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EFFECTS OF EMULSIFYING AGENTS ON THE MICROSTRUCTURE AND OTHER CHARACTERISTICS OF PROCESS CHEESE – A REVIEW

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Abstract

Sodium phosphates, polyphosphates, and citrates are melting salts (emulsifying agents) most commonly used in the manufacture of process cheese either alone or in mixtures. Their role during processing is to sequester calcium in the natural cheese, to solubilize protein and increase its hydration and swelling, to facilitate emulsification of fat, and to adjust and stabilize pH.

Changes taking place in natural cheese during processing can be studied by microscopy. Micrographs demonstrating the emulsification of fat, presence of salt crystals, and partial solubilization of protein in laboratory-made and commercial process cheeses have been used to illustrate the various effects of the emulsifying agents. Optical, particularly polarizing and fluorescence microscopy provides rapid information. Electron microscopy reveals greater detail. In combination with energy dispersive spectrometry, electron microscopy can be used to analyze the chemical composition of salt crystals in the process cheese. However, detailed studies of the relationships existing among the microstructure of the process cheese, its composition, and physical properties such as consistency, spreadability, capability of remelting etc. have yet to be carried out.

Introduction

Process cheese has a relatively short history with the first attempt to develop it dating back to 1895. The first patent was granted to a German cheese company in 1899 but at that time the cheese was processed only with heat and no additives were used. In 1912, citric acid was introduced in Switzerland as a melting salt. After this important discovery, industrial production of process cheese commenced in Europe in 1919 (9). A combination of citrates and phosphates was used to develop process cheese independently in the USA in 1917 (49). The introduction of phosphates as melting salts resulted in a marked increase in the production of process cheese.

The original idea of processing cheese was to increase its keeping quality (49) and also to utilize cheese which would otherwise be difficult to sell, e.g. remnants from cheese-cutting operations or cheese containing minor defects such as deformations, over-ripening, localized incidence of molds, etc. Later the producers found that a wide assortment of novel products could be made using various types of cheese (ripened to different degrees), by incorporating other dairy products such as skim milk powder, whey powder, whey protein concentrate, cream, butter, flavourings, and emulsifying agents, and by varying the processing conditions. In most countries, the production of process cheese has been steadily on the increase because of the many variations in flavour, consistency, size, and shape of the product. These properties make it simple and attractive to use process cheese in the preparation of meals both at home and in public dining establishments.

Melting salts are of great importance to cheese processing because they affect the chemical, physical, and microbiological properties of the finished product. Melting salts are not emulsifiers in the strict sense, i.e. they are not surface-active substances, yet they are commonly included in the group of ingredients called 'emulsifying agents' (49, 61). True emulsifiers such as mono- and diglycerides are used in combinations with the melting salts by some process cheese producers. It is generally recognized that there are inconsistencies in the nomenclature of the 'emulsifying agents'.

The objective of this review is to compile information on the effects of the most commonly used emulsifying agents such as citrates and phosphates on selected properties, including microstructure, of process cheese.

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KEY WORDS: Cheese; Citrates; Emulsifying agents; Light microscopy; Melting salts; Microstructure of cheese; Phosphates; Process cheese; Scanning electron microscopy; Transmission electron microscopy.
Principles of process cheese production

Process cheese is produced by blending shredded natural cheeses of various types and degrees of maturi-
ty with emulsifying agents (which consist of melting salts and may include other ingredients, such as pectin, modified starch and/or mono- and diglycerides), and by heating the blend under reduced pressure, with constant stirring, to produce a smooth and homogeneous mass. Kosikowski (40) suggests melting temperatures in the range from 71 to 80°C for process cheese and from 87 to 90.6°C for process cheese spreads. In contrast, the cheese blend is heated under pressure to 140°C for several seconds in continuous process cheese operations. This treatment destroys all clostridia but has no detrimental effect on cheese protein (49). Water is added to the cheese blend as a vehicle for the emulsifying agents and to adjust the dry matter content in the final product. The operations are carried out in the following order:

SELECTION OF CHEESE → COMPUTATION OF THE INGREDIENTS → BLENDING → TRIMMING → SHREDDING → ADDITION OF EMULSIFYING AGENTS AND OTHER INGREDIENTS → HEATING (PROCESSING) → PACKAGING → COOLING → STORAGE → RETAIL OF PROCESS CHEESE.

Table 1. INGREDIENTS USED IN THE MANUFACTURE OF PROCESS CHEESE

<table>
<thead>
<tr>
<th>CHEESE BASE:</th>
<th>EMULSIFYING AGENTS:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shredded natural cheese</td>
<td>Melting salts</td>
</tr>
<tr>
<td></td>
<td>Glycerides</td>
</tr>
</tbody>
</table>

MILK PROTEIN INGREDIENTS:
- Skim milk powder
- Whey powder
- Whey protein concentrate
- Coprecipitates
- Previously processed cheese

FAT INGREDIENTS:
- Cream
- Butter
- Butter oil

PRESERVATIVES:

COLOURING AGENTS:

FLAVOURING AGENTS:

WATER
SALT

MUSCLE FOOD INGREDIENTS:
- Ham
- Salami
- Fish

VEGETABLES AND SPICES:
- Celery
- Mushrooms
- Mustard
- Onions
- Paprika
- Pepper
- Tomatoes

BINDERS:
- Locust bean gum
- Pectin
- Starch

In addition to natural cheese, other ingredients are also used; some of them are mandatory such as emulsifying agents and water, and others may be optional as indicated in Table 1.

