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Influence of Calcium Lactate and Tripotassium Citrate on the Production of Stable and Acceptable Calcium-Enriched Soymilk

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Authors' contributions

This work was carried out in collaboration among all authors. Author VCK helped in conceptualization, methodology, investigation and wrote the original draft of the manuscript. Author BM wrote, review & edited the manuscript and perform formal analysis. Author SS wrote, review & edited the manuscript and helps in analysis. Author YH helped in conceptualization, supervised and funding acquisition. Author CZ administered the project and help in searching resources. All authors read and approved the final manuscript.

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ABSTRACT

In the current study, calcium lactate (ca-lactate) was used as a calcium source and tripotassium citrate (TPC) as chelating agents to produce calcium-enriched soymilk with calcium content equivalent to cow's milk (120 mg/100mL). Physicochemical properties of calcium-enriched soymilk, including nutritional composition, pH, titratable acidity, particle diameter, sedimentation, viscosity, ion conductivity, and sensory evaluation were investigated. Our results showed significant differences ($P<.05$) in moisture, ash content, titratable acidity, and ion conductivity after calcium and TPC were added. Moreover, the addition of calcium decreased the pH of soymilk from 6.69 to 6.21-

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6.51. The higher concentration of calcium also increased the calcium content, particle diameter, sedimentation, and viscosity, while the reverse results were shown when TPC was added. The intensities perceived of mouthfeel, visual appearance, and overall acceptability were greatly varied among calcium-enriched soymilks. The exact proportion of ca-lactate and TPC were able to produce calcium-enriched soymilk regarding higher stability during storage and great acceptability of the final product.

Keywords: Calcium-enriched soymilk; calcium lactate; tripotassium citrate, storage.

1. INTRODUCTION

Soymilk is an aqueous liquid extracted from soybean, which has a balanced nutritive value and rich in isoflavones. It is also frequently considered as milk substitute for lactose intolerance patients, due to the lack of lactose and cholesterol [1,2]. However, despite the benefits of soymilk, it was also reported to having lower calcium content. Calcium is an essential mineral for the human body; responsible for developing bones, teeth, nerves, muscles, and enzyme functions [3]. The calcium amount in soymilk (25mg/100mL) which was reported to account about one-sixth of the calcium in cow's milk (120mg/100mL) and slightly lower than human breast milk (32 mg/100 ml) [4,5].

Over the last few decades, the direct addition of calcium salts has gained more attention as an effective method to improve the calcium content of soymilk. There are many calcium salts which are typically used as calcium sources in soymilk, both inorganic and organic calcium salts. Prior to selecting the appropriate calcium salts, some factors should be considered, such as calcium content, solubility, taste, and bioavailability [6]. The most common calcium salt used in beverages is calcium lactate (ca-lactate). It is an organic salt with high bioavailability, high solubility in liquid, and neutral taste [3,6].

Previous research by Prabharaksa et al. [5] used ca-lactate as a calcium source in soymilk, but they provided only 60% (74.7mg/100g) of calcium content in cow's milk and coagulated after a few days of storage. Pathomrungsiyounggul et al. [7] also tried to add ca-lactate in soymilk as the same concentration of cow's milk and coagulated right after pasteurization. Visible coagulation occurred when ca-lactate was added in soymilk because organic calcium salts contain a lower concentration of elemental calcium than other inorganic calcium salts [6], so it is harder to maintain the stability. Therefore, a suitable combination of chelating agents (CA) such as tripotassium citrate (TPC) is believed to improve stability; since citrate salts are known to have an excellent effect on the stability and characteristics of soymilk [8].

Specifically, this study aims to enhance the calcium of soymilk to be equivalent to cow's milk without changing its characteristics during storage. The acceptability of the calcium lactate enriched soymilk was also investigated because not many studies explored about this. It is expected that this current study can refine the previous study [5] in order to produce consumable stable calcium-enriched soymilk without compromising quality during storage time.

Table 1. List of abbreviations that appears in the manuscript along with their nomenclature

Abbreviations	Nomenclature
AOAC	Association of Official Analytical Chemist
CA	Chelating agents
Ca-lactate	Calcium lactate
DLS	Dynamic light scattering
DMRT	Duncan multiple range tests
ISO	International Organization of Standarization
TPC	Tripotassium citrate
TTA	Tritatable acidity

2. MATERIALS AND METHODS

2.1 Materials

Dry soybeans (Glycine max) were purchased from a local market (Wuxi, China) and stored in room with controlled temperature (25^{o}C). The calcium salt used was ca-lactate ($CaC_6H_{10}O_6$, food-grade), purchased from Wangwang Biological Technology Co., Ltd., China; TPC ($K_3C_6H_5O_7$, general-purpose grade, Sinopharm Chemical Reagents Co., Ltd., China) was used as a CA. Other reagents used in this study were analytical grade and purchased from Sinopharm Chemical Reagents Co. Ltd., China.

