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# Mineral Balance in Milk Heated Using Microwave Energy

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Milk heated to 75 and 85 °C in a water bath or in a microwave oven was assayed for changes in salt partitioning after cooling to room temperature. To properly to assess differences and draw valid comparisons, the two heating methods used in the experiment were applied to samples for identical exposure times, and the samples were heated to attain the same final temperatures. Although the soluble Ca and $P_i$ contents were lower in the heated milk samples, no significant differences in salt partitioning were found between microwave and conventional heating. Ionic calcium levels in the milk samples pasteurized using microwave energy were very close to the levels in the samples heated in a conventional water bath (~90% of the level in the untreated milk samples). The microwave heating-induced changes were completely reversed after storage at 20 °C for 24 h. The coagulation properties of the heated milk samples were also examined, and the coagulation time was longer and the curd formation rate slower in the microwave-heated milk than in the raw milk. Still, the experimental results demonstrated that microwave heating was no more detrimental to the milk than conventional heating and could thus be used for pasteurization purposes.

**KEYWORDS: Microwave; calcium; milk; mineral balance; heating**

## INTRODUCTION

Numerous studies have dealt with the application of microwaves as an alternative method to conventional heat treatment of foods (*1*). Investigations on one such application, the pasteurization of milk by microwave heating, have been carried out over the past decade. Studies on the microwave heating of milk have monitored different aspects of the efficacy of this procedure (*2*−*8*).

Pasteurization has today become common in cheese-making in the manufacture of a large number of commercial cheese varieties, in which it is used to destroy pathogenic microorganisms and the native flora of the raw milk before inoculation of specific starter cultures. A problem with pasteurization that is commonly encountered is fouling of the heat exchanger over time, due to the high surface temperatures employed. Fouling reduces heat transfer and leads to flavor changes as the fouling material decomposes. Microwave heating could provide a means of avoiding fouling by eliminating the steep temperature gradient that occurs in conventional pasteurization and of heating fluids directly and efficiently. Using a domestic microwave oven to pasteurize raw milk, Thompson and Thompson (*6*) reported a significant reduction in bacterial contamination with no appreciable damage to the organoleptic characteristics of the product. More recent studies have confirmed that microwaved milk, compared with milk heated in a plate heat exchanger, had a longer shelf life (*5*) and microwave heating had no adverse effects on the flavor characteristics of milk (*7*).

A number of workers have reported different rates of whey protein denaturation in milks depending on the severity of the microwave heat treatment (*3*, *4*, *8*). It is well-known that the complex between denatured $\beta$-lactoglobulin and $\kappa$-casein that forms when milk is heated increases rennet coagulation time. However, at pasteurization temperatures the extent of thermal denaturation observed after microwave heating has been found to be similar to (*2*) or lower than (*3*, *4*) that produced by conventional systems. Furthermore, microwave pasteurization causes less thermal damage to milk constituents than conventional pasteurization, enhances the survival of native proteolytic enzymes important to cheese ripening, and produces milk that retains desirable cheese-making attributes (*8*).

It is also well-known that changes in the mineral balance can also alter milk rennet clotting properties (*9*). However, no data have been reported on the calcium status—essential to coagulation of milk by rennet—of microwave-heated milk. Microwave heating is accomplished both by absorption of the microwave energy by rotation of the dipolar water molecules andby translation of the ionic components of the food. Whereas dipolar rotation is the more frequently discussed means of microwave heat generation, ionic conduction plays a major role in food systems. Thus, both the water content and the dissolved ion content are predominant factors in the microwave heating of foods. These alterations could affect the mineral balance and the ionic calcium content in milk and, subsequently, susceptibility of the milk to coagulation by rennet. The role of calcium

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and minerals in the coagulation of heated milk has been thoroughly explored for conventional heating (*9*, *10*) but not for microwave heating, so further work is required. Consequently, the objective of this study was to evaluate the effect of microwave energy on the mineral balance and on changes in the ionic calcium content after microwave treatment and to compare the results to those for conventional heating.

