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THE EFFECT OF PROCESSING TEMPERATURES ON THE MICROSTRUCTURE AND FIRMNESS OF LABNEH MADE FROM COW'S MILK BY THE TRADITIONAL METHOD OR BY ULTRAFILTRATION

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Abstract

The types of Labneh were made from full-fat cow's milk: (a) traditional Labneh was produced by straining cold yoghurt at 7°C in a cloth bag, and (b) UF Labnehs were produced by ultrafiltration (UF) of warm yoghurt at 35°, 40°, 45°, 50°, and 55°C. The UF Labnehs contained 22.7-23.9% total solids, 7.8-8.3% protein, and 10.6-11.3% fat as compared to 25.3%, 9.1%, and 11.9%, respectively, in traditional Labneh.

Homogenization of the experimental Labneh samples in an ALM homogenizer using the D-170 or D-280 heads made the products smoother than unhomogenized Labnehs. Scanning electron microscopy revealed that the largest and least uniform pores were present in traditional unhomogenized Labneh, where the protein clusters were relatively compact. Homogenization reduced the dimensions of the large pores and opened the structure of the protein clusters.

Ultrafiltration of Labneh at elevated temperatures of 35° to 55°C resulted in an increase in the dimensions of the casein particles forming the protein matrix of the Labneh, evidently as the result of extended fermentation. Formation of complex casein particle chains, as observed by transmission electron microscopy, was associated with increased firmness of Labneh samples concentrated at temperatures above 45°C.

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Introduction

Labneh (yoghurt concentrated to 22% total solids contents) can be produced in various ways (Tamime *et al.*, 1989a, 1989b, 1991a; Salji, 1991). It is manufactured mainly by concentrating plain yoghurt, *i.e.*, yoghurt which is not sweetened or fortified. This type of yoghurt is made from full-fat milk prewarmed to 60°C, homogenized at 17 MPa, heated to 90°-95°C for 5 min, and cooled to 42°C. The milk is subsequently inoculated with a mixed starter culture consisting of, *e.g.*, *Streptococcus salivarius*, subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, and is incubated at 42°C. The resulting yoghurt is cooled to 5°-7°C.

Traditional Labneh is produced by using a cloth bag for straining the cold yoghurt. This method of manufacture is not suitable for large processing dairy plants because it: (a) needs a long processing time (2-3 days), (b) is labor-intensive, (c) requires a large floor space under refrigeration, (d) is unhygienic, and (e) gives a low yield.

More recently, the traditional method of manufacture has been replaced by mechanized processes where the warm yoghurt is concentrated directly after the fermentation to the desired level of solids by using a mechanical separator or by ultrafiltration (UF).

The applications of UF technology in the dairy industry have been reviewed by Glover (1985) and Cheryan (1986). In brief, some of the factors which can influence the performance of UF membrane when ultrafiltering full-fat milk, skim-milk, or whey are: (a) operating pressure and temperature, (b) flow velocity over the membrane, (c) degree of fouling of the UF membrane, and (d) level of concentration. A wide range of studies on UF of milk and whey have been published (Glover, 1985; Cheryan, 1986; Renner and Abd El-Salam, 1991), but only limited data are available on UF of warm yoghurt for the manufacture of Labneh.

The objectives of this study were to manufacture Labneh from full-fat milk by the traditional method and by ultrafiltering the fresh warm yoghurt at temperatures ranging from 35° to 55°C. Effects of these processing conditions on the microstructure of the individual Labnehs were also studied.

Materials and Methods

Preparation of Labneh

Full-fat cow's milk was obtained from the farm of the Scottish Agricultural College, Auchincruive, in November 1988 and August 1989. This milk was used for the production of Labneh (traditional and UF) as described by Tamime *et al.*, 1989b). The manufacturing stages are shown in Fig. 1.

The specifications of the ALM homogenizer and the Alfa-Laval pilot scale UF plant (type PM2-50 series No. 6 PL 1256S membranes having a total filtering area of 1.3 m² and the molecular mass cut-off of 50 kD) have been described by Tamime *et al.* (1989a, 1989b, 1991a). Portions of each type of Labneh were smoothed in the ALM homogenizer using the D-170 or D-280 homogenizer heads.

The milk was fermented by using freeze-dried thermophilic lactic acid bacteria (MYO-87, Marschall-Eurozyme Ltd., Cheshire, UK) consisting of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus*.

Microscopic Analysis

The Labnehs were sampled using a glass tube, 7.0 mm in diameter. Sample columns, approx. 10 mm long, were fixed in a 2.8% glutaraldehyde solution and mailed to Ottawa, Canada, for electron microscopy (Allan-Wojtas, 1984). After arrival, the yoghurt columns were prepared for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) according to the methods described by Tamime *et al.* (1991a).

For SEM, the fixed Labneh columns were cut into prisms, 1x1x10 mm, and dehydrated in a graded ethanol (20, 40, 60, 80, 95, and 100%) series, defatted in chloroform, and returned into ethanol. Then the samples were frozen in Freon 12 at -150°C and freeze-fractured under liquid nitrogen. The frozen fragments were thawed in absolute ethanol and critical-point dried from carbon dioxide. The fragments, sputter-coated with gold, were examined in an ISI DS-130 scanning electron microscope operated at 20 kV.

