

Production and Evaluation of a High Protein Version of Non-fat Yogurt

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Abstract: High-protein non-fat yogurts were manufactured from skim milk fortified with a combination of skim milk powder and skim milk retentate in various ratios. Different yogurt mixes with protein content ranged from 5.77% - 8.57% were prepared. Control was made from skim milk fortified only with skim milk powder to approximately 4.84% protein content. Mixes were well blended and heat treated either at 80°C for 20 min or at 90°C for 10 min, and fermented with a yogurt culture at 42°C. The effects of fortification method and heat treatment on rheological properties of yogurts in terms of apparent viscosity and firmness, as well as syneresis were investigated. The yogurt to which skim milk retentate had been incorporated exhibited; higher apparent viscosity; higher firmness and lower syneresis as well as higher protein and lower lactose content in comparison with the yogurt fortified only with skim milk powder. Yogurts from low heated mix had a lower viscosity and firmness than that from high heated mix. Sensory analysis showed that yogurts added with skim milk retentate up to 6.7% protein, particularly that subjected to a low heat treatment were evaluated as acceptable as control yogurt.

Key words: formulation, heat treatment, non-fat yoghurt, ultrafiltration.

INTRODUCTION

Consumers' interest in high protein non-fat dairy products including yogurt has increased recently. The texture and rheological properties of these products are different from normal products. In order to improve the rheological properties of these products, various fortification methods were applied to modify the milk composition used for production. Non-fat yogurt is one of the products in which milk solids must be traditionally increased to overcome the problems associated with weak body, poor texture, and excessive whey separation in the final product.

All fortification methods are primarily aimed to increase the solids content in milk for yogurt manufacture. However, fortification methods for yogurt milk increase not only the solids content, but also the protein content (casein in particular) as a percentage of milk solids. Fortification with skim milk powder (SMP) is the common practice to increase the solid content in conventional yogurt manufacture, but when enrichment the protein content is the main target, the amount of SMP that can be added to provide extra protein content becomes limited, since too high levels of SMP can lead to a powdery taste and high lactose content, which ultimately results in a highly acidic product. Moreover, the thermal degradation of proteins during SMP production can be very important in these cases, diminishing the nutritional value of the yogurt.

An alternative approach of fortification of the milk for yogurt manufacture is by ultrafiltration. Ultrafiltration has been described as an adequate technique for milk concentration and much research has been done to evaluate the rheological properties of UF skim milk concentrate^[19,3,7]. Protein concentrates produced by ultrafiltration have better nutritional value than that produced with traditional methods. Another advantage of UF milk is that it contains higher level of protein with lower level of lactose than regular milk. Therefore, UF milk has been used in the manufacture of various dairy product, including yogurt to increase protein content without substantially increasing lactose content^[5,11]. This approach eliminates defects, which can occur when SMP is used for fortification. However, UF concentration of milk for yogurt manufacture lead to an excessively firm coagulum and a more viscous product than conventional SMP fortification^[17]. This is may be the most important limitation for employing UF milk to boost the protein content in yogurt. Thus, using UF milk as an ingredient in manufacture of a high protein non-fat yogurt may require some formula adjustments for further increase of the protein content in yogurt premix to the desired level.

On the other hand, the gel strength and viscosity of yogurt are greatly influenced by the extent of heat treatment the yogurt mix receives prior to fermentation^[21,16,10,8]. The present study investigated formulation and heat treatments to enhance texture and viscosity properties of a high protein version of non-fat

yogurt utilizing SMR separated by membrane technology in combination with SMP for fortification. Previous studies have emphasized either ultra high temperature UHT (135-150°C) or little lower temperatures (100-130°C). This study evaluated the effects of moderate temperatures (80-90°C), which are commonly applied in yogurt manufacture.

MATERIALS AND METHODS

Fluid Skim Milk: Pasteurized fresh skimmed cow's milk (63°C for 30 min) was used as a stock of raw material throughout this experiment to avoid variation of milk composition.

Skim Milk Powder: Spray-dried medium heated skimmed milk powder (Mlekpól, Poland) was used in this study.

