Characterisation of Milk Proteins in Confectionery Products

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CONFECTIONERY PRODUCTS

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Abstract

The proteins from milk play an important part in the structural properties of confectionery products. These properties will in turn influence texture and flavour. Electron microscopy techniques have been used to examine milk chocolate, caramel and fudge and to locate and characterise the milk proteins within their structures. Following scanning electron microscopy examination of the products at low temperature, thin sectioning and freeze-fracture were used to resolve the fine ultrastructure of casein and whey proteins.

A chocolate prepared from milk crumb could be distinguished from one made with a dry milk powder on the basis of a more even distribution of protein and a high level of association between milk protein and other ingredients. Caramel was seen to be composed of an amorphous sugar support matrix containing numerous fat globules. Milk protein was found frequently associated with fat although the level of association depended on whether casein or whey protein was present.

The development of crystalline sugar in fudge caused the milk protein to concentrate within the remaining amorphous sugar regions. Fat/protein associations were not as obvious as those observed in caramel.

Introduction

The various constituents of milk form an important part of confectionery products. They are thought to impart characteristic texture, flavour and appearance to milk chocolate, caramels and fudge. The proteins in milk, although representing only a small percentage (3-4%) of its total composition are considered to possess unique structural and functional properties which will influence these final quality parameters as well as affecting processing. Of particular importance in confectionery are organoleptic, hydration and surface properties. These properties will differ depending on whether the casein or whey protein fractions of milk are considered.

The source and form of milk proteins available to the confectioner is increasing every year. In order to use them to their maximum effectiveness, there is a need to understand precisely, the roles they play in confectionery manufacture. One approach is through a study of their microstructure.

Microscopy has been applied mainly to the study of isolated milk proteins or to raw ingredients rather than the complex manufactured products. Brooker (1979) provides a good review of how light microscopy and electron microscopy has been used to study milk and a range of dairy products. A further review by Kalab (1981) outlines the techniques suitable for such studies with reference to both scanning electron and transmission electron microscopy (TEM). A number of workers have used SEM to study milk powders and Kalab (1979) gives a summary of the findings together with technique details. Spray dried powders, for example were seen as spherical in nature and frequently highly porous. Increased humidity turned the initial glassy appearance of the surface lactose into crystalline α-hydrate. TEM was used to examine similar substrates by Muller (1964) using thin sectioning and by Buchheim (1981) with a modified freeze-fracture technique. Both authors were able to discriminate between casein and whey milk proteins and to identify fat globules within the powders.

Buchheim and Knoop (1971) applied freeze-fracture techniques to examine chocolate and were able to identify specific features such as

fat, sugar and proteins. The fat in chocolate has also been studied by microscopy (Berger et al. 1979) and thin sectioning was used by Cruickshank (1976) to visualise cellular debris, protein and the position of sugar crystals within milk chocolate. In studies on the conching process of chocolate, Niediek and Babernics (1981) used SEM in examining particulates. Dimick and Hoskin (1981) describe some of the chemo-physical aspects of conching also using SEM in their studies which concerned mainly dark rather than milk chocolate. Of particular importance in milk chocolate manufacture is the distinction between milk crumb- or a dry milk powder-based chocolate. In the former, all ingredients are mixed in a liquid state and the mass dried to around 2% prior to mixing with fat. By comparison a powder chocolate contains ingredients which are dried independently and then together added to fat.

Scanning electron microscopy when used to examine caramels (DeBruin and Keeney 1973) indicated that sugar was the continuous support which contained fat globules and protein. Differences measured in mechanical values were reported to be related to water, protein and butterfat variables but could all be explained in terms of the influence each of these had upon the sugar phase.

The purpose of this study has been firstly to identify and compare the form and location of milk proteins in three different confectionery products. Secondly, to investigate how proteins from different milk sources and various processes have performed in the products. Following initial light microscopy studies, electron microscopy has been used to examine the proteins in milk chocolate, caramel and fudge. The higher resolving power of transmission electron microscopy, in particular thin sectioning and freeze-fracture techniques have been used to characterise the milk protein components within the structures.

Materials and Methods

Milk chocolate, caramel and fudge products were studied, together with their corresponding milk sources. Included were liquid and powdered forms of milk representing both casein and whey-rich fractions. All are commercially available as follows: sweetened condensed milk (SCM), skimmed sweetened condensed milk, full cream milk powder (FCMP) - sometimes referred to as whole milk powder, skimmed milk powders (SMP) representing low, medium and high heat treatments. Milk chocolates examined were milk crumb-based and dry powder products which incorporated full, skimmed or whey milk powders. Caramels were prepared with milk, vegetable fat and sugar cooked at 115°C to a final moisture of 10%. Fudges examined were commercial milk and chocolate fudge products together with their corresponding pre-mix material.

Light Microscopy

Sections of milk chocolate were cut at 20°C to a thickness of 8 μm using a 1130 Reichert microtome. Sections were picked up on glass microscope slides which were coated with gum Apathy as adhesive and allowed to equilibrate for one hour at room temperature.

Light Microscopy Staining

Eosin Y (yellow shade) was used as a general stain to locate protein in the milk chocolate samples. Sections were first defatted by immersion in 1,1,1-trichloroethane for five minutes. They were then stained in alcoholic Eosin (2%) for 30 seconds-1 minute. Following a wash with absolute alcohol, sections were mounted in Euparal and covered with a glass cover slip. Specimens were viewed by crossed polars with the inclusion of a 3rd order retardation filter. This allowed the examination of stained protein together with the birefringent sugar crystals.

Scanning Electron Microscopy (SEM)

Samples of chocolate, caramel and fudge were prepared for examination at low temperatures using SEM. Small cubes (3mm sides) of caramel and fudge were supported between two copper rivets prior to flash freezing in liquid nitrogen slush (-210°C). Preliminary studies indicated that freezing chocolates from ambient temperature resulted in fractures through the continuous fat phase only. It was necessary therefore to melt the fat in the chocolate to 40°C in order to reveal the particulate components. Once melted the samples were placed in pre-warmed rivets or support stubs before freezing.