For TEM, the Labneh samples were cut into 0.5 mm cubes, postfixed in a 2% osmium tetroxide solution in a 0.05 M veronal-acetate buffer, pH 6.75, and embedded in medium hard Spurr's low-viscosity medium (J. B. EM Service, Inc., Pointe Claire-Dorval, Quebec, Canada). Sections, approx. 90 nm thick, were stained with uranyl acetate and lead citrate solutions and examined in a Philips EM-300 transmission electron microscope operated at 60 kV.

Chemical Analysis

The total solids, fat, protein, and ash contents in different milks and Labnehs were determined as described by Tamime *et al.* (1987).

Firmness Analysis

A Stevens LFRA Texture Analyzer (C. Stevens & Son Ltd., Hertfordshire, UK) equipped with a cylindrical probe (type TA3-TFE 105-504), 25 mm in diameter and 35 mm long, was used to measure the firmness of Labneh. The probe penetrated the samples to a depth of 15 mm at a speed of 0.5 mm/s and the force exerted on the probe was recorded. Averages of two readings of Labneh samples were taken at 7°C in each trial and the results were expressed in newtons (N).

Results and Discussion

The Labnehs under study were made in November 1988 and August 1989 from two batches of full-fat milk. On an average, these batches contained 12.4% total solids, 3.2% protein, 4.0% fat, and 0.7% ash (Table 1).

Composition

The composition of Labnehs is summarized in Table 1. The total solids losses in the permeate and the cloth bag filtrate ranged between 5.3 and 5.8% (Tamime *et al.*, 1991b), and these values were similar to the data reported by Tamime *et al.* (1989b) and Mahdi (1990). The solids losses in the permeate/filtrate consisted mainly of lactose, organic acids, and minerals.

It is evident from the data shown in Table 1 that the total solids, including the fat and protein contents, have been increased in all Labnehs. The concentration factors of the protein and fat in UF and traditional Labnehs were in the range of 2.4 to 2.8 and 2.7 to 3.0, respectively. These slight variations depended on the chemical composition of the original milk (*i.e.*, seasonal variation - Walstra and Jenness, 1984) and the degree of concentration of Labneh. Similar figures were reported by Tamime *et al.* (1989b), but lower figures have been reported by El-Samragy and Zall (1988) for the production of Labneh from ultrafiltered milk (21% total solids).

The highest values for total solids, protein, and fat were found in traditional Labneh (25.3%, 9.0%, and 11.8%, respectively) whereas slightly lower figures were found in UF Labnehs made at different temperatures (22.6-23.9%, 7.8-8.3%, and 10.6-11.3%, resp.). The pH of all the Labnehs after refrigeration fluctuated within a very narrow range of 4.17 to 4.23 (Table 1).

Firmness

The firmness of different types of Labneh is shown in Table 2 and the results suggest that the rheological property of the product was influenced by: (a) method of production (traditional vs. UF), (b) processing temperature during ultrafiltration, and (c) passage of the product through the ALM homogenizer including the different types of homogenizer heads used.

The firmness of UF Labnehs, which were not homogenized, ranged between 1.7 and 3.9 N (Table 2) despite the fact that the total solids contents were within a very narrow range of 22.7 to 23.9% (Table 1). The duration of concentration and the differences in the firmness readings of UF Labnehs depended on the processing temperature. By increasing the temperature of ultrafiltration from 35° to 55°C, the firmness of UF Labneh was practically doubled (Table 2) and the processing time was halved (Tamime *et al.*, 1991b). The higher firmness readings of UF Labneh concentrated at 55°C could be attributed to partial agglomeration of the casein particles similar to what happens to yoghurt when heated after the fermentation stage in the absence of hydrocolloids (Tower, 1984; Foley and Mulcahy, 1989).

Firmness of traditional Labneh (3.3 N) was very close to Labnehs ultrafiltered at 50° and 55°C. Passage of all the Labnehs through the ALM homogenizer using the D-170 head resulted in marked decrease in firmness when compared with the use of the D-280 head (Table 2). The latter

