Rheology and Microstructure of Labneh (Concentrated Yogurt)

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ABSTRACT

Labneh was produced by concentrating milk to approximately 23% total solids (wt/vol) by the traditional cloth bag method (control), ultrafiltration, reverse osmosis, or direct reconstitution. For ultrafiltration and reverse osmosis, membrane processing was carried out either before or immediately after fermentation. Dynamic rheological studies revealed that the physical behavior of labneh was heavily dependent on the protein concentration and the severity of mechanical agitation during membrane treatment. Scanning electron microscopy showed that the higher protein content samples had more compact structure and smaller voids than their lower protein content counterparts. Also, reverse osmosis and ultrafiltration of warm fermented milk had clearly detrimental effects on gel structure, producing thicker casein strands than in the traditional sample. No major differences were observed in the other test samples except gel densities, which varied with the different casein concentrations. In general, ultrafiltration of warm, fermented milk is a promising treatment for the manufacture of good quality labneh. (**Key words**: rheology, microstructure, concentrated yogurt, labneh)

Abbreviation key: **G*** = complex modulus, **RO** = reverse osmosis, **RO-AF** = RO after fermentation, **RO-BF** = RO before fermentation, **SCEM** = scanning electron microscopy, **tan** δ = loss tangent, **UF-AF** = ultrafiltration after fermentation, **UF-BF** = UF before fermentation.

INTRODUCTION

There have been numerous studies on the relationships between structure and texture of yogurt and yogurt-like products (5, 6, 8, 9, 20). The rheological properties of natural set and stirred yogurts have been widely investigated (14, 17, 18) as have the effects of processing variables such as heat treatment, concentration of TS, and type of starter bacteria on rheology and microstructure (2, 12, 15). However, until recently, such rheological and microstructural examination had not been carried out with labneh, a concentrated yogurt that is popular in the Middle East and Balkan regions (19). The recent increase in the popularity of labneh in Europe has led to more interest in the structure of labneh, especially in relation to milk species and concentration techniques (19, 21). In those studies, UF was proposed as a better alternative to the traditional labneh-making process, which is uneconomical and unhygienic (19). Recent studies in that laboratory (10, 11) have investigated the rheology of labneh produced by a range of techniques for increasing TS and have concluded that UF could be used to produce gel properties similar to the traditional product. The present study aims to extend that work by comparing the physical and microstructural properties of labneh produced by traditional methods, direct reconstitution, and membrane techniques [both UF and reverse osmosis (**RO**)] using dynamic rheological techniques and scanning electron microscopy (**SCEM**). In addition, a separate experiment was carried out in which labneh was prepared from UF retentates with varying protein contents. Rheological studies on these samples were used to assess the role of protein concentration regardless of method of concentration.

MATERIALS AND METHODS

Membrane Processing

Both UF and RO were carried out using tubular systems supplied by Paterson Candy International (PCI Membranes, Whitchurch, United Kingdom). The UF membranes were ES 625 (polyether sulfone) with a surface area of 0.8 m^2 and a molecular mass cutoff of 25,000 Da and were operated at inlet and

Received June 26, 1998.
Accepted November 20, 1998.

outlet pressures of 0.3 and 0.1 MPa, respectively. The RO membranes were ZF 99 (polyether sulfone) with a surface area of 1.2 m^2; the operating pressure was 2 MPa.

