

1986

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### Recommended Citation

Green, M. L.; Langley, K. R.; Marshall, R. J.; Brooker, B. E.; Willis, A.; and Vincent, J. F. V. (1986) "Mechanical Properties of Cheese, Cheese Analogues and Protein Gels in Relation to Composition and Microstructure," *Food Structure*: Vol. 5 : No. 1 , Article 19.

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# Mechanical Properties of Cheese, Cheese Analogues and Protein Gels in Relation to Composition and Microstructure

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MECHANICAL PROPERTIES OF CHEESE, CHEESE ANALOGUES AND PROTEIN  
GELS IN RELATION TO COMPOSITION AND MICROSTRUCTURE

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Abstract

Samples of commercial Cheddar cheese, experimental Cheddar cheeses made from heated, ultrafiltration-concentrated milks, processed cheese analogues and whey protein gels of defined composition were examined microscopically and some fracture and deformation properties were determined. Surfaces of cheese prepared by critical point drying and those examined frozen were comparable in microstructure. As the concentration factor of the milk used for experimental cheesemaking increased, the cheese became more resistant to reversible deformation, the work required to cut with a wire or break with a hammer increased and the microstructure showed that the protein matrix was coarser. The force to deform or work to cut cheese analogues depended on the composition. The work to deform and break whey protein gels depended on the composition, microstructure and testing direction. In general, gels containing more  $\beta$ -lactoglobulin were less easy to break and formed tighter gels. Microscopy of surfaces formed by impact fracture indicated that the combination of cutting and cracking may vary between samples.

Introduction

Food texture broadly describes the consumer's mouthfeel. The handling properties, especially the ease of cutting, packaging and transport, are also important in practice. The texture of a food must result from its composition and microstructure.

The various components of the physical properties of a food can be estimated directly by subjective sensory means, but instrumental measurements are preferable as they are easier to standardize and reproduce and require fewer trained people. Instrumental measurements are also potentially more useful as they can generate absolute rheological data provided care is taken to understand the types of forces exerted and the outputs obtained. Such data can be used to interpret the mechanics of the structures.

Other types of instrumental measurement are really useful only if they can be directly correlated with some sensory or structural feature of the food. This applies to a number of empirical measurements of individual foods (Bourne, 1982). However, to be of general application, measurements should probably reflect the actual circumstances of consumption. This may involve cutting and certainly involves mastication, in which food is usually deformed rapidly under a high force and to beyond the point of failure. In such instances, the texture may depend on the fracture mechanism (Jowitt, 1979; Hamann, 1983).

The deformation of three types of cheeses under equilibrium conditions has been studied by applying an oscillating force of known frequency (Taneya et al. 1979). The relaxation times became larger as the cheeses matured, reflecting the formation of a rigid structure. Processed cheeses had a relaxation time distribution extending to shorter times, reflecting the weaker network structure. As heat-denatured protein gels are less brittle than cheese, conventional compression measurements are often made under equilibrium conditions, so providing useful structural data. More rapid aggregation of the protein tends to result in microstructures in which the strands and pores of the network are

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Initial paper received February 15 1986  
Manuscript received May 15 1986  
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**Key Words:** Cheese, cheese analogues, whey protein gels, scanning electron microscopy, deformation, fracture

coarser (Tombs, 1974) and firmer, less elastic textures in compression (Hermansson, 1982). For soy protein gels, the peak force measured by compression could be related to the number of linkages and the evenness of the gel observed by SEM (Furukawa et al., 1979).

When hard cheese is compressed to the point of failure, the sample cracks vertically (Culioffi and Sherman, 1976) or obliquely (Green et al., 1985). However, the mechanism of fracture of Cheddar and Cheshire cheeses in compression is clearly different from that in cutting or biting (Green et al., 1985). The cracks occur between the grains in compression, but are mostly through the grains when the cheese is cut or bitten.

The present paper describes preliminary work extending that of Green et al. (1985). Both fracture and reversible deformation of cheeses, cheese analogues and protein gels have been considered in relation to their compositions and microstructures. The work has included microscopic examination of fracture surfaces.

#### Materials and Methods

##### Samples

Cheddar cheese used for the examination of fracture surfaces was mild Irish Cheddar obtained from a commercial source, and probably matured for less than 6 months. Experimental Cheddar cheeses were made from milks ultrafiltered (UF) at 50°C at their natural pH to increase the concentration of fat and protein by 2.67- and 3.14-fold, and then heated in a flow-through plant at 90°C for 15 s. Conventional cheesemaking methods were used, except that the starter and rennet levels were adjusted to give a pH decrease and a coagulation time equal to the control. The cheeses were ripened for 5 months at 13°C before assessment. Processed cheese analogues were made by mixing skimmed milk powder (SMP), sodium caseinate and lactic acid to a final pH value of 5.7 with an emulsion of butteroil in water stabilised by Tween 80 at 45-60°C. Air was removed by vacuum treatment and the mixture poured into moulds. These were placed at 5°C after 30 min and held overnight before sampling. The components of the mixture were adjusted so that only the fat and water concentrations varied significantly during the experiment. Heat-denatured protein gels were prepared from whey protein powders, produced by ion exchange (Skudder, 1983). These were dissolved in water at pH 6.5, poured into 15 mm (i.d.) glass tubes and heated at 80°C for 10 min. The gels were cooled to room temperature prior to testing.

##### Determination of chemical compositions

Fat, moisture, salt and pH in cheeses and cheese analogues were determined as described by Green (1985). The protein compositions of whey powders were determined as described by Langley et al. (1986).

##### Scanning electron microscopy (SEM)

Samples which were prepared conventionally

for SEM were fixed for 2 h in 3% glutaraldehyde adjusted to pH 7.2 with 0.1M phosphate buffer. They were washed several times in phosphate buffer and post-fixed for 1.5h in 1% OsO<sub>4</sub> at pH 7.0. After dehydration in acetone, specimens were critical point dried, attached to aluminium stubs and coated with gold in an Edwards S150 sputter coater. They were then examined in a Hitachi S 570 scanning electron microscope.

Some samples were examined by SEM whilst still hydrated, using an Emscope cryopreparation unit and a low temperature microscope specimen stage. Specimens were frozen by rapidly plunging into nitrogen slush which had been prepared by evacuating a closed container of liquid nitrogen. They were then sputtered with gold and examined in the electron microscope using an accelerating voltage of 1-5kV. Where indicated, uncoated frozen samples were etched at -40°C using a specimen stage heater whilst observed with the microscope. When sufficient water had been sublimed, the specimen was cooled to liquid nitrogen temperature, coated with gold and re-examined.

Since the electron scattering properties were poor compared with their secondary electron emission, TEM of whey protein gels did not provide adequate information and thin sections of these specimens were examined by SEM. Gels were fixed as described above and were then embedded in Araldite. Sections (1 µm thick) were cut using a glass knife and a Reichert OmU2 ultramicrotome, mounted on coverslips and the Araldite removed using ethyl alcohol saturated with sodium hydroxide as described by Brooker and Wells (1984). Sections were critical point dried, mounted on stubs and coated with gold before examination in the microscope.