Binding agents such as tragacanth, locust bean gum, caraya, pectin, or starch are frequently used along with emulsifying salts in the production of process cheese. Their role is to act as water-binding agents which prevent the drying out of the cheese, and as thickening agents to provide a suitable texture. They are used, in particular, in the manufacture of process cheese spreads.

Depending on the ingredients used, three kinds of product are distinguished: block process cheese, process cheese food, and process cheese spread (49). Their characteristics are summarized in Table 2. Only block process cheese and process cheese spread are being dealt with in this review.

Block process cheese and process cheese food are firm and have a low moisture content (45%) and low acidity (pH = 5.6-5.8) (29); process cheese spreads are soft (spreadable), have a higher moisture content (55%) (70) and should have a higher acidity (pH = 5.2) than the two former products (29).

The emulsifying agents (melting salts) are used to provide a uniform structure during the melting process. Phosphates, polyphosphates, and citrates (22, 29, 41, 45, 70) are most common. Other salts may also be used, e.g., sodium potassium tartrate (41, 70) or complex sodium aluminum phosphates of the general formula:

\[ X \text{Na}_2O \cdot Y \text{Al}_2O_3 \cdot 8 \text{P}_2\text{O}_5 \cdot Z \text{H}_2\text{O}, \]

where \( X = 6 \) to 15, \( Y = 1.5 \) to 4.5, and \( Z = 4 \) to 40 (70).

Other emulsifying agents such as sodium potassium tartrate, trihydroxyglutaric acid, or diglycolic acid are rarely used and are not discussed further in this review. A list of emulsifying agents most commonly used, their chemical formulae, molecular mass, \( \text{P}_2\text{O}_5 \) content, solubility in water at 20°C, and pH values of 1% aqueous solutions is presented in Table 3.

A thorough yet concise review of the process cheese principles was recently published by Shimp (61).

Effects of emulsifying agents on the production of process cheese

The essential role of the emulsifying agents in the manufacture of process cheese is to supplement the emulsifying capability of cheese proteins. This is accomplished by:

1. removing calcium from the protein system,
2. peptizing, solubilizing, and dispersing proteins,
3. hydrating and swelling proteins,
4. emulsifying fat and stabilizing the emulsions,
5. controlling pH and stabilizing it, and
6. forming an appropriate structure after cooling.

The ability to sequester calcium is one of the most important functions of the emulsifying agents. For simplicity, casein in cheese may be viewed as molecules which have one end nonpolar and thus lipophilic, whereas the other end, which contains calcium phosphate, is...
PROCESS CHEESE - A REVIEW

Table 2.
SOME CHARACTERISTICS OF PROCESS CHEESE, PROCESS CHEESE FOOD, AND PROCESS CHEESE SPREADS (40, 70)

<table>
<thead>
<tr>
<th>Type of product:</th>
<th>Ingredients:</th>
<th>Cooking temperature:</th>
<th>Composition:</th>
<th>pH:</th>
<th>Author:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process cheese</td>
<td>Natural cheese, emulsifiers, NaCl, colouring</td>
<td>71-80°C</td>
<td>Moisture and fat* contents correspond to the legal limits for natural cheese</td>
<td>5.6-5.8</td>
<td>Kosikowski (40)</td>
</tr>
<tr>
<td>Process cheese food</td>
<td>Same as above plus optional ingredients: milk, skim milk, whey, cream, albumin, skim milk cheese; organic acids</td>
<td>74-85°C 79-85°C</td>
<td>45% moisture</td>
<td>5.2-5.6</td>
<td>Thomas (70)</td>
</tr>
<tr>
<td>Process cheese spread</td>
<td>Same as process cheese food plus gums for water retention</td>
<td>88-91°C 90-95°C</td>
<td>44% and no more than 60% moisture</td>
<td>&lt;5.2</td>
<td>Kosikowski (40)</td>
</tr>
</tbody>
</table>

* 1% higher for Cheddar cheese.

hydrophilic (Fig. 1). This arrangement makes the casein molecule function as an emulsifier (14, 61). As the amount of calcium phosphate is decreased, the solubility of casein in water is increased and so is its emulsifying capability. When calcium in the Ca-paracaseinate complex in the natural cheese is removed by the ion exchange reaction initiated by the addition of melting salts, insoluble paracaseinate is converted into a soluble form. This soluble form is most frequently Na-caseinate; monovalent sodium is provided by the emulsifying agent.

