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Full Length Research Paper

Effect of calcium salts and tri-sodium citrate on certain properties of calcium-fortified soy milk

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Soybean milk was fortified with calcium salts to increase its calcium contents and improve its nutritional value. The addition of calcium fortifiers gave rise to a significant drop of pH and a marked rise of calcium ion concentration. Addition of calcium salts to soy milk had a pronounced negative effect on its heat stability against heat-induced and calcium-induced coagulation. Tri-sodium citrate was added to improve the heat stability of Ca-fortified soy milk as a result of lowering ionic calcium concentration and increasing pH. Twenty five millimolar (25 mM) calcium lactate, calcium chloride and calcium gluconate were added to soy milk with no coagulation when 0.7, 1.2 and 1.4% tri-sodium citrate were added in combination. Dialysis was applied to investigate how pH and ionic calcium changed at high temperature and evaluate the influence of sterilization on certain properties of Ca-fortified soy milk. The pH and calcium ion concentration significantly decreased at high temperature but recovered after cooling.

Key words: Soy milk, calcium, calcium salts, tri-sodium citrate, heat stability.

INTRODUCTION

Soybean foods have been paid more attention by consumers in recent years because of their high nutritional value and healthy beneficial bioactive components such as isoflavones, lecithin, phytates, protease inhibitors, phenolic acids, phytosterols and omega-3 fatty acids. These important components promote soybean products, playing an important role in protecting people against chronic diseases including cancer, cardiovascular disease and osteoporosis. Moreover, soybean can also help women to alleviate menopausal symptoms (Omoni and Aluko, 2005). Soybean foods consist of non-fermented oriental products such as soy milk, “tofu”, soybean sprouts, soy flour and fermented oriental products like soy sauce, Chinese Jiang, Japanese “Miso”, “tempeh”, “natto” and “sufu” (Liu, 1997).

Soy milk, as an alternative to dairy milk, provides adequate proteins, iron, unsaturated fatty acids and other nutrients but contains low fat, carbohydrates and calcium as well as no lactose or cholesterol. Soy milk is better for persons who are dairy milk intolerant and people in areas where the supply of animal milk is insufficient. Although soy milk is a healthy food, calcium content in it is too low to achieve the standard of the recommended daily allowance (RDA) of calcium dietary intake. The calcium amount in a typical soy milk, cow’s milk and human milk is 15, 100 and 35 mg/100 g, respectively (Liu, 1997). Chaiwanon et al. (1999) mentioned that 100 ml cow’s milk and soy milk contains 120 and 20 to 30 mg calcium. Prabharaksa et al. (1989) stated that the calcium content in soy milk (20 mg/100 ml) is approximately one-sixth of the calcium in cow’s milk (120 to 125 mg/ml) and a little more than a half of calcium in human milk (34 mg/100 ml). The RDA recommends 800 and 1200 mg of calcium per day for children and adults in the United States (USAID, 1989). Institute of Medicine suggests that calcium daily intake for children 4 to 8 years and 9 to 18 years old should be 800 and 1300 mg, while for adults 19 to 50 years and over 51 years old is 1000 and 1200 mg. Milk and milk products can confer over 40% of calcium intake to adults as the major source of calcium in daily diet. Sufficient calcium intake is able to achieve peak

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bone mass (PBM) and reduce the risk of hypertension, colon cancer, kidney stones, lead absorption as well as reduce losses of bone mineral and prevent osteoporosis (Singh et al., 2006; Theobald, 2005). Moreover, calcium is vital for bone health and normal growth and development of teeth, muscle and enzyme functions as a key mineral in the human body (Gerstner, 2003). Therefore, soy milk is usually fortified with calcium salts to avoid its calcium deficiency and improve its nutritional value. In addition, Lacey et al. (2004) believed that soy milk fortified with calcium is able to enhance calcium and vitamin D nutrition, which is beneficial for the health of people, particularly students, who are allergic to lactose or who are sun-avoiders.

