combination with that of milk phosphatidyl inositol (6). Difficulties in separating the phosphatidyl serine from other phospholipids may account for inadequacies of data regarding its quantitation and fatty acid composition.

The data presented in Table 2 are for samples gathered in May and June. A sample of phosphatidyl serine isolated from mixed herd milk in October contained higher levels of saturated fatty acids than reported for the samples in the table. The ratio of stearic to oleic acid in this October sample was 1:0.61, compared to a ratio of approximately 1:1.25 for the samples in Table 2.

We have not recorded in our analyses low levels of C₂₀ and longer fatty acids, including 2 or 3% of arachidonic acid which occurs in the phosphatidyl ethanolamine of cow's milk. However, a concerted effort to reveal any of these acids in two of our serine preparations failed, even when hydrogenation of the methyl esters was used to accumulate the C₂₀ and C₂₂ components.

VIRGINIA E. BOATMAN, STUART PATTON, and JOHN G. PARSONS. Lipids Laboratory, Division of Food Science, Pennsylvania State University, University Park.

Acknowledgment

This research was supported in part by a grant (HE 03632) from the U.S. Public Health Service and was authorized for publication on July 11, 1968, as Paper no. 3442 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

References


Identification of Amino Compounds Derived from k-Casein During Storage

Abstract

Low molecular weight amino compounds derived from k-casein by sterilization and during storage were identified with an amino acid analyzer and by thin-layer chromatography. Ammonia and a small amount of cysteic acid were detected. Ammonia was derived mainly from amides by heat degradation, and cysteic acid by oxidation of cystine in k-casein. Also, a decrease in histidine residues in the protein fraction of stored k-casein was observed.

1 Supported by an Operating Grant A-3641 of the National Research Council of Canada.

It was reported previously that compounds with a molecular weight of approximately 160 were produced from k-casein by heat sterilization and storage (10, 11). However, the identification of these compounds was not carried out at that time. According to Kirchmeyer (7), phenylalanine, threonine, serine, and aspartic acid as additional terminal amino acids and free phosphoric acid were observed in whole casein as a result of heat degradation, indicating a cleavage of peptide linkages. It was assumed that a similar degradation occurs in k-casein as well. The objectives of this study were to identify amino compounds derived from k-casein during storage and to explain the decrease in stabilizing action of the stored k-casein.
Experimental Procedures

The preparation, sterilization, and storage of κ-casein were reported (10).Low molecular weight amino compounds separated on a Sephadex G-50 column (10), freeze-dried and dissolved in citrate buffer pH 2.2, were analyzed with a Phoenix Micro Amino Acid Analyzer, Model M 6800, Moore-Stein System. Cystine in the protein fraction of κ-caseins was determined as cysteic acid (13) and as S-carboxymethyl cysteine (2). Excess performic acid was removed by freeze drying after oxidation. The amino compounds were dissolved also in 10% isopropanol and analyzed for amino acids by thin-layer chromatography (3, 6).

The protein fractions separated from κ-casein by acid coagulation (10) were freeze-dried, hydrolyzed for 24, 72, and 96 hours with 6 N glass-distilled HCl, and analyzed for amino acids. Amides were analyzed according to the method reported by Hayashi, Fukushima, and Mogi (5). Protein was estimated by the Kjeldahl method.

Free ammonia in κ-casein was determined by the indophenol method (9). To 1 ml of 2% κ-casein diluted with 5.2 ml of water were added 0.6 ml of a digesting reagent (6.7 ~ ~H~SO, containing 4% of K~SO~ and 0.09% CuSO~· 5 H~O) and 3.2 ml of 5% phenol. The solution was centrifuged for 5 min at 1,200 × g to remove protein. Five milliliters of the supernatant were neutralized with 1 ml of 2 N NaOH and 4 ml of carbonates (1:1 mixture of saturated NaHCO~ and M Na~CO~). The color developed by mixing the above mixture to 1.2 ml of Na~Cl, NF-grade, was measured at 610 m/z.

Results

Amino acids in low molecular weight amino compounds. Analysis of the low molecular weight amino compounds from stored κ-casein revealed a prominent ammonia peak and a peak at a retention volume of phosphoserine or cysteic acid (4), and other amino acids were undetectable on the Amino Acid Analyzer. Thin-layer chromatography for identification of cysteic acid. Analysis of the low molecular weight amino compounds by Fahmy's method of chromatography (3), without oxidation of the amino acids, revealed a spot of cysteic acid but not of O-phosphoserine. The Rf and the color stained with the pyridine-isatin reagent (1) were the same as cysteic acid. The color of spots for cysteic acid and for O-phosphoserine were violet and pinkish orange, respectively. Cysteic acid was also confirmed from the Rf value on a Jones's cellulose plate (6), in combination with the color developed by spraying ninhydrin-Cu(NO~)~ indicator (8).