Skim Milk Retentate: Pasteurized fresh skimmed milk was ultrafiltered at 50°C to a three times volumetric concentration factor by CARBOSEP module UF-unit (Type 2 S 151 tubular, France) using zirconium oxide membrane with a total area of 6.8 m². The inlet and outlet pressures were 5 and 3 bar respectively. The resultant retentate contained 15.3 % T.S. After the concentration process, skim milk retentate was pasteurized at 63°C for 30 min, rapidly cooled to approximately 5°C, and frozen stored at - 23°C. Before use in experiments, the retentate was thawed in a water bath at 30°C for 60 min.

Preparation of Yogurt Premix: Pasteurized fresh skimmed milk was fortified with SMP and SMR in various ratios to formulate the premixes with protein content ranging from 5.77% to 8.57%. The experiment consisted of four treatments. A control mix fortified only with SMP was prepared for comparison. Yogurt mix formulations used for yogurt manufacture are listed in (Table 1). The final mixes were blended at 45°C for 5 min using a high speed blender. Samples were taken for chemical analysis.

Yogurt Manufacture: The mix of each formula was divided into two separated batches. The first batches were heat treated at 80°C for 20 min. The second were heated at 90°C for 10 min.

Each batch rapidly cooled to 42°C, and inoculated with 2% yogurt culture. Inoculated yogurt premix was dispensed into 250-mL sterilized glass containers, covered with aluminum foil, incubated at 42°C until complete coagulation. After coagulation, the containers were stored 5 ± 1°C in the cold refrigerator for 14-d. Two replicate of non-fat yogurt trials were undertaken

on two separate days. Apparent viscosity, firmness and syneresis of the yogurts were evaluated after 5 days of cold storage and sensory evaluation was carried out for yogurt samples when fresh and at the end of storage period.

Compositional Analysis: Total solids of yogurt mixes were determined by oven drying, total protein by the Semi-macro-Kjeldahl and lactose was determined with a lactose/D-galactose enzymatic BioAnalysis kit (Scil Diagnostica, Martinsried, Germany).

Apparent Viscosity: Apparent viscosity was measured with a Fungilab viscometer (model Visco star-L; GR. Scientific Ltd., Bedfordshire, UK) using spindle L4 at a low, steady shear analysis (50 s⁻¹ rpm). For each sample three readings were taken and their mean was reported. The temperature of yogurt samples was maintained at 5 ± 1°C throughout the experiment by a cooled water bath. Three samples were measured for each treatment. An average of 3 readings was calculated for each sample.

Firmness: The firmness of yogurts was measured with a Koehler penetrometer (Instrument Company, Inc., New York, USA) using the cone penetration test ASTM D217 with a 100-g shaft weight. With this test, results are expressed in millimeters. The softer the sample is then the deeper the penetrant will sink into the sample and thus the higher the penetration number will be. The depth of penetration was measured at 20 sec at a product temperature of 5 ± 1°C.

Sensory Evaluation: The sensory evaluation was carried out by a trained panel consisting of 5 judges. They were instructed about the process of evaluating the different parameters of sensory quality. The sensory evaluation of produced yoghurts was done on a 5 point hedonic scale (1- the worst; 5- the best). The following quality properties were evaluated: appearance, flavor, consistency and syneresis. Samples of yoghurt for sensory evaluation were presented in glass containers of a volume of 250-mL coded, about half an hour after being taken out of refrigerator at 20°C. The judges first evaluated the overall appearance, and syneresis in the settled gel. Next, with a spoon they gently pressed on the gel in order to assess on the firmness. The consistency of the product was also evaluated by placing spoon vertically into the product. Flow properties of the product were observed on the other side of the spoon. Then they stirred the gel with the spoon until they obtained a uniform consistency. After stirring, flavour and mouth feeling of samples were then evaluated.

Table 1: Yogurt mix formulations of high protein non-fat yogurt made from fluid skim milk fortified with SMP and SMR.