Cow's Labneh Made at Different Temperatures

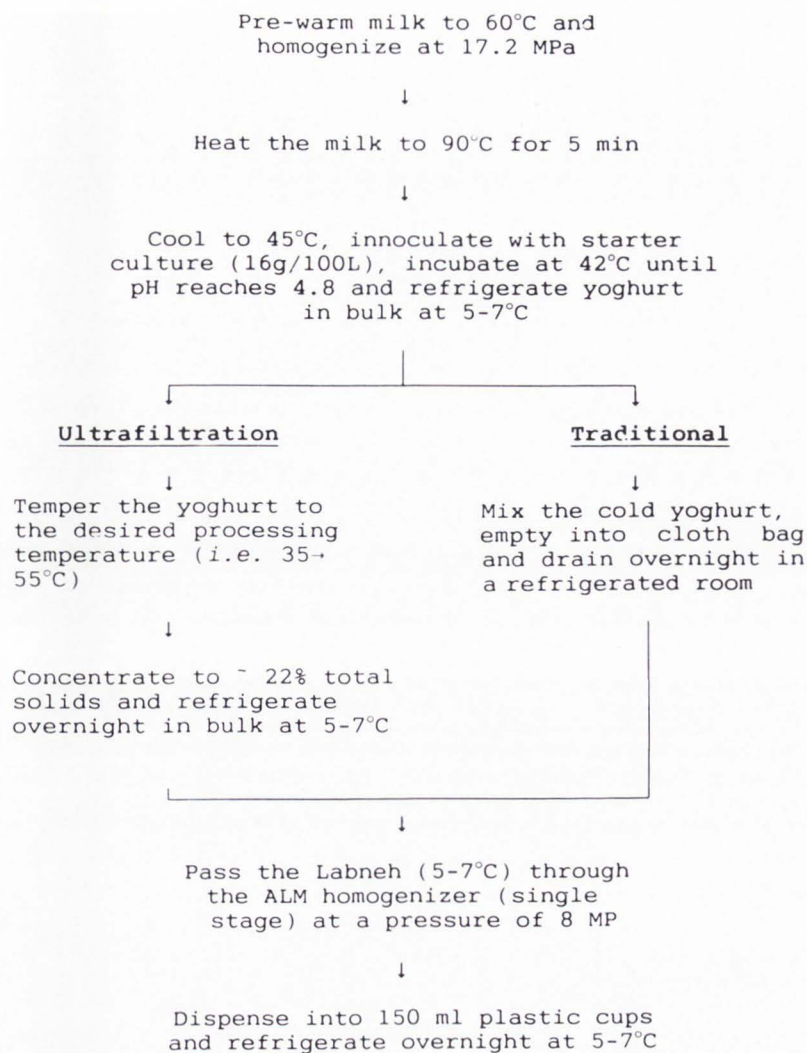


Fig. 1. A generalized scheme illustrates the production of Labneh (traditional procedure and ultrafiltration).

homogenizer head had a slightly less detrimental effect on the Labnehs because it contains fewer restrictions or grooves, and as a result causes a less turbulent flow.

The results of the firmness of UF Labneh are influenced by the chemical composition of the product and the processing conditions (e.g., UF temperature, homogenization of the product and type of homogenizer head used). The data shown in Tables 1 and 2 were analyzed statistically using the Gen-Stat 5 computer program (copyright Lawes Agricultural Trust). The interaction of some of the parameters that showed any degree of significance regarding the firmness of the product could be summarized as follows:

No homogenization vs. homogenization using head D-170 ($P < 0.01$);

No homogenization vs. UF temperature ($P < 0.001$);

Homogenization (head D-170) vs. total solids ($P < 0.01$);

Homogenization (head D-170) vs. fat ($P < 0.05$).

The role of fat in the development of firmness of Labneh (total solids vs. fat, $P < 0.01$) could be partially explained by the microstructure of the product where homogenized fat globules are embedded in the protein matrix (Tamime *et al.*, 1989a) in contrast to cheese curd where unhomogenized fat globules are not part of the matrix (Gavarić *et al.*, 1989). Another explanation may be the effect of concentration by ultrafiltration. The yoghurt is subjected to pressure as it passes through the UF module causing a similar effect on the fat globules as homogenization. Since a high degree of significance is shown between

Table 1. Chemical composition (% w/w) of milk and Labneh processed at different temperatures¹

Product/ proc. temp. (°C) ²	Total solids	Protein	Fat	Ash	pH
Milk	12.40	3.21	3.95	0.74	ND
UF Labneh					
55	23.95	8.16	11.33	0.70	4.17
50	22.83	7.80	10.59	0.69	4.21
45	23.48	8.18	11.17	0.67	4.23
40	22.65	7.82	10.78	0.70	4.23
35	23.10	8.31	10.89	0.69	4.21
Traditional					
7	25.26	9.08	11.87	0.63	4.23

¹ Results are the means of two trials, whereby each sample was analyzed in duplicate.

² Processing temperature during the production of Labnehs.

ND: Not determined.

Table 2. Firmness (N) of different Labnehs¹

Labneh/proc. temp. (°C) ²	Un- homogenized	Homogenized ³ D-170	D-280
Ultrafiltration			
55	3.9	1.1	1.4
50	3.0	0.8	1.6
45	2.6	0.8	1.6
40	2.2	0.7	1.1
35	1.7	0.7	1.3
Traditional			
7	3.3	1.9	2.7

¹ Results are the means of 2 or 3 readings at 7°C in each of 2 parallel trials using the Stevens-LFRA texture analyzer.

² Processing temperature during the manufacture of different Labnehs.

³ Type of head used with the ALM homogenizer. (Head D-280 caused a less turbulent effect than head D-170).

the UF temperature and the firmness of unhomogenized Labneh, the regression response (adjusted $R^2 = 79.2\%$) is illustrated in Fig. 2.