##### Light microscopy and transmission electron microscopy

Light micrographs, magnification x 320, were prepared from rapidly frozen cheese samples sectioned and stained with carbolfuchsin. The mean coarseness of the matrix of pairs of micrographs was determined by counting the number of times any line on a 28 x 19 rectangular grid was visible through any hole in the network (Green et al., 1981). Transmission electron micrographs, magnification x 7800, were prepared from 60-90nm sections of cheese samples fixed in glutaraldehyde, post-fixed in 1% OsO<sub>4</sub>, stained in 1% uranyl acetate, dehydrated and embedded in Araldite. The fat/protein interfacial area was determined stereologically as the mean from 3 micrographs using a 16 x 11 line rectangular grid (Green et al., 1981).

##### Determination of mechanical and sensory properties

Impact testing was carried out at 10 ± 3°C using the Charpy pendulum method with a wedge-shaped hammer. For the cheeses, the hammer energy required to fracture the sample was determined. For gels, the average velocity of the hammer on contact with the sample was

about  $0.7 \text{ m s}^{-1}$ . The impact strength was determined by calculating the energy absorbed per unit distance of penetration of the sample.

All other mechanical tests used an Instron food tester (Table Model 1140 at IFR or 4202 in Biomechanics Group). The stiffness of cheese was determined by compressing 15 mm cubes in triplicate to a point where linearity of response just disappeared (i.e., the onset of a yield) then cycling the displacement between that point and zero until the results were repeatable (i.e., all plastic deformation had disappeared). Some 50% hysteresis remained. The strain to this yield was about 2% at  $0.033 \text{ mm s}^{-1}$ . The compressive Young's modulus was estimated from the initial slope of the stable loading curve. The work of fracture of the experimental cheeses was determined in triplicate as the area under the force/distance curve when 10 mm cubes were cut with a 25  $\mu\text{m}$  diameter tungsten wire at  $0.083 \text{ mm s}^{-1}$ . For cheese analogues, the work of fracture was determined using 20 mm wide blocks in sextuplicate cut at  $0.083 \text{ mm s}^{-1}$  by tungsten wires 25–100  $\mu\text{m}$  diameter tensioned to constant stress. The Young's modulus and the stress at fracture were estimated from the initial slope and the first peak of force/distance curves of axial compression of individual cylinders between flat plates at  $0.83 \text{ mm s}^{-1}$ . A total of 16 cylinders (17 mm diameter x 15 mm high), 8 cut so the cylinder axis would have been vertical in the moulded sample and 8 cut so the cylinder axis would have been horizontal were tested. For compression testing, whey protein gel cylinders were cut into 15 mm lengths and compressed between plates coated with fine grade emery cloth at  $0.83 \text{ mm s}^{-1}$  in quintuplicate. The Young's modulus and stress at fracture were determined as for the cheese analogues. Tensile testing was carried out as described by Langley et al. (1986).

The elastic moduli of whey protein gels compressed along or perpendicularly to the cylinder axis were determined from the equations:

Along the axis:

$$h/L = h_0/E\pi a_0^2 - h/E\pi a_0^2 \quad (1)$$

$$\text{Perpendicularly to the axis:} \quad \Delta D = \quad (2)$$

$$\left(4p/3\right) \left[ (1-v^2)/\pi E \right] + 4p \left[ (1-v^2)/\pi E \right] \ln \left[ (DE)^{1/2} / 1.075p^2 \right]$$

where  $h_0$  is the initial length,  $h$  is the vertical displacement at an applied load  $L$ ,  $a_0$  is the initial radius,  $E$  is the elastic modulus,  $D$  is the initial diameter,  $\Delta D$  is the change in diameter,  $P$  is the applied force per unit length of cylinder and  $v$  is Poisson's ratio. Equation (2) is taken from Roark (1965) and  $\Delta D$  was determined from photographs during compression.

Sensory firmness and graininess of cheeses were assessed by panels as described by Green (1985).

### Results

#### Cheddar cheese fracture surfaces

Green et al. (1985) used critical point dried specimens to examine the surfaces formed

when Cheddar cheese was fractured in various ways. Here the two parts formed when the sample was fractured have been prepared for microscopy in different ways, so as to compare the structures observed on the complementary surfaces. One surface was frozen and etched and the other critical point dried. Some fat globules can be differentiated on the critical point dried surface but not on the frozen surface (Fig. 1). In 4-point manual breaking, in which fracture results in irregular surfaces with tearing and stepped cleavage planes, these are observed using both preparation methods. Conversely when the sample was cut, the surfaces prepared by both methods were relatively smooth.

#### Cheddar cheeses made from heated, UF-concentrated milks

Cheddar cheeses were made from milks which had been concentrated by UF then heated at  $90^\circ \text{C}/15 \text{ s}$ . As the concentration factor (CF) was increased the moisture-in-non-fat solids and fat-in-dry matter contents of the cheeses decreased (Table 1). At 5 months of age, the degree of proteolysis in the experimental cheeses was comparable with the control. The microstructures differed from the control in that the protein networks increased in coarseness (Fig. 2), with a corresponding decrease in the fat/protein interfacial area, as the CF increased (Table 1).

The stiffness, or resistance to reversible deformation, was higher at  $18^\circ$  than  $24^\circ \text{C}$ , consistent with some melting of the fat over this range of temperature (Table 1). Comparison of the changes in stiffness and sensory firmness with CF indicate that these 2 measurements are related to each other but not directly to composition. The energy required to cut the cheese with a wire, the work of fracture, and the force required to break a cylinder, the impact force, both increased with CF. It seems probable that these measurements do relate to the microstructure, and possibly also the composition of the cheese. The requirement for more energy to fracture a coarser network is in accord with general fracture mechanics theory (Kinloch and Young, 1983).