Labneh Manufacture

Full fat, medium heat-treated milk powder supplied from Adams Food Ingredients Ltd. (Leek, United Kingdom) was used in the preparation of labnehs. The method of yogurt manufacture proposed by Tamime and Robinson (22) was applied. Milk powder was reconstituted at 40°C by a high speed mixer (Silverson Machines Ltd., Chesham, United Kingdom) to the desired TS concentration before further treatment. The milks were heated to 85°C for 20 min and then were cooled to 42°C in an ice-cold water bath. Samples were inoculated with starter culture (CH-1; Chr. Hansen's Laboratory, Reading, United Kingdom) at a rate of 2% (wt/wt). The starter culture was a blend of *Streptococcus thermophilus* and *Lactobacillus delbruckii* sp. *bulgaricus* in equal proportions. The desired TS content of the labneh samples at the end of the manufacture was approximately 23% (wt/vol), which is typical of traditional labneh from the Middle East (22). Incubation was halted when the pH dropped to 4.3 for samples that were concentrated postincubation and to 4.0 for previously concentrated samples; the final pH was 4.0 for all samples. Samples were stored overnight in 200-g plastic cups at 4°C and were allowed to equilibrate to 25°C in an incubation room before rheological measurements were performed the following day. Six different manufacturing methods were employed, and 8 replicates of each batch were prepared for analysis.

1. Traditional labneh. To produce traditional stirred-type labneh (control), yogurt with 16% TS (wt/vol) (pH 4.3) was drained after overnight refrigeration following manufacture in double layer cheesecloth bags until the desired TS concentration (~23%, wt/vol) was reached. Drainage was achieved at 4°C, and the volume of whey separated was measured periodically. Total drainage time was 18 to 20 h.
2. Stirred-type labneh produced by UF after fermentation (**UF-AF** labneh). Ultrafiltration was applied immediately after incubation of fermented milk (16% TS, wt/vol) was complete (pH 4.3). The temperature was maintained at 42°C by circulating cold water around the feed tank when necessary. After the desired concentration of TS was reached (~23% wt/vol), the sample was filled into yogurt pots and stored in the refrigerator until analysis.
3. Stirred-type labneh produced by RO after fermentation (**RO-AF** labneh). The procedure just described for UF-AF was followed except that RO was applied.
4. Set-type labneh produced by UF before fermentation (**UF-BF** labneh). Milk with 16% TS (wt/vol) was concentrated by UF to 23% TS (wt/vol) at 50°C. The standard yogurt manufacturing procedure was then followed. Incubation was stopped at pH 4.0.
5. Set-type labneh produced by RO before fermentation (**RO-BF** labneh). The same procedure described for UF-BF labneh was followed except that RO membranes were applied.
6. Direct reconstitution, set-type labneh. The procedure for the control was followed except that milk powder was dissolved to a concentration of 23% TS (wt/vol). Incubation was halted at pH 4.0.

The fermentation process took approximately 3.5 h for the control, UF-AF, and RO-BF labnehs and took 5 to 6 h for the direct reconstitution, UF-BF, and RO-BF labnehs.

After overnight refrigeration at 4°C, the pH values of the samples were around 4.0.

Gels with Varying Protein Concentrations

Milks were prepared with protein concentrations ranging from 1 to 9% by UF with appropriate dilution of retentates to the appropriate protein concentration with the same UF permeate to maintain the same ionic environment. Standard yogurt manufacture was carried out as just described.

Chemical Analysis

Protein, TS, fat, and ash were determined by the methods of the British Standards Institution (10).

Rheological Measurements

Rheological properties of labnehs after overnight storage at 4°C were monitored using an RTI controlled-stress dynamic rheometer (Rheo-Tech Int., Ltd.; Camtel Ltd., Royston, United Kingdom). The rheometer was set up with a parallel plate geometry (10-mm radius and 1-mm gap setting), and the temperature of the samples was maintained at 25°C by a circulating water system. Labneh samples were

evaluated rheologically by conducting stress and frequency sweep tests. The frequency and amplitude ranges were 10^{-3} to 10^{1} Hz at 0.07 mNm torque and 1.5×10^{-2} to 1.5×10^{-1} mNm at 0.25 Hz, respectively. Labnehs were kept in a room maintained at 25°C to equilibrate before being loaded into the rheometer. Samples were allowed to relax (5 min) prior to assessment of their amplitude and frequency behavior.