## MATERIALS AND METHODS

**Samples.** Raw bovine milk samples were obtained from mixed bulk milk originating on farms in the region of Madrid, Spain. Freshly drawn milk was shipped to the laboratory in isothermal containers, and sodium azide was added (0.1 g/L) as preservative.

**Microwave Oven.** The microwave system was a model A-ET3 2L oven (Moulinex, Caen, France) with a programmable timer and nine power settings (ranging from 150 to 900 W), equipped with a revolving plate. Magnetron frequency was 2450 MHz.

**Heat Treatments.** Milk was heated conventionally in a water bath and in a microwave oven. Portions of the milk were heated to 75 and 85 °C in a water bath for 60 and 70 s, respectively, in tightly sealed Pyrex glass tubes. Preliminary trials were carried out under differing conditions to determine the heating times needed to reach each final temperature. The microwave oven heating parameters were also established. Conditions selected were treatment at 500 W for 60 and 70 s in order to reach similar final temperatures of 75 and 85 °C using the same heating times (60 and 70 s) as for the conventional water bath heat treatment. The milk volume heated by microwave energy was 70 mL. Similar temperatures in the same heating times in the water bath could be attained only for 10-mL milk samples.

Three independent experiments were carried out in the conditions finally selected for each heat treatment. In all cases, after heating, the samples were immediately cooled to room temperature in an ice−water bath. For the soluble Ca, Mg, and $P_i$ determinations, the samples were held for 10 min at room temperature before ultracentrifugation. Samples were also held for 10 min before testing of the renneting properties and measurement of ionic calcium levels.

Aliquots of the same sample held at 20 °C without heating were also ultracentrifuged to determine the soluble mineral content in unheated milk for purposes of comparison.

**Analytical Methods.** The soluble phase of the heated and unheated milk was separated by high-speed centrifugation in a Sorvall Combi Plus ultracentrifuge (Wilmington, DE). An amount of 30 mL of milk in polypropylene threaded Oak Ridge bottles with a shoulder and screw cap sealing assembly was centrifuged (100000*g*) using a 50-RT-1250 rotor at 20 °C for 1 h. The supernatant was carefully removed and vacuum-filtered through Whatman No. 40 paper. Inorganic phosphorus ($P_i$) was determined colorimetrically at 750 nm by the molybdenum blue method, and Ca, Mg, Na, and K were determined by flame atomic spectrometry, both after deproteinization of the supernatant with trichloroacetic acid (*11*). Ionic calcium was measured using an ion-selective electrode as described by Geerts et al. (*12*). All analyses were performed in triplicate.

**Determination of Renneting Properties.** Coagulation time and curd characteristics were analyzed using a model D thromboelastograph (Hellige GmbH, Freiburg, Germany). This thromboelastograph consists of two modules and draws a firmness versus time diagram as clotting proceeds. A service module heats the sample cuvette and the milk, controls instrument temperature, and houses the on/off controls for the recorder module. The recorder module consists of a two-channel recording system. Each channel comprises a pendulum with counterbalanced damper and an adjacent optical system. Common to both channels are the sample oscillator system, flashing light unit, and strip-chart recorder.

An amount of 25 mL of each sample was allowed to equilibrate to 32 ± 0.1 °C, and 2% $CaCl_2$ was added (4% v/v). After 10 min at this temperature, a 2.5% solution of animal rennet (0.4% v/v) (Chr. Hansen, Hørsholm, Denmark; strength = 1:50000) was added. The mixture was stirred, and, immediately, 0.8 mL was taken up by syringe and placed in each thromboelastograph cuvette. The samples were then brought into contact with the pendulum loops, and the recorder module was started up. While the milk remains uncoagulated, insufficient force to cause the pendulum to move is transmitted from the linearly oscillating milk. When coagulation occurs, the resulting increase in viscosity and curd formation cause synchronous motion of the pendulum, and light flashes reflected from the pendulum mirrors are recorded at different lateral positions on the chart.

