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Relationship between the Emulsifying Capacity of Cheese and the Size of Casein during Ripening

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The emulsifying capacity of cheese and the extractability of proteins from cheese were studied with various melting salts. The size of the extractable proteins from cheese was also estimated by SDS polyacrylamide gel electrophoresis. The emulsifying capacity of cheese decreased during ripening, while the extractability of proteins from cheese increased with all of the melting salts tested. Three fractions were found on the gel of the extract from cheese ripened for 35 days. Molecular weights of them were 37,000, 28,000 and 16,000. During ripening, the amounts of the fractions having higher molecular weights decreased, while those having lower molecular weights increased. In addition, the fractions having intermediate molecular weights appeared. No significant difference was found between the electrophorograms of the extracts with the varying melting salts tested.

Natural cheese undergoes a variety of chemical and physical changes during ripening. Cheese body loses its firmness and toughness and becomes soft and mellow, while insoluble paracasein in cheese is converted to water-soluble nitrogenous compounds due to proteolysis (Schormüller, 1968). So, it is needed to select of the proper age and characteristics of flavor to ensure a uniform product in process cheese manufacturing.

It is known that melting salts[a)] stabilize the emulsion which consists of milk fat, water and casein in a process cheese. It has also been suggested that the effect of melting salts on the emulsifying capacity of cheese varies concomitantly with the alteration of the properties of cheese casein during ripening. However, it remains uncertain whether or not melting salts always interact with cheese casein in the same manner, when the properties of cheese casein vary during ripening.

In the present study, we investigated the emulsifying capacity of cheese, the extractability of cheese proteins, and SDS polyacrylamide gel electrophoresis of the extractable proteins from cheese with various kinds of melting salts, and discussed the interrelationship between the emulsifying capacity of cheese and the extent of fragmentation of cheese proteins during ripening.

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a) Melting salts mean the emulsifier in process cheese manufacturing.

MATERIALS AND METHODS

Materials

Gouda cheese was divided into blocks (8×6×15 cm) and coated with paraffin. Each block was stored at room temperature for 10 days, and then stored at about 10°C and at 60–80 % in humidity until it was used for the experiments.

For determining the emulsifying capacity and extractability four types of melting solutions were used: (1) 0.1 % sodium carbonate, 0.1 % sodium citrate and 3 % sodium phosphate, dibasic, pH 10.5; (2) 0.1 % sodium carbonate, 0.1 % sodium citrate and 3 % sodium pyrophosphate, pH 11.1; (3) 0.1 % sodium carbonate, 0.1 % sodium citrate and 3 % sodium tripolyphosphate, pH 10.6; (4) 0.1 % sodium carbonate, 0.1 % sodium citrate and 3 % sodium hexametaphosphate, pH 7.1.

Emulsifying capacity

Five grams of cheese was weighed in a homogenizer cup and then melted at 80°C. To the melted cheese 2 ml of the melting solution preincubated at 80°C was added, and mixed with a Waring Blendor for a few seconds. A definite amount of cotton seed oil preincubated at 80°C was added to the mixture and then homogenized for 1 min with a Waring Blendor at 80°C. Addition of cotton seed oil and homogenization were repeated alternately until unemulsified oil appeared in the homogenizer cup after homogenization. The end point of the emulsification reaction was taken to be the time when the unemulsified oil appeared. Emulsifying capacity was expressed as added oil (ml)/cheese protein (g).

Extractability

Cheese proteins were extracted by homogenizing 2 g of cheese with 20 ml of the melting solution and then filtering the mixture through Whatman 40 filter paper using a Zartorius membranefilter holder. Extractability was obtained by multiplying the volume of the extract by its protein concentration.

SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was done according to the method of Weber and Osborn (1969). The extract from cheese with the melting solution was dialyzed against the solution containing 0.1 % SDS, 0.1 % β-mercaptoethanol and 0.01 M sodium phosphate (pH 7.0). The solution for electrophoresis was prepared by boiling the dialyzed extract in the solution containing 1 % SDS, 1 % β-mercaptoethanol, 0.1 M sodium phosphate (pH 7.0) and 25 % glycerol, and adding a small volume of 0.05 % Bromophenol Blue. Electrophoresis (10 % gel) was run at 8 mA per each tube for 5 hr. Gels were stained with 0.25 % Coomassie Brilliant Blue in 45.4 % methanol and 9.2 % acetic acid for 2 hr. Destaining was made in 7.5 % acetic acid and 5 % methanol with several changes.

Protein concentration

Protein concentration of cheese and the extracts with the melting solutions was determined by the micro-Kjeldahl method using a nitrogen factor of 6.38.

