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K. Fleming
R. Jenness
H. A. Morris
R. Schmidt

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PROPERTIES OF CALCIUM CASEINATES WITH DISPARATE PERFORMANCE IN IMITATION CHEESE

*Department of Food Science and Nutrition
**Department of Biochemistry
University of Minnesota
St. Paul, Minnesota 55108
***Department of Food Science and Human Nutrition
University of Florida
Gainesville, Florida 32611

Abstract

Two commercial lots of calcium caseinate preparations differing in performance in imitation cheese were analyzed for various characteristics. A imitation process cheese manufacturing classified the caseinates as good or poor based on appearances of the finished cheese, emulsification of fat, oiling-off during sheeting and melting properties. The sample which exhibited good functional properties in imitation cheese, had slightly higher calcium (16.4 mg/g protein), non-casein protein (2.87%) and γ-casein contents, a higher formol titre (8.95 mg free amino N/g protein), lower water absorption (138 mg/100 g protein), and lower stability to added calcium (8.0 min) than did the other sample (14.6 mg/g, 1.66%, 8.02 mg/g, 129 mg/100 g, and 15 min, respectively), which was described as having poor functionality according to the criteria of the imitation cheese producer.

Introduction

Calcium caseinates are being widely used in the manufacture of imitation cheeses and other food products. Functional properties of caseinates have been investigated in protein dispersions and in model systems (Hermansson, 1972, Hermansson and Akesson, 1975) and in imitation cheese systems (Hokes 1982, Hokes et al., 1982). Moreover, the relationship between casein structure and functionality has been reviewed (Morr 1979). Unfortunately, caseinates vary markedly in how they function in imitation cheese blends during manufacture and in their influence on the properties of the finished products. Development of a satisfactory formula and processing method does not always ensure a marketable product. Calcium caseinate exhibiting poor functionality may produce rough appearing imitation cheese that oils-off during sheeting, and does not melt satisfactorily. Further research data are needed to relate the properties of calcium caseinate to its performance in imitation cheese and to provide insight as to why variations occur. Therefore, the purpose of this investigation was to study selected physical and chemical properties of two commercial calcium caseinate samples that have been shown to have different functionalities in imitation cheese manufacture.

Materials and Methods

Caseinate Dispersions

Two calcium caseinates produced by two different manufacturers were chosen for this study. One caseinate exhibited good functionality in imitation cheese while the other was described as having poor functionality according to the criteria of an imitation cheese producer. Functionality evaluations were based on amount of oiling-off, melting properties, appearance of the product, mixing and emulsification problems. The good sample is of Dutch origin and is probably from Fresian (Holstein cows) and the poor sample is from New Zealand and probably from Jerseys. Caseinate solutions were prepared by dispersing caseinate powders in double distilled, deionized water at 35-40°C and by stirring for at least 30 min, with a magnetic stir bar.
Compositional Analyses

Moisture. One g samples were weighed into dried, preweighed, porcelain ashing dishes. Samples were dried in a vacuum oven at 70°C to a constant weight.

Ash and Mineral Determination. Following moisture determination, the casein samples were ashed overnight at 600°C cooled and weighed. Ash solutions were prepared by moistening the ash with water, dissolving in 5 ml of 1 N HCl, and adding distilled water to a final volume of 100 ml. Aliquots from these solutions were analyzed for phosphorus by the colorimetric method of Sumner (1944) and for calcium using the EDTA titration method of Jenness (1953).

Nitrogen Determination. The total, non-casein, and nonprotein nitrogen contents were determined using the semi-micro Kjeldahl method of Larson and Jenness (1950). Total nitrogen was determined directly on the casein sample. Non-casein and nonprotein nitrogen were determined on a 3% dispersion. A factor of 6.32 was used to calculate protein from the nitrogen content (Walstra and Jenness, 1984).

Soluble Calcium, Soluble Phosphorus, and Lactose Determinations. Fifteen ml of water was dialyzed against 1500 ml of 3% casein (or caseinate) dispersion (pH 7.0) at 4°C. After 24 h, the protein dispersion was changed and dialysis continued for an additional 24 h. The dialysate was then analyzed for lactose by the method of Marier and Boulet (1959), for phosphorus as previously described, and for calcium by the Nickerson et al. method (1964).