Various emulsifiers have varying affinity, i.e. sequestering ability, for calcium. This affinity increases, for example, in the following order: Na₂H₂PO₄, Na₂H₃P₂O₇, Na₃H₂P₂O₇, Na₄P₂O₇, Na₅P₃O₁₀. Polyvalent anions (phosphates, citrates) have a high water-adsorption ability. They become bound by calcium to protein molecules in the cheese and provide them with a negative charge. The addition of basic salts also increases the pH value of the cheese. The increases

Table 3.
EMULSIFYING SALTS USED IN THE PROCESSING OF CHEESE (40, 70)

<table>
<thead>
<tr>
<th>Group:</th>
<th>Emulsifying salt:</th>
<th>Formula:</th>
<th>Molar mass:</th>
<th>P₂O₅ content (%)</th>
<th>Solubility at 20°C (%)</th>
<th>pH value (1% soln.):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrates</td>
<td>Trisodium citrate</td>
<td>(2Na_3C_6H_5O_7·11H_2O)</td>
<td>714.31</td>
<td>-----</td>
<td>High</td>
<td>6.23-6.26</td>
</tr>
<tr>
<td>Orthophosphates</td>
<td>Monosodium phosphate</td>
<td>(NaH_2PO_4·2H_2O)</td>
<td>156.01</td>
<td>59.15</td>
<td>40</td>
<td>4.0-4.2</td>
</tr>
<tr>
<td></td>
<td>Disodium phosphate</td>
<td>(Na_2HPO_4·12H_2O)</td>
<td>358.14</td>
<td>19.80</td>
<td>18</td>
<td>8.9-9.1</td>
</tr>
<tr>
<td>Pyrophosphates</td>
<td>Disodium pyrophosphate</td>
<td>(Na_2H_2P_2O_7)</td>
<td>221.94</td>
<td>63.96</td>
<td>10.7</td>
<td>4.0-4.5</td>
</tr>
<tr>
<td></td>
<td>Trisodium pyrophosphate</td>
<td>(Na_3H_2P_2O_7·9H_2O)</td>
<td>406.06</td>
<td>34.95</td>
<td>32</td>
<td>6.7-7.5</td>
</tr>
<tr>
<td></td>
<td>Tetrasodium pyrophosphate</td>
<td>(Na_4P_2O_7·10H_2O)</td>
<td>446.05</td>
<td>31.82</td>
<td>10-12</td>
<td>10.2-10.4</td>
</tr>
<tr>
<td>Polyphosphates</td>
<td>Pentasodium tripolyphosphate</td>
<td>(Na_5P_3O_{10})</td>
<td>-----</td>
<td>57.88</td>
<td>14-15</td>
<td>9.3-9.8</td>
</tr>
<tr>
<td></td>
<td>Sodium tetrapolyphosphate</td>
<td>(Na_6P_4O_{13})</td>
<td>-----</td>
<td>60.42</td>
<td>14-15</td>
<td>9.0-9.5</td>
</tr>
<tr>
<td></td>
<td>Sodium hexametaphosphate</td>
<td>((NaPO_3)_n)</td>
<td>-----</td>
<td>69.60</td>
<td>infinite</td>
<td>6.0-7.5</td>
</tr>
</tbody>
</table>
of both the negative charge and the pH value result in a higher water absorption by the proteins. Analytical determination of calcium and phosphorus in process cheese showed that the concentration of these elements was twice as high in the insoluble portion of the process cheese as in the initial natural cheese. The reactivity between the emulsifier and protein is defined by the ratio of insoluble to total proteins in the initial natural cheese and in the process cheese (22). Lee et al. (44) investigated protein disaggregation and structure of process cheese and found that the affinity of protein for the cations and anions of the melting salts was determined by the valency of such ions. Ideal emulsifying characteristics are possessed by salts which consist of a monovalent cation and a polyvalent anion. Some salts have better emulsifying effects than other salts but may have inferior calcium-sequestering abilities, or may not sufficiently solubilize and hydrate protein. To achieve excellent emulsifying and melting characteristics simultaneously, it is necessary to combine two or more salts into mixtures.

The pH value is important for several reasons. It affects the protein configuration and solubility as well as the extent to which emulsifying salts bind calcium (61). In process cheese, pH is maintained between 5.0 and 6.5. At the lower limit of pH 5.0, which is close to the isoelectric point of cheese proteins, the cheese texture may become crumbly. The crumbliness is probably caused by weakened protein-protein bonds and the onset of the fat emulsion breakdown because the interactions of the protein molecules with other protein as well as fat molecules are reduced. At the upper limit of pH 6.5, the cheese becomes excessively soft. Microbiological problems, which affect the shelf life, may also be encountered. However, shelf life may be prolonged by sterilization or drying of the process cheese produced (8).

The effect of pH on the texture of process cheese was demonstrated by Kariadhan (31), who used monosodium, disodium, and trisodium phosphates. The respective pH values of their 1% solutions were 4.2, 9.5, and 13.0. Cheese made with monosodium phosphate (low pH) was dry and crumbly whereas cheese made with trisodium phosphate (high pH) was moist and elastic; texture of cheese made with disodium phosphate was in between. Similar pH effects apply to other emulsifiers (61).

Emulsifying agents vary in their bacteriological effects. Specific bacteriostatic action was observed with monophosphates and was even more pronounced in higher phosphates and polyphosphates (40, 70). Citrates lack such effects and may, in contrast, even themselves be the subject of bacterial spoilage. As the usual heat treatment of the cheese during processing is rather mild, process cheese is not sterile. Although the final product contains no viable bacteria, it contains viable spores, probably even including clostridia, which may originate from the natural cheese or from the spices added (9, 40, 70). Tanaka et al. (67) reported that orthophosphate suppressed the germination of Clostridium botulinum spores in process cheese whereas citrates were without effect. Differences in the processing conditions such as the kind of emulsifier used, pH, moisture level, etc. also affect spore germination.