In a typical diagram of firmness versus time, $t_r$ represents the time to gel formation. The value of $t_r$ was determined by measuring the distance from the origin to the point where the baseline begins to increase in width. The time from the start of gel development until a width of 20 mm was reached on the chart is shown as $K_{20}$. This equates with a curd firmness suitable for cutting of the cheese curd. $A_m$ is the measurement of the greatest width in millimeters between both lines and represents curd firmness.

**Statistical Analysis.** Analysis of variance was carried out using the Statgraphic statistical system (Rockville, MD). The level of significance was $p < 0.05$.

**Table 1.** Soluble Ca, Mg, $P_i$, Na, and K (Milligrams per Liter) Contents in Bovine Milk Pasteurized by Heating in a Water Bath (70 s to 85 °C) and in a Microwave Oven (70 s at 500 W to 85 °C) and Then Stored for 15 min at 20 °C[a]

| milk | Ca | $P_i$ | Mg | K | Na |
|---|---|---|---|---|---|
| unheated | 407 ± 12[a] | 338 ± 16[a] | 77.3 ± 1.2[a] | 1498 ± 25[a] | 391 ± 7[a] |
| microwave | 395 ± 8[ab] | 323 ± 6[ab] | 76.6 ± 0.5[a] | 1530 ± 9[a] | 403 ± 3[a] |
| water bath | 381 ± 7[b] | 301 ± 10[b] | 75.2 ± 0.4[a] | 1503 ± 10[a] | 385 ± 5[a] |

[a] Mean values for three sample batches and triplicate determinations for each batch. Means in the same column with differing superscripts were significantly different $p < 0.05$.

**Table 2.** Ionic Calcium Content (Percent of That in Unheated Milk) in Milk Pasteurized in a Water Bath (70 s to 85 °C and 60 s to 75 °C) and in a Microwave Oven (70 s at 500 W to 85 °C and 70 s at 500 W to 75 °C) at Different Storage Times at 20 °C[a]

| storage time (min) | microwave (85 °C) | water bath (85 °C) | microwave (75 °C) | water bath (75 °C) |
|---|---|---|---|---|
| 15 | 85.0 ± 1 | 88.7 ± 1 | 94.2 ± 1 | 85.0 ± 3 |
| 30 | 90.0 ± 1 | 91.8 ± 1 | 93.3 ± 1 | 88.3 ± 3 |
| 45 | 89.4 ± 2 | 91.8 ± 2 | 95.8 ± 1 | 86.6 ± 2 |
| 60 | 90.9 ± 1 | 90.7 ± 1 | 93.3 ± 1 | 90.8 ± 2 |

[a] Mean values for three sample batches and triplicate determinations for each batch.

## RESULTS

**Table 1** summarizes the effects of conventional and microwave heating of milk on the soluble mineral concentration. Soluble Ca, Mg, and $P_i$ levels in the heated samples were lower than in the untreated milk. Soluble Ca, $P_i$, and Mg values were slightly higher in the microwave-heated milk. However, no statistically significant differences between the two methods were found in the heated samples. No significant changes in the soluble K and Na levels were recorded after milk heating, and neither heating method tested altered the distribution of these two elements, which for the most part remained in the soluble phase.

The concentration of ionic calcium decreased when the calcium phosphate precipitated in the heated milk. Comparison of short-term changes in the ionic calcium content in milk heated to 75 and 85 °C by both test procedures indicated similar trends (**Table 2**). Under the experimental conditions assayed the ionic calcium values in both heat-treated milks were significantly different from those in the unheated milk. One hour after heating, ionic calcium levels were ∼90% of the level in the

