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GRITTIENESS IN A PASTEURIZED CHEESE SPREAD: A MICROSCOPIC STUDY

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Abstract

Coagulation of pasteurized (63°C for 30 min) milk and blending of the resulting curd with high-fat cream followed by heat treatment (44° to 80°C for 10 min) of the blend in preparation of a hot-pack cheese spread led to the formation of a gritty product. Microscopic examination of hard particles causing the grittiness indicated that they consisted of compacted protein. Staining for calcium failed to detect any elevated concentration of this element in the particles. They were amorphous and contained no crystalline structures.

Encapsulation of the gritty curd in agar gel tubes made it possible to freeze-fracture the small hard particles and to examine their internal structure by scanning electron microscopy. The hard or gritty particles consisted of compacted protein. This was confirmed by transmission electron microscopy of thin sections of the product embedded in a resin.

The unpasteurized (unheated) cold-pack cheese spread prepared from the same ingredients was smooth with no grittiness defect. Based on the findings, grittiness was avoided in the hot-pack product by using curd obtained by coagulating milk which had previously been heated to 90°C for 10 min.

Introduction

The manufacture of some dairy products is based on mixing cultured or acid curd with high-fat cream and homogenizing the mixtures [10, 11] to form suspensions and emulsions. The consistency of the product depends on its moisture and fat content. Thickening agents such as gelatin, starch gel, carrageenan, alginates, or various gums may be used to stabilize the suspensions or emulsions and increase their water-holding capacity and viscosity.

Occasionally, grittiness develops in a newly formulated product, which means that the presence of small hard particles can be detected by sensory evaluation. In dairy products, particles which may cause grittiness originate from interactions between protein and various ingredients such as carrageenan, alginates, and other thickening agents [9], from the development of crystalline inclusions such as calcium phosphate and calcium lactate [4], or amino acid crystals [6], from the occurrence of compacted protein particles, and from other unidentified sources.

In this study, optical fluorescence microscopy and electron microscopy were used to examine grittiness in milk curd used in the manufacture of a spreadable milk product.

Materials and Methods

Preparation of curd

Milk (3.25% protein, 3.7% fat), obtained from the Greenbelt Farm of Agriculture Canada, was vat pasteurized at 63°C for 30 min. The milk was cooled to 27°C and inoculated with 2% lactic acid-producing starter culture. A mixed lactic acid and diacetyl producing culture (Code 5270) was obtained from the Rosell Institute, Inc. (Montreal, Quebec, Canada) and used in most of the experiments. An alternate single-strain culture, designated C₂, was also used to see if the grittiness was culture related.

Twenty minutes after filling the cheese vat with approximately 200 L of milk, 4.2 mL (diluted 20:1 with distilled water) of single strength calf rennet (Chr. Hansen's Laboratory, Inc., Milwaukee, WI) was added. The pH of the mixture was monitored by means of a Radiometer Model 26 pH-meter equipped with a Jena Thalamid combination electrode. After the pH had decreased to 5.85, approximately

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4 h and 20 min after starter addition, the coagulated milk was cut using 1.25 cm knives. Immediately after cutting, the vat was cooled to 10°C and left overnight under quiescent conditions. Whey was drained from the vat on the following morning. The curd was placed in 20 kg hoops and allowed to drain for an additional period of 24 h before use.

Acid curd was prepared by the same procedure except rennet was omitted and the curd was not cut until the pH had declined to 4.02 (11 h after starter addition). Flow diagrams for both types of curd are shown in Fig. 1.

Preparation of cheese spread

The cheese spread product was formulated to contain approximately 85% curd (approximately 35% solids), 8 to 10% cream (50 to 60% milkfat), 5% sucrose, and 0.2% sodium alginate. Cream was added to a dry blend of sugar and stabilizer contained in a Groen processing kettle (Model TDC/TA-20SP). The mixture was pasteurized at 80°C for 10 min and then cooled to 30°-35°C. Cheese curd was then blended with the cream, sugar, and stabilizer mixture by means of a Polytron blender, Model 4 STE. The mixture, with the exception of the control treatment, was pasteurized by heating to 63°C. After holding for 30 min at 63°C, the product was homogenized in a two-stage Gaulin homogenizer, Model 769441, at total pressure of 17.2 MPa. The product was packaged directly into 100-ml containers and refrigerated at 4°C. In the subsequent discussion, the pasteurized product will be referred to as the "hot-pack product" whereas the control or unpasteurized cheese will be referred to as the "cold-pack product". Fig. 2 shows the flow diagram for both the cold-pack and the hot-pack products.