2.2 Preparation of Soymilk

Soybeans were washed three times using tap water then deionized water to remove physical contaminants. The cleaned soybeans were soaked in soybean: water with the ratio of 1:5 (w/w) for 10 hours at room temperature in a dark room. The swollen soybeans were sieved and rinsed with distilled water; then, ground with laboratory self-made oxygen-insulated grinding equipment [9]. Later, the raw soymilk was heated using a flash heating method at 120°C for 30 seconds to inhibit enzymatic oxidation reaction. The cooked soymilk was centrifuged with a high speed refrigerated centrifuge (Hitachi Himac CR21GII, Japan) at 5160 rpm for 15 min with controlled temperature (20°C).

2.3 Preparation of Calcium-Enriched Soymilk

Soymilk was heated to 45°C in a water bath before adding TPC and kept stirring using a heated magnetic stirrer (Pioway Medical 79-1, China) for 10 min at 50°C. Ca-lactate was then added to soymilk and kept as condition as above. The different formulations of ca-lactate and TPC are given in Table 2. A sample with neither ca-lactate nor TPC added was also prepared as a control. The calcium-enriched soymilk was pasteurized in sterilized glass bottles at 95°C for 15 minutes and cooled to room temperature. The samples were then stored at 4°C for 21 days.

2.4 Nutritional Composition

Association of Official Analytical Chemist (AOAC) Method [10] were used to determine moisture, protein, ash, calcium, and potassium content of calcium-enriched soymilk. Moisture content was determined using a hot air oven (105°C); protein content was determined by the Kjeldahl method using the conversion factor of 6.25; ash content was determined using a muffle furnace at 550°C (Chamber Electric Furnace, Shanghai Kang Lu Instrument Co. Ltd, China). Fat content was determined using chloroform-methanol extraction [11]. Carbohydrate content was determined by the difference of total weight with the sum of moisture, protein, fat, and ash content [12]. Both calcium and potassium content was determined using an atomic absorption spectrometer (Spectra AA 220, Varian, USA) reading at 422.7 nm and 766.5 nm of absorbance.

2.5 Particle Diameter

The mean particle diameter of samples was measured using dynamic light scattering (DLS), respectively (Nano Brook Omni, Brookhaven Instrument, USA). Each sample was diluted 100 times in deionized water and tested at 25°C. Water was selected as the dispersant, and protein (1.450 refraction index) was selected as materials.

2.6 Dry Sedimentation

Dry sedimentation in soymilk was determined according to the modified method by Pathomrungsiyounggul et al. [13]. A sample was pipetted to a 50 mL polypropylene centrifuge tube and centrifuged for 10 min at 4200 rpm in a centrifuge (Hitachi Himac CR21GII, Japan) with controlled temperature (25°C). The centrifuge tubes were put upside down before the supernatant was discarded to obtain wet sediment. Wet sediment was transferred to an aluminum round dish and then dried at 105°C in a hot air oven until the dry sediment's stable weight was obtained.

2.7 Viscosity

The viscosity of samples was measured using a controlled-stress rheometer (MCR 301, Anton Paar Tru Gap TM Instrument, Austria) equipped with parallel plate geometry (25 mm diameter, 1 mm gap). Shear rates from 0.1 to 300/s at temperature 25°C were used to measure shear stress of each sample. The model used to fit the flow behavior data was Power-law [14], represented by this following formula:

$$\eta = \frac{\sigma}{\gamma} = K(\gamma)^n$$

where η is the apparent viscosity (Pa.s), σ is the shear stress (Pa), γ is the shear rate (s-1), K is the flow consistency index ($Pa.s^n$), and n is the flow behavior index (dimensionless).

2.8 pH and Titratable Acidity

A digital pH meter (FiveEasy Plus FE28, Mettler Toledo, China) was used to measure pH of the sample at 25°C, and results were expressed as pH units. Titratable acidity (TTA) of samples was determined according to Tyl and Sadler [15]. The results were expressed as % lactic acid.