Formal Titration. Formal titration for free-amino nitrogen followed the procedure of Kuehler and Stine (1974). Three 5-ml aliquots of each 2.5% casein dispersion were titrated with 0.0183 N NaOH following the formaldehyde addition. Free-amino nitrogen was calculated as follows: mg free-amino N = (ml NaOH to pH 8.5) x (0.0183 N NaOH) x (14 mg N/meq. N).

Fat Determination. Fat content was determined by Soxhlet extractions using petroleum ether according to AOAC method 24.005 (1980).

Caseinate Properties

Buffering Capacity. Titration curves (buffering capacities) of 1, 2 and 3% caseinate dispersions were determined by titration with 1 N HCl from approximately pH 7.0 to pH 5.5. A Beckman 3550 digital pH meter was used to measure the pH one minute after each 20 ml addition of HCl. Measurements were taken at 22°C with constant agitation.

Water Absorption. The absorptive capacity of the calcium caseinates was determined using the Brabender farinograph and the Baumann capillary device. The farinograph was used according to the constant flour weight method (Locken et al., 1972). The method, using 300 g of flour on a 14% moisture basis, was modified by replacing 15 g of the flour with calcium caseinate. The required water was added within 30 sec and the absorption was determined at 30°C. Sample absorption was calculated as follows: Absorption (ml H2O/100 g sample) = 100(x-y)/g protein in 15 g sample where x is the amount of water required in the caseinate determination, y is the amount of water absorbed by 300 g of flour (14% moisture basis), and (x-y) is the amount of water absorbed by 15 g of sample.

The dough stability time was measured and is defined as the difference in time between the point where the curve is first centered on the 500 Brabender units (B.U.) line and the point where the curve begins to leave the 500 B.U. line.

Water uptake using the Baumann capillary device was determined as described by Wallingford and Labusa (1983). Thirty to thirty-five mg of caseinate were used for each of four replicate determinations at 18°C. The absorption due to the filter paper (0.0204 ml) was subtracted from the water uptake prior to plotting.

Stability in the Presence of Calcium and Phosphate. The stability of the caseinates in the presence of calcium and phosphate ions was determined using a method described by Thompson et al. (1969) with several modifications. Stock caseinate dispersions were treated with varying levels of CaCl2 (0-20 mM) or K2HPO4 (0-100 mM), centrifuged, and the supernatant protein concentration determined as follows:

1) Four ml of stock calcium caseinate solution (5 mg/ml) were added to a 20 ml centrifuge tube containing 5 ml of 0.1 M imidazole-HCl buffer (pH 7.0) and 1 ml of CaCl2 or K2HPO4 solution.

2) The tubes were inverted twice and incubated for 30 min at 30°C in a water bath.

3) The protein dispersions were centrifuged at 1500 x g for 15 min in an International Equipment IEC centrifuge, and the supernatant protein concentration determined using a standard curve based on total nitrogen as determined by semi-micro Kjeldahl. Supernatant protein was expressed as percent soluble protein.

Solvent. The solvation studies were performed in duplicate and at two centrifugal forces. The procedure, adapted from Thompson et al. (1969), was as follows:

1) Dry and weigh cellulose nitrate tubes (w0).

2) Pipette 5 ml of 35 mg/ml caseinate dispersion into the preweighed tubes.

3) Centrifuge at 68,000/140,000 x g for 30 min at 20°C.

4) Remove the top 2 ml of supernatant and analyze for protein by the modified Lowry method (Hartree, 1972).

5) Drain tubes inverted for 5 min.

6) Weigh the tubes with pellets (w1).

7) Freeze-dry the tubes and pellets for 20 hr and reweigh (w2).