Analytical procedures

Fat and nitrogen were determined according to the AOAC procedures [2] and total solids were determined using a CEM Model AVC-80 microwave moisture oven. Nitrogen was converted to protein using a factor of 6.38.

Evaluation of grittiness

Grittiness of various degrees which developed in several experimental variations was evaluated subjectively by mouthfeel and by visual examination under a low-magnification dissecting microscope.

Fluorescence microscopy

The cheese samples were fixed with 2.5% glutaraldehyde for 24 h, embedded in Histo Prep (Fisher Scientific Co., Fair Lawn, NJ, USA) support medium for cryo-sectioning, mounted on cold object disks, and frozen immediately at -25°C. Frozen sections, 4-6 µm thick, were obtained using a cryo-microtome (Reichert-Jung Scientific Instruments, Belleville, Ontario, Canada) and affixed to glass slides for subsequent staining and microscopic examination.

To reveal the distribution of gritty particles in the cheese preparations, the frozen sections were stained with 0.1% (w/v) aqueous Fast Green FCF according to a method similar to that described by Chayen et al. [5]. Alternatively, the sections were stained with 0.1% (w/v) Acridine Orange [14].

To detect the presence of calcium-containing structures in the cheese spread, the sections were

treated with 5% (w/v) silver nitrate followed with 5% (w/v) sodium thiosulphate according to the Van Kossa's procedure described by Thompson [13] and Yiu [14].

All stained sections were rinsed with distilled water, air-dried, and mounted with non-fluorescent immersion oil under cover slips. Stained sections were then examined using a Zeiss Universal Research Photomicroscope (Carl Zeiss Ltd., Montreal, Quebec, Canada) equipped with polarizing, bright-field, and fluorescence optics.

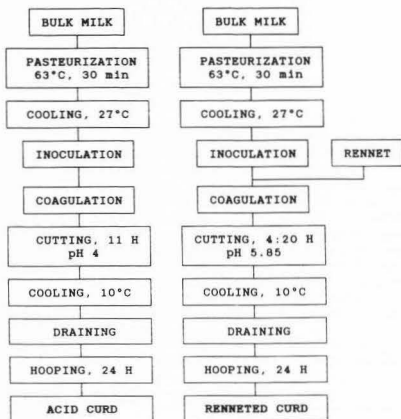


Fig. 1. Flow diagrams for acid and renneted curd.

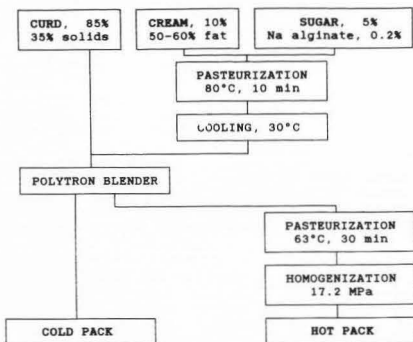


Fig. 2. Flow diagram for the cold-pack and hot-pack cheese spread products.

A fluorescence filter combination with a dichromatic beam splitter and an exciter/barrier filter set for maximum transmission at 450-490 nm/>520 nm was used for the fluorescence analysis. Micrographs were recorded on 35 mm Ektachrome 400 daylight diapositive film from which colour prints were obtained.

Scanning electron microscopy (SEM)

The samples were encapsulated in agar gel tubes (inner diameter of 1.0 mm) using a method described earlier [1, 7], fixed in a 2.5% glutaraldehyde solution for 24 h, dehydrated in a graded ethanol series, frozen in melting Freon 12 at -150°C, and freeze-fractured under liquid nitrogen. The fragments were thawed in absolute ethanol at 20°-25°C and were critical-point dried from carbon dioxide. Dry fragments were mounted on aluminum stubs, sputter-coated with gold, and examined in an ISI DS-130 microscope which had been equipped with an external oscilloscope [3], and operated at 20 kV. Micrographs were taken on 35-mm 125-ASA film.

Transmission electron microscopy (TEM)

Also for TEM, the samples were first encapsulated in agar gel [7] in order to prevent them from disintegrating during preparatory steps. The encapsulated samples were fixed in a 2.5% glutaraldehyde solution for 24 h, postfixed in a buffered (0.05 M veronal-acetate buffer, pH 6.75) 2% osmium tetroxide solution for 2 h, dehydrated in a graded ethanol series, and embedded in a Spurr's low-viscosity resin (J. B. EM Service, Inc., Pointe Claire-Dorval, Quebec, Canada). Sections stained with uranyl acetate and lead citrate solutions were examined in a Philips EM-300 electron microscope operated at 60 kV.

