Study of the physical properties that confer string cheese with its texture and taste

Jungeun Kim*, Keigo Nagao**and Akihito Yamano**

1. Introduction

String cheese, which has a texture similar to that of jerky, the taste of fresh cheese, and strands that can be split apart, is a type of immature natural cheese. Natural cheese is generally prepared by adding lactic acid bacteria and rennet to milk, followed by adding salt to the coagulated milk protein to solidify it. String cheese is made from natural mozzarella cheese. After soaking the mozzarella cheese in hot water at 80℃, it can be stretched into a bar shape at room temperature and folded in half. This folding process is repeated to prepare the fibrous string cheese[(1)].

There is limited information about the history of string cheese. Some documents indicate that it was prepared in homes in Europe for a long time as a way of preserving fresh cheese, and/or it spread mainly as a menu item in pubs along the US coast. As a Japanese product, string cheese was developed by the Cheese Research Laboratory in Kobuchizawa in Yamanashi Prefecture in Japan, and string cheeses of various flavors are now on sale[(2)]. According to an article by the Food Industry newspaper on July 27, 2019, annual domestic consumption of cheese is 350,000 tons, of which household consumption is 140,000 tons, and total cheese consumption is increasing every year[(3)]. String cheese ranks high for its unique texture and strands, and its popularity has rapidly increased. However, there is a problem with insufficient milk supply due to an increase in the consumption of dairy products. For example, six pieces of string cheese, 1.5 cm in diameter and 10 cm in length each, require about 200 g of mozzarella cheese, which is prepared from 2 L of milk, corresponding to one-tenth of the daily production of a cow. The BBC News has criticized European laboratories for research into the efficacy of piercing a cow's body to inject feed directly into its stomach to increase milk yield[(4)]. Research and development on substitutes for string cheese are urgently needed.

Substitutional foods reproduce the texture, such as chewiness and softness, and the taste of original food. However, studying relevant parameters that reflect the characteristics of the original food is not simple because the texture and the taste of food generally depend on subjective factors such as individual differences in sense of taste and smell, changes in taste related to age, and amount of chewing and smelling. On the other hand, there are some objective factors that can be approached scientifically, such as internal structure, degree of crystallinity, crystal phase, and melting and crystallization process. X-ray measurement is one of the critical tools used to study these parameters. X-rays penetrate the material, are absorbed, and interact with the atoms and molecules that constitute the material to cause a diffraction phenomenon. Using the characteristics of X-rays, the internal structure at the micrometer ($1\,\mu m = 10^{-6}$ mm) to nanometer ($1\,nm = 10^{-9}$ mm) or Angstrom ($1\,Å = 10^{-10}$ mm) level can be visualized without destroying the material. The components of the material can be identified and quantified from the X-ray diffraction data. In addition to X-ray analyses, thermal analysis is also a powerful tool to study texture and taste. It allows the investigation of phase transition, melting, and crystallization temperature of materials, and so on.

In this study, we observed the internal structure of string cheese at the micrometer level and the periodic structure at the molecular level using X-ray analyses. The key features that were strongly related to the characteristics of texture and taste of string cheese are studied and the question of why string cheese splits is discussed in conjunction with an examination of the results of thermal analysis.

2. Observation of the Internal Structure of String Cheese by X-ray Microscopy

In food science, observing the internal structure of materials is often performed *via* electron microscopy, such as Scanning Electron Microscope (SEM) or Transmission Electron Microscope (TEM). However, pre-processing such as freezing, drying, staining, and slicing is necessary to observe the internal structure; thus, the images are often deformed or contaminated. An X-ray microscope can observe the internal structure without destroying the sample. A Cu source is especially appropriate for visualizing the internal structure with high contrast, even for foods composed of light elements.

A 3D image of a piece of string cheese[A] having a diameter of about 2 mm was obtained by computed tomography (CT) from images of 600 sections measured for 2.4 seconds per slice. Figure 1(a)–(c) show orthogonal images sliced from different directions. The center of the intersection on each image represents the same coordinates. White and black colors show high- and low-density regions. The string cheese includes

* Product Service Division, Rigaku Corporation.
** Application Laboratories, Rigaku Corporation.