Frozen samples were transferred to the preparation chamber of a Hexland Cryo-system attached directly to a JSM 35-CF SEM. Samples were held at -170°C and fractured using a microtome blade directly on the sample or by separation of the two rivets. Fractured surfaces were diode sputter coated with a layer of gold and transferred under vacuum into the specimen chamber of the SEM. Samples were examined on the pre-cooled stage (-180°C) and using an electron beam operating at 10 or 15 kV. An anti-contamination plate held at -196°C above the

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Figure 1. Freeze-fracture preparation of skimmed SCM showing aggregated casein particles (P) and, a fat globule (F) in an amorphous matrix. Bar = 200 nm.

Figure 2. Skimmed SCM showing aggregated casein particles (P), sugar crystal (S) and needle-crystals (N). Bar = 200 nm.

Figure 3 a-c. Thin section of skimmed milk powders a) low heat b) medium heat c) high heat - showing submicellar (arrows) and aggregated casein particles (P). Bar = 200 nm.

Figure 4. Light micrograph of milk crumb chocolate showing a homogeneous distribution of protein (P) and regular shaped sugar crystals (S). Bar = 50 μm.

Figure 5. Light micrograph of milk powder chocolate showing discrete, densely staining proteinaceous regions (P) and fractured sugar crystals (S). Bar = 50 μm.

Figure 6. Scanning micrograph of crumb chocolate. Outline of a sugar crystal (S) in close contact with remaining ingredients. Bar = 10 μm.

Figure 7. Scanning micrograph of milk powder chocolate showing clean crystals (S) and milk powder particles (MP). Bar = 10 μm.
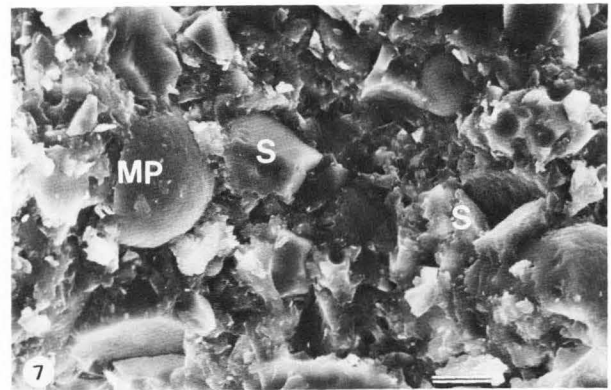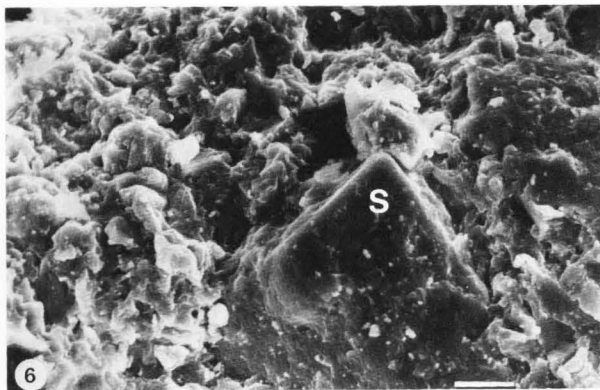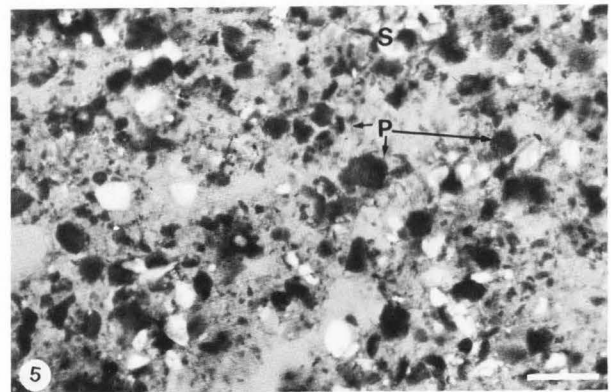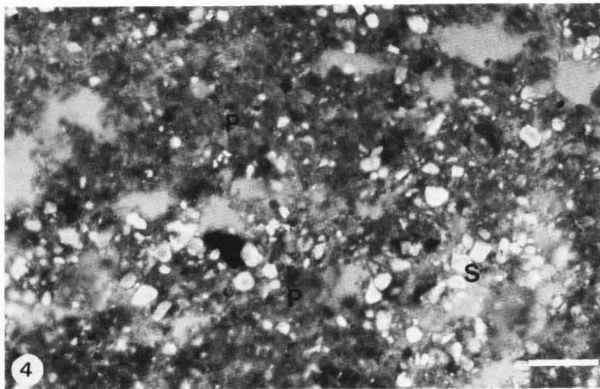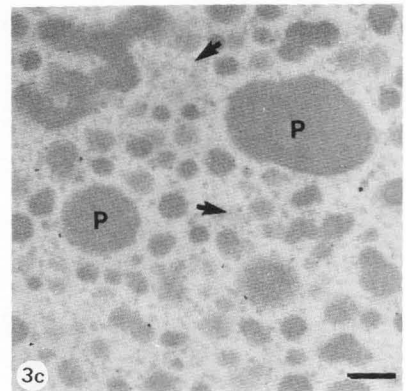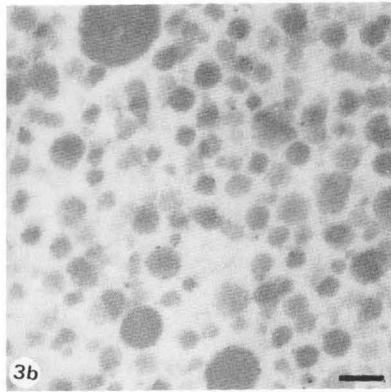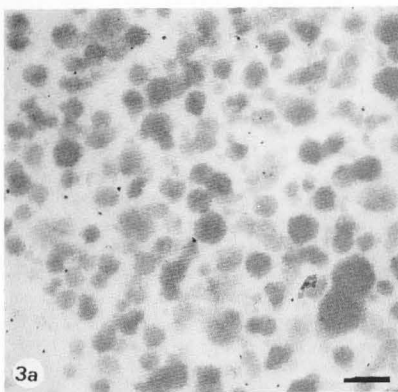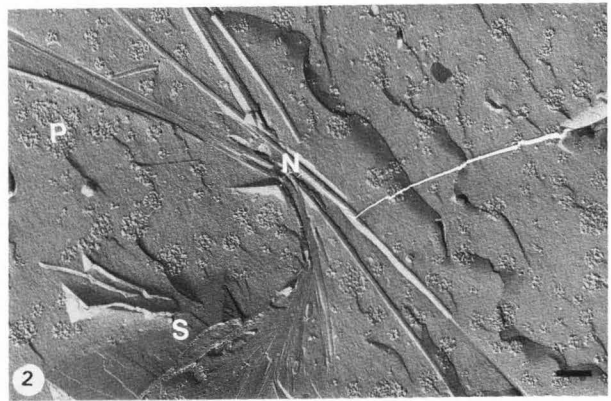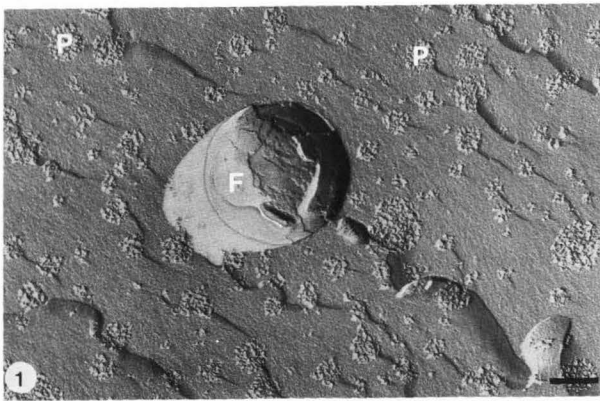
stage prevented condensation or contamination of the sample.