SCEM Studies

Preparation of thick sections for SCEM. Labneh samples were prepared for SCEM studies according to the method proposed by Brooker and Wells (4). Specimens of labneh were fixed and solidified by addition of 25% SCEM grade glutaraldehyde at a ratio of glutaraldehyde to labneh of 1:7 (wt/vol). This mixture was poured onto a petri dish as a thin layer and left for 30 min at 4°C to solidify before 1-mm cubes were cut with a razor blade. Because the RO-AF labneh remained too soft to handle after this treatment, the cubes were coated with 3% aqueous agar to protect them and were stored overnight in 3% glutaraldehyde in 175 m*M* sodium cacodylate-HCl buffer (pH 7.2).

After rinsing in water, the specimens were dehydrated with three changes of acidified dimethoxy propane over 3 h and embedded in araldite resin. Two-micrometer sections of labnehs were cut with a glass knife on an ultramicrotome (Reichert Ultracut E; Leica UK, Milton Keynes, United Kingdom), placed on a drop of 10% acetone on a circular 10-mm diameter cover slip, and dried by gentle heating.

Etching of sections. A stock solution of saturated NaOH in absolute ethanol was prepared by the method of Lane and Europa (7) and was allowed to stand for about 1 wk until the solution became dark brown. The coverslip was immersed in stock solution that had been diluted 1:1 (vol/vol) with ethanol immediately before the sample was placed on a hot plate (40°C) for 10 min. The progress of etching was followed using a binocular microscope. Complete removal of the resin was considered to have occurred when the boundary of the section was no longer visible.

Preparation of etched sections for SCEM. When etching was judged to have reached a desirable stage, the coverslips were transferred rapidly to ethanol (to prevent ethanol evaporation, which would lead to NaOH crystallization on the surface) and washed several times with absolute ethanol. The coverslips were then transferred to 100% acetone for 10 min before critical point drying (Polaron E3000; Polaron Equipment Ltd., Watford, United Kingdom) with liquid CO_2 and were mounted on aluminum stubs using silver conducting paint and then coated with gold in a vacuum sputter coater (Edwards High Vacuum S150; Crawley, Sussex, United Kingdom). The sections were placed 20 mm from the gold electrode and were sputtered for 1 to 3 min (depending on thickness) under a vacuum of 20 Pa using 2.5-kV high tension and a discharge current of 20 mA. Sections were examined in an SCEM (Hitachi L750; Nissei Sangyo Co. Ltd., Tokyo, Japan) operating at accelerating voltages from 5 to 30 kV. The results were recorded on Kodak plus X 120 film (Kodak Ltd., Hemelhempstead, United Kingdom).

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition of the labnehs are summarized in Table 1. The traditional (control) and UF-

TABLE 1. Chemical composition of labnehs.[1]

Labneh[2]	TS		Protein		Lactose[3]		Fat		Ash	
	(mg/kg)									
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Traditional (control)	23.31	0.20	9.20	0.14	4.16	0.05	9.18	0.10	0.79	0.05
UF-AF	22.64	0.33	8.80	0.24	4.53	0.34	8.45	0.10	0.86	0.02
RO-AF	22.22	0.15	6.38	0.10	8.24	0.22	6.60	0.30	1.00	0.05
UF-BF	22.24	0.21	9.00	0.11	4.26	0.09	8.20	0.13	0.78	0.01
RO-BF	23.22	1.03	6.82	0.48	9.07	0.02	6.25	0.07	1.08	0.03
Direct reconstitution	22.50	0.19	6.38	0.34	8.72	0.31	6.10	0.14	1.30	0.02

[1]n = 3.

[2]Labneh manufactured with UF-AF = (UF after fermentation), UF-BF = (UF before fermentation), RO-AF = [reverse osmosis (RO) after fermentation], or RO-BF = (RO before fermentation).

[3]Determined by difference.

treated labnehs (UF-AF and UF-BF labnehs) had higher protein and fat contents than did the remaining test samples, which was to be expected as the double-layer cloth bags and UF membranes allowed the separation of lower molecular mass compounds, such as lactose and minerals, into the permeate or filtrate while retaining protein and fat. Conversely, with RO and direct reconstitution, all constituents were concentrated in proportion to the concentration factor. The latter samples had much higher lactose contents than the traditional and UF-treated samples. There were no major differences in chemical composition between labnehs that were concentrated before and after fermentation.