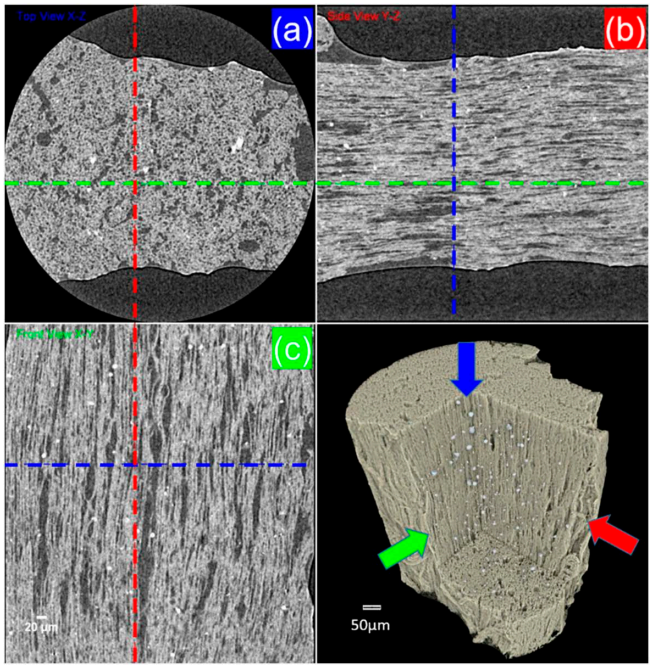

Fig. 1. Sliced CT image of string cheese[A].
(a) Top view (b) side view (c) front view.

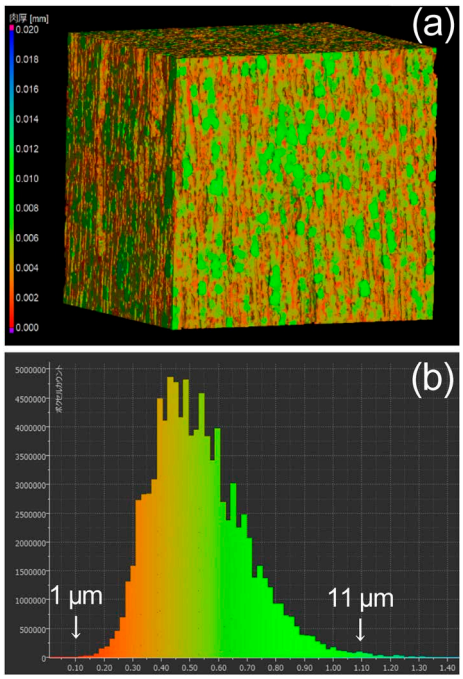

Fig. 2. (a) 3D view of the fibrous part colored by thickness and (b) thickness histogram.

irregularly distributed high-density ingredients of spherical shape considered to be salt, and low-density ingredients of irregular shape assumed to be oil and water. The fibrous part shown in the side and the front view has a net-like pattern in the top view; it is considered to be milk proteins. The fibrous part was extracted from the CT image of cheese by 3D image processing and was colored by thickness (Fig. 2(a)). The thickness of the fibrous part ranged from 1 μm to 11 μm, as shown in Fig. 2(b). The 3D image colored by thickness revealed clearly that the fibrous part consisted of aggregated milk proteins oriented in the vertical direction, lacking the shape of a single fiber.

3. Observation of Water Evaporation and Milk Fat Melting by TG-DTA and DSC

Differential thermal analysis (DTA) measures the temperature difference between a reference material and a sample with heat applied, while thermogravimetry (TG) measures the change in weight of a sample. The combination of these two thermal analyses can be used to obtain information such as the melting point, crystallization temperature, water evaporation, and specific heat capacity. These properties are strongly related to texture and taste, including melting in the mouth and smooth texture; thus, they are indispensable for food research. In addition, thermal analysis is often used to investigate the conditions in a tempering process in which the crystallization temperature is intentionally changed to obtain the best taste and flavor.

Figure 3 shows the results of TG-DTA of string cheese[A], and the surface photographs of the cheese acquired in real-time at 22℃ during the early stage and at two points where the DTA curve changes. The

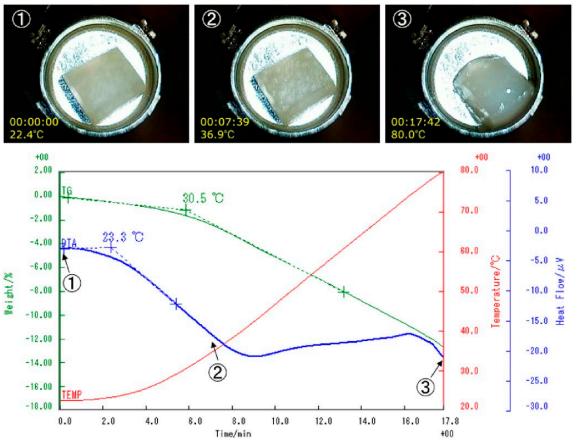

Fig. 3. TG-DTA result and simultaneously recorded surface photograph of string cheese[A].