Determination of amides. Under a mild condition of hydrolysis, amide linkages of glutaminyl and asparaginyl residues liberate ammonia. It is possible, therefore, that the ammonia peak from stored κ-casein was derived from these amides. The ammonia peak was found to increase and the amides concentration to decrease by sterilization and during storage. Amides and free ammonia in the control, sterilized, 45-day and 90-day stored κ-caseins were 13.2, 12.7, 12.1, and 11.8 ~ for amides and 0.08, 0.49, 0.60, and 0.75 ~ for ammonia per 10,000 g of κ-casein, respectively.

Although the ammonia in the nonprotein fraction of stored κ-casein accounted for only approximately 50% of the decrease in concentration of amides, there appears to be a negative correlation between the concentration of amides in the protein fraction and the concentration of ammonia in the nonprotein fraction of stored κ-casein. It is possible that the phosphate buffer used with κ-casein (10) did not trap all the ammonia produced from κ-casein during storage.

Decrease in amides in κ-casein during storage would explain faster mobility of stored κ-casein than the control by polyacrylamide-gel electrophoresis at pH 9.3 (12).

Amino acids in the protein fraction of κ-casein. Amino acid composition in the protein fraction separated from κ-caseins is indicated in Table 1. Decreases were significant in histidine and methionine.

Table 1. Amino acid composition of the protein fraction of κ-caseins.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sterilized</th>
<th>90-day stored</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M/10,000 g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>7.5</td>
<td>7.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>8.3</td>
<td>8.1</td>
<td>7.8</td>
</tr>
<tr>
<td>Serine</td>
<td>7.3</td>
<td>7.4</td>
<td>7.0</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>16.1</td>
<td>16.0</td>
<td>15.7</td>
</tr>
<tr>
<td>Proline</td>
<td>11.2</td>
<td>10.7</td>
<td>10.7</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Alanine</td>
<td>8.7</td>
<td>8.6</td>
<td>8.7</td>
</tr>
<tr>
<td>1/2 Cystine</td>
<td>1.3</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Valine</td>
<td>6.9</td>
<td>6.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>7.0</td>
<td>7.0</td>
<td>6.9</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.3</td>
<td>5.4</td>
<td>5.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>5.1</td>
<td>4.8</td>
<td>5.0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.5</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.5</td>
<td>5.5</td>
<td>5.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.8</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
</tr>
</tbody>
</table>

* Determined as cysteic acid following performic acid oxidation (13).

J. DAIRY SCIENCE Vol. 52, No. 2
tidine and cystine in addition to a tendency of a slight decrease in heat-labile lysine, threonine, and serine. Cystine in the protein fraction by the S-carboxymethylation method was 1.2, 0.4, and 0.6 residues for the control, sterilized, and 90-day stored κ-caseins, respectively. This method yielded the same results as the performic acid method for the control, but not for sterilized and stored κ-caseins. This may be due to deeper masking of cystine residues by sterilization and storage.

Low molecular weight amino compounds cleaved from κ-casein molecules were identified as ammonia, with a slight contamination of cysteic acid. The molecular weight of 160 previously calculated (11) should be interpreted as ammonium salts produced from κ-casein in the presence of phosphates during storage (10). Most of the ammonia was derived from amides by decomposition and partly from amino acid residues such as histidine by degradation. Cysteic acid presumably has been produced by an oxidation of cystine during storage, since ampules of κ-casein were sealed without evacuation. A calculation indicated the amount of cysteic acid produced during storage for 90 days to be 1.7 mm per 10,000 g of κ-casein. A part of degradation products of cystine consists of the cysteic acid. Destruction of cystine may result in decrease in molecular weight of κ-casein; however, aggregations probably through noncovalent interactions counteract to this degradation. Thus, the effect of destruction of cystine on molecular size of κ-casein may not be obvious as indicated by gel filtration (10). A decrease in histidine in the protein fraction of κ-casein conforms to a previous report of histidine determination by colorimetry in stored κ-casein (12). Histidine residues in stored κ-casein seem to help explain the decrease in the stabilizing action of κ-casein. However, the significance of the liberation of ammonia and the exposure of free carboxyl groups from amides, which account for approximately 13 moles as compared to 1.8 moles of histidine per 10,000 g of κ-casein, remains to be elucidated by further investigation.

Acknowledgment
We are indebted to Mrs. J. J. Perrin for her skilful assistance as a laboratory technician.

P. C. TATTO and S. NAKAI, Division of Animal Science, University of British Columbia, Vancouver

References

Present address, Fraser Valley Milk Producers' Association, Vancouver.

All correspondence to be addressed to S. Nakai.

Now with the Department of Food Science.