8) Calculate solvation by the following formula: Solvation g H2O/g caseinate = w1-w2/w2-w0

Ion Exchange Chromatography

Caseins were alkylated and chromatographed as described by Davies and Law (1977) except that...
the flow-rate was maintained at 20 ml/hr with a peristaltic pump. The NaCl gradient was supplied from an eight-chambered gradient former with each chamber holding approximately 125 ml. Chambers 1–4 initially contained buffer with 0.03 M NaCl while chambers 5–8 contained buffer with 0.22 M NaCl. Column eluate was collected in 5.0–5.5 ml volumes using a Gilson microfractionator. Approximately 900 ml were collected and the absorbance of each fraction determined at 280 nm relative to buffer. Fractions were pooled according to the elution profile, dialyzed against distilled water, and freeze-dried. Peaks were characterized by disc gel electrophoresis according to Groves (1975) at pH 9.5 with 4 M urea. Duplicate samples were separated by chromatography. Protein recovery was calculated from the absorbance of a 1% solution at 280 nm in a 1 cm cell (A280), the volume, and the absorptivity according to Rose et al. (1969). Scanning Electron Microscopy (SEM) of Calcium Caseinates
A piece of double sticky tape was attached to an aluminum SEM stub and a small amount of dry calcium caseinate was applied to the tape. Fractured particles were prepared by slicing through the powder with a razor blade. Loose particles were removed from the tape by a stream of air. Silver paint was applied to the tape edge providing a conductive surface between the metal-coated particles and the metal stub. The stubs were coated with Au/Pd by vacuum evaporation and viewed under a Philips 500 scanning electron microscope at 12 kV.

Results and Discussion
Compositional Analyses
The results of the compositional analysis are given in Table 1. The caseinate exhibiting good functionality in imitation cheese had higher levels of ash, fat, lactose, noncasein protein, calcium (total and soluble), and phosphorus (total and soluble) than the poor caseinate. Formal titration data are given in Table 2. The poor caseinate had a titration value reasonably close to the theoretical calculation based on lysine content of bovine casein and noncasein protein. In contrast, the good caseinate had a considerably larger amount of free amino groups. This higher value may suggest a conformational difference, presence of more protein fragments of low molecular mass or a higher degree of protein hydrolysis. A study by Creamer and Matheson (1977) in New Zealand showed that when casein curd is dissolved in alkali at high pH and temperature, the protein is extensively damaged by hydrolysis of some peptide bonds and modification of some individual amino acids. Examination of 43 commercial samples showed amino acid modification to be absent from 27 New Zealand sodium and calcium caseinate samples. Six caseinates from other sources, including two calcium caseinates from The Netherlands, contained substantial amounts of lysinoalanine resulting from modified amino acids and indicating excessive alkali treatment during manufacture. Thompson and Farrell (1973) noted that exposure to high pH, as

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Casein Protein (%)</th>
<th>Noncasein Protein (%)</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>7.27</td>
<td>3.64</td>
<td>81.2</td>
<td>2.87</td>
<td>2.62</td>
</tr>
<tr>
<td>Poor</td>
<td>6.82</td>
<td>3.42</td>
<td>85.3</td>
<td>1.66</td>
<td>1.22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lactose (%)</th>
<th>Total Soluble Calcium (mg/g casein)</th>
<th>Total Soluble Phosphorus (mg/g casein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>0.14</td>
<td>16.4</td>
<td>2.85</td>
</tr>
<tr>
<td>Poor</td>
<td>0.02</td>
<td>14.6</td>
<td>1.19</td>
</tr>
</tbody>
</table>

* Walstra and Jenness (1984)

Figure 1. Titration curves (buffering capacities) of 1, 2, and 3% calcium caseinate dispersions. (100 ml of solution was titrated.)
Properties of Good and Poor Calcium Caseinates

Buffering Capacity

Titration curves for 1, 2, and 3% caseinate dispersions are shown in Fig. 1. In the pH range 5.5 to 7.0, the good caseinate required less acid to achieve any given pH at the three protein levels titrated. The higher level of soluble calcium (2.85 mg/g casein) in the good sample may potentially result in the screening of ester phosphate groups and, therefore, in the suppression of the protonation of primarily phosphoserine residues. Comparison of titers of the two samples is complicated by differences in their contents of calcium and phosphate.

