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STRUCTURE AND RHEOLOGY OF STRING CHEESE

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Abstract

String cheese samples ranging in pH from 5.0 to 5.9 were prepared by kneading Mozzarella curd and stretching it 2 to 80 times. Rheological properties were examined by compression, stress relaxation, and flow property tests. Stringiness was tested by a device specifically designed for this purpose: a standardized string was pulled from the sample at a direction perpendicular to the orientation of the curd and the amount of fibres resulting from this process was quantitated using digital image analysis.

Force-deformation curves implied that the curd prepared by kneading may be considered to be an incompressible viscoelastic body similar to rubber. Curd made at pH 5.0 flowed more easily than the other curds. Changes in pH had greater effects on rheological properties than the moisture content. The effect of temperature on the flow properties should be considered when pumping the curd through pipes, extruding from nozzles, and in stretch processing. Stringiness was low at 5 to 10 °C but was markedly increased at 25 °C, at which temperature string cheese should be consumed. Optimal stringiness was obtained in cheese curd samples having a pH of 5.4.

Light microscopy and electron microscopy revealed that stringiness is associated with a uniform longitudinal orientation of the protein matrix. Moderate stringiness occurred in a network of casein subunits having diameters equal to those of casein submicelles. Cryo-SEM showed that the state of water in string cheese was affected by kneading.

Introduction

String cheese has a characteristic structure which allows the cheese to be torn in one direction like crab meat or scallops. String cheese is usually consumed as a snack [1]. It reveals a firm texture when chewed. The product is marketed in the form of sticks which have been obtained by cutting Mozzarella curds. Manufacturing of string cheese is similar to that of Mozzarella cheese except that the cheese is stretched (elongated) in hot water to form ropes which acquire a fibrous structure, all oriented in one direction.

The quality of string cheese is evaluated from the amount of fibrous material noticeable when the cheese is torn. We refer to this quality as "stringiness" [2]. Stringiness is more important to string cheese than shreddability and meltability; these two latter properties are important to Mozzarella cheese. Stringiness is conventionally judged by graders, who tear each sample several times with fingers, examine them visually, and note the amount of the fibrous material and its thickness. Cheese with copious amounts of fine fibrous material on the torn surface is given a high rating.

Although stringiness is an attribute which determines quality, no methodology suitable to objectively evaluate manufacturing conditions leading to desired stringiness has yet been established. In this study, the effects of the manufacturing conditions have been clarified on the basis of rheological properties of string cheese over a wide range of pH values. This study has also resulted in the development of a method for objective evaluation of string cheese. The structure of string cheese is elucidated and the mechanism by which it is formed is discussed.

Materials and Methods

Sample Preparation

String cheese was prepared according to the Mozzarella preparation process described by Kosikowski [8, p. 196]. Partially skimmed milk (3% fat, pH 6.55) was pasteurized at 75 °C for 15 minutes, and then cooled to 30 °C. At this point, starter (1.5% of mesophilic lactic bacterial culture consisting of *Streptococcus lactis* and

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Key Words: String cheese, Mozzarella cheese, stringiness, compression test, flow tester, stress relaxation, network structure, light microscopy, scanning electron microscopy, transmission electron microscopy.

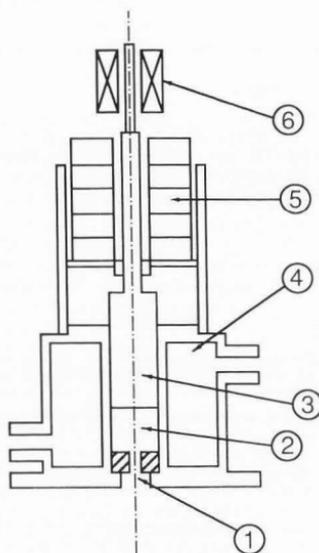


Figure 1. Schematic diagram of flow tester showing capillary (1), sample (2), plunger (3), jacket (4), weight (5), and transducer (6).

Table 1. Chemical Composition of Cheese Curds. (constituents in weight percent)

pH	water	fat	non-fat solids
5.9	48.7	27.0	24.3
5.4 (normal)	42.0	30.9	27.1
5.0	43.6	29.7	26.7

Streptococcus cremoris and calcium chloride (0.01%) were added. Calf rennet (33 ppm, Chr. Hansen's Laboratorium, Als, Denmark) was added one hour later. After an additional 35 minutes, the coagulating curd was cut into cubes using a curd knife having 12 parallel steel wires with a pitch of 10 mm. The curd cubes were gently agitated and heated to 38 °C. When the pH value of the curd had fallen to 6.0, the whey was drained off and the curd was matted. Matted curd was kept at 38 °C to permit mild acid ripening to further lower the pH value. As the pH was decreasing, samples were collected at pH 5.9, 5.4, and 5.0. The decrease from pH 5.9 to 5.4 needed one hour and another hour was required to lower pH of the curd from 5.4 to 5.0. Curd produced commercially usually has pH 5.2 to 5.4.

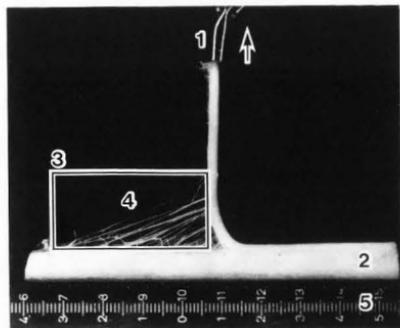


Figure 2. Measurement method for stringiness of cheese. 1: clamp; 2: sample; 3: window; 4: stringy material; 5: sample holder with a scale.