Calcium salts include inorganic salts such as calcium carbonate, calcium chloride, calcium phosphate and organic salts such as calcium lactate, calcium gluconate, calcium lactate gluconate and tricalcium citrate. In this study, calcium chloride, calcium lactate and calcium gluconate are selected as calcium fortifiers to fortify soy milk. Calcium chloride contains a high calcium amount, that is, 36% calcium, and shows high solubility properties, but its taste is bitter and salty. Calcium lactate and calcium gluconate are highly soluble and neutral in taste and flavor but contain comparably low calcium, which are, 13 and 9% calcium, respectively. Nevertheless, calcium lactate may display adverse effect on certain sensory properties, like becoming bitter when it is used in high concentrations. Calcium gluconate has low water content, neutral taste properties and good dissolution ability. Considering economic factors and bioavailability, inorganic calcium salts like calcium chloride have lower price and higher calcium amount but have lower nutrient effectiveness than organic salts including calcium lactate and calcium gluconate (Gerstner, 2003, 2004). Ranjan et al. (2005) stated that milk fortified with calcium gluconate had the highest heat stability and bioavailability of calcium.

A significant problem caused by calcium fortification of soy milk is calcium-induced coagulation process. Soy protein is highly sensitive to calcium ions and may precipitate after addition of calcium to soy milk. Coagulation may occur when Ca-fortified soy milk is heat processed and even happen without heat treatment as calcium may react with protein to reduce the stability of soy milk. Similar destabilization problem has been reported and described by Singh et al. (2006), Pathomrungsiyounggul et al. (2007, 2009), Yazici et al. (1997), Mangino and Hansen (1997) and Ono et al. (1993). Consequently, process conditions must be carefully controlled as the addition of calcium salts can destabilize food system and soybean protein molecules. Gerstner (2003) also stated that, it is difficult to achieve higher amounts of calcium in calcium fortified products with no control of pH or chelating agents added. In order to reduce or eliminate coagulation and precipitation effect, chelating agent and stabilizing agent are often used in combination with calcium salts to enhance stability of Ca-fortified soy milk. Chelating agents are very important for stabilizing food via stable complexes formation between sequestrants and metallic and alkaline earth ions reactions. Chelating agents used for calcium fortification of soy milk include sodium hexametaphosphate (SHMP), disodium hydrogen orthophosphate (DSHP), tri-sodium citrate (TSC) and disodium salt of ethylenediamine tetraacetic acid (EDTA-Na_2) (Lindsay, 1996; Pathomrungsiyounggul et al., 2009). This study only uses tri-sodium citrate (TSC) as a chelating agent to stabilize Ca-fortified soy milk to prevent coagulation. According to Pathomrungsiyounggul et al. (2009), TSC has good effect on producing stable soy milk fortified with calcium lactate through binding to calcium ions.

Heat treatment condition for sterilization of Ca-fortified soy milk is 121°C for 15 min. This process is carried out to kill pathogenic microorganisms, ensure food safety and prolong the shelf life of products. However, McKinnon et al. (2008) stated that chemical compositions such as proteins in milk may have obvious changes during heat treatment. α-Lactalbumin, β-lactoglobulin, the immuneglobulins and bovine serum albumin become unfolding and reacting with casein to form heat-induced protein coagulation when milk is heated above 65°C. Moreover, the stability of the casein micelles, the pH values and the quantity of the casein in the supernatant reduce with calcium ions been added to heated milks. The solubility and heat stability of milk powder are influenced both by the heat treatment and calcium ions addition (Vyas and Tong, 2004). Furthermore, several studies have described how heat treatment influences physical and chemical properties of soy milk. Results indicate that pH of soy milk decreases from 7.5 to 6.0 after sterilization (Pathomrungsiyounggul et al., 2009). However, there is little report on the effect of sterilization on the properties of Ca-fortified soy milk. This study uses dialysis to evaluate how pH and ionic calcium changed with temperature in soy milk. Dialysis was used to measure pH and Ca ion concentration in milk at heat treatment temperatures in the region of 80 to 120°C in a study. It was found that pH and ionic Ca decreased significantly when milk was heated at high temperatures. However, their values increased again during cold storage (Pathomrungsiyounggul et al., 2009). Therefore, the measurement of pH and ionic calcium at sterilization conditions plays an important role in evaluating heat stability.