**Table 3.** Soluble Ca, Mg, $P_i$, and Ionic Ca (Milligrams per Liter) Contents in Bovine Milk Pasteurized by Heating in a Microwave Oven (70 s at 500 W to 85 °C) and Then Held at 20 °C for Different Storage Times[a]

| | | time from heating | | |
|---|---|---|---|---|
| | unheated | 15 min | 6 h | 24 h |
| Ca | 392 ± 7 | 376 ± 3 | 376 ± 9 | 397 ± 2 |
| ionic Ca | 101 ± 3 | 73 ± 7 | 96 ± 2 | 102 ± 1 |
| Mg | 79.4 ± 1.5 | 77.2 ± 0.8 | 77.5 ± 0.4 | 80.6 ± 0.9 |
| $P_i$ | 375 ± 7 | 355 ± 4 | 363 ± 4 | 374 ± 7 |

[a] Mean values for three sample batches and triplicate determinations for each batch.

**Table 4.** Renneting Properties of Milk after Heating to 75 and 85 °C in a Microwave Oven or a Water Bath Followed by Cooling to 32 °C[a]

| | unheated | microwave (75 °C) | water bath (75 °C) | microwave (85 °C) | water bath (85 °C) |
|---|---|---|---|---|---|
| $t_r$ (min) | 1.9 ± 0.5[a] | 2.9 ± 1.1[a] | 2.6 ± 0.7[a] | 5.0 ± 0.7[b] | 5.8 ± 0.2[b] |
| $K_{20}$ (min) | 20.8 ± 4.5[a] | 24.5 ± 3.7[a] | 26.8 ± 1.3[a] | 31.4 ± 2.6[b] | 32.4 ± 3.6[b] |
| $A_m$ (mm) | 21.5 ± 1.4[a] | 21.1 ± 1.4[a] | 19.6 ± 1.0[a] | 22.2 ± 1.8[a] | 22.1 ± 0.7[a] |

[a] Mean values for three sample batches and triplicate determinations for each batch. Means in the same column with differing superscripts were significantly different $p < 0.05$.

unheated milk. Although the observed differences between microwave- and water bath-heated milks were small, the highest ionic calcium content was recorded for the microwave-heated milk at 75 °C.

Longer term availability of ionic calcium and other soluble minerals was checked by holding samples of the microwave-heated milk at 20 °C for 24 h. The results are presented in **Table 3**, which shows that the soluble mineral levels after 24 h of storage were similar to those in the unheated milk.

The rennet clotting time ($t_r$), which included both the enzymatic and aggregation stages, the time to curd firmness ($K_{20}$), and curd firmness ($A_m$) were determined in unheated milk samples and in the milk heated by the two different methods tested (**Table 4**). Both $t_r$ and $K_{20}$ increased with the heating temperature. No statistically significant differences were observed between the conventionally heated and microwave-heated samples. Conversely, $A_m$ was not affected by the different treatments.

## DISCUSSION

Although the mechanisms involved in microwave heating differ from those of conventional heat treatment, the mineral balances observed afterward were similar in all of the samples (**Table 1**). Previous studies have reported that on cooling of conventionally heated milk, reversal of the changes in mineral partitioning was very largely complete within the first minute or so, and this was followed by a slower recovery to levels resembling those in raw milk. In comparison with untreated milk, only a slight depletion in soluble minerals has been measured after cooling of heat-treated milk. Law (*13*) reported that the distribution of Ca and $P_i$ between soluble and colloidal phases in heat-treated milk was similar to that in raw milk after storage of milk heated to different temperatures at 20 °C for 22 h. These findings indicate that the composition of calcium phosphate, its dissolution behavior, and the amount of Ca bound directly to casein remained largely unaffected by heating below 90 °C, provided heating was followed by sufficiently prolonged storage. The slow re-equilibration in the colloidal calcium phosphate composition that takes place after heating was attributed by Pouliot et al. (*14*) to a complex equilibrium between calcium phosphate and citrate salts.

