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The Role of Ca Equilibrium on the Functional Properties of Cheese: A Review

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Abstract

The functional properties of cheese, such as texture and melt, are known to be controlled by several important parameters. Recently, the characteristics of Ca in cheeses, especially the form of Ca (insoluble (INSOL) or soluble) and the shift in Ca equilibrium (i.e., from INSOL to soluble Ca), during aging has received a lot of attention. The INSOL form of Ca, which is present as a structural form in casein, plays a critical role in determining the functional properties of cheese during the early period of ripening (~1 mon). It seems that there is always a reduction of INSOL Ca content in cheese during ripening and there are also factors that can affect the shift in Ca equilibrium. These factors may include the composition of cheese milk, cheese manufacturing pH, acid development during aging, adopting curd-washing in various methods, pre-acidification of milk, etc. There have been many studies showing that the rheological and melting properties of cheese during ripening were significantly ($p<0.05$) affected by the shift in Ca equilibrium. Therefore, for cheese makers, it is now possible to predict/ manage the functional properties of cheese by monitoring and controlling Ca equilibrium in cheese during aging.

Key words: Ca equilibrium, insoluble Ca, cheese functionality

Introduction

Cheese is now widely used in all over the worlds due to its versatile adaptability as a food ingredient. To prepare each food, different functional properties of cheese are required to obtain specific attributes of each food. For example, to get a smooth and creamy texture of tiramisu, mascarpone cheese is normally used while fresh Mozzarella cheese is commonly used for caprese salads to give a curdy texture. Therefore, understanding the functional properties of cheese, such as texture and melting behavior when heated, becomes a great importance to manipulate the proper functionality of each cheese. To accomplish this purpose, there have been tremendous studies to understand the physico-chemical properties of cheese from the manufacture to its end-use (e.g., Lucey *et al*., 2003).

During last decades, there was a remarkable progress in understanding the role of Ca in the functional properties of cheeses (Hassan *et al*., 2004; Johnson and Lucey, 2006; Lucey and Fox, 1993; Lucey *et al*., 2005). Until early 90es, it was perceived among cheese makers that most of Ca in cheese milk is dissolved from casein-structure during manufacture and that major controlling factors in the functional properties of cheeses during ripening is proteolytic activity either by enzyme or bacteria (Lawrence *et al*., 1987). Nowadays, it is believed by many cheese researchers and cheese makers that Ca in cheese also can determine the physico-chemical and functional properties of cheese as a reasonable amount of Ca turned out to be present inside of casein after cheese making. In this review, some key perspectives of Ca equilibrium in cheese and its role on the functional properties of cheeses will be discussed.

Ca in Milk and Cheese

The total Ca concentration in milk is ~26-32 mM, which is approximately 117 mg/100 g of milk (Schmidt, 1980; Walstra, 1990). The concentration of Ca and other minerals in milk can vary according to the stage of lactation, nutritional status of the cows and environmental and genetic factors (Roginski *et al*., 2003). Mean concentrations of the major minerals in cows' milk are shown in Table 1. It is estimated that around 32% of total Ca is present in serum phase as soluble complexes (10 mM)

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Table 1. Mean concentrations of the main macrominerals in cows' milk (Walstra *et al*., 1999)

Minerals	Content (mg/100g of milk)
Sodium	53
Potassium	136
Chloride	97
Calcium	112
Phosphorus	89
Magnesium	11

and free ions (3 mM). The remainders of Ca is associated with casein micelles (i.e., exists as structural form) and called colloidal calcium phosphate (CCP) or insoluble (INSOL) Ca. The total calcium content of cheese varieties varies mostly due to differences in the manufacturing pH values, especially pH at whey draining (Lee *et al*., 2005; Lucey and Fox, 1993; Yun *et al*., 1995). Table 2 shows Ca content in various cheeses. The high calcium content of Emmental cheese is obtained by having high (~6.0) draining pH while low calcium content of cottage cheese is mainly due to low (~5.2) draining pH.