Results and Discussion

The curd used in the preparation of the cheese spread contained approximately 35% solids, 17-18% fat, and 14% protein and had a pH of 4.5 ± 0.1 . The addition of cream and sugar increased the total solids and fat contents to approximately 40% and 20%, respectively, in the finished cheese spread. There was about 12% protein and 10% fat in the final product and its pH was 4.60 ± 0.05 .

The texture of the cheese spreads produced by this process varied from smooth for the cold-pack product to gritty for the hot-pack spread. Reasons for the development of the gritty texture during the initial part of this experimentation remain unclear. A reduction in the temperature of heat treatment, after the curd was blended with the pasteurized cream, sugar, and stabilizer mixture, failed to resolve the problem: grittiness was observed even when the product was held at 44°C for 30 min. Attempts to reduce the grittiness through double or even triple homogenizing were only partially successful. Other measures taken, including the use of buffering salts such as trisodium phosphate (Na_3PO_4) to raise the pH to 5.8 prior to heating, using acid-coagulated curd rather than renneted curd, as well as changing from the Rosell 5270 mixed culture to the C_2 culture failed to resolve the grittiness problem.

From the fact that the cold-pack cheese spread was smooth, it is apparent that the heat

treatment at 44° to 63°C for 30 min may have led to the formation of small hard particles causing the hot-pack product to be gritty. However, pasteurization of the cheese spread is necessary to extend the shelf life of the product.

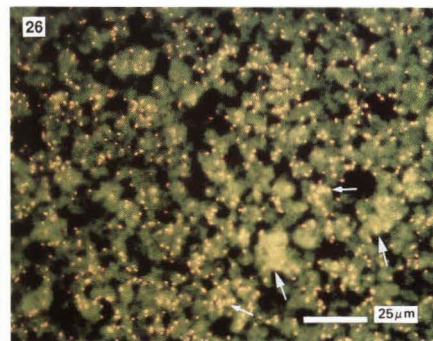
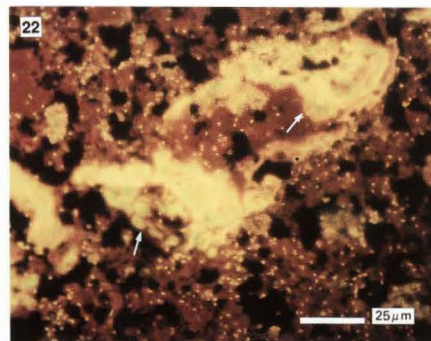
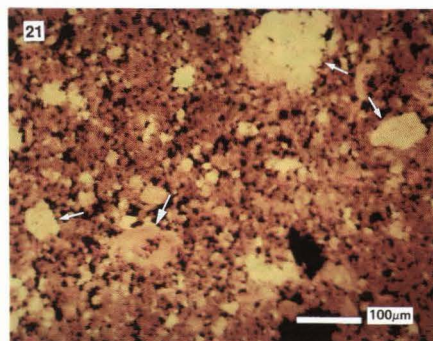
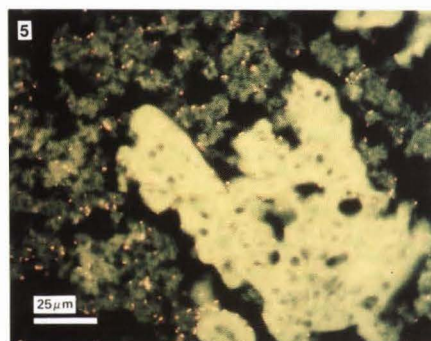
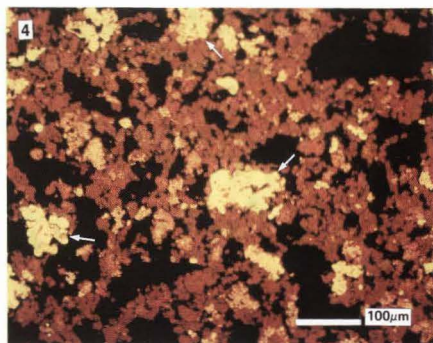
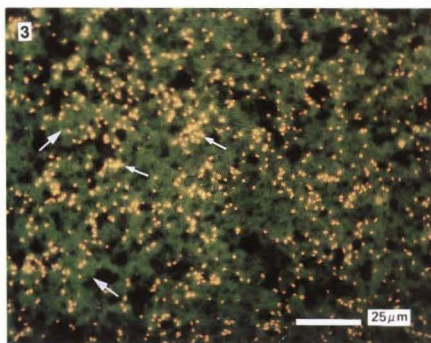
The presence of hard particles which caused grittiness in the hot-pack cheese spread was easily recognized by sensory evaluation, in particular by mouthfeel, and also visually by viewing a smear under a dissecting microscope.

