

# MEASUREMENT OF CALCIUM OF MILK BY ATOMIC ABSORPTION SPECTROPHOTOMETRY IN THE PRESENCE OF MAJOR INGREDIENTS OF DETERGENTS<sup>1, 2</sup>

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(Received for publication August 1, 1973)

## ABSTRACT

Twelve chemical ingredients commonly used to formulate detergents were tested to determine their interferences with measurement of calcium in milk by atomic absorption spectrophotometry. For concentrations of 0.01, 0.1, and 1.0% milk, the mean amounts of calcium measured for all ingredients were 0.20, 1.85, and 15.89 mg/l, respectively. Statistical analysis showed that milk accounted for 97.3% of the variation in spectrophotometric response with temperature and concentration of ingredient being of little practical importance. However, increasing the temperature caused the instrument to indicate increased amounts of calcium in solutions containing sodium metasilicate (anhydrous), EDTA plus sodium hydroxide, sodium gluconate, and phosphoric acid. Changing the concentration of ingredient did not interfere substantially with the amount of calcium measured except for sodium metasilicate (anhydrous) where an increase in concentration reduced the amount of calcium measured. From the data it can be concluded that an atomic absorption spectrophotometer shows considerable promise for use in monitoring removal of residue from a milk contact surface.

The mineral constituents of the residue remaining on a milk contact surface, after the processing cycle is completed, has been determined in several studies. Lyster (3) found that ash from milk deposits on ultra high temperature processing equipment contained 35% calcium, 58% phosphate, and 7% other minerals. Shere (6) observed that deposits from heated surfaces, where several products were processed at various temperatures, contained from 2.5 to 46.5% calcium oxide (CaO). Heinz et al. (1) reported that dry milk film on a milk storage tank, before cleaning, con-

tained 113.2 mg of calcium per 100 cm<sup>2</sup> of tank surface. After the contact surface was rinsed, the film contained 18.2 mg/100 cm<sup>2</sup> which indicated that 95 mg/100 cm<sup>2</sup> were removed during the rinse phase of the cleaning cycle. The alkaline cleaning phase removed an additional 9.5 mg/100 cm<sup>2</sup>. Therefore, assuming that all milk constituents are simultaneously removed, calcium, because it is present in milk residue, should be usable as a tracer in exhausted cleaning solutions to monitor removal of residue from a milk contact surface.

The calcium content of various solutions can be determined by using an atomic absorption spectrophotometer with little, if any, sample preparation. The principle limitation of the atomic absorption technique in chemical analysis is the failure of some metals to dissociate completely. The nitrous oxide-acetylene flame overcomes many of the chemical interferences found with conventional flames, and concentrations of calcium are not influenced by 100 times their concentration of phosphorus (7). The high temperature flame gives a high metal dissociation and greater freedom from condensed phase interference than other flames, but ionization of the element does occur, which reduces analytical sensitivity. Addition of an easily ionized compound, such as potassium chloride, will eliminate ionization interference (2).

The objective of this research was to determine whether ingredients used in formulating a detergent interfered with measurement of calcium by an atomic absorption spectrophotometer. The long range objective is to develop a system for monitoring the rate of removal of constituents of residue from milk contact surfaces.

## MATERIALS AND METHODS

### Materials

The detergent ingredients tested and the range of concentration (as normally found in a formulated commercial detergent) of each ingredient are presented in Table 1. Percentages of ingredients used in formulating commercial detergents

<sup>1</sup>Contribution from the University of Missouri Experiment Station. Journal Series No. 6739.