Treatment	Ingredients (%)			Composition (%)		
	FSM	SMP	SMR	T.S	Protein	Lactose
Control	95	5	0	13.40 ± 0.23 ^c	4.84 ± 0.10 ^c	7.40 ± 0.14 ^a
1	76	4	20	13.82 ± 0.17 ^d	5.77 ± 0.08 ^d	6.76 ± 0.10 ^b
2	57	3	40	14.24 ± 0.26 ^c	6.70 ± 0.12 ^c	6.12 ± 0.18 ^c
3	38	2	60	14.66 ± 0.31 ^b	7.64 ± 0.05 ^b	5.48 ± 0.03 ^d
4	19	1	80	15.08 ± 0.12 ^a	8.57 ± 0.04 ^a	4.84 ± 0.12 ^c

Mean values of duplicate experiments. Mean values with the same superscript letter in the same column are not significantly different ($P < 0.05$).

Statistical Analysis: Experiments were replicated. Results of yogurt mixes composition were submitted to ANOVA using the general linear model (GLM) procedure of SAS. Mean comparison was conducted using the technique of Duncan, where $P < 0.05$. Correlations between the protein content and each of gel firmness and viscosity of yogurts were also computer calculated.

RESULTS AND DISCUSSION

Chemical Composition: The mean values of chemical composition for yogurt mixes are shown in Table 1. Significant differences ($P < 0.05$) among yogurts mixes as to T.S, protein, and lactose contents were observed. The incorporation of SMR in the manufacturing of yogurt has contributed to the increase of the T.S content in favour of protein and to the decrease of the lactose content of the yogurt mixes, because of the chemical composition of retentate. The maximum protein content was 8.57%; corresponding to 15.08% total solids. This was equivalent to approximately 56.8% protein on the basis of dry matter compared with 36.1% for the control.

Apparent Viscosity: The apparent viscosity of yogurt samples is shown in (Fig.1). Differences in the apparent viscosity were observed among different yogurt samples. These differences might be attributed to the differences in protein content associated with differences in heat treatment. As expected, yogurts made with SMR added had in all cases a higher viscosity than SMP fortified yogurt (control). The higher the SMR added to yogurt premix, the higher was the viscosity. Many authors reported significant higher gel firmness and viscosity in yogurts produced from UF milk, than in yogurts produced by addition of milk powder^[2,3,2,15,4].

Becker and Puhon^[2] demonstrated that UF results in firmer non-fat yogurts with higher viscosity than those prepared to the same SNF content by addition of SMP. They attributed the lower firmness for yogurt

with SMP to the partial denaturation of the whey proteins during the manufacture of milk powder. Savello and Dargan^[15] found that the greater gel strength and viscosity of UF yogurts occurred despite the higher average total solids in the SMP yogurts (12.98% vs. 11.43%). Recent studies also reported that addition of dairy ingredients, which increases the dry matter content, increased the rheological parameters of fermented milk. The texturing capacity was directly related to the dairy ingredient protein content. It was higher for milk protein concentrate than for skim milk powder^[18,6].

In yogurt sample from mix of 8.57% protein content, the influence of the higher protein content of yogurt was particularly evident. The higher protein content, in the case of this sample, resulted in a too viscous product, with a firmer texture resembling a fresh cheese. Similar results were found by Alvarez *et al.*^[11] and Magenis *et al.*^[11] who found greater viscosity of yogurts with higher protein content.

As illustrated in Fig. (1), the differences in viscosity become more pronounced when the viscosity is related to the heat treatment for premix prior to fermentation. Heat treatment of yogurt mix at 90°C for 10 min caused almost higher viscosity compared to mix treated at 80°C for 20 min. High heat treatment of the yogurt mix increases the hydrophilic properties of the coagulum and the stability of the yogurt gel due to the denaturation of whey proteins and association with k-casein. Similar observations were reported by Thomopoulos *et al.*^[21] who concluded that, preheating of skim milk fortified either with skim milk powder or milk protein concentrate at 95°C for 5 min gave more viscous yogurts than that treated at 65°C for 15 min.

