Changes in the Mineral Components in Cheese Juice (Aqueous Phase of Cheese) during Ripening

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Abstract
Four different types of cheeses were measure detailed changes in the mineral concentrations of cheese–serum during ripening. Concentrations of minerals in cheese juice were measured. The pH value using the low pH method (LPM) cheese was significantly (p<0.05) lower than that of other cheeses. Similarly the total Ca, S, Mg, and P contents of LPM cheese were significantly lower in than those of other cheeses. Ca, S, Mg, and P remained in coloidal form, while other minerals were mostly in soluble forms after 1 day. The minerals associated with the structure of cheese (i.e., casein or colloidal calcium phosphate) remained largely insoluble even after 1 month of ripening.

Keywords
cheese, cheese juice, ripening, minerals, insoluble Ca

Introduction
The serum (aqueous) phase of cheese, called cheese juice, is considered as the medium where a variety of biochemical processes involved during maturation of cheese takes place (Broome and Limstowtin, 2002). In early 1900’s cheese juice was extracted with hydraulic pressure from various cheeses and the juice was used to measure soluble nitrogen, salts, total solids, lactose and lactic acid (Barthel et al., 1928; Sandberg et al., 1930; McDowall and Dolby, 1936). These parameters were used to predict cheese properties. Monib (1962) measured some minerals (Ca and P) along with other soluble components in cheese juice and concluded that if cheese was highly diluted with water and there was a different concentration of Ca compared to cheese juice. Morris et al. (1988) measured the concentration of various minerals in cheese juice from Cheddar cheese aged 1 mo. Morris et al. (1988) considered that there was the nucleation and growth of calcium phosphate crystals on cheese as the cheese serum was supersaturated with calcium phosphate.

Broome and Limstowtin (2002) prepared an artificial cheese aqueous phase buffer similar to (cheese juice) to perform an initial screening of potential adjunct microorganisms that could be used for ripening of Cheddar cheese. O’Mahony et al. (2006) reported that the concentration of INSOL Ca in cheese can be modified by incubating cheese in synthetic cheese buffers with various concentrations of Ca.

There have been only a few studies on the changes in mineral components of cheese juice during ripening. In the previous chapters, the calcium concentration in cheese juice
was measured. Therefore, the objective of this chapter was to examine the changes in the composition of other mineral components in cheese during ripening by analysis of cheese juice.

Materials and Methods

1. Cheese manufacturing

Four types of full fat cheese described as high pH method (HPM), low pH method (LPM), high pH method with lactose added (HPML) and high pH method with washing (HPMW) were manufactured. Cheeses were made on two occasions by licensed cheese makers at the University of Wisconsin-Madison Dairy Plant. The detailed make-up procedure for these cheese is shown in the Table 1. Two cheese trials were performed over a 2-mo period. A mixed strain starter culture consisting of Lactococcus lactis ssp. cremoris and Lactococcus lactis ssp. lactis was inoculated into the milk with the rate of 1,490 g/226 kg of milk. Double strength chymosin (Chymax Extra, Chr. Hansens, Milwaukee, WI) was added at the rate of 17 mL/226 kg of milk at 32°C. The coagulum was cut with 0.63 cm knife and the curd was given a 5-min healing time before gentle agitation of 10 to 15 min. The curd–whey mixture was heated slowly from 32 to 39°C and continuously stirred at 39°C until the pH of curd reached ~6.2.

For the HPMW cheeses, ~23 kg of cold water (~10°C) was added immediately (within 2 min time) to the cheese curd after the first draining. After adding the cold water, the curd was held in cold water for 5 min with stirring. After draining, curd was salted at the rate of 0.72 kg/226 kg of milk. Salted curd was packed in 9-kg-Wilson-style hoops and pressed for 3 h at ambient temperature. Cheese was kept overnight at ambient temperature before vacuum packaging. After vacuum packaging the cheese was stored at 6°C for further analysis. Two 9-kg blocks of cheese were made from each treatment in each trial.