measurement temperature range was 20℃ to 80℃. 80℃ is the water temperature required to prepare string cheese, and a temperature at which milk protein does not denature. The heating rate was 5℃/min. TG decreased as temperature increased, and its slope rapidly changed near 30℃. DTA showed one endothermic reaction at 23℃ and a second close to 80℃. String cheese contains milk protein, milk fat, water, and salt. The melting point of salt is about 800℃, well beyond the upper limit of the measurement temperature range. The decrease in TG observed as soon as the measurement started is likely due to the evaporation of water from the cheese. This suggests that the water in the cheese begins to evaporate as soon as the package is opened. A liquid separated from the cheese is observed in Fig. 3-② obtained at 37℃: the liquid that was not observed at 22℃ shows

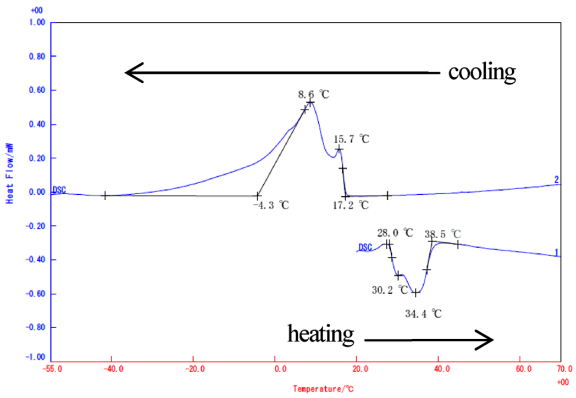

Fig. 4. DSC result of string cheese[A].

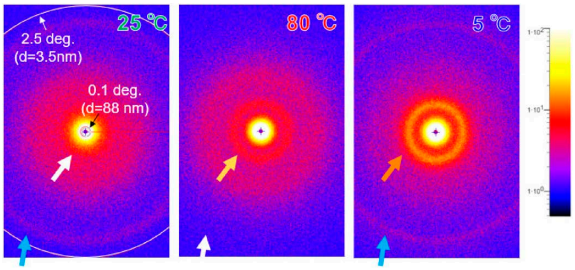

Fig. 5. 2D images of the string cheese[A] measured at 25℃ of early-stage, 80℃ after heating, and 5℃ after cooling.

on the cheese surface. It seems to be a result of an oil-off phenomenon, in which fat globules and water are released. The amount of liquid consisting of separated fat and water increased with heating. The cheese slipped due to the increased liquid in the container at around 80℃. This explains the change in the DTA curve.

To study the endothermic reaction at 23℃ in detail, the sample holder was completely sealed and differential scanning calorimetry (DSC) was carried out. The result is shown in Fig. 4. The sample was heated from 20℃ to 80℃ at 5℃/min, and then cooled to −55℃ at the same cooling rate. The DSC shows two endothermic peaks beginning at 28℃ during the heating process, and multiple exothermic peaks with two or more superposed at about 17℃ during the cooling process. The two endothermic peaks can be attributed to the melting of two kinds of milk fats with different fatty acids. The melted milk fat crystallizes on cooling. The exothermic reactions at 4℃ or lower may relate to the crystallization of water (ice) inside the container that continuously evaporates from the cheese. The milk fats of cheese are not simple. Among milk fat that melts at temperatures between 28℃ and 38℃, there is capric acid with a melting point of 31℃. However, triacylglycerol (TAG) molecules, which consist of an ester bonded with a glycerol and three fatty acids, are also possible components of milk fat. Milk fat melting at a temperature lower than human body temperature could produce the soft texture.

From the results of X-ray microscopy and thermal analysis, it can be inferred why the string cheese splits. When mozzarella cheese is kneaded in hot water at 80℃, the water in the cheese flows out, and the melted milk fat forms a membrane on the cheese surface. When the kneaded cheese is folded at room temperature, the water and milk fat form a gap between milk proteins. The gap formed by the milk fat and water has a weaker cohesive force than the fiber constituted by the milk protein aggregation, and the cheese splits along the gap between fibers.