Curd samples (each weighing 1 kg) were wrapped in plastic film, frozen in a freezer, and stored at -18 °C until needed.

Moisture in the samples was determined using the atmospheric oven method [4] and the fat content was determined by the Rose-Gottlieb method [5]. Composition of the samples is presented in Table 1. The pH values of the cheese curd were measured using a needle-type glass electrode and a pH-meter (Model PH82, Yokogawa, Japan).

In preparation for rheological measurements, the frozen curd samples were thawed in a 55 °C water bath and kneaded by hand to remove air and excess whey. This process yielded a compacted mass of plastic curd. The curd was then formed into cylinders for rheological measurements. Sample preparation took 5-7 minutes.

During kneading in hot water, the water was turned white as soluble components of the curd, such as cream, leached out. The kneaded curd samples were placed in a water bath adjusted at a preset temperature for subsequent measurements.

Preliminary experiments showed that freezing and thawing of string cheese or the cheese curd did not significantly affect stringiness or rheological properties. Also Cervantes *et al.* [2] reported that consistent and statistically significant effects of the freeze-thaw cycle on the characteristics of Mozzarella cheese could not be detected using sensory or mechanical tests.

For microscopic examination, samples having various elongation ratios were prepared by stretching the curd to elongation ratios of twice, 8-times, and 80-times the original length [7]. The matted thawed curd was kneaded by hand in water at 60 °C to make plastic curd. A plastic curd sample (400 g) was formed into a cylinder 30 cm long and 4.0 cm in diameter. The curd was stretched by hand to double its length (so-called "double-elongated" sample). The 8-times elongated sample

was prepared by firstly elongating a curd sample 4-times, immersing it into hot water making a single compacted rope. This rope was then doubly elongated to produce the 8-times (4 x 2) elongated sample. The 80-times elongated sample was prepared in a similar way (4 x 4 x 5). After elongation, the curd was immersed in cold water (7 °C) to harden; the hardened curd was cut into samples of the required length.

Rheological Measurements

Compression Tests: A rheometer (Rheoner, Model RE-3305, Yamaden, Japan) was used for the compression tests. Plastic curd, prepared in a 55 °C water bath, was placed in a plastic pipe, 3.5 cm in diameter and 4.5 cm in length. Both ends were flattened by pressing with a plastic plate. Then, the curd was immersed for 15 minutes in a water bath, adjusted to a given temperature. The curd was then taken out of the pipe and loaded into the sample chamber bath attached to the rheometer. Compression tests were carried out on the sample immersed in the water bath. Because the manufacturing process involves kneading and cooling in water, evaporation of water and fluctuation of temperature can be minimized.

Compression tests were carried out at 0.05 cm/s at 5, 15, 25 and 30 °C. The force-strain curve was recorded. Young's modulus was calculated from the slope of the tangent to the initial part of the curve.

Stress Relaxation The rheometer was also used to measure stress relaxation. Samples were prepared in the same way as for the compression tests. A strain of 4 to 8% was applied by compressing at 1 cm/s and held for 5 minutes. The stress relaxation curve was recorded. The measurements were carried out at 5, 15, 25 and 30 °C. An eight-element mechanical model was applied to analyze the curve.

Flow Tester Flow properties of the string cheese curd were examined using a flow tester. The schematic diagram of the flow tester is shown in Figure 1. Plastic curd, prepared in a 55 °C water bath, was formed into a cylinder using a plastic pipe. Then, the sample was loaded into the sample chamber (2). The sample was extruded from a capillary (1) by pressing with a plunger (3). A capillary, having an inner diameter of 0.16 mm and a length of 0.5 cm, was used. The pressure applied to the capillary was adjusted by applying weights (5). The plunger speed was measured using a transducer (6), attached to the plunger head, while varying the pressure between 1370 and 41000 Pa. The flow volume was calculated from the plunger speed and the cross-sectional area of the capillary. Measurements were made at 45, 50, 55, 65 and 70 °C while the whole apparatus was immersed in a water bath. Preliminary experiments showed that extremely high pressure had to be applied to extrude the curd from the capillary and a great amount of "oiling off" was observed if the temperature of measurement was below 45 °C. Consequently, the measurements were carried out at a higher temperature of 45 °C. The power law equation [8] was applied to analyses of

the data obtained.

Generally, the end effect was not negligible. A correction was made according to Bagley's method [1]. Three capillaries, each having radii (r) of 0.2 cm and being 0.5, 1.0, and 1.5 cm in length (l), were employed to correct the end effect. The l/r was 2.5, 5.0 and 7.5, respectively. The correction factor (ν) was estimated from the relation between the shear rate ($\dot{\gamma}$) and pressure (P). Shear stress at the wall surface (τ_R) was calculated by applying the following equation and using the corrected capillary length (l_c).

$$l_c = l + \nu r \quad (1)$$

$$\tau_R = P r / (2 l_c) \quad (2)$$

Stringiness The same kind of samples which had been subjected to rheological measurements were used to measure stringiness. A sample measuring 10 x 20 x 90 mm was cut out with a steel wire. Then, the sample was wrapped in plastic film and immersed in a water bath adjusted to a given temperature for 30 minutes. One end of the sample was cut 5 mm deep and 2 mm wide. The sample was fixed in a holder after pinching the end in a clamp. The clamp was pulled up vertically at constant speed to tear the sample apart as shown in Figure 2. The length of the torn part was read from the scale on the holder. The area occupied by fibrous material was measured using a digital image analyzer (PIAS LA-555, Japan); the visible area was limited by a rectangular window [5]. The measurements were carried out at room temperature (26 °C) in five repetitions. Each measurement took 3 minutes to complete.

