EFFECTS OF SODIUM CHLORIDE SALTING AND SUBSTITUTION WITH POTASSIUM CHLORIDE ON WHEY EXPULSION OF CHEESE

by

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ABSTRACT

Effects of Sodium Chloride Salting and Substitution with Potassium Chloride on Whey Expulsion of Cheese

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The rate and extent of syneresis (whey expulsion) strongly affects cheese composition and quality. During salting, curd syneresis is influenced by the combined effect of both osmotic pressure and protein hydration. Our objective is to examine how cheese composition and whey expulsion are influenced by dry salting curd at various intervals, levels, applications, and potassium chloride (KCl) substitution, or change in calcium or sodium level in test solution (i.e., whey-brine).

Four sets of unsalted fresh Cheddar curds were salted with different methods, with at least 3 replicates of each set on separate days. Set A was salted with 30 g/kg NaCl over 3 applications, either 5 or 10 min apart. Set B was salted with 30, 25, and 20 g/kg NaCl over 3 applications 5 min apart. Set C was salted with 20 g/kg NaCl using 1, 2, or 3 applications. Set D received salt consisting of a 2:1 molar ratio of NaCl and KCl over 3 applications 5 min apart. Whey was collected every 5 or 10 min until 30 or 40 min after the start of salting and subsequently pressed for 3 h. Using 10-min intervals delayed whey syneresis but after pressing there was no significant influence on final cheese composition. Decreasing salt levels significantly reduced the amount of whey expelled prior to pressing and resulted in cheeses with higher moisture and slightly lower pH. Adding salt over different applications did not significantly affect cheese composition. Partial substitution with KCl did not affect the amount of whey expelled or cheese moisture composition.

Salted milled Cheddar cheese curd was immersed at 22°C for 6 or 18 h in test solution, with the addition of 1, 5, 10, or 20 g/L calcium, and 15 g/L salt. After immersion, curd weight change, moisture, pH, sodium, serum calcium and total calcium levels were measured. When calcium levels in solution increased, curd moisture, pH, and weight gain decreased while serum and total calcium levels increased significantly. Similarly, unsalted milled Cheddar cheese curds were immersed at 22°C for 6 h in test solution with 30, 60, 90, or 120 g/L salt in addition to 6 g/L calcium. The salt level in solution was inversely proportional with weight change, moisture, and salt level of curd.

(78 pages)

PUBLIC ABSTRACT

Ying Lu

Heart disease and stroke are leading causes of death in America. In 2008, not only are more than 0.6 million people dying of heart disease, but it is estimated that almost 1 in every 4 Americans with heart disease die. Consuming excess salt has been associated with increasing risk of high blood pressure, heart attacks, and stroke. Americans consume 40% more salt than the USDA recommended level -- 1500 mg. Therefore, it is important to reduce the amount of salt in foods such as cheese to reduce salt consumption that may lead to heart disease.

However, simply reducing salt level in cheese produces many defects in cheese for flavor, texture, and shelf life; for example, reduced sodium cheese is very soft, bitter, less salty, and has a strong off-flavor and shorter shelf life. Potassium chloride (KCl) has a similar structure and salty flavor as sodium chloride (salt) and partial replacement of salt with potassium chloride can be used to lower salt content in foods. Moreover, potassium can lower the blood pressure and risk of heart disease or stroke. Thus, potassium chloride is a potential substitution for salt in cheese.

Our study aimed to investigate effects of different salting levels, rates, and substitution with KCl on cheese composition and whey expulsion. Whey expulsion is the process of water coming out from cheese. It influences cheese texture and composition by affecting the amount of water in cheese. We found that 1) decreasing salt levels caused less whey expulsion and increase cheese moisture, 2) applying salt at a slower rate delayed the whey expulsion, and 3) partial replacement of salt with KCl did not change whey expulsion, moisture, or pH of cheese.

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Ying Lu

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LIST OF ABBREVIATIONS

S/M = Salt in Moisture

Na/M = Na in Moisture

Insol Ca/Solids = the ratio between insoluble Ca and solids in cheese

AWC = Adjusted Weight Change

LITERATURE REVIEW

2010 Dietary Guidelines

The 2005 Dietary Guidelines for Americans (USDA/USDHHS, 2005) suggested a daily sodium consumption of 2,300 mg (USDA/USDHHS, 2005). However, the newly released 2010 Dietary Guidelines (USDA/USDHHS, 2010) recommended not only reducing the sodium intake to less than 2,300 mg per day, but also further diminishing it to 1,500 mg among people who are 51 or older, African Americans, patients of hypertension, diabetes, stroke or chronic kidney disease. More than two thirds of United States (U.S.) adults belong to the latter group (CDC, 2009; Labarthe, 2009).

Cheese and Diet

Cheese is a fermented dairy product that contributes many nutrients to the American diet, including 9% of protein, 11% of phosphorus, and 27% of calcium (Johnson et al., 2009). According to the 2010 Dietary Guidelines for Americans (USDA/USDHHS, 2010), the consumption of milk and milk related products can improve bone health, especially for children and adolescents. It was also reported that cheese intake was associated with a lower risk of cardiovascular diseases (Wang et al., 2008), type 2 diabetes (Choi et al., 2005), and lower blood pressure in adults (Wang et al., 2008). In addition, there is the potential for cheese to be fortified with additional nutrients, such as the cheese supplemented with probiotics (Buriti et al., 2005; Pereira et al., 2010), vitamin D (Banville et al., 2000; Wagner et al., 2008), omega-3 fatty acids (Murphy et al., 2007), and antioxidants (Hala et al., 2010).

Excess sodium intake has been linked to an increasing risk of high blood pressure, cardiovascular disease, and stroke, of which the latter 2 are the leading causes of death in the U.S. (Appel et al., 2003; Cook et al., 2007; Forshee, 2008; Appel et al., 2011). High sodium consumption is also associated with increased urinary calcium excretion, a higher risk of osteoporosis (Heaney, 1993, 2006), and occurrence of kidney stones (Massey, 1995, 2005).

Americans of age 2 and over have an average daily sodium consumption of 3,330 mg (USDA, 2010), which is 40% more than the 2010 Dietary Guidelines' recommendation — 2,300 mg (USDA/USDHHS, 2010). In the U.S., about 77% of sodium comes from packaged food, of which Cheese is the second largest source (Jacobson, 2005). The regular Cheddar cheese contains ~6200 mg/kg of salt, which in a 50-g serving size contributes almost 13.5% of daily recommendation (FDA-DHHS, 2010; USDA/USDHHS, 2010).

Role of Salt in Cheese Manufacture

Salting is an important step in cheese manufacture. There are 3 salting methods that are commonly used. Dry salt can be mixed in with the curd prior to pressing (i.e. Cheddar cheese), rubbed onto the surface of a cheese block, or immersion of cheese block in salt brine (Guinee, 2004; Bintsis, 2007).

Salt is added to cheese for several purposes: controlling microbial growth and enzyme activities, promoting curd syneresis (whey expulsion), modifying flavor, texture and other physical properties (Guinee, 2004; Johnson et al., 2009). In general, bacterial and enzyme activities decrease as salt concentration increases (Guinee, 2004). Salt to moisture (**S/M**) level is significantly correlated with cheese quality (Guinee, 2004;

Guinee and Fox, 2004; Lawrence et al., 2004). For Cheddar cheese, S/M levels of 4.5% to 5.5% have been reported to produce the best quality cheese (Guinee, 2004). The high ratio of S/M reduces the bacterial growth and enzyme activity. Therefore it can decrease the rate of pH dropping and proteolysis during cheese ripening, and slow flavor development (Guinee, 2004; Lawrence et al., 2004). At low level of S/M, there is a high rate of microbial growth, proteolysis, leading to the incidence of bitterness and off-flavors (Guinee, 2004; Lawrence et al., 2004; Upreti and Metzger, 2007).

Reduced Sodium Cheese

Unsalted Cheddar cheese curd has a sodium level of ~240 mg/kg while the typical salted Cheddar cheese contains ~6,200 mg/kg (Johnson et al., 2009). In the U.S., reduced sodium cheese should have at least a 25% reduction of sodium, which is equivalent to a sodium level of ~4,650 mg/kg. Low sodium cheese can have no more than 140 mg sodium per 50 g cheese, which corresponds to 2,800 mg/kg (FDA-DHHS, 2010).

Cheeses salted at different levels have been evaluated (Lindsay et al., 1982). The unsalted Cheddar cheese aged over 60 days was unacceptable to most consumers due to its very soft body, high bitterness, low acidity, low Cheddar cheese flavor, low saltiness, remarkable off-flavor, more rapid aging and a shorter shelf life compared to salted Cheddar cheese. The reduced sodium cheese (containing 4,300 mg/kg) was reported as flat, bland and acidic, while the intermediate sodium cheese (containing 5,300 mg/kg) was considered to have a fuller and better flavor.

Schroeder et al. (1988) assessed Cheddar cheeses with sodium levels from 275 to 5,667 mg/kg. These cheeses were salted at different rates, aged and evaluated over 7 mo. Cheeses containing sodium of more than 4,408 mg/kg received significantly higher scores for flavor than cheeses with less sodium. However, this was not attributable to salt content alone as the cheeses with lower sodium also had higher acidity and moisture, presumably because of the impact of salt on whey syneresis during processing. In general, there is a lower Cheddar intensity, more pronouncing bitterness and more disagreeable aftertaste associated with the low sodium cheeses.

In a more recent study of consumer perception regarding salt reduction (Drake et al., 2011), sodium reductions of 8% in cottage cheese (3663 mg/kg), 14% in cheese sauce (6375 mg/L), and 15% in milk-based soup (11057 mg/kg) were noticeable and correctly identifiable. The acceptance scores of cottage cheese significantly decreased with 12% sodium reduction (3504 mg/kg). Customers are used to the saltiness of cheese, so large reductions of sodium may lose the customers' loyalty. In addition, simply reducing sodium in cheese causes defects such as higher rates of proteolysis, water activity, acidity, bitterness as well as decreasing firmness and saltiness (Lindsay et al., 1982; Fitzgerald and Buckley, 1985; Ayyash and Shah, 2010; Ayyash et al., 2011; Drake et al., 2011; Gomes et al., 2011).

Instead of adding less salt, sodium content in cheese can also be reduced by substituting salt (i.e., NaCl) with KCl or other minerals (Johnson et al., 2009). The most commonly investigated sodium replacer is KCl, which has the most similar chemical structure to sodium chloride (Johnson et al., 2009).

Potassium is considered as one of the concern nutrients for Americans, especially for African Americans and individuals with hypertension (USDA/USDHHS, 2010). Typical American diets usually reach only 56% of the potassium adequate intake for adults (4700 mg per day) (USDA, 2008). Randomized trials have shown that increasing potassium intake in the diets is linked with lower cardiovascular mortality (He and MacGregor, 2008; He et al., 2010), lower blood pressure (He et al., 2005), and is likely to prevent or at least slow down the progression of renal disease (He and MacGregor, 2008). In addition, potassium may promote renal calcium retention, resulting in a more positive calcium equilibrium (Lemann et al., 1993; Zhu et al., 2009). So overall, potassium contributes to greater bone mineral density, especially for elder people (Tucker et al., 1999; Whiting et al., 2002).

Partial replacement of NaCl with KCl appears feasible for producing cheeses without significant change of quality. Katsiari et al. (1998) made Kefalograviera cheese with 50% replacement of NaCl by KCl. This cheese was not significantly different from the normal salted cheese in composition (moisture, fat and protein), chemistry (pH and water activity) and texture. Similar observations have also been made by Fitzgerald and Buckley (1985) in Cheddar cheese, Katsiari et al. (2000a, b) in Feta cheese, Karagözlü et al. (2008) in white pickled cheese, and Dorosti et al. (2010) in Iranian white cheese. However, cheeses containing KCl have been reported as having more lipase activity and proteolysis, less saltiness and acidity, more bitter flavor and softer body (Lindsay et al., 1982; Fitzgerald and Buckley, 1985; Katsiari et al., 1998; Karagözlü et al., 2008; Johnson et al., 2009; Gomes et al., 2011).

Cheese Syneresis

In cheese manufacture, the process of whey being expelled out of cheese curds is called syneresis (Pearse and Mackinlay, 1989). The rate and extent of syneresis strongly affect the cheese processing method, the loss of fat and protein in whey, cheese moisture, acidification, and proteolysis, and therefore strongly influences the cheese composition and quality (Daviau et al., 2000; Dejmek and Walstra, 2004; Everard et al., 2008). There are several factors that influence cheese syneresis including physical (mechanical pressure and homogenization) and chemical factors (temperature-induced changes, pH and ionic strength).