Rapid screening of the structure, size, and distribution of the gritty particles present in the cheese samples was conducted by fluorescence microscopy using Fast Green FCF and Acridine Orange as staining reagents. The structure of the smooth cold-pack cream cheese spread is shown in Fig. 3. Fast Green FCF provided the best visual contrast between the particles causing grittiness and the surrounding matrix when the samples were excited at the 450-490 nm wavelength. The gritty particles imparted a yellow fluorescence against the red fluorescing background of the matrix. Positive interactions between Fast Green FCF and the gritty particles as well as their surrounding matrix suggested that both the gritty particles and the matrix contained protein. At a low magnification, the particles appeared as irregular clusters, varying in size and shape, embedded in a homogeneous matrix (Fig. 4). The particle sizes ranged from 10 to 100 μm in diameter and their shapes varied from being elongated, near-spherical, to irregular. They were distributed at random throughout the cheese spread samples. At a higher magnification, they appeared as densely aggregated masses which lacked visible microstructural details (Fig. 5). The different colour fluorescence detected after staining with Fast Green FCF indicated that the gritty particles differed from the surrounding protein matrix in terms of their affinity for the dye. Changes in the dye-binding property may imply chemical modification of the protein curd resulting from the heating process or they may reflect a difference in chemical composition such as the presence of components other than protein.

No birefringence was noticed when the particles causing grittiness were examined under polarized light, indicating that the particles were not crystalline. They also failed to give a positive reaction with a silver nitrate solution in the Von Kossa method which is recommended for the detection of calcium phosphate crystals in cheese [4, 14]. Thus, the grittiness of the hot-pack cheese spread was not caused by the presence of crystalline mineral salts or salts containing calcium.

A higher resolution such as that provided by a scanning electron microscope was used to reveal more detail of the gritty particle structure.

In order to establish the nature of the particles, their internal structure was examined. Encapsulation of the viscous samples in agar gel tubes made it possible to handle them as solid samples, i.e., to fix them, dehydrate, and freeze-fracture them. Freeze-fracturing of the samples impregnated with absolute ethanol produced smooth fracture planes (Fig. 6) running through the casein particles, thus opening their interior for examination. The smooth gels (cold-pack) had relatively uniform protein matrices (Fig. 7) which



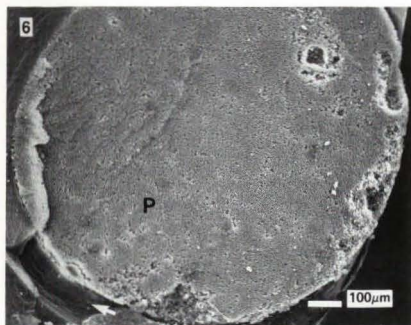


Fig. 6. Encapsulation in an agar gel tube (arrow) makes it possible to examine a freeze-fractured cold-pack cheese spread sample at a low magnification using conventional SEM. Freeze-fracturing produces a smooth fracture plane (P).

Fig. 7. SEM of a smooth cold-pack cheese spread made from pasteurized (63°C, 30 min) milk shows a protein matrix to be composed of relatively uniform small particles.

Fig. 8. Detail of the fluffy particles constituting a smooth cold-pack cheese spread made from pasteurized (63°C, 30 min) milk.

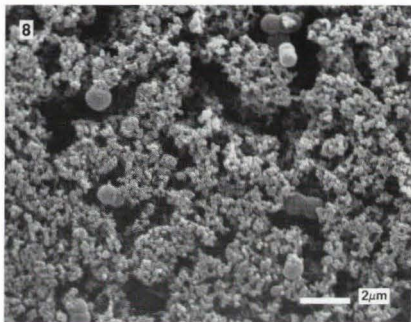
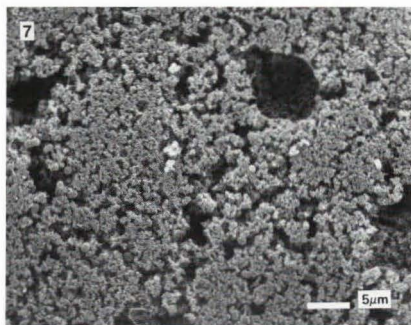


Fig. 3. Fluorescence micrograph of a smooth cold-pack cheese spread made from pasteurized (63°C, 30 min) milk. Staining with Acridine Orange shows a uniform protein matrix (green fluorescence, large arrows) with evenly distributed lactic acid bacteria (yellow fluorescence, small arrows).