Table 2. The combination ratios of ca-lactate and TPC to produce calcium-enriched soymilk

Sample codes	Calcium lactate (% w/v)	Tripotassium citrate (% w/v)	Remarks
CS-0	0	0	Control
CS-1	0.8	1	Calcium-Enriched Soymilk
CS-2	0.8	1.2	Calcium-Enriched Soymilk
CS-3	0.8	1.4	Calcium-Enriched Soymilk
CS-4	1	1	Calcium-Enriched Soymilk
CS-5	1	1.2	Calcium-Enriched Soymilk
CS-6	1	1.4	Calcium-Enriched Soymilk

2.9 Ion Conductivity

Ion conductivity of soymilk samples was determined using an electric conductivity (DDS-307, Shanghai Tianda Instrument Co. Ltd., China) at 25^{o}C. The probe was immersed in each sample until a fixed value was observed. The results were expressed as ms/cm.

2.10 Sensory Evaluation

Quantitative descriptive analysis was used to describe the gustatory properties of calcium-enriched soymilk products acceptable to the consumers. The ISO standard [16] was applied regarding the qualification level, selection, and panelist's skills. Each panelist held a similar qualification level and selected after involving in training from a group of experts. This sensory evaluation was evaluated by fifteen trained panelists (nine males and six females) from Jiangnan University who are familiar with soymilk. The age of panelists is between 20 and 35 years old. Six samples of calcium-enriched soymilk and control soymilk were prepared for assessment as described above. Each panelist was requested to evaluate the mouthfeel, flavor, visual appearance, and overall acceptability of every sample. Soymilk samples were dispensed in plastic cups and coded with three-digit random numbers. The evaluation data were recorded and calculated mean scores of each attribute compared with samples. The 9-hedonic scales [17] with anchor point 1-dislike extremely and 9-like extremely was used to describe the panelist's preferences.

2.11 Statistical Analysis

All experiments were carried out at least twice in triplicate. The data were analyzed using SPSS for Windows (version 19.0, SPSS Inc., IL). The data shown are represented as means values ± standard deviations and were compared using Duncan multiple range tests (DMRT) at $P \leq .05$.

3. RESULTS AND DISCUSSION

3.1 Main Nutritional Composition of Calcium-Enriched Soymilks

The nutritional composition of control and calcium-enriched soymilk are shown in Table 3. The result showed that the addition of ca-lactate influenced the moisture and ash content of various soymilk samples. The moisture content of CS-0 (89.78%) was significantly higher (P<.05) compared to other samples with added calcium (88.10-88.60%). On the other hand, it shows the opposite results on ash content, where the ash content of the control sample (0.47%) is the significantly least (P<.05) than other samples (1.05-1.23%). Similar findings were also reported by Yazici et al. [18], where calcium-fortified soymilk has lower moisture content and higher ash content than control soymilk. The increase in ash content is probably due to the direct addition of ca-lactate and TPC, as reported by Irshad et al. [19]. No marked change was identified for carbohydrate, fat, and protein content from each sample, even though the results were quite varied.

In this study, the addition of calcium lactate as a calcium source was able to enhance the calcium content of soymilk to be equivalent to cow's milk (120 mg/100 g). As shown in Table 3, the addition of 0.8% ca-lactate significantly increased (P<.05) the calcium content in soymilk (120.02 to 121.60 mg/100 gr), while 1% ca-lactate increased the calcium content in soymilk to be more than calcium content in cow's milk (146.35–153.82 mg/100 g). However, the more calcium added makes the soymilk more unstable, so it is required to add more TPC as CA to maintain its stability. There was a significant difference (P<.05) in potassium content between control

(130.11 mg/100 g) and other samples (185.78-287.34 mg/100 g). Potassium is another essential mineral that has an essential role in electrolyte regulation, nerve function, muscle control, and blood pressure; the required daily intake of potassium for an adult (either male or female) is 4700 mg/day [20]. However, excess amounts of potassium are harmful to those who are unable to secrete it or have kidney problems, which may cause muscle weakness, stomach pain, irregular heartbeat, or even heart problems [20]. The addition of TPC as CA needs to be controlled not to harm human health because CA may not only bind calcium but also react with other metal ions, such as magnesium and iron [8].