Usually, mechanical pressure (such as cutting, stirring and cheddaring) promotes curd syneresis (Lodaite et al., 2000; Dejmek and Walstra, 2004; Geng et al., 2011). Small curd sizes usually result in a high syneresis rate (Grundelius et al., 2000; Dejmek and Walstra, 2004) although it has been reported that the initial syneresis rate increases proportionally to curd thickness (Lodaite et al., 2000). Homogenization and recombination of milk hinder the syneresis process (Pearse and Mackinlay, 1989; Dejmek and Walstra, 2004), although the effect of homogenization on curd firmness can be reduced by the application of microfiltration (Thomann et al., 2008).

Increasing temperature within a certain range can promote curd syneresis (Pearse and Mackinlay, 1989; Van Vliet et al., 1991; Geng et al., 2011). It was observed that increasing heating temperature from 20 to 35°C promoted one-dimensional gel shrinkage (Van Vliet et al., 1991). In a more recent study, a temperature rise from 32°C to 40°C strongly stimulated curds shrinkage (Geng et al., 2011). However, higher temperature (≥ 70°C) could denature whey proteins and decrease the syneresis rate (Pearse and Mackinlay, 1989; Dejmek and Walstra, 2004).

Reducing pH can increase syneresis rate (Pearse and Mackinlay, 1989; Grundelius et al., 2000; Lodaite et al., 2000; Dejmek and Walstra, 2004; Thomann et al., 2008). This occurs because at a low pH, the net micelle charges and electrostatic repulsion are diminished, and thus, more whey is expelled as a stronger attractive forces will cause the gel to shrink (Pearse and Mackinlay, 1989; Dejmek and Walstra, 2004). It should also be noted that at the casein isoelectric pH (~pH 5), electrostatic bonds are quite strong and cause casein molecules to aggregate (Dejmek and Walstra, 2004).

Generally, salting of Cheddar cheese curd (i.e., increasing ionic strength) promotes syneresis and results in a decreased moisture level (Kindstedt et al., 1992; Pastorino et al., 2003a; Agarwal et al., 2008). Although in some cases (such as cheese with low calcium levels), it was observed that adding salt does not lead to moisture loss (Cervantes et al., 1983; Paulson et al., 1998).

When dry salt is applied to milled cheese curds, salt dissolves slowly in the moisture on curd surfaces and forms a thin highly saturated salt solution. The osmotic pressure difference between the saturated solution on curd surfaces and the serum inside curd particles is considered as the driving force of the salt diffusion. Sodium chloride ions and water molecules respond to different osmotic pressure by traveling through the serum portion inside protein matrix of curds. The salt diffusion creates a salt concentration gradient inside the curds with the highest level on surfaces and the lowest at the center of curds (Guinee, 2004; Guinee and Fox, 2004).

Syneresis is also influenced by the response of protein matrix to different salt levels. Pastorino et al. (2003a) injected a 20% (wt/wt) NaCl solution into Muenster cheese and found after 5 injections, the moisture level of cheese decreased from 41% to 38%. Scanning electron micrographs showed that 84% of the uninjected cheese matrix was occupied by protein and the rest 16% was occupied by fat/serum pockets. After 5 injections, the salt-injected cheese had a 4% increase in protein matrix (88% of cheese matrix) with 12% being occupied by fat/serum. This indicates that the protein hydration ability was increased by NaCl solution injection, although there was also a moisture loss during 40-d storage of cheese that had been injected 5 times with the NaCl solution.

The effect of $CaCl_2$ addition is still under controversy. Cheeseman (1962) reported a reduction in curd syneresis rate with 10, 50, and 100 mM $CaCl_2$ addition to fresh or reconstituted milk prior to cheese manufacture. Fagan et al. (2007) also observed that at the beginning of adding up to 18 mM $CaCl_2$ to milk, curds syneresis rate slightly decreased due to the increasing curd rigidness. In a more recent study, 1.5 mM $CaCl_2$ was added to milk and mixed well before cheese making. Compared to curds made from milk without $CaCl_2$ addition, curds make from $CaCl_2$-added milk were firmer and had a lower syneresis rate (Geng et al., 2011). However, it was also reported that in a synthetic milk solution without phosphate, the syneresis was slightly enhanced by increasing calcium addition amount from 5.4 to 11.25 mM, and then diminished significantly with further calcium addition (Aiyar and Wallace, 1970). Marshall (1982) observed that curds syneresis was stimulated by 2 mM addition of $CaCl_2$ and also 4 mM $CaCl_2$ if cutting times were reduced. Pastorino et al. (2003b) injected 40% (w/w) $CaCl_2$ solution under high pressure to Mozzarella cheese causing a drop in pH and moisture level, as well as a more compact protein matrix with larger fat/serum void space. Cheese with 5 injections reached a Ca level of 1.4% and lost about 12% moisture. This indicates that the Ca addition reduces casein hydration and promotes protein-protein interactions. The variable effect on syneresis may depend on the amount of $CaCl_2$ added (Pearse and Mackinlay, 1989), and the time after $CaCl_2$ addition at which parameters are measured (van den Bijgaart, 1988; Dejmek and Walstra, 2004).

Role of Calcium in Cheese

The concentration of calcium in milk is about 117 mg/100 g, in which about 31% is present as soluble cation in the serum, while the rest forms calcium phosphate nanoclusters and bind to the phosphoserine domains of the proteins to stabilize the casein micelle (Figure 1) (Lucey and Fox, 1993; Horne, 1998; McMahon and Oommen, 2008; Lucey and Horne, 2009). The dynamic equilibrium of soluble and insoluble calcium in cheese can be expressed as (Lucey and Horne, 2009):

$$3\ Ca^{2+} + 2\ HPO_4^{2-} \longleftrightarrow Ca_3(PO_4)_2 \downarrow + 2\ H^+$$

Curd with high Ca content has a more cross-linked and rigid protein network and a decreased hydration state of protein (Pastorino et al., 2003b; McMahon et al., 2005). Both scanning electron and transmission electron micrographs showed that nonfat Mozzarella cheese containing 0.6% Ca had more protein folds and serum pockets compared to the cheese with 0.3% Ca (McMahon et al., 2005). This indicates that calcium strengthens the protein interactions and dismisses moisture from protein matrix.

Lucey and Fox (1993) suggested that it may be the insoluble Ca amount other than the total Ca level in cheese which affects protein-protein interactions, structure and texture. They found that a dispersion of young Cheddar cheese in distilled water presented a strong buffering capacity during the titration from its initial pH (~5.2) to pH 2.0 with subsequent titration to pH 11.0. When titrated with acid, the increasing solubility of insoluble calcium in cheese generates larger amount of HPO_4^{2-}, leading to a strong buffering capacity indicated by a peak in the titration curve. Conversely, when back titrated to pH 11, the precipitation of $Ca_3(PO_4)_2$ and release of H^+ present buffering ability. Hassan et al. (2004) support this hypothesis by demonstrating a decrease of the

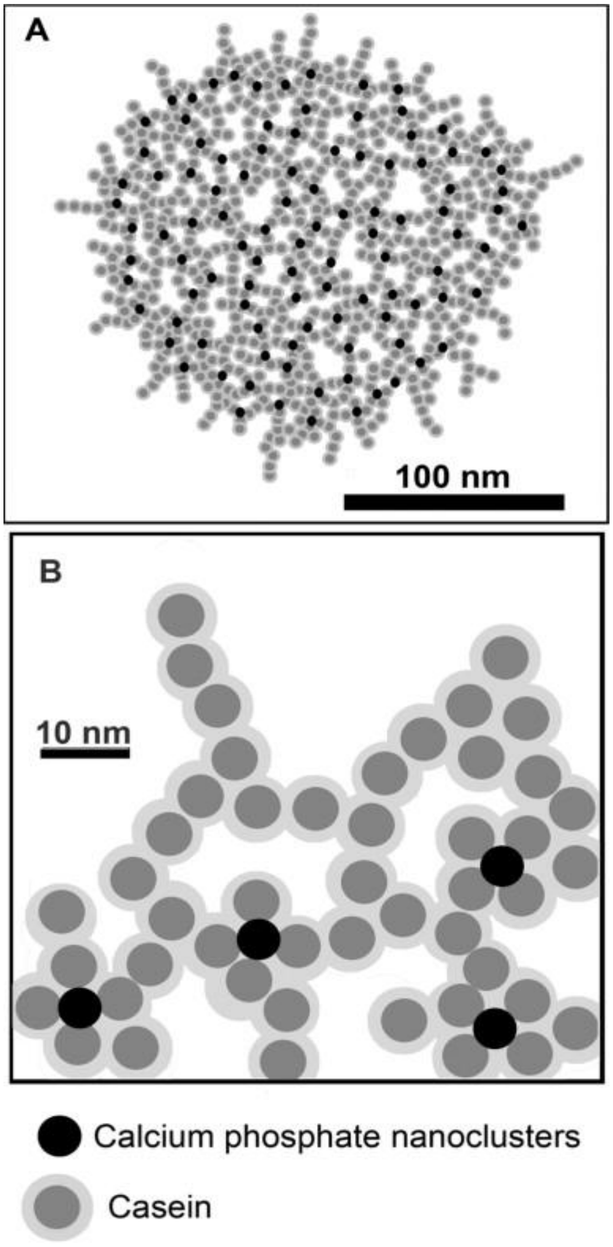

Figure 1. Schematic diagram of an interlocking lattice model of the casein micelle with casein-calcium phosphate aggregates throughout the entire supramolecule and chains of proteins extending between them. Drawn as cross-sectional scaled views of (A) the complete Schematic diagram of an interlocking lattice model of the casein micelle with casein-calcium phosphate aggregates throughout the entire supramolecule and chains of proteins extending between them. Drawn as cross-sectional scaled views of (A) the complete supramolecule, and (B) a portion of the supramolecule periphery. Calcium phosphate nanoclusters are shown with a diameter of 4.8 nm and approximately 18 nm apart, and caseins are shown with a hydrodynamic diameter of 8 nm. (Reprinted from McMahon and Oommen, 2008, Supramolecular structure of the casein micelle, J. Dairy Sci. 91:1709-1721, with permission from Elsevier.)

insoluble Ca level in Cheddar cheese from ~73% to ~58% aging from the 1st to the 4th mo, indicating the solubilization of insoluble Ca during cheese ripening. The insoluble Ca was further correlated with the texture change of cheese during early stages of ripening (Lucey et al., 2005; O'Mahony et al., 2006). This also occurs in milk, in which a decreasing insoluble Ca reduces the extent of casein micelles crosslinking and promotes swelling and hydration (McMahon et al., 2005; Choi et al., 2007; Choi et al., 2008; Lucey and Horne, 2009).

HYPOTHESES AND OBJECTIVES

The hypotheses of this study were:

1. Whey syneresis from Cheddar cheese curd is influenced by the method and amount of salt application.
2. Syneresis of sodium reduced cheese can be increased by adding other minerals.
3. Whey syneresis from Cheddar cheese curd is influenced by the amount of insoluble calcium in cheese.

The objectives of this study were:

1. To determine the effect of rate of salt application, salt levels, and 33% KCl molar substitution of NaCl on Cheddar cheese curd syneresis.
2. To determine the importance of insoluble calcium in brine on whey expulsion of immersing Cheddar cheese curd.
3. To determine the concentration of NaCl that causes a contraction of Cheddar cheese curd.

MATERIALS AND METHODS

Dry Salting of Cheddar Cheese Curd

Unsalted milled Cheddar cheese curd was purchased from the Gary Haight Richardson Dairy Products Laboratory (Utah State University, Logan, UT) and cheese curds were manufactured following the method of Rogers et al. (2010) (Appendix A). Four sets of curd were salted with different methods, with at least 3 replicates of each set on separate days.

Salting Interval. Separate 3-kg [1]aliquots of cheese curd (Set A) were salted at a total rate of 30 g/kg using NaCl dividing into 3 equal applications, spaced either 5 or 10 min apart, and whey was collected every 5 or 10 min until 30 or 40 min after salting (Table 1). Curd was placed in a container and whey was collected in small bottles (Figure 2). Curds were weighed before and after salting. Salted curds were placed in a cloth-lined

Table 1. Different salting levels, intervals, applications, and salting agents applied to four sets of unsalted milled Cheddar cheese curd at 20ºC.

Sets	Salting Levels (g/kg)	Intervals (min)	Number of Applications	Salting Agents
A	30	5 or 10	3	NaCl
B	20, 25, or 30	5	3	NaCl
C	20	5	1, 2, or 3	NaCl
D	30	5	3	NaCl or NaCl / KCl (2:1 molar ratio)

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[1] In the first of three trials, 1.5-kg aliquots were used.

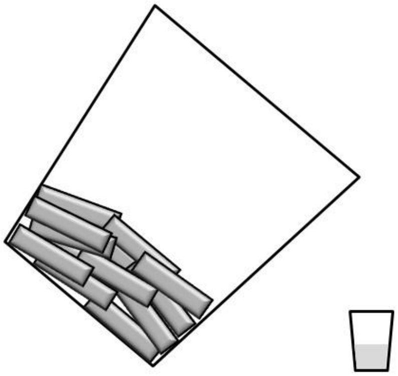

Figure 2. Schematic picture showing the collection of whey when cheese curd was dry salted in a bucket at room temperature. Cheese curd is shown in a grey rectangle and whey is shown with light grey in the small bottle on the right.

round plastic hoop, a plastic bag to collect whey placed around the hoop, then the hoops were placed in a vertical press and pressed at 103.4 kPa for 3 h at room temperature (~20ºC). The weight, pH, salt and moisture of pressed cheese were measured, as well as the weight of expressed whey.