Fig. 4. Fluorescence microscopy of a gritty hot-pack cheese spread made from pasteurized (63°C, 30 min) milk. Particles causing grittiness (arrows) appear bright yellow on a reddish background of the uniform protein matrix. Staining with Fast Green FCF.

Fig. 5. Detail of a particle causing grittiness in hot-pack cheese spread made from pasteurized (63°C, 30 min) milk. Conditions for fluorescence microscopy were the same as in Fig. 3 except for a higher magnification.

Fig. 21. Fluorescence microscopy of a hot-pack cheese spread [made from heated (90°C, 10 min) milk] containing a stabilizer (large arrow) shows a heterogeneous structure. Casein particle aggregates (small arrows) vary in dimensions. Staining with Fast Green FCF.

Fig. 22. Fluorescence microscopic detail of stabilizer particles (arrows) present in a hot-pack cheese spread sample. Staining with Fast Green FCF.

Fig. 26. Homogenization of the curd which contained casein particle aggregates led to a uniform structure of the product (green fluorescence; large arrows). In this sample stained with Acridine Orange, yellow dots (small arrows) indicate lactic acid bacteria.

consisted of fluffy particles as was demonstrated at a higher magnification (Fig. 8). In contrast, the gritty samples (hot-pack) had structures, the coarseness of which was visible even at a low magnification (Fig. 9). Examination of the larger particles in greater detail showed them to be compact (Fig. 10) with a higher incidence of lactic acid bacteria (Fig. 11) than the surrounding medium consisting of smaller fluffy particles. This increase in the incidence of the bacteria may have been caused by the contraction of the porous protein matrix.

TEM confirmed the findings made by SEM concerning the presence of compact protein particles in the gritty hot-pack cheese spread. Fig. 12 shows both kinds of structure in the cheese spread. The body of the product consisted of casein particles aggregated in the form of chains and clusters with relatively evenly distributed pores filled with the liquid phase (whey). Only the porous structure was present in the smooth cold-pack cheese spread. The hard particles causing the grittiness problem in the hot-pack cheese spread, however, were compact, as had already been shown by SEM.

Since grittiness was found in the hot-pack cheese spreads made using either acid-coagulated

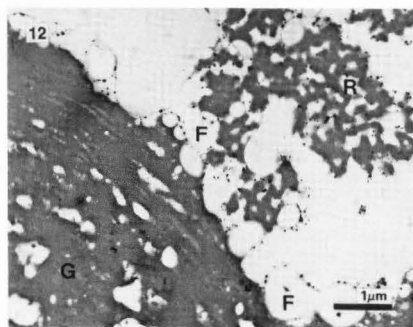
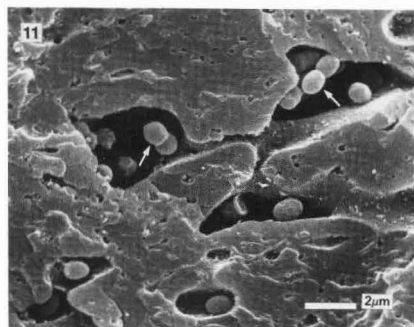
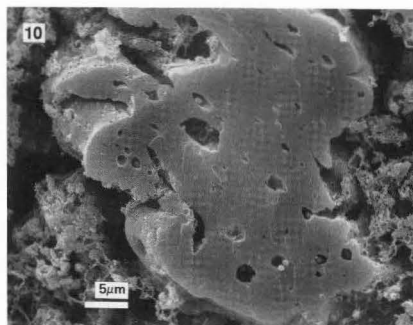
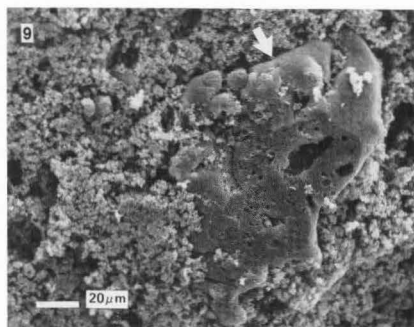


Fig. 9. SEM of a gritty hot-pack cheese spread made from pasteurized (63°C, 30 min) milk shows a coarse structure of the protein matrix. Arrow points to a compact particle.

Fig. 10. Detail of a compact particle present in the gritty hot-pack cheese spread made from pasteurized (63°C, 30 min) milk.