Colloidal Calcium Phosphate (CCP)

CCP is also known as nanocluster and the exact structural form of CCP is still controversial. It is believed that the molar ratio of Ca bound to inorganic phosphate (Ca/P) is ~1.5-1.6 (Pyne and McGann, 1960; Walstra and Jenness, 1984). It has been reported that CCP in milk has form of $3Ca_3(PO_4)$•CaH citrate; $Ca_9(PO_4)_6$; CaHPO•$2H_2O$, which is acidic, amorphous, and reminiscent of brushite (Holt *et al*., 2003; Schmidt, 1980). The total amount of CCP is about 7 g/100 g dry casein (Walstra *et al*., 1999). CCP is one of the minor constituents of the casein micelles (Fox and McSweeney, 1998) but CCP is one of the principal structural elements (Horne, 1998). CCP acts as a neutralizing or cross linking agent in casein micelles, i.e., it helps in maintaining the integrity of casein micelles. CCP is being positively charged (Schmidt, 1980) and binds to negatively charged phosphoserine clusters. This leads to reduce the charges of protein to the level to make the attractive interactions dominated between hydrophobic regions of casein (Horne, 1998). Dissolving CCP by decreasing milk pH (completed by pH ~5.0) disrupts the stability of casein micelles (Dalgleish and Law, 1989; Lucey *et al*., 1997; Pyne and McGann, 1960).

Table 2. Calcium content in various cheese (Kosikowski and Mistry, 1997)

Cheese variety	Content (mg/100g of milk)
Cottage	80
Camembert	400
Cheshire	550
Edam	750
Cheddar	800
Gouda	820
Emmental	920

The form of Ca in cheese and changes in Ca equilibrium during cheese ripening

Cheese making is a dehydration process of milk in which casein and fat are concentrated about 10 folds (for Cheddar cheese) (Lucey *et al*., 2003). During cheese making, most of serum Ca is lost during draining and casein-bound Ca is dissolved when the draining pH is lowered (Lucey and Fox, 1993). Until recently, it was believed that the CCP in milk was completely solubilized during manufacture of cheese and that Ca in cheese was only present in serum phase as most of INSOL Ca in milk is dissolved with pH <5.2 (Choi *et al*., 2007; Pyne and McGann, 1960). The early attempts to quantify soluble Ca (that fraction that is in serum phase) and INSOL Ca (that fraction that is attached to casein) in cheeses were performed by Lucey and Fox (1993) and Morris *et al*. (1988). Both studies suggested that there were a large amount of INSOL Ca still remained after cheese making. Morris *et al*. (1988) reported that ~56% of total Ca in cheese was present as colloidal forms in 1 mon Cheddar cheese in the form of Ca associated with para-casein or in the form of Ca phosphate crystals. Lucey and Fox (1993) reported that both cheese pH and the amount of CCP remained after cheese manufacture had important effects on the cheese texture. Lucey and Fox (1993) also reported that the buffering capacity of cheese was primarily determined by the amount of CCP remained after cheese manufacture. Therefore, cheese manufacturing conditions, such as the rate of acid development and draining pH, are important in determining the buffering capacity of cheese since the concentration of CCP is altered by these manufacturing conditions. However, changes in this INSOL Ca during cheese ripening and their effects on the functional properties of cheese were not investigated extensively until recently.

Hassan *et al*. (2004) developed two robust methods to quantify the INSOL and soluble Ca content in Cheddar cheese during ripening. It is now possible to monitor the proportion of INSOL and soluble Ca as a percent of the total Ca content of cheese during ripening using either of these two methods. One approach is called cheese juice method (Hassan *et al*., 2004; Lee *et al*., 2005; Lee, 2007;

Lucey and Fox, 1993; Monib, 1962; Morris *et al*., 1988). In cheese juice method the serum phase of cheese is extracted by hydraulic pressure and this extracted juice was used for Ca measurement. Another method is the acid-base titration method, which measures the buffering capacity contributed by residual CCP using acid-base titration and this buffering capacity is used to quantify the amount of Ca associated with casein micelles (Hassan *et al*., 2004; Lucey *et al*., 2005; O'Mahony *et al*., 2005). By using these two methods it has been demonstrated that there are significant changes/reduction in INSOL Ca content of Cheddar cheese during ripening and that the proportion of INSOL Ca in Cheddar cheeses decreased from ~70 to 56% during the first 3 mon of ripening (Hassan *et al*., 2004; Lucey *et al*., 2005). It was also observed that most of the decrease in the INSOL Ca content of cheese occurred during the first month and after this period no large changes were observed (Hassan *et al*., 2004; Lee *et al*., 2005; Lee, 2007; Lucey *et al*., 2005) (Fig. 1).