Salting Level. Set B was salted with a total rate of 20, 25, or 30 g/kg using NaCl divided into 3 equal applications with 5 min apart (Table 1). Whey was collected and weighed every 5 min until 30 min after salting. Salted curds were then pressed into a block and whey collected as described above.

Salting Application. Set C was salted with 20 g/kg NaCl applied using 1, 2, or 3 applications (Table 1). Multiple applications were performed 5 min apart and whey was collected every 5 min until 30 min after salting. Salted curds were then pressed into a block and whey collected as described above.

Salting Substitution. Set D was salted at a rate of 30 g/kg salt using either 100% NaCl or with a 33% KCl molar substitution using 3 equal applications spaced 5 min apart(Table 1). Salted curds were then pressed into a block and whey collected as described above.

Brine Salting of Cheddar Cheese Curd

Different Calcium Levels. Salted Cheddar curds were accurately weighed to 100 ± 0.3 g and placed into a plastic container filled with 300 g of test solution. The moisture, pH, soluble Ca amount and the total Ca amount of the cheeses were measured before being placed in the containers, which were sealed and kept at 22ºC (Figure 3). After 6 or 18 h storage, the containers were opened, observations made on the condition of cheese, the cheese sample weighed, and the weight and density of test solution in the container measured. Serum Ca and total Ca amount, salt, pH and moisture of the cheese samples were measured. The procedure was outlined in Figure 4.

Different Salt Levels. Unsalted Cheddar curds were accurately weighed to 100 ± 0.07 g then immersed in 300 g test solution in a plastic container at room temperature (~22ºC) for 6 h. The pH, salt, moisture and weight change of curds were measured after the 6-h immersion. Adjusted Weight Change (**AWC**) was calculated by the weight change of curd minus salt level of cheese. The procedure was outlined in Figure 5.

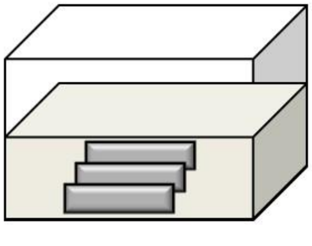

Figure 3. Schematic picture showing the immersion of cheese curd in test solution in a sealed container. Cheese curd is shown in a grey rectangle and test solution is shown with light grey.

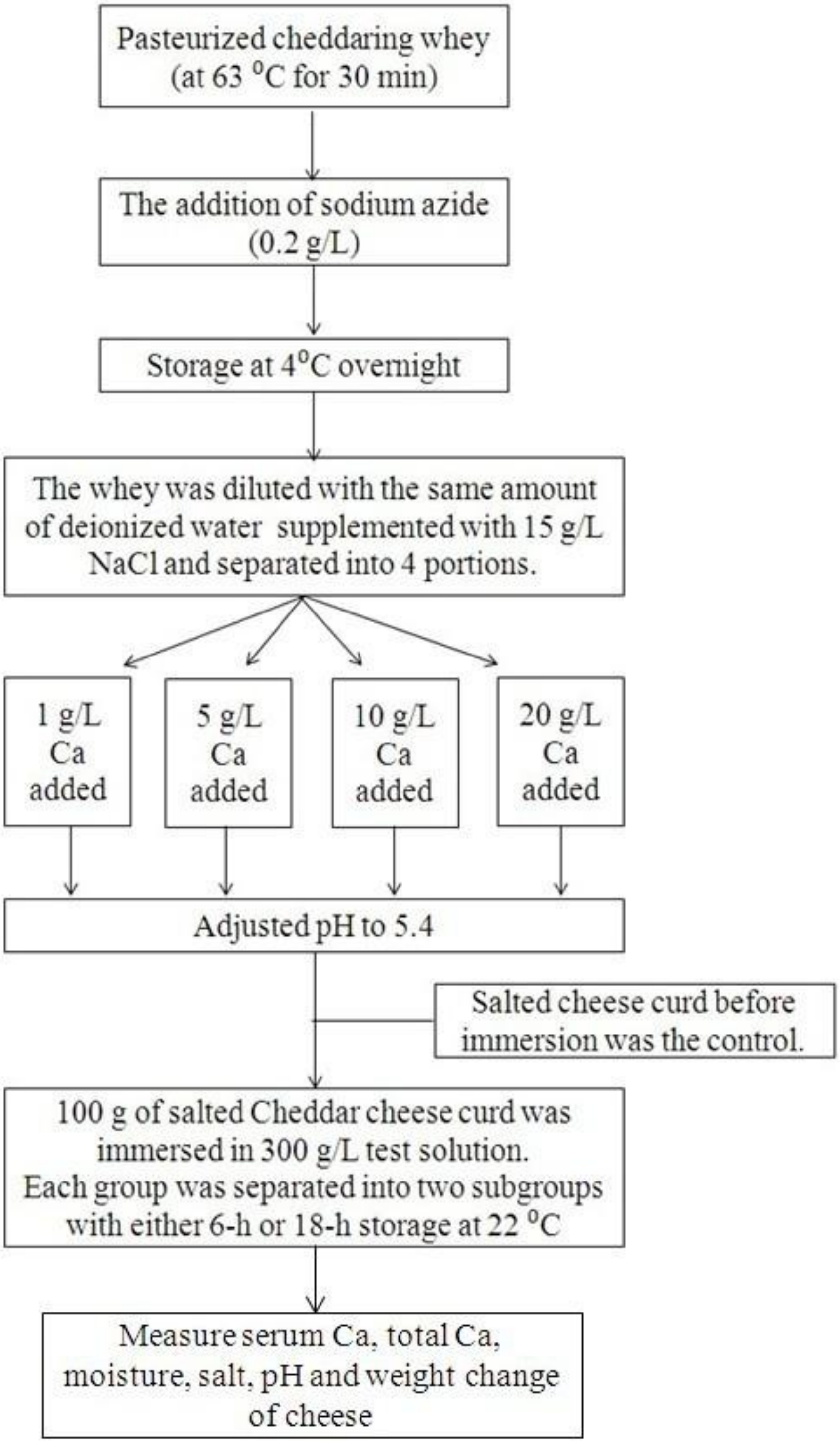

Figure 4. Flow of process steps for brine salting with different calcium levels.

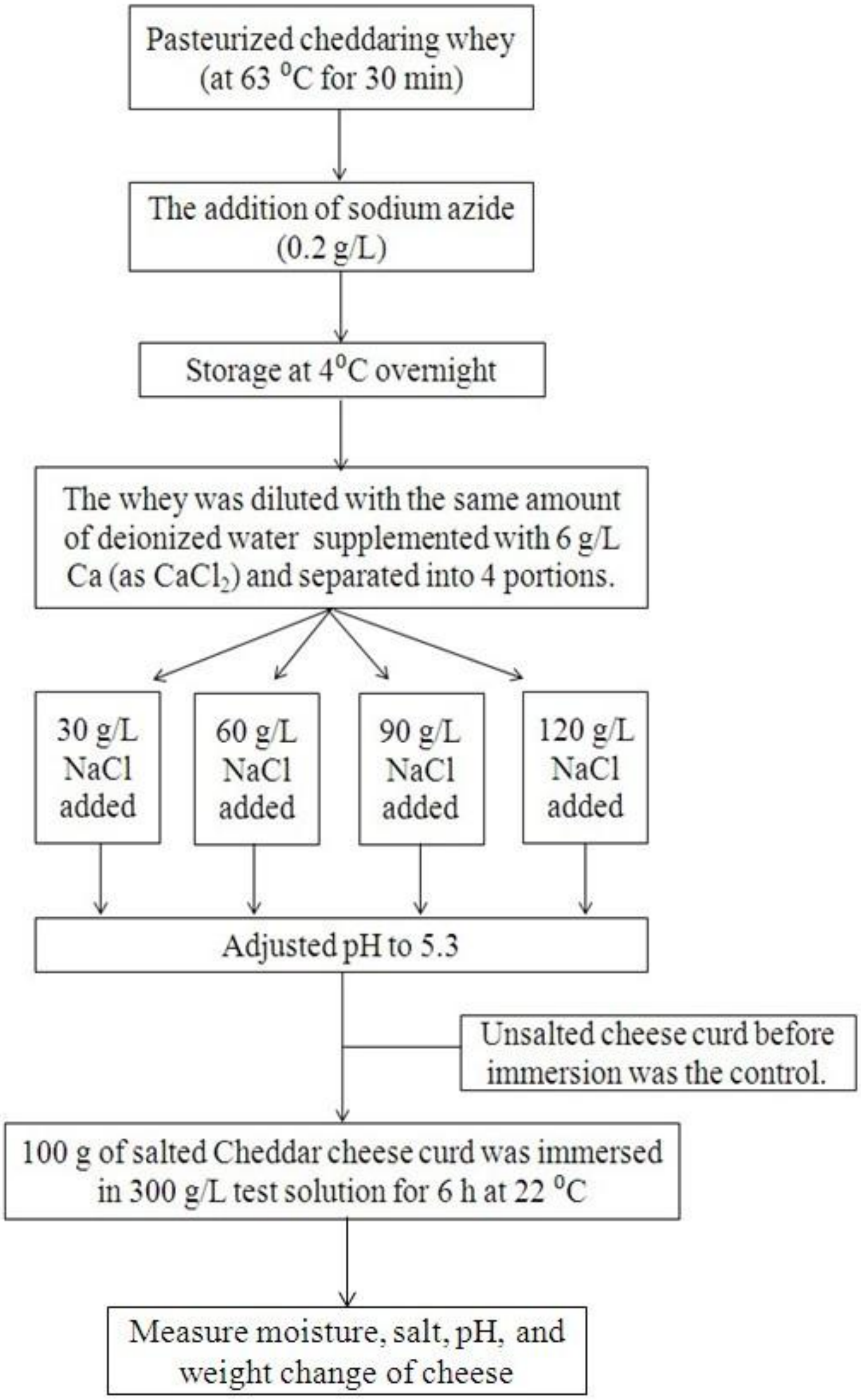

Figure 5. Flow of process steps for brine salting with different salt levels.

Cheese Manufacture and Test Solution Preparation

Unsalted and salted Cheddar cheese curds were both obtained from the Gary Haight Richardson Dairy Products Laboratory (Utah State University, Logan, UT) and cheese curds were manufactured following the method of Rogers et al. (2010) (Appendix A). The test solution was a 1:1 dilution of cheddaring whey with deionized water supplemented with different levels of NaCl and Ca (as $CaCl_2$), and adjusted to the pH of cheese curds before immersion. The cheddaring whey was collected, pasteurized at 63ºC for 30 min, added 0.2 g/L sodium azide, and stored at 4ºC overnight before the immersion. When brine salting with different calcium levels, the test solutions consisted of 15 g/L of NaCl, and 1, 5, 10, or 20 g/L of Ca, and were adjusted with sodium hydroxide to the pH of 5.4 (i.e., the pH of salted curd before immersion); while when brine salting with different salt levels, the test solutions were supplemented with 6 g/L Ca, and 30, 60, 90, or 120 g/L of NaCl, then adjusted to the pH of 5.3 (i.e., the pH of unsalted curd before immersion).

Cheese pH, Moisture and Salt Measurement

Cheese pH was measured using a plastic electrode after stomaching 20 g of grated cheese and 10 g of deionized water at 260 rpm for 1 minute in a Stomacher 400 (Seward, London, UK). Moisture was analyzed by weight loss in triplicate using a microwave oven (CEM Corp., Indian Trial, NC) (McMahon et al., 2009). In the experiment of brine-salting with different calcium levels, total salt content was determined by the mineral analysis of ash from 50 g of grated cheese at 500˚C in a muffle furnace (model 550-126, Fisher Scientific, Hanover Park, IL). In other experiments, total salt content was measured by stomaching 5 g of grated cheese with 98.2 g of deionized water, filtering the slurry through Whatman #1 filter paper, and analyzing the filtrate using a chloride analyzer (model 926; Corning Scientific, Medfield, MA). All measurements were made in triplicate.

Total and Soluble Calcium Measurement

Total Ca was determined by ashing 50 g of grated cheese at 500 ˚C in a muffle furnace (model 550-126, Fisher Scientific, Hanover Park, IL). The ash was sent to a commercial laboratory (Analab, Fulton, IL) for mineral analysis by inductively-coupled plasma spectroscopy. Soluble Ca was determined by blending 5 g of grated cheese with 50 g of deionized water in the stomacher. After sitting for 10 min, the slurry was filtered through Whatman #42 filter paper. The filtrate was sent to the Analab laboratory. Mineral concentration of test solutions before cheese immersion was also measured after filtering by Whatman #42 filter paper. Measurements of cheese sample were made in triplicate.

