TECHNICAL NOTES

INFLUENCE OF CALCIUM CONCENTRATION IN MILK UPON COOKED FLAVOR DEVELOPMENT

Sulfide liberation from milk has been found to be decreased by the addition of salts of copper, silver, iron, or sodium (7) or by the addition of calcium chloride or disodium phosphate (1). The removal of calcium by ion exchange did not alter the sulfhydryl production, however (1). The importance of calcium to certain milk protein reactions has been demonstrated in rennet coagulation and in protein stability in evaporated milk. The protein stability of frozen milk has been increased by reducing the calcium concentration (2). Certain casein fragments have been shown to bind calcium; others do not (8). The sulfhydryls of milk have been shown to have their origin in the \( \beta \)-lactoglobulin. This protein has been shown to react with \( \kappa \)-casein, especially where the mixture is heated (4, 9). Sawyer et al. (6) have shown that the sulfhydryl group is implicated in this reaction.

The present report deals with the relationship between the calcium concentration, the volatile sulfhydryls, and the cooked flavor of milk.

EXPERIMENTAL PROCEDURE

All raw milk samples used in these experiments were obtained from mixed milk at the University Creamery on the morning the sample was to be heated. Calcium determinations were made by the method described by Kamal (3). Experiment I was designed to determine the influence upon flavor of the direct addition to milk of calcium in the form of \( \text{CaCl}_2 \cdot 2 \text{H}_2\text{O} \) powder. Following the addition of the calcium, the pH of the milk was adjusted to 6.7 with 0.1 N NaOH. Pint samples of milk with added calcium, as well as a control sample having no added calcium, were heated at 155°F for 30 min with constant agitation supplied by a steady flow of nitrogen through the milk. After heat treatment the milk was stored approximately 2 hr at 40°F, then tasted by four experienced judges. The milk was scored 0, 1, 2, 3, or 4, the higher numbers signifying a progressively greater degree of cooked flavor.

Experiment II was designed to determine the influence upon cooked flavor in milk of adding or removing calcium by use of ion exchange resins. For adding calcium, one liter of raw milk was agitated 10 min with 50, 25, or 12 g of Dowex 50W X12 in the calcium form. The decanted milk was adjusted to pH 6.7 with 0.1 N KOH, then heated as described above, tasted, and the calcium determinations conducted. KOH was used instead of NaOH, inasmuch as the latter tended to give a salty taste under similar conditions. For removing calcium, one liter of milk was exposed to 50 or 25 g of resin in the sodium form. Those samples which had the greatest removal of calcium increased in pH to about 7.0, but no adjustments were made.

Experiment III was designed to measure the influence of added calcium upon the production of volatile sulfhydryls from milk. Raw milk was analyzed for calcium concentration and to one portion of the sample \( \text{CaCl}_2 \cdot 2 \text{H}_2\text{O} \) was added. Both samples were then heated at 194°F for 30 min in an apparatus described by Townley and Gould (7). At the end of the heating period the milk was cooled to below 158°F by addition of cold water to the water bath and

![Graph](image.png)

**Fig. 1.** Relationship between calcium concentration and intensity of cooked flavor in milk.
the nitrogen flow continued for 30 min. The zinc acetate receiving tubes were immersed in ice water at the end of the nitrogen aspiration period and later analyzed by the method used by Townley and Gould (7) on a Bausch and Lomb colorimeter at 620 nm. Nitroprusside tests were made by the method described by Patton and Josephson (5).

Experiment IV was conducted with milk exposed to a calcium or sodium resin. The milk was then heated and analyzed as described in Experiment III.

RESULTS

The relationship between added calcium and flavor scores (Experiments I and II) is shown in Figure 1, with hand-fitted lines to denote the trend. Occasionally the judges noted a slightly salty flavor at the high calcium level. A smaller concentration of calcium is necessary to decrease cooked flavor when the calcium is added by means of ion exchange rather than by means of direct addition. Data were not collected on the alteration in concentration of other cations (e.g., Na, K, Mg) by the resin treatment. They are, however, decreased by calcium resin treatment; the K, Mg, and Ca are decreased by a sodium resin treatment. The calcium resin and sodium resin treatment both reduced the cooked flavor below those samples receiving the same heat treatment but not undergoing resin treatment. The average correlation coefficient between the flavor score and the calcium concentration in the added calcium was -0.91, and in resin-treated milk, -0.89. Analysis of variance of the flavor scores on milk receiving the same heat treatment but having different concentrations of calcium showed the calcium concentration to have a significant influence (P < 0.05) upon flavor score.