Structure

At low magnifications, SEM showed all Labneh samples to have smooth microstructures. The microstructures were similar to each other irrespective of the ways in which the Labnehs were produced or whether they were homogenized or not. The only minor difference was the appearance of the freeze-fracture planes at very low magnification. These planes were smooth in most samples except the Labneh samples ultrafiltered at 35° and 50°C and homogenized using the D-280 head, and the samples ultrafiltered at 40°C which had not been homogenized. The microstructure of

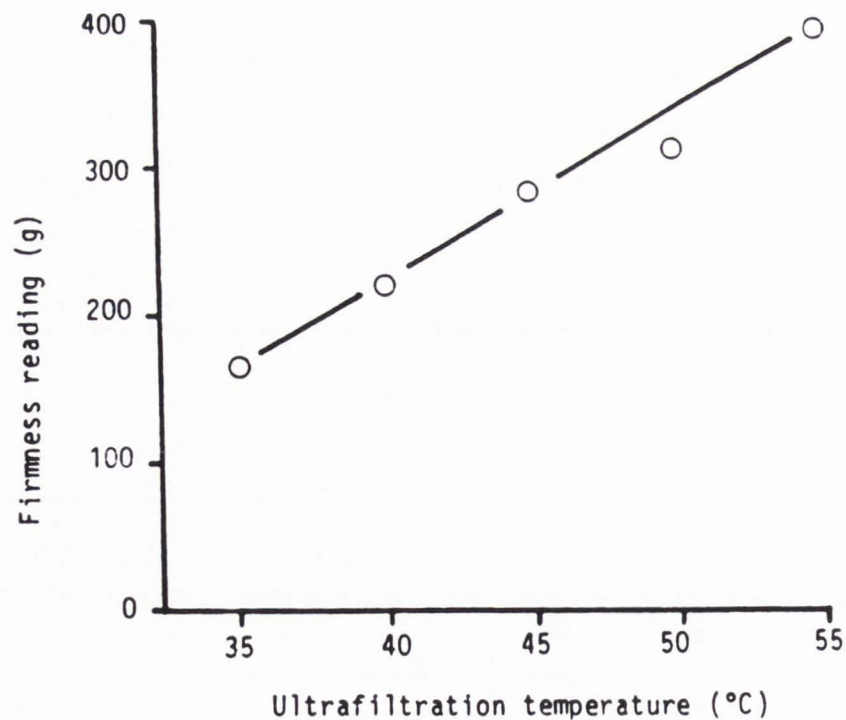


Fig. 2. Relationship between firmness of unhomogenized UF Labneh and concentration temperature.

The results are the means of two trials. Adjusted regression response $R^2 = 79.2\%$.

the protein matrices, however, was uniform in all the Labneh samples.

Long casein micelle chains, seen at higher magnification to form the structure of Labneh, confirmed that they originated in yoghurt (Kaláb *et al.*, 1975, 1983; Harwalkar and Kaláb, 1981; Parnell-Clunies *et al.*, 1987). There were, however, marginal differences which may be explained as follows: (a) partial removal of the liquid phase, which increased the total solids in the products, made the protein matrices denser (Fig. 3). Compared to the 3.21% protein content in the original yoghurt milk, the Labnehs under study contained between 7.8 and 9.1% protein (Table 1); (b) homogenization reduced the dimensions of the void spaces in the Labnehs, particularly those surrounding bacterial colonies (described by Harwalkar and Kaláb, 1986), and redistributed the bacteria.

Whereas the dimensions of large void spaces between clusters were reduced by homogenization, the initially compact protein clusters became slightly more open (Figs. 4a and 4b). Overall, the void spaces (pores) were more uniform in homogenized Labnehs.

Fat/protein and protein/protein interactions were evident in the different Labneh samples examined by TEM (Figs. 5, 6, and 7). Minute fat particles can be seen embedded in the protein particles and, as a result, are integrated in the Labneh matrix. A similar observation was reported by Tamime *et al.* (1991a). The protein chains appear longer and more complex in UF Labneh ultrafiltered at 55°C than at 35°C (Figs. 5 and 6) as compared to simple chains in traditional Labneh (Fig. 7). Increased dimensions of the casein particles in yoghurt fortified with sodium caseinate have been reported by Modler and Kaláb (1983) and Tamime *et al.* (1984) who attributed them to a higher casein concentration in the yoghurt milk. In the present study, the protein content of UF Labnehs ranged between 7.8 and

