Effect of protein concentration on the properties and structure of concentrated yogurts

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Concentrated yogurts were produced by traditional (control), direct reconstitution, ultrafiltration and reverse osmosis techniques. The membrane techniques were applied either before or soon after incubation. The physical properties of the samples were monitored using a penetrometer (set yogurt) and viscometer (stirred yogurt), and the results indicated that different manufacturing techniques led to differences in the rheology of the concentrated yogurts. As expected, samples with high protein contents had greater gel strengths. Also, the concentration techniques caused large differences between the samples, even at the same protein level. The rheological properties correlated well with the microstructure as monitored by confocal laser scanning microscopy. In general, larger compartments in the network were associated with a weaker structure.

INTRODUCTION

Yogurt gels are particulate structures, mainly composed of caseins.[1] Their structure has been studied widely, as have the effects of processing conditions, such as heat treatment, type of starter culture or level of total solids, on the rheology of both set and stirred yogurts.[2-4] In addition, the formation of yogurt gels has been monitored using electron microscopy and dynamic oscillatory rheometry.[5,6] Depending on the processing conditions, continuously connected protein strands produce a heterogeneous three-dimensional gel network which holds free water. Any factors which affect the properties of the gel network by changing the nature and number of protein interactions will also affect the water holding capacity of the gel. The gel structure is known to involve both covalent (thiol/disulfide interchange) and non-covalent bonds.[7] Dickinson[8] claimed that the physical characteristics of particulate gels are determined by both strong permanent bonds (covalent bonds) formed during the aggregation, and subsequent rearrangements of protein particles. Furthermore, the final gel structure is also dependent upon the nature of weak reversible interactions between the particles prior to formation of the permanent bonds; therefore, the balance between strong and weak bonds controls the rheology.[8] Another factor affecting the physical characteristics of yogurt type gels is the distribution of protein–protein bonds over the gel network.[9] Several studies have investigated the relationship between protein concentration, distribution of protein–protein bonds and rheology of resulting gels.[10,11] In the case of homogeneous cross-linked particulate gels, all particles contribute to the network moduli equally.[11] However, in non-homogeneous gels like yogurt thick protein nodes including more than one protein junction point are evident, and their contribution to the elasticity of a gel decreases as the number of stress carrying strands decreases.

The use of membrane techniques in the manufacture of concentrated yogurt has gained popularity,[12] but knowledge of the gel characteristics of concentrated yogurts is limited.[13-15] Consequently, the aim of the present study was to prepare concentrated yogurts by different methods, and determine whether apparent contrasts in rheology would be confirmed by confocal laser scanning microscopy.

MATERIALS AND METHODS

Materials

Full cream milk powder (Adams Food Ingredients Ltd, Leek, Staffs) was used in the production of all the yogurts, as was a freeze dried yogurt culture (coded-CH1) from Chr Hansen's Laboratory (Reading, UK). The starter was a blend of *Streptococcus thermophilus* and *Lactobacillus delbruckii* subsp *bulgaricus* in equal proportions, and resuscitation and routine subculturing were carried out according to the procedure described by Tamime.[16]

Methods

Yogurt was manufactured according to the method described by Ozer *et al*,[14] and the standard milk base (16 g l^{-1} total solids (TS)) was prepared by reconstituting the required amount of milk powder in tap water. Six different concentration techniques were applied

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in order to generate two groups of concentrated yogurts, namely set (concentration before fermentation) and stirred (concentration after fermentation):

Traditional concentrated yogurt (control) was produced by holding batches of yogurt (5 kg, 16 g l^{-1} TS) in bags of double layer cheese cloth at 4°C for 18–20 hours.

Concentrated yogurt (direct reconstitution) was manufactured by reconstituting the required amount of milk powder in water at 40°C to give 5 kg of endproduct with 23 g l^{-1} TS.

Concentrated yogurt (ultrafiltration (UF)-after fermentation) 16 g l^{-1} TS fermented milk (pH 4.3) was concentrated by UF at 42°C immediately after fermentation. The pH value of finished product was around 4.0.