Microscopic Observation

Light microscopy Samples measuring 7 x 7 x 15 mm were cut out from elongated string cheese in order to check their uniform orientation and then fixed in a 10% formaldehyde solution for 20 hours. Fixed samples were washed several times with distilled water and then 10 μ m sections were cut using a cryo-microtome (ERMA Optical Instruments, Japan) equipped with a freezing unit (PIKA SEIKO Ltd., Japan). Fat and protein in the sections were stained using Sudan IV and Methylene Blue, respectively, as follows:

1. Sections were washed in water for 2 to 5 minutes, then immersed in propylene glycol for 3 to 5 minutes.

2. The sections were immersed in a Sudan IV solution saturated in 80% propylene glycol for 1 hour, then immersed in 80% propylene glycol for 2 to 3 minutes.

3. After washing with water, the sections were immersed in Methylene Blue for 3 to 5 seconds.

4. After washing with water, the sections were mounted on glass slides and sealed with gelatin. Micrographs at magnifications of 40x to 400x were obtained using a light microscope (Apophot, Nikon, Japan).

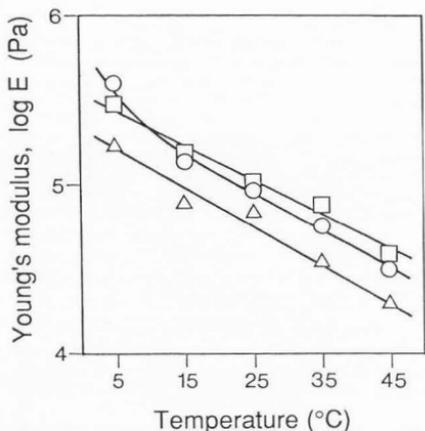


Figure 3. Temperature dependency of Young's modulus E of curd. Young's modulus are calculated from initial stage of force-strain curves. Curd pH: 5.9 (squares); 5.4 (circles); and 5.0 (triangles).

Critical Point Drying, Scanning Electron Microscopy (SEM) Samples were fixed with 1% glutaraldehyde (pH 6.0, cacodylate buffer) for over 20 hours. Dehydration was carried out using a series of ethanol solutions of 50, 70, 80, 90, 95%, and absolute ethanol. Each step was performed for 20 minutes with stirring. After dehydration, absolute ethanol was replaced with isoamylacetate and the samples were dried in a critical point dryer (HCP-2, Hitachi, Japan) using liquid carbon dioxide. The dried samples were mounted on aluminum sample holders using a water-soluble adhesive (White bond, Konishi, Japan) and then given a 15 nm thick platinum coating in an ion coater (IB-5, Eiko Engineering, Japan). The specimens were examined in an S-800 Hitachi scanning electron microscope (Hitachi, Japan).

Cryo-SEM Samples measuring $1 \times 1 \times 5$ mm were cut out and placed on a cryo-stat sample holder and frozen by immersing into liquid nitrogen for 20 to 30 seconds without any chemical fixation. The frozen samples were immediately loaded into the cryo-SEM and fractured with a cooled blade. Images of the fractured surface were observed immediately after fracture, without any further treatment. Then the sample temperature was raised to -80°C to sublime the ice, after which the surface was coated with gold to prevent charge build-up and enhance contrast. This method enables the detection of water by comparing the appearance of the fractured surfaces before and after sublimation.

Freeze-Fracturing, Transmission Electron Microscopy (TEM) Cheese samples measuring $1 \times 1 \times 5$ mm were cut out and mounted on sample holders and

then immersed in liquid nitrogen for 40 seconds. Immediately after freezing, the samples were loaded into a freeze-fracture unit (JFD9000, JEOL, Japan) and fractured with a blade at -170°C . The samples were then subjected to ice sublimation at -80°C and were rotary-coated with platinum and carbon at an angle of 60° by electron beam evaporation followed by carbon coating to produce a replica film. The replicas were cleaned by immersion in hot water for 10 minutes followed by defatting in acetone and a mixture of methanol and chloroform (1:1). Then the replicas were placed again into acetone followed by immersion in a 5% sodium hypochlorite solution for 60 minutes and washing with 3 changes of distilled water. Dried replicas were examined in a HU-12A (Hitachi, Japan) transmission electron microscope operated at 100 kV.

Results and Discussion

Force-Deformation Curve

Young's modulus, the modulus of elasticity E , obtained from the compression tests, is shown in Figure 3. The logarithm of Young's modulus decreased linearly as the temperature increased. Curd prepared at pH 5.0 showed a lower Young's modulus than other curds in this experimental series. The curds prepared at pH 5.4 and pH 5.9 showed similar Young's moduli.