The objective of this study was to use 25 mM calcium chloride, calcium lactate and calcium gluconate to fortify soybean milk which will have similar calcium contents as cow's milk. Appropriate concentrations of tri-sodium citrate are added to prevent coagulation and enhance heat stability of Ca-fortified soy milk so that heat-stable products can be obtained. Calcium ion concentration, pH and coagulation conditions of Ca-fortified soy milk are analyzed before and after sterilization. Dialysis is also

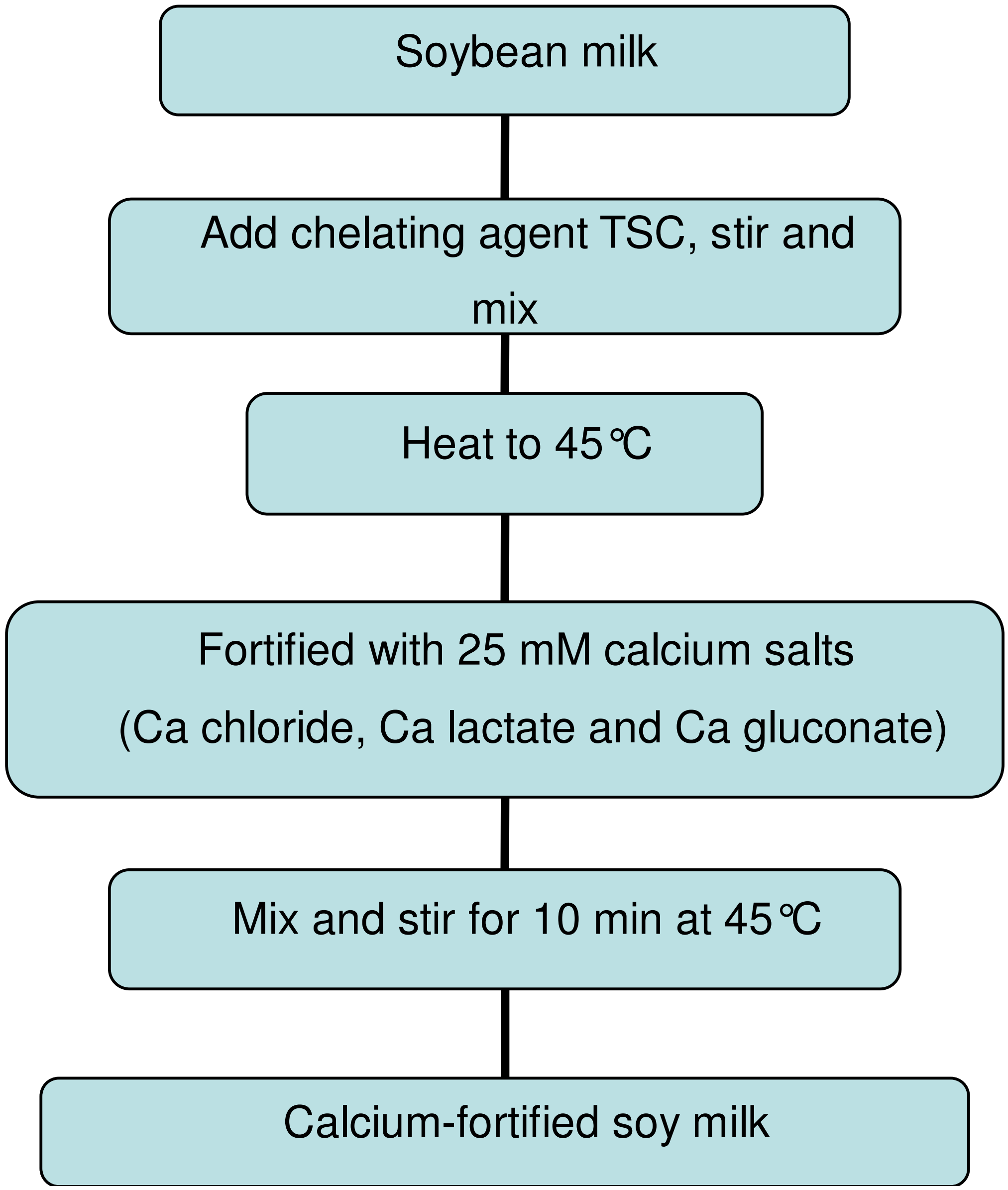

Figure 1. Preparation procedures of calcium-fortified soybean milk.

applied to investigate the effect of the additives on soy milk stability and properties at heating temperature.