Statistical Analysis

A randomized block design was used to investigate the effect on cheese curd syneresis of salting time intervals, salting levels, and 33% KCl molar substitution of NaCl (Appendix B). Two-way ANOVA was used to analyze the effect of different Ca levels (1, 5, 10, or 20 g/L) in test solution and storage time (6 or 18 h) on curds (Appendix C). A completely randomized design was applied to analyze the effect of different salt levels in test solution to curds, and to compare the curd immersed in test solution with control (Appendix C).

All data were analyzed for statistical significance at the 95% level using the proc glm or proc mixed function in Statistical Analysis Software (SAS) version 9.3 (SAS Institute, Inc. Cary, NC). The data of experiments in which cheese dry salted was square-root transformed to meet the normality and homoscedasticity assumptions before significance testing. Post-hoc means comparisons were made based on p-values ($\alpha = 0.05$) using the Tukey-Kramer adjustment to obtain differences of least means squares (Appendix B).

RESULTS AND DISCUSSION

Salting Interval

Cheese curd had a typical moisture content of 420 g/kg. After salting with 5-min or 10-min intervals followed by 6-h pressing, there was no significant difference ($P >$ 0.4) between cheese composition with mean (±SD) values of moisture 362 ± 6.98 g/kg, salt 18.6 ± 1.31g/kg, S/M 48.9 ± 3.68 g/kg, and pH 5.2 ± 0.06.

When the typical level of salt was added to Cheddar cheese curd (i.e. 30 g/kg), there was very little whey expelled (~2%) prior to the second salt addition in both 5-min and 10-min intervals (Figure 6). Applying the salt at 5-min intervals caused faster syneresis than using 10-min intervals, with major syneresis occurring at 15 and 25 min

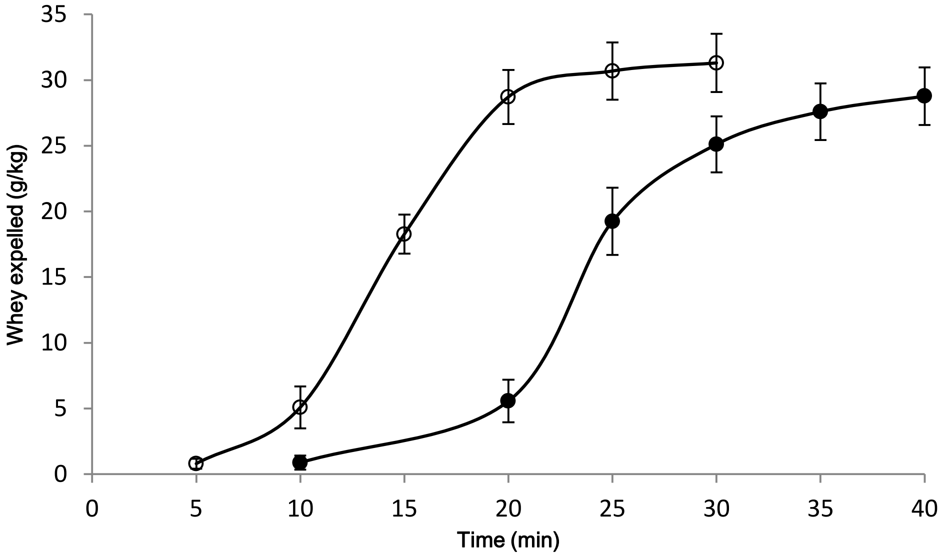

Figure 6. Whey expulsion over time from the first salt application of milled Cheddar curd with 30 g/kg salt added over 3 applications using either 5-min (○) or 10-min (●) intervals. Bars = SE.

after the start of salting, respectively. Thus, there was significantly ($P < 0.001$) different whey expulsion observed at both 20 min and 25 min when considered from the start of salting. Even when considered as the time after the third salting (e.g., 20 min after the third salting), there was still a trend ($P = 0.054$) for slightly more whey expulsion when using the 5-min intervals than the 10-min intervals.

As seen from the shape of the whey expulsion curves in Figure 6, when using a 10-min salting interval, the last portion of the whey was expelled more slowly. With the 5-min intervals, 39.3% of the whey was expelled within the first 15 min after the third salting while only 34.5% was expelled in the first 15 min after the third salting with 10-min intervals.

Curd syneresis is strongly influenced by protein matrix hydration state (Paulson et al., 1998; Pastorino et al., 2003a; Guinee, 2004, Guinee and Fox, 2004; McMahon et al., 2005; McMahon et al., 2009). When salt is applied to curd, a portion of the salt is dissolved in the moisture located on and slightly within the surface of the curd. As the salt dissolves, potentially reaching a saturated solution at the curd surface, there is movement of water based on osmotic forces, to the surface causing a dehydration of surface protein matrix (Guerts et al., 1974; Guinee, 2004, Guinee and Fox, 2004). Water inside the curd pieces continues to drive outwards in response to osmosis pressure as the sodium and chloride ions from the dissolved salt diffuse inwards. This forms a salt gradient with extremely low levels at the center of the curd pieces and gradually increasing as towards curd surfaces.

Monovalent cations such as Na have a salting-in effect at low S/M (< 5%) that causes increased protein solubility, and a salting-out effect at higher S/M resulting in decreased protein solubility, contraction, and dehydration of the protein matrix (Paulson et al., 1998; Guinee and Fox, 2004; McMahon et al., 2005). The low S/M portion of the curd interior (i.e., volume A as shown in Figure 7) would have a tendency to expand and hold water, while the surface area (volume B in Figure 7) would contract and expel water as described by Fucà et al. (2012). At the beginning of salting, the ratio between the exterior (B) and interior (A) curd portions (B/A) is relatively small, therefore the curd particles will retain moisture with very little whey expulsion occurring. Then as salting continues through the third application, more salt diffuses into the curd particles, increasing both the S/M concentration and the distance of salt diffusion into the curd, so B/A increases and more whey is expelled from the curd.

Salting using 5-min intervals apparently causes a faster increase in salt concentration and diffusion through the curd particle exterior portion, so that the B/A ratio increases more rapidly resulting in faster whey expulsion. By 20 min after the third salt application (i.e., 30 or 40 min after commencement of salting for 5 and 10-min salting intervals, respectively), the amount of whey being expelled reaches a plateau. This suggests that the B/A ratio reached a constant level that would depend upon the salting levels used. The combined driving forces of osmotic movement of water molecules in

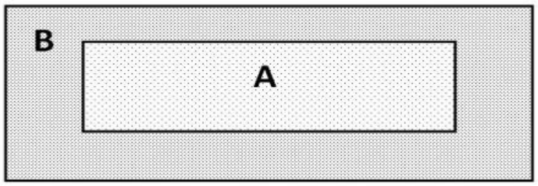

Figure 7. Schematic cross section of a curd particle showing (A) the central interior portion with low salt-in-moisture (S/M) concentration and (B) the surface exterior portion that has high S/M concentration.

response to the presence of a salt gradient and contraction/expansion of the curd protein network in response to salt concentration have reached an equilibrium state and whey expulsion ceases. It does appear from Figure 6 that there may have been a little more whey expulsion occurring after the 20 min, but usually at this time the curd has been hooped and pressing started.

Salting Level

Unsalted curds were obtained with moisture of 41% and pH of 5.4. Applying salt with increasing salting levels (i.e. 20, 25, and 30 g/kg) significantly influenced cheese composition by decreasing moisture ($P < 0.001$), increasing pH ($P < 0.006$), salt level ($P < 0.001$), and S/M ($P < 0.001$) (Table 2). This result was in agreement with McMahon et al. (2009) and Fucà et al. (2012).

When salting with levels of 20, 25, and 30 g/kg, there was little whey expelled (less than 5%) before the third salting (Figure 8). The major syneresis occurred at 15 min after the start of salting, and reached 84.3%, 90.8%, and 90.5% within 10 min, respectively. The increasing salting levels significantly enhanced whey expulsion at 15

Table 2. Composition of cheese after salting with different levels of salt (20, 25, and 30 g/kg) over 3 applications with 5 min apart, and pressed for 3h at 22ºC.

Salting levels	Moisture	Salt	S/M[1]	pH
----------------	----------------g/kg----------------			
20	370^{a}	15.4^{a}	40.0^{a}	5.2^{a}
25	359^{b}	17.6^{b}	46.7^{b}	5.3^{b}
30	356^{b}	18.9^{c}	50.4^{c}	5.3^{b}

[1] S/M: salt-in-moisture

$^{a\text{-}c}$Means within a column with the same superscript letter were not significantly different

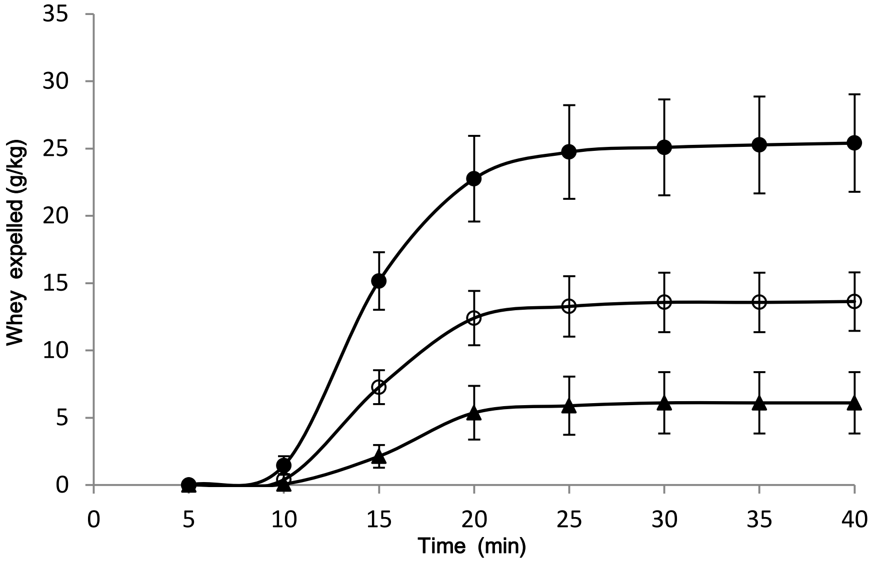

Figure 8. Whey expulsion over time from the first salt application of milled Cheddar curd with 30 g/kg (●), 25 g/kg (○), or 20 g/kg salt (▲) added over 3 applications using 5-min intervals. Bars = SE.

min (*P* < 0.001), 20 min (*P* < 0.001), and prior to pressing (*P* < 0.001). The addition of 1/3 less salt (20g/kg versus 30 g/kg) reduced average syneresis before pressing by 80% from 25.4 g/kg to 6.1 g/kg.

Cheese composition (i.e. moisture, salt, S/M, and pH) was influenced by the salting levels (Schroeder et al., 1988; Pastorino et al., 2003a; Guinee, 2004; Guinee and Fox, 2004; Fucà et al., 2012). The increase in salting levels promotes syneresis and decreases moisture (Schroeder et al., 1988; Pastorino et al., 2003a; Guinee and Fox, 2004; Fucà et al., 2012). Salt absorption of cheese increases with the salting level in brine (Guinee, 2004; Fucà et al., 2012). Ragusano cheese salted in brine of from 2% to 26% NaCl had increasing salt levels from 108 g/kg to 807 g/kg (Fucà et al., 2012). Low syneresis rate is associated with decreased pH in cheese (Guinee, 2004).

When dry salt is distributed over surface of milled curds, salt dissolves in the moisture around surfaces and forms a supersaturated salt solution on cheese surface (Guinee, 2004). The movement of water from curd to surface is caused by the osmotic pressure difference between curd and saturated brine solution on surface (Guinee, 2004). When curds were salted at increasing levels of 20, 25, and 30 g/kg, the salt diffusion and water movement are caused by the increasing osmotic pressure. The ratio (B/A) was increased by rapid salt diffusion using high salting levels, giving rise to more protein shrinkage and whey expulsion (Figure 7). Therefore, whey expulsion was significantly promoted as higher salting levels. Since less water was held in matrix, cheese with more whey expulsion tends to be low in moisture (Schroeder et al., 1988; Pastorino et al., 2003a; Guinee and Fox, 2004; Fucà et al., 2012).

Salting Application

Unsalted Cheddar curds were obtained with mean moisture of 42% and pH 5.4. After salting with 1, 2, and 3 applications for a total of 20 g/kg NaCl and pressing, cheese composition had no significant difference ($P \geq 0.2$) with mean (± SD) values of moisture 375 (± 3.35) g/kg, salt 16.5 (± 1.01) g/kg, S/M 42.0 (± 2.52) g/kg, and pH 5.2 (± 0.03).