However, the impact fracture surfaces of the 3 cheeses appear to be very different (Fig. 3). The control cheese was indented by the hammer for some 7 mm into the sample. Beyond this, the sample was cracked, with an irregular surface. The cheese from the 2.67-fold concentrated milk was indented for about the first 6 mm of penetration, though some pieces may also have been knocked from the surface. The further 2 mm penetrated by the hammer appeared to cause irregular cracking and loss of material. Beyond this, the sample showed a very irregular surface suggesting that cracking had occurred both round and through cheese grains. The cheese from the 3.14-fold concentrated milk was possibly cut for about the first 5 mm of penetration (not shown in Fig. 3). The hammer penetrated for about a further 1 mm, and in this section and beyond it

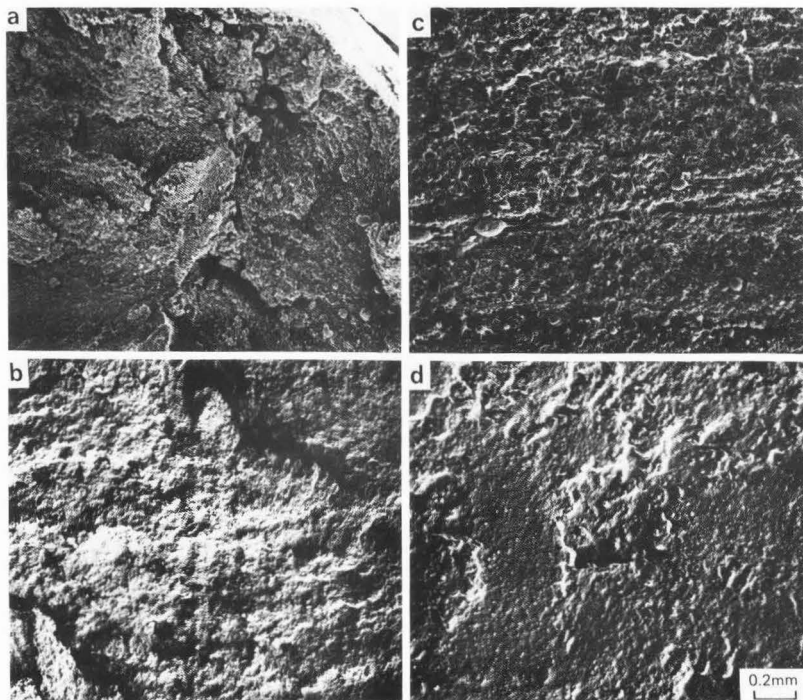


Fig. 1. Comparison of critical point drying and etching after freezing for examination of Cheddar cheese fracture surfaces. a, c, critical point dried; b, d, etched after freezing; a, b, broken manually; c, d, cut. In each instance the fracture was from top to bottom of the micrograph.

appeared that the sample was mostly cracked along grain boundaries rather than through the grains.

#### Cheese analogues

Processed cheese analogues with 5-20% fat and 50-60% moisture in non-fat solids (MNFS) have been prepared by mixing SMP and sodium caseinate into a butteroil emulsion. The structures were not stable to fixing by glutaraldehyde followed by critical point drying, so SEM samples were examined frozen with etching to a constant appearance. The microstructure of a surface of the 5% fat analogue prepared by cutting with a wire is shown in Fig. 4. The surface was fairly smooth, comparable to that in Cheddar cheese prepared in a similar way.

The Young's modulus and the stress at fracture of analogues varying in composition were measured by compression applied at a

medium rate. Both properties were highly correlated with a model containing both fat and MNFS contents ( $P < 0.001$ , >80% of variance accounted for). The effects of composition are shown in 3-dimensional graphs (Figs. 5 and 6). The Young's modulus decreased as the fat content was increased at low MNFS (Fig. 5). However, at low fat levels, increasing MNFS caused a steady decline in Young's modulus. There was a central region, 13-20% fat and 50-60% MNFS, where the Young's modulus hardly altered. The stress at fracture was high at low fat and MNFS and decreased as either parameter increased (Fig. 6). However, above 10% fat and 55% MNFS, further change in composition had little effect on the stress at fracture.

For cheese analogues, the work to fracture was determined using wires of a range of diameters. The work done increased linearly

Mechanical properties, composition and microstructure

Table 1  
Mechanical and structural properties of 5 month-old Cheddar cheeses made from heated, UF-concentrated milks

	Control	Concentrates heated 90°C/15 s x 2.67                      x 3.14	
<u>Composition</u>			
% Moisture in non-fat solids	57.3	56.6	54.9
% Fat in dry matter	56.9	49.1	41.2
% Salt in moisture	4.2	4.6	4.2
pH	5.1	5.4	5.2
<u>Microstructure</u>			
Fat/protein interfacial area/unit vol., $\mu\text{m}^{-1} \times 10^3$	9.1 <sup>a</sup>	6.7 <sup>a</sup>	4.2 <sup>b</sup>
Coarseness of protein network	2.5 <sup>c</sup>	3.8 <sup>d</sup>	4.6 <sup>d</sup>
<u>Sensory properties</u>			
Firmness	-0.2 <sup>e</sup>	-0.4 <sup>e</sup>	1.1 <sup>f</sup>
Graininess	-0.1 <sup>g</sup>	1.3 <sup>h</sup>	2.4 <sup>i</sup>
<u>Mechanical properties</u>			
Stiffness at 18°C, $\text{kNm}^{-2}$	640	600	1070
Stiffness at 24°C, $\text{kNm}^{-2}$	370	320	640
Work of fracture, $\text{Jm}^{-2}$	13	17	32
Impact energy to break, J	0.33±0.06	0.57±0.08	0.79±0.08

Superscripts: Values for microstructure and sensory properties having different superscripts differ with a probability of at least 5%.

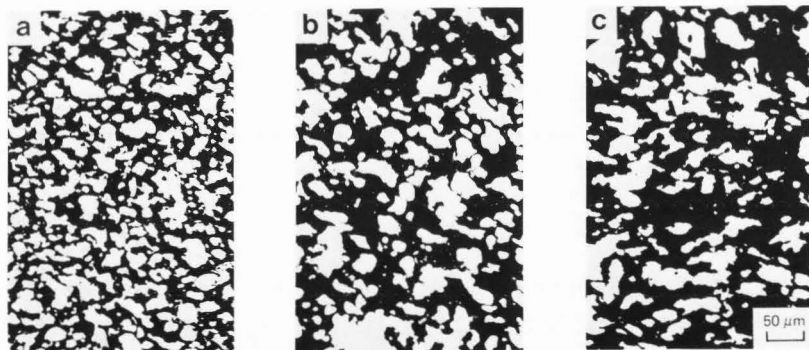


Fig. 2. Light micrographs of 5 month-old Cheddar cheeses made from heated, UF-concentrated milks stained for protein. a, control; b, milk concentrated x 2.67 and heated 90°C/15 s; c, milk concentrated x 3.14 and heated 90°C/15 s.

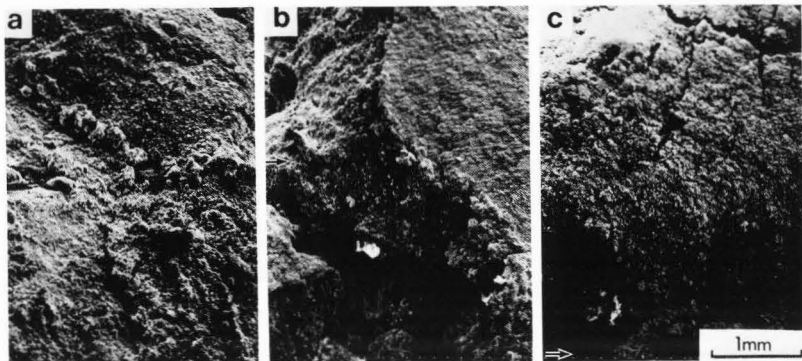


Fig. 3. Scanning electron micrographs of critical-point dried samples of 5 month-old Cheddar cheeses made from heated, UF-concentrated milks showing part of surfaces fractured in impact tests, where the fracture travelled from bottom to top. a, control; b, milk concentrated x 2.67 and heated 90°C/15 s; c, milk concentrated x 3.14 and heated 90°C/15 s;  $\rightarrow$ , level of penetration of hammer.