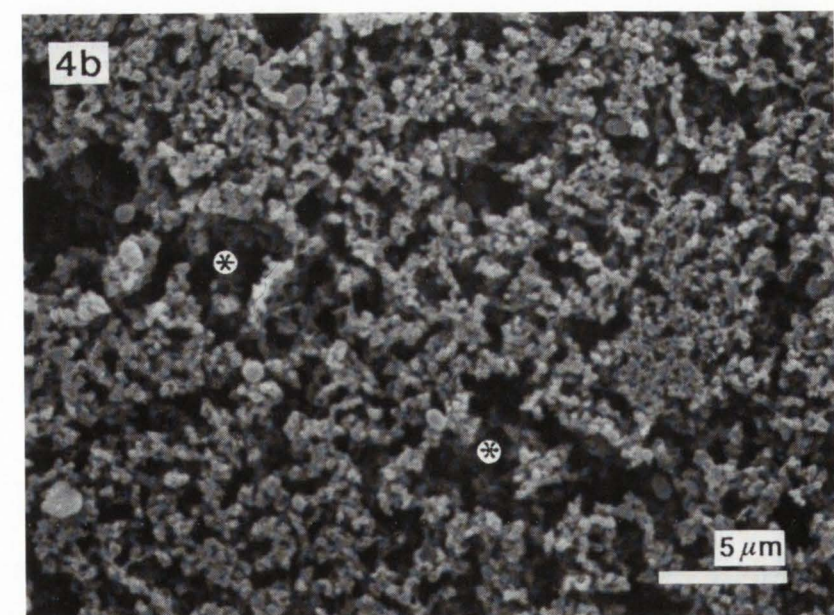
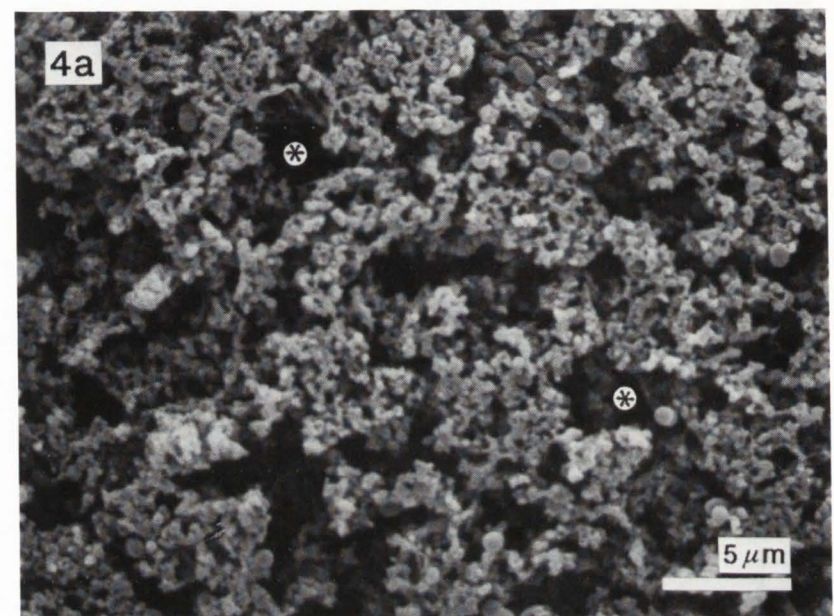
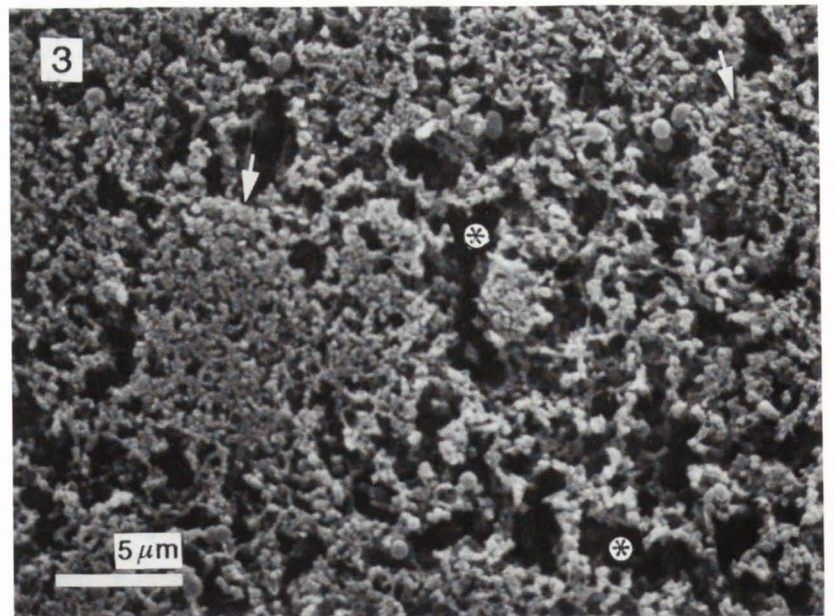


Fig. 3. Unhomogenized traditional Labneh consists of relatively compact protein clusters (arrows) and void spaces (asterisks).

Fig. 4. The sizes of void spaces (asterisks) in unhomogenized UF Labneh ultrafiltered at 55°C (Fig. 4a) are slightly larger than in the more open structure of the same product after passage through the ALM homogenizer using head D-170 (Fig. 4b).