MATERIALS AND METHODS

Unsweetened soya-bean milk was purchased from TESCO supermarket in Reading, England. The following calcium salts were used: Calcium chloride ($CaCl_2{\cdot}2H_2O$, 147.02 g/mol, BDH Laboratory Supplies, U.K.), calcium L-lactate ($[CH_3CH(OH)COO]_2Ca{\cdot}xH_2O$, 218.22 g/mol, SIGMA Ltd., U.K.) and calcium gluconate ($C_{12}H_{22}CaO_{14}{\cdot}H_2O$, 448.35 g/mol, Laboratory reagent grade, Fisher Scientific Ltd., U.K.). TSC (tri-sodium citrate, $Na_3C_6H_5O_7{\cdot}2H_2O$, 294.10 g/mol, Fisher Scientific Ltd., U.K.) was used as a chelating agent as well as a stabilizer to improve the heat stability of Ca-fortified soy milk.

Preparation of Ca-fortified soy milk

Soy milk, with the addition of different concentrations of TSC, was heated to 45℃ on a heated magnetic stirrer. Calcium chloride, calcium lactate and calcium gluconate were then added into soy milk separately at a concentration of 25 mM. TSC was added at concentrations of 0.2, 0.8, 1.0, 1.1 and 1.2% (wt/wt) into Ca chloride fortified soy milk, 0.2, 0.6, 0.7 and 0.8% (wt/wt) into Ca lactate fortified soy milk and 0.2, 0.7, 1.3 and 1.4% (wt/wt) into Ca gluconate fortified soy milk. The mixtures were stirred for 10 min on the heated magnetic stirrer at a constant temperature of 45℃ (Figure 1). Each Ca-fortified soymilk was divided into three parts. The first part was sterilized at 121℃ for 15 min via retorting. The second portion was sterilized at the same condition with dialysis bags which were filled with 10 ml deionized water. The samples used for sterilization were put into baby cans and each can contained approximately 100 ml of soy milk. After heat treatment,

Table 1. Thresholds of coagulation of Ca-fortified soy milk.

Calcium fortifier	Coagulation	No Coagulation
Calcium Lactate fortified soymilk	0.6% TSC*	0.7% TSC
Calcium Chloride fortified soymilk	1.1% TSC*	1.2% TSC
Calcium Gluconate fortified soymilk	1.3% TSC*	1.4% TSC

* Sample showed weak coagulation; TSC, tri-sodium citrate.

Table 2. Effect of TSC on pH and [Ca^{2+}] of soy milk fortified with calcium lactate.

TSC conc. (%)	Ph			Ca ion concentration (mM)		
	Before sterilization	With dialysis	After sterilization	Before sterilization	With dialysis	After sterilization
0 (control)[a]	6.96	6.48	6.91	-	-	-
0 (control)[b]	6.01**	5.61	6.13**	2.53**	1.96	2.38**
0.2	6.22**	5.80	6.21**	2.00**	0.48	1.58**
0.6	6.56*	6.06	6.57**	0.36*	0.22	0.32**
0.7	6.64	6.10	6.64*	0.25	0.083	0.21*
0.8	6.84	6.26	6.84	0.16	0.0062	0.14

Each result was an average of three replicated experimental readings. **Sample showed strong coagulation;*sample showed weak coagulation; [a]control sample was soy milk without any additives; [b]control sample was Ca-lactate fortified soy milk without TSC addition.

samples were cooled to room temperature and stored in fridges for analysis. The remaining part of Ca-fortified soy milk was without sterilization. Control samples without any treatment or additives addition and control Ca-fortified soy milk without stabilizers were also prepared and analyzed. All samples were prepared in triplicate.

Analysis of samples

Ionic calcium concentration and pH values of all samples were analyzed and coagulation conditions were examined visually. Each parameter was tested in triplicate. Samples with weak and strong coagulation were all centrifuged before analysis.