<sup>2</sup>Trade names and names of commercial companies are used in this publication solely to provide specific information. Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

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TABLE 1. DETERGENT INGREDIENTS AND CONCENTRATIONS

| Ingredient  | Concentrations<br>(%) (w/v) |      |      |      |
|---|-----------------------------|------|------|------|
|   | A                           | B    | C    | D    |
| Basic Alkalis   |                             |      |      |      |
| 1. Sodium hydroxide (NaOH)  | 0.0                         | 0.45 | 0.9  | 1.35 |
| 2. Sodium metasilicate anhydrous (Na <sub>2</sub> SiO <sub>3</sub> )          | 0.0                         | 0.3  | 0.6  | 0.9  |
| 3. Sodium carbonate (Na <sub>2</sub> CO <sub>3</sub> )                        | 0.0                         | 0.3  | 0.6  | 0.9  |
| Phosphates  |                             |      |      |      |
| 4. Trisodium phosphate (Na <sub>3</sub> PO <sub>4</sub> · 12H <sub>2</sub> O) | 0.0                         | 0.75 | 1.50 | 2.25 |
| 5. Sodium hexametaphosphate (Na(PO <sub>3</sub> ) <sub>6</sub> )              | 0.0                         | 0.3  | 0.6  | 0.9  |
| 6. Sodium tripolyphosphate (Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub> )  | 0.0                         | 0.3  | 0.6  | 0.9  |
| 7. Tetrasodium pyrophosphate (Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> ) | 0.0                         | 0.3  | 0.6  | 0.9  |
| Chelating agents  |                             |      |      |      |
| 8. Tetrasodium ethylenediaminetetraacetic acid (EDTA)                         | 0.0                         | 0.21 | 0.42 | 0.63 |
| 9. Sodium gluconate (C <sub>6</sub> H <sub>11</sub> O <sub>7</sub> Na)        | 0.0                         | 0.21 | 0.42 | 0.63 |
| Wetting agent   |                             |      |      |      |
| 10. Wetting agent DC-161  | 0.0                         | 0.06 | 0.12 | 0.18 |
| Acids   |                             |      |      |      |
| 11. Gluconic acid 50% (C <sub>6</sub> H <sub>12</sub> O <sub>7</sub> )        | 0.0                         | 0.6  | 1.2  | 1.8  |
| 12. Phosphoric acid 75% (H <sub>3</sub> PO <sub>4</sub> )                     | 0.0                         | 0.6  | 1.2  | 1.8  |

were obtained from detergent formulators and knowledgeable authorities. Detergent ingredients were supplied by Economics Laboratory, Inc., St. Paul, Minnesota.

Water used in the milk-ingredient-water (MIW) solutions was softened with a commercial ion-exchange water softener. The raw whole milk used was obtained from the University of Missouri-Columbia dairy plant and was analyzed for fat by the Mojonnier, ether-extraction method and for total solids by the gravimetric method (5).

A standard stock solution of 2000 mg calcium/l was prepared by dissolving 4.9945 g of dried CaCO<sub>3</sub> (analytical grade) in 6 N HCl and diluting with distilled water to one liter.

A Varian Techtron, Model 1000, atomic absorption/flame emission spectrophotometer, equipped with a calcium, hollow cathode lamp, was used to measure calcium. Dimensions of the nitrous-oxide burner were 6.0324 cm by 0.457 mm. Slit width was 0.5 nm, and lamp current was 6 ma. Acetylene was the fuel gas, and nitrous oxide was the support gas. The millivolt signal from the spectrophotometer was fed into a 10-inch chart, Honeywell Electronik 194 laboratory recorder.

Individual water baths were used to temper samples to 20, 45, and 70 C before analysis.

#### Methods

The experimental design was a 3 × 4 × 4 factorial in randomized complete block with two replications. Three temperatures (20, 45, and 70 C), four concentrations of detergent ingredients, and four concentrations of milk (0.0, 0.01, 0.1, and 1.0%) were used.

Standard curves were prepared from solutions that contained 0, 5, 10, and 15 mg calcium/l. These solutions were aspirated into the spectrophotometer at room temperature, and their peak values were recorded. The peak values were used to prepare a standard curve which was used to quantitate the peak response in amount of calcium obtained during the analysis of the MIW solutions. A new standard curve was prepared for each replication in the analysis of each ingredient.

Each of four concentration (Table 1) of each ingredient was prepared in 12 BOD bottles (300 ml), and milk was added to provide concentrations of 0.0, 0.01, 0.1, and 1.0% (v/v). Each milk concentration occurred four times within each ingredient concentration, thus providing one set of 16 samples for use at each temperature. Bottles were inverted, shaken,

and placed in the appropriate water bath. When solutions reached the required temperature ( $\pm 2$  C), they were analyzed for calcium content.