Transmission Electron Microscopy (TEM)

Resin sectioning. Samples of chocolate, caramel and milk powders were processed for thin sectioning.

Chocolate: Cubes (1-2mm sides) of chocolate were fixed in 2% Palade's osmium tetroxide vapour (Palade 1952) at 4°C for a minimum of 21 days. Samples were dehydrated through a graded series of acetone solutions and finally infiltrated and embedded in low viscosity Spurr resin (Spurr 1969).

Caramel: 2 g samples of caramel were dispersed in 3cm^3 of distilled water. Samples of the dispersion were mixed with equal quantities of a 2% solution of molten agar and the agar allowed to solidify. Cubes (1-2 mm sides) of agar were fixed in 3% glutaraldehyde in phosphate buffer (pH 7.3) and post-fixed in osmium tetroxide (4°C). Dehydration and embedding was carried out as described previously.

Milk powders: Samples of milk powder were mixed with molten agar which was then allowed to solidify. Specimens were fixed in osmium tetroxide prior to preparation as above.

In all cases, sections were cut on a Reichert-Jung Ultracut to a maximum thickness of 50 nm and collected on uncoated copper grids. Staining was carried out for 30 minutes in 1% uranyl acetate followed by 2 minutes in lead citrate solution.

Freeze-fracture/etching

Samples of liquid milk preparations, chocolate, caramel and fudge were prepared for freeze-fracture/etching. Small aliquots of milk and melted chocolate (40°C) were placed on Balzers 3 mm specimen holders and flash frozen by immersion in liquid nitrogen slush (-210°C) and stored under liquid nitrogen before use.

It was not possible to take very small samples of caramel or fudge without introducing changes during the sampling process. Bulk samples (cubes 1mm sides) of each product were taken therefore and flash frozen and then fractured under nitrogen. All samples were transferred onto the pre-cooled table (-150°C) of a Balzers 400D unit with the pre-fractured specimens having their fracture face uppermost. When a vacuum better than 10^{-6} Torr was reached and all frost removed, these specimens were shadowed with platinum and coated with carbon by electron beam evaporation. The time to reach this vacuum probably resulted in a low level of surface etching. For the remaining samples, specimens were warmed to -100°C and then fractured using the pre-cooled (-196°C) microtome knife and immediately coated with platinum and carbon. After removal from the vacuum chamber, samples were immersed in water or acetone and the replica then cleaned in alcoholic sodium hydroxide.

For all TEM specimens, grids were carbon coated and examined using a JEOL 1200 EX TEM operating at 80 kV.

Results

Milk Products

Transmission electron micrographs reveal the characteristic structural details of milk products. Using freeze-fracture, the condensed milk preparations showed the typical granular structure of the casein protein associated into distinct micelles (Figures 1 and 2). The micelles, in turn, associated into small aggregates. The size and size distribution of these showed some variation between samples. Maximum size varied between 50 and 300 nm. Numerous fat globules were present in the whole milk showing the characteristic layering of the crystalline fat. A few fat globules were found in the skimmed sweetened condensed milk (Figure 1) but the prominent feature was the casein aggregates. In this case aggregates ranged from 10-200 nm. Also apparent were fractures through crystals, the larger more angular shapes representing sugar, probably lactose and the long needle-like structures - calcium phosphate or calcium citrate (Figure 2).

The fine detail of the protein was also shown in sections of milk powders. Figures 3 a-c represent the internal structure of SMP particles with low, medium and high heat treatments respectively. The same particulate nature of the micelles was observed but there appeared to be slight changes in the size distribution of the aggregated units. This ranged from a fairly uniform distribution in the low heat SMP to a distinctly wider range in the high heat with a higher number of larger (>200 nm) aggregates being observed (Figure 3c).

Milk Chocolate

Figures 4 and 5 represent sections of chocolate as viewed by light microscopy. In the crumb-based chocolate (Figure 4), the protein is seen to be distributed homogeneously throughout the sample. The majority of the sugar crystals appear regular in shape and in close association with the protein. In contrast, chocolate prepared from a dried milk powder showed, first,

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Figure 8. Thin section of crumb chocolate showing sugar 'spaces' (S), milk protein (P), cell wall fragments (CW) and polyphenolic material (M). Bar = 1 μm.

Figure 9. Crumb chocolate section showing close association of cocoa debris (CW) and milk protein (P). Bar = 500 nm.

Figure 10. Full cream milk powder chocolate section showing spherical milk powder particles (MP) and cell wall fragments (CW). Bar = 5 μm.

Figure 11. Skimmed milk powder chocolate section showing milk powder particle (MP). Bar = 1 μm.

Figure 12. Skimmed milk powder chocolate showing sugar (S), milk particles (MP), cocoa debris and a possible protein network (arrow). Bar = 2 μm.

Figure 13. Peeling of outer layers (arrows) of a milk particle in chocolate containing high-heat skimmed milk powder. Bar = 1 μm.