Ionic calcium concentration of samples was determined by using a Ca^{2+}/pH analyzer (Model 634, Ciba Corning Diagnostics, Essex, U.K.) according to the description by Lin et al. (2006). The millivolt (mV) values of standard solutions were measured directly from the analyzer to obtain a calibration curve (log Ca ion concentration as a function of mV). Seven ionic Ca standards were used: 0.25, 0.50, 1.00, 1.50, 2.00, 2.50 and 3.00 mM. The analyzer was calibrated each time before measurement of samples. The pH of samples was measured by a pH meter (Model 420A, Orion Research Inc., U.S.A.) at 25°C.

RESULTS AND DISCUSSION

Effect of tri-sodium citrate concentration on heat stability of Ca-fortified soy milk

Coagulation was found in Ca-fortified soy milk without tri-sodium citrate addition. TSC was added as a chelating agent to prevent coagulation. However, coagulation was still observed in some samples with calcium salts and TSC addition. Table 1 shows that, various concentrations of tri-sodium citrate were required to eliminate co-agulation of soybean milk fortified with different calcium salts. Soy milk fortified with calcium gluconate needed the most tri-sodium citrate amount (1.4% TSC) whereas soy milk fortified with calcium lactate needed the least tri-sodium citrate concentration (0.7% TSC) and soy milk fortified with calcium chloride needed 1.2% TSC to avoid coagulation. It was indicated that TSC was the most effective chelating agent to improve stabilization of soy milk fortified with Ca-lactate.

Effect of tri-sodium citrate on pH and Ca ion concentration of calcium fortified soy milk

Tables 2, 3 and 4 presents changes of pH and Ca ion concentration in calcium fortified soybean milk with different concentrations of tri-sodium citrate addition. It was found that calcium salts addition gave rise to strong coagulation in soy milk and significantly decreased the pH and increased calcium ion concentration of products both before and after sterilization ($P < 0.05$). However, with the increase of TSC addition, pH progressively went up and ionic calcium concentration gradually decreased. Similar results have been reported by Yazici et al. (1997), Pathomrungsiyounggul et al. (2007, 2009). Moreover, control samples which were fortified with calcium salts but without TSC and samples with little TSC added showed strong coagulation. Yazici et al. (1997) also found that calcium-fortified soy milk was unstable and had poor heat stability unless sequestering agents were added. With the increase amounts of TSC addition, the certain

Table 3. Effect of TSC on pH and [Ca^{2+}] of soy milk fortified with calcium chloride.

TSC conc. (%)	pH			Ca ion concentration (mM)		
	Before sterilization	With dialysis	After sterilization	Before sterilization	With dialysis	After sterilization
0 (control)[a]	6.96	6.48	6.91	-	-	-
0 (control)[c]	5.80**	5.56	5.84**	4.51**	3.93	4.41**
0.2	5.87**	5.58	5.95**	4.08**	3.02	3.79**
0.8	6.56	6.01	6.54**	0.65	0.28	0.52**
1.0	6.64	6.05	6.62*	0.36	0.21	0.30*
1.1	6.69	6.14	6.72*	0.12	0.067	0.11*
1.2	7.16	6.39	7.10	-	-	-

Each result was an average of three replicated experimental readings. **Sample showed strong coagulation; *sample showed weak coagulation; [a]control sample was soy milk without any additives; [c]control sample was Ca-chloride fortified soy milk without TSC addition.

Table 4. Effect of TSC on pH and [Ca^{2+}] of soy milk fortified with calcium gluconate.

TSC conc. (%)	pH			Ca ion concentration (mM)		
	Before sterilization	With dialysis	After sterilization	Before sterilization	With dialysis	After sterilization
0 (control)[a]	6.96	6.48	6.91	-	-	-
0 (control)[d]	6.17**	5.48	6.09**	2.99**	2.20	2.68**
0.2	6.22**	5.66	6.14**	2.89**	1.64	2.60**
0.7	6.50*	6.10	6.46**	0.49*	0.23	0.44**
1.3	6.80	6.25	6.61*	0.14	-	0.14*
1.4	6.93	6.48	6.86	0.10	-	0.063

Each result was an average of three replicated experimental readings. **Sample showed strong coagulation; *sample showed weak coagulation; [a]control sample was soy milk without any additives; [d]control sample was Ca-gluconate fortified soy milk without TSC addition.

level, the coagulation disappeared.