RESULTS

It is known that the emulsifying capacity of cheese depends on the effect of the melting salts used for the emulsification. In preparing the melting solutions various phosphates were added to the common solution containing 0.1% sodium carbonate and 0.1% sodium citrate. So, the emulsifying capacity of cheese might reflect the effect of each phosphate. Figure 1 shows the emulsifying capacity of cheese during ripening. At the 21st day of ripening, the effect of the melting salts on the emulsifying capacity decreased in the order of hexametaphosphate, pyrophosphate, tripolyphosphate and orthophosphate. This order of the effect of these phosphates on the emulsifying capacity of cheese was almost similar to the case of the more ripened cheese, although the emulsifying capacity decreased significantly during ripening. This result is consistent with the result of Schultz and Hetzel (1960) which denoted that the mixed addition of hexametaphosphate and reclaimed cheese is most effective for process cheese manufacturing. As shown in Fig. 1, the emulsifying capacity of cheese decreased rapidly during the 21st to 35th day of ripening, and thereafter decreased slightly. Over the 77th day no decrease was found in the

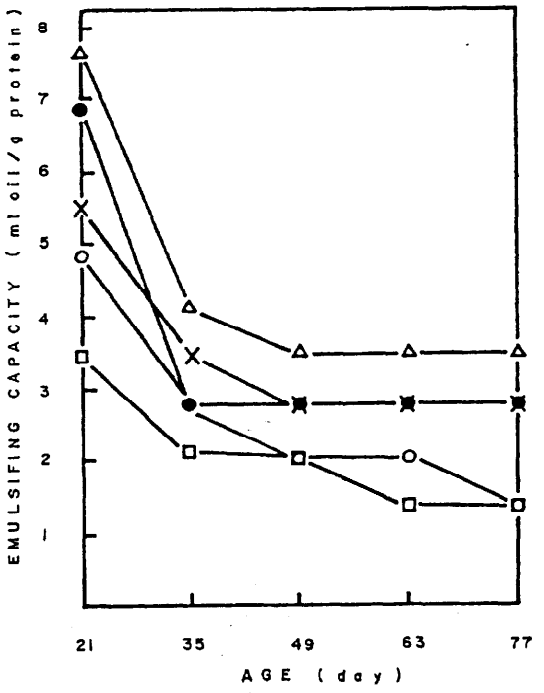

Fig. 1. Effect of melting salts on the emulsifying capacity of cheese during ripening. Emulsifying capacity of cheese was determined using the following melting solutions: △, 0.1% sodium carbonate, 0.1% sodium citrate and 3% sodium hexametaphosphate; ×, 0.1% sodium carbonate, 0.1% sodium citrate and 3% sodium tripolyphosphate; ●, 0.1% sodium carbonate, 0.1% sodium citrate and 3% sodium pyrophosphate; ○, 0.1% sodium carbonate, 0.1% sodium citrate and 3% sodium phosphate, dibasic; □, distilled water.

emulsifying capacity. When distilled water was used for emulsification reaction, the emulsifying capacity of cheese was much less than that with melting solutions.

Extractability of proteins from cheese with the melting solutions is shown in Fig. 2. At the 21st day of ripening, the extractability with the solution containing hexametaphosphate showed the highest value (90 %), while the solution containing orthophosphate showed the lowest (67 %). The extractability with the solutions containing tripolyphosphate and pyrophosphate was equally 80 %. This order of the effect of the melting solutions on the extractability was quite similar to that for the emulsifying capacity. The extractability of proteins with these solutions increased significantly until the 77th day of ripening. However, little increase was found in the extractability for more ripened cheese. The extractability with distilled water at the 21st day of ripening was only 10 %, but increased gradually until the 63rd day of ripening and increased markedly during the 77th to the 142nd day of ripening. This result is consistent with the result of Nakanishi and Tokita (1958). The results shown in Figs. 1 and 2 also indicate that both the emulsifying capacity of cheese and the extractability of proteins from cheese reflect the effect of each melting solution, although each solution has a different pH value.

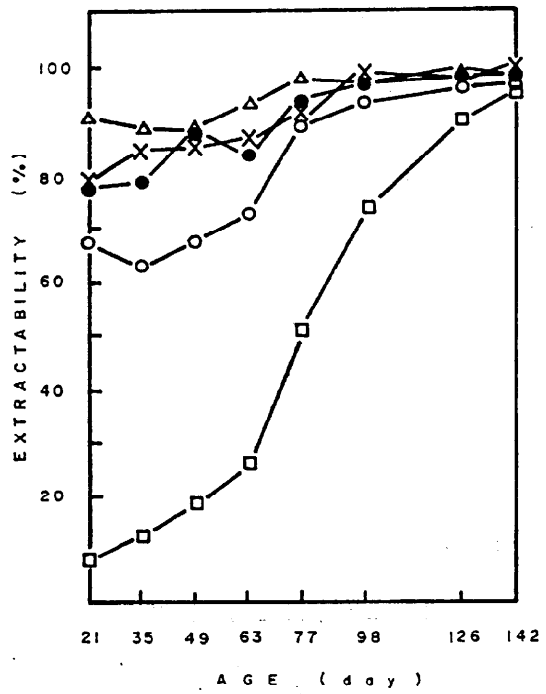

Fig. 2. Effect of melting salts on the extractability of proteins from cheese. The symbols in this figure are the same as those in Fig. 1.