Fig. 4. Scanning electron micrograph of surface of 5% fat cheese analogue cut with a wire. The sample was frozen and etched. The sample was cut from top to bottom.

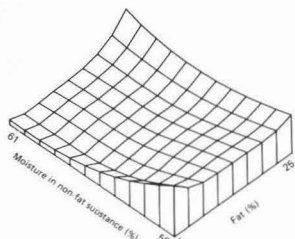


Fig. 5. Three-dimensional graph of the variation of the Young's modulus in compression with the composition of cheese analogues.

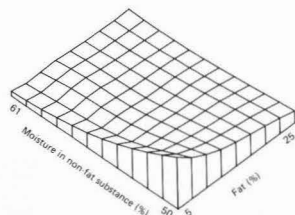


Fig. 6. Three-dimensional graph of the variation of the stress at fracture in compression with the composition of cheese analogues.



Table 2:  
Energy to cut cheese analogues containing 50% MNFS but varying in butterfat content

Fat content %	Work of cutting $\text{Jm}^{-2}$	Work of deformation, $\text{Jm}^{-3} \times 10^{-4}$
5	7.5	0.17
12	4.4	0.12
20	2.0	0.13

with wire diameter. This is consistent with a model where the work to fracture is made up of 2 terms, the work used in deforming the sample and the work involved in creating the two new surfaces on either side of the cut (Atkins and Vincent, 1984). The first term would be some function of wire diameter and the second would be a parameter of the material. Thus, the work to make an infinitely thin cut through the cheese could be determined by projection to zero wire diameter. Using such a projection, it was found that both the work to cut a sample and the work to deform it decreased as the fat content increased (Table 2). This is presumably because the fat is softer than the aqueous matrix.

#### Heat-denatured protein gels

Whey protein gels, formed as cylinders, were tested to failure in compression and tension. In compression, strong samples failed by cracking obliquely forming a shear fracture. In tension, failure occurred across the sample forming a convex and a concave surface. The irregular stepped fracture surface (Fig. 7) indicated that failure occurred across a weak part of the protein network (Beachem, 1968).

The composition of the whey protein powder determined both the microstructure (Fig. 8) and the mechanical properties of the heat-denatured protein gel (Table 3). The cracks and voids in the microstructure are probably artefacts of the preparation. There were relatively few in Gel 35 compared with the other gels, presumably reflecting the high tensile strength. The tight, homogeneous matrix of Gel 12 could have been the cause of the relatively high resistance to compression. Gel 19 was stronger in compression than Gel 35, presumably because part of the matrix was tight and compact.

For powders containing less than 2%  $\alpha$ -lactalbumin, the tensile strength and impact force to fracture increased with  $\beta$ -lactoglobulin concentration (Fig. 9). The fracture surfaces resulting from impact differed between gels of different composition and across the gels (Fig. 10). Both surfaces were irregular, without evidence of tearing indicating that they were formed as a result of



Fig. 7. Scanning electron micrograph of the concave fracture surface of a whey protein gel failed in tension.

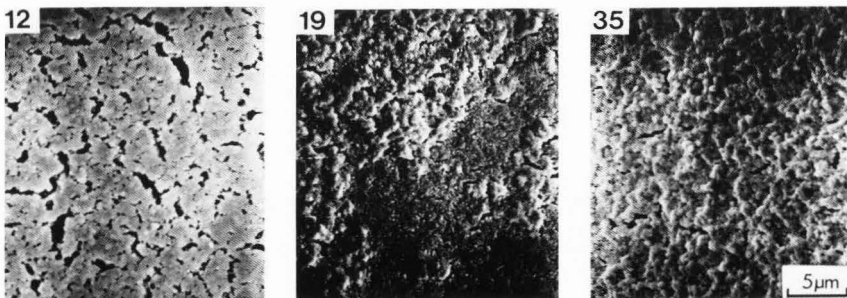


Fig. 8. Scanning electron micrographs of the 15% whey protein gels described in Table 3.

Table 3:  
Composition and mechanical properties of 15% gels formed by heating whey protein solutions

Powder	12	19	35
<u>Composition %</u>			
$\alpha$ -lactalbumin	0	14	9
$\beta$ -lactoglobulin	70	40	55
Casein-derived protein	30	46	36
<u>Mechanical properties, kNm<sup>-2</sup></u>			
Tensile strength	9.5 $\pm$ 1.5	9.4 $\pm$ 1.6	20.3 $\pm$ 1.2
Stress at fracture in compression	91.2 $\pm$ 14.3	43.2 $\pm$ 1.3	27.5 $\pm$ 1.4
Young's modulus in compression	24.0 $\pm$ 1.2	10.0 $\pm$ 0.6	3.0 $\pm$ 0.4

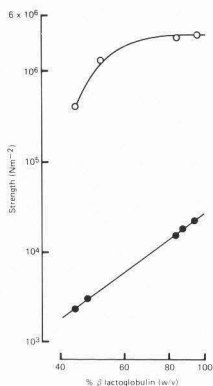


Fig. 9. Tensile strength (●) and impact force to fracture (○) of 15% gels made from whey protein solutions containing  $\beta$ -lactoglobulin and casein derived protein only.

cracking rather than cutting. Both gel surfaces contained craters, presumably originally containing ungelled liquid. These tended to be larger in Gel D than in Gel A, presumably because Gel D contained the larger proportion of casein-derived protein. Samples containing entirely casein-derived protein did not gel at all. Such an explanation probably explains the more open structure of Gel D compared with Gel A.

The craters were concentrated in the centre on both gel surfaces, the parts nearer the outside having a tighter, more homogeneous network structure. This is probably because there was a temperature gradient from the outside towards the inside, so that setting occurred first on the edge and the non-gelling material was concentrated in the centre. The anisotropic nature of the gel cylinders is

shown by the difference between the elastic moduli when they are compressed along or perpendicularly to the cylinder axis (Fig. 11). This is consistent with a tighter, more rigid structure on the outside of the gel cylinder, such as observed on a radial motor tyre.

#### Discussion

A well-known problem in many kinds of microscopy is that specimen preparation methods may generate artefacts. This could well apply to the examination of surfaces formed in a hydrated state and observed after drying, as was carried out by Green et al. (1985) on fractured cheeses. In the present work, similar surfaces were examined in the hydrated state by a cryo technique. Although this did not give as much detail as the dried specimens, the general structure and regularity of the complementary surfaces was comparable, indicating that critical point drying did not generate excessive artefacts.