Characteristics of individual melting salts and their mixtures have been studied to a great extent for the effects which they have on process cheese during manufacture and storage (1, 9, 10, 30, 32, 33, 62-64, 71). Effects of some selected emulsifying agents on process cheese are summarized in Table 4.

### Table 4. CHARACTERISTICS OF EMULSIFIERS MOST COMMONLY USED IN THE MANUFACTURE OF PROCESS CHEESE AND RELATED PRODUCTS (40)

<table>
<thead>
<tr>
<th>Emulsifier*</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium citrate</td>
<td>Versatile; produces cheese with good melting properties; inexpensive; best qualities.</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>Good firming, buffering, and melting properties, poor creaming properties. Least expensive.</td>
</tr>
<tr>
<td>Trisodium phosphate</td>
<td>Highly alkaline; improves sliceability when used in combination with other emulsifiers; good buffering ability; used at low concentrations.</td>
</tr>
<tr>
<td>Sodium hexametaphosphate (Graham's salt)</td>
<td>Produces tartish flavour and a very firm body; product does not melt easily; least soluble of all; bacteriostatic.</td>
</tr>
<tr>
<td>Tetrasodium diphosphate</td>
<td>Good creaming properties; strong buffering capacity; high protein solubility; excellent ionic exchange; tartish flavour.</td>
</tr>
</tbody>
</table>

* Other emulsifiers permitted by the U.S. Federal Standards of Identity are: sodium acid pyrophosphate, sodium potassium tartrate, tetrasodium pyrophosphate, dipotassium phosphate, potassium citrate, calcium citrate, and sodium aluminum phosphate.
with polyphosphate. Similar findings were made by Daclin (12). Sodium polyphosphates were found to be superior to ortho- and metaphosphates in the manufacture of process cheese from mixtures of PDO and AOC cheeses. Lien and Alais (42) reported that the concentration of soluble nitrogen was increased with an increased concentration of the polyphosphate added in the range from 1 to 3%. Hydrolysis of polyphosphates into ortho-phosphates in the process cheese was clearly evident after cooling.

Ney and Garg (52, 53) compared cyclic polyphosphates having 3 and 4 phosphorus atoms with linear polyphosphates: calcium sequestering was markedly lower with sodium metaphosphate than with sodium tetrametaphosphate; a smooth homogeneous process cheese was obtained with the latter salt. Kirchmeier et al. (37) studied the flow properties of process cheese manufactured by various techniques (continuous kettle, blender, or the UHT process) using various phosphates. Differences in depolymerization of casein and changes in the flow properties were attributed to differences in calcium complexation in mono- and tetrapolyphosphates.

Nakajima et al. (51) varied the polyphosphate-to-pyrophosphate ratio in condensed phosphates used as melting salts. Melting rate, ultrafiltrable calcium content of the cheese, and various properties such as stress relaxation, hardness, gumminess, and elasticity in the finished product were affected more by varying the condensed phosphate than the polyphosphate concentrations.

Borrelli (4) assumed that the theory about the ion-exchange effect of polyphosphates, whereby Ca-paracaseinate is converted into Na-paracaseinate, has been confirmed by the finding that ethylenediaminetetraacetic acid (EDTA), which has a high affinity for calcium, can be used successfully in the manufacture of process cheese. Scharpf (60) suggested that the emulsifying effect of chain phosphates is associated with their interaction with paracasein in such a way that phosphate anions form bridges between protein molecules.

In the USSR, Zakharev et al. (76) investigated the possibility of reducing the customary quantity of phosphates because phosphorus from the added emulsifiers increases the P:Ca ratio in the process cheese and this is considered to be nutritionally detrimental. Monoglycerides as true emulsifiers were used to partly replace phosphates. Process cheese of good quality was produced using 1% of a surface-active monoglyceride preparation. Results confirmed by the finding that ethylenediaminetetraacetic acid (EDTA) was used to combine the best effects of their individual components.

Some results obtained earlier (33, 63) seem to favor citrate in the melting salt combinations but more recent studies emphasize the desirable effects of phosphates. According to Shubin (63), a combination of sodium...
Some earlier results of Kiermeier (33) had shown that orthophosphates and pyrophosphates were generally unsatisfactory whether employed alone or in a combination, but citrate was useful to a limited extent. Polyphosphates proved to be satisfactory in every respect.

Thomas et al. (71) produced process cheese with a 3% addition of disodium phosphate, tetrasodium

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**Fig. 2.** Curd granule junctions (small arrows) in Brick cheese. Dark areas (large arrows) are void spaces (air pockets) in the cheese.

**Fig. 3.** Curd granule junctions (small arrows) and milled curd junctions (large arrows) in Cheddar cheese form characteristic patterns. Depending on the orientation of the milled curd particles, cheddar curd granules are displayed in cross sections (c) or longitudinal sections (l).

**Fig. 4.** Light (fluorescence) microscopy of a curd granule junction (light area between large arrows). Dark structures (f) are fat globules. Small dark arrows point to calcium phosphate crystals and light arrows point to lactic acid bacteria appearing as very light points. Courtesy of S. H. Yiu.

**Fig. 5.** SEM of a curd granule junction (dark area between light arrows) indicates that the junction is devoid of fat globules as evident from the low incidence of empty cavities which contained fat before it had been extracted with chloroform.