Coagulation is undesirable in Ca-fortified soy milk products and it can be inhibited by the addition of tri-sodium citrate. Two mechanisms of protein aggregation including isoelectric precipitation and calcium precipitation can give rise to coagulation (Lee and Rha, 1978). Protein molecules often precipitate at their isoelectric points (pI) as they are insoluble at this pH. Coagulation may occur when the pH values of Ca-fortified soy milk are in a region near the pI (4.2) of soy protein (Liu et al., 2004). Therefore, coagulation could be reduced by increasing pH in order to make pH of Ca-fortified soy milk to be far away from the pI of soy protein. Calcium-induced coagulation is mainly because of the cross-link between calcium ions and the anionic groups of soybean protein molecules (Lee and Rha, 1978). According to Pathomrungsiyounggul et al. (2009), the coagulation process contains the heat-induced denaturation of protein and calcium ions promoting hydrophobic coagulation. The authors stated that 25 mM calcium chloride addition to soy milk led to a clear precipitation of soybean protein in control samples (without TSC). A good curd could be formed if the calcium content was in the range of 10 to 100 mM. Soy curd formation involves two main storage proteins, that is, 7S and 11S globulin. Calcium ions can bind with soy protein and phytate in soy milk. Interaction between calcium ions with protein particles, fat globules and soluble proteins forms the coagulation of soy milk (Pathomrungsiyounggul et al., 2007). According to Ono et al. (1993), the proteins in soy milk contain particulate proteins and soluble proteins. The soluble proteins precipitate at higher calcium concentrations than that of the particulate proteins. This precipitation occurs at higher pH with the presence of calcium than the absence of calcium.

Tri-sodium citrate is an effective stabilizer to ensure heat stability of enriched soy milk with calcium salts. Singh et al. (2006) argue that tribasic sodium citrate was used to enhance heat stability of milk as a buffer salt and sodium citrate was used as a stabilizer for ultra-high temperature (UHT) treated skim milk with calcium fortifycation. Buffering agents such as disodium phosphate and/or citrates are necessary to be used to alleviate the effect of fortifying agents on pH of dairy products and the heat stability of milk protein molecules. Yazici et al. (1997) states that calcium gluconate, a partially ionized and soluble calcium salt, combined with chelating and stabilizing agents can achieve calcium fortification of

Table 5. Effect of Ca salts on pH and [Ca^{2+}] of soy milk.

Calcium Salt addition	pH			Ca ion concentration (mM)		
	Before sterilization	With dialysis	After sterilization	Before sterilization	With dialysis	After sterilization
0 (control)[a]	6.96	6.48	6.91	-	-	-
Calcium lactate**	6.01	5.61	6.13	2.53	1.96	2.38
Calcium chloride**	5.80	5.48	5.84	4.51	3.93	4.41
Calcium gluconate**	6.17	5.56	6.09	2.99	2.20	2.68

Each result was an average of three replicated experimental readings. **Sample showed strong coagulation; [a]control sample is soy milk without any additives.

soy milk with less heat stability problems.

The effect of tri-sodium citrate on pH and Ca ion concentration of soy milk fortified with calcium lactate, calcium chloride and calcium gluconate was similar. No matter the amount of calcium salts added, the trend of changes in pH and ionic calcium contents was the same. Increase in TSC addition caused a rise of pH and a reduction of Ca ion concentration. A recent study by Pathomrungsiyounggul et al. (2009) showed that in cow's milk, an increase in TSC, SHMP and DSHP amounts also resulted in a drop of Ca ion concentration. In soy milk and raw cow milk, pH went up with increase in the levels of chelating agent DSHP and TSC addition. Their previous study also found that the formation of a Ca-phosphate complex derived from the combination of phosphate of SHMP and calcium is a possible reason for the drop of ionic calcium in calcium fortified soy milk after SHMP addition (Pathomrungsiyounggul et al., 2007). They stated that, TSC can combine with calcium ions so that less hydrogen ions are released to soy milk. Consequently, pH increases and Ca ion concentration decreases with the addition of tri-sodium citrate. According to experimental results in this study, adequate tri-sodium citrate addition to calcium fortified soy milk could help pH recover to the value in the original soybean milk. Therefore, tri-sodium citrate had good effect on maintaining initial properties of soy milk.