Figure 14. Thin section of chocolate containing whey powder (W). Fragments of casein-containing particles are also present (MP). Bar = 1 μm.

Figure 15. Freeze-fracture preparation of crumb chocolate. S = sugar crystals, F = fatty matrix, P = protein. Bar = 2 μm.
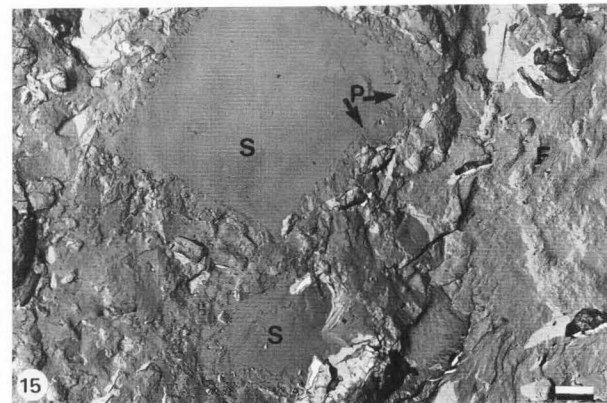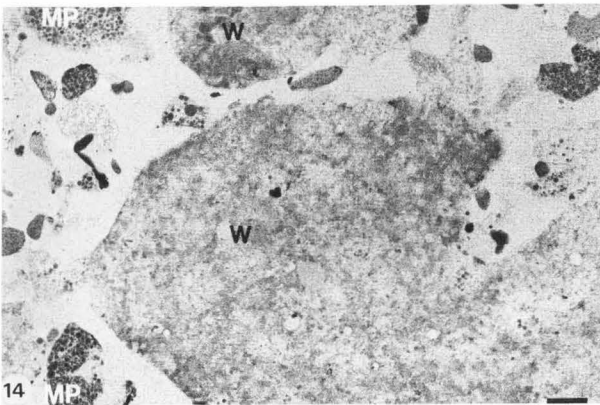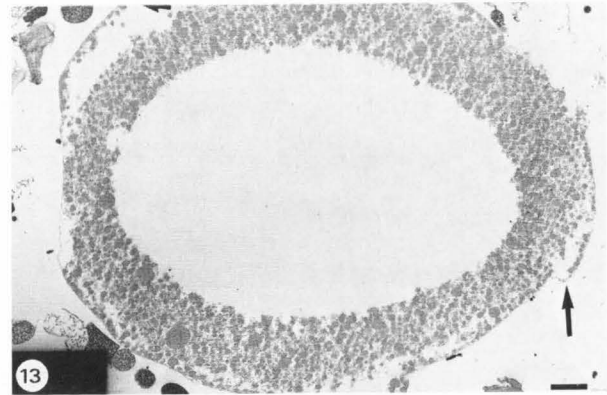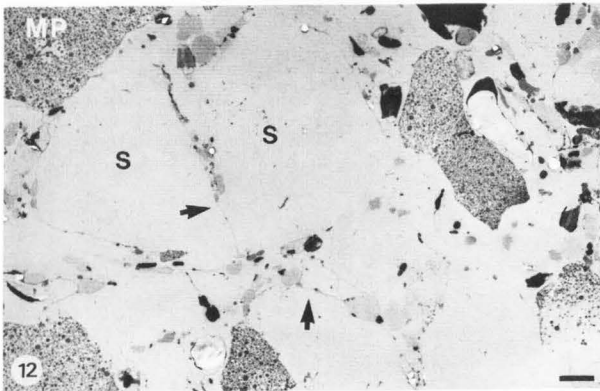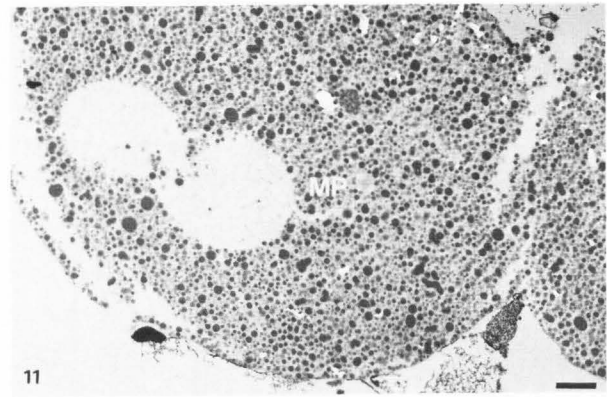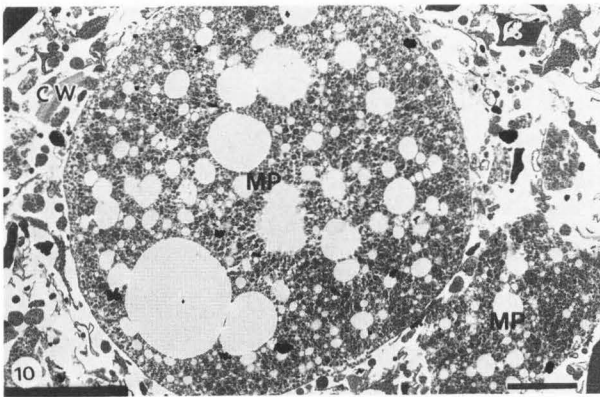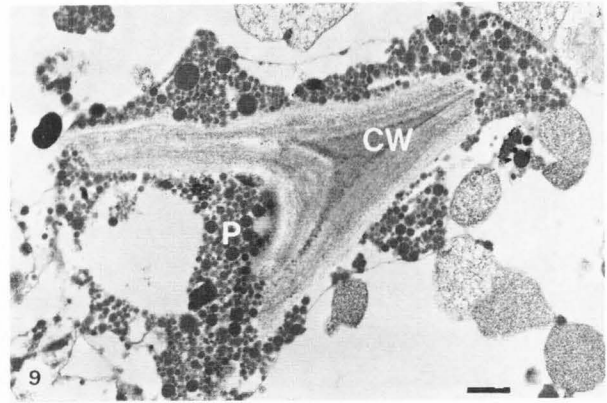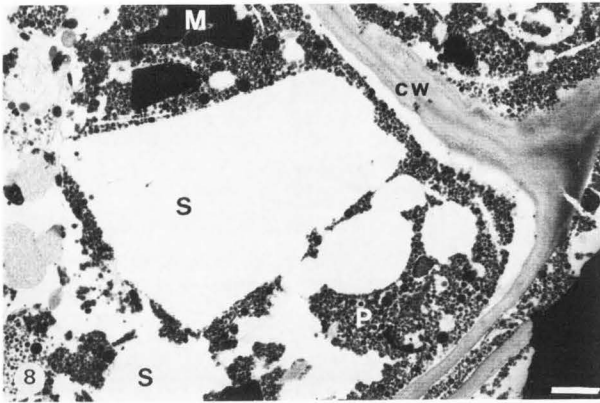
a granularity in the appearance of the background protein. The second, more prominent feature was the presence of discrete, densely staining proteinaceous regions (Figure 5) which ranged in size from ≅ 5-50 μm. Pores were also seen occasionally within the proteinaceous structures. Sugar crystals in this case had a cleaner and more fractured and fragmented appearance indicating the sugar had been added in a dry, pre-milled form.