The important role of the ionic calcium content in rennet coagulation in the aggregation stage and in curd structure during cheese-making is well-known. High levels of this cation are conducive to the aggregation process and help to decrease $t_r$, whereas low concentrations could hinder coagulation. In this study the decreases in ionic calcium following heating (∼10% of the original level) were too low to be regarded as an appreciable reduction.

No previous studies have been reported on alterations in ionic calcium levels as a consequence of microwave heating. According to the present findings ionic calcium levels recovered completely after storage for 24 h (**Table 3**). Reversion to initial ionic calcium levels in milk subjected to conventional heat treatment is still a matter of controversy. Kocak et al. (*15*) monitored ionic calcium after heating to temperatures of up to 85 °C and concluded that values do not recover completely to initial levels. Using raw milk from different species, de la Fuente et al. (*16*) found that recovery to initial values was affected by the severity of heating. Nevertheless, in both of these studies ionic calcium levels were monitored for only 5 h of storage. A study on dried milk (*17*) reported that the ionic calcium content largely reverted to its original level if sufficient time for equilibration was allowed.

As pointed out under Results, no significant differences in the renneting properties were observed between conventional and microwave heating. Few studies on the effect of microwave heating on renneting properties are available in the literature. Villamiel et al. (*8*) compared the effect of different heating methods (either a plate heat exchanger or a microwave oven) on the renneting properties of goat's milk pasteurized at 72.5 and 80.1 °C for a few seconds. The renneting properties were quite similar after both heat treatments, despite the higher levels of whey protein denaturation found in milk heated using the plate heat exchanger. Vasavada et al. (*2*) found that cow's milk discontinuously heated in a microwave oven exhibited better coagulation properties than conventionally heated milk, even though the degrees of whey protein denaturation were similar in both cases. However, the times needed to reach the final heating temperature reported by these authors differed. Heating times for samples heated using the water bath were ∼4 min as compared to just 2 min for samples heated using the microwave oven. The exposure time for conventionally heated samples was thus double the exposure to microwave energy, and this could account for the longer $t_r$ and $K_{20}$ values observed in those samples. To circumvent this shortcoming, in the present study the heat treatments employed took into account sample exposure time. Accordingly, the final temperatures attained were also the same in both heat treatments, and instead sample volume exposed to heating was varied to achieve the desired time and temperature values. Thus, the effects recorded for each heat treatment were more directly comparable.

Although ionic calcium values did not immediately recover completely to initial levels after microwave heating, they were quite close to those measured after conventional heat treatment. The decrease in ionic calcium after microwave heating and subsequent storage would be too small to exert any appreciable influence on the milk clotting process. Additionally, short microwave treatment at high temperatures is well-known to produce less whey protein denaturation than conventional water-bath heating (*3*−*5*, *8*).

Previous studies have demonstrated the effectiveness of microwave pasteurization of milk in terms of microbial inactivation and unaltered taste and odor after and during treatment and subsequent storage (*5*, *7*). Furthermore, the low degree of whey protein denaturation after microwave heating *(4*) and the only slight decrease in the ionic calcium content observed in the present study are also indicators that microwave heating could be a promising method of pasteurizing milk. Microwave heating is no more detrimental to milk renneting properties than conventional heating, and hence the low level of alteration of the milk constituents would seem to make this method highly suitable for preparing milk for subsequent use in cheese-making.

In conclusion, the changes in the mineral balance brought about by microwave heating did not differ from the changes produced by conventional heating. Microwave ovens offer the added advantages of rapid heating, more uniform thermal distribution, and a reduction in the size of the hot surface area in contact with the milk. Conductive procedures tend to be poorer conductors of heat and thus require time to heat the whole product mass. Microwave heating involves more direct absorption of energy by the material being heated, and hence the procedure is more efficient than conventional heating. Furthermore, the milk never comes into direct contact with heated metal surfaces, thereby minimizing the problems of product burn-on and equipment fouling. As a consequence, the results of this study suggest that microwave heating could profitably be used instead of conventional heating procedures to pasteurize milk for cheese-making.

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