Concentrated yogurt (UF-before fermentation) was made by concentrating freshly reconstituted milk (16 g l^{-1} TS) to approximately 23 g l^{-1} TS at 50°C prior to fermentation.

Concentrated yogurts (reverse osmosis (RO)-after and before fermentation) were prepared as the UF products except that RO systems replaced UF.

Both UF and RO were carried out using tubular systems supplied by Peterson Candy International (PCI Membranes, Whitchurch, Hants, UK). The UF membranes were ES 625 (polyether sulfone), surface area 0.8 m^2, MWt cut-off 25000 Da, operated at inlet and outlet pressures of 0.3 and 0.1 MPa respectively. The RO membranes were ZF 99 (polyether sulfone), surface area 1.2 m^2, operating at a pressure of 2 MPa.

All the samples (~ 150 g) were dispensed into (stirred yogurt) or incubated in (set yogurt) polystyrene cartons, and the pH values of the samples were measured using a pH meter (model Kent EIL 7045/46) fitted with a standard combination of glass electrode.

Assessment of the physical properties

The gel strength was measured with a standard penetrometer (Stanhope Seta Ltd, Camberley, Surrey, UK) using a probe of 67 g, a diameter of 2.5 cm and an apical angle of 90°; the penetration time was 5 s. In all cases, the temperature of the yogurt was equilibrated at 10°C, and duplicate pots from each batch were assessed.

After standardizing the temperature at 10°C, the apparent viscosity was measured with a Brookfield Viscometer (Model LVT with Helipath Attachment) (Brookfield Engineering Inc, Stoughton, USA) fitted with a T-bar spindle (D) rotating at 0.6 rotations per minute; the readings were converted to centipoises using the factor supplied by the manufacturer.

Statistical analysis

The results were analysed in Exel (Windows 95) to obtain mean values and standard errors, and the physical properties were compared with a standard *t* test.

Confocal laser scanning microscopy

Yogurt samples were prepared at least 24 h prior to examination, and a thin 'slice' taken with a scalpel was placed on a clean slide. After staining for 5–10 minutes with 0.3% fast green (BDH/Merck, Poole, UK), the slide was examined under a Zeiss LSM II Confocal laser scanning microscope (Zeiss, Welwyn Garden City, UK) using oil immersion optics (numerical aperture = 1.41). The protein was imaged by excitation of the fast green using a helium/neon laser emitting at a wavelength of 633 nm.

RESULTS AND DISCUSSION

The chemical compositions of test samples are given in Table 1. In general, while UF treatment to both fresh milk and fermented milk led to an increase in the protein and fat content, a large decrease in lactose was seen. However, in the RO treated samples and direct reconstitution concentrated yogurt, the concentrations of all the components rose proportionally.

The comparative gel strengths and viscosities are shown in Table 2, and it was notable that the gel strength of the traditional product, although stirred after leaving the cloth bag, was much higher than the rest. However, by contrast, the product made from milk concentrated by UF prior to fermentation gave

TABLE 1

Chemical compositions of the reconstituted milk and the concentrated yogurts. Results are the means and standard errors of duplicate samples taken over three separate runs and expressed as g kg^{-1} of sample

Samples	*Total solids*	*Protein*	*Lactose*[a]	*Fat*	*Ash*
Milk base	160.0 ± 1.8	43.6 ± 0.08	61.6 ± 0.9	45.0 ± 1.5	9.8 ± 0.3
Traditional yogurt	233.1 ± 2.0	92.0 ± 1.4	41.6 ± 0.5	91.8 ± 1.0	7.9 ± 0.5
UF-after fermentation yogurt	226.4 ± 3.3	88.0 ± 2.4	45.3 ± 3.4	84.5 ± 1.0	8.6 ± 0.2
RO-after fermentation yogurt	222.2 ± 1.5	63.8 ± 1.0	82.4 ± 2.2	66.0 ± 3.0	10.0 ± 0.5
UF-before fermentation yogurt	222.4 ± 2.1	90.0 ± 1.1	42.6 ± 0.9	82.0 ± 1.3	7.8 ± 0.1
RO-before fermentation yogurt	232.2 ± 10.3	68.2 ± 4.8	90.7 ± 0.2	62.5 ± 0.7	10.8 ± 0.3
Direct reconstitution yogurt	225.0 ± 1.9	63.8 ± 3.4	87.2 ± 3.1	61.0 ± 1.4	13.0 ± 0.2

