC and guar did not have any effect on sensory aroma. The average aroma score of the experimental samples was  $3.5 \pm 0.07$ .

Overall acceptability of the product was not affected by different levels of WPC (Figure 5). Considering that WPC decreased color and flavor scores of the product, reduction in acceptability was expected, but was not the case. The profound positive impact of utilizing WPC on the texture score of product made the overall acceptability score remain at the desired level. Similar result was reported by Pinto *et al.* (2007) regarding the preference of imitation cheeses manufactured with or without WPC. The overall acceptability of cheeses increased with increasing level of guar used; the difference was found to be significant for samples containing guar gum at 0 and 0.6% levels only. The highest overall acceptability score (3.81-3.82) was associated with cheese containing guar gum at 0.6% level, with WPC present at 0 or 3.0% level.

## CONCLUSIONS

Production of imitation cheese based on Na-caseinate with desired functional and sensory properties is possible utilizing some

modifications in the formulation. When using WPC to substitute part of Na-caseinate at 1.5 and 3.0% levels in imitation cheese formulation, little difference in the sensory and textural characteristics was observed. WPC, when used at 3.0% level, reduced the lightness and greenness, but increased the yellowness of the product significantly compared to the control sample. Increasing the level of WPC from 1.5 to 3% led to slight decrease in the melting ability but increased the hardness of the product. Thus, it is recommended to substitute Na-caseinate with WPC at 1.5% level only, taking into account the sensory characteristics of the product. Guar gum increased the melting ability of cheese product favorably, but had a slight negative effect on the hardness of the product. Inclusion of guar gum improved the sensory and color properties of the product; guar gum is recommended to be used at a level of 0.3% in the formulation. If other polysaccharides that could form a thermoreversible and elastic gel are used in protein replacement, functional properties of Na-caseinate-based imitation cheeses might be improved. While a variety of processing methods are employed by different manufacturers to produce cheese analogues without taking into account the preference of Iranian consumers, this study could enable cheese industry to assign different batches of imitation cheese based on Na-caseinate to the most appropriate end users.

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## اصلاح خصوصیات عملکردی پنیر تقلیدی بر پایه کازئینات سدیم با استفاده از پروتئین آب پنیر و تثبیت کننده

م. حسینی، م. ب. حبیبی نجفی و م. محبی

### چکیده

صمغ گوار و کنسانتره پروتئینی آب پنیر به عنوان افزودنی های کاربردی برای بهبود ویژگی های عملکردی (سختی و قابلیت ذوب) پنیر تقلیدی بر پایه سدیم کازئینات استفاده شدند. همچنین، تغییرات در ساختار، پذیرش حسی، رنگ و بافت ناشی از این ترکیبات مورد بررسی قرار گرفت. پنیر تقلیدی با سه سطح WPC (۰، ۱.۵ و ۳ درصد) و سه سطح صمغ گوار (۰، ۰.۳ و ۰.۶ درصد) در فرمولاسیون پنیر فرموله شد. پنیرهایی با گوار بیشتر و WPC کمتر نرم تر بودند و به مقدار بیشتری ذوب شدند. استفاده از دو افزودنی غذایی در سطوح افزایشی مقدار پروتئین را بطور معنی داری کاهش داد ( $P < 0.05$ ). WPC برخلاف گوار مقدار pH را افزایش داد. رنگ پنیر بطور مثبتی تحت تاثیر صمغ گوار و بطور منفی تحت تاثیر WPC قرار گرفت ( $P < 0.05$ ). پذیرش کلی پنیر تحت تاثیر سطوح WPC نبود اما با افزایش سطح صمغ گوار استفاده شده در فرمول بهبود یافت. با در نظر گرفتن، تاثیر سوء WPC بر رنگ و قابلیت ذوب و تاثیر تا حدی منفی صمغ گوار بر سختی پنیر تقلیدی، استفاده از WPC در سطح ۱.۵ درصد و گوار در سطح ۰.۳ درصد در فرمولاسیون پنیر تقلیدی توصیه می شود.