4. Observation of Periodic Structure of Milk Fat and Milk Protein by Small-angle X-ray Scattering

To study the properties related to the texture and taste of string cheese from the perspective of the periodic structure at the molecular level, a small-angle X-ray scattering (SAXS) measurement was carried out. SAXS is effective for studying the periodic structure of macromolecules such as milk fat and milk protein, and the average particle size and its degree of distribution. The NANOPIX, a small-angle X-ray scattering device, can perform high-intensity measurements in a short period of time. The camera distance from the sample to the detector can be freely changed to measure the region of the target periodic structure with high resolution. Using a Cu source with a tube voltage of 40 kV and a tube current of 30 mA, a two-dimensional (2D) camera was installed at 1345 mm to observe the periodic structure from 3.5 nm to 88 nm. The temperature was controlled by a Peltier device with a transmission sample holder. The samples were heated and cooled with a temperature interval of 10℃ and exposed at each set temperature for 10 minutes. Figure 5 shows the 2D diffraction images of string cheese[A] measured at 25℃ during the early stage, 80℃ after heating, and 5℃ after cooling. The Debye ring was observed on the outside (blue arrow) in the image at 25℃. At 80℃ after heating, the outer Debye ring disappeared, and the inner Debye ring appeared (orange arrow). At 5℃ after cooling, the outer Debye ring appeared again and the inner Debye ring is more intense than that in the image at 80℃. The outer and inner Debye rings showed a uniform intensity distribution, with no preferred orientation of molecules in a specific direction. These results, discrete X-ray diffraction patterns at different temperature, suggest that the outer and inner Debye rings are derived from different crystals.

Figure 6 shows the diffraction profiles converted to 2θ-intensity (I) to study in detail the change in 2D images as temperature changes. The outer Debye ring observed at 25℃ in Fig. 5 is the peak at $2\theta=2.15°$. This peak disappeared at 35℃ or higher during heating, and became two crystalline peaks at around 20℃ during cooling. This result is consistent with the DSC results. Therefore, we concluded that the peak around $2\theta=2.15°$ was derived from the milk fat crystals. Of these two diffraction peaks, $2\theta=2.15°$ on the low angle side corresponds to a periodic structure of about 4 nm. Assuming that the string cheese is a fleshy type of natural cheese and that milk fat is not broken down

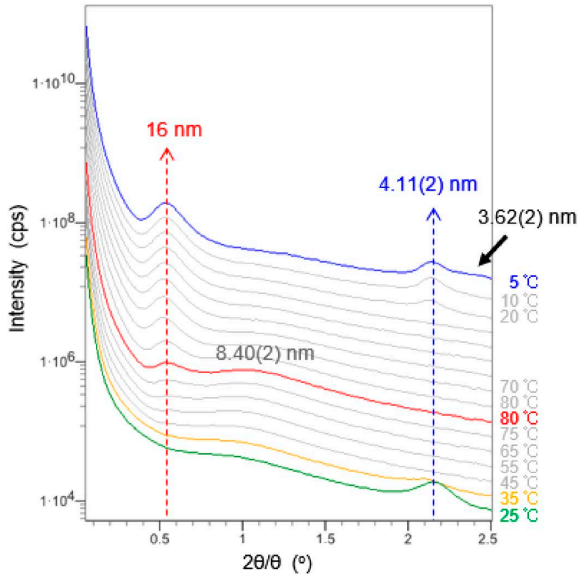

Fig. 6. Temperature dependent of diffraction profiles converted to 2θ-I from the 2D images of string cheese[A].

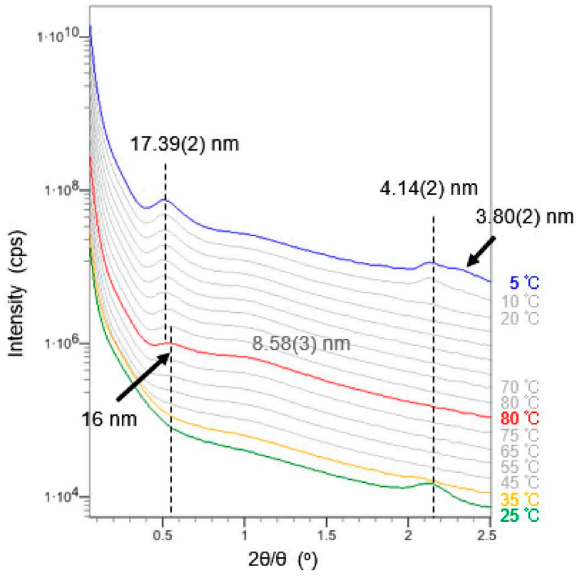

Fig. 7. Temperature dependent of diffraction profiles converted to 2θ-I from the 2D images of string cheese [B] having a low elasticity and a mild taste compared to [A].

into individual fatty acids, it can be inferred that the milk fat that constitutes the cheese is composed of TAG molecules. A TAG molecule that melts completely at 35°C and has a length of 4 nm is a POP molecule in which two palmitic acids and one oleic acid are bound[5, 6].