2. Compositional analysis

All compositional tests were done at least in triplicate. Milk was analyzed for total solids, casein, fat and protein content (Marshall, 1992). Rennet whey was made from cheese milk on the same cheese making day and total Ca of milk and rennet whey was measured by inductively coupled argon plasma emission spectroscopy (Vista-MPX Simultaneous ICP-OES: Varian, Palo Alto, CA). Cheese and cheese juice were analyzed for moisture, fat, protein (Marshall, 1992), Lactose and lactic acid (both D- and L-lactate) of milk and cheese were determined by enzymatic methods (Severn et al., 1985; IDF, 1993). Major minerals in cheese were measured by inductively-coupled argon plasma emission spectroscopy (Vista-MPX Simultaneous ICP-OES: Varian, Palo Alto, CA).

Wavelengths used for measurement were 422.5 nm for Ca, 214.9 nm for P, 285.2 nm for Mg, 181.9 nm for S, 239.6 nm for Fe, 766.5 nm for K, 257.6 nm for Mn, 223.0 nm for Cu and 568.8 nm for Na (Park, 2000).

Salts were analyzed by Corning Salt Analyzer (Marshall, 1992). During cheese ripening, cheese pH (Marshall, 1992), and the INSOL mineral contents of cheese were analyzed (juice method: Hassan et al., 2004) at 1 d, 1 wk and 1 mo.

The INSOL or colloidal form of the mineral content of cheese and cheese juice were calculated based on the methods of Morris et al. (1988).

Results and Discussion

The cheese making methods are shown in Table 1. The renneting, milling, and draining pH values were ~6.6, ~6.3, and ~5.6 for HPM, HPML and HPMW cheeses. The renneting, milling, and draining pH was ~6.5, ~6.1, and ~5.4 for LPM cheeses. The manufacturing pH value was kept constant within HPM cheeses regardless of whether there was added lactose to milk or washing (Table 1).

1. Mineral composition of cheese and cheese juice

Cheese and cheese juice had the same pH values in all cheeses (Table 2) suggesting that cheese juice is close to being in thermodynamic equilibrium with the cheese serum (Morris et al., 1988). In Table 7, the measured values were compared with published values.

The actual concentration of minerals in cheese or cheese
juice is shown in Table 3 and Table 4. The calculation of the proportion of these minerals in the colloidal forms is shown in Tables 5 and 6. The K content of cheese ranged from 34 to 139 mg/100 g of cheese. In Morris et al. (1988), the K content of 1 mo old Cheddar cheese was 96 mg/100 g of cheese. The Na content of cheese ranged from 400 to 700 mg/100 g of cheese. In Morris et al. (1988), the Na content of 1 mo old Cheddar cheese was 798 mg/100 g of cheese. In another study, the Na content of 12 mo cheese was 1,500 mg/100 g of cheese (Broome and Limsowtin, 2002). The Na content of cheese can be easily varied by changing the amount of salt added during cheese making.

In both trials, the amount of Cu, Fe and Mn content in cheese juice or cheese were very low. Broome and Limsowtin (2002) reported that the Mn content in 12 mo old cheese juice was 0.079 mg/100 g of cheese juice. A significant increase in the concentrations of was observed Ca, Mg and S in cheese juice all cheeses from both trials.

Table 5 and Table 6 shows the mineral concentration in cheese as colloidal form (as % of the total mineral concen-
**Table 3.** Composition of minerals in cheese and cheese juice (CJ) (Trial I)

| Minerals Mg/100 g in cheese or in CJ | High pH method | Low pH method | High pH method with lactose added | High pH method with washing |
|-------------------------------------|----------------|---------------|-----------------------------------|-----------------------------|
|                                     | Cheese | CJ at 1 D  | CJ at 1 wk | CJ at 1 mo | Cheese | CJ at 1 D  | CJ at 1 wk | CJ at 1 mo | Cheese | CJ at 1 D  | CJ at 1 wk | CJ at 1 mo |
| Ca                                  | 771    | (15)        | 405       | (4)        | 633       | (4)        | 672       | (13)       | 725    | (19)        | 483       | 743       | 719       | (5)        | 783    | (8)        | 444       | 631       | 604       |
| Mg                                   | 36.5   | (0.2)       | 47.8      | (2.0)      | 67.3      | (1.7)      | 75.3      | (4.1)       | 35.0   | (1.1)       | 49.3      | 69.7      | 69.9      | (1.4)      | 38.3   | (0.3)      | 57.2      | 71.4      | 65.5      |
| Na                                   | 668    | (32)        | 1,425     | (194)      | 1,447     | (166)      | 1,331     | (32)         | 426    | (13)        | 1,124     | 1,125     | 1,048     | (37)       | 590    | (10)       | 1,349     | 1,247     | 1,265     |
| P                                    | 496    | (6)         | 179       | (7)        | 142       | (3)        | 186       | (5)          | 485    | (12)        | 219       | 173       | 241       | (2)        | 507    | (5)        | 167       | 151       | 154       |
| S                                    | 11.6   | (1.8)       | 17.0      | (2.2)      | 29.1      | (4.5)      | 29.9      | (2.7)        | 10.3   | (0.4)       | 16.7      | 26.2      | 24.0      | (0.3)      | 14.6   | (1.7)       | 17.2      | 21.3      | 25.3      |