Data were analyzed by analysis of variance (ANOVA). Duncan's New Multiple Range Test (DNMRT) was used to determine significant differences between means of the variables and pertinent interactions. Sums of squares, attributable to regression, were determined.

#### RESULTS AND DISCUSSION

Mean amounts of calcium measured (mg/l) for each concentration of milk averaged over four concentrations of ingredient at three temperatures are given in Table 2. Concentrations of calcium observed in samples containing no milk and 0.01% milk were at the limits of the sensitivity of the instrument. This accounts, therefore, for the failure to obtain significant differences in concentrations of calcium in samples containing these concentrations of milk. The measured amount of calcium in samples containing no milk was subtracted from values for samples with 0.01, 0.1, and 1.0% milk for the respective ingredients to obtain changes in the amounts of calcium concentrations that resulted from changes in concentrations of milk.

Numbers in parentheses in Table 2 show net quantities of calcium measured as a result of changes in concentrations of milk. The overall mean quantity of measured calcium as a result of the addition of 1.0% milk was 15.89 mg/l. Normal cow's milk contains 1.25 g calcium/l, which means that a 1.0% concentration of milk would be expected to contain 12.5 mg/l (4). The difference between 15.89 and 12.5 mg calcium/l was probably a result of suppression of ionization of calcium by the ingredients and sodium in the water. The low readings for sodium metasilicate

cate (anhydrous), 11.90 mg calcium/l, and sodium tripolyphosphate, 12.26 mg calcium/l, at a 1.0% concentration of milk may have been caused by formation of thermally stable compounds containing silicates and phosphates. Standard solutions (5, 10, and 15 mg calcium/l) prepared with and without 1000 mg potassium/l, as KCl, indicated that this concentration of potassium reduced ionization of calcium by 36%.

Individual detergent ingredients did not interfere greatly with spectrophotometric measurement of calcium at concentrations of 0.01, 0.1, and 1.0% milk. A tenfold increase in the concentration of milk theoretically should have produced a tenfold increase in amount of calcium in samples. Means averaged over all ingredients of detergent showed that an increase in concentration of milk from 0.01 to 0.1% caused a 9.3-fold increase in measurable calcium (from 0.20 to 1.85 mg/l). The next tenfold increase in concentration of milk from 0.1 to 1.0% caused an 8.6-fold increase in measurable calcium (from 1.85 to 15.89 mg/l). Thus, limited interference probably occurred

as concentration of milk was increased. Milk accounted for an average of 97.3% of the variation in the data; the range was from 91.9 to 99.6%.

For a 0.01% concentration of milk, the net amount of measured calcium varied from a low of 0.06 mg/l for sodium metasilicate (anhydrous) to a high of 0.47 mg/l for gluconic acid; the overall mean for this concentration was 0.20 mg calcium/l. No significant differences ( $P < 0.05$ ) were noted in amounts of calcium measured between 0.0 and 0.01% concentrations of milk for any individual ingredient. This indicates considerable variation at the lowest concentration of milk. For a 0.1% concentration of milk, the measurable amount of calcium varied from a low of 1.10 mg/l for sodium metasilicate (anhydrous) to a high of 2.67 mg/l for sodium carbonate with an overall average of 1.85 mg calcium/l. For a 1.0% concentration of milk, the range was from 11.91 to 19.45 mg/l for sodium metasilicate (anhydrous) and sodium carbonate, respectively; the overall mean was 15.89 mg calcium/l.