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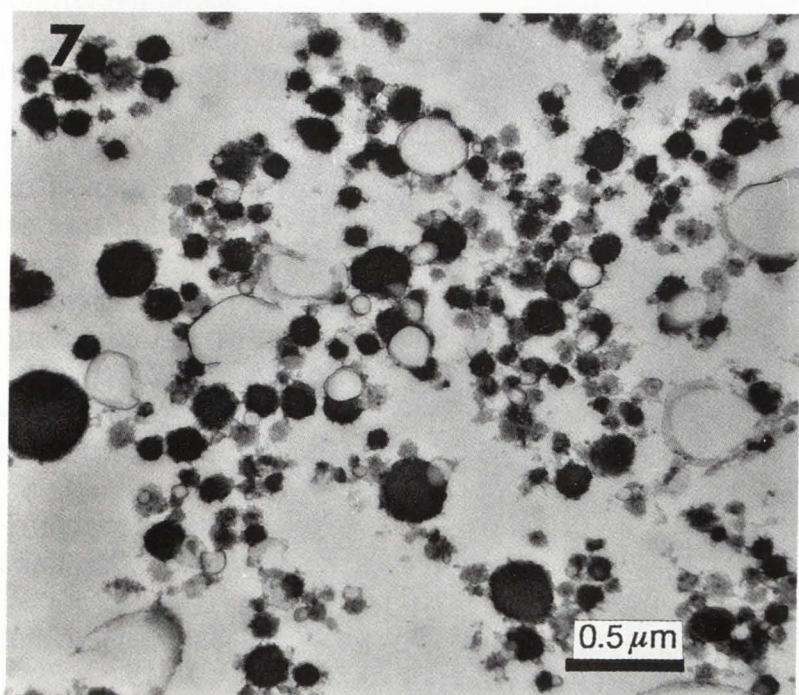
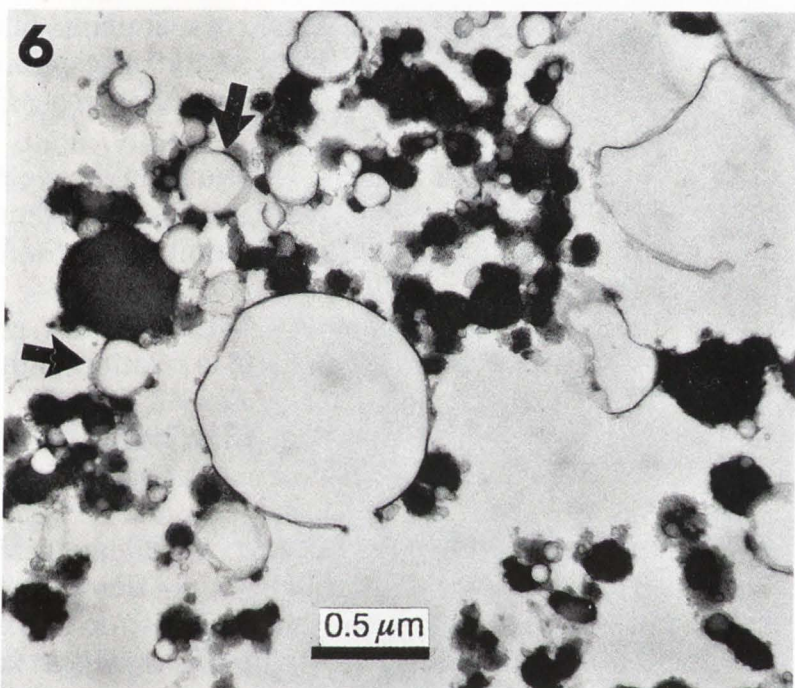
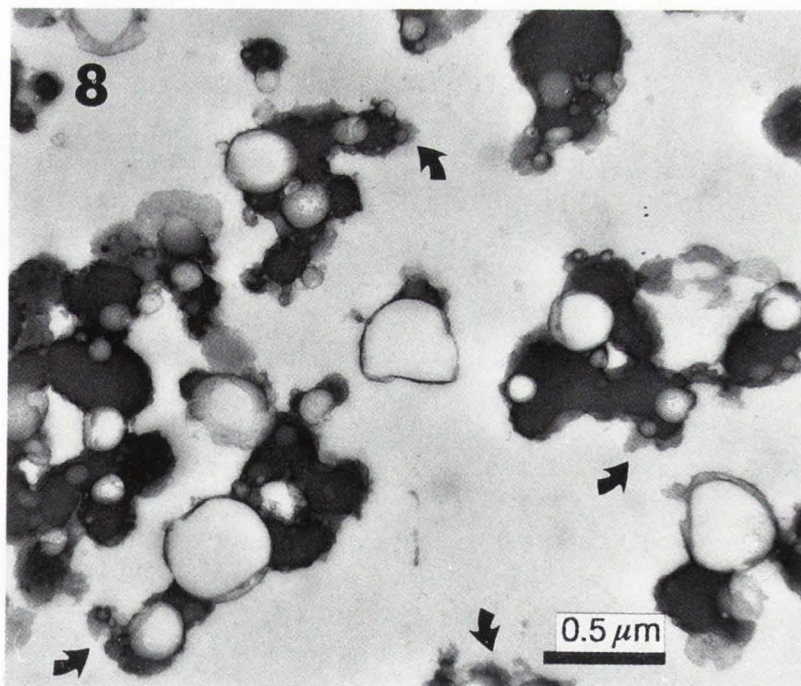
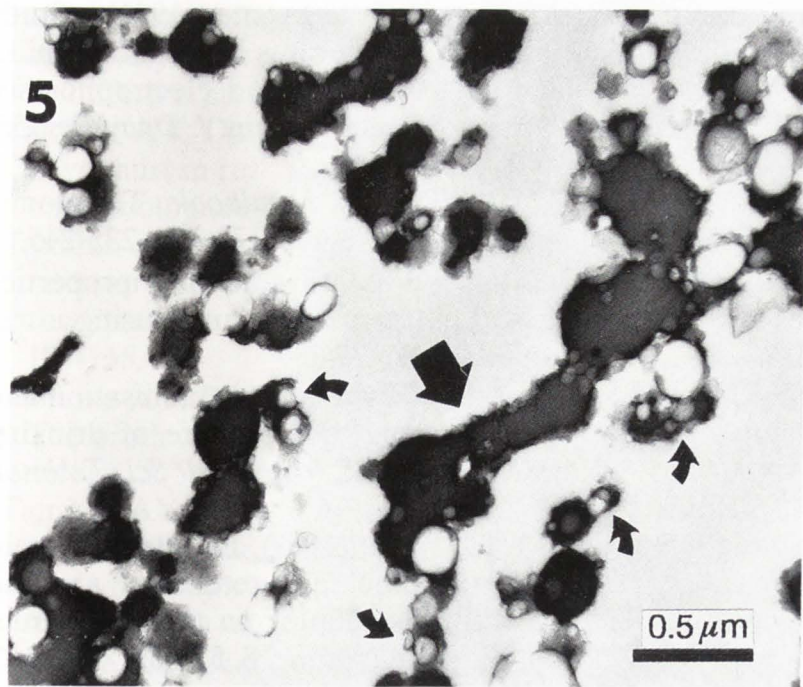