[a]Lactose was determined by difference.
After Ozer *et al.*[15]

TABLE 2

Physical properties of the different yogurts measured as viscosity (centipoise/1000) and gel strength (penetration in mm) after overnight storage at 4°C

Product	*Viscosity*	*Gel strength*
Traditional	65.80 ± 1.2^{a}	78.30 ± 9.3^{a}
UF-after ferment.	27.50 ± 1.5^{b}	33.10 ± 1.3^{b}
UF-before ferment.	60.10 ± 1.0^{a}	31.70 ± 1.7^{b}
RO-after ferment.	5.60 ± 0.6^{c}	25.00 ± 1.0^{b}
RO-before ferment.	13.90 ± 1.0^{d}	30.30 ± 0.5^{b}
Direct Reconstit.	13.20 ± 0.9^{d}	28.30 ± 3.4^{b}

Each reading is the average of three separate trials, and means within a column sharing a common superscript do not differ significantly ($p > .05$).

a viscosity measurement close to the traditional, and the micrographs (Figs. 1a and 1d) showed that the two samples were similar, especially in terms of the size and structure of the voids. Exactly why the gel strength of the UF-before fermentation sample was significantly lower than the traditional product was not established, but it may have been because the cloth bag method allowed for the establishment of more protein–protein bonds. Thus, once the 5 l of yogurt were poured into the bag, it would have taken several hours for the temperature to reach 4°C and during this time the physical compression of the protein might have encouraged aggregation of the casein micelles and increase the extent of chemical bonding. In the membrane treated milk, by contrast, the extent of bonding would have been no different from that in any high solids yogurt, which would explain the similarity in gel strength between all the membrane concentrated yogurts.

In the absence of stabilizers, viscosity depends on total protein concentration and the size of the whey filled spaces, and there was an excellent visual correlation between viscosity and the dimensions of the void spaces (see Fig. 1). Thus the dense protein networks of the traditional and UF-before fermentation yogurts confirm the expectation of high viscosity, while the damage to the gel inflicted by membrane processing, especially by the high pressure associated with the RO treatments led to considerable breakdown of the gel structure (see viscosity measurements in Table 2) and the emergence of large whey filled spaces (Figs. 1c and 1e).

A contrast between the gels with different protein levels was apparent also for, in general, as the total solids increased, so the chains of casein particles became shorter, the dimensions of the voids diminished and the density of the matrix increased.[17] In the traditional UF-after and UF-before fermentation concentrated yogurts (Figs. 1a, 1b and 1d) much denser structures were observed compared to samples with lower protein contents (the RO-before fermentation, RO-after fermentation and direct reconstitution concentrated yogurts (Figs. 1c, 1e and 1f).

Overall, the study confirmed that damage done to the coagulum has a major impact on the viscosity of concentrated, stirred yogurts, and that the larger the undisturbed aggregations of casein and smaller the whey filled spaces, the higher the viscosity of the endproduct. Gel strength, by contrast, is probably more dependent on the extent of protein–protein bonding, and factors that may encourage these interactions are clearly important.