Further, the cryo technique was essential for the examination of fracture surfaces in cheese analogues, since the specimens were not stable to fixing and drying. The technique is also likely to be useful for delicate, highly hydrated specimens such as protein gels, although attention will need to be paid to the freezing rate to avoid distortion of the network (Hermansson and Buchheim, 1981).

When Cheddar cheeses were subjected to impact testing, the surfaces formed suggested that the structures had failed in different ways. Thus, the fracture mechanisms varied across the range of samples, so the impact energies were not strictly comparable and could not be used to give information on the variations in microstructure. Further, failure must occur at a weak plane, which can be through or round the grains in the case of cheese. Thus, the surface formed is not necessarily typical of the material as a whole. These observations suggest that it is important to take account of the method used to measure fracture properties when the results of failure tests on different materials are

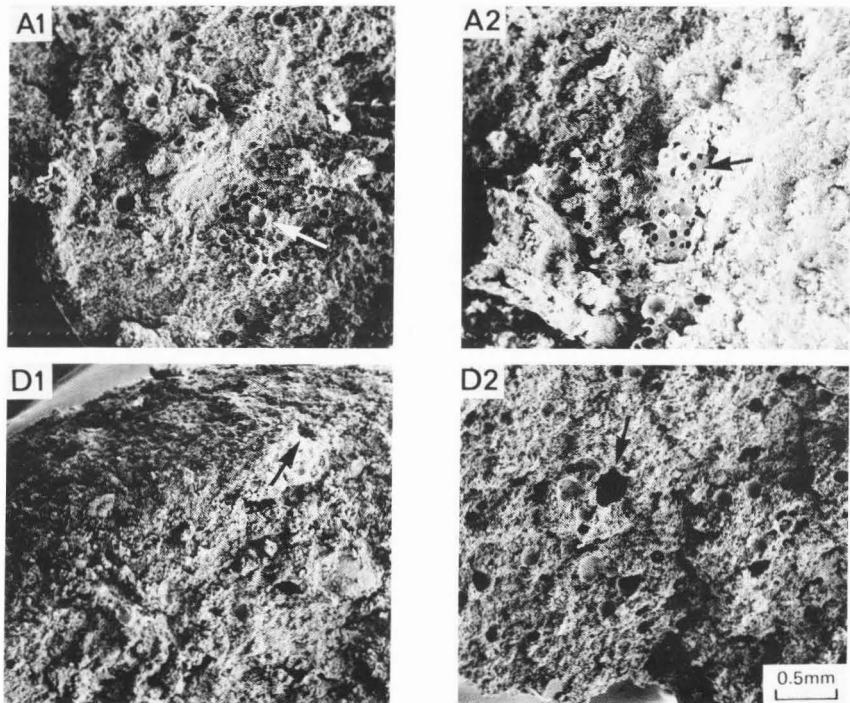
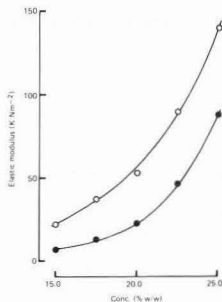


Fig. 10. Scanning electron micrographs of impact fracture surface of 15% whey protein gels at 2 points across the surface. The whey protein powder for Gel A contained 83%  $\beta$ -lactoglobulin + 17% casein-derived protein and that for Gel D contained 44%  $\beta$ -lactoglobulin + 56% casein-derived protein. The direction of fracture was from left to right.  $\rightarrow$ , crater. Surface locations: 1, at edge where impact occurred; 2, in centre.

compared.

The results obtained here suggest that controlled cutting of a sample with a wire may be a useful mechanical property to study. The morphology of the surface created confirms that the plane of cutting was not dependent on weak planes within the sample. The work with cheese analogues reported here and that of Emmons et al. (1980) with experimental cheeses suggest that the results are simple to interpret and can be related to the composition of the sample. However, only rather slow rates of cutting appear to have been used so far in work with foods. This may be useful in relation to

Fig. 11. Elastic moduli of gel cylinders of the concentration of whey protein shown compressed along (●) and perpendicular to (○) axis of cylinder.



handling of the materials, but is unlikely to provide good simulation of biting. For this, cutting rates of the order of 20 mm s<sup>-1</sup> are likely to be required (Bourne, 1982), which could raise problems not found at lower rates.

Provided there is no failure of the specimen, equilibrium deformation properties would be expected to be closely related to both the composition and the microstructure. However, the results with all the types of samples tested here indicates that the relationships were not simple, though they tended to follow the expected trends. Hermansson (1982) made a similar observation finding that whey protein gels having a denser, more aggregated structure tended to be firmer.

#### Acknowledgements

The authors thank Dr A.C. Smith of AFRC Institute of Food Research, Norwich Laboratory, for assistance with the use of his impact tester, Misses R. Oakman and E.C. Pope and Messrs J.C.D. Taylor and P. Dunthorne for skilled technical assistance and Mr E. Florence and his staff for gross chemical analyses.

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#### Discussion with Reviewers

Reviewer 1: The authors discuss surfaces of cheese, cheese analogues and whey protein gels. If the surfaces were created prior to preparation by critical point drying and freezing the question arises how the surface structure was affected by the various steps during preparation?  
Authors: We have no definitive answer. However, the complementary fracture surfaces prepared in the two ways and shown in Fig. 1 are recognizably similar, suggesting that the structures were not too much affected by the preparation.

Reviewer 1: The results mainly indicate that impact fracture could result in a combination of cutting and cracking due to the conditions during fracture as well as to the sample tested. Will it be possible to explain the relationships between the different mechanical properties of the samples and variations in their microstructure by work at higher magnifications?

Authors: We have not found this very useful. A general appearance of the whole fracture

surface has enabled us to detect the various characteristics of the different types of failure. At higher magnification the details of the structure tend to obscure the major features. However, there is obviously the possibility of gaining information about specific features by examination at a range of magnifications.

D. E. Carpenter: You mention that the degree of proteolysis was the same in control and experimental cheese. How was this assessed? Since less coagulant is added for the concentrated milk cheese, isn't this unexpected? (Green, 1985).

Authors: Proteolysis was assessed by polyacrylamide gel electrophoresis, measuring casein disappearance, and by concentration of trichloroacetic acid - soluble N, measuring peptides and amino acids. Less coagulant was used for concentrated milk cheese than for controls, but more than for unheated concentrates. As the proportion of cheese: whey is increased by the use of concentrates, a higher proportion of coagulant may be retained in the curd than for the controls. It seems that the differences approximately balanced out in the present instance.

D. E. Carpenter: Could microstructural coarseness be quantified with units for frequency of troughs/peaks so that data could be related from various papers?

Authors: Yes, but what constitutes a peak or a trough, since there are variations in the background stain level across light micrographs? This method has been tried (Green et al., 1981) but is no quicker and less widely applicable than the method used in the present paper. Neither method is absolute, so the data are necessarily comparative. However, the ratios of two values should be the same regardless of the quantitation method used.