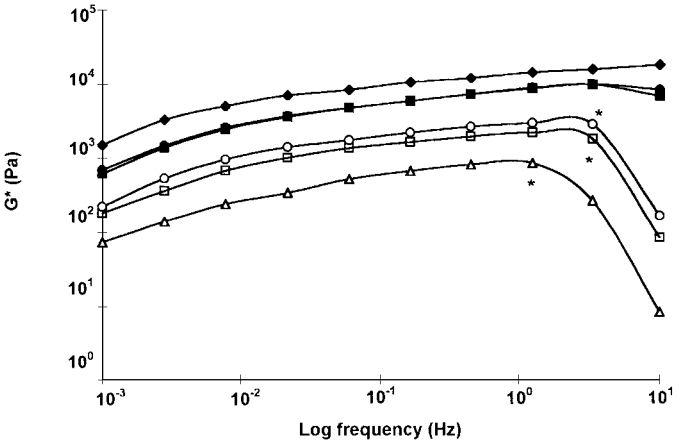

Figure 1. Frequency sweep pattern of labnehs tested. Results are the means of eight separate runs repeated five times (n = 8). Standard errors are less than ±8% of the mean values and smaller than symbol dimensions. Asterisks indicate the complex modulus (G*) after gel structure degradation (outside the linear viscoelastic region). Traditional labneh (♦; control); labneh manufactured with reverse osmosis (RO) after fermentation (△), UF after fermentation (■), UF before fermentation (●), direct reconstitution (○), and RO before fermentation (□).

Dynamic Rheological Properties

The physical properties of the labnehs were examined by conducting frequency sweep tests (Figure 1). The different manufacturing techniques led to differences in the physical properties of the resulting products. Within the linear viscoelastic region, the traditional sample had the greatest complex modulus (**G***), followed by UF-AF and UF-BF samples, direct reconstitution, RO-BF, and RO-AF labnehs, respectively. Structural degradation occurred at some point in the frequency range in all except the traditional labneh. Both the rheological differences between the samples within the linear viscoelastic region and structural degradation indicate that the same interaction forces take place in the formation of labneh gels but to different degrees. Similarly, within the linear viscoelastic region, the loss tangent (**tan** δ = loss modulus/storage modulus) values of the samples were not significantly different, indicating that the nature and type of the interaction forces were similar (data not shown). The storage modulus and loss modulus are similarly related to the spatial distribution and the number of protein-protein bonds, which, therefore, suggests that tan δ is related to the nature of the protein bonds (13). A slight frequency dependency within the linear viscoelastic region was apparent, which seemed to be independent of the method by which the labnehs were manufactured. The increase of G* with frequency suggests a relaxation of bonds over the time scale of the measurements (13) with greater numbers of individual bonds relaxing over time. This type of rheological behavior is typical of particle gels such as yogurt (1).

A yogurt gel network is primarily built of casein and denatured whey protein complexes, and, to study the role of protein concentration (independent of method of concentration) in gel formation, UF milks were adjusted to various protein concentrations using the same UF permeate to keep the same ionic environment of each sample. The effect of protein concentration on the dynamic modulus G* and tan δ is illustrated in Figure 2. A strong dependence of G* and tan δ on the protein concentration was evident. The minimum protein concentration at which a gel could be formed was 3%, which is presumably due to the lower number of protein contacts in the larger solvent concentration. Structural degradation occurred at protein concentrations up to 7% but not at 9%. With increased protein, G* increased. These findings are consistent with previous studies (3, 16, 23), which relate gel strength to the casein concentration. Bremer et al. (3) proposed that the total length of the stress carrying strands per unit volume is a decisive factor for the gel strength of yogurt and that the nature and position of the strands in the network also determine the rheological properties of the gel. Ross-Murphy (16) stated that, ideally, there should be a direct relationship between the number of molecules participating in the junction zones of a protein network and the gel strength. Also, Walstra et al. (23) reported that the number of contact points between the casein clusters is independent of their size but dependent on casein concentration.