The clear distinction between these chocolates was also apparent following examination by SEM. The outline of sugar crystals could be identified but in the case of crumb-based chocolate, these were in close contact with the remaining ingredients. Figure 6 shows a central, large (30 μm) sugar crystal covered with an amorphous, probably proteinaceous layer. By comparison, numerous clean, fractured crystals (average 15 μm) were observed in the powder milk chocolate (Figure 7). The fracture face also revealed distinct spherical milk powder particles.

The location and appearance of the milk protein was shown more clearly using TEM preparations. Figure 8 represents a thin section through a milk-crumb particle within milk chocolate. A regular sugar "space" (6 μm long) within the particle corresponded to the location of sugar which had been dissolved away during preparation. This was in close association with milk protein, identified as densely packed regions of casein micelles. Plant debris from the cocoa bean was evident in the form of fragments of cell wall material and highly electron dense regions corresponding to polyphenols. The strong association of components within a crumb-based chocolate is also illustrated by Figure 9. A smaller fragment of crumb was observed to be composed of a piece of cell wall material embedded within the milk protein. Spherical features in the surrounding matrix probably represent vesicles from the cocoa bean.

The prominent feature in sections of chocolate made from full cream milk powder was the presence of large (5-50 μm) frequently spherical proteinaceous particles (Figure 10). These particles showed a high density of packing of casein micelles which surrounded fat globules (0.5-2 μm in diameter) and air pores. Smaller milk powder fragments and pieces of plant debris were apparent in the surrounding media though they appeared to show little association of individual components.

The spherical nature of the milk particles was again apparent in chocolate prepared from skimmed milk powder. The particles (average 10 μm) were characterised by a uniform distribution of the casein within a structureless matrix (Figure 11). There was no evidence of fat globules in the structure. Pores, a few microns in diameter, representing air bubbles, were found within some of the particle fragments. Broken fragments of SMP particles are also shown in Figure 12. This section prepared from medium heat treated SMP showed clear sugar "spaces" corresponding to fragmented sugar crystals. For all powder-based chocolates, fractured sugar

spaces, milk particles and plant debris could be recognised and in each case all components were separate from each other and dispersed within the fatty matrix. Another interesting feature in these samples was the presence of a thin layer of protein surrounding the particles perhaps representing a protein network. Figure 12 shows this protein present as a line of delineation between individual sugar crystal "spaces".

A comparison of the three different heat treated SMP chocolates showed the same discrimination that was apparent with the corresponding dried powders and based on the extent of protein aggregation (Figure 3 a-c) within the particles. A further observation in the high heat treated sample was the apparent peeling away of the very outer layer of the particles (Figure 13).

The presence of whey powder in a milk chocolate was recognised by the fine microstructural detail of the protein. Fragments of the powder were found which showed a much finer protein structure and only loose aggregations of the molecules (Figure 14). As with other powder chocolates, these fragments showed little association with other components. It was interesting to observe additional milk powder fragments in the preparation. These were identified as containing casein, probably representing skimmed milk powder.

Freeze-fracture preparations of chocolate also highlighted the differences between crumb- and powder-based samples. A crumb-based chocolate (Figure 15) showed regular sugar crystals (average ≅ 10 μm) within an outer fatty support matrix. Associated with the sugar was a layer of protein around the periphery of individual crystals and acting as a sandwiching layer between them. A powder-based chocolate was seen to contain clean, fractured crystals and the separate dense protein regions corresponding to milk particles (Figure 16).

Caramel

Low temperature preparation procedures for

Figure 16. Freeze-fracture preparation of powder chocolate. S = sugar crystals, MP = milk powder particles. Bar = 1 μm.

Figure 17. Scanning micrograph of caramel showing fat globules (F) and cavities (C) within an amorphous sugar matrix (A). Bar = 10 μm.

Figure 18. Freeze-fracture preparation of caramel showing fat (F) and associated layers of protein (P). Bar = 2 μm.

Figure 19. Caramel showing fat globules (F) and associated protein (P). Bar = 500 nm.

Figure 20. Freeze-fracture preparation of a whey caramel showing irregular shaped fat regions (F) and whey protein (W). Bar = 2 μm.

Figure 21. Whey caramel showing fat (F) and whey protein (W). Bar = 500 nm.

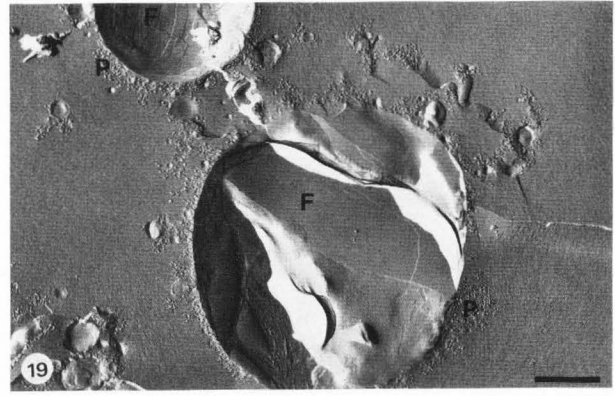Figure 22. Thin section of whey caramel showing whey protein aggregates (WA) and ring of protein (arrow). Bar = 500 nm.