Measurements of pH and calcium ion concentration in dialysis bags have shown changes in the properties of Ca-fortified soy milk at high temperatures. It was found that both pH and Ca ion concentration markedly decreased when Ca-fortified soy milk were sterilized. However, the values went up to near their original amounts after cooling. According to Lin et al. (2006), ionic calcium and pH of milk reduced immediately after sterilization but recovered after cooling. Chaplin and Lyster (1988) demonstrated that the loss of CO_2 gave rise to an initial increase in the pH of milk which was heated to a high temperature and then the pH reduced as a result of the Maillard reaction. A recent study by McKinnon et al. (2008) proves that pH of reconstituted skim milk decreased with increased temperature. When the skim milk was heated, the pH changed from 6.7 to 6.3 with the temperature changing from 25 to 80℃. They claimed that the pH at heating temperature rather than the initial pH is an important parameter to control the heat stability of milk and affect the heat-induced changes in milk. The pH during heating decreases with calcium addition and this drop significantly influences the reduction in heat stability of milk with calcium addition ($P < 0.05$).

Effect of calcium salts on pH and Ca ion concentration of soy milk

Table 5 summarizes how calcium salts including calcium chloride, calcium lactate and calcium gluconate influences pH and Ca ion concentration of soy milk. Experimental results demonstrate that calcium salts addition led to an apparent decrease in pH and an increase in calcium contents of soybean milk. It was not able to detect calcium content in original soy milk, however, calcium ions could be measured easily after calcium salt addition. Therefore, most of the Ca ions in Ca-fortified soy milk were derived from added calcium salts, which were also reported by Pathomrungsiyounggul et al. (2009). Singh et al. (2006) revealed that addition of calcium chloride, calcium lactate and calcium gluconate to milk gives rise to a drop of pH and strong coagulation of milk. Vyas and Tong (2004) believed that, changes in heat stability are correlative with changes in pH of reconstituted skim milk and heat stability decreases with the addition of calcium. This study results also demonstrate that calcium chloride had the most apparent effect on pH and calcium ion concentration of soy milk.

Influence of calcium salts on pH and ionic calcium of soy milk have been reported by several scientific studies and reasons for their changes have also been interpreted. Lin et al. (2006) stated that, the pH decreased and ionic calcium increased with calcium chloride addition. Shun-Tang et al. (1999) found that 14 mM calcium addition using calcium chloride decreased the pH of soy milk from 6.7 to 5.8. All these results have shown the same trend with this study result. There are two reported mechanisms to explain how hydrogen ions are released to soy milk to cause the reduction of pH (Pathomrungsiyounggul et al., 2009). The hydrogen ions and calcium ions have the same binding sites on the

