

Stabilization and sensory evaluation of calcium-enriched soymilk prepared using different chelating agents

^{1,2,3,*}Kaharso, V.C. and ^{2,3}Hua, Y

¹Department of Food Technology, Faculty of Agricultural Technology, Widya Mandala Surabaya Catholic University, Surabaya, East Java, Indonesia

²School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, People's Republic of China

³State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, People's Republic of China

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Abstract

It is generally acknowledged that calcium lactate (ca-lactate) fortification of soymilk to achieve the equivalent calcium level in cow's milk (1.2 mg/g) causes instability in soymilk. In order to produce acceptable calcium-enriched soymilk with great stability, three different kinds of chelating agents were added, namely tripotassium citrate (TPC), trisodium citrate (TSC), and sodium hexametaphosphate (SHMP). In this study, the addition of ca-lactate was found to reduce the pH (6.62 to 5.37) and increase the particle diameter (268.66 to 1,222.81 nm) and sedimentation (0.19 to 8.75%) of soymilk. Meanwhile, the addition of TPC and TSC produced calcium-enriched soymilks with smaller particle diameter (295.21-452.22 nm and 297.61-461.80 nm) and lower sedimentation levels (0.25-1.55% and 0.26-1.58%). The pH of calcium-enriched soymilk was also increased when both chelating agents were added. The application of SHMP as a chelating agent was not as effective as TPC and TSC because the particle diameter (527.98-703.40 nm) and sedimentation level were still relatively higher (4.24-5.68%) than the unfortified soymilk. Even though both TPC and TSC showed no significant difference ($P>0.05$) in stability, the sensory evaluation showed different results. Soymilk with TSC added was saltier and sourer than samples with TPC added. The study recommends the use of TPC as the chelating agent in soymilk enriched with ca-lactate.

1. Introduction

Calcium enrichment is a common technique applied to improve calcium content in food. Generally, calcium is fortified in beverages to fulfil the daily calcium intake of consumers, especially those who cannot consume dairy products. Some beverages that are normally enriched with calcium salts are mineral water, soymilk, energy drink, nectar, or juice (Gerstner, 2003). In order to produce a calcium-enriched product similar to cow's milk, soymilk is preferable since it has balanced nutrition and characteristics like cow's milk. However, calcium fortification in soymilk caused coagulation due to the interaction between calcium and soymilk protein (Prabharaksa *et al.*, 1989; Yazici *et al.*, 1997; Pathomrungrungsiyounggul *et al.*, 2007; Kaharso *et al.*, 2020). One possible calcium salt that can be used as a calcium source is calcium lactate (ca-lactate). Ca-lactate has excellent solubility and a bland taste (Gerstner, 2003; Trailokya *et al.*, 2017). The use of ca-lactate as the

calcium source in soymilk is still infrequent due to the challenges in maintaining soymilk stability upon calcium addition. Previous studies by Prabharaksa *et al.* (1989) and Pathomrungrungsiyounggul *et al.* (2010a) used ca-lactate as the calcium source in soymilk. However, both studies failed to maintain stability due to the coagulation after sterilization and pasteurization, respectively.

Chelating agent addition can help to stabilize soymilk upon ca-lactate addition due to their ability to link with metallic or alkaline earth ions and form stable complexes (Lindsay, 1996; Flora *et al.*, 2015). Several chelating agents that have been employed in calcium-enriched soymilk are tripotassium citrate (TPC) (Yazici *et al.*, 1997; Chaiwanon *et al.*, 2000), trisodium citrate (TSC) (Prabharaksa *et al.*, 1989), and sodium hexametaphosphate (SHMP) (Rasyid and Hansen, 1991; Pathomrungrungsiyounggul *et al.*, 2007). Based on those previous studies, chelating agents are usually added between 0.3 to 1.5% (w/v) and have been reported as

*Corresponding author.

Email: vckaharso@ukwms.ac.id

good stabilizers of calcium-enriched soymilk. However, the comparison among those chelating agents to stabilize soymilk with ca-lactate fortification has not been studied yet. The effect of different chelating agents on the sensory properties of calcium-enriched soymilk has also not been studied. Therefore, this study was aimed to determine the suitable chelating agents to stabilize calcium-enriched soymilk with ca-lactate as the calcium source. The effects of TPC, TSC, and SHMP on the stability and sensory properties of calcium-enriched soymilk were also investigated.

2. Materials and methods

2.1 Materials

Soybeans (*Glycine max*) Heinong 54 were harvested in 2020, purchased from a local market (Wuxi, Jiangsu, China), and stored in a room with a controlled temperature (25°C). A food-grade ca-lactate was purchased from Wangwang Biological Technology Co., Ltd., China. Chelating agents used in this study were TPC (general-purpose grade, Sinopharm Chemical Reagents Co., Ltd., China), TSC (general-purpose grade, Sinopharm Chemical Reagents Co., Ltd., China), and SHMP (food-grade, Zhejiang Yinuo Biological Technology Co., Ltd., China).