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citrate and trihydroxyglutarate produced the best results as emulsifying agents in the manufacture of process cheese. A mixture of these salts (10-20%) with disodium phosphate (80-90%) yielded a high-quality process cheese, as far as the solubility of protein, emulsification of fat, and the pH value of the finished product were concerned.
diphosphate, pentasodium triphosphate, or trisodium citrate or with the addition of a mixture of equal quantities of sodium polyphosphate and tetrasodium phosphate. General acceptability was about the same for all process cheeses. However, cheeses made with disodium phosphate, tetrasodium diphosphate, or pentasodium triphosphate had an elevated content of water-soluble nitrogen compared to cheeses made with trisodium citrate or the mixture of sodium polyphosphate and tetrasodium phosphate. By decreasing the amount of the melting salts to 2% or by increasing it to 4%, no differences were detected in the water-soluble nitrogen fraction in the process cheeses or in their stickiness, crumbliness, sliceability, or general acceptability.

Csók (11), working with a commercial emulsifying agent, reported that an increase in the melting time up to 1080 s resulted in an increase of the total bound water as well as osmotically bound water.

In general, polyphosphates had superior effects on the structure and keeping quality of process cheese (1) compared to other emulsifying agents. This has been attributed by the authors to the ability of polyphosphates to solubilize calcium paracaseinate because of their high calcium-sequestering capacity. Pyrophosphates and, in particular, orthophosphates have been found to introduce unfavourable sensory attributes to the process cheese. Citrates were as efficient emulsifiers as polyphosphates but lacked their bacteriostatic effect.

Sood and Kosikowski (64) investigated the possibility of replacing cheese solids with plain or enzyme-treated skim milk retentates in the manufacture of process Cheddar cheese. Casein in the retentates is mostly insoluble, for which reason the retentates cannot be used alone for processing. However, process cheese containing up to 60% of retentate solids (treated with food grade fungal protease and lipase preparations) had better sensory attributes than the reference process cheese. Of a variety of melting salts tested, a combination of sodium citrate (2.7%) and citric acid (0.3%) was best suited to produce the retentate-containing process cheese. Increasing the retentate content to 80% resulted in an unacceptable product having a hard, long-grained texture.

Microstructure of process cheese

Optical as well as electron microscopy have been used to select natural cheese for processing, to check the progress of processing, and to evaluate the finished product.

Boháč (3) examined the suitability of cheese for processing by using a polarizing microscope equipped with a hot stage. Cheese slices were heated to 85-95°C within 8-10 min and the interface between the cheese and the melting salt solution was observed. The following phenomena were noted: Some samples disintegrated along the curd granule junctions after a temperature of 70°C was reached. A diffuse zone containing protein and fat globules released from the cheese was formed around most samples or their fragments. The suitability of the cheese for processing was assessed from the dimensions of the fat globules and the amount of fat released and from the temperature at which it melted. Ripe and overripened cheeses sometimes rapidly diffused into the salt solution even before the melting temperature had been reached. At 60-70°C, some cheese samples rapidly contracted, remained unchanged until a temperature of 95-98°C was reached, and then melted.

The behaviour of melting salts in an aqueous medium or in the presence of cheese was studied using the same microscope: small glassy crystals dissolved relatively slowly or occasionally only after firstly aggregating. Instantized salts formed minute globules which dissolved more rapidly than regular salts. Fine bubbles of carbon dioxide sometimes developed and facilitated the dispersion of the salt crystals in the cheese mass. Time lapse photography using a movie camera showed the changes in succession.

The microstructure of process cheese resembles, to some extent, the microstructure of the natural cheese.
Fig. 9. A large calcium phosphate crystal (ph) in process cheese (SEM). Fat globules around the crystal (arrows) show signs of distortion.

Fig. 10. Calcium phosphate (ph) crystals and void spaces (ci) in a process cheese blend cooked for 10 min. The void spaces are the imprints of sodium citrate crystals which had dissolved in aqueous glutaraldehyde during fixation for SEM. Fat (f) is in the process of emulsification. b = Bacterium. From Rayan et al. (56).

Fig. 11. Light microscopy of calcium phosphate crystals (ph) specifically stained for calcium with Alizarin Red. Courtesy of S. H. Yiu (75).

Fig. 12. Crystallization takes place with disodium phosphate used as the melting salt in the preparation of process cheese and is indicated by needle-shaped outgrowth (arrows). From Rayan et al. (56).

Fig. 13. Crystallization takes place also with tetrasodium pyrophosphate (arrows). b = Bacterium. From Rayan et al. (56).

Fig. 14. Detail of cavities (arrows) indicating that sodium citrate was used as the melting salt in the preparation of this process cheese. f = Cavities initially occupied by fat particles.
However, there are several differences. Some can be studied by light microscopy and others may be studied by electron microscopy.

Natural cheese is made by pressing curd granules, consisting mostly of insoluble calcium caseinate and fat droplets, into a homogeneous mass. The sites, where the granules fuse with each other, are called curd granule junctions (23-26, 46, 54, 59, 69, 75). At a low magnification, they are seen to form characteristic patterns. Compared to simple patterns in stirred-curd cheeses such as Brick cheese (Fig. 2), the patterns are complex in Cheddar cheese (Fig. 3) because an additional type of milled curd granule junction develops as the result of milling cheddared curd and pressing milled curd. Curd granule junctions are areas depleted of fat globules, as is evident from optical (Fig. 4) as well as SEM micrographs (Fig. 5). Their development was described earlier (27, 28).