Fig. 11. Lactic acid bacteria (arrows) were associated with the compact particles in the gritty hot-pack cheese spread made from pasteurized (63°C, 30 min) milk.

Fig. 12. TEM shows that a gritty particle (G) consisted of compacted protein whereas the surrounding casein matrix (R) was porous. F = fat.

or renneted curd, it appeared that the reasons for grittiness to develop in the product should be sought in the treatment of milk prior to its coagulation. It is known that curd made from unheated milk is composed of large clustered casein particles. This structure favours exclusion of whey and rapid compaction of the casein particle clusters which is important in cheese manufacture. In contrast, the structure of curd made from milk heated to a minimum of 85°C consists of smaller casein particles which are coagulated in the form of chains. This structure is more resistant to syneresis than the former structure and is thus the basis of yoghurt where it is important to retain the liquid phase [8].

In another series of experiments, therefore, the milk was heated to 90°C in an APV-UHT Pilot Plant Apparatus (APV-Gaulin, Inc., Everett, MA, USA) and held for 10 min at that temperature (Fig.

13). This heat treatment of milk [12] successfully prevented the development of grittiness in the final product, *i.e.*, in the hot-pack. The curd, which was made using one half of the amount of rennet used in the first series, consisted of grains (Fig. 14) which had a uniform structure formed by casein particle chains apparent at higher magnifications (Figs. 15 and 16). This structure differed from the structure of curd made from pasteurized milk in that the latter curd consisted of casein particle clusters (Figs. 7 and 8). Blending of the curd made from milk heated at 90°C with high-fat cream in the Polytron blender resulted in the disintegration of the curd grains (Fig. 17). Homogenization in the two-stage Gaulin homogenizer produced a smooth cheese spread, the microstructure of which was uniform (Fig. 18). At a higher magnification, however, a corpuscular microstructure was apparent (Figs. 19 and 20).

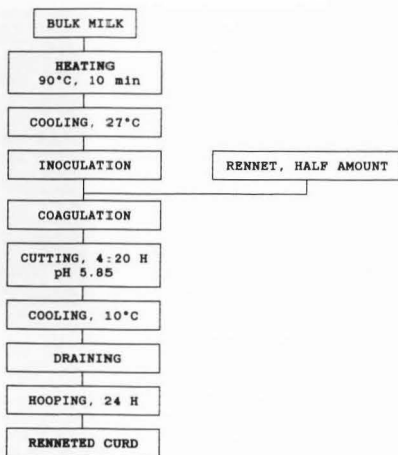


Fig. 13. Flow diagram showing the preparation of curd made from milk heated at 90°C for 10 min.

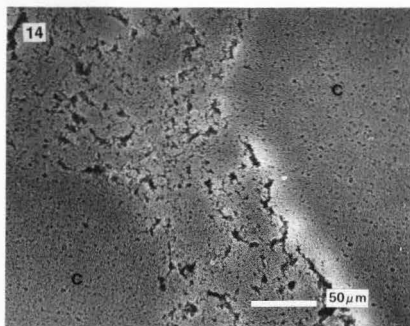
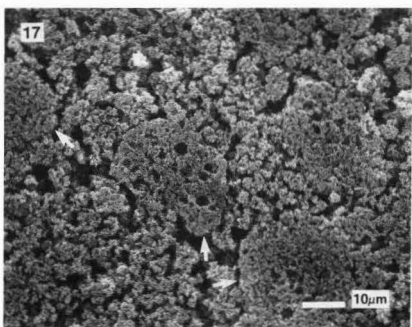
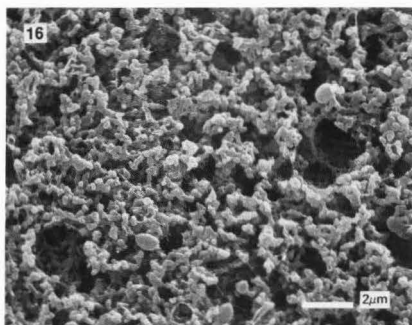
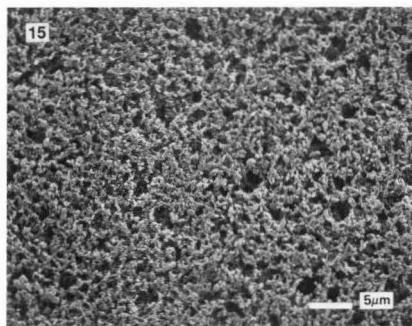


Fig. 14. Curd made from heated (90°C, 10 min) milk consisted of grains (C) having a uniform structure.