The viscosity of yogurts from high heat treated mix were higher than those of yogurt from low heat treated mix at the same levels of protein. The 8.57% protein content mix yogurts, for each heat treatment, exhibited the highest viscosity, whereas the 4.84% protein content low heated yogurt mix had the lowest viscosity. Yogurt from high heat treated mix with %8.57 protein had the highest viscosity. Similarly, for

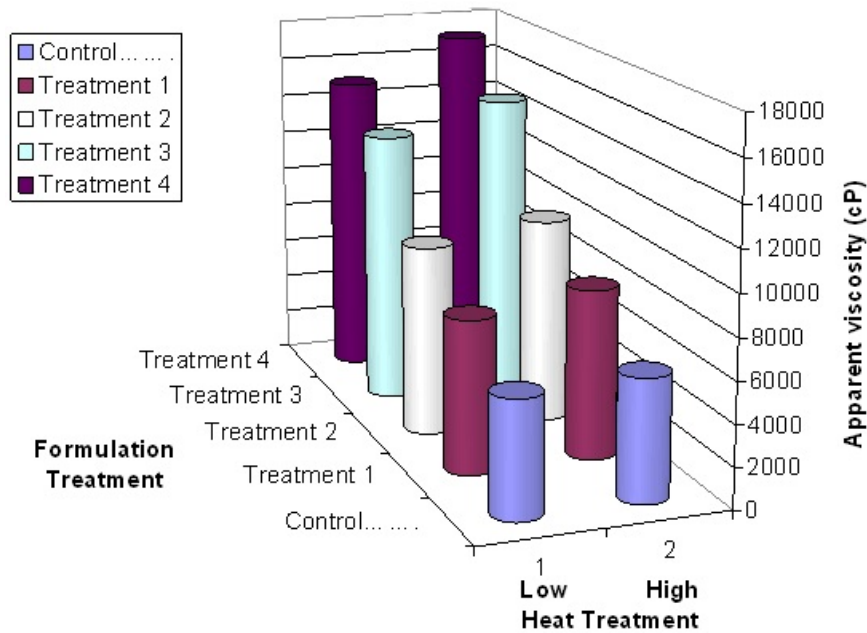


Fig. 1: Apparent viscosity of high-protein non-fat yogurts fortified with a combination of SMP and SMR with respect to heat treatment.

the yogurts made from low heat treated mix, the viscosity of the 8.57% protein sample was the highest. The viscosity of yogurt from low heat treated mix with 8.57% protein content was similar to that of high heat treated mix with 7.64% protein content.

Firmness: Incorporation of SMR in yogurt premix contributed to the increase of gel firmness when compared with the control yogurt fortified only with SMP (Fig. 2). Each incremental increase in SMR required significantly greater force to penetrate the yogurt gel, reflecting the effect of increased milk protein content (casein in particular) on gel firmness. The higher protein content, the higher the firmness of yogurts. The reason for the somewhat lower firmness for yogurt fortified only with SMP, compared with that made with SMR added may be due to the partial denaturation of the whey proteins during the manufacture of milk powder. Although TS content did not greatly vary among experimental yogurts, protein content was greatly higher in yogurts with SMR added which may have resulted in a higher firmness in these yogurts. Increasing the total solids, specially milk protein, in yogurt increases the density of the gel network and reduce pore size. Consequently, water is bound more firmly in the product, increasing the firmness of the yogurt. White ^[24] reported that increased protein content in yogurt resulted in an increase in the level of bound water (water of hydrated proteins) and led to firm and viscous yogurts.

The firmness of yogurt is dependent on T.S content and on the protein content of the product as well as on the type of protein^[2,12,5,22,13,14,8].

Oliveira *et al.*^[13] found lower firmness of the fermented milk enriched with whey. Puvanenthiran *et al.*^[14] reported that decreasing the casein : whey protein ratio in milk destined for yogurt manufacture by substituting whey protein concentrated caused a lower firmness of the final yogurt. Magenis *et al.*^[11] reported that the addition of whey retentate to milk retentate in the manufacturing of yogurt had contributed to lower firmness and viscosity than yogurt manufactured only with milk retentate. They suggested that the lower protein content of the product and the type of protein of the whey retentate may be responsible for the decrease in firmness of yogurt.