The distribution of fat in natural cheese was studied by optical (3, 13, 75) as well as electron microscopy (2, 18, 19, 23-27, 34, 39, 43, 45, 55, 56, 58, 69). Most fat globules have been found to have their fat globule membranes preserved; this can be best observed by transmission electron microscopy (TEM) of thin sections (Fig. 6) (23, 24, 27, 34).

During processing, both the curd granule junctions and the fat globule membranes vanish as the result of heating, melting, and stirring the cheese. The fat melts and forms particles several micrometers in diameter. The relatively insoluble protein in the natural cheese is partially solubilized by the action of the calcium-sequestering melting salts and is converted into a smooth and homogeneous mass. The salts increase the natural emulsifying properties of the cheese proteins, and the fat disperses in the form of minute globules.

Rayan (55) and Rayan et al. (56) used SEM and TEM to study the emulsification of fat during the processing of Cheddar cheese. The melting salts used were sodium citrate, disodium phosphate, tetrasodium pyrophosphate, and sodium aluminum phosphate. Depending on the processing conditions, the initially large fat particles were emulsified into smaller droplets (Figs. 7 and 8).

In addition to the dispersion of fat, electron microscopy also documents the presence of crystalline

![Figs. 15-18. Experimental process cheese made from a blend of Feta, Gouda, Kachkaval, and White cheeses using the following emulsifiers: Fig. 15. Commercial emulsifier consisting of 5% sodium phosphate (SP) and 49% sodium polyphosphate (SPP): Fig. 16. Laboratory-made mixture of 61% SP and 39% SPP; Fig. 17. A mixture of 15% SP, 70% SPP, and 15% modified starch; Fig. 18. A mixture of 10% SP, 65% SPP, 15% modified starch, and 10% mono- and diglycerides (1:1, w/w). The distribution of fat (f) differs from cheese to cheese (see the text).](image-url)
Figs. 19-21. Commercial process cheeses: Fig. 19. Processed Cheddar cheese with the declared use of sodium phosphate, sodium-aluminum phosphate, sodium triphosphate, and sodium citrate contains only a small number of calcium phosphate crystals (arrows). Fat particles (f) vary widely in dimensions. Fig. 20. Processed Gruyère cheese A made with sodium-calcium citrate. Fat particles (f) are considerably smaller than in processed Cheddar cheese. Large crystals and their clusters (arrows) are abundant. Fig. 21. Processed Gruyère cheese B made with sodium citrate. Fat is emulsified into minute globules. Crystals (arrows) are abundant.
Fig. 22. Diagram of energy dispersive spectrometric analysis of a crystal in commercial processed Cheddar cheese. Peaks of aluminum (Al), phosphorus (P), sulphur (S), and calcium (Ca) are identified. Courtesy of S. H. Yiu.

Fig. 24. (Below) Diagram of energy dispersive spectrometric analysis of a crystal in commercial processed Gruyère cheese. Peaks of sodium (Na), phosphorus (P), sulphur (S), and calcium (Ca) are identified. Courtesy of S. H. Yiu.

fixed protein matrix (26).

Cheddar cheese forms the base for most process cheeses in the USA, Canada, and the United Kingdom, but Gruyère, Gouda, and Emmental cheeses are used the most in continental Europe. In our experiments, a mixture of Feta, Gouda, Kachkaval, and White cheeses (20% fat, 58% moisture, pH = 5.6) was processed to assess the effects of various emulsifying agents such as (a) a commercial emulsifier consisting of 51% sodium phosphate (SP) and 49% sodium polyphosphate (SPP) (Fig. 15), (b) a laboratory-made emulsifier consisting of 61% SP and 39% SPP (Fig. 16), (c) an emulsifier consisting of 15% SP, 70% SPP, and 15% modified starch (Fig. 17), and (d) an emulsifier consisting of 10% SP, 65% SPP, 15% modified starch, and 10% of a monoglycerides and diglycerides mixture (1:1, w/w) (Fig. 18). The emulsifiers were added to the shredded cheese at a concentration of 3%, and the cheese blend was heated by direct steam at 95°C for 600 s (including the warming of the blend to 95°C) with stirring at 90 rpm. The finished products were stored at <10°C and examined 24 h after production.

Compared to other process cheeses presented in this review, these experimental samples were almost completely free of salt crystals; their absence is probably due to a rapid dissolution of the melting salts because direct steam was used to heat the cheese blend. Samples (c) and (d), which contained modified starch, had more compact microstructures than samples (a) and (b). The appearance of the fat globules suggested that the emulsification process had been completed only in sample (c): the fat globules were spherical with few signs of their continuing separation. In contrast, the emulsification process was still in progress in the other samples when their processing had been terminated. This is evident from elongated fat particles or strings of fat particles not yet separated from each other.