1 The number in parenthesis is the standard deviation.
2 CJ = cheese juice.

**Table 4.** Composition of minerals in cheese and cheese juice (CJ) (Trial II)

| Minerals Mg/100 g in cheese or in CJ | High pH method | Low pH method | High pH method with lactose added | High pH method with washing |
|-------------------------------------|----------------|---------------|-----------------------------------|-----------------------------|
|                                     | Cheese | CJ at 1 D  | CJ at 1 wk | CJ at 1 mo | Cheese | CJ at 1 D  | CJ at 1 wk | CJ at 1 mo | Cheese | CJ at 1 D  | CJ at 1 wk | CJ at 1 mo |
| Ca                                  | 729    | (14)        | 391       | (6)        | 658       | (15)       | 732       | (12)       | 680    | (13)        | 540       | 706       | 728       | (5)        | 728    | (9)        | 429       | 641       | 635       |
| Mg                                   | 32.5   | (3.3)       | 42.2      | (1.0)      | 62.9      | (1.3)      | 63.6      | (4.4)       | 29.0   | (1.0)       | 54.7      | 64.1      | 67.9      | (2.0)      | 30.4   | (0.6)       | 49.0      | 62.2      | 63.3      |
| Na                                   | 462    | (8)         | 1,219     | (104)      | 1,223     | (76)       | 1,076     | (75)         | 418    | (15)        | 1,160     | 1,235     | 1,186     | (36)       | 450    | (36)       | 1,390     | 1,385     | 1,126     |
| P                                    | 472    | (2)         | 176       | (2)        | 181       | (7)        | 176       | (3)          | 445    | (5)         | 203       | 176       | 211       | (2)        | 461    | (7)         | 195       | 167       | 172       |
| S                                    | 10.0   | (0.6)       | 16.3      | (0.9)      | 21.3      | (0.6)      | 25.6      | (1.5)        | 9.5    | (0.8)       | 21.3      | 25.2      | 31.4      | (0.8)      | 8.6    | (1.7)       | 17.4      | 24.9      | 24.1      |

1 The number in parenthesis is the standard deviation.
2 CJ = cheese juice.
tration of cheese). Generally LPM cheese had a significantly \( p < 0.05 \) lower total amounts of Ca, Mg, P and S compared to other cheeses (Table 3). All the other minerals did not have any specific trends among cheeses made from the different manufacturing methods. When presented as colloidal form, only Ca, Mg, P and S had significant proportions in the colloidal form; all the other minerals were mostly in the soluble form (i.e., entirely in the serum phase) at 1 d (Table 5 and Table 6). Morris et al. (1988) also reported that virtually all the lactose, galactose, Na, K and Cl in the 1 mo old cheese can be extracted into the cheese juice when the concentration of those components in cheese and cheese juice were expressed per unit weight of water.