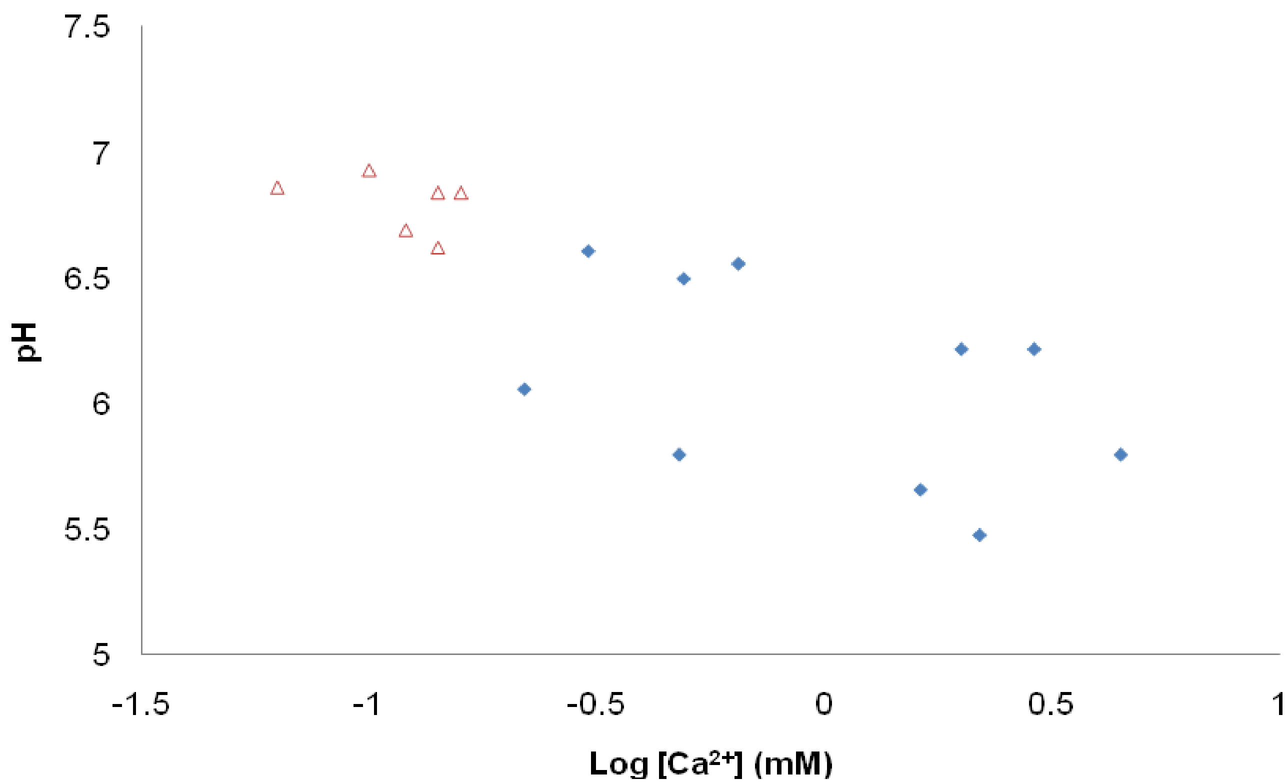

Figure 2. Relationship between log [Ca^{2+}] and pH. △ Non-coagulated and ◆ coagulated sample.

protein molecules. When calcium is added to soy milk, these two types of ions compete with each other to bind with protein, which results in the liberation of hydrogen ions and the pH decrease gradually. The side-chain of the imidazole group of histidine residues, the side-chain of the carboxyl groups of the aspartic and glutamic acid residues are all calcium binding sites with proteins. The existence of calcium ions gives rise to more dissociation of hydrogen ions (Kroll, 1984). Hydrogen ions are also released through the formation of Ca phytate complex which is produced by the interaction between calcium and phosphate group of phytate in soymilk. There is a linear relationship between the decrease in pH and the addition of calcium. Calcium phytate formation is considered as the main reason for pH drop upon the calcium addition (Ono et al., 1993). Therefore, Lin et al. (2006) recommended that both the addition of hydrogen ions and calcium salts are able to increase ionic calcium.

Relationship between Ca ion concentration and pH

Figure 2 presents the relationship between Ca ion concentration and pH. In order to better interpret their relationship, log Ca ion concentration instead of calcium ion concentration was explored as a function of pH. It was found that Ca ion concentration increased with a decrease in pH. This observation is similar to the results reported by Pathomrungsiyounggul et al. (2007, 2009).

According to Pathomrungsiyounggul et al. (2007), soy milk samples were susceptible to coagulation when their Ca ion concentrations were over 0.4 mM or pH was less than 5.74. Coagulation could be prevented if pH of soy milk was adjusted to a higher at 5.79 or Ca ion concentration was lower than 0.40 mM. However, the regions of coagulation are a little bit different in their other report. Pathomrungsiyounggul et al. (2009) stated that, soy milk did not coagulate when its pH was between 5.6 and 7.1 and its Ca ion concentration was from 0.28 to 1.12 mM. In this study, experimental results indicate that almost all non-coagulated Ca-fortified soy milk had log Ca ion concentrations below -0.80, that is, Ca ion concentration of 0.16 mM and coagulated products had ionic calcium concentrations exceeding -0.66 (or Ca ion concentration of 0.22 mM). No coagulation occurred when the pH of soy milk fortified with calcium was above 6.62 and samples were susceptible to coagulation when pH was lower than 6.61.

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