Fig. 5. Protein structure (large arrow) of unhomogenized UF Labneh concentrated at 55°C contains embedded minute fat globules (small arrows).

Figs. 6 and 7. Unhomogenized Labneh made by ultrafiltration at 35°C (Fig. 6) and the traditional procedure (Fig. 7). TEM shows simple aggregates of protein particles (dark structures) as compared with Fig. 5. Fat globules droplets (arrows) are associated with the casein particle chains.

Fig. 8. Casein particles in UF Labneh concentrated at 55°C and subsequently homogenized (ALM homogenizer, head D-170) have appendages (small arrows) projecting irregularly from the surface.

8.3% (Table 1). The complex micellar chains (Fig. 5) were influenced mainly by processing conditions (e.g., ultrafiltration at 55°C). The occurrence of a simple micellar structure in UF and traditional Labnehs (Figs. 6 and 7 as compared with Fig. 5) could be the result of using a lower temperature such as 35°C or 7°C during the concentration stage. The differences in the structure of Labnehs made by the traditional procedure and the UF at 35°-55°C can be attributed to the fact that the starter microorganisms continued fermentation in the yoghurt during ultrafiltration and, thus, this structure is 'more advanced' (compare Figs. 3 and 4a). The formation of complex micellar chains shown in Fig. 5 could be attributed to agglomeration of the casein particles in fermented milk subjected to heating (see the Firmness section).

The differences in the structural appearances of the micellar chains of UF Labnehs viewed by TEM suggest that there is a relationship between firmness and the temperature at which the Labnehs were concentrated (Table 2 and Fig. 2): UF Labnehs concentrated at 55°C were firmest (3.9 N) and had more complex micellar chains than other Labnehs, in particular the Labneh concentrated at 35°C, which was the softest (1.7 N). However, the differences in the firmness of UF Labnehs (35° and 45°C) and traditional Labneh could be attributed to processing conditions (i.e.,

concentration under pressure vs. gravitational concentration) despite the similarities of their micellar chain structure.

No major differences in the structure of all Labnehs could be observed before or after passage through the ALM homogenizer. However, homogenization improved their overall appearance possibly due to the formation of appendages at the surface of the casein particles (Fig. 8). According to Mottar *et al.* (1987, 1989), casein particles in fermented milk heated to 100°C retained their smooth uninterrupted contours as viewed by TEM. When the same fermented milk was sonicated, the casein particles became ornamented with appendages which projected irregularly from the surface and there was no evidence of fusion between the appendages from adjacent casein particles. Thus, sonication of heated fermented milk increased the hydrophobicity of the casein particles and improved the appearance of the product due to changed spatial configuration, *i.e.*, the formation of appendages at the surface of the casein particles. It is possible to suggest that the firmness of UF Labneh concentrated at >50°C and the effect of the ALM homogenizer may have caused similar changes in the micellar casein structure to those reported by Mottar *et al.* (1987).