3.2 Particle Diameter

Based on the results present in Fig. 1, it was found that there was a significant difference (P<.05) between each sample. The CS-0 sample has the lowest mean particle diameter (243.69 nm), while the addition of calcium increases the particle diameter. By adding calcium in soymilk, Ca^{2+} binds with soy protein (β-lactoglobulin and α-lactalbumin) and reduces electrostatic repulsion between molecules and increases the possibility for intermolecular association and aggregation between protein and oil droplets [21-23]. As a result, the quaternary structures of β-lactoglobulin and α-lactalbumin may have changed from original to more associated structures, before the adsorption on the droplet surface occurs [24]. The transport of the protein to the interface may be affected and possibly not undergo the protein spreading and rearrangement effectively; therefore, some smaller oil droplet which has higher surface free energy coalesced into larger droplets, since the lower amount of protein being available to cover the new surface area of droplets [24].

The addition of CA improved the stability by lowering particle diameter, which was enlarged due to the addition of calcium; increasing the concentration of CA led to the reduction in particle diameter, which was also reported by Pathomrungsiyounggul et al. [13]. This reduction indicating that sequestering Ca^{2+} with CA avoided droplets to form aggregation by reducing the availability of calcium to promote instability in soymilk [25] and caused the repulsive force of negatively charged protein molecules to become predominant [26]. The duration of storage also has a significant effect (P<.05) on the particle diameter of each sample. After seven days of storage, the particle size increased and showed a more considerable increase on the 21st day of storage, which indicates the instability of soymilk emulsion. This probably due to more interaction between droplets during storage and led to higher aggregation formation, resulting in a higher increase in soymilk particle diameter.

3.3 Dry Sedimentation

In this study, dry sedimentation was measured as an indicator of the stability of calcium-enriched soymilk by applying a centrifugal force (Fig. 2). For CS-2 and CS-3 samples, the sedimentation rates were 0.53% and 0.29%, respectively and has no significant different (NS) with control sample (0.19%). Pathomrungsiyounggul et al. [13] stated that a low dry sediment level indicates good stability, while about 5% indicates destabilization and led to coagulation. Coagulation was only shown in the CS-4 with 6.00% of dry sedimentation. The direct addition of ca-lactate led to the interaction between calcium and soy protein, resulting in ca-protein complex formation, which causes coagulation in soymilk [27]; the presence of citrate as polyanion stabilized the protein aggregation by competing with other anion and avoid aggregation to occur [28]. Almost all samples except sample with CS-4, remained stable with sedimentation value lower than 5%. The sedimentation value of nearly all samples was showed significantly (P<.05) increased during the 21st day of storage, excluding CS-5. The above results indicate the desired conditions to minimize sedimentation to achieve maximum stability in calcium-enriched soymilk during storage.

3.4 Ion Conductivity

Ion conductivity or electric conductivity is used to measure the ability of substances to transmit electric charge and generally expressed in Siemens per centimeter (S/cm) [29]. Fig. 3 shows the ion conductivity of control and calcium-enriched soymilks during storage. The ion conductivity of the CS-0 sample was 1.85 ms/cm lower than other samples. A significant increase in ion conductivity was observed when ca-lactate added since calcium is a divalent anion that can transmit electric charge. Rasyid and Hansen [30] were reported to found a similar finding; the addition of calcium-n-saccharic acid increased ionic strength. A study by Jensen and Unangst [31] was found that ca-lactate has moderate ionic conductivity (3.79 ms/cm) in 0.1 N concentration, compared to other calcium salts which generally uses in beverages, such as calcium chloride (8.62 ms/cm) and calcium gluconate (2.52 ms/cm). Higher ionic conductivity results may also be affected by calcium salts solubility since ca-lactate also has excellent solubility. The addition of TPC also improved the ionic conductivity of calcium-added soymilks. This arises occurred because of the ionic nature of TPC, which increases conductivity in an aqueous medium [27]. The ionic conductance of some samples was found to increase during seven days of storage. This increase may occur because of the degradation of chemical components, such as protein [27]. During 21 days of storage, ion conductivity of each sample was found to decrease; this was probably caused by a decrease in ion mobility due to an increase in samples viscosity, resulting reduction in ion ability to transmit electric charge.