In addition, it can be seen also that, at the same level of protein, yogurts from high heated mix had higher firmness than those of the corresponding low heated ones (Fig. 2). This may be due to the difference in the extent of whey protein denaturation in yogurt premix for each type of heat treatment. The 8.57% protein content mix yogurts, for each heat treatment, exhibited the highest firmness, whereas the 4.84% protein content low heated yogurt mix had the lowest firmness. As the viscosity, the firmness of yogurt from low-heat treated mix with 8.57% protein content was close to that of 7.65% protein from the high-heat treated mix.

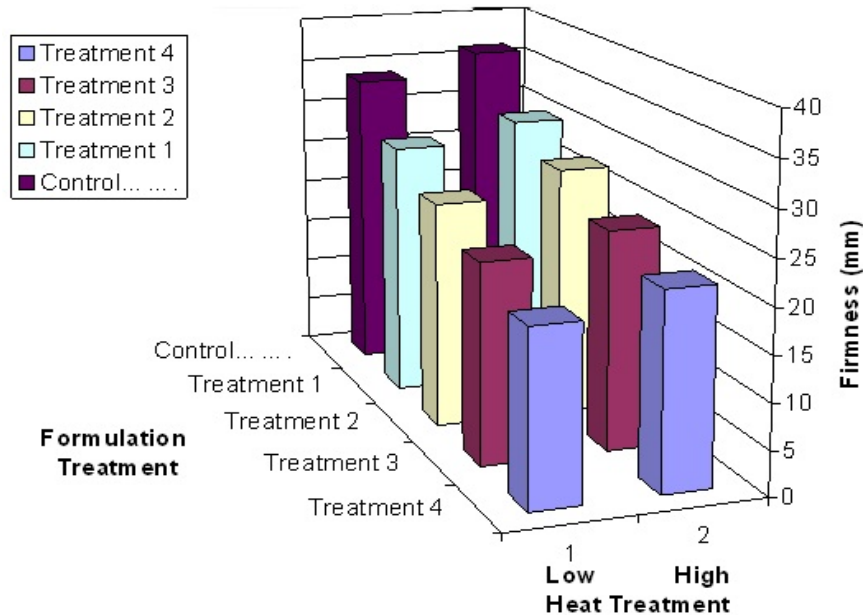


Fig. 2: Firmness values of high-protein non-fat yogurts fortified with a combination of SMP and SMR with respect to heat treatment
-The lower the value, the firmer the texture

Correlation analysis of gel firmness and viscosity values at different levels of protein with respect to heat treatment used, showed a linear fit. The equations describe the relationships were computer calculated and displayed on the graphs as shown in Fig. 3. Apparent viscosity and firmness in this study were highly correlated with the protein level. The correlation coefficient values (R^2) approach 1. This indicates that the protein content is one of the responsible factors for the firmness of the product, increasing the viscosity dramatically.

The positive relationship between the milk base protein content and the yogurt texture has been emphasized by other authors^[9,18,8].

Syneresis: Yogurts with added SMR displayed minimal free whey, whereas the control yogurt was criticized for whey separation. This behaviour may be attributed to the higher solids content particularly protein content of yogurt made with SMR^[16,20]. The addition of milk protein to yogurt through the incorporation of SMR in its formula may also have contributed to a decrease in syneresis. SMR serves as a stabilizer in nonfat yogurts to improve texture and reduce whey separation. It has been shown in other studies that yogurts prepared with casein-supplemented milk had low whey separation because of water binding by casein. Savello and Dargan^[16] underlined that concentration of milk for yogurt with UF significantly diminishes susceptibility of yoghurt to syneresis.

Sensory Evaluation: Yogurt produced from mix with 6.7% protein received the best flavour score among the experimental yogurts when evaluated freshly and after 2 wk of storage. This treatment also was judged by the sensory panel to have proper firmness and viscosity, which was indicated by the rheological properties. Treatments made from mixes with higher protein levels were regarded as too firm and lacked flavour and developed bitterness after 2 wk of storage. These treatments contained lower lactose (Table 1) and consequently, had a lower flavour development than control. The major criticism for the control and high SMP level supplemented yogurts after 2 wk of storage was the presence of too much acid. In yogurts with higher SMR, acid production could be controlled because lower initial lactose levels. The best results of all the experiments made were those corresponding to yogurt sample, which obtained when 3% SMP combined with 40% SMR in yogurt mix to achieve 6.7% protein and the mix was subjected to 80°C for 20 min prior to fermentation.