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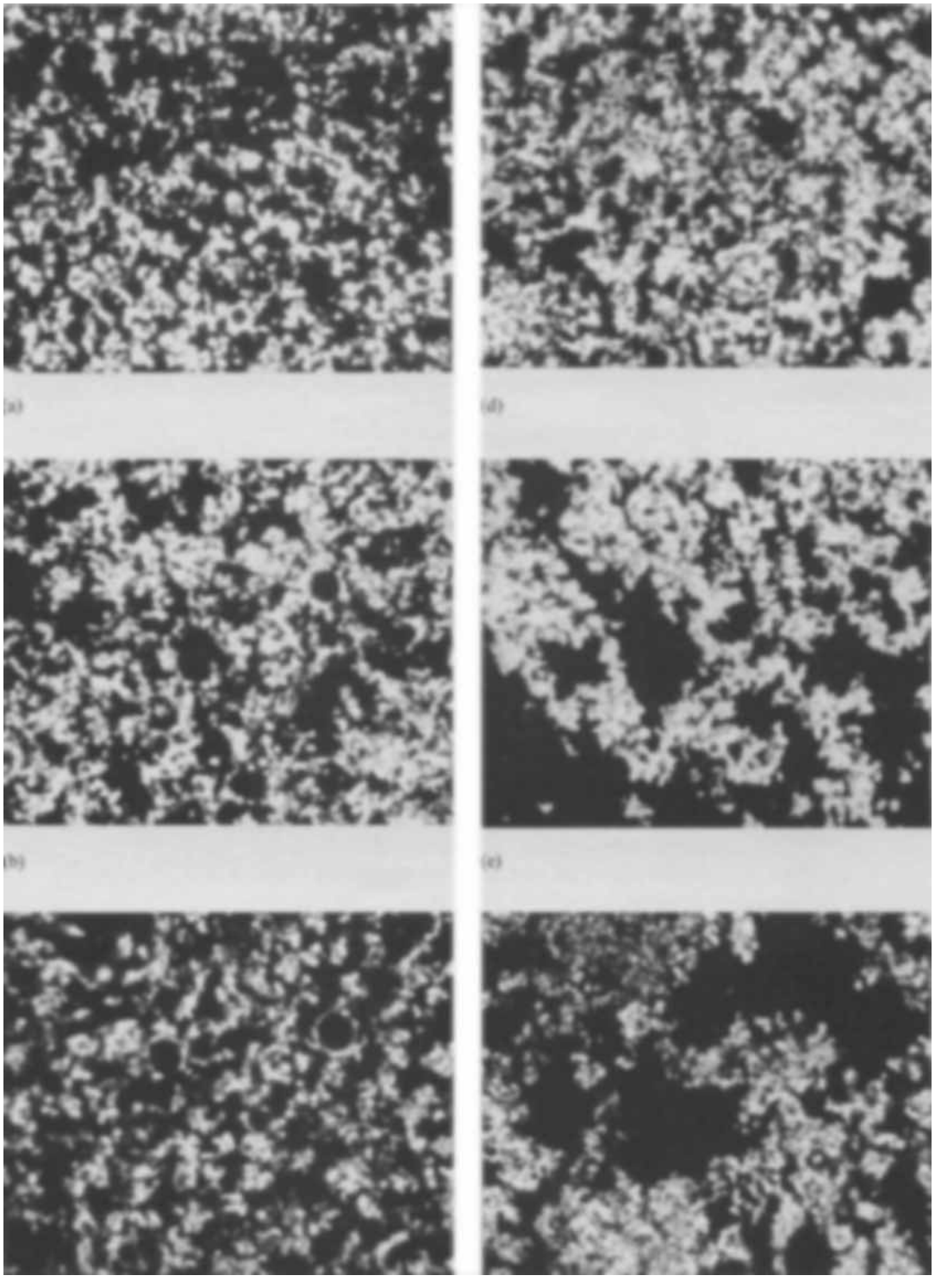

(c) (f)

Fig. 1. Confocal laser scanning micrographs of protein distribution in stirred concentrated yogurts (23 g l^{-1} total solids). The protein was stained with fast green and imaged by excitation at 633 nm. Whey filled voids in the structure were seen by negative contrast. The circular voids are fat globules, and this conclusion was confirmed by staining with nile-blue with excitation at 488 nm. The samples were: (a) traditional product, (b) UF-after fermentation, (c) RO-after fermentation, (d) UF-before fermentation, (e) RO-before fermentation and (f) direct reconstitution.