Figure 23. Freeze-fracture preparation of a low-fat caramel showing even distribution of protein (P). Bar = 1 μm.
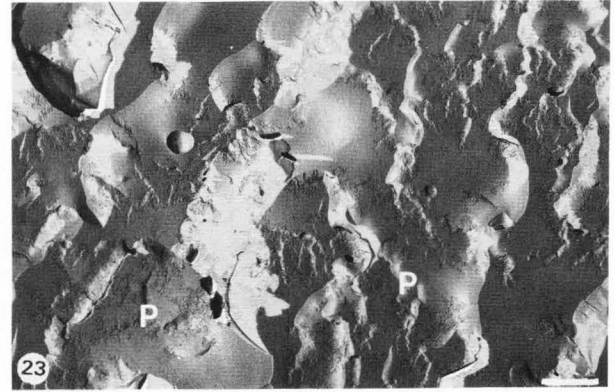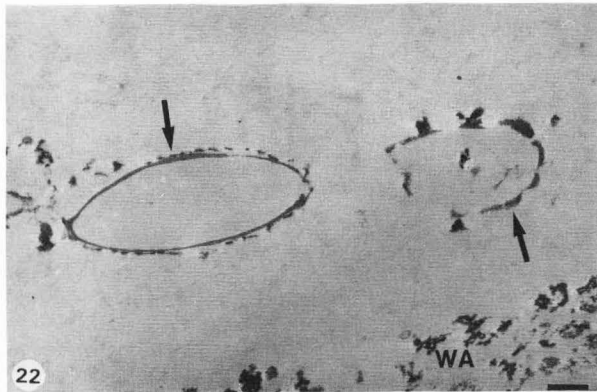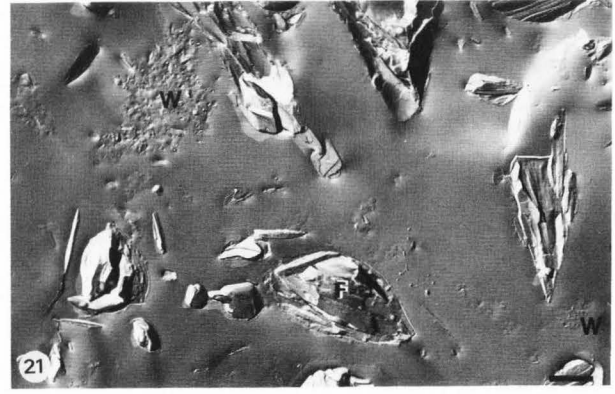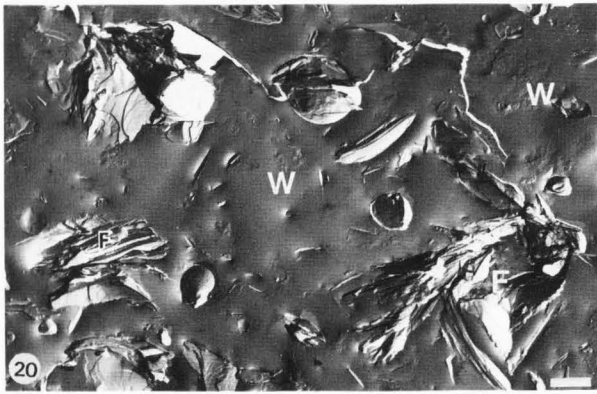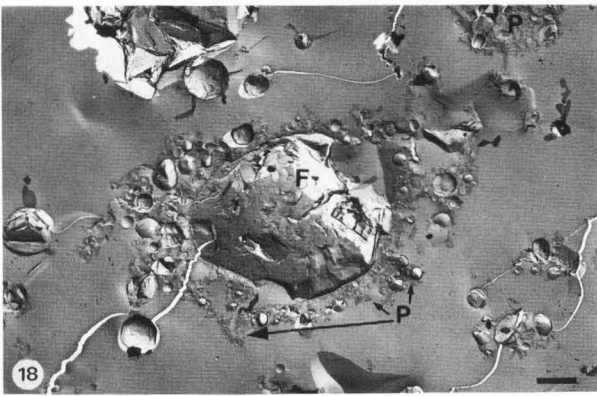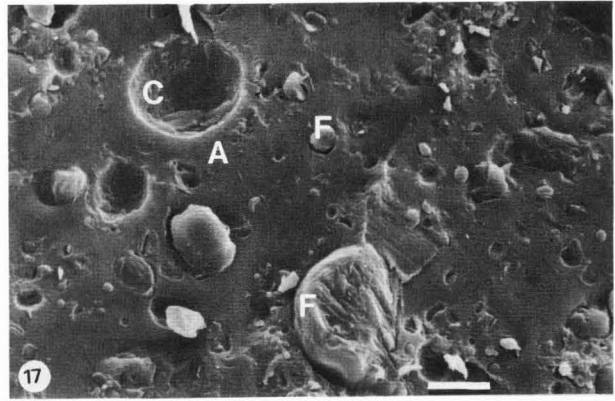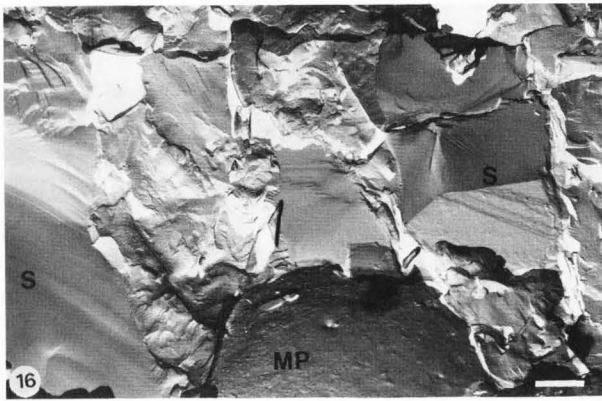
the SEM (Figure 17) revealed the general morphology of the caramel. The fracture face showed a structureless matrix containing numerous fat globules. These globules were seen as predominantly spherical in form and ranging in size from 1-20 μm. Pores were also observed which represented both air bubbles and the cavities where fat had been pulled out during the fracturing procedure. A common feature was the presence of small ($\cong$ 1 μm) globules forming a layer around central, larger (15 μm) ones.