In most instances the type or concentration of de-

TABLE 2. MEAN AMOUNTS OF CALCIUM (mg/l) FOR FOUR CONCENTRATIONS OF MILK AVERAGED OVER FOUR CONCENTRATIONS OF DETERGENT INGREDIENT AND THREE TEMPERATURES

| Detergent ingredient            | Concentration of milk (%)   |                     |        |         |
|---------------------------------|-----------------------------|---------------------|--------|---------|
|                                 | 0.0                         | 0.01                | 0.1    | 1.0     |
|                                 | —[Calcium measured (mg/l)]— |                     |        |         |
| Sodium hydroxide                | 0.22 <sup>a</sup>           | 0.33                | 2.05   | 16.84   |
|                                 |                             | (0.11) <sup>b</sup> | (1.83) | (16.62) |
| Sodium metasilicate (anhydrous) | 0.14                        | 0.20                | 1.24   | 12.05   |
|                                 |                             | (0.06)              | (1.10) | (11.91) |
| Sodium carbonate                | 1.19                        | 1.35                | 3.86   | 20.64   |
|                                 |                             | (0.16)              | (2.67) | (19.45) |
| Trisodium phosphate             | 1.77                        | 1.93                | 3.56   | 17.58   |
|                                 |                             | (0.16)              | (1.79) | (15.81) |
| Sodium hexametaphosphate        | 0.82                        | 1.00                | 2.79   | 16.90   |
|                                 |                             | (0.18)              | (1.97) | (16.08) |
| Sodium tripolyphosphate         | 0.78                        | 0.99                | 2.74   | 13.04   |
|                                 |                             | (0.21)              | (1.96) | (12.26) |
| Tetrasodium pyrophosphate       | 0.85                        | 1.12                | 3.01   | 17.49   |
|                                 |                             | (0.27)              | (2.16) | (16.64) |
| EDTA plus sodium hydroxide      | 0.53                        | 0.75                | 2.36   | 16.47   |
|                                 |                             | (0.22)              | (1.83) | (15.94) |
| Sodium gluconate                | 0.28                        | 0.45                | 2.19   | 18.68   |
|                                 |                             | (0.17)              | (1.91) | (18.40) |
| Wetting agent DC-161            | 0.51                        | 0.82                | 2.60   | 18.96   |
|                                 |                             | (0.31)              | (2.09) | (18.45) |
| Gluconic acid 50%               | 1.13                        | 1.60                | 2.39   | 15.95   |
|                                 |                             | (0.47)              | (1.26) | (14.82) |
| Phosphoric acid 75%             | 0.15                        | 0.26                | 1.76   | 14.47   |
|                                 |                             | (0.11)              | (1.61) | (14.32) |
| Mean                            | 0.70                        | 0.90                | 2.55   | 16.59   |
|                                 |                             | (0.20)              | (1.85) | (15.89) |

<sup>a</sup>Each entry is an average of 24 readings; 12 are from each replication.

<sup>b</sup>Numbers in parentheses represent the change in average calcium concentrations that were a result of changes in concentrations of milk.

<sup>c</sup>Underscored values indicate no significant differences at 5.0% level of probability according to Duncan's New Multiple Range Test.

TABLE 3. MEAN AMOUNTS OF CALCIUM (mg/l) FOR FOUR CONCENTRATIONS OF DETERGENT INGREDIENT AVERAGED OVER FOUR CONCENTRATIONS OF MILK AND THREE TEMPERATURES