D. E. Carpenter: The composition and pH of cheese made from concentrated milk must play a large role in texture, i.e., x 2.67 cheese has a pH of 5.4, and the x 3.14 cheese has a low fat content which must contribute to its firmness.

Authors: Yes. In cheesemaking experiments it is difficult to precisely control composition, especially when using modified milks. The main purpose of using cheese analogues was to control composition and reduce the variables resulting from the use of biological materials.

D. E. Carpenter: What effect would the 90°C, 15s milk heat treatment have on the textural properties of the experimental cheese? One would expect some whey protein denaturation at this temperature with resulting textural/microstructural changes.

Authors: This is under investigation currently.

D. D. Hamann: Many of us are not very experienced with the interpretation of SEM photographs of foods as related to texture.

Has the field developed to the point that a systematic approach of what to look for has been established for specific foods such as Cheddar cheese? If not can the start of such a system be based on the results of this paper?

Authors: We now have considerable information on some of the structural influences on the texture of cheese. The distribution of fat and protein clearly affects some aspects of mouthfeel (Green et al., 1981), and their orientations influence some rheological properties (Taranto, MV, Wan, PJ, Chen, SL, Rhee, KC. (1979) Scanning Electron Microsc. 1979; III: 273-278). Incomplete fusion of the protein matrix appears to cause crumbliness in Cheshire cheese (Marshall, RJ (1986) J. Dairy Res. 53: 313-322). The degree of interaction between curd particles at curd junctions, which probably relates to the presence of cracks and voids, influences the uniformity of Cheddar cheese body (Lowrie, RJ, Kalab, M, Nichols, D. (1982) J. Dairy Sci. 65: 1122-1129). However, most of this information is qualitative, and we really have no means yet of assessing the degree of importance of different structural factors. The problem is exacerbated by the difficulty of making useful measurements of cheese texture.

M.A.Tung: In the small strain compressive tests of Cheddar cheese cubes the loading curves were said to be linear while repeated loading and unloading curves exhibited about 50% hysteresis which implies that the curves form a closed loop with the area under the unloading curve being half the area under the loading curve. How would you explain the mechanism of this behaviour based on the observed microstructures of these cheeses?

Authors: We would suppose this behaviour is due to the viscous and elastic components of both the matrix and the fat. Both phases would be expected to deform to a limited extent, the deformation of the fat phase depending on the ratio of liquid to crystalline components. This could be checked directly by comparing the microstructure before and after such deformation, and it would seem a good idea to do this.

Reviewer 1: In the "Materials and Methods" section the authors state that "Since the electron scattering properties were poor...TEM of whey protein gels did not provide adequate information and thin sections of these specimens were examined by SEM." It is difficult to understand why primary transmitted electrons did not give adequate information with regard to whey protein gels. Thin sections of whey protein gels generally give good contrast and these sentences are therefore better omitted.

Authors: Sections do give good contrast but the images obtained are probably from only part of the total organic matrix. The scattering power depends on the attachment of electron dense atoms to protein molecules. There is no guarantee that this will occur in such a way as to render the entire molecule visible. TEM of protein gels suffers from this. The secondary electron emission, especially after coating with gold, is from the entire organic matrix.

as in the original cream sample. In contrast, a cream having a coffee stability of 55°C (cream sample No. 4 after 2 months of storage) showed a pronounced aggregation of fat globules when added to a coffee solution of 45°C (Fig. 8).

Further information on the fine structures of large floccules of cream is obtained by applying the freeze-deep-etching technique. This preparatory modification requires, however, the isolation of cream floccules and the removal of soluble constituents (e.g. by washing with distilled water). Figs. 9a and 10a give representative views of parts of large floccules which formed in a coffee solution of 90°C with those 2 cream samples which have already been compared above at 45°C, i.e. sample No. 6 (Fig. 9) and No. 4 (Fig. 10). Although both samples differ in their coffee stability values (No. 6: 80°C and No. 4: 55°C) the overall appearance of the fine structure of the floccules is similar. It is a network of strongly aggregated fat globules consisting of strands of globules of approx. 1  $\mu$ m in thickness. It appears as if the individual globules have largely retained their original size, i.e., that destabilization phenomena occur at least rarely. At higher magnification the fine structure of the interfacial protein layer can be inspected in more detail (Figs. 9b and 10b). The globules appear to be predominantly covered, similar to the original cream, by a smooth and very thin protein film to which occasionally somewhat larger protein aggregates ('casein micelles') are attached. Pronounced differences between the 2 samples under study could not be detected with the experimental conditions used.

#### Creaming phenomena

The formation of compact layers (plugs) at the top of a cream sample during storage is an only rarely occurring phenomenon. Such layers are highly undesirable because they will generally not disperse when the cream is added to a coffee solution. Electron microscopic examination of such cream plugs of one cream sample revealed that they consisted of very densely aggregated fat globules (Fig. 11) and even contained larger particles of destabilized fat (Fig. 12). The cream sample showing this peculiar phenomenon exhibited a major degree of clustering of fat globules (Fig. 13) although the average size of individual globules was typical for a well homogenized product.

A pronounced clustering of fat globules in a homogenized cream may be a result of inadequate homogenizing conditions such as a non-optimum temperature or an imperfectly operating homogenizing valve. Furthermore the formation of cream layers during storage is facilitated by a low degree of homogenization, i.e., (volume - moment average) fat globule diameters ( $d_{43}$ ) higher than ca 0.7  $\mu$ m.

#### Sediment formation

The formation of a rather compact sediment layer is another undesired phenomenon which may occur, although rarely, in UHT-treated coffee

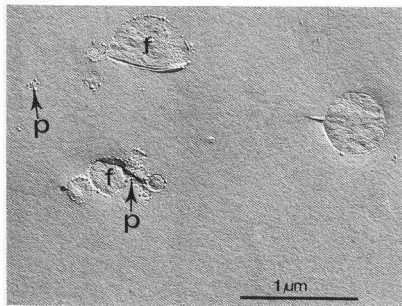


Fig. 7. Freeze-fracture preparation of a mixture of cream (No. 6; coffee stability value: 80°C) with a coffee solution of 45°C showing very little aggregation of fat globules (f); p: protein particles.

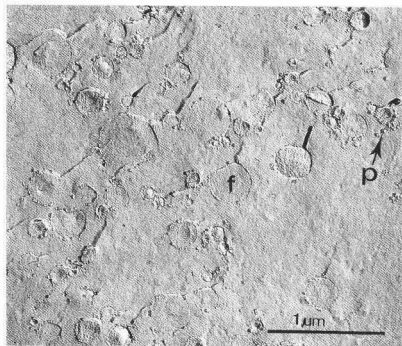


Fig. 8. Freeze-fracture preparation of a mixture of cream (No. 4; coffee stability value: 55°C) with a coffee solution of 45°C showing a pronounced, but visually not detectable aggregation of fat globules (f).

creams. According to our experience such sediments develop slowly and may not become visible until 1-2 months of storage but then increase during further storage. The sediment may consist of a solid layer, up to approx. 1 mm in thickness, which will not dissolve when the cream is added to hot coffee. In water (at room temperature) small pieces of such a sediment may remain intact even after intensive stirring.