Shear modulus (G) was measured at 25°C using Van Holde's shear viscometer [12] to estimate Poisson's ratio (σ). The value was 3.44×10^6 Pa for curd prepared at pH 5.4. The following relationship exists [12] between Young modulus, shear modulus (G) and Poisson's ratio (σ):

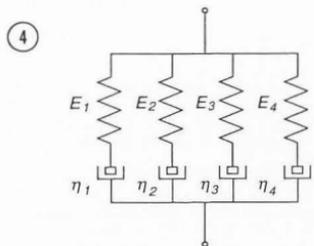
$$\sigma = (E - 2G) / 2G \quad (3)$$

Substituting the values of Young's modulus and shear modulus into equation (3), yielded $\sigma = 0.493$. This value implies that curd, prepared by kneading to remove the air and excess whey, may be considered as being an incompressible viscoelastic body, similar to rubber.

Stress Relaxation

Linearity was confirmed up to 10% strain from the stress-strain curve. Four to eight percent strain was then applied to obtain the stress relaxation measurements. The stress relaxation curve was analyzed using an eight-element mechanical model consisting of four Maxwell models combined in parallel [10]. The four Maxwell element model is shown in Figure 4, where E_1 , E_2 , E_3 and E_4 are relaxation moduli; η_1 , η_2 , η_3 and η_4 are relaxation viscosities; and τ_1 , τ_2 , τ_3 and τ_4 are relaxation times and the order of magnitude is $\tau_1 > \tau_2 > \tau_3 > \tau_4$.

The temperature dependence of the relaxation modulus, relaxation viscosity and relaxation time for curd of pH 5.4 are shown in Figure 4. All decrease as temperature increases. The relaxation modulus E_1 , for



Four Maxwell Element Model

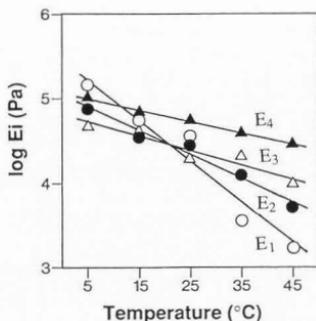
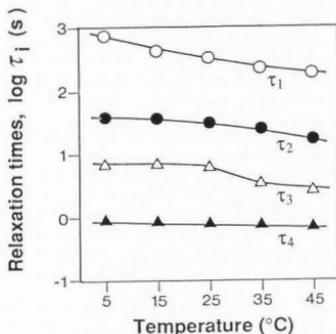
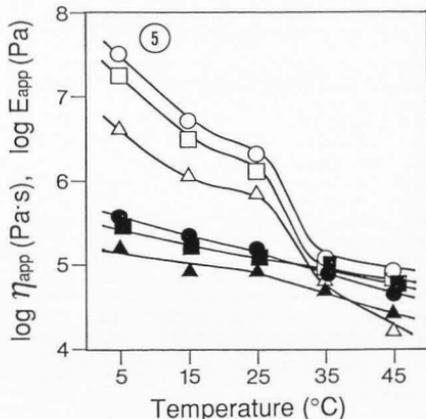
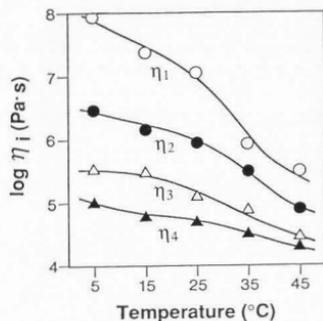


Figure 4 (above). Rheological parameters on Maxwell module in pH 5.4 curd. E_1 to E_4 : relaxation moduli; η_1 to η_4 : relaxation viscosities; τ_1 to τ_4 : relaxation times.

Figure 5 (at right). Temperature dependence of apparent relaxation modulus E_{app} and viscosity η_{app} for curds. The rheological model used here consists of only one Maxwell model. The parameters are calculated as apparent modulus and viscosity. η_{app} (pH 5.9): hollow squares; η_{app} (pH 5.4): hollow circles; η_{app} (pH 5.0): hollow triangles; E_{app} (pH 5.9): solid squares; E_{app} (pH 5.4): solid circles; and E_{app} (pH 5.0): solid triangles.

which the relaxation time is the longest, showed a pronounced decrease. Temperature dependence decreased in the order of E_2 , E_3 , E_4 . The same tendency was observed for the relaxation viscosity, η_1 , for which the relaxation time is the longest, showed a remarkable temperature dependence. On the other hand, the relaxation time showed less temperature dependence than the relaxation viscosity and modulus. Especially, τ_4 , for which the relaxation time is the shortest, showed no temperature dependence. These tendencies were observed for



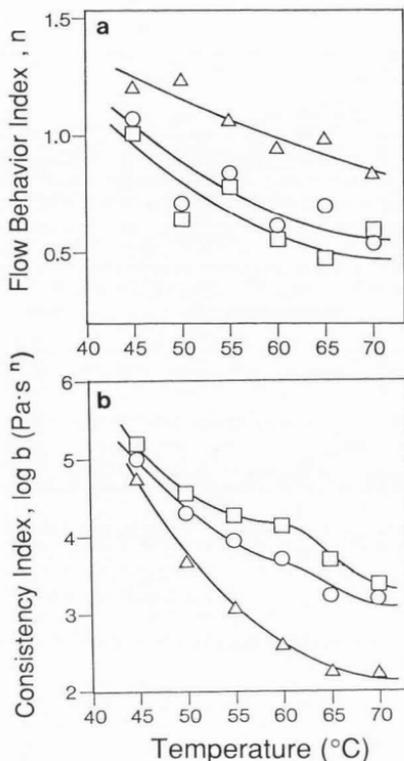


Figure 6. Flow behavior index (n) and consistency index (b) versus temperature of curds. pH of curds: pH 5.9: squares; pH 5.4: circles; pH 5.0: triangles.

the curd having a pH of 5.0 and 5.9.