Fig. 15. Uniform structure of curd grains at a higher magnification.

Fig. 16. Detail of the casein particle matrix in curd made from milk which had been heated at 90°C for 10 min.

Fig. 17. Blending of curd made from heated (90°C, 10 min) milk with cream resulted in the disintegration of the curd grains into smaller particles (arrows).



This structure is the result of the disintegration of the considerably larger grains by blending and homogenization. It is important to note that all casein particle clusters were porous and that no compact particles were found either by sensory evaluation or by SEM of the hot-pack product.

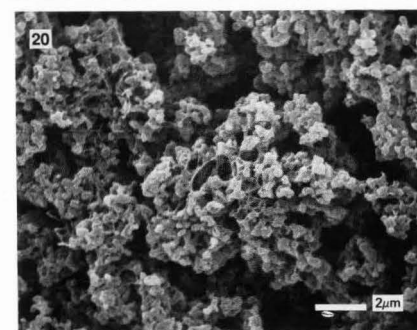
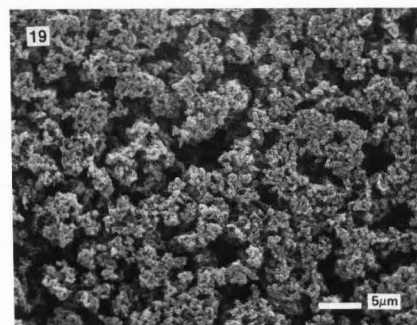
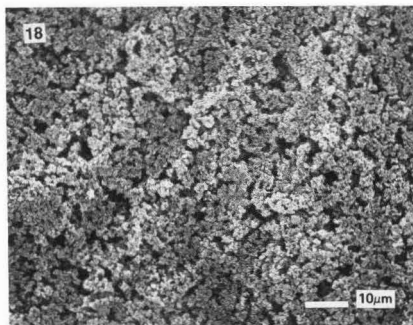


Fig. 18. Homogenization of the curd and cream blend produced a cream cheese spread which had a uniform structure.

Fig. 19. At a higher magnification, the disintegration of the uniform grain matrices into small

An examination of the effects of stabilizers on the microstructure showed that some stabilizers, although not causing grittiness, may have contributed to the development of a coarse texture of the product. One such example was a stabilizer based on a red seaweed (*Gelidium*) extract which contained galactomannans. Its use in the preparation of the cheese spread resulted in a heterogeneous microstructure which, although not noticeable by the mouthfeel, was clearly detected by fluorescence microscopy. This heterogeneity was characterized by the presence of a low concentration of casein particle aggregates (Fig. 21) and also fibrous particles of the stabilizer (Fig. 22). The aggregates included fat globules and were neither as compact (Figs. 23 and 24) nor as large as the particles which caused grittiness (Figs. 9-11). TEM confirmed the less compact internal structures of the aggregates (Fig. 25). The casein particle aggregates developed in the presence of the stabilizer were susceptible to partial disintegration by homogenization (Figs. 26 and 27). The mouthfeel of the resulting hot-pack cheese spread was smooth.

In conclusion, the development of grittiness in the products described was found to be related to the heat treatment of milk. Fluorescence microscopy was used to rapidly detect hard particles causing grittiness. SEM contributed to the solution of the grittiness problem because it helped to establish that the gritty particles were composed of compacted protein. The examination of their internal structure was made possible following the encapsulation of the viscous product in agar gel tubes and freeze-fracturing. TEM of thin sections confirmed the SEM findings.

Acknowledgments

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casein particle clusters is noticeable leading to the formation of a spreadable product.

Fig. 20. Detail of casein particle clusters in a homogenized hot-pack cheese spread made from heated (90°C, 10 min) milk.