As can be seen from Figure 2b, within the linear regions, tan δ values were independent of casein (pro-

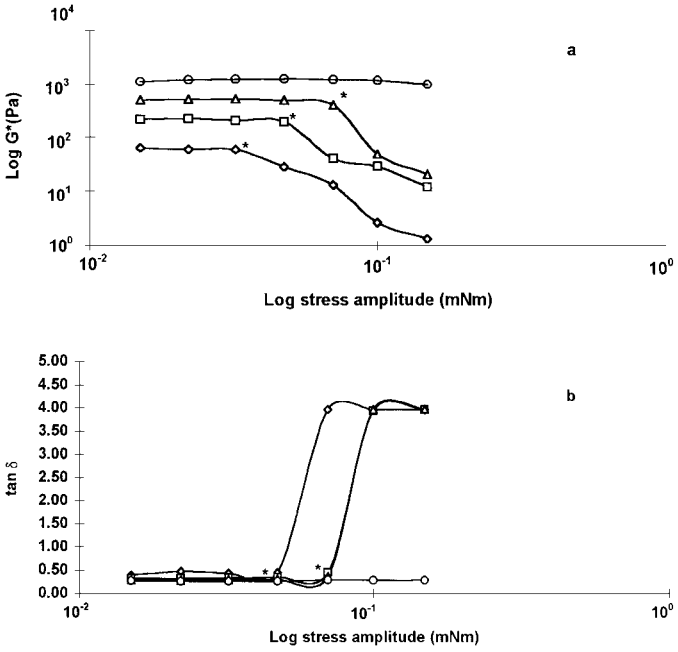

Figure 2. Effect of protein concentration [3% (◊), 5% (□), 7% (△), or 9% (○)] on the complex moduli (G*) (a) and the loss tangent (tan δ) (b) of the samples examined. Results are the means of eight separate runs repeated five times (n = 8). Standard errors are less than ±8% of the mean values and are smaller than symbol dimensions. Asterisks indicate the complex modulus (G*) after the gel structure has degraded (outside the linear viscoelastic region).

tein) concentration, which implies that the nature of the protein-protein bonds was the same. Because the dependence of G* on the protein concentration was nonlinear, it can be concluded that the number of stress-carrying strands was not proportional to volume fractions of casein particles, and so the network formed was very heterogeneous.

Microstructure

The SCEM of traditional and stirred-type labnehs are presented at low and higher magnifications in Figures 3 and 4, respectively, and the set-type labnehs are shown in Figure 5. The microstructure of the samples was determined by the mechanical treatments and the order of application of concentration technique (before or after fermentation). At low magnification (2500×), the traditional labneh (Figure 3a) appeared to have a more compact structure than did the other two stirred-type labnehs (UF-AF and RO-AF labnehs; Figure 3, b and c, respectively). Surprisingly, SCEM failed to reveal any notable difference between the two membrane-treated, stirred-type labnehs (Figure 3, b and c) despite the considerable