Water Absorption

The two methods used to determine water absorption - the farinograph and the Baumann capillary device - represent entirely different experimental conditions. The farinograph measures the viscosity of a dough containing approximately 5% caseinate. Water absorption in this system is due to several factors including the competitive absorption by flour. The Baumann capillary device, on the other hand, measures the water uptake to the capillary suction pressure and swelling of the particles. Variations in particle porosity and swelling ability of the caseinates may be observed using this method.

Results from the Brabender farinograph experiment are shown in Table 3. The dough containing the good calcium caseinate absorbed more water than the poor sample and had a considerably shorter stability time, 8 min, compared to 15.0 min. Water-soluble constituents are known to have an effect upon the mixing characteristics of the dough. The higher level of soluble calcium in the good sample may have resulted in more protein calcium interaction thus causing water exclusion and a lower viscosity.

The mean absorption data of four replicate determinations for poor and good caseinates are plotted in Fig. 2. The poor sample, contrary to the farinograph data, absorbed more water throughout the 100 min test period. Microscopy data would have been helpful to observe the swelling and breakdown of the particles. It is tempting to speculate that the lower water uptake by the good sample may be due to a faster rate of particle breakdown. Our limited data suggest that the capillary device may be useful in distinguishing between caseinates with different swelling properties. Particle size and variations in particle porosity caused by different drying methods may influence swelling behavior.

Stability in the Presence of Calcium and Phosphate

Data presented in Fig. 3 show that the good sample was less soluble in the presence of increased levels of CaCl\(_2\) than was the poor sample possibly reflecting a difference in protein aggregation. The phosphate ion stability experiment (Fig 4) shows that both caseinates were quite stable over a wide range of phosphate concentrations. The good caseinate, consistently exhibited lower (approximately 5% lower) stability than the poor sample.

Solvation

Results from the solvation experiments are tabulated in Table 4. At a centrifugal force of 68,000 x g good calcium caseinate had more protein in the pellet and a higher degree of solvation. However, unlike the poor sample, the good caseinate did not form an intact pellet which made complete draining difficult. At 140,000 x g both samples were compacted to the point of excluding water and were easily drained. In this case, the poor caseinate was slightly more solvated at 3.64 g H\(_2\)O/g solid compared to 3.39 g H\(_2\)O/g solid for the good one. The good sample again had more protein in the pellet suggesting a higher amount of unstable casein material or larger protein aggregates in the dispersion.

Sedimentable Matter

After centrifugation at 300 x g for 10 min the good caseinate dispersion had a mean sedimentation volume of 2.67 ml/10 ml of 5% dispersion. The poor caseinate had no visible sedimentation. After an additional 10 min at 300 x g, the poor sample had an average of 0.5 ml sediment/10 ml dispersion. The good caseinate showed an increase to 0.1 ml sediment after the additional 10 min centrifugation. According to Roeper (1977) volumes of 2.77 and 0.5 ml/10 ml represent approximately 83 and 15% respectively of the weight of the powder. The high amount of sedimentable matter in the good caseinate may partly explain the lower soluble protein values in the calcium and phosphate stability experiments. The amount of sedimentable matter has been used as a criterion for assessing the rate and completeness of conversion of casein into caseinate and the amount of unstable material in reconstituted spray dried calcium caseinate. Roeper (1977) discusses manufacturing variables that account for large sedimentation (> 1.0 ml/10 ml) volumes. Our data again suggest the possibility of manufacturing differences between the two caseinates.

Ion-Exchange Chromatography

Figure 5 shows the elution profiles for the alkylated calcium caseinates when subjected to ion-exchange chromatography. Protein recovery was greater than 92% for both samples. Elution began at approximately 30 ml and stopped at approximately 730 ml. Based on the profile and
Properties of Calcium Caseinates

Figure 2. Water absorption or swelling behavior of calcium caseinates as determined by the Baumann capillary suction method.

Figure 3. Stability of calcium caseinates in the presence of calcium ions. Plots are mean values from four experiments.

Figure 4. Stability of calcium caseinates in the presence of phosphate ions.

Figure 5. The fractionation of alkylated calcium caseinates by ion-exchange chromatography.