3.5 Viscosity

The viscosity of all samples was analyzed using a controlled stress rheometer, which in principle is the ratio of shear stress to shear rate. The viscosity results at shear rate ranging from 0.1 to 300 s^{-1} during 21 days of storage are shown in Fig. 4. The addition of calcium increased the viscosity of calcium-enriched soymilk throughout the shear rate of 0.1-300 s^{-1}. An increase in viscosity as the concentration of calcium increased was also found in the previous study by Prabharaksa et al. [5]. The increased viscosity in calcium-enriched soymilk was caused by calcium-protein interaction, which led to curd formation and coagulation. In this study, coagulation occurred after pasteurization, consistent with Kinsella [32] statement, where heating protein dispersion to pasteurization temperature (70-80^{o}C) after the addition of calcium increased the viscosity. However, based on the results, increasing the concentration of TPC reduces the viscosity in soymilk samples. The same results were also reported by some researchers [18,27]; both studies used TPC as CA and successfully decreased the viscosity of soymilk after the addition of calcium lactogluconate and tricalcium phosphate, respectively. This was indicated that CA bind to Ca ions and decreased the extend of globules aggregation [25] to avoid viscosity increment. The viscosity of all samples at different shear rates was significantly increased (P<.05) when stored in 4^{o}C for 21 days. The increase probably occurred because samples show signs of spoilage, which is characterized by a decrease in pH and an increase in acidity, which led to the destabilization of soymilk samples.

All samples were found to have shear thinning behavior due to the disruption of protein-protein interaction caused by increasing shear rate, resulting in lower protein capability to bind water [14]. In order to understand the rheological behavior of calcium-added soymilk, data were fitted to power-law fluid, shown in Table 4. All samples, including control and calcium-added soymilk during storage, were found to have flow behavior index (n-values) less than 1 (0.08-0.26). This indicates that samples were typical non-Newtonian fluids (pseudoplastic), which were favorable properties for soymilk, since they influence the sensory properties, flavor release to food, high shelf stability of emulsifying and suspending [14]. The flow consistency index (K) of each sample were ranging from 0.07 to 4.83.

3.6 pH and TTA

According to the study results shown in Table 5, all samples with added calcium had lower pH than CS-0 (6.69). The addition of calcium significantly reduced (P<.05) pH in soymilk. This reduction possibly happened because the direct addition of calcium led to the competition between calcium ions with hydrogen ions to bind protein molecules [33] and the phosphate group of phytate in soymilk [34], resulting in the liberation of hydrogen ions. The reduction of pH below 6.0 led to precipitation of soy protein [35]. In order to prevent coagulation occurred, TPC was added to soymilk samples and significantly increased (P<.05) the pH of samples depending on the level of Ca added. On CS-1, CS-2, and CS-3 samples no significant difference (NS) are shown when TPC was added; but on CS-4, CS-5 and CS-6, all samples had a significant difference (P<.05). The increase in pH probably happened due to calcium ions combined with TPC, resulting in fewer hydrogen ions were liberated since fewer calcium ions bound with protein and phytate in soymilk [13].

TTA generally used to determine the total acid concentration in food and has an inverse correlation with pH. The TTA results are shown in Table 3. There was no significant difference (NS) among calcium-enriched soymilks but had a significant difference (P<.05) if compared with the CS-0. TTA of the control sample is 0.011%, and other samples are around 0.014-0.015%. This increase was probably due to the liberated hydrogen ions in calcium-enriched soymilk were titrated by NaOH, thereby increasing the acid concentration.

Table 3. Main nutritional composition of control and calcium-enriched soymilks

Samples	Moisture (%)	Carbohydrate (%)	Protein (%)	Fat (%)	Ash (%)	Calcium (mg/100 g)	Potassium (mg/100 g)
CS-0	89.78±0.57[b]	2.80±0.53[a]	4.04±0.13[a]	2.91±0.09[a]	0.47±0.14[a]	19.07±7.22[a]	130.11±26.42[a]
CS-1	88.60±0.43[a]	3.13±1.12[a]	4.05±0.21[a]	3.14±0.76[a]	1.08±0.16[bc]	121.60±8.55[b]	194.20±12.91[bc]
CS-2	88.50±0.62[a]	3.82±0.59[a]	3.80±0.38[a]	2.78±0.20[a]	1.11±0.09[bc]	120.02±14.34[b]	246.15±53.49[cd]
CS-3	88.18±0.51[a]	3.73±0.44[a]	3.98±0.49[a]	2.96±0.26[a]	1.15±0.09[bc]	120.78±3.99[b]	287.34±77.57[d]
CS-4	88.43±0.49[a]	3.82±0.50[a]	3.90±0.05[a]	2.80±0.08[a]	1.05±0.06[b]	150.10±15.90[c]	185.78±39.66[b]
CS-5	88.27±0.48[a]	3.85±0.50[a]	3.98±0.07[a]	2.80±0.06[a]	1.11±0.11[b]	153.82±14.21[c]	243.67±30.60[cd]
CS-6	88.10±0.48[a]	3.69±0.56[a]	3.95±0.11[a]	3.04±0.22[a]	1.23±0.11[d]	146.35±6.90[c]	272.64±33.99[d]