The impact of some manufacturing variables, i.e., different pH values during manufacture, altered lactose content of cheese milk, washing and different ways of curd washing was also investigated. Cheddar cheese with low manufacturing (renneting and draining) pH and with two different lactose contents (4.5 and 5%) in cheese milk was made to find out the changes in Ca equilibrium during ripening. The reduction of INSOL Ca content of cheese was accelerated with elevated lactic acid level (due to low manufacturing pH) during ripening (Lee *et al*., 2005). Surprisingly, there was a still significant amount of INSOL Ca content of cheese (more than 40% as a % of total Ca content of cheese) remained after 3 mo ripening even in cheeses with pH 4.7 (Lee *et al*., 2005). This indicates the attainment of "pseudo-equilibrium" in cheese during ripening.

O'Mahony *et al*. (2006) reported that precipitation occurred when the concentration of Ca in a synthetic

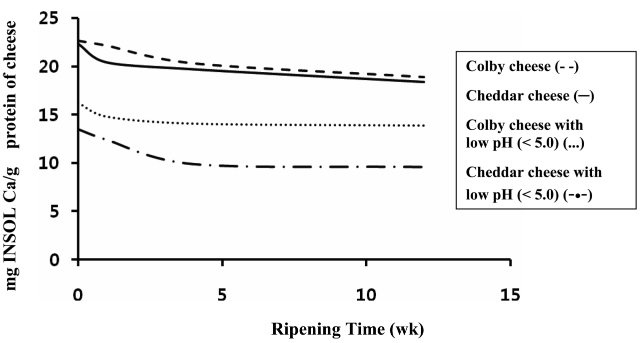

Fig. 1. Changes in INSOL Ca content of various cheeses during ripening (approximation only).

Cheddar cheese aqueous phase solution exceeded 900 mg /100 g (they made this solution targeting 4 mon old cheese juice). It has been suggested that establishment of a pseudo-equilibrium between INSOL and soluble forms of Ca in the cheese matrix is a driving force contributing to the solubilization of CCP in cheese during ripening (Hassan *et al*., 2004; Lucey *et al*., 2005; O'Mahony *et al*., 2006). The observed increase in solubility of Ca and precipitation of Ca in cheese juice may reflect this attainment of a new stable pseudo-equilibrium between soluble and INSOL Ca in cheese (Hassan *et al*., 2004; Lee *et al*., 2005; O'Mahony *et al*., 2006). It is also possible that there are other limiting factors that influence the solubility of Ca phosphate in cheese, such as pH, interactions with other ions, and high ionic strength.

Lee (2007) investigated the changes in INSOL Ca content of Colby (washed curd) cheese during ripening; there was also a significant reduction of INSOL Ca content of cheese during the first mon of ripening. With curd-washing, it was possible to prevent extreme drop of cheese pH during ripening especially for the cheese with elevated lactose content of cheese milk or with low manufacturing pH. Changes in INSOL Ca content of Colby cheese were also significantly ($p<0.05$) affected by the different ways of curd-washing, such as batch and continuous washing (Lee, 2007). It was also observed that there was a reduction of INSOL Ca content of cheese during ripening even if there was an increase in cheese pH, which indicates that a decrease in cheese pH is not necessary to have a shift of INSOL to soluble form of Ca (Lee, 2007).

The Role of Ca in cheese on textural and melting properties of cheese

During ripening texture of cheese generally changes from curdy (short) to smooth and soft structure (Lawrence *et al*., 1987). Also more flow and higher meltability of cheese during heating are commonly observed with aging (Lucey *et al*., 2005). For a long time it was believed that the proteolysis (either by enzymatic or by bacterial activity) occurred during ripening is the major contributing factor in textural changes of cheese during ripening (Lawrence *et al*., 1987). It was also generally recognized that lower total Ca content of cheese gives softer and more meltable cheeses (Lucey and Fox, 1993). But recently, it seemed that the textural changes of cheese during early period of ripening (~1 mon) are more correlated with the reduction of INSOL Ca content than with proteolysis (Lucey *et al*., 2005; O'Mahony *et al*., 2006). Also there were differences in melting behavior of cheese even with similar total Ca content of cheese indicating that total Ca is not a precise predictor of textural and melting properties of cheese during aging (Lee, 2007).