Three commercial process cheeses purchased in retail stores were examined for comparison. Composition of the cheeses and the emulsifiers used were declared (in descending order) by the manufacturers as follows: processed Cheddar cheese (28% fat, 44% moisture, Na phosphate, Na-Al phosphate, Na triphosphate, Na citrate) (Fig. 19), processed Gruyère cheese A (25% fat, 43%
Protein strands (arrows) are present in the protein matrix of a process cheese of the hard type (block process cheese) made with 2.2% sodium polyphosphate. Courtesy of T. Kimura.

The existence of the string-like protein structures was confirmed by Heerste et al. (20). Investigating the submicroscopic structure of process cheese, Tynyakov (72) reported microvules with shape and size variations dependent upon the type of cheese. Cheeses produced with sodium citrate were found to have a fibrous structure.

Fig. 26. Protein strands (arrows) are present in the protein matrix of a process cheese of the hard type (block process cheese) made with 2.2% sodium polyphosphate. Courtesy of T. Kimura.

Fig. 27. Protein is in the form of single particles in the protein matrix of a process cheese of the soft type (process cheese spread) made with a mixture of 1% sodium citrate and 1.5% sodium polyphosphate. Courtesy of T. Kimura.

Protein is in the form of single particles in the protein matrix of a process cheese of the soft type (process cheese spread) made with a mixture of 1% sodium citrate and 1.5% sodium polyphosphate. Courtesy of T. Kimura.

Conclusions

Process cheese is a complex system composed of protein, fat, mineral salts, and other ingredients. Its properties are affected by many variables such as the composition and nature of the initial natural cheese, the nature and amount of the emulsifying agents, the manufacturing regimen, and additional factors. Emulsifying agents play one of the most important roles. Although a large number of such agents has been tested, citrates and phosphates have been used most frequently in process cheese manufacturing practice. To be used commercially, emulsifying agents must perform several functions at the same time and must not adversely affect the sensory attributes of the product. Because some emulsifying agents may perform better than others as far as individual functions are concerned, such emulsifying agents are combined in mixtures. In spite of their favourable technological properties, phosphates and polyphosphates have been raising the concern of nutritionists, because these salts introduce sodium and phosphorus into process cheese. In recent years, there has been a trend to reduce the concentration of sodium in foods. Effects of additives such as modified starch or mono- and diglycerides have been explored on an experimental scale.

Chemical composition and consumer acceptance are the ultimate criteria for process cheese. However, microscopy is very useful in examining the initial raw materials such as the natural cheese blend and, in particular, the effects of processing on the finished product. Optical as well as electron microscopy can reveal whether the amount of the emulsifying agents used is appropriate or excessive. The presence of large differences in the protein matrices of soft and hard process cheeses were studied by Kimura and Taneya (35), Kimura et al. (36), and Taneya et al. (68) using thin-sectioning and freeze-fracturing techniques for electron microscopy. The soft type process cheese had been made using a mixed emulsifying agent (1% sodium citrate and 1.5% polphosphate). The cheese exhibited predominantly single particles in the protein matrix, whereas the hard process cheese (made with 2.2% polphosphate) consisted of a network structure containing long protein strands (Figs. 26 and 27). The authors assumed that the protein strands contributed to the ability of hard process cheese to retain its shape on heating. The existence of the string-like protein structures was confirmed by Heerste et al. (20). Investigating the submicroscopic structure of process cheese, Tynyakov (72) reported microvules with shape and size variations dependent upon the type of cheese. Cheeses produced with sodium citrate were found to have a fibrous structure.
amounts of emulsifying salt crystals indicates that such undissolved crystals do not participate in the emulsifying process and that the concentration of the emulsifying agent should be reduced. Fat globule dimensions are indicative of the extent of emulsification. The fat globule dimensions diminish as emulsification advances. Also the microstructure of the protein matrix is indicative of the extent of emulsification, during which casein first disaggregates and subsequently forms string-like structures.

It is assumed that future trends in process cheese research will be concerned with the development of new types of emulsifiers better acceptable from the nutritional viewpoint than the sodium phosphates used presently. Microscopy will play an increasingly important role in this research: conventional optical microscopy using specific staining techniques and fluorescence microscopy will be used to check the presence of salt crystals and the distribution of fat in the finished product. SEM in conjunction with energy dispersive spectrometry will make it possible to analyze the crystals. In conjunction with digital image analysis, SEM will be used to evaluate the distribution of fat globules in the product in greater detail. TEM is assumed to provide the solution to problems associated with the melting or the lack of it in cheese already processed. Defects in process cheese are a separate set of problems, to the solution of which all kinds of microscopy will contribute.

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Phosphates


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Discussion with Reviewers

B. E. Brooker: How can the identity of crystals in cheese (Figs. 8-10) be so certain simply from the use of morphological features - especially in view of the results obtained by energy dispersive spectrometry (EDS) which seem to suggest that crystals with the same morphology as those purported to be sodium citrate do in fact contain Na, P, Ca, and S? More information should be given about the EDS analysis. How were the samples prepared and are Figs. 22 and 24 the results obtained from point or area analyses?

D. B. Emmons: EDS has indicated the presence of Ca and P in crystals assumed to be sodium citrate in the processed Gruyere cheese. Please comment.

Authors: Differences in the appearance of crystals in micrographs obtained with natural cheese and in micrographs of the same cheese to which a specific melting salt such as sodium citrate had been added have been attributed to the presence of the added salt. Preliminary results obtained by EDS have been shown only to demonstrate the potential of this technique. Fixed, dehydrated, defatted, and critical-point dried samples were mounted on carbon disks and coated with carbon. Area analyses were carried out at magnifications at which the crystals under study covered the SEM screen. EDS analysis of authentic salt crystals added to cheese is in progress in order to investigate the requirements of this technique.