Means ± standard deviation in the same column followed by the different letter are significantly different (P<.05)

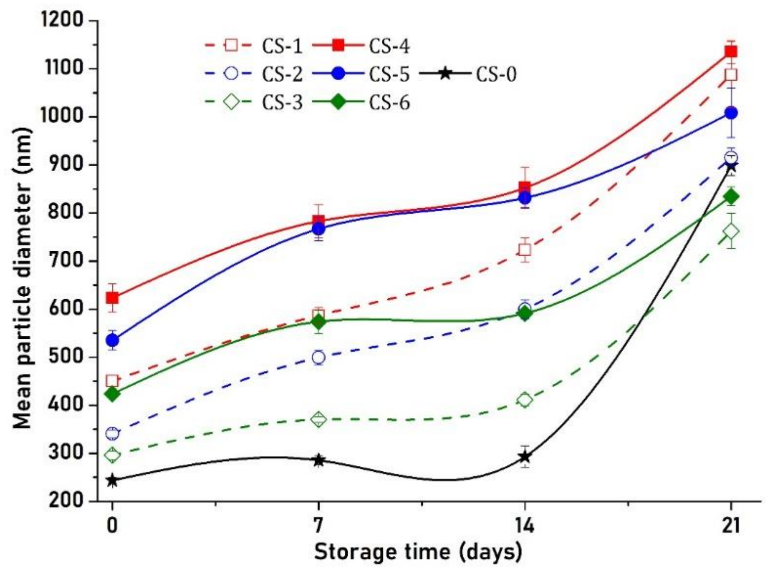

Fig. 1. Changes in mean particle diameter of control and calcium-enriched soymilks during storage time

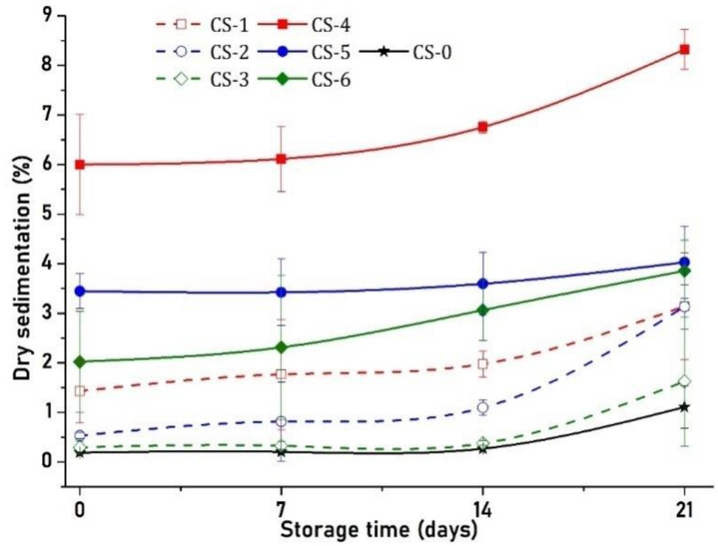

Fig. 2. Changes in dry sedimentation of control and calcium-enriched soymilks during storage time

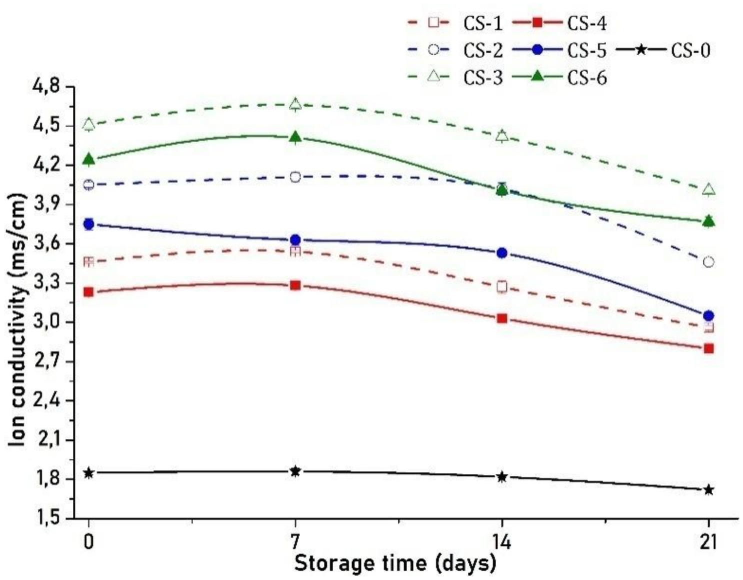

Fig. 3. Changes in ion conductivity of control and calcium-enriched soymilks during storage time