| Detergent ingredient            | Concentration of detergent ingredient <sup>a</sup> |                      |         |         |
|---------------------------------|--|----------------------|---------|---------|
|                                 | A  | B                    | C       | D       |
|                                 | —[Calcium measured (mg/l)]—                        |                      |         |         |
| Sodium hydroxide                | 4.52 <sup>b</sup>                                  | 5.43                 | 4.69    | 4.80    |
|                                 |  | ( 0.91) <sup>c</sup> | ( 0.17) | ( 0.28) |
| Sodium metasilicate (anhydrous) | 4.24   | 3.98                 | 2.94    | 2.47    |
|                                 |  | (-0.26)              | (-1.30) | (-1.77) |
| Sodium carbonate                | 6.55   | 6.66                 | 6.77    | 7.07    |
|                                 |  | ( 0.11)              | ( 0.22) | ( 0.52) |
| Trisodium phosphate             | 4.61   | 6.27                 | 6.36    | 7.59    |
|                                 |  | ( 1.66)              | ( 1.75) | ( 2.98) |
| Sodium hexametaphosphate        | 4.96   | 5.20                 | 5.60    | 5.75    |
|                                 |  | ( 0.29)              | ( 0.64) | ( 0.79) |
| Sodium tripolyphosphate         | 3.57   | 4.13                 | 4.74    | 5.11    |
|                                 |  | ( 0.56)              | ( 1.17) | ( 1.54) |
|                                 | 4.60   | 5.25                 | 6.24    | 6.38    |
|                                 |  | ( 0.65)              | ( 1.64) | ( 1.78) |
| EDTA plus sodium hydroxide      | 4.73   | 4.99                 | 5.08    | 5.31    |
|                                 |  | ( 0.26)              | ( 0.35) | ( 0.58) |
| Sodium gluconate                | 5.14   | 5.30                 | 5.64    | 5.52    |
|                                 |  | ( 0.16)              | ( 0.50) | ( 0.38) |
| Wetting agent DC-161            | 5.86   | 5.93                 | 5.30    | 5.81    |
|                                 |  | ( 0.07)              | (-0.56) | (-0.05) |
| Gluconic acid 50%               | 5.08   | 5.29                 | 5.04    | 5.67    |
|                                 |  | ( 0.21)              | (-0.04) | ( 0.59) |
| Phosphoric acid 75%             | 4.03   | 3.99                 | 4.19    | 4.43    |
|                                 |  | (-0.04)              | ( 0.16) | ( 0.40) |
| Mean                            | 4.44   | 5.20                 | 5.22    | 5.50    |
|                                 |  | ( 0.76)              | ( 0.78) | ( 1.06) |

<sup>a</sup>Concentrations of individual ingredients were calculated from values given in Table 1. For a 3.0% total concentration of detergent of sodium hydroxide the highest concentration (Column D) was calculated as  $45\% \times .03 = 1.35\%$ .

<sup>b</sup>Each entry is an average of 24 readings; 12 are from each replication.

<sup>c</sup>Numbers in parentheses represent the change in average calcium concentration that are a result of changes in the concentrations of detergent.

<sup>d</sup>Underscored values indicate no significant differences at 5.0% level of probability according to Duncan's New Multiple Range Test.

tergent ingredient did not substantially affect the measured quantities of calcium (Table 3). However, the B concentration of sodium hydroxide (0.03%) gave a significantly higher reading than the other concentrations. The metasilicates apparently interfered with analyses, because quantities of calcium observed decreased with increasing concentrations of this detergent component. The decrease may have been caused by formation of thermally stable compounds containing calcium during evaporation of the solvent. When the concentration of trisodium phosphate, sodium hexametaphosphate, tetrasodium pyrophosphate, EDTA plus sodium hydroxide, or phosphoric acid was increased, an increase in the amount of calcium measured was noted. This increase in detected calcium may have been caused by an increase in the suppression of ionization of the calcium.

Numbers in parentheses in Table 3 represent average changes in concentrations of calcium caused by changes in concentrations of detergent ingredients.

Calcium measured decreased with increasing concentrations of sodium metasilicate (anhydrous), but quantities increased with increasing concentrations of sodium carbonate, trisodium phosphate, sodium hexametaphosphate, sodium tripolyphosphate, tetrasodium pyrophosphate, and EDTA plus sodium hydroxide. However, these increases were not significant. The ingredients apparently suppressed ionization of calcium.

Mean amounts of calcium for the three temperatures averaged over the four concentrations of each detergent ingredient and the four concentrations of milk are presented in Table 4. Temperature did not significantly affect calcium measurements when the ingredient was sodium hydroxide, trisodium phosphate, sodium tripolyphosphate, or phosphoric acid. For sodium metasilicate (anhydrous), EDTA plus sodium hydroxide, sodium gluconate, and phosphoric acid significantly more calcium was measured as the temperature increased from 20 to 70 C. An increase in