The relaxation curve could be represented perfectly if the number of elements in the mechanical model were increased. However, the physical meaning would become ambiguous and an explanation, from the practical point of view, would become difficult. We consequently tried to summarize the stress relaxation curve using a few elements. One Maxwell mechanical model was assumed, from which the apparent relaxation modulus, viscosity and relaxation time were calculated. The apparent relaxation modulus (E_{app}) was estimated from the initial stress and the apparent relaxation time (τ_{app}) was estimated from the time when the stress became one third of the initial stress. The apparent viscosity (η_{app})

was estimated by the following equation:

$$\eta_{app} = E_{app} \times \tau_{app} \quad (4)$$

The temperature dependence of the apparent relaxation modulus and viscosity are shown in Figure 5. Both had a large temperature dependence with the apparent viscosities decreasing drastically at around 30 °C.

Flow Properties

A slight yield was observed in the curd at 45 °C, but no yield was observed at temperatures over 50 °C. The relationship between shear stress (S) and shear rate ($\dot{\gamma}$) can, therefore, be expressed by the following equation:

$$S = b (\dot{\gamma})^n \quad (5)$$

where n : flow behavior index, b : consistency index which represents the degree of "flowability".

The temperature dependence of n for the three samples is shown in Figure 6a. The value was almost unity below 55 °C, but decreased for higher temperatures. The temperature dependence of n could be explained as follows. The interaction forces between proteins, such as hydrogen bonds and van der Waals' force, are thought to weaken as the temperature increases. Such weak bonds would be broken and/or be regenerated when the sample is deformed [9]. The value of n may be influenced by the phenomena mentioned above. The flow properties for temperatures between 50 and 60 °C are the main concern in the production of string cheese. The curd may be considered as exhibiting Newtonian flow in this temperature range.

The relationship between the consistency index b and temperature is shown in Figure 6b. The consistency index b has the dimension of viscosity when $n = 1$. The value of b decreased drastically as the temperature increased. The curd having a pH of 5.9 gave the highest value. The value decreased as the pH of the curd decreased. These results suggest that curd having a lower pH can flow more easily and that the temperature of the curd is a very important factor in controlling the flow properties such as when pumping through pipes, extruding from nozzles and in stretch processing.

Evaluation of Stringiness

Dependence of area of fibrous material upon tearing speed and temperature is shown in Figures 7a, b. The sample, 1 cm in thickness, was torn along a 4 cm length and measured at 5 °C and 25 °C. The error bar shown in Figure 7 shows the standard deviation. Figure 7a indicates a tearing speed dependence. The tearing speed did not have a significant effect. Measurement required a few seconds at any tearing speed. The tearing speed employed was 1 cm/s, which can be observed visually. The relationship between sample temperature and the area of fibrous material is shown in Figure 7b. The data spread was large. However, a tendency to increase with temperature was observed, reaching a maximum at 25 °C and then decreasing. Fat in the curd was

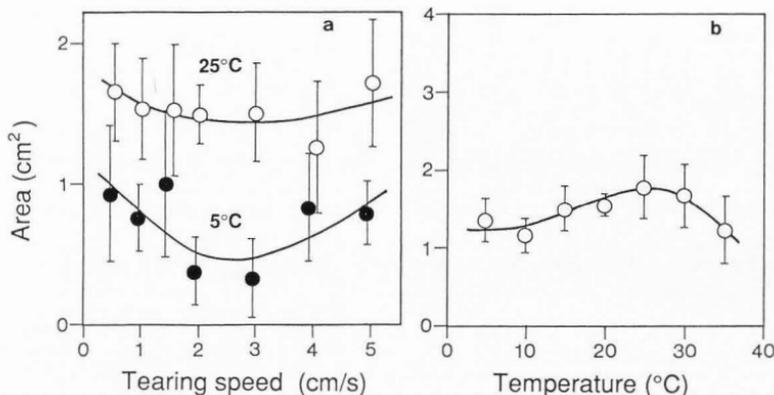


Figure 7. Effects of tearing speed and temperature of samples on area of stringy materials. Sample of 1 cm in thickness was torn 4 cm in length, and the area of stringy materials was measured. (a) Tearing speed dependency; (b) temperature dependency.

observed to oil off at 35 °C because the structure of the curd is disrupted at this temperature.

String cheese is normally stored in a refrigerator. At such storage temperatures between 5 and 10 °C, stringiness remains low due to the fibrous material snapping easily. Since string cheese is characterized by its stringiness, to maximize the stringiness, its temperature should be increased to 25 °C before consumption.

Structure of String Cheese

Figures 8a, b, c show longitudinal sections of the curd samples, stained and observed with a light microscope. The fibrous structure of the doubly elongated curd is shown in Figure 8a. A deformation in the stretching direction (indicated by a pair of arrows) was clearly visible. Although a lump-like structure still exists, an elongated structure was observed to be dominant. Fat was deformed in the longitudinal direction and dispersed between the protein strands. It was assumed that fat globules were ruptured during heating and stretching, and that they coalesced.