HPMW cheese had slightly lower \( (p < 0.05) \) amounts of total Ca and Mg compared to other cheeses presumably due to curd washing. Most changes in the solubilization of Ca, Mg and S occurred during first wk of ripening in both

| Table 5. Composition of minerals in cheese as colloidal form (% of insoluble minerals as % of total minerals) (Trial I) |
|---|
| Minerals Mg/100 g in cheese or in CJ | High pH method | Low pH method | High pH method with lactose added | High pH method with washing |
|  | 1 D 1 wk 1 mo | CJ at 1 D CJ at 1 wk CJ at 1 mo | CJ at 1 D CJ at 1 wk CJ at 1 mo | CJ at 1 D CJ at 1 wk CJ at 1 mo |
| Ca (0) | 79 (0) 66 (0) 63 (0) | 74 (1) 58 (0) 57 (0) | 77 (0) 67 (0) 68 (0) | 70 (0) 62 (0) 62 (1) |
| Mg (2) | 51 (2) 26 (1) 14 (2) | 44 (2) 16 (2) 12 (2) | 39 (1) 24 (3) 25 (1) | 25 (2) 15 (2) 16 (2) |
| P (1) | 86 (1) 88 (0) 84 (0) | 82 (0) 85 (0) 79 (0) | 86 (0) 87 (0) 87 (0) | 83 (0) 86 (0) 85 (1) |
| S (7) | 44 (1) 10 (1) 3 (1) | 34 (1) 0 (0) 0 (0) | 54 (2) 42 (2) 30 (2) | 39 (3) 9 (0) 7 (0) |

1 The number in parenthesis is the standard deviation.
2 CJ = cheese juice.

| Table 6. Composition of minerals in cheese as colloidal form (% of insoluble minerals as % of total minerals) (Trial II) |
|---|
| Minerals Mg/100 g in cheese or in CJ | High pH method | Low pH method | High pH method with lactose added | High pH method with washing |
|  | 1 D 1 wk 1 mo | CJ at 1 D CJ at 1 wk CJ at 1 mo | CJ at 1 D CJ at 1 wk CJ at 1 mo | CJ at 1 D CJ at 1 wk CJ at 1 mo |
| Ca (1) | 78 (1) 61 (1) 56 (1) | 68 (1) 59 (1) 54 (1) | 77 (1) 64 (0) 64 (1) | 73 (1) 65 (0) 63 (0) |
| Mg (1) | 46 (3) 19 (1) 17 (1) | 25 (0) 9 (0) 2 (0) | 38 (4) 18 (1) 13 (4) | 38 (0) 25 (1) 21 (0) |
| P (0) | 85 (0) 84 (0) 83 (0) | 82 (0) 84 (0) 80 (0) | 84 (0) 85 (0) 85 (0) | 83 (1) 86 (0) 85 (0) |
| S (4) | 32 (1) 9 (0) 0 (0) | 17 (0) 3 (0) 0 (0) | 0 (0) 0 (0) 0 (0) | 34 (2) 22 (0) 23 (0) |

1 The number in parenthesis is the standard deviation.
2 CJ = cheese juice.
trials (Table 5 and Table 6); which agrees with other studies.

The extent of the changes in colloidal forms of Mg and S in cheese varied in cheeses from both trials (Table 5 and Table 6), indicating that the changes from INSOL to soluble form of these minerals were also dependant on cheese processing variables.

It seems that various minerals associated with colloidal calcium phosphate (CCP) (Horne, 1998) decreased gradually as CCP was solubilized, although some remained in the colloidal form even at 1 mo of ripening. Morris et al. (1988) also reported that in 1 mo Cheddar cheese, 57% of total Ca, 88% of total P, 23% of total Mg and 63% of sulphate were still in the colloidal form. Morris et al. (1988) reported that Ca, P, acid soluble P and citrate might be expected to be present in the cheese either as part of protein matrix or as crystals of Ca phosphate or Ca citrate, which might form during ripening. They also reported that Mg and citrate may be partly adsorbed to Ca phosphate crystals as exemplified by the presence of these ions in CCP of milk (Horne, 1998).