2.2 Preparation of calcium-enriched soymilk

Soymilk was prepared using our self-made laboratory equipment (anaerobic grinding equipment, Wuxi, Jiangsu, China). Calcium-enriched soymilks were prepared according to Kaharso *et al.* (2020). Soymilk was heated to 50°C before adding TPC, TSC, and SHMP at different concentrations of 1%, 1.2%, and 1.4% w/v, then stirred for 15 mins. Later, 0.8% w/v of ca-lactate was added to each sample to reach the equivalent level of calcium in cow's milk (1.2 mg/g) and stirred again for 15 mins at 50°C. The calcium-enriched soymilk samples with different chelating agents were then pasteurized in sterilized glass bottles at 95°C for 15 mins and cooled to room temperature. Unfortified soymilk (S) and calcium-enriched soymilk without chelating agent addition (CaS) were also prepared.

2.3 pH

pH was measured using a digital pH meter (FiveEasy Plus FE28, Mettler Toledo, China). The probe was immersed in each sample at 25°C and the results were expressed as pH units.

2.4 Effective particle diameter

The effective particle diameter was measured using dynamic light scattering (DLS; Nano Brook Omni, Brookhaven Instruments, USA). Samples were diluted

100 times before being tested at 25°C. Water was used as the dispersant, while protein was selected as the material (1.450 refraction index).

2.5 Dry sedimentation

Dry sedimentation was determined according to the modified method by Pathomrungsyounggul *et al.* (2007). Each sample was pipetted to a 50 mL polypropylene centrifuge tube and centrifuged for 10 mins at 25°C, 4200 rpm in a centrifuge (Himac CR21GII, Hitachi, Japan). The supernatant was discarded and the tube was placed upside down for 15 s to obtain wet sediment and remove the remaining supernatant. The wet sediment was then transferred to an aluminium round dish and dried at 105°C in a hot air oven until a stable weight was obtained.

2.6 Sensory evaluation

A total of ten trained panellists (6 males and 4 females) from Jiangnan University participated in the determination of the sensory attributes of the samples. The attributes tested were viscosity, sweetness, saltiness, bitterness, and sourness. The panellists' age ranged from 24 to 33. Samples were served in plastic cups and were coded with a random three-digit number. A scoring system of 1-10 points (Zhou *et al.*, 2019) was used, where 1 was defined as the lowest intensity of the attributes and 10 was the highest intensity. The reference standard for sensory evaluation is shown in Table 1.

2.8 Statistical analysis

All experiments were analyzed at least twice in triplicate and represented as mean values \pm SD. Statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., USA) and compared with Duncan Multiple Range Test (DMRT) at $P < 0.05$.

3. Results and discussion

3.1 Changes in pH of calcium-enriched soymilk

As shown in Figure 1, soymilk without the addition of calcium and chelating agents (S) has a pH of 6.62 and significantly decreased ($P < 0.05$) to 5.37 when calcium was added (CaS). The reduction of soymilk pH was caused by the liberation of hydrogen ions due to its competition with calcium ions in the same binding sites of soy protein molecules and the phosphate group in soymilk phytate (Kroll, 1984; Ono *et al.*, 1993). As the result of pH reduction, coagulation occurred since soy protein is coagulated when the pH is reduced to about 6.0 (Lu *et al.*, 1980). However, this pH reduction can be avoided by adding chelating agents. As presented in Figure 1, the three chelating agents used in this study (TPC, TSC, and SHMP) were found to significantly

Table 1. The reference standard of sensory evaluation

Attribute	Definition	Reference standard
Viscosity	The flow rate of approximately one teaspoon of fluid on the tongue	Water = 1 Sweetened condensed milk = 10
Sweetness	The typical basic taste of sucrose in water	1% sucrose solution = 2 5% sucrose solution = 10
Saltiness	The alkali taste exhibited by sodium chloride	2% NaCl solution = 10
Bitterness	The fundamental taste factor of caffeine in water	0.1% caffeine solution = 2 0.5% caffeine solution = 10
Sourness	The acid taste exhibited by citric acid	1% citric acid solution = 10

Attributes definition by Navicha *et al.* (2018). The reference standard was adopted from Drake *et al.* (2003), Meilgaard *et al.* (1999), and Russell *et al.* (2006).

increase ($P < 0.05$) soymilk pH compared to the CaS sample. The pH increases because chelating agents link with calcium ions and form complex ring-like structures called chelates (Lindsay, 1996; Flora *et al.*, 2015) that fewer hydrogen ions are liberated from the protein molecules and phytate (Pathomrungsyounggul *et al.*, 2007).