When a reduced amount of salt (i.e. 20 g/kg) was applied to Cheddar cheese curd, the mean amount of whey syneresis was lower (i.e., ≤ 6 g/kg) with large variations (Figure 9). Applying salt using one application caused faster syneresis than using 2 or 3 applications, with major syneresis occurring at 10 or 15 min after the start of salting, respectively. Even when considered as the time after the last salting, the whey expulsion reached 81.1% within 5 min when using one application, while only achieved 3% and 45.6% using 2 or 3 applications, respectively. Using 2 applications, whey expulsion

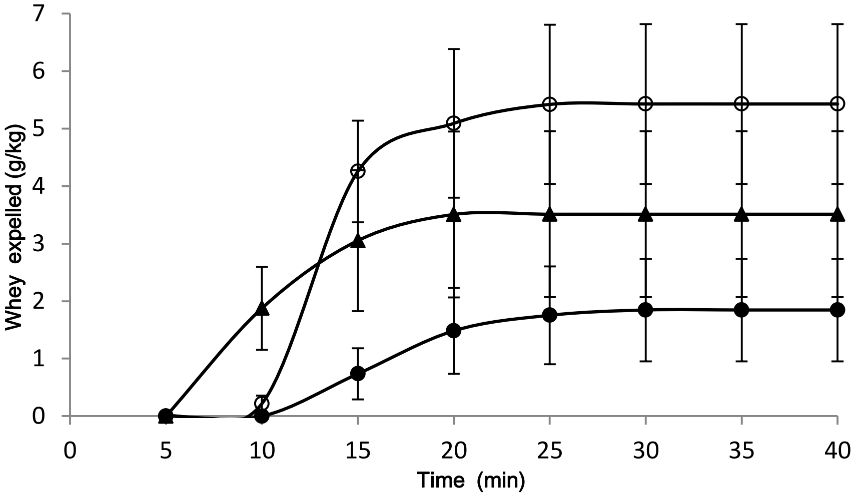

Figure 9. Whey expulsion over time from the first salt application of milled Cheddar curd with 20 g/kg salt added over 1 (▲), 2 (○), or 3 (●) applications using 5-min intervals. Bars = SE. Note the smaller Y-axis scale.

was significantly greater than using 3 applications at 15 min ($P < 0.001$), 20 min ($P < 0.003$), and before pressing ($P < 0.003$) (Table 3). Using 3 salting applications, whey expulsion at 15 min was significantly lower than using 1 application ($P < 0.04$), but no significant difference at 20 min ($P > 0.14$) or prior to pressing ($P > 0.2$). No significant differences were found in whey expulsion using 1 or 2 salt applications ($P > 0.08$). As seen from the percentage and shape of whey expulsion curves in Figure 9, the last portion of whey was expelled slowest using 2 applications, followed by 3 applications, with the fastest using one application. With one salt application, last 18.9% of whey was expelled in 10 min; with 3 applications, last 15.8% was expelled in 20 min; while with 2 applications, last 14.6% was expelled in 30 min.

Salting with 3 applications apparently causes a slower increase in salt concentration and diffusion across the curd exterior part and therefore the B/A ratio increases more slowly resulting in less and more retarded whey expulsion. By 20 min

Table 3. Whey expulsion after the start of salting at 15 min, 20 min, and before pressing. Cheese was salted with levels of 20g/kg using 1, 2, or 3 applications with 5 min apart.

Applications	15 min	20 min	Before pressing
	--------------------------	g/kg	------------------------
1	3.1^{a}	3.5^{ab}	3.5^{ab}
2	4.3^{a}	5.1^{a}	5.4^{a}
3	0.7^{b}	1.5^{b}	1.8^{b}

[a-b]Means within a column with the same superscript letter were not significantly different

after the last salt application, whey expulsion reaches a plateau in all three groups.

Salting with one application leads to the fastest increase in salt level and diffusion rate in cheese and causes the fastest whey expulsion at 5 min after the start of salting. However, the increased whey expulsion causes high level of salt lost (Guinee, 2004). Large amount of salt on curd surface is washed off from curd surface before diffusing into curds. Therefore, the amount of salt diffuses into curds is greatly reduced, causing the increase of ratio B/A not proportional to the salting speed and thus no significant difference in amount of whey expulsion compared to using 2 salting applications.

Salt Substitution

Unsalted curds were obtained with moisture of 42% and pH of 5.4. After salting with 30 g/kg salt or salt mixture consisting of a 2:1 molar ratio of NaCl and KCl, there was no significant difference ($P > 0.05$) in cheese composition with mean (± SD) values of moisture 356 (± 2.94) g/kg, salt 20.6 (± 1.26) g/kg, S/M 54.5 (± 3.41) g/kg, and pH 5.3 (± 0.04). Similar results were found by others (Lindsay et al., 1982; Fitzgerald and

Buckley, 1985; Katsiari et al., 1998, 2000a, b; Karagözlü et al., 2008; Dorosti et al., 2010; Ayyash et al., 2011; Grummer et al., 2012).

When using salt or KCl/NaCl mixture, little whey was expelled prior to the third application (Figure 10). Major syneresis occurred at 15 min after the start of salting and reached 94.2% and 97.1% within 10 min, respectively. The amount of whey prior to pressing was not significantly different (*P* = 0.19), although syneresis was slightly faster at 15 min and 20 min when using KCl/NaCl mixture. This result was in agreement with the fact that no significant moisture change in cheese after pressing. No significant difference between cheese composition and whey expulsion prior to pressing between salting with salt or KCl/NaCl mixture implies that the cation K has the same effect as Na on cheese composition and syneresis (Fitzgerald and Buckley, 1985; Katsiari et al., 1998, 2000a, b; Dorosti et al., 2010; Ayyash et al., 2011; Grummer et al., 2012).

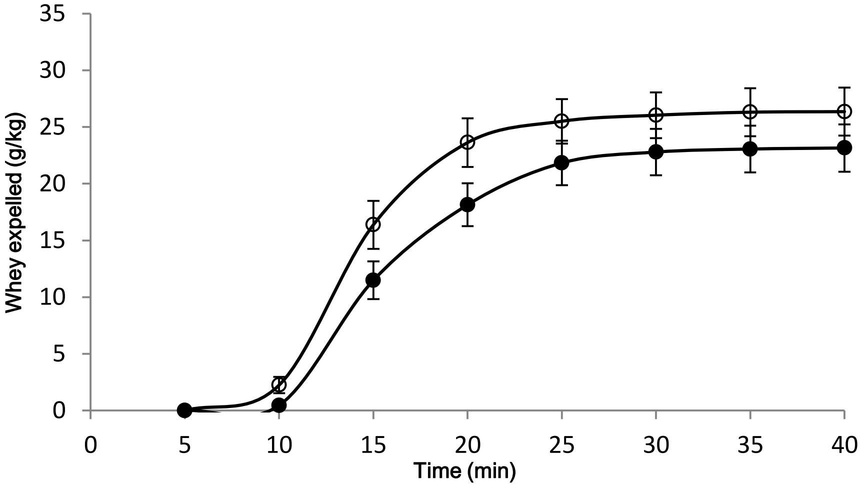

Figure 10. Whey expulsion over time from the first salt application of milled Cheddar curd with 30 g/kg salt (●) or salt mixture consisting of a 2:1 molar ratio of NaCl and KCl (○) added over 3 applications using 5-min intervals. Bars = SE.

Immersion with Different Ca Level

Curd Composition and Brine. Salted Cheddar curd was obtained at pH of 5.35 and its composition was analyzed before immersion (Table 4). When test solutions were prepared with undiluted whey, the precipitation of calcium phosphate occurred after the addition of more than 2 g/L Ca (as $CaCl_2$). The 50% substitution of whey with deionized water allowed adding more Ca (up to 27 g/L) without precipitation. When phosphate had been added to the test solutions, the precipitation of calcium phosphate occurred, and high Ca levels in the test solutions could only be achieved by not adding any phosphate. As the calcium level was increasing, test solutions became more turbid, presumably from some precipitation of calcium with phosphate already present in the whey. This explains why soluble calcium concentrations in test solutions were 1.2, 4.7, 8.3, and 15.4 g/kg, respectively, for the solutions to which were added 1, 5, 10, and 20 g/L Ca. In order to reduce the diffusion of sodium between cheese serum and test solutions, all solutions

Table 4. Cheddar curd composition before immersion.

Variable	Mean
Moisture (g/kg cheese)	405
Na (g/kg cheese)	6.2
Na/M[1] (g/kg)	15.1
Total Ca (g/kg cheese)	6.9
Serum Ca (g/kg serum)	4.0
Insoluble Ca[2] (g/kg cheese)	5.2
Insol Ca/Solids[3] (g/kg solids)	8.8

[1] Na/M: Na in moisture = Na / (Na + Moisture)

[2] Insoluble Ca = Total Ca – Serum Ca

[3] Insol Ca/Solids = Insoluble Ca × 1000/ (1000 – Moisture)

were prepared by adding 15 g/L NaCl and the mean level in the final solutions was 13.1 ± 1.2 g/kg.

The calcium level in test solution significantly affected cheese composition and weight change (Table 5). Immersion time (i.e., 6 or 18 h) significantly influenced moisture, serum Ca, total Ca, and weight change. The interaction of these two factors significantly affected cheese composition and weight change except insoluble Ca and the ratio of insoluble Ca to cheese solids (**Insol Ca/Solids**).

Moisture and Calcium. Both Ca levels of test solutions and immersion time significantly affected cheese moisture (Table 5). Compared to control, cheese moisture increased after immersion at test solutions of 1, 5, and 10 g/L Ca (Table 6). The moisture level of curd decreased as Ca content of test solution (Figure 11) and immersion time increased (Table 6). This suggests that in these cheeses there was a swelling of the curd particles with a concomitant absorption of moisture into the curd. When curd was immersed in the 20 g/L Ca solution, there was no change in moisture.

Calcium content of the test solutions significantly affected all aspects of Ca in cheese (Figure 12) but immersion time was only a significant factor on soluble and total Ca levels and did not significantly affect insoluble Ca (Table 5). When immersed in 1 Ca solution for 18h, there was a decrease in soluble Ca in the curd (Table 7) as Ca from the curd diffused into the surrounding solution. The equilibrium of Ca in curd can be expressed as (Lucey and Horne, 2009):

$$3\ Ca^{2+} + 2\ HPO_4^{2-} \longleftrightarrow Ca_3(PO_4)_2 \downarrow + 2\ H^+$$

To maintain this equilibrium, when the soluble Ca decreases there will be a subsequent dissolving of insoluble Ca that is bound to proteins. In both cheese and milk, a decreasing

Table 5. Statistical results (α = 0.05) of cheese composition, weight change, and calcium level after immersed at test solution of 1, 5, 10, and 20 g/L Ca at room temperature for 6 or 18 h.

Source of Variation	Ca	Time	Ca × Time
Moisture	< 0.001	0.004	0.012
Na	< 0.001	0.946	< 0.001
Na/M	0.003	0.373	0.001
pH	< 0.001	0.561	0.005
Weight change	< 0.001	< 0.001	< 0.001
Serum Ca	< 0.001	< 0.001	< 0.001
Total Ca	< 0.001	< 0.001	< 0.001
Insoluble Ca	< 0.001	0.620	0.907
Insol Ca/Solids	< 0.001	1	0.826

Table 6. Mean moisture, Na, Na/M, pH, and weight change of Cheddar cheese curd after immersion in whey test solutions containing 1, 5, 10, or 20 g/L added calcium and 15 g/L added sodium.

	Added calcium (g/L)			
Parameter	1	5	10	20
After 6 h				
Moisture (g/kg cheese)	455^{f}*	437^{e}*	423^{cd}*	409^{b}
Na (g/kg cheese)	6.56^{b}*	6.47^{ab}	6.48^{ab}*	6.59^{b}*
Na/M (g/kg)	14.2^{a}*	14.6^{ab}	15.1^{abc}	15.9^{c}
pH	5.27^{d}	5.17^{c}*	5.05^{b}*	4.95^{a}*
Weight change (%)	8.9^{f}*	5.0^{d}*	3.1^{c}*	1.4^{b}*
After 18 h				
Moisture (g/kg cheese)	458^{f}*	433^{de}*	418^{bc}*	395^{a}*
Na (g/kg cheese)	7.17^{c}*	6.53^{b}	6.30^{ab}	6.09^{a}
Na/M (g/kg)	15.4^{bc}	14.9^{ab}	14.9^{ab}	15.2^{bc}
pH	5.32^{d}	5.14^{c}*	5.06^{b}*	4.90^{a}*
Weight change (%)	9.8^{e}*	3.7^{c}*	1.7^{b}*	-0.5^{a}*

^{abcdef} Means for the same parameter with the same letter were not significantly different, α=0.05
*Means of parameter are significantly different from Cheddar cheese curd before immersion

Table 7. Mean total Ca, serum Ca, insoluble Ca, and insol Ca/Solids of Cheddar cheese curd after immersion in whey test solutions containing 1, 5, 10, or 20 g/L added calcium and 15 g/L added sodium.