The fibrous structure of a curd sample, elongated eight times, is shown in Figure 8b. As the elongation ratio increased, the proteins were elongated and the fibrous structure became clearer. It appeared that the gap between the fat and the protein narrowed, resulting in the appearance of finer fibrous material as the elongation increased. This could be seen in curd elongated eighty times, as shown in Figure 8c. The fibrous material indicated by arrow "a" in Figure 8c was 10 μm in width.

The elongated cheese curds shown in Figures 8b, and 8c had high elasticity in the longitudinal direction, and were easy to tear in the perpendicular direction.

When the cheese curd was torn apart, a lot of fibrous material appeared on the torn surface.

Many void spaces were observed as white areas in the three micrographs of Figure 8. Cryo-SEM was used to show which components were present in the original samples and which components had been removed during sample preparation. The cryo-SEM images of the cheese elongated eight times are presented in Figure 9. The curd had been cut in the longitudinal direction and the images of the same areas were taken immediately after cutting before freeze-etching (Figure 9a) and again after freeze-etching (Figure 9b). The arrows in Figures 9a and 9b show the same points confirming that both micrographs are of the same area. There were no void spaces in the cheese shown in Figure 9a and only fat globules and elongated protein were observed. In contrast, void spaces appeared between protein and fat following freeze-etching (Figure 9b). It may be concluded that water was initially present in those spaces and was removed by the sublimation of ice. The water may have originated from whey or from water dispersed in the cheese during its kneading in hot water. The state of the water is different from water present in process cheese or in natural cheese, where it is hydrating the proteins.

A cross-sectional view of the fibrous structure is shown in Figure 10. The sample was prepared by critical point drying and examined by SEM. The fat in the cheese was extracted since the sample was treated with ethanol during the dehydration process. Consequently, only protein was observed with no indication that fat or water were present in the cheese. It can be seen from this micrograph that the cheese structure consisted of protein fibers, all oriented in one direction.

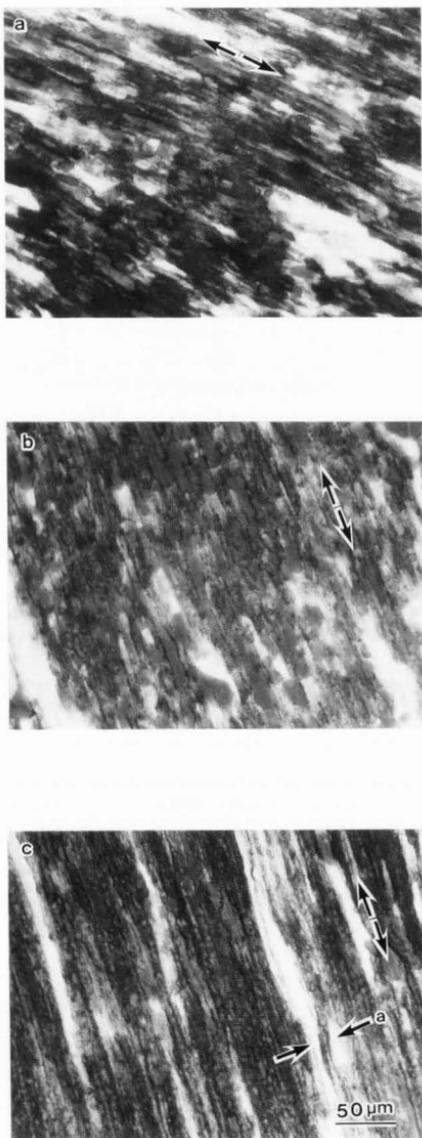


Figure 8. Longitudinal section of curds of various elongation ratios. The arrow pairs show the longitudinal direction. a) Doubly elongated curd; b) 8 times elongated curd; and c) 80 times elongated curd. Arrow "a" (in c) shows fibrous material with 10 mm width.

Reddish yellow: Fat; Blue: Protein

The fibrous material that appears when the cheese is torn is shown in Figure 11. The sample was prepared by critical point drying. The surface of the protein layer was not smooth. There were many marks (arrow a) caused by the presence of fat and water. Arrows (b) in Figure 11 show the fractured part of the protein layers that appeared when the cheese was torn with the fingers.

Casein matrices of three kinds of curds having different pH values are shown in Figure 12. Curd samples with 2 to 3 times elongation were prepared. A sample was prepared by the freeze fracturing method and observed with TEM. The casein matrix consisted of subunits of 10 nm in diameter. The diameters were equivalent to those of casein submicelles. The subunits appeared to be connected to each other. In curd having pH of 5.4, the diameters of the subunits were less than 10 nm, some of which appeared to have formed a network structure. In curd having pH of 5.0, the network structure was not as well defined as that of curd having pH of 5.4.

By observing the network structure, we concluded that curd having pH of 5.4 was suitable for stretching.

Conclusions

The relationship between preparation conditions and fiber formation during elongation was studied.

(1) The rheological properties of cheese curds are highly dependent on temperature. Therefore, temperature is a very important factor in the manufacture of string cheese.

(2) Cheese curds lose their flowability and harden below 35 °C. Therefore, the forming process during manufacture should be performed above this temperature.

(3) Cheese curd may be considered as being an incompressible viscoelastic body. The relationship between shear rate and shear stress was well represented by the power law equation. The flowability of cheese curds of various pH values decreased in the order of pH 5.9, 5.4, 5.0.