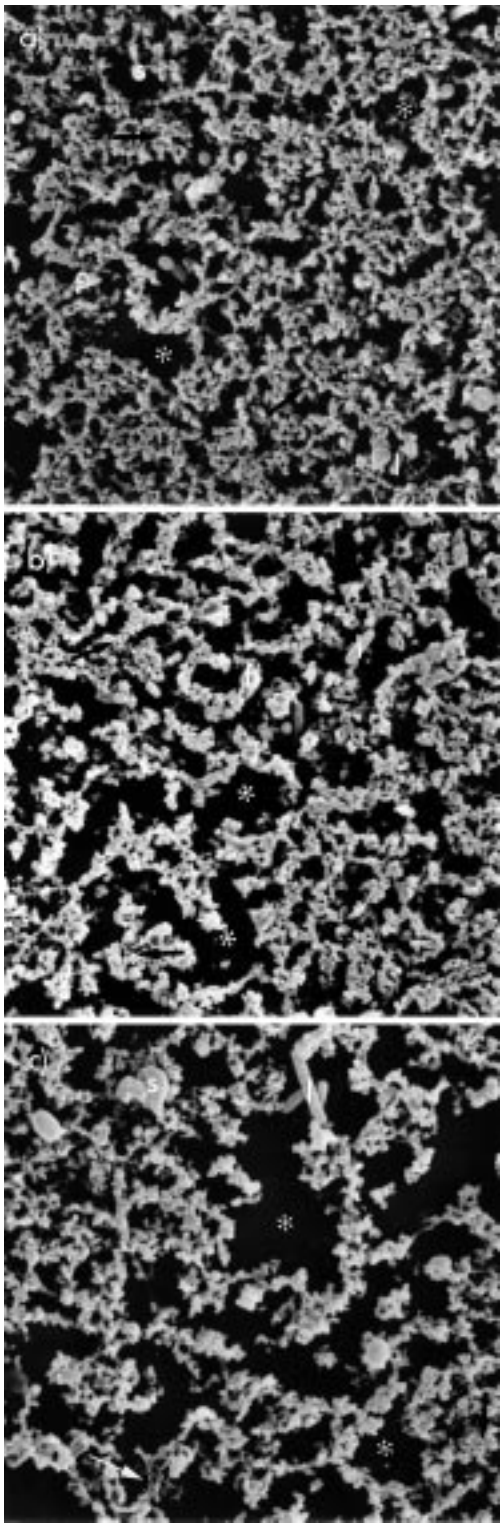

Figure 3. Scanning electron micrographs of stirred-type labnehs: traditional (control) (a), manufactured by UF after fermentation (b), and manufactured by reverse osmosis after fermentation (c). Continuous casein micelles (black arrows) and thread-like structures (white arrows) can be observed between casein strands. Magnification: 2500×. Legend: lactobacilli = l, streptococci = s, fat globules = f, and voids = *.