	Added calcium (g/L)			
Parameter	1	5	10	20
After 6 h				
Total Ca (g/kg cheese)	5.9^{a}*	7.4^{b}*	9.0^{c}*	11.6^{d}*
Serum Ca (g/kg serum)	3.6^{a}	5.2^{b}*	7.6^{c}*	11.5^{d}*
Insoluble Ca (g/kg cheese)	4.1^{a}*	4.7^{ab}*	5.2^{bc}	5.9^{c}
Insol Ca/Solids (g/kg solids)	7.5^{a}*	8.4^{ab}	9.1^{bc}	10.0^{c}
After 18 h				
Total Ca (g/kg cheese)	5.6^{a}*	7.8^{b}*	9.8^{c}*	13.2^{e}*
Serum Ca (g/kg serum)	3.4^{a}*	6.9^{c}*	10.8^{d}*	18.0^{e}*
Insoluble Ca (g/kg cheese)	4.1^{a}*	4.8^{ab}*	5.4^{bc}	5.9^{c}
Insol Ca/Solids (g/kg solids)	7.5^{a}*	8.5^{ab}	9.3^{bc}	9.7^{bc}

^{abcd} Means for the same parameter with the same letter were not significantly different, α=0.05

*Means of parameter are significantly different from Cheddar cheese curd before immersion, α=0.05

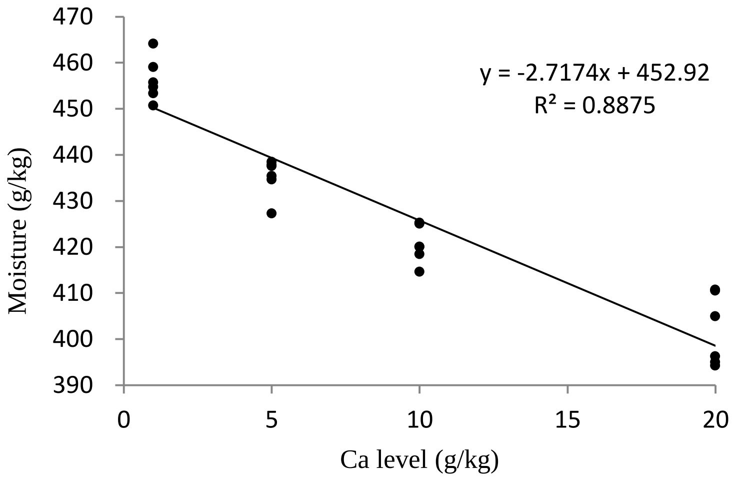

Figure 11. Effect of calcium concentration (with linear regression) on the moisture of cheese curd after 6-h and 18-h immersion (pooled data) at 22°C in whey-brine solutions (containing 13.1 ± 1.2 g/kg sodium).

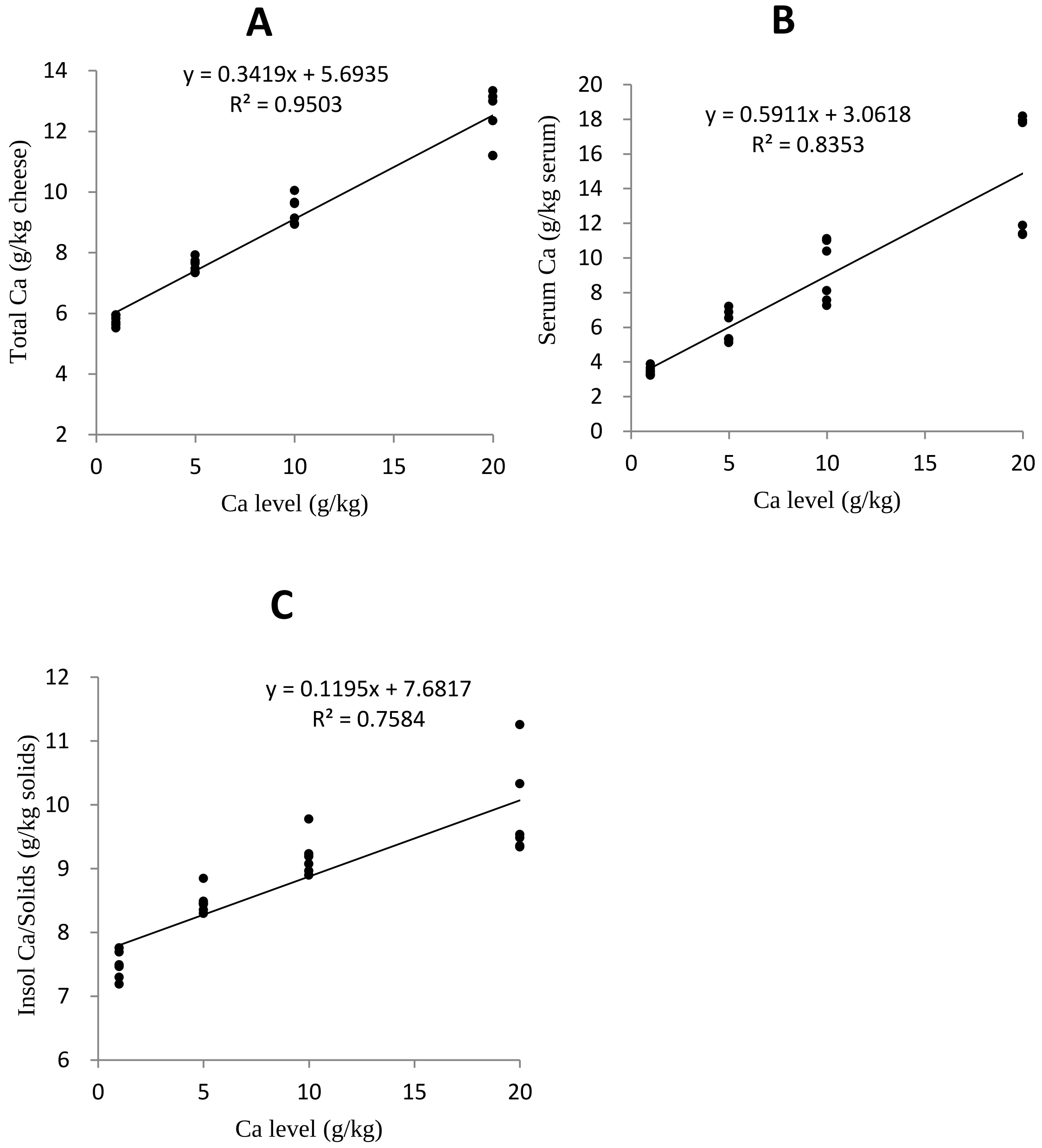

Figure 12. Effect of calcium concentration (with linear regression) on (A) total Ca (g/kg cheese), (B) serum Ca (g/kg moisture), and (C) Insol Ca/Solids (g/kg solids) of cheese curd after 6-h and 18-h immersion (pooled data) at 22°C in whey-brine solutions (containing 13.1 ± 1.2 g/kg sodium).

insoluble Ca reduces the extent of casein micelles crosslinking and promotes swelling and hydration (Lucey and Fox, 1993; Horne, 1998; Hassan et al., 2004; McMahon et al., 2005; Choi et al., 2007, 2008; Lucey and Horne, 2009). The weaker cross-linked proteins in cheese curd would be expected to allow more changes taking place during immersion such as swelling of the protein matrix and absorption of water from test solutions.

When immersed in the 10 g/L Ca solution, the level of soluble Ca increased but the Insol Ca/Solids ratio did not change significantly (Table 7). However, in the 20 g/L Ca solution, the Ca balance in curd was changed sufficiently that the Insol Ca/Solids ratio increased from 8.8 g/kg before immersion to 10.0 g/kg after 6 h immersion. Curd with high Ca content has been associated with a more cross-linked and rigid protein network that has reduced protein hydration (McMahon et al., 2005; O'Mahony et al., 2006), and this was observed with a small (2.5%) but significant decrease in cheese moisture (Table 6).

In these experiments, it was necessary to increase the serum Ca above that originally present in the curd to maintain a similar Insol Ca/solids ratio, and to prevent expansion of the protein network. This was attributed to the Ca being bound to the proteins as calcium phosphate and the test solutions having a low level of phosphate. When phosphate had been added to the test solutions, precipitation of calcium phosphate occurred and high Ca levels in the test solutions could only be achieved by not adding any phosphate.

Na and pH. Both pH and Na content of cheese were significantly affected by Ca content of the test solution but not by immersion time (Table 5). There were some significant Ca x time interactions, however. After 6-h immersion, Na and Na-in-Moisture

(**Na/M**) levels did not significantly change, although Na/M significantly increased in 20 g/L Ca solution (Table 6). With continued immersion for 18 h, [Na] in cheese was inversely proportional with [Ca] in solutions, while no significant change in Na/M levels. The Na/M concentration of the cheeses was still close to that before immersion. The increase of Na content after immersion was primarily caused by the increase in moisture (and its accompanying Na ions).

The pH of curd decreased with an increased [Ca] in the test solutions (Figure 13 and Table 6). During cheese manufacture, the curd was milled at pH 5.40 and then salted over a 20-min period and there was virtually no change in the curd immersed for 6 or 18 h with the pH remaining at 5.3. As the [Ca] was increased the curd pH decreased proportionally with curd immersed in the 20 g/L Ca solution dropping to pH 4.9. Similar effects of adding Ca to cheese were reported by Pastorino et al. (2003b) who showed that

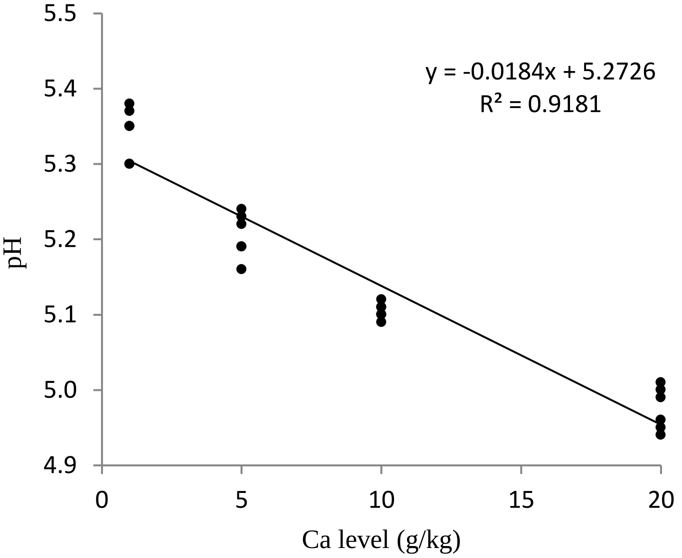

Figure 13. Effect of calcium concentration (with linear regression) on pH of cheese curd after 6-h and 18-h immersion (pooled data) at 22°C in whey-brine solutions (containing 13.1 ± 1.2 g/kg sodium).

the pH of part skim Mozzarella cheese dropped from 5.5 to 4.6 after 5 high-pressure injections of 40% $CaCl_2$ solution. Part of pH drop is also attributed to the growth of starter bacteria in cheese, because generally, the pH of Cheddar cheese dropped to 5.2 after pressing.

Curd Weight. The weight of curd was significantly affected by calcium content of the test solution, immersion time, and there was a significant calcium x time interaction (Table 5). After 6-h and 18-h immersion, weight increase was inversely proportional with [Ca] in test solutions (Figure 14). After 6-h immersion all of the curd pieces gained weight with the greatest weight change occurring with the lowest Ca content (Table 6). With continued immersion for 18 h, the curd in the 1 g/L Ca test solution continued to gain weight (with a final weight increase of 9.8%) while those with higher Ca solutions lost weight. For the curd in the 20 g/L added Ca solution this resulted in a net 0.5% loss of weight.

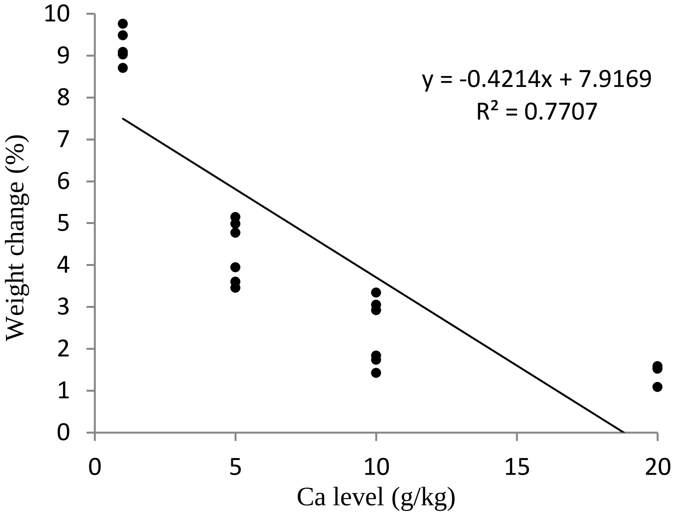

Figure 14. Effect of calcium concentration (with linear regression) on weight change of cheese curd after 6-h and 18-h immersion (pooled data) at 22°C in whey-brine solutions (containing 13.1 ± 1.2 g/kg sodium).