Table 3. Effect of good and poor caseinates on water absorption of doughs determined using the Farinograph

<table>
<thead>
<tr>
<th>Dough with</th>
<th>Water Absorption (%) (mg/100g protein)</th>
<th>Stability (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no caseinate</td>
<td>62.7</td>
<td>498</td>
</tr>
<tr>
<td>good caseinate</td>
<td>118</td>
<td>138</td>
</tr>
<tr>
<td>poor caseinate</td>
<td>113</td>
<td>129</td>
</tr>
</tbody>
</table>

Table 4. Solvation of calcium caseinates

<table>
<thead>
<tr>
<th>Force (x g)</th>
<th>Calcium caseinate</th>
<th>Solvation</th>
<th>Supernatant protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H₂O g</td>
<td>H₂O g solid g protein g</td>
</tr>
<tr>
<td>68,000</td>
<td>good</td>
<td>5.81</td>
<td>6.17</td>
</tr>
<tr>
<td></td>
<td>poor</td>
<td>5.15</td>
<td>5.35</td>
</tr>
<tr>
<td>140,000</td>
<td>good</td>
<td>3.39</td>
<td>3.65</td>
</tr>
<tr>
<td></td>
<td>poor</td>
<td>3.64</td>
<td>3.83</td>
</tr>
</tbody>
</table>

Centrifugal force (x g)
K. Fleming, R. Jenness, H. A. Morris and R. Schmidt

Figure 6. Disc gel electrophoretic patterns for charged good calcium caseinate or peaks 1a, 1b, 2-6 as shown in Figure 5; and B) alkylated caseinate (AC) and for is shown in Fig. 5.

Figure 7. SEM micrographs of calcium caseinate powders: A) and B) are intact particles of the good caseinate; C) is a fractured particle from the good caseinate; D) and E) are particles of the poor caseinate and F) is a fracture particle from the poor caseinate.

data presented by Davies and Law (1977), the eluate was pooled into seven peaks for the good caseinate designated 1a, 1b, 2, 3, 4, 5, and 6, and into six peaks for the poor sample designated 1 through 6. The profiles show a number of main features common to both samples; however, the good caseinate had an initial elution of two peaks (1a and 1b) whereas the poor caseinate only had one initial peak. The numbers shown on the profiles correspond to the peaks subjected to disc gel electrophoresis (Fig 6). The gel pattern for peak 1a of the good sample shows the elution of some minor protein components. No distinct \( \gamma \)-casein bands were seen in 1a on this gel despite the application of a relatively large amount of protein. The eluate from peak 1b contained \( \gamma \) - and \( \kappa \) -caseins. In contrast, the poor caseinate eluate from peak 1 contained \( \gamma \) - and \( \kappa \) -caseins. The patterns for peaks 2-6 are identical for both samples. Peaks 2 and 3 consisted of \( \kappa \)-casein, peak 5 of \( \beta \)-casein, and peak 6 of \( \alpha _S \)-caseins. Peak 4 is probably some type of modified \( \beta \)-casein, possibly dephosphorylated \( \beta \)-casein, as suggested by Davies and Law (1977).

Although the elution profiles for both samples are essentially the same, except for peak 1b, the absence of a distinct \( \gamma \)-casein band in peak 1a of good caseinate and the elution of two peaks initially suggest some differences in minor protein components or fragments. A study by Hokes et al. (1982) on the curd formation of calcium caseinates showed that following the formation of a protein aggregate, the remaining supernatant was "rich" in \( \gamma \)-casein and minor milk proteins. The authors suggested that further research was necessary to determine if the differences in the functional performance of different calcium caseinates are related to a variable amount of the proteins found in the supernatant. Our ion-exchange data support the need for further research in this area.

SEM

Figure 7 shows some obvious structural differences between good and poor calcium caseinates. The good caseinate had more spherical particles which were generally less indented. Approximately 60% of the particles were less than 10 \( \mu \)m in diameter. In contrast, the poor caseinate particles were imploded to a higher degree and had a more even particle size distribution with approximately 50% of the particles in the 11-30 \( \mu \)m range. Inside, the particles appeared to be similar. Whether or not these differences have an influence on functionality would need to be explored more fully.