Lindqvist and Storgårds (1957) found a variable decomposition of casein: α-casein preferable in Svecia and Finnish Emmentaler, β-casein mainly in Port Salut and Camembert. Nakanishi and Tokita (1958) also found that protein contained in unripened Gouda cheese were α-, β- and γ-casein, and the electro-

phoretic patterns of the water-insoluble proteins changed during ripening; α-casein decomposed more rapidly than β-casein in cheese. Nakajima *et al.* (1972) also observed that α-casein decomposed more rapidly than β-casein during ripening. They also tried to estimate the size of the extracted proteins from cheese by means of gel-filtration with Sephadex G-50 using 0.05 M Tris-HCl buffer (pH 8.0) containing 7 M urea as a elution buffer, but no clear data were obtained. In addition, little information was so far obtained about the comparison of the constituents of the extracts from cheese with various melting salts. In order to elucidate how cheese protein decomposes during ripening, we investigated SDS polyacrylamide gel electrophoresis of extractable proteins from ripened cheese with various melting solutions. Figure 3 shows the electrophorograms of the extract from cheese with the melting solution containing 0.1% sodium carbonate, 0.1% sodium citrate and 3% sodium tripolyphosphate. As shown in Fig. 3, three bands were found on the gels applied the extract from cheese ripened for 35 days, and their molecular weights were approximately

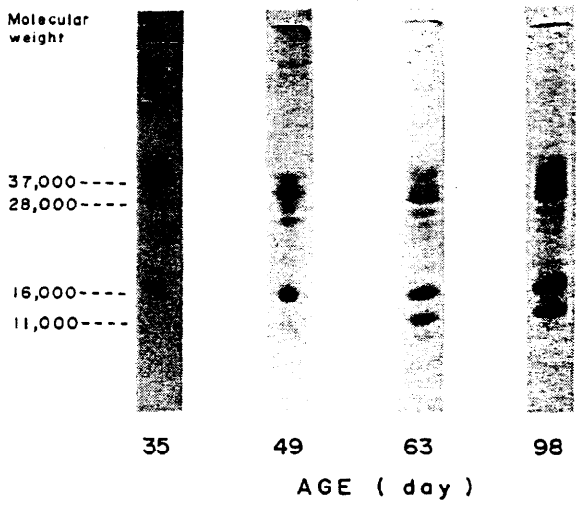

Fig. 3. SDS polyacrylamide gel electrophorograms of the extract from cheese with the melting solution containing 0.1% sodium carbonate, 0.1% sodium citrate and 3% sodium tripolyphosphate.

37,000, 28,000 and 16,000. The band having a molecular weight of 28,000 might be the band of rennet casein, because rennet casein shows a band whose molecular weight was approximately 28,000. The main component of the extract whose molecular weight is 37,000 may be a product produced during a process of cheese manufacturing, while the band whose molecular weight was 16,000 may be a proteolytic fragment of calcium paracaseinate in cheese. At the 49th day of ripening, a new band having much lower molecular weight (11,000) appeared. The amounts of the bands having higher molecular weights (37,000 and 28,000) decreased during ripening, whereas the bands having lower molecular weights (16,000 and 11,000) increased. In addition, several bands having intermediate molecular weights (28,000-16,000) appeared in the extracts from ripened cheese. Similar results were also obtained in the case of the other

melting solutions (not shown). These results indicate that the insoluble calcium paracaseinate in cheese is degradated into small fragments during cheese ripening.

DISCUSSION

It is known that the insoluble calcium paracaseinate in natural cheese is converted into the soluble sodium or potassium paracaseinate due to the function of melting salts during process cheese manufacturing. In young cheese, almost all of the proteins are the insoluble calcium paracaseinate. As shown in Figs. 1 and 2, at the 21st day of ripening the melting solution containing hexametaphosphate is most effective for both the emulsifying capacity of cheese and the extractability of protein from cheese, while the effect of the melting solution containing orthophosphate is much less than that with tripolyphosphate and pyrophosphate. These results indicate that the effect of melting salts on the emulsifying capacity of cheese is related to the solubilizing effect of the melting salts on the insoluble calcium paracaseinate.

It is also known that cheese proteins decompose and become soluble even in water during ripening. The extractability of proteins from cheese increases during ripening (Fig. 2), indicating that the insoluble paracaseinate in cheese decomposes and becomes soluble in the melting solution during ripening. However, this decomposition rate of casein during ripening seems to be much slower than the decreasing rate of the emulsifying capacity (Figs. 1 and 3). It has been found that the calcium binding capacity and the phosphorus content of cheese casein decreased rapidly in early stage of ripening (Nakajima *et al.*, 1972). Therefore, it appears that the emulsifying capacity of cheese proteins might be greatly affected only by a minor change of the properties of the cheese proteins, such as a charge distribution, and it may not be necessary to degradate the cheese proteins into small fragments for decreasing the emulsifying capacity.

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