Most of weight gain was caused by water absorption, with a small portion from diffusion of Ca into the curd. As discussed before, in the lower Ca solutions (i.e., 1 and 5 g/L), the dissolution of insoluble Ca produced a more solubilized and hydrated protein matrix resulting into an expansion of the protein network and infusion of test solution into the curd. At higher Ca levels (i.e., 10 and 20 g/L), insoluble Ca increased in the curd due to the precipitation of Ca from the test solution diffusing into the curd, resulting in a more cross-linked and rigid protein matrix. Therefore, water diffusion is limited and less cheese weight gain occurs, with shrinkage of the curd occurring at the highest Ca level. This is shown with weight change inversely proportional to Insol Ca/Solids ratio (Figure 15). Such contraction of the protein network takes time and is slower than the transition of Ca from the solution to be bound to the proteins. This is shown with the

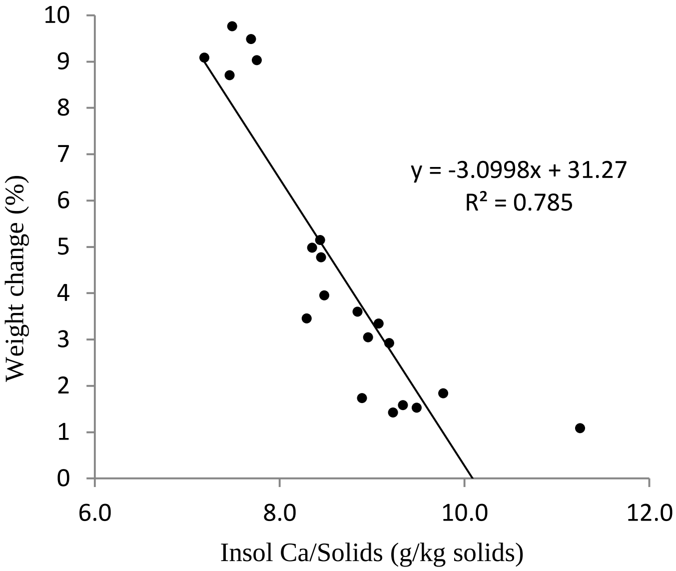

Figure 15. Cheese weight change (%) as a function of Insol Ca/Solids (g/kg solids) after 6-h and 18-h immersion at 22°C (pooled data) in whey-brine solutions (containing 1, 5, 10, and 20 g/L added calcium and 13.1 ± 1.2 g/kg sodium).

Insol Ca/Solids ratio reaching its equilibrium level after 6 h and an initial swelling of the curd followed by a decrease in weight occurring between 6 and 18 h (Figure 16).

Immersion with Different Salt Level

<u>Composition.</u> Unsalted Cheddar cheese curd was obtained with mean moisture and pH of 409 g/kg and 5.3, respectively. Moisture ($P < 0.001$), salt ($P < 0.001$), S/M ($P < 0.001$), and pH ($P = 0.025$) of curd were significantly affected after 6-h immersion in test solutions of 30, 60, 90, or 120 g/L NaCl in addition to 6 g/L Ca (Table 8). The moisture of the curd was inversely proportional with the salt level in test solutions (Figure 17-A), with 2.4% increase in 30 g/L NaCl solution, but 1.7%, 6.8%, and 9.5% drop in solution of 60, 90, and 120 g/L NaCl, respectively. This suggests that there was a contraction of curd particles with an accompanying water expulsion towards the adjacent brine solution.

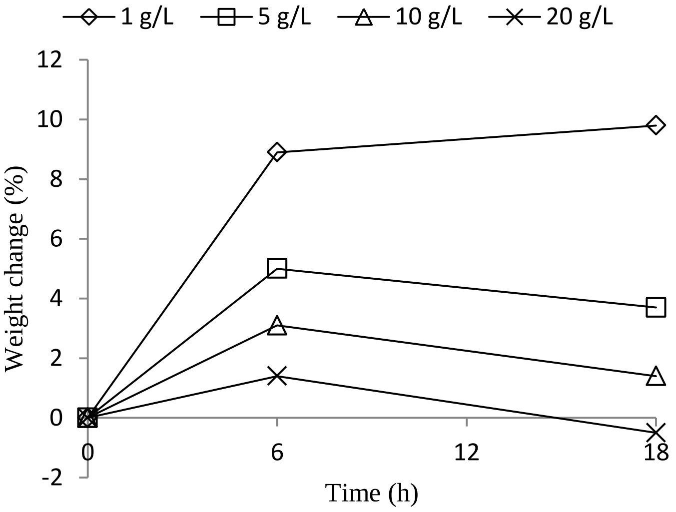

Figure 16. Curd weight change over time after 6-h and 18-h immersion (pooled data) at 22°C in whey-brine solutions (containing 1, 5, 10, and 20 g/L added calcium and 13.1 ± 1.2 g/kg sodium).

Table 8. Weight change and composition of curd after 6-h immersion in solutions of 30, 60, 90, or 120 g/L NaCl in addition to 6 g/L Ca at 22ºC.

NaCl level (g/L)	30	60	90	120
Moisture (g/kg)	419^{d*}	402^{c}	381^{b*}	370^{a*}
Salt (g/kg)	13^{a}	19^{b}	25^{c}	29^{d}
S/M[1] (g/kg)	30^{a}	44^{b}	61^{c}	72^{d}
pH	5.08^{b*}	5.10^{b*}	5.07^{ab*}	5.05^{a*}
Weight change (%)	4.4^{d}	1.4^{c}	-1.4^{b}	-4.0^{a}
AWC[2] (%)	3.0^{d}	-0.5^{c}	-3.9^{b}	-6.7^{a}

[abcd] Means for the same parameter with the same letter were not significantly different, α=0.05
*Means of parameter are significantly different from Cheddar cheese curd before immersion, α=0.05
[1] S/M: salt-in-moisture
[2] AWC: adjusted weight change as a function of water absorption. AWC = Weight change – Salt level of cheese

Salt and S/M of curd increased proportionally with salt level in test solutions (Table 8 and Figure 17-B), indicating that salt was diffused from the surrounding test solution towards the serum in curds. Cheese pH dropped from 5.3 to 5.0~5.1 after immersion (Table 8). The pH drop was primarily caused by the growth of starter bacteria from cheese since generally, the pH of cheese dropped to 5.1~5.2 after pressing.

When immersed in solutions of 5 and 10 g/L Ca, the insol Ca/Solids did not change significantly (P > 0.2), but the total Ca in cheese increased significantly (P < 0.001) from 6.9 g/kg to 7.4 and 9.0 g/kg, respectively. In order to maintain the same insol Ca/Solids without excessive change in total Ca content, 6 g/L of Ca was added to the test solutions for immersing with different salt levels. At low S/M concentration (i.e., < ~50 g/L), Na has a salting-in effect in casein which leads to an increasingly hydrated protein matrix (Paulson et al., 1998). Instead, at high S/M level (i.e., > ~50 g/L), the salting-out

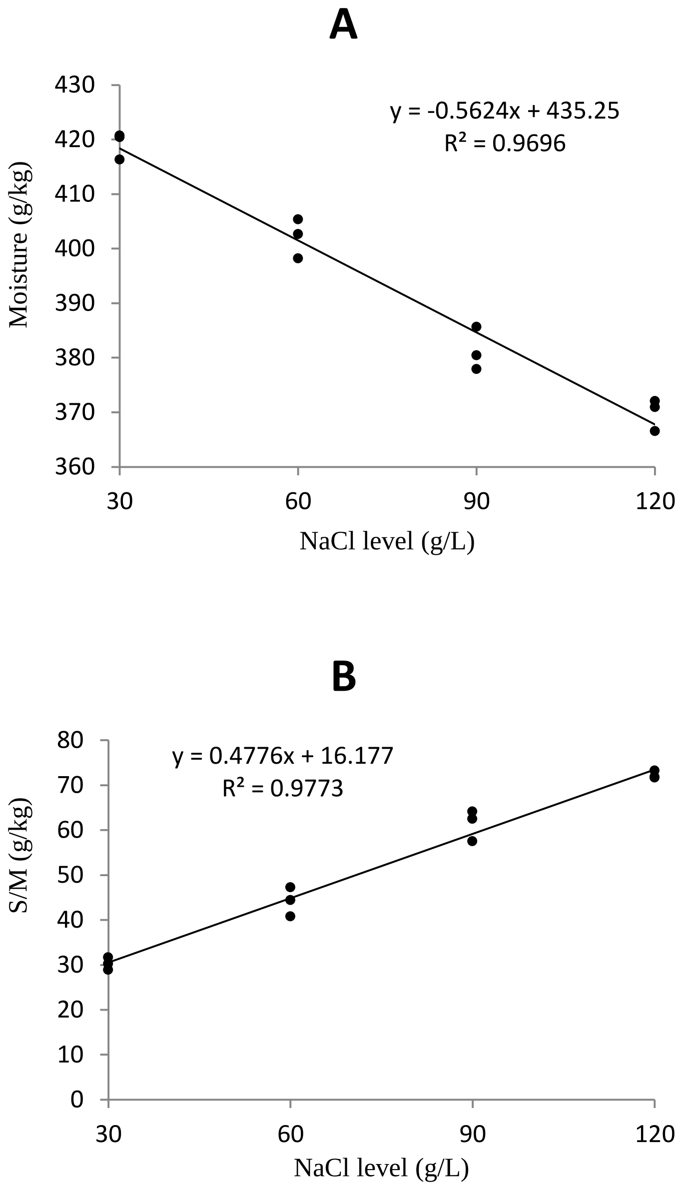

Figure 17. Effect of brine salt concentration (with linear regression) on (A) moisture, and (B) salt-in-moisture (S/M) of cheese curd after 6-h immersion at 22°C in whey-brine solutions (containing 6 g/L calcium).

effect of Na causes a less soluble protein matrix (Guinee and Fox, 2004). When immersed in 30 g/L NaCl solution, there was an increase in salt and S/M levels of curds as salt from the solution diffused into curd in response to the osmosis pressure of salt between curd serum and surrounding test solutions. The low S/M of curd (i.e., 30 g/L) caused by salt diffusion leads to a more soluble protein matrix with the absorption of moisture into the curd. Besides, the decrease of pH from 5.3 to 5.1influences the Ca balance, decreases the insoluble Ca level and therefore leads to a less cross-linked and more hydrated protein matrix. Thus, these combined factors promote protein hydration in curd which was shown with the moisture increase after immersion.

When immersed in solution of 60 g/L NaCl, the combined effects of attenuated salting-in effect at increasing S/M of curd (i.e., 44 g/L) and the increased osmosis pressure of salt between curd serum and adjacent solutions lead to a slightly dehydrated protein matrix, which was shown with a significant but small (1.7%) drop in moisture. With further increasing salt level in solutions, the S/M of curd increased sufficiently (i.e., 61 and 72 g/L in corresponding solutions of 90 and 120 g/L NaCl), possibly causing a salting-out effect, together with the increasing moisture loss in response to osmosis force, resulting in a less soluble and contracted protein matrix as evidenced by a 9.5% decrease in moisture after 6-h immersion.

Curd Weight. Both Cheese weight ($P < 0.001$) and adjusted weight change (AWC) ($P < 0.001$) were inversely proportional to NaCl level in test solutions. Cheese weight increased by 4.4% and 1.4% in 30 and 60 g/L salt solution, but dropped 1.4% and 4.0% in 90 and 120 g/L salt solution, respectively (Table 8). Similarly, AWC of curd increased 3% but lost 0.5%, 3.9%, and 6.7% in solutions of 30, 60, 90 and 120 g/L salt, respectively (Figure 18).

Cheese weight change is related to the diffusion of both NaCl and moisture around cheese. AWC is curd weight change neglecting salt absorption, and thus implies solely the curd hydration extent. At a low salt level in solution (i.e. 30 g/L), both cheese weight and AWC increased due to water absorption in response to the salting-in effect of protein matrix. At 60 g/L salt solution, AWC changes marginally (5%), suggesting the extent of protein hydration is relatively constant. Curd absorbs water in response to the attenuated salting-in effect, but also expels slightly more water in response to the osmosis force. Meanwhile, cheese is still gaining weight due to the salt absorption, which is shown with 1.4% increase in curd weight change after immersion. At 90 and 120 g/L NaCl test solution, the salting-out effect promoted syneresis and reduced the moisture of

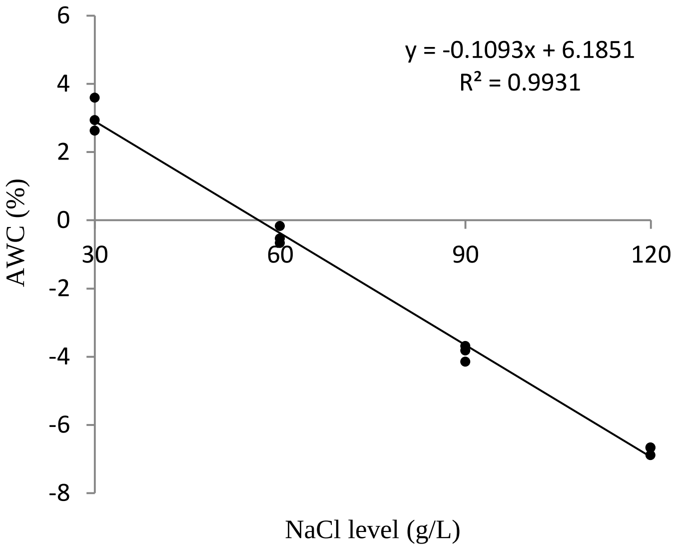

Figure 18. Effect of brine salt concentration (with linear regression) on adjusted weight change (AWC) of cheese curd after 6-h immersion at 22°C in whey-brine solutions (containing 6 g/L calcium).

curd after immersion, which is shown by the drop in both cheese weight and AWC after immersion (Schroeder et al., 1988; Kindstedt et al., 1992; Pastorino et al., 2003a; Guinee and Fox, 2004).