Addition of calcium to milk decreases the nitroprusside value as well as the volatile sulfur production (Figure 2). The amount of volatile sulfur was calculated on the basis of one sulfur atom per molecule of methylene blue formed. The total amount of volatile sulfur evolved and the calcium concentration has a correlation coefficient of -0.9.

These experiments demonstrate that additional calcium in milk will reduce the cooked flavor, whether the calcium is added directly or whether it is exchanged by ion exchange resin for some other cation in the milk, and that additional calcium will retard the production of sulfhydryls as measured by the nitroprusside test or by formation of methylene blue from its precursor. Likewise, the removal of calcium from milk by a sodium ion exchange resin will cause a larger amount of sulfur present in milk to become volatile. The specific action of the calcium in preventing the liberation of sulfhydryl groups cannot be stated definitely; however, the data indicate the possibility that the calcium may enter into a bond-

Fig. 2. Influence of calcium concentration upon volatile sulfhydryl production in milk.

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QUANTITATIVE ANALYSIS OF THE MAJOR FREE FATTY ACIDS IN BLUE CHEESE

Fatty acids are important in the characteristic flavor and odor of Blue cheese. However, the literature lacks data on the absolute amount of fatty acids present in this cheese. Coffman, Smith, and Andrews (3) used gas chromatography to determine the relative percentages of free fatty acids isolated from dry Blue cheese, but made no attempt to determine the individual acids. Acetic and butyric acids have been measured by Sjöström and Willart (7) and Morris et al. (6) determined the butyric and total moles of higher fatty acids in Blue cheese. This investigation was undertaken to determine the major individual free fatty acids in several cheeses.

Four Blue-vein type cheeses were examined. Two, A and B, were domestic Blue cheese obtained as 5-lb wheels from a local plant. The two other cheeses, one a domestic Blue (Sample C), the other an imported Roquefort (Sample D), were small retail packages purchased from a local market.

Results indicated that samples from the edge of the wheels of cheese varied considerably in fatty acid content when compared to the center of the wheel. To obtain reproducible results it was necessary to remove a wedge from the wheel and mix it thoroughly. A sample of the blended cheese was then used for analysis. In the small packages, two packages were blended and a portion used for analysis.

The column of Wiseman and Irvin (8) was used for determining acetic acid. Fifteen grams of cheese and 30 grams of silicic acid were acidified to pH 1.9 with 50% $\text{H}_2\text{SO}_4$ and ground to a homogenous mixture in a mortar and pestle. Two grams of the mixture were used as cap material for the column. Eluting solvents were employed as suggested by Wiseman and Irvin (8).

The Keeney column (5) was used for quantitation of butyric acid and the total moles of higher fatty acids. Two grams of cap material prepared as just described were used.

Fractions eluted from both columns were titrated with approximately 0.01 N isopropylanolic KOH, using 0.5% phenolphthalein as an indicator. Carbon dioxide free air (prepared by passing through 20% KOH) was used for agitation of the samples, to prevent fading end points.

The method of Bills and Day (1) was used to determine the higher fatty acids. A 50-g sample of cheese was ground with sufficient 50% $\text{H}_2\text{SO}_4$ to reduce the pH to 1.9. After adding the internal standards, 12.5 mg heptanoic and 125 mg heptadecanoic acid in 5 ml hexane, the cheese was packed in 50-ml stainless steel centrifuge tubes and placed in a 40 C water bath for 30 min. After centrifugation at 30,000 × $g$ for 20 min the fat layer was removed carefully. The free fatty acids were then converted to their methyl esters, extracted, and concentrated as previously reported (2).

The total moles of higher fatty acids determined by the Keeney column were distributed according to their molar ratios, as determined by gas liquid chromatography (1).

As shown in Table 1 there was considerable variation in the free fatty acid content of the cheeses examined. This variation was evident in the flavor and odor of the cheeses. Sample B was mild in flavor and was criticized for not having typical Blue cheese flavor. The flavor of the Roquefort sample was considerably different from that of the Blue cheese samples, in that it was lacking in butyric acid and more characteristic of the $C_6$, $C_8$, and $C_{10}$ acids. This might be expected in view of the fatty acid composition of sheeps' milk. Hilditch (4) states that sheeps' milk is low in butyric acid and has high proportions of octanoic and decanoic acids, compared to cows' milk. Calculations of the mole per cent of the free acids found in the cheeses indicate that octanoic and