The rheological properties of Cheddar cheese during heating (represented by storage modulus and maximum loss tangent) were more highly correlated with the INSOL Ca content of cheese than with the level of 4.6-soluble nitrogen which represent the extend of primary proteolysis during 9 mon aging (Lucey *et al*., 2005). There was a significant (p<0.05) reduction in hardness of Cheddar cheese in large deformation test (Texture Profile Analyzer) during first 21 d of ripening when hydrolysis of a_{s1}-casein at Phe_{23}-Phe_{24} was completely blocked or inhibited at all stage of ripening (180 d) by the addition of pepstatin (a potent inhibitor of chymosin) with a concentration of 10 μmol/L of cheese milk during cheese making (O'Mahony *et al*., 2005).

It seems that lower INSOL Ca content of cheese gives more meltable and softer cheese (Choi *et al*., 2007; Hassan *et al*., 2004; Lee, 2007; Lucey and Fox, 1993; Lucey *et al*., 2005; O'Mahony *et al*., 2005 and 2006) (Fig. 2). O'Mahony *et al*. (2006) varied the concentration of INSOL Ca in cheese from 1.36 to 2.36 g Ca/100 g of protein in cheese by incubating 4 mon old Cheddar cheese slices in synthetic Cheddar cheese aqueous phase solutions containing various levels of Ca. With a significant increase in INSOL Ca content of cheese the LT_{max} value (meltability index) decreased significantly (p<0.05) from 0.7 to 1.7 and the force to compress the cheese by 5, 20 and 75% of its original height increased significantly (p<0.05) in large deformation test (O'Mahony *et al*., 2005).

Choi *et al*. (2007) reported that INSOL Ca content of cheese was varied either pre-acidification of cheese milk with lactic acid or chelating Ca in cheese milk with ethylenediamine tetraacetic acid (EDTA). The INSOL Ca content of cheese was varied from 337 to 823 mg/100 g of cheese with pre-acidification of cheese milk and from 375 to 511 mg/100 g of cheese with the addition of EDTA to cheese milk, respectively. As INSOL Ca content of cheese decreased in both EDTA and pre-acidification treatment, the LT_{max} value (melatability index) was increased from 1.52 to 4.93 and 1.45 to 3.24, respectively. This result indicates that cheese has more flexible (less cross-linking) casein network with less amount of INSOL Ca.

In Lee (2007), the total Ca content of 4 types of Colby cheese from different curd-washing method was not significantly (p<0.05) different (~700 mg/100 g of cheese). For the first 2 wk, the INSOL Ca content of cheese, however, was varied from 18 to 23 mg INSOL Ca per g protein in cheese resulting in significantly (p<0.05) higher LT_{max} value for the cheese with lower INSOL Ca content (ranging from 0.82 to 2.13). This result shows that the amount of INSOL Ca in cheese can be the determining factors in melting behavior of cheese when total Ca content, pH, cheese moisture, and the level of 4.6 soluble nitrogen of cheese are not significantly (p<0.05) different (Lee, 2007).

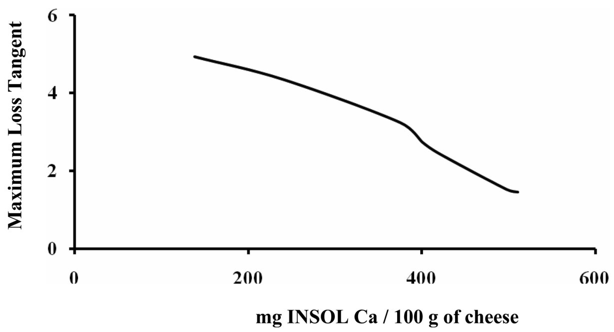

Fig. 2. Maximum loss tangent value (meltability index) during heating with various amount of INSOL Ca content of cheese (approximation only).

Conclusion

The scientific and precise ways to monitor the quantity of INSOL Ca content of cheese, such as cheese juice and titration methods during aging were developed during last two decades. It seems that the solubility of INSOL Ca in milk and in cheese is different due to the differences in the amount of serum phase present. It was found that INSOL Ca content of cheese is a crucial factor to affect cheese functionality (such as texture and melting) during early period of ripening. It was also found that shift in Ca equilibrium in cheese i.e., shift from INSOL to soluble form of Ca, can be controlled or affected by the cheese manufacturing variables, such as lactose content of cheese milk, acid development during cheese making, curd-washing and curd-washing methods as well as the level of lactic acid at post manufacture. It is now possible for cheese-makers and cheese researchers to check the INSOL Ca content of cheese using newly developed method and use that information as one of the key parameters to predict the functional properties of cheeses.

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