Contrastingly, the diffraction peak at $2\theta = 0.6°$ (the inner Debye ring in Fig. 5) was not observed near room temperature, but the intensity changed significantly after the heating and the cooling processes. This peak, which corresponds to 16 nm when converted to the length of the periodic structure, represents the periodic structure of the milk protein in which no melting or crystallization is observed in the temperature range.

Milk protein consists of a macromolecular aggregation called casein[5,7]. The casein molecules are reported to occur in 4 variations, namely, α_{s1}, α_{s2}, β, and κ casein. Compared to the hydrophobic α and β caseins, κ casein has amphipathic properties. The crystal structure of casein has been studied using TEM and small-angle synchrotron radiation. However, definitive evidence of the casein structure has not been reported yet, and the debate continues. Nanocluster[8] and sub-micelle models[9] have been reported as typical casein models. In Japan, numerous reports support the sub-micelle model. The sub-micelle model purports that casein forms an aggregate to form sub-micelles, and micelles are formed by sub-micelles that are linked by calcium phosphates. In contrast, the nanocluster model purports that casein binds to calcium phosphate nanoclusters, and the aggregations associate to form micelles. In both models, κ casein constitutes the micelle surface and calcium phosphate is bound to caseins. The average size of the casein sub-micelle was reported as 20 nm[10]. Recently, the water domains within casein micelles were reported using TEM[11]. Takagi *et al*. carried out analyses of SAXS results using the water domain model, and they found a domain size of approximately 16 nm[12]. Holt *et al*. have applied the nanocluster model to the small-angle neutron scattering results and reported that colloidal calcium phosphate (CCP) with a diameter of about 5 nm was distributed at about 18 nm intervals[13]. Although clarifying the origin of the length equating to 16 nm is difficult through this study alone, the 16 nm due to the evaporation of water indicates a part of the structure of milk protein. Atomic and/or electronic level crystallographic studies would have to be performed considering all of the parameters such as the particle size of the casein aggregates, the structural factors of the water domain, and the periodic structure of CCP to clarify the casein structure.

To study the relationship between the diffraction peaks derived from milk protein and milk fat crystals, and the texture and taste, 2D diffraction images of a string cheese[B] having relatively low elasticity and a mild taste were measured under the same temperature conditions (Fig. 7). String cheese[B] shows that the peak position derived from milk protein moved slightly to the lower angle side through the heating and cooling processes and extended by about 1 nm. However, the behavior of the diffraction peak for the milk fat is similar to that of string cheese[A]. Table 1 shows the values of full-width-half-maximum (FWHM) and integrated intensities (I_{int}) of diffraction peaks measured at 5°C after cooling. The I_{int} and FWHM are related to the weight of the component and the degree of crystallinity, respectively. The diffraction peaks derived from milk protein of cheese[A] and [B] show almost the same width. However, the I_{int} of cheese[A] is twice as high as that of [B]. On the other hand, the peak by the milk fat shows that cheese[B] has a wider FWHM and a higher I_{int}, compared to [A]. These results indicate that cheese[B] has a lot of milk fat crystals with low crystallinity. Cheese[A] has a lot of milk

Table 1. FWHM and I_{int} of the diffraction peaks at 0.55° and 2.15° in 2θ, which is measured at 5°C after cooling.

cheese	Peak at 2θ≈0.55°		Peak at 2θ≈2.15 °	
	FWHM(°)	I_{int} (a.u.)	FWHM(°)	I_{int} (a.u.)
A	0.1753(8)	1.853(11)	0.149(5)	0.146(12)
B	0.1649(7)	0.851(4)	0.177(8)	0.173(13)

protein crystals, if these peaks of 2θ≈0.55° indicate the periodic structure of milk protein, not a structure factor of particle size. The SAXS results suggest that the elasticity and mild taste of string cheese is strongly related to the amounts and the crystallinity of the milk protein and the milk fat crystals.

5. Conclusions

The internal structure of string cheese at the micrometer level was observed using X-rays. The physical properties related to the texture and taste of string cheese were studied using thermal analysis and observations of the periodic structure at the nanometer level. Although the crystal structure of the milk protein and milk fat could not be identified, the study showed that it is possible to evaluate texture and taste using X-rays, which have rarely been applied to food research thus far. X-ray-based research will help identify key parameters useful for the development of foods such as casein-free and gluten-free foods, low-fat alternative foods, functional nutrition foods, and space foods in the future.

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