Table 7. Ranges of minerals in cheese and cheese juice (CJ) from two trials

| Concentration (mg/100 g) | Cheeses from this study | Morris et al. (1988) | Broome and Limstowtin (2002) |
|--------------------------|-------------------------|----------------------|-----------------------------|
|                          | Cheese | CJ at 1 D | CJ at 1 wk | CJ at 1 mo | Cheese | Cheese juice of 1 mo Cheddar | Cheese juice of 20 mo old Cheddar cheese |
| Ca                       | 680–783 | 391–548 | 612–743 | 604–732 | 688 | 708 | 530 |
| K                        | 34–139  | 175–353 | 131–328 | 123–353 | 96  | 244 | 190 |
| Mg                       | 29–38   | 42–67   | 58–71   | 59–75   | 31  | 59  | 45  |
| Na                       | 413–668 | 1,108–1,425 | 1,073–1,472 | 1,048–1,345 | 798 | 1895 | 1500 |
| P                        | 445–507 | 167–219 | 147–203 | 154–241 | 499 | 140 | 320 |
| S                        | 8–15    | 16–21   | 21–29   | 18–31   | Not measured/detected | Not measured/detected | Not measured/detected |

measured and it was hard to tell the proportion of P from total P as colloidal calcium phosphate. Further investigation is needed in this area.

2. Chemical composition of milk and cheese

The lactose content of milk with lactose added was significantly higher than normal milk (Table 2). The moisture content of all HPM cheeses (Table 2) was ~39%, which was significantly ($p<0.05$) higher than LPM cheese. Cheeses with low manufacturing pH values usually had lower moisture contents compared to cheese from high manufacturing pH methods. The protein contents of cheeses were not significantly different ($p>0.05$, Table 2).

The residual (1 d) lactose content of LPM (~0.3%) and HPMW (~0.2%) was significantly lower ($p>0.05$, Table 2) than the lactose content of other cheeses (0.6~0.8%). The lower level of lactose content in cheese with a low manufacturing pH method or curd-washing has already been reported.

The lactic acid content of cheese at 1 d ranged from 1.0 to 1.3% method (Table 2). HPMW cheese exhibited little change in lactic acid (Table 2) during ripening due to the curd-washing. There have been other studies that have reported that the lactic acid content in cheese during ripening can be altered depending on the initial lactose content in cheese (if the lactose is fermented to lactic acid) (Huffman and Kristoffersen, 1984; Shakeel-Ur-Rehman et al., 2004).
3. Cheese pH

The pH of all cheeses at 1 d was significantly higher (p<0.05) compared to the cheese pH values at 1 mo (Table 2) except HPMW cheeses. There was no significant change in HPMW cheese pH (Table 2). It is very common to have very small decrease in cheese pH when curd-washing is adopted.

An increase or little change in cheese pH during ripening in cheeses with curd-washing or whey dilution has been reported in other studies (Huffman and Kristoffersen, 1984; Shakeel-Ur-Rehman et al., 2004).

4. Ca equilibrium in cheese

Total Ca content of cheese ranged from 704 to 756 mg/100 g of cheese (Table 2). The total Ca content of HPMW cheese was significantly lower (p<0.05) compared to other HPM cheeses (Table 2). This is very typical for the cheese with washing step (Johnson and Lucey, 2006). During the washing step, soluble Ca was washed out resulting in lower Ca content compared to cheese made without a washing step (Table 2).

There was decrease in INSOL Ca content of all cheese during ripening (Table 2). A significant decrease in INSOL Ca during ripening has been reported in other studies (Hassan et al., 2004; Lucey et al., 2005).

The initial (1 d) INSOL Ca content was significantly lower (p<0.05) in LPM cheeses compared to other cheeses (Table 2). Lower manufacturing cheese pH values resulted in not only lower total Ca contents but also lower INSOL Ca content of cheese (Table 2). The extent of the decrease in the INSOL Ca content of HPMW cheese was smaller than the other cheese types (Table 2). This was presumably due to the lower formation of lactic acid in this cheese compared to all the other cheeses (Table 2) due to the washing step.

Conclusions

Different cheese manufacturing methods resulted in different levels of residual lactose, lactic acid, INSOL Ca and minerals in cheese and cheese juice. Cheese juice contained various minerals. The total Ca, P, Mg and S content was significantly lower in LPM cheeses compared to other cheeses. Lower proportions of colloidal Ca and Mg at 1 d were observed in HPMW cheeses presumably due to washing removing some of these components. Minerals which were not associated with CCP, such as Na and K, were almost completely in the serum phase of cheese at 1 d. Those minerals which were part of colloidal calcium phosphate were largely retained as a structural component; these minerals exhibited gradual decrease in colloidal form during 1 mo ripening but some amount were still in the colloidal form at 1 mo.

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