(4) Stringiness is well defined by the area of fibrous material that appears when cheese samples of 1 cm in thickness are torn for 4 cm at a speed of 1 cm/s.

(5) The protein matrix in string cheese is arranged in the longitudinal direction. It constitutes the continuous phase, with water and fat dispersed throughout this phase.

(6) The fibrous material, that appears when the cheese is torn, arises from the breakage of the protein

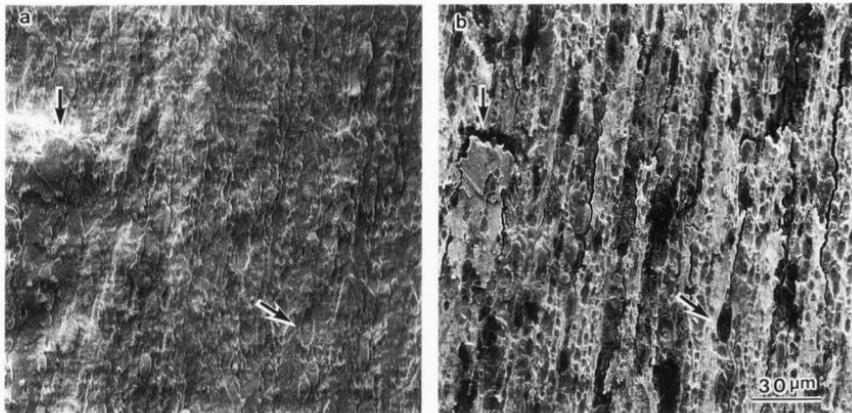


Figure 9. Longitudinal section of curd before and after freeze-etching. These images were taken with cryo-SEM. The arrows in the micrographs (a) and (b) show the same points to confirm that the same area was being examined. (a) Before freeze-etching (immediately after cutting); (b) after freeze-etching and coating.

matrix arranged in the longitudinal direction, and the release of water and fat dispersed within the matrix.

(7) Electron microscopic examination of cheese curds revealed networks consisting of casein subunits at pH 5.9 and 5.4 but not at pH 5.0. The network was more clearly noticeable in curd having pH of 5.4.

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Discussion With Reviewers

W. Buchheim: The freeze-etch micrographs of Fig. 12 demonstrate the fine structure of the protein matrix at a rather high magnification and with some differences as to the graininess of the background. The question arises to what degree experimental conditions (e.g., etching, replication) effect the appearance of the protein matrix and to what extent this appearance may vary on one replica, e.g., because of local variation of the inclination of the plane of fracture.

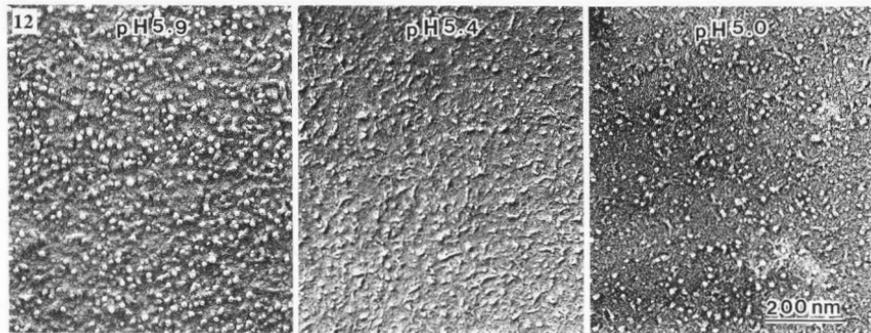
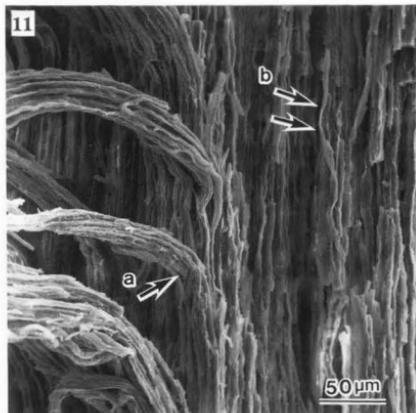
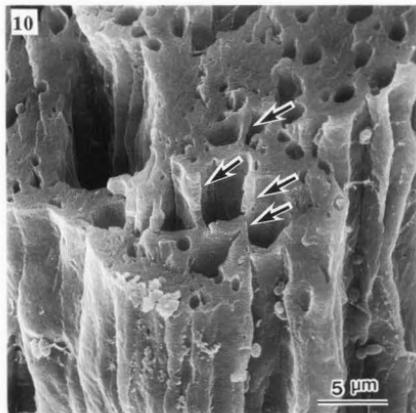


Figure 10 (top left). Cross-section of curd. Sample was prepared by critical point drying and observed with SEM. Arrows point to the thin protein layers where breakage would occur.

Figure 11 (top right). Fibrous protein matrix. Sample was torn along longitudinal direction, prepared by critical point drying and observe with SEM. Arrow (a) indicates the marks caused by the presence of fat and water; arrows (b) point to the fracture part of the protein layer.

Figure 12 (bottom). Casein matrix of cheese curd of 3 to 5 times elongation. Samples were prepared by freeze-fracture method and observed with TEM.