Fig. 14 shows the typical fine structure of such a sediment layer. It consists mainly of comparatively large protein aggregates (up to 1  $\mu$ m in diameter) which are rather densely attached to each other. There are also many small protein particles present between these aggregates.

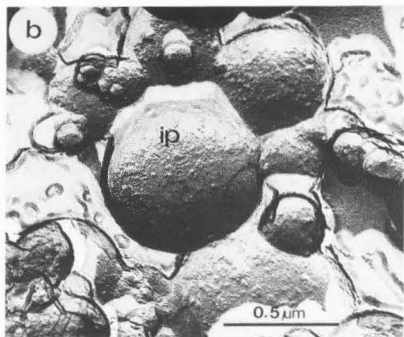
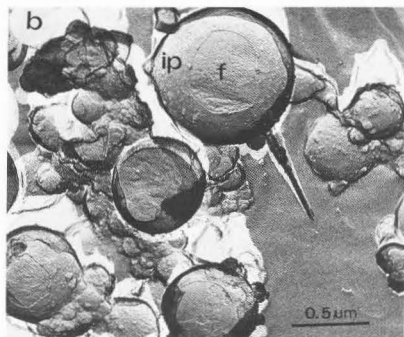
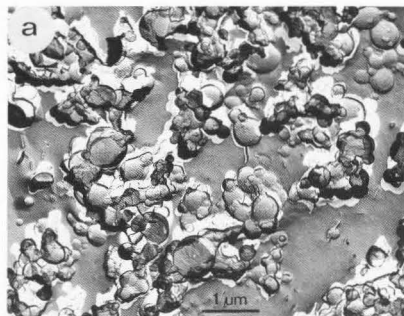
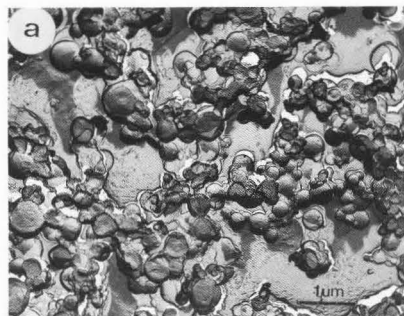


Fig. 9. Freeze-deep-etched floccules from a cream (No.6) after feathering. a: general views of the network of aggregated fat globules. b: detailed views showing the interfacial protein layers (ip). Sometimes the fat phase becomes partially exposed as a result of local cleavage during freeze-fracturing (f).

Fig. 10. Freeze-deep-etched floccules from a cream (No.4) after feathering. a: general views of the network of aggregated fat globules. b: detailed views showing the interfacial protein layers (ip). Sometimes the fat phase becomes partially exposed as a result of local cleavage during freeze-fracturing (f).

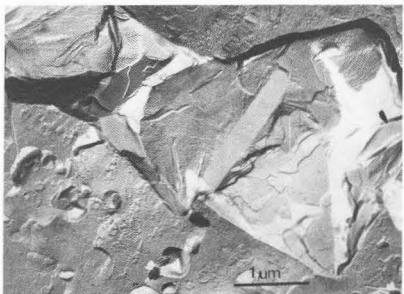
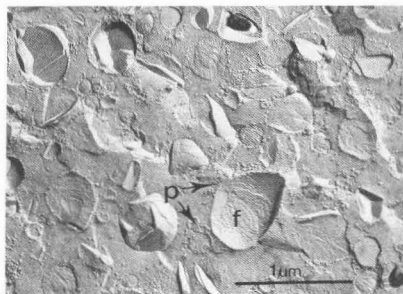


Fig. 11. Aggregated complexes of fat (f) and protein (p) of a cream plug.

Fig. 12. Destabilized fat within a cream plug.

The coffee cream sample which showed this peculiar sedimentation phenomenon had an elevated pH-value (pH 7.05). According to the information which could be obtained from the manufacturer, stabilizing salts (a phosphate-citrate mixture) had been added continuously to the cream immediately before it underwent the UHT-treatment (homogenization was done after the thermal treatment under aseptic conditions). Possibly such processing conditions favour the formation of large protein aggregates at the high temperatures ca 140°C occurring in the UHT plant. A direct injection of a highly concentrated solution of basic salts immediately before the heating step will probably not allow an equilibrium state of all milk salts to build up and may furthermore result in an uneven distribution of salts (and pH-value) within the cream flow in the heating section.

### Gelation

It is well known that UHT-treated, homogenized cream as well as UHT milk show gelation phenomena after prolonged storage. These phenomena are generally ascribed to the action of proteolytic enzymes which have not completely been inactivated during the heat treatment, and also to purely chemical mechanisms, such as the Maillard type of reaction (3, 9).

The viscosity measurements made from the 7 commercial coffee cream samples (Fig. 1) demonstrate that the pH-value of the cream appears to be an additional factor leading to a quicker gelation at higher pH-values.

Electron microscopical studies were made on two gelled samples of UHT-treated coffee cream (12 % fat) after a storage period of 10 months at room temperatures. Fig. 15 is from a product made without any stabilizing salts having a pH-value of 6.72. The gel consisted primarily of chains and loose aggregates of fat-protein complexes. Fig. 16 is from a product to which basic phosphates and citrates had been added resulting in a pH-value of 7.05. The fine structure of the gel is distinctly different, i.e. it appears to consist mainly of interconnected protein particles, smaller protein aggregates and, to a lesser extent, fat globules (fat-protein complexes). In some parts of Fig. 16 the small protein particles (subunits) appear to be arranged in small rows indicating that the formation of thin protein strands (fibres) play a major structural role for the gel network. The decisive differences between the gel structures of both cream samples may be explained by the differences in the protein-fat interaction. Whereas in the cream without stabilizing salts most of the protein (casein and denatured whey protein) is generally adsorbed to fat globules (compare to Fig. 2) the addition of pH-elevating stabilizing salts results in a disintegration of protein aggregates (casein micelles), a distinct decrease of protein adsorption and, consequently, an increase in 'free' (i.e. non-adsorbed) protein in the serum (compare to Fig. 3). This may be the reason that in the cream sample with the higher pH-value these 'free' protein particles are the major

constituents of the gel network whereas in the other cream sample, in which 'free' protein particles are comparatively rare, the fat-protein complexes interact and constitute the network structure.

### Conclusion

The results of the comparative studies on commercial UHT-treated coffee creams have shown that this type of product requires further research in order to develop optimum processing conditions. The resistance against feathering in hot coffee solutions appears to be still a major problem.