During storage at 4°C for 21 days, pH was found to significantly decrease (P<.05) from 6.21-6.69 to 6.08-6.57, while TTA significantly increased (P<.05) from 0.011-0.015 to 0.015-0.019. These results are similar to a study which were reported by Navicha et al. [14]. The changes in pH and TTA might be due to acidic hydrolysis of reducing into non-reducing sugar in soymilks [36].

3.7 Sensory Evaluation

The sensory evaluation results of calcium-enriched soymilk are shown in Table 6. The intensities perceived of mouthfeel and visual appearance were greatly varied among calcium-enriched soymilk samples. CS-1 and CS-4 samples showed the lowest score (P<.05) in mouthfeel analysis, while CS-3 showed no significant difference (NS) with control soymilk. These results were influenced by particle diameter since it is another critical factor in beverages that affects the frictional resistance to describe mouthfeel sensation in terms of smoothness, chalky, and grainy [37]. The increment of soymilk viscosity also affected panelist's preferences resulting in unfamiliar texture, which able to be observed visually and decrease the sensory score.

The addition of ca-lactate and TPC has no significant difference (NS) on the soymilk flavor. This happened because calcium lactate is known as calcium salt with a neutral taste [3,6]. Similar results were also found by Daengprok et al. [38] when calcium lactate addition has no effect on flavor perception. Potassium citrate tended to increase the saltiness of the product as reported by other researchers [39], however in this study, the increase in saltines was not able to detect by panelists. Overall acceptability score of calcium added soymilks showed significant (P<.05) varied results. CS-0 and CS-3 were acceptable by panelists with the same average score (7.3), which means that panelists were not able to detect the difference between these two samples. In contrast, other samples showed a significant (P<.05) difference in the panelist's acceptability.