Authors: The appearance of the network structure varies with the etching time. Increasing the etching time results in more of the network structure being exposed. The network structure is formed by interconnected casein particles. As the etching time is extended, the strings of the structure appear longer. The etching times for the micrographs in Figure 12 were almost the same. Sample preparation was repeated several times to confirm that a similar structure was obtained.

The inclination of the fracture plane tends to vary. It is rare to find two fracture planes with the same inclination. These differing inclinations result in a different thickness of metal coating being laid down. This, in turn, affects the contrast of the protein particles and the graininess of the background.

P. S. Kindstedt: "Stringiness" as defined in this paper is an attribute that determines, in part, the quality of unmelted Mozzarella cheese. However, from the stand-

point of food services, which uses low-moisture Mozzarella as an ingredient for pizza, shreddability is a more important rheological quality attribute. Is there a correlation between stringiness as defined in this paper and shredding properties?

Authors: We have not examined the correlation between stringiness and shreddability. String cheese is eaten as a snack. Therefore, stringiness is an important attribute for string cheese, more than shreddability or meltability as required for Mozzarella cheese.

P. S. Kindstedt: Freezing Mozzarella cheese under certain conditions has been shown to cause large physico-chemical and rheological changes in the thawed cheese. What assurance do you have that you did not induce such changes by freezing your samples before analysis? With respect to your objective method for assessing stringiness, did you analyze a variety of samples with different degrees of stringiness, and did the objective measurements correlate with your subjective assessment of stringiness? For example, could the test distinguish between a sample that was "very stringy" and one that was "slightly stringy"?

Authors: Our main concern is the stringiness of string cheese. We confirmed that stringiness was not affected by freezing and thawing, and could survive extended periods of freezing. We did not measure the effect of freezing on rheological properties of string cheese. We believe that freezing the curd has little effect on rheological properties because the thawed curd was first kneaded in hot water (to simulate the actual manufacturing conditions of string cheese) to make it a homogeneous body.

We measured several samples having different degrees of stringiness. For example, one sample scored 3 to 4 while another sample scored 7 to 8 by sensory evaluation on a ten-point scale. The areas of fibrous material in samples were measured when a length of 4 cm was torn. The areas were 0.5 and 1.7 cm² respectively. These results suggest that poor to excellent stringiness may be evaluated from the area. However, to establish a concrete relation between sensory evaluation and objective methods, more repetitive experiments should be carried out using several kinds of samples.

P. S. Kindstedt: The moisture levels in your curd samples were extremely low, much lower than is typical for Mozzarella curd prior to stretching. How will this affect your rheological measurements and the interpretation of your data?

Authors: The water content of the string cheese curd tended to vary with the cutting size of the coagulating curd. Generally, the rheological parameters of cheeses, such as firmness, become higher as the water content decreases. Also, a few papers have dealt with the effect of pH on the rheological properties of cheeses. In our experiments, samples of pH of 5.4 and 5.0 contain almost the same amounts of water and fat. We concluded that the pH of the cheese curd had a greater effect on the rheological properties, such as Young's modulus, than

the water content.

P. S. Kindstedt: Did you replicate your cheese-making experiments enough times to draw statistically valid conclusions regarding the effect of curd pH on the rheological properties studied?

Authors: We did not repeat the cheese-making for this particular experiment. In practice, string cheese is made routinely. In fact, the quality of string cheese fluctuates to some extent. It is partly because of a slight difference in activity of rennet and starter which affect the rate of pH decrease and pH level. The quality standard in house is set for string cheese; we used typical string cheese which met the quality standard for this experiment. The effect of pH on the rheological properties was deduced from the results of the compression tests, stress relaxation and flow tests.

P. S. Kindstedt: Your cheese curds had extremely high fat-in-dry-matter (FDM) and contained no added salt. Several reports have shown that the oiling-off tendency of Mozzarella cheese increases as FDM increases and salt content decreases. Therefore, I would expect that your samples showed considerable oil separation when melted. Such separation can introduce artifacts into rheological measurements, particularly with capillary rheometry. Did your melted samples experience oil separation, and if so, how it influenced your measurements?

Authors: This experiment was originally aimed at establishing the manufacturing conditions of string cheese that involves kneading in hot water. When a frozen sample was thawed in hot water to provide samples for rheological measurement, the water became white because the soluble components of the cheese curd leached out. Then, the rheological measurement was performed. No oiling off was observed by capillary rheometry. This is because the unstable oil leached out when the cheese was kneaded in the hot water.

D.G. Pechak: You mention several times that fat and water are dispersed in a continuous protein phase. I would contend that the water/protein is a single phase in which fat droplets (round or elongated) are dispersed. Your interpretation, while easily visualized from the obvious "structure" of the protein component does not account for the interactions of water soluble components (protein, ions, etc.) with the structural proteins. Could you speculate on what components may be present in the water phase and how they may impact stringiness?

Authors: Many void spaces in string cheese were observed (Fig. 8). We concluded, from cryo-SEM observation (Fig. 9), that these spaces were originally occupied by the aqueous phase. The aqueous phase may consist of whey. The water/protein is a single continuous phase in case of ripened cheese. String cheese is not ripened. It can be considered to be a compact mass of fused curd particles. In the curd, aqueous phase (whey) may exist between curd particles. Stringiness is affected by the arrangement of the fat and aqueous phase because breakage would occur at such void spaces as shown in Figure 8 when string cheese is torn apart with fingers.