Conclusion: High protein-nonfat yogurt can be made from skim milk fortified with SMP, and SMR. Additional stabilizers are not needed to prevent whey separation, which is the common defect in non-fat yogurts. To produce acceptable high protein non-fat yogurt fortified with SMR, the protein content must be not more than 6.7% in the yogurt mix for proper viscosity and firmness. When the protein content was

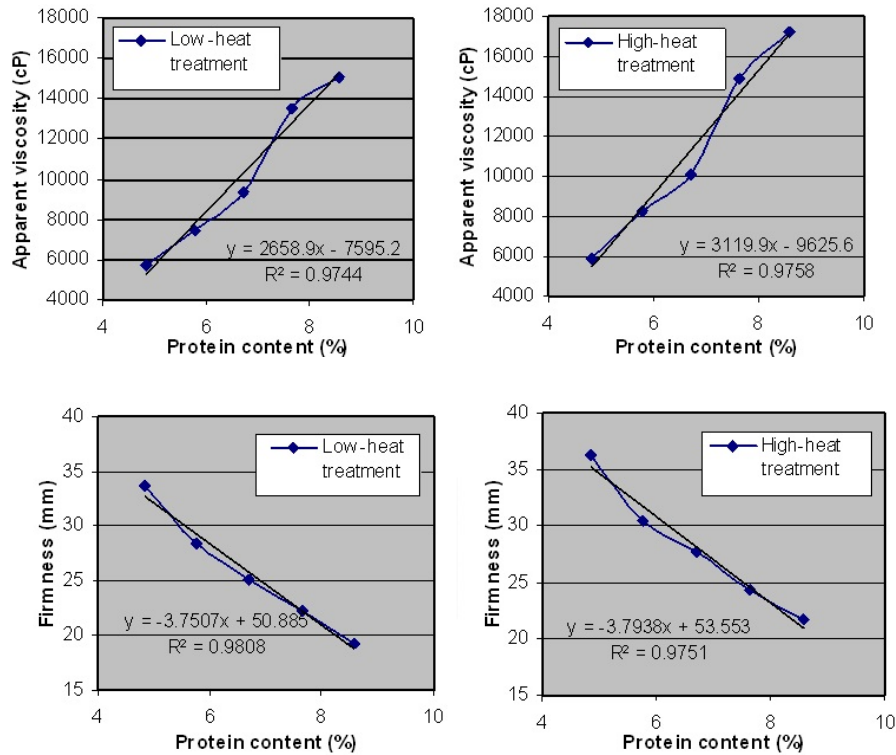


Fig. 3: Correlation between protein content and both of viscosity and firmness means of high protein non fat yogurts with respect to heat treatment

increased, the firmness and viscosity of yogurt increased. Less viscous and firmer coagulum resulted from low-heat treatment compared with high-heat treatment. At the same level of protein, the firmness of low heated mix yogurt maintained lower than the high heated yogurt. Lower heat treatment prior to fermentation could create a texture more similar to traditional products. However, at the highest protein level (8.57%) used in this study, the firmness and viscosity of the low-heat treated mix yogurt were still too high. Using of SMR together with SMP in fortification of non-fat yoghurt can assist product developers working with UF skim milk to optimize high protein-nonfat yogurt textural properties and create body and texture more similar to traditional products. These can be used also to minimize the need for milk powder addition. That means that a non-fat yogurt with high protein content can be obtained from skim milk by controlling the SMR and SMP formulation and subjecting to heat treatment at 80°C /20 min prior to fermentation. Therefore, SMR could be combined with SMP to enrich the protein content without depressing yogurt quality.

Abbreviation Key: FSM = fluid skim milk, SMP = skim milk powder, SMR = skim milk retentate

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