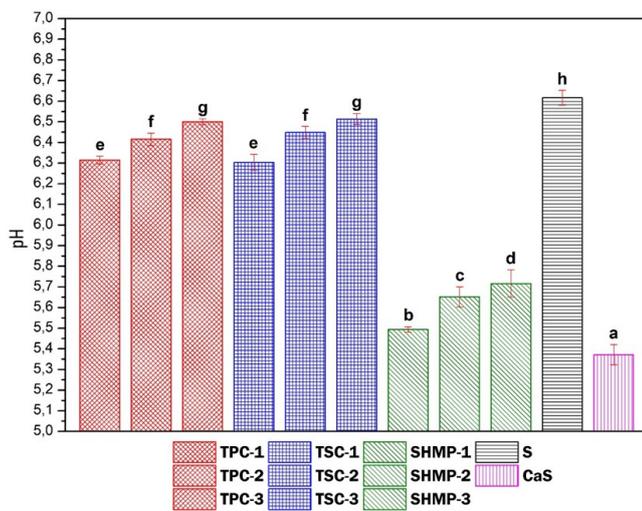


Figure 1. pH of soymilk and calcium-enriched soymilk prepared using different chelating agents

The addition of TPC and TSC showed a greater increase in soymilk pH because both salts are classified as citrate salts (alkaline salts) that are commonly used for pH control in the food industry (Lindsay, 1996). These results were in agreement with several studies conducted by Yazici *et al.* (1997) and Pathomrungsyounggul *et al.* (2010b). Compared to each other, there was no significant difference ($P > 0.05$) in the pH values between TPC and TSC enriched soymilk; this indicates that TPC and TSC have a similar effect as a chelating agent. Meanwhile, the use of SHMP was not as effective as TPC and TSC because the pH of soymilk was still lower than 6.0 (5.49-5.72). Similar results were also reported by Pathomrungsyounggul *et al.* (2010b), where the addition of 1% SHMP was found to slightly increase the pH of soymilk from 5.66 to 5.92. These results contradict those reported by Yazici *et al.* (1997) since the addition of 0.3 – 1.5% SHMP reduced the pH of soymilk from

7.31 to a pH lower than 7.0. It indicates that the use of SHMP as the chelating agent caused varied results. The pH can be increased and also decreased, depending on the initial pH of the sample.

3.2 Stability of calcium-enriched soymilk

In this study, the particle diameter and sedimentation were measured to identify the stability of soymilk upon calcium addition. As shown in Table 2, the particle diameter and sedimentation were significantly increased ($P < 0.05$) from 268.66 to 1,222.81 nm and 0.19 to 8.75%, respectively. These increments were due to the interaction between calcium and protein, resulting in curd formation. As explained by Ono (2003), curd formation is related to the behaviour of protein particles, oil globules, and soluble protein in soymilk. When calcium salt is added to soymilk, calcium ions neutralize the negatively charged protein and the hydrophobic interaction between neutralized protein molecules becomes more predominant (Liu, 1997). The calcium-protein interaction caused the reduction of electrostatic repulsion between molecules (Horne and Dalgleish, 1980). The protein molecules combined with oil globule, then the bound globules aggregate with each other while

Table 2. The stability parameters of soymilk and calcium-enriched soymilk prepared using different chelating agents

Samples	Effective particle diameter [nm]	Sedimentation [% sediment]
S	268.66±4.44 ^a	0.19±0.02 ^a
CaS	1,222.81±58.46 ^h	8.75±1.58 ^c
TPC 1	452.22±10.20 ^d	1.55±0.12 ^b
TPC 1.2	331.92±15.96 ^c	0.61±0.10 ^{ab}
TPC 1.4	295.21±10.16 ^b	0.25±0.13 ^a
TSC 1	461.80±17.22 ^d	1.58±0.04 ^b
TSC 1.2	335.81±20.91 ^c	0.59±0.03 ^{ab}
TSC 1.4	297.61±10.77 ^b	0.26±0.10 ^a
SHMP 1	703.40±15.08 ^g	5.68±0.44 ^d
SHMP 1.2	603.14±9.75 ^f	5.00±0.97 ^{cd}
SHMP 1.4	527.98±22.32 ^d	4.24±0.98 ^c

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different ($P < 0.05$).

carrying round water (Ono, 2003), resulting in the enlargement of particle diameter and curd formation. The increase in particle diameter and sedimentation level indicates instability of soymilk upon calcium addition.