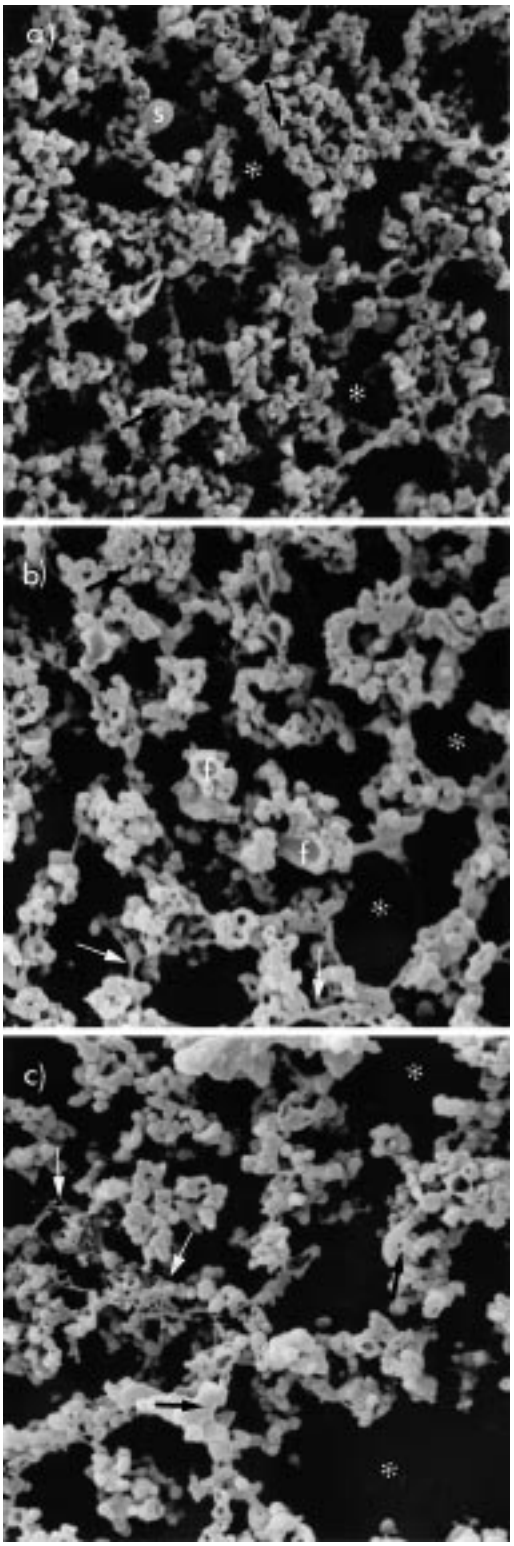

Figure 4. Scanning electron micrographs of stirred-type labnehs: traditional (control) (a), manufactured by UF after fermentation (b), and manufactured by reverse osmosis after fermentation (c). Continuous casein micelles (black arrows) and deformed (ripped-off) casein strands (white arrows) are evident. Magnification: 12,600×. Legend: streptococci = s, fat globules = f, and voids = *.

differences in the rheological properties and protein concentrations of these samples.

The RO-BF labneh had a structure similar to the direct reconstitution labneh (Figure 5) and is not shown. Damage was not detected in the structures of