D. B. Emmons: Could the absence of salt crystals in the process cheese made by the authors (Figs. 15-18) be due to using cheeses low in calcium and phosphate (acid cheeses such as White cheese)?

Authors: This is one possibility and the other is the use of direct steam to heat the cheese blend. Interestingly, the presence of White cheese in the process cheese was detected by preliminary TEM studies (unpublished) by the observation of the characteristic core-and-lining ultrastructure (80).

I. Heertje: It is mentioned that the suitability of the starting cheese for processing was assessed from the dimensions of the fat globules and the amount of fat released and from the melting temperature. Are these considered to be proper criteria in view of the fact that at that stage of observation no melting salts have been added?

Authors: The tests referred to were carried out by V. Rhee (Department of Cheese Technology, Dairy Research Institute, Tábor, Czechoslovakia), who has adapted a polarizing microscope specifically for the studies of cheese processing. Release of the fat globules and their dimensions were studied in relationship to the temperature of the cheese in the presence of melting salts.

D. P. Dylewski: What is "compact microstructure"? Can it be determined using morphometry or stereology?

Authors: Compact microstructure is characterized by the absence of void spaces resulting from the presence of air or whey pockets. We believe that morphometric analysis, particularly of cheese fixed with imidazole-buffered osmium tetroxide to retain fat will be useful in evaluating the compactness of the cheese protein matrix.

I. Heertje: Do all fat globules appear as cavities (Fig. 14)? Is this caused by the preparation procedure?

Authors: Fat globules were removed from the process cheese samples by extraction with chloroform prior to freeze-fracturing and, therefore, cavities as seen in the micrographs were fat globules that had been in the cheese. Not all cavities, however, originate from the removal of fat. Whey pockets and air bubbles also appear as void spaces. Fat globules may be retained in the sample by fixation with imidazole-buffered osmium tetroxide (78, 80).

D. P. Dylewski: How important are ultrastructural immunocytochemical studies of process and natural cheese? Would knowledge of protein distribution and interactions during cheese development be important?

Authors: Immunocytochemical studies of cheese and process cheese may be important to better understand allergies to these milk products. Then attempts can be made to locate individual proteins in the cheese matrix by immunoelectron microscopy. Concerning the distribution of proteins and their interactions during cheese development and processing, very little is known about these phenomena. Proteolysis in Meshanger cheese was studied by fluorescence and interference light microscopy and by electron microscopy (79). By electron microscopy, protein in process cheese was found to form matrices having different ultrastructures depending on whether the product was soft or hard. String-like structures were
present in hard process cheese (35, 36, 68). Heertje et al. (20) assumed that such structures resulted from an association mechanism at the molecular level; they supported their assumption by reports that other proteins such as ovalbumin, insulin, and lysozyme produced similar structures on gelling under the effect of heat. The authors (20) consider string-like structures to be formed by unfolding of the protein molecules, followed by their non-random aggregation into continuous network structures.

I. Heertje: It is very striking that of the four investigated samples, only the modified starch product (Fig. 17) shows proper fat globules. Can you offer an explanation for this behaviour, considering that the starch will not act as an emulsifier?

Authors: Apart from mentioning that starch binds water and reduces the amount of free water in the cheese, we cannot comment until additional experiments are carried out using various cheese blends and melting salts in the presence or absence of modified starch.

I. Heertje: Is it likely that the distortion of the fat globule shapes in Fig. 9 is caused by some preparation artefact or by the image formation? The phosphate crystal appears to form the bottom of a crater and the 'distorted' globules are at the slopes of the crater.

Authors: A pair of stereo micrographs had not been taken to confirm your assumption that there is a slope between the crystal and the body of the cheese. It is probable that such a slope really exists although it should not. The fixed cheese sample under study had been dehydrated in ethanol, defatted in chloroform, impregnated with ethanol, and freeze-fractured. This procedure usually produces flat and smooth fracture planes. There is evidently an exception to this rule as shown in Fig. 9.

B. E. Brooker: The experimental process cheeses produced from Cheddar cheese and different combinations of emulsifying agents showed variation in the degree of fat dispersion. What effect does this have on the mechanical and textural properties of the cheese?

Authors: Processed Cheddar cheese was of commercial origin. Experimental cheeses were made from a mixture of Feta, Gouda, Kachkaval, and White cheeses. In general, fat emulsified into fine globules makes a firmer process cheese than fat present in the form of large globules. The total surface of very finely dispersed fat globules may be so high that there would not be enough protein to cover all the fat. The excess fat would separate as oil during processing and leave a hard nonmeltable cheese. This effect may be caused by tetrasodium pyrophosphate (with a high affinity for calcium), whereas sodium hexametaphosphate (with a lower affinity for calcium) produces a hard cheese without oil separation. Disodium phosphate (with a low affinity for calcium) produces a soft and meltable cheese which has large fat globules (81). In addition to the emulsifying salts used, the composition of the cheese blend and, in particular, heating of the blend with direct steam were other important factors which affected the physical properties of our experimental cheeses.

Additional References