There is no doubt that various compositional factors (e.g. fat content, fat/protein ratio, mineral balance) and processing parameters (e.g. homogenization and heating conditions) influence the final physico-chemical properties. From the results obtained it appears the composition and structure of the interfacial layers of fat globules are mainly responsible for the extent of flocculation in hot coffee solutions. Therefore special attention will be given to this aspect during further studies.

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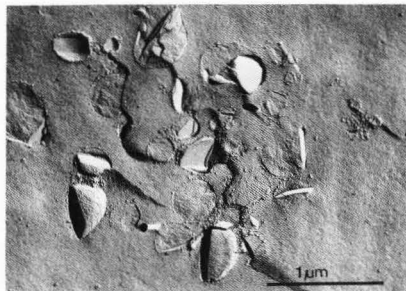


Fig. 13. Fat globule cluster in a cream sample which had formed a cream plug.

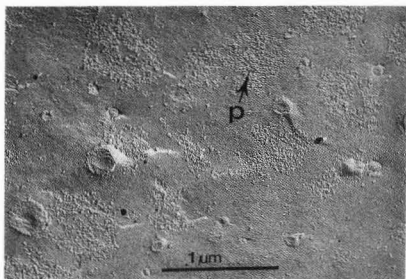


Fig. 14. Sediment layer from a coffee cream sample (pH 7.05) showing the presence of larger and obviously interconnected protein aggregates (p).

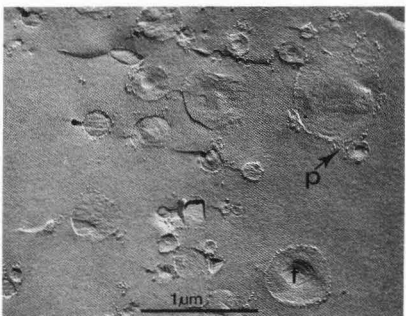


Fig. 15. Gelled coffee cream sample (pH 6.72). The network appears to be formed by loosely aggregated complexes of fat globules (f) and protein (p).

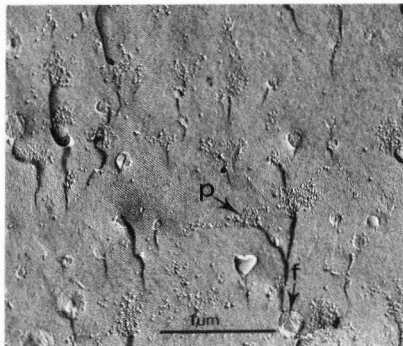


Fig. 16. Gelled coffee cream sample (pH 7.05). The network appears to consist primarily of loosely aggregated protein (p) and occasionally fat globules (f).

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#### Discussion with Reviewers

B.E. Brooker: As a result of these observations, I wonder if you would care to comment on possible mechanisms to explain the heat instability of the fat-casein complex in UHT cream. This appears to be one of the central questions in this problem.

Authors: Because of the numerous factors which have been found to be related to the resistance of a homogenized, UHT-treated cream against feathering it is still very difficult to give a satisfying explanation of this phenomenon.

I. Heertje: Is there not a danger that under the influence of the washing regime in the deep-etching technique a redistribution of protein occurs, leading to wrong interpretations? It may be argued that in particular larger aggregates attached to the interface layer have a chance to be removed (Ca concentration during washing is 0).  
Authors: It is justified to suggest that the washing in distilled water might eventually lead to a redistribution of protein. We are therefore trying to improve the washing procedure, e.g. by using at first buffered solutions and also to introduce a chemical fixation of the protein (by glutaraldehyde).

I. Heertje: Sediment formation and gelation appear to be related phenomena. Should you not consider the possibility that the difference in behaviour between the pH 6.6 and the pH 7.05 samples should be ascribed to the difference in the heat induced association of whey proteins (in particular beta-lactoglobulin) and casein micelles. At pH 7.05 there will be no interaction, whereas at pH 6.5 the interaction is nearly complete. This may lead to an enhanced possibility for interaction of caseins with itself at high pH and perhaps to an enhanced possibility for interaction of the beta-lactoglobulin-casein complex with the interfacial layer at lower pH. What is your comment?

Authors: It remains questionable whether sediment formation and gelation are related phenomena. It should be pointed out that the formation of unusually large protein aggregates which sedimented during storage (see Fig. 14) took place only irregularly at one manufacturer who added pH-elevating salts to his product. Normally, aggregated protein (casein micelles) disintegrates as a result of calcium complexing salts at pH-values above ca 6.9. The pH-dependent differences in the heat-induced association of whey proteins and caseins at low and high pH-values (6.5 v. 7.0) might well explain the observed differences in the rate of gelation.

W.G. Soucie: What was the time allowed for the coffee stability testing? Are you assuming that a lower temperature will slow aggregation but not stop it?

Authors: When a hot coffee solution is poured over the cream (or vice versa) the phenomenon of feathering occurs almost immediately. A pronounced increase in the average size of floccules does not take place in the mixture of coffee and cream except when larger floccules rise up to the surface (see Fig. 6). At a temperature lower or equal to the 'coffee stability' value visible flocculation will generally not occur also after a longer time.

W.G. Soucie: How did you determine that 15-20 min is long enough for glycerol to diffuse into the

fine structure areas of the aggregated samples?  
Authors: It is common experience that the diffusion of glycerol into small volumes of such types of samples is completed within such a period. An insufficient penetration of the cryoprotectant would result in freeze-artifacts, i.e., ice crystals which are easily detectable on the micrographs.

W.G. Soucie: The 'free' salts and protein are in equilibrium with the fat globule membrane. Did the washing procedure affect the fat globule membrane and was there any evidence of fat release?

Authors: There was no evidence that the washing procedure used resulted in a destabilization of fat globules. It appears, however, necessary to study the effects of washing on the structure of interfacial layers systematically in order to avoid misinterpretations.

W.G. Soucie: In your particle size determination how did you account for the fact that the fracture lines will slice through the spheres at different levels and not only at the maximum diameter? How do your fat globule sizes compare to published results using similar homogenization conditions?

Authors: The conversion of apparent (two-dimensional) size distributions into true (three-dimensional) size distributions is described in the literature (see e.g. Ref. 13). The fat globule sizes (e.g.  $d_{32}$  and  $d_{43}$ ) which have been found for the various cream samples compare well with published results for commonly used homogenization conditions (ca 200 bar).

W.G. Soucie: Addition of salts, changes in pH and temperature, all affect the zeta potential of particles and consequently stability to aggregation. How might your results be interpreted when considering the effect of these factors on the zeta potential of the fat globule surfaces?  
Authors: It appears as if the composition and structure of the interfacial protein layers, i.e. complexes formed by casein, denatured whey protein and calcium phosphate, are mainly responsible for the degree of stability of the homogenized creams under the acidic conditions of hot coffee solutions. Hence, the zeta potential of the fat globule surfaces might be an important factor. So far as I know zeta potential measurements of such systems have not yet been published.