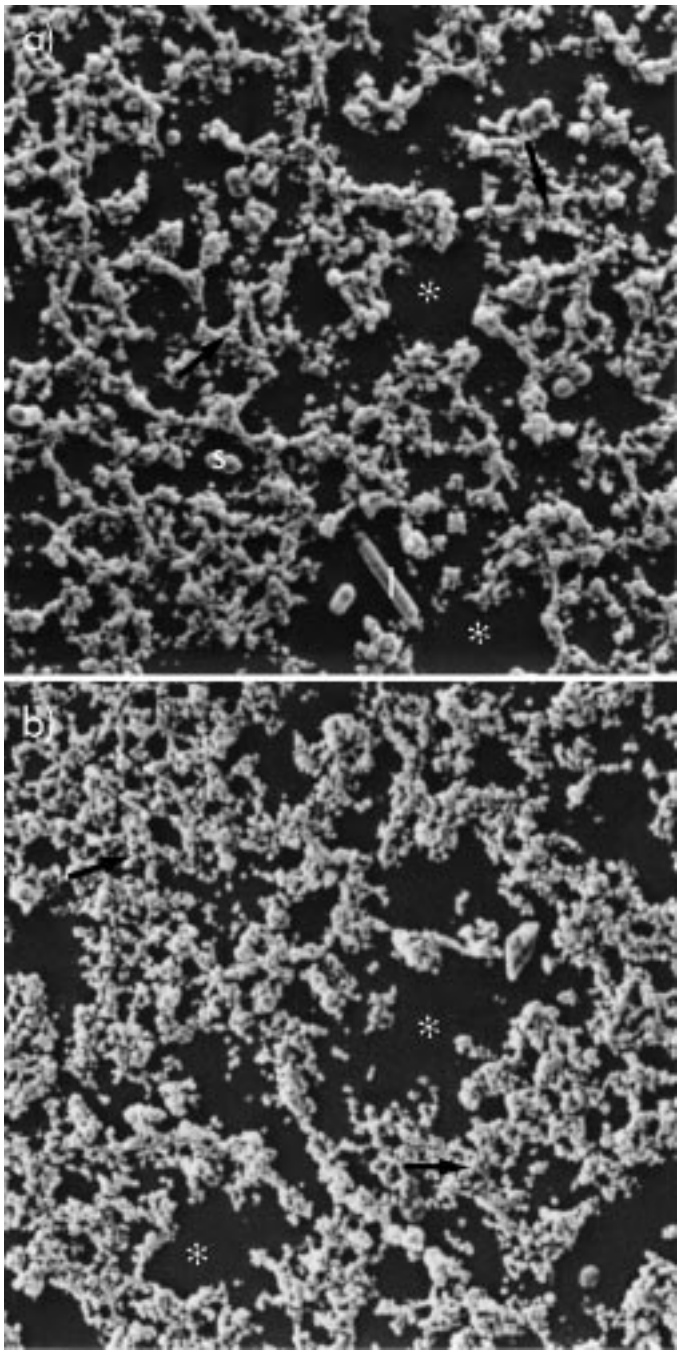

Figure 5. Scanning electron micrographs of set-type labnehs: manufactured with UF before fermentation (a) or by direct reconstitution (b). Continuous nondeformed structure is evident (black arrows). Magnification: 2500×. Legend: lactobacilli = l, streptococci = s, and voids = *.

UF-BF and direct reconstitution labnehs; the voids and protein structure were relatively evenly distributed.

The labnehs generally displayed continuity of structure except for RO-AF and, to a lesser extent, UF-AF labneh. The discontinuity in the membrane-treated, stirred-type labnehs is probably linked to the detrimental effect of RO and UF on the delicate gel structure. The casein clusters were thicker in the membrane-treated samples, perhaps as a result of pressure forcing the casein aggregates to come together during the early stages of the membrane processes. This compression is particularly notable at higher magnification in Figure 4, a and b, which shows that the thicker casein strands in UF-AF labneh occupied less space in the casein network than did the traditional product, although the protein concentrations were the same. However, the same membrane treatments to milks (i.e., before fermentation) did not produce such structures; instead, fine, continuous microstructures were evident (Figure 5a).

Small, thread-like structures were visible between the strands in both the RO-AF and UF-AF labnehs, which may be the result of stretching and shearing of casein aggregates during the later stages of UF and RO when viscosity is increased. More separate particles were present in the network in the RO-AF labneh than were in the UF-AF counterpart. The lower protein content of the RO-AF labneh would be expected to have resulted in considerably larger compartments compared with the higher protein content samples (traditional or UF-AF). However, fairly close network densities were evident in both membrane-treated, stirred labnehs (UF-AF and RO-AF Figure 3, b and c, respectively). One explanation is that the high pressure RO application to fermented milk might have caused a ripping of the casein aggregates, but the lower pressure during the UF process might have stretched the casein strands and broken a lower number of bonds between the casein aggregates. This difference may be connected to the wide polysaccharide-like structures in the UF-AF labneh compared with the tiny thread-like structures in the RO-AF seen at higher magnification (Figure 4, b and c, respectively). Because nonpolysaccharide-producing yogurt culture was used in the manufacture of the labnehs, these structures are unlikely to be polysaccharide materials.

It is possible that these unusual structures may be the result of artifacts or that the apparently separate particles may in some cases actually be the front view of a casein chain that has a continuous structure. However, these structures were not seen in the traditional (control) labneh (Figure 4a), which was not agitated, and there was reasonable agreement between the rheology and microstructure of samples tested. It is, therefore, concluded that the separate particles seen in the membrane-treated samples could be broken protein aggregates (UF-AF labneh) and broken or ripped-off casein strands (RO-AF).

CONCLUSIONS

The large differences in the rheological properties of the labnehs seemed to be dependent on the level of protein and TS elevation technique. Electron microscopy revealed that the application of the concentration techniques to the fermented milk had detrimental effects on the structure. The amount of damage to the casein strands seemed to be related to the shearing effect of the UF and RO membranes. However, in terms of the physical properties of the labnehs, the UF-AF and UF-BF labnehs had similar characteristics. Through changes in the processing variables (e.g., pH at the beginning of concentration, transmembrane pressure, and operating temperature), a material might be produced that has a less damaged structure and a better texture. In summary, UF treatment to the warm fermented milk seems to be a promising technique in the manufacture of good quality concentrated yogurt.

ACKNOWLEDGMENT

Author Ozer thanks the Turkish Higher Educational Council and Harran University (Sanliurfa, Turkey) for financial support.

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