CONCLUSIONS

Salt influences the whey expulsion of cheese by affecting its protein hydration state. Dry salting Cheddar cheese curd using a 5-min salting interval produces cheese with same composition as using a 10-min interval. The addition of 1/3 less salt (20g/kg versus 30 g/kg) reduces initial syneresis by 80% with final cheese moisture increasing from 36% to 37%. Whey expulsion (when adding salt at 20 g/kg) does not increase by adding salt all at once rather than over 3 applications. Substituting KCl for 1/3 of NaCl produces the same whey syneresis as when only NaCl was used.

Brine salting Cheddar curd at 22°C and various salt levels (with sufficient Ca to maintain the same Insol Ca/Solids ratio) demonstrated that at a salt concentration of 60 g/L, a contraction occurs of the cheese curd. Immersion of Cheddar curd at increasing Ca levels (1, 5, 10, and 20 g/L Ca with 13 g/kg salt) at 22°C also promotes protein interaction and thus decreases moisture. Understanding the effect of salting on whey expulsion prior and during pressing can help in designing process systems for the manufacture of cheeses with reduced sodium content. The lack of syneresis occurring with a salt reduction can be overcome by substituting KCl for NaCl.

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APPENDICES

Appendix A. Cheese Manufacture

Full fat Cheddar cheese curds were manufactured following the method of Rogers et al. (2010). Cold milk was transported to the Gary Haight Richardson Dairy Products Laboratory at Utah State University (Logan) and 700-kg batches were standardized to ~0.83 protein to fat ratios. The milk was pasteurized (73°C for 16 s) and pumped into a Tetra Scherping horizontal cheese vat (Tetra Pak Cheese & Powder Systems Inc., Winsted, MN) and heated to 31°C. After 10 min, starter culture was added to milk at the rate of 21g/100 kg and the milk was allowed to ripen for 10 min. $CaCl_2$ (32% wt/wt), annatto (single strength) and chymosin (double strength) were added at 12, 7, and 7 mL/100 kg, respectively. Followed by 2-min stirring, then milk was allowed to coagulate without stirring until a firm set was reached (~30 min).The curd was cut at 10, 11, and 12 rpm for 1 min each, reversed for 30 sec, and then cut at 14 rpm for 1 min. The curd was allowed to rest for 5 min, stirred starting at 8, 9, 10, 11, and 12 rpm of 1 min each, cut at 14 rpm for 1 min, stirred at 10 rpm for 9 min, cut at 14 rpm for 1 min again, and stirred at 12 rpm for 14 min. Sixty-five min after renneting, the curd and whey were heated to 39°C over 30 min. After stirring for 35 min, the curd and whey were pumped to a drain table (Kusel Equipment Co., Watertown, WI) and stirring continued until the curd pH reached 6.3, and whey drained. After draining the whey, the curd was formed in packs of 8 to 10 cm deep and cut into slabs about 15 cm wide after 5 min. The curd slabs were flipped over and stacked, and curd had dropped to ~34°C. Cheeses were milled at pH 5.4. Then unsalted curd was collected or the curd was salted at a total rate of 30 g/kg divided into 3 applications with 5 min intervals.

Appendix B. Analysis of Variance — Dry Salting Cheddar Cheese Curd

Salting Interval

Moisture

Effect	Num DF	Den DF	F Value	Pr > F
Interval	1	21	0.62	0.4392

Salt

Effect	Num DF	Den DF	F Value	Pr > F
Interval	1	21.1	0.54	0.4712

S/M

Effect	Num DF	Den DF	F Value	Pr > F
Interval	1	21	0.56	0.4622

pH

Effect	Num DF	Den DF	F Value	Pr > F
Interval	1	21	0.17	0.6825

The amount of whey expulsion prior to pressing

Effect	Num DF	Den DF	F Value	Pr > F
Interval	1	21	4.21	0.0529

Salting Level

Moisture

Effect	Num DF	Den DF	F Value	Pr > F
Level	2	14	46.66	<.0001

Differences of Least Squares Means									
Effect	Level	_Level	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Level	1	2	-3.4667	1.5424	14	-2.25	0.0412	Tukey-Kramer	0.0975
Level	1	3	-14.2833	1.5424	14	-9.26	<.0001	Tukey-Kramer	<.0001
Level	2	3	-10.8167	1.5424	14	-7.01	<.0001	Tukey-Kramer	<.0001

Salt

Effect	Num DF	Den DF	F Value	Pr > F
Level	2	14	25.58	<.0001

Differences of Least Squares Means									
Effect	Level	_Level	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Level	1	2	1.3000	0.4947	14	2.63	0.0199	Tukey-Kramer	0.0490
Level	1	3	3.5000	0.4947	14	7.07	<.0001	Tukey-Kramer	<.0001
Level	2	3	2.2000	0.4947	14	4.45	0.0006	Tukey-Kramer	0.0015

S/M

Effect	Num DF	Den DF	F Value	Pr > F
Level	2	14	35.96	<.0001

Differences of Least Squares Means

Effect	Level	_Level	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Level	1	2	3.5000	1.2609	14	2.78	0.0149	Tukey-Kramer	0.0372
Level	1	3	10.5000	1.2609	14	8.33	<.0001	Tukey-Kramer	<.0001
Level	2	3	7.0000	1.2609	14	5.55	<.0001	Tukey-Kramer	0.0002

pH

Effect	Num DF	Den DF	F Value	Pr > F
Level	2	14	7.64	0.0057

Differences of Least Squares Means

Effect	Level	_Level	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Level	1	2	0.006667	0.01048	14	0.64	0.5349	Tukey-Kramer	0.8029
Level	1	3	0.03833	0.01048	14	3.66	0.0026	Tukey-Kramer	0.0068
Level	2	3	0.03167	0.01048	14	3.02	0.0091	Tukey-Kramer	0.0233

The amount of whey expulsion prior to pressing

Effect	Num DF	Den DF	F Value	Pr > F
Level	2	14	18.28	0.0001

Differences of Least Squares Means

Effect	Level	_Level	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Level	1	2	1.3408	0.4816	14	2.78	0.0146	Tukey-Kramer	0.0366
Level	1	3	2.9092	0.4816	14	6.04	<.0001	Tukey-Kramer	<.0001
Level	2	3	1.5684	0.4816	14	3.26	0.0057	Tukey-Kramer	0.0148

Salting Application

Moisture

Effect	Num DF	Den DF	F Value	Pr > F
Application	2	22	0.91	0.4159

Differences of Least Squares Means

Effect	Application	_Application	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Application	1	2	0.1222	1.3843	22	0.09	0.9304	Tukey-Kramer	0.9957
Application	1	3	1.5556	1.3843	22	-1.12	0.2732	Tukey-Kramer	0.5100
Application	2	3	1.6778	1.3843	22	-1.21	0.2384	Tukey-Kramer	0.4588

Salt

Effect	Num DF	Den DF	F Value	Pr > F
Application	2	24	0.37	0.6972

Differences of Least Squares Means

Effect	Application	_Application	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Application	1	2	0.1000	0.4865	24	-0.21	0.8389	Tukey	0.9770
Application	1	3	0.3000	0.4865	24	0.62	0.5433	Tukey	0.8125
Application	2	3	0.4000	0.4865	24	0.82	0.4191	Tukey	0.6932

S/M

Effect	Num DF	Den DF	F Value	Pr > F
Application	2	24	0.38	0.6874

Differences of Least Squares Means

Effect	Application	_Application	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Application	1	2	1.1111	1.2733	24	-0.87	0.3915	Tukey	0.6624
Application	1	3	0.5556	1.2733	24	-0.44	0.6665	Tukey	0.9008
Application	2	3	0.5556	1.2733	24	0.44	0.6665	Tukey	0.9008

pH

Effect	Num DF	Den DF	F Value	Pr > F
Application	2	22	1.85	0.1813

Differences of Least Squares Means									
Effect	Application	_Application	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Application	1	2	0.01778	0.01224	22	-1.45	0.1604	Tukey-Kramer	0.3323
Application	1	3	0.02222	0.01224	22	-1.82	0.0830	Tukey-Kramer	0.1877
Application	2	3	0.00444	0.01224	22	-0.36	0.7199	Tukey-Kramer	0.9301

The amount of whey expulsion prior to pressing

Effect	Num DF	Den DF	F Value	Pr > F
Application	2	22	7.99	0.0025

Differences of Least Squares Means									
Effect	Application	_Application	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Application	1	2	2.3186	0.5816	22	-3.99	0.0006	Tukey-Kramer	0.0017
Application	1	3	1.0129	0.5816	22	-1.74	0.0955	Tukey-Kramer	0.2126
Application	2	3	1.3057	0.5816	22	2.25	0.0351	Tukey-Kramer	0.0856

Salting Substitution

Moisture

Effect	Num DF	Den DF	F Value	Pr > F
Substitution	1	17	2.68	0.1201

Salt

Effect	Num DF	Den DF	F Value	Pr > F
Substitution	1	17	0.00	1.0000

S/M

Effect	Num DF	Den DF	F Value	Pr > F
Substitution	1	17	0.17	0.6879

pH

Effect	Num DF	Den DF	F Value	Pr > F
Substitution	1	17	4.35	0.0523

The amount of whey expulsion prior to pressing

Effect	Num DF	Den DF	F Value	Pr > F
Substitution	1	17	1.87	0.1897

Appendix C. Analysis of Variance — Brine Salting Cheddar Cheese Curd

Immersion with Different Ca Level

Moisture

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Ca	3	9505.188333	3168.396111	234.07	<.0001
Time	1	157.081667	157.081667	11.60	0.0036
Ca*Time	3	205.728333	68.576111	5.07	0.0118

Na

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Ca	3	0.99447917	0.33149306	14.89	<.0001
Time	1	0.00010417	0.00010417	0.00	0.9463
Ca*Time	3	0.99474583	0.33158194	14.89	<.0001

Na/M

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Ca	3	2.43458333	0.81152778	7.27	0.0027
Time	1	0.09375000	0.09375000	0.84	0.3731
Ca*Time	3	3.01458333	1.00486111	9.00	0.0010

pH

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Ca	3	0.43340000	0.14446667	339.92	<.0001
Time	1	0.00015000	0.00015000	0.35	0.5608
Ca*Time	3	0.00818333	0.00272778	6.42	0.0046

Weight Change

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Ca	3	265.9321125	88.6440375	1383.17	<.0001
Time	1	5.3110042	5.3110042	82.87	<.0001
Ca*Time	3	7.0686458	2.3562153	36.77	<.0001

Total Ca

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Ca	3	143.0195458	47.6731819	652.16	<.0001
Time	1	2.2022042	2.2022042	30.13	<.0001
Ca*Time	3	2.7373125	0.9124375	12.48	0.0002

Serum Ca

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Ca	3	424.1825000	141.3941667	1591.98	<.0001
Time	1	45.8160667	45.8160667	515.85	<.0001
Ca*Time	3	35.5779000	11.8593000	133.53	<.0001

Insoluble Ca

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Ca	3	11.06764583	3.68921528	42.42	<.0001
Time	1	0.02220417	0.02220417	0.26	0.6203
Ca*Time	3	0.04744583	0.01581528	0.18	0.9072

Insol Ca/Solids

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Ca	3	18.71666667	6.23888889	26.64	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Time	1	0.00000000	0.00000000	0.00	1.0000
Ca*Time	3	0.21000000	0.07000000	0.30	0.8257

Immersion with Different Salt Level

Moisture

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NaCl	3	4318.993333	1439.664444	134.59	<.0001

Salt

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NaCl	3	434.109167	144.703056	112.83	<.0001

S/M

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NaCl	3	3083.583333	1027.861111	166.68	<.0001

pH

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NaCl	3	0.00449167	0.00149722	5.44	0.0247

Weight change

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NaCl	3	117.9786000	39.3262000	591.08	<.0001

AWC

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NaCl	3	161.7712250	53.9237417	573.45	<.0001