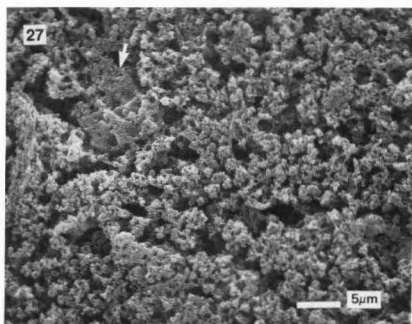
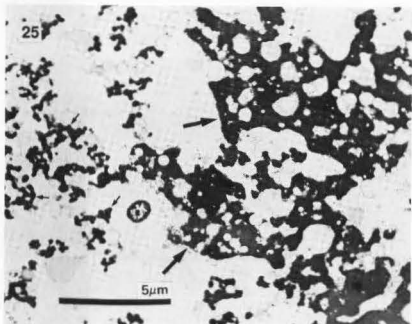
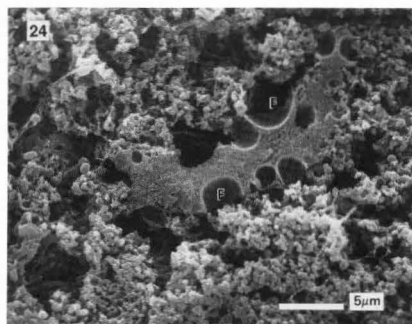
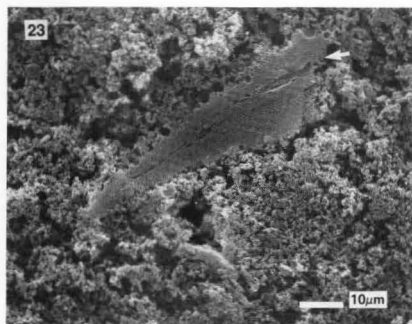


Fig. 23. SEM of a casein particle aggregate (arrow) in a hot-pack cheese spread [made from heated (90°C, 10 min) milk] containing a stabilizer.

Fig. 24. The presence of fat globules (F) in a casein particle aggregate is evident from globular void spaces which developed in the aggregate as a result of preparing the sample for SEM.

Fig. 25. TEM confirms the formation of casein particle aggregates (large arrows) in the porous protein matrix (small arrows) in a curd containing a stabilizer.

Fig. 27. SEM confirmed that casein particle aggregates (arrow) were markedly disintegrated as the result of homogenization.

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Discussion with Reviewers

R. T. Marshall: Since the curd, which produced a smooth cheese spread, was made with one half as much rennet as the curd giving a gritty spread, this introduces two variables, i.e., heat treatment and rennet concentration. How was it determined that heat alone was the causative factor?

Authors: Several measures to reduce the grittiness were taken and have been mentioned, e.g., lower temperature of pasteurization, triple homogenization, use of another bacterial starter culture, and use of acid-coagulated curd instead of renneted curd. All of these measures failed to prevent grittiness from developing. Since heating of the milk to 90°C eliminated the defect, insufficient heating of the milk prior to coagulation was identified as the factor causing grittiness in the product under study.

R. T. Marshall: That homogenization reduced the sizes of larger grains is not apparent in comparison of Figs. 16 and 20. These are of the same magnification and appear to have the same sizes of casein micelles. Fig. 20 appears to show a higher degree of aggregation of the micelles and other particulates. This point needs clarification.

Authors: Fig. 16 shows detail of a large grain, featured in Fig. 14 and marked with letter C. These grains have a uniform microstructure consisting of a continuous matrix of casein micelle chains and clusters. As a result of homogenization, the grains were broken into considerably smaller particles. These particles are shown in Fig. 20. Homogenization also led to some compaction of the matrix but the casein micelle dimensions remained unaffected.

M. L. Green: You state that the porous structure of the spread contains whey-filled pores. Where is the relatively large amount of fat in this product located? As you have extracted the fat during the preparation of most samples, can you be sure that fat is not involved in the formation of the gritty particles?

Authors: Preliminary examination of the gritty product by fluorescence microscopy pointed to protein rather than to fat as the cause of grittiness. Fat was in the form of small globules, the distribution and dimensions of which are evident from Fig. 12. This micrograph was obtained by TEM of a thin section.

D. N. Holcomb: You state that grittiness was evaluated subjectively by mouthfeel. Was such evaluation done by a trained panel or by expert cheesemakers? Was it done by sensing particles between the tongue and palate or between the teeth? Can you provide enough details so a reader could repeat this evaluation? In my experience, "experts" may disagree as to whether or not a product is gritty.

Authors: Grittiness was detected by examining the cheese spread between the tongue and the palate. It was so pronounced in the initial product that it was not necessary to use the services of a trained panel.

D. N. Holcomb: The authors note that bacteria were associated with the compact particles in hot-pack cheese with a higher incidence than in the surrounding medium. Can they postulate an explanation as to why the bacteria should be preferentially associated with the particles? Could the bacteria contribute to formation of the compact particles?

Authors: Gritty particles originated by the shrinkage and compaction of larger areas of curd. During this act of shrinking, the bacteria were concentrated into the smaller volume of the compact particles and gave the appearance of being in higher numbers per unit volume.

D. N. Holcomb: Is there any correlation between particle size and grittiness? Would the same correlation hold for compact protein particles, crystals, and other particles?

Authors: Probably there is a correlation, but it was not the subject of this study.