In conclusion, there were marked differences in the firmness of UF Labnehs ultrafiltered at different temperatures in spite of their similar compositions. Labnehs made by ultrafiltration (50° and 55°C) and by the traditional method were the firmest. The ALM homogenizer, used to smoothen the products, markedly decreased the firmness; if homogenization is carried out, it is recommended to use the D-280 head in order to minimize the changes in firmness.

Ultrafiltration carried out at elevated temperature (>45°C) increases the fouling rate of the UF membranes (Attia *et al.*, 1991a, 1991b) which may affect the processing conditions in large-scale operations where the equipment requires to be washed more frequently. However, ultrafiltration of yoghurt at such high temperatures was successfully used to produce Labneh within the shortest processing time of all the manufacturing methods and the firmness of the product was similar to traditional Labneh.

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Discussion with Reviewers

R. W. Martin: The correlation of temperature with the firmness of Labneh is well illustrated in this work. How do you think homogenization pressures variation would affect firmness?

Authors: The ALM homogenizer used operates at one pressure (8 MPa). Using different heads consisting of different restrictions alters the turbulent flow of the product through the homogenizer. As illustrated in Table 2, the homogenizer head D-170 caused a greater back pressure and turbulent effect as compared with D-280 and as a result, the firmness of Labneh was reduced. In our opinion, the ALM machine should be referred to as a 'structurizer' rather than homogenizer.

H. D. Goff: You attributed the increase in firmness as a result of ultrafiltration at 55°C to the formation of casein complexes and the increase in firmness as a result of homogenization to the formation of 'appendages' on the surface of the casein complex. Also you attributed the differences in firmness of the ultrafiltration products vs. the traditional products to gravitational drainage. I am curious about the role of whey proteins in all these processes. Was the amount of whey protein measured between the ultrafiltered and traditional process and could this account for differences? Also, do denatured whey proteins contribute to the formation of the higher-temperature complexes and to the formation of the homogenization appendages?

Authors: The role of denatured whey proteins during the manufacture of fermented milks including yoghurt is well established and for further reference see Tamime *et al.* (1991a). The amount of protein in the UF permeates and the traditional filtrates were 0.24% and 0.36%, resp. (Tamime *et al.*, 1991b), and such values could be considered very similar. In milk heated to >85°C, β -lactoglobulin reacts with κ -casein on the surface of the casein micelles and forms a complex which prevents the casein micelles from forming large complexes. Consequently, micellar chains rather than clusters are formed (Knoop and Peters, 1975).

Sonication of acidified milk gel in an ultrasonic disintegrator led to the development of appendages on the casein micelle surfaces. As a result, there was no evidence that appendages were involved in fusion of adjacent casein micelles (Mottar *et al.*, 1987). In the present study, the yoghurt was ultrafiltered at 55°C and then homogenized using the ALM homogenizer. The processing conditions were different from those reported by Mottar *et al.* (1987). Differences in the microstructure of this UF Labneh before and after the homogenization stage are evident in Figs. 5 and 8. Fusion of the casein particles is less noticeable in the latter figure probably because of the turbulent effect introduced by the ALM homogenizer and the development of appendages on the surface of the casein particles.

R. Olsen: The authors suggest that the higher firmness readings of UF Labneh concentrated at 55°C could be attributed to partial agglomeration of casein particles. At lower operating temperatures, the processing time is greatly increased. Is it possible that an increased exposure to shear effects, because of the increased processing time, could influence the microstructure more than the relatively low operating temperatures?

Authors: Yes, increased exposure to shear effects during ultrafiltration and the effects of the ALM machine are most likely to affect the microstructure of the product. Dimensions of the void spaces in the protein matrix appear to be somewhat increased and micelle fusion is less apparent.

R. Olsen: Authors have ascribed differences in degree of concentration in part to seasonal variation of the original milk. How much did the original milk compositions vary between November and August? Would it be useful to use milk collected closer in time?

Authors: The chemical composition of milk in November and August, respectively, was: total solids 12.40 and 12.39%, protein 3.10 and 3.32%, fat 4.12 and 3.77%, and ash 0.76 and 0.71% (Tamime *et al.*, 1991b). Although the composition of both batches of milk was quite close, we agree that future trials should be conducted closer in time.

R. Olsen: Authors have mentioned that the role of fat in the firmness of Labneh can be partially explained by the fat globule being embedded in the protein matrix. Can they propose a mechanism by which embedded fat contributes to the firmness of this product?

Authors: Fat that has been homogenized contributes to a higher firmness of curd where this effect was demonstrated by TEM (Gavarić *et al.*, 1989). Homogenization of the yoghurt milk ruptures the fat globule membranes and the protein present in the milk or yoghurt becomes attached to the newly formed small fat particles. Consequently, the fat particles become an integral part of the protein matrix. In contrast, in unhomogenized milk or yoghurt, the fat globules do not interact with the protein matrix and are dispersed as individual entities. Yoghurt made from unhomogenized milk has a weaker gel firmness because the fat separates in the product during the fermentation stage and as a result, the majority of the fat globules would not be embedded in the casein structures. Recently, the effects of fat on the structure of milk products were studied by Xiong and Kinsella (1991).