The location of the protein within the structure was revealed by freeze-fracture. The preparations confirmed the presence of spherical fat globules within a more or less structureless or amorphous support matrix. The protein was identified as the fine granular structure characteristic of casein micelles. Figure 18 shows the protein within the amorphous sugar regions but strongly associated with the fat. The central feature is a fat globule (10 μm diameter) surrounded by numerous smaller (< 1 μm) droplets, all showing a layer of protein around them. This fat/protein association is shown more clearly in Figure 19. Two fat globules were observed each showing a distinct protein layer around their periphery.

The replacement of milk by whey protein in a caramel was recognised by the clear difference in the ultrastructure of the protein. Figures 20 and 21 represent freeze-fracture preparations of a whey caramel and contrast strongly with Figures 18 and 19. The whey protein was seen to have a finer appearance and the proteins appeared quite tightly aggregated. Fat and amorphous sugar were identified but relatively few of the fat globules were regular spheres and there was a wide size distribution (< 1 μm-20 μm). In Figure 21, a proportion of the fat is seen as distinct, regular crystals. It was also observed that significantly less aggregated protein was present around the fat globules (Figure 21).

The same sample prepared for thin sectioning showed regions of aggregated proteins characteristic of fine whey protein (Figure 22). A further feature was the presence of a proteinaceous layer around the smaller fat globules. This, however, was only a thin, diffuse film around easily deformed fat globules.

A caramel prepared with reduced levels of fat showed the characteristic micellar structure of the casein. The protein, however was well distributed throughout the sugar (Figure 23) rather than the localised associations characteristic of a normal caramel (Figure 18).

Fudge

The immediate distinction between the structure of fudge and caramel was the presence of crystalline rather than amorphous sugar. The fracture face of fudge as revealed by SEM (Figure 24) showed a crystalline support matrix which contained regions of fat, contrasting strongly with the clean fracture through caramel and predominantly spherical fat globules (Figure 17). A few spherical fat regions were observed in fudge although a large proportion of fat was present as irregular, distorted structures. Air

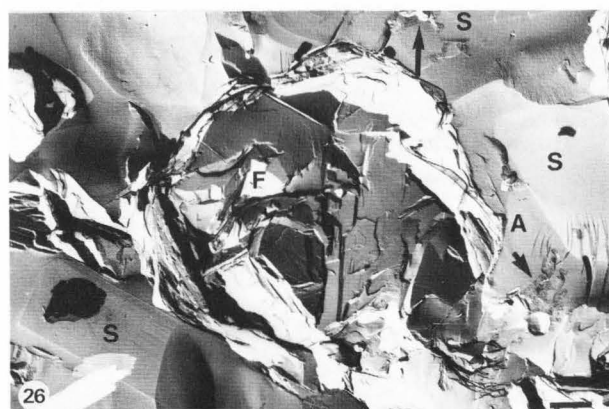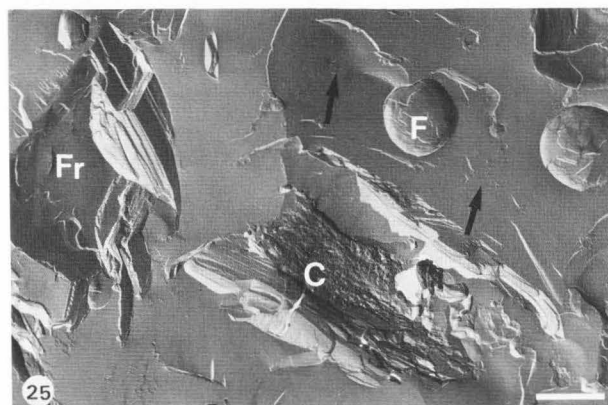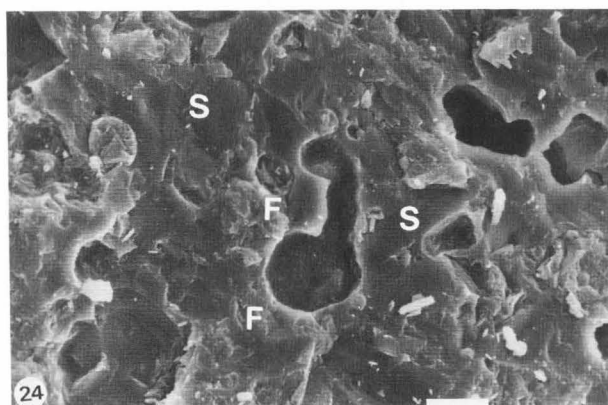Figure 24. Scanning micrograph of fudge showing crystalline sugar (S) and fat (F). Bar = 10 μm.
Figure 25. Freeze-fracture preparation of chocolate fudge pre-mix showing fat globules (F) and fat-rich regions (Fr), cocoa debris (C) and protein (arrows). Bar = 1 μm.
Figure 26. Freeze-fracture preparation of fudge showing sugar crystals (S), amorphous sugar (A), fat (F) and protein (arrows). Bar = 1 μm.

bubbles were also irregular in form. It was again necessary to use freeze-fracture to resolve the fine detail of the protein. Figure 25 is a freeze-fracture preparation of a fudge pre-mix, in this particular case, of chocolate fudge. The sugar support matrix was still amorphous at this stage and the fat seen as spherical fat globules together with more irregular fatty-rich regions. Associated with one of the large areas of fat was a structure showing a rough surface appearance. This is likely to represent the inclusion of cocoa plant debris in this chocolate fudge. The milk protein was observed as small aggregates of casein micelles dispersed fairly evenly through the continuous sugar phase. There was evidence of some association with fat although this tended to be with the smaller, regular shaped globules.

A freeze-fracture preparation of finished fudge showed that the protein remained associated with the amorphous sugar regions (Figure 26). The concentration of the protein has increased within such regions due to the development of numerous regular sugar crystals within the sugar matrix. Figure 26 shows a large fat-rich region surrounded by highly crystalline sugar. Amorphous sugar still remains as a layer around the individual sugar crystals and in contact with the fat-rich regions. The protein, although the development of the crystals has concentrated it within the remaining amorphous sugar, still does not appear to have a strong association with the fat.

Discussion

Sugar, fat and milk are all common basic ingredients to chocolate, caramel and fudge confectionery products. Depending on how they are introduced into the system and depending on subsequent processing, products of very different structural and textural properties are formed. Milk proteins are particularly important. Changes in only these components produce significant changes even within a single confection.

The scanning microscope allowed the effect of such changes to be seen although without resolving the proteins themselves. TEM complemented these observations and enabled more precise identification of the protein to be made.