The stability of calcium-enriched soymilks was increased when chelating agents were added, marked by the reduction of the particle diameter and sedimentation. The reduction of particle diameter and sedimentation was due to the interaction between chelating agents and calcium ions to form complex ring-like structures called chelates and caused the repulsive force among protein molecules hence decreasing aggregation (Lindsay, 1996; Pathomrungsyounggul, *et al.*, 2010a; Flora *et al.*, 2015). Similar to pH analysis results, TPC and TSC were more effective than SHMP. Both chelating agents decreased the sedimentation to be less than 1.6% and there was no significant difference ($P>0.05$) when compared with the S sample upon an increase in the concentration of chelating agents to 1.2% and 1.4%. The addition of 1% TPC and TSC significantly reduced ($P<0.05$) the particle diameter (452.22 and 461.80 nm) and continued to decrease when 1.2% (331.92 and 335.81 nm) and 1.4% chelating agent (295.21 and 297.61 nm) was added. The results showed that the higher the concentration of chelating agents, the smaller the particle diameter of the sample.

Simultaneously, the addition of SHMP also significantly decreased ($P<0.05$) the sedimentation and particle diameter in calcium-enriched soymilk, but was still significantly higher ($P<0.05$) than other samples with TPC and TSC added. A study about soy protein by Molina and Wagner (1999) could explain this. They presumed that the addition of citrate salt caused the dissociation of calcium-protein interaction due to its sequestering effect on cations, so fewer calcium ions are available for interaction with soy proteins. On the other hand, the presence of phosphate increases the turbidity of dispersion due to the formation of protein-calcium-phosphate aggregates, resulting in the decrease of dispersion stability.

3.3 Sensory evaluation

In this analysis, the samples tested were S samples and samples with TPC and TSC added, because other samples were not eligible for sensory testing. According to Figure 2, there were no significant differences ($P>0.05$) among all the samples in terms of sweetness and bitterness attributes. On the viscosity attributes, the most viscous sample described by panellists was TSC-1 (5.90), but there was no significant difference ($P>0.05$) when the results were compared to those of TPC-1 and TPC-2. However, results from both TPC-1 and TPC-2 were not significantly different ($P>0.05$) from other

samples, including the S sample. These results indicate that the addition of 1% TSC was not effective enough to produce soymilk with viscosity similar to the initial soymilk. Furthermore, the addition of TPC and TSC increased the saltiness of calcium-enriched soymilk. This observation is obvious because both salts are known to have a saline taste (Doores, 2011; National Center for Biotechnology Information, 2021). The highest intensity of saltiness was observed on samples with the addition of TSC, while samples with TPC added were significantly ($P<0.05$) less salty. This result indicates that TPC has lower salinity since potassium salt is generally used to replace sodium salt in low-or no-salt products (U.S International Trade Commission, 2020). The addition of both chelating agents also increased the sourness in calcium-enriched soymilk. However, the highest intensity was detected on samples with TSC added. A report by the US International Trade Commission (U.S International Trade Commission, 2020) reported that TSC produces an acidic taste so this might be the cause for the higher increment in sourness attributes.

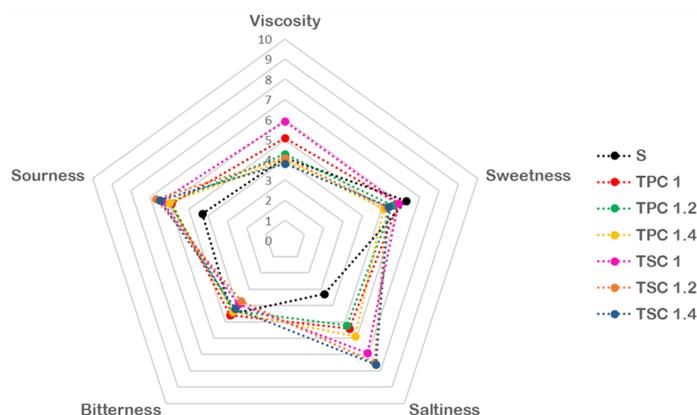


Figure 2. Sensory evaluation of calcium-enriched soymilk prepared using different chelating agents

4. Conclusion

Calcium-enriched soymilk can be stabilized by using different kinds of chelating agents, namely TPC, TSC, and SHMP. Among those chelating agents, TPC and TSC showed greater effects on soymilk stability, marked by the reduction in particle diameter and dry sedimentation of soymilk after calcium addition. The addition of TPC and TSC also increased the pH of soymilk to 6.30 – 6.51, so the coagulation in soymilk can be avoided. However, the use of TSC increased the saltiness and sourness of soymilk, which might reduce consumers' preferences. Meanwhile, SHMP also showed similar effects on stability and pH, but the effects were not as effective as TPC and TSC. Thus, it is recommended to use TPC as the chelating agent due to its capability to stabilize calcium-enriched soymilk and fewer effects on the taste of calcium-enriched soymilk.

Conflict of interest

The authors declare no conflict of interest.

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