Chemical and functional analysis of calcium caseinates with disparate functionalities in imitation cheese has shown differences in the following respects:

- amount of soluble minerals
- amount of free-amino nitrogen determined by formol titration
- elution profile when separated by ion-exchange chromatography
- water absorption determined with the Baumann capillary device
- amount of sedimentable matter in a 5% dispersion
Since a limited sample size was used, a larger number of samples would be necessary to determine the significance of the differences observed. Further study would also be necessary to determine whether the solvation or calcium ion stability data are useful in characterizing the caseinates.

The calcium caseinate described as having good functionality in imitation cheese appeared to have been prepared under conditions resulting in a higher degree of hydrolysis as indicated by formol titration and ion-exchange chromatography, and/or under conditions resulting in incomplete conversion as suggested by the amount of soluble magnesium in milk and milk fractions with ethylenediamine tetraacetate. Anal. Chem. 25, 966-968.

Acknowledgements

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References


Properties of Calcium Caseinates


Discussion with Reviewers

M. Kalab: The authors have suggested that differences in manufacturing may have been responsible for the differences in the chemical composition and physical properties of the two calcium caseinate preparations; it would be useful to describe, even briefly, the manufacturing of calcium caseinate and the possible technological differences. It would be also useful to briefly describe the manufacturing of imitation cheese using calcium caseinates, including the relative amounts of the caseinates used.

Authors: The conversion of casein curd to calcium caseinate is a slower, more sensitive process than the manufacture of sodium caseinate. Calcium caseinate dispersions are more sensitive to heat. Problems that may arise during processing (and reconstitution of the dried powder) include sedimentation, precipitation, and gelatin (Roeper, 1974; Hayes et al., 1968).

Calcium caseinate is formed by neutralizing an acid casein dispersion with calcium hydroxide. The reaction product is a white, colloidal dispersion of relatively low viscosity which can be spray dried. The completeness of conversion is often measured by the level of sedimentable matter. Roeper (1977) studied some of the factors affecting the rate of conversion of acid casein curd into a dispersion with only 1-2% (by weight) sedimentable solids. He concluded that best results were obtained by reacting finely-milled, well-hydrated "soft" curd with alkali at or below 40°C. Heating above 40°C during the conversion adversely affected the solubility of the resulting caseinates.

Dispersions of calcium caseinate are unstable upon heating and may precipitate, especially as the pH is lowered or the soluble calcium content increases.

Roeper (1977) also noted reversible gelation upon heating calcium caseinate prior to spray drying. Reversible gelation appears to be peculiar to calcium caseinate.

Imitation process cheeses are made essentially the same as conventional process cheeses. The blends, of course, are different. Kosikowski (1977) provides compositions for some imitation process cheeses. The total protein he shows is 23.5% and the soluble protein is 1.7% for a Cheddar analog. The soluble protein is usually sodium caseinate and a large amount of the rest of the protein (21.8%) is calcium caseinate. Blend formulas are not readily available because they are proprietary.

H. E. Swaisgood: Comparison of the composition data and the calcium solubility data also deserves some comment because of the seeming inconsistency. Thus, the "good" sample contains less casein protein and more noncasein protein and yet it is less soluble in the presence of calcium. Perhaps the authors could expand their discussion to include their thinking as to the cause of this inconsistency.

Authors: If the proteins present in the "non-casein" fraction are, in fact, products from partial hydrolysis, it doesn't necessarily follow that they should be more soluble with respect to CaCl₂ addition. Depending upon which casein is partially hydrolyzed and depending upon where in the primary sequence the hydrolysis took place, it is conceivable that the calcium solubility profile could be extensively altered. For example, partial hydrolysis could result in two fractions: a low molecular weight fraction (la and lb) and a modified larger molecular weight fraction, which being largely hydrophobic in nature (if from β-casein or α-casein), would tend to aggregate more readily than prior to hydrolysis. Thus, the total "system" would be more susceptible and would exhibit lower solubility in the presence of CaCl₂. While the elution profiles and electrophoretic data show little differences in the larger casein components, it is possible that these techniques are not sensitive enough to show subtle alterations in structure.

Additional References