A milk chocolate prepared from milk crumb was readily distinguished from a dry milk powder product. Differences were related to the homogenous distribution of the milk protein in a crumb-based chocolate and to the strong association of protein with the other main particulate ingredients, namely sugar and cocoa. This can be recognised right back at the first stages of chocolate manufacture in the formation of the intermediate crumb product. This process as described by Minifie (1979) is critical in producing the 'milk chocolate' and caramel flavours in the chocolate. The components are allowed to mix and react together prior to being dried down to a solid mass which is then broken up and incorporated into chocolate. Milk powder chocolate represents the other extreme where milk is dried first to a powder and the sugar and cocoa milled to fine particles before all ingredients are mixed into the chocolate. The structure of protein within the finished chocolate is therefore dependent on the form of the starting powder and FCMP, SMP and whey fragments can readily be identified within the structure of the product. A difference in the heat treatment of SMP was recognised in the aggregation of the protein both in the powder and followed through to the chocolate. This can be related to the increased level of protein denaturation in the high heat sample and association of whey with casein proteins. Buchheim (1981) found no corresponding difference in structure using freeze-fracture preparations of these powders.

In caramels, proteins are likely to influence both emulsion formation (Kinsella 1970) and water sorption (De Bruin & Keeney 1973). A standard caramel made from milk and therefore containing mainly casein, is known to have a firm, chewy texture by comparison to the softer more runny texture of a whey-containing product (Webb 1970). This could be explained by the location of the protein in the different products. In the former, the caramel represents an emulsion of fat in sugar and the casein was seen to stabilise the emulsion droplets forming a solid and rigid outer layer. In the whey caramels, this layer when present around the fat is thin and easily deformed. The whey product also exhibits the phenomenon of 'cold-flow' upon storage which again may be related to the flexibility of the protein. These observations agree with those made recently in studies on toffees (Dodson et al 1984 a & b) where again milk was replaced by protein fractions, including whey.

The development of crystalline sugar in a fudge appears to result in the concentration of milk protein in the remaining non-crystalline regions of sugar. There does not seem to be any specific association of the protein with the crystals although from observations with milk crumb, the removal of water and the crystallisation of sugar can lead to layering of milk proteins around their periphery. This layering probably occurs in the final drying stages of the crumb process and water is less than 10%. The more interesting observation in fudge is the lack of association of protein with the fat which is a further distinction from the caramel system.

Acknowledgements

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Discussion with Reviewers

D. M. Manning: What is the result of using 2% Palades osmium tetroxide vapour and what effect, if any, would variations in the 21 day exposure create?
Author: Due to the high fat content, conventional fixation with aldehyde fixatives and short exposures to osmium were insufficient to retain the structural integrity of the samples. Following experimentation and reference to previous studies (eg. Cruickshank 1976), exposure to osmium over extended time periods was considered most suitable. This is quite a drastic treatment and it is likely some fixation artefacts result. Routinely, therefore, a complementary range of techniques are used to examine any one sample.

D. F. Lewis: Do you think that the difference in casein aggregation in SMP could be used diagnostically to deduce the level of heat treatment?
Author: Where a common method of drying is employed, differences in the casein aggregation could be used to indicate the level of heat treatment. Where, however, an alternative method and/or rate of drying is used, it is likely that the state of hydration of the protein is different, which in turn produces a difference in microstructure.

D. M. Manning: Do you feel that peeling of the outer layers in the high heat treated sample is due to thermal effects or could it be the exterior layer of protein is different in nature? How would such peeling affect milk protein functionality in a product?
Author: The peeling effect only becomes obvious once the powders are incorporated into chocolate and the difference between the powders does indicate that it is a thermal effect. It is interesting to consider whether these outer layers of material are contributing to the protein network. The presence of a network is frequently associated with an increased viscosity and the texture is described as sticky or cloying.

D. F. Lewis: Is the milk powder chocolate subjected to roller refining or a similar process which could bring about breakdown of solid particles?
Author: Yes, a milling stage is involved which leads to breakdown not only of milk powder but also the sugar crystals and cocoa solids. The size and compactness of structure of the powders appears to influence the way this breakdown occurs producing fragments of different size and shape. This, I believe, you have also shown to be the case in studies on milk powders in compound chocolates.

D. M. Manning: What effect would amorphous sugar edges have on the accumulation of protein in Figure 12?
Author: It is not clear whether accumulation of protein and the formation of a network is influenced more by surface chemistry of particles or simply by their size and shape. The sugar here has been added in a dry, crystalline state and unlikely to include amorphous sugar. It is, however, an interesting question and I would anticipate a stronger attraction of protein onto amorphous sugar surfaces when they are present.

M. Saltmarch: In the discussion on 'cold flow', do you mean flexibility of the whey protein or solubility of the protein?
Author: It seems unlikely that the solubility of whey is altered in the finished product which contains a maximum of 10% moisture. The degree of hydration of the protein probably does change, however, which will in turn affect its flexibility.

D. F. Lewis: In caramels, do you observe signs of broken casein/whey membranes and "free fat"? What effect do you think that damage to these membranes will have?
Author: Yes, broken and disrupted membranes have been observed and the presence of unbound fat which has an irregular rather than regular, globular form. The effect of damage seems dependent on the stage in the process at which it occurs. During cooking, when the system still has low viscosity, there is a chance membranes will reform. When membranes remain disrupted, pools of free fat form. In the extreme case, a caramel of very low viscosity is produced which is prone to both cold-flow and fat expression.

M. Saltmarch: Can you expand on the unique structural and functional properties of milk proteins which influence the characteristics of confectionery products?
Author: The key properties of the proteins when incorporated into confectionery are probably:- organoleptic - relating to colour and flavour; hydration - wettability, gelling viscosity etc.; surface - emulsification, foaming; structural - eg. aggregation, cohesiveness, network formation.   There are numerous factors which influence these properties but environmental and process variables are really of greatest importance as they are to some extent, under the control of the confectionery manufacturer.

D.F. Lewis: I am surprised by the apparently low level of emulsification at the pre-mix stage of this product. Is it possible to explain this in terms of the recipe? Possibly the use of light microscopy on agar dispersions of both caramel and fudge at stages of production would help present a clearer picture.
Author: I regret my inability to provide confidential information regarding the recipe!