TABLE 4. MEAN AMOUNTS OF CALCIUM (mg/l) FOR THREE TEMPERATURES AVERAGED OVER FOUR CONCENTRATIONS OF MILK AND FOUR CONCENTRATIONS OF DETERGENT INGREDIENTS

| Detergent Ingredient            | Temperature (C)            |      |      |
|---------------------------------|----------------------------|------|------|
|                                 | 20                         | 45   | 70   |
|                                 | Calcium measured<br>(mg/l) |      |      |
| Sodium hydroxide                | 4.76 <sup>a</sup>          | 4.92 | 4.90 |
| Sodium metasilicate (anhydrous) | 2.99                       | 3.23 | 4.07 |
| Sodium carbonate                | 5.40                       | 7.28 | 7.60 |
| Trisodium phosphate             | 5.34                       | 6.26 | 7.03 |
| Sodium hexametaphosphate        | 4.99                       | 5.62 | 5.57 |
| Sodium tripolyphosphate         | 4.17                       | 4.59 | 4.41 |
| Tetrasodium pyrophosphate       | 4.98                       | 5.71 | 6.16 |
| EDTA plus sodium hydroxide      | 4.59                       | 5.10 | 5.40 |
| Sodium gluconate                | 4.55                       | 5.62 | 6.03 |
| Wetting agent DC-161            | 5.25                       | 5.73 | 6.19 |
| Gluconic acid 50%               | 4.75                       | 5.54 | 5.52 |
| Phosphoric acid 75%             | 3.62                       | 4.30 | 4.56 |
| Mean                            | 4.63                       | 5.33 | 5.62 |

<sup>a</sup>Each entry is an average of 32 readings; 16 are from each replication.

<sup>b</sup>Underscored values indicates no significant differences at 5.0% level of probability according to Duncan's New Multiple Range Test.

temperature from 20 to 45 C caused significant increases in amounts of calcium measured for the other five ingredients tested. The overall mean quantities of calcium observed at 20, 45, and 70 C were 4.63, 5.33, and 5.62 mg/l, respectively. Differences in concentrations of calcium measured at the three temperatures were probably caused by decreases in viscosity and surface tension (3) which increased aspiration rates.

## CONCLUSION

The following were our conclusions: (a) major ingredients used in formulation of commercial detergents do not significantly interfere with the measurement of calcium, (b) the analytical procedure was sensitive to concentrations of calcium of about 0.2 mg/l and above, (c) an increase in temperature of the solution caused an increase in amount of calcium measured, and (d) milk accounts for most of the variation in the data, with concentrations of detergent ingredients and temperature being of little importance. Because variable other than concentration of milk were relatively insignificant, we concluded that the atomic absorption spectrophotometer shows considerable promise for use in monitoring the rate of removal of residue from a milk contact surface.

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## PFIZER AWARD GIVEN TO JAMES A. ELLIOTT

The 1973 recipient of the Pfizer Award, for cheese research, Dr. James A. Elliott, was born in 1923 in Wawota, Saskatchewan, Canada, and in 1951 received his B.S.A. degree from the University of Saskatchewan with a major in dairy science. He crossed the border to start graduate study in dairy industry at the University of Wisconsin and received the M.S. degree in 1952 and the Ph.D. in 1955. He accepted a position with the Canada Department of Agriculture as a research scientist to conduct investigations in dairy microbiology in what is now the Food Research Institute. He has been a member of the staff ever since, presently serving as Head, Microbiology Section.

Most of Elliott's working life has been spent on various aspects of cheese research. His chief interests have been in the microbiology of flavor development in Cheddar cheese, in starter efficiency, and in the mechanism of enzymes in milk coagulation. His numerous scientific papers on these subjects are respected by other scientists for their originality,

soundness of experimental methodology, and significance to the dairy industry.

Among the major discoveries in which Elliott participated are clarification of the role of starter culture strains in bitter flavor development of cheese, production of fatty acids from amino acids, and origin of fruity flavors. Using his background on the serology of cheese bacteria, he developed the passive indirect hemagglutination test to detect rennin in cheese and showed its possible application to other enzymes, a technique of considerable importance to control agencies. He also has collaborated in originating tests for the quantitative determination of enzymes in mixtures and has shown the role of natural agglutinins in sedimenting susceptible strains of starters thus leading to sluge formation in cottage cheese. At the Food Research Institute, he pioneered use of sophisticated taste panels and statistical analyses in flavor research.

Elliott is a modest, quiet, and thoughtful researcher who is always willing to assist others and share his knowledge.