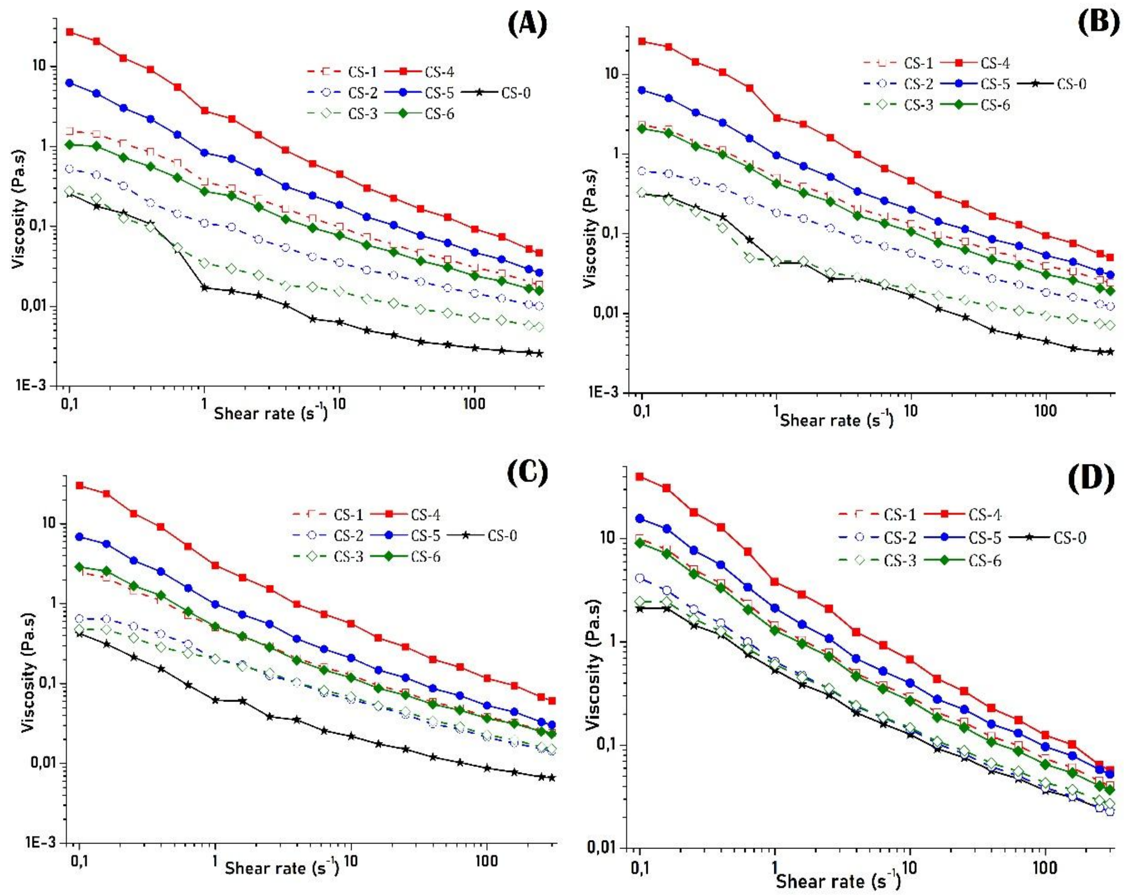

Fig. 4. Viscosity of control and calcium-enriched soymilk as function of shear rate at different storage time; (A) fresh, (B) 7 days, (C) 14 days and (D) 21 days

Table 4. Soymilk flow behavior (n) and flow consistency indexes (K) in control and calcium-enriched soymilks during storage time

Samples	n value				K value (Pa.s^n)			
	Fresh	7 Days	14 Days	21 Days	Fresh	7 Days	14 Days	21 Days
CS-0	0.20± 0.04$^{c(a)}$	0.20±0.03$^{bc(a)}$	0.23±0.03$^{d(a)}$	0.18±0.01$^{d(a)}$	0.07±0.02$^{a(a)}$	0.12±0.06$^{a(a)}$	0.18±0.12$^{a(a)}$	0.99±0.07$^{a(b)}$
CS-1	0.20± 0.01$^{c(b)}$	0.19±0.01$^{bc(b)}$	0.19±0.01$^{c(b)}$	0.14±0.00$^{b(a)}$	0.77±0.14$^{b(a)}$	1.02±0.04$^{f(b)}$	1.01±0.07$^{b(b)}$	2.55±0.15$^{b(c)}$
CS-2	0.23±0.02$^{de(b)}$	0.22±0.01$^{cd(b)}$	0.23±0.01$^{de(b)}$	0.16±0.01$^{c(a)}$	0.28±0.01$^{a(a)}$	0.40±0.02$^{e(ab)}$	0.46±0.10$^{a(b)}$	1.20±0.14$^{a(c)}$
CS-3	0.25±0.04$^{e(b)}$	0.26±0.03$^{d(b)}$	0.25±0.01$^{e(b)}$	0.19±0.00$^{d(a)}$	0.12±0.02$^{a(a)}$	0.16±0.01$^{d(a)}$	0.43±0.03$^{a(b)}$	1.15±0.04$^{a(c)}$
CS-4	0.09±0.01$^{a(b)}$	0.09±0.04$^{a(b)}$	0.10±0.02$^{a(b)}$	0.08±0.01$^{a(a)}$	4.53±0.12$^{d(c)}$	3.16±0.30$^{ab(a)}$	3.79±0.37$^{d(b)}$	4.83±0.52$^{d(c)}$
CS-5	0.15±0.01$^{b(a)}$	0.16±0.03$^{b(a)}$	0.15±0.02$^{b(a)}$	0.13±0.00$^{b(a)}$	1.58±0.19$^{c(a)}$	1.75±0.60$^{bc(a)}$	1.81±0.52$^{c(a)}$	3.59±0.16$^{c(b)}$
CS-6	0.21±0.02$^{cd(a)}$	0.20±0.04$^{bc(a)}$	0.17±0.01$^{c(a)}$	0.14±0.00$^{b(a)}$	0.58±0.07$^{b(a)}$	0.84±0.40$^{c(ab)}$	1.03±0.23$^{b(b)}$	2.31±0.03$^{b(c)}$

[a] Means ± standard deviation in the same column followed by the same letter are not significantly different (P > 0.05)
[(a)] Means ± standard deviation in the same row followed by the same letter are not significantly different (P > 0.05)

Table 5. pH and TTA of control and calcium-enriched soymilks during storage time

Samples	Soymilk pH				Soymilk TTA (% lactic acid)			
	Fresh	7 Days	14 Days	21 Days	Fresh	7 Days	14 Days	21 Days
CS-0	6.69± 0.06$^{e(b)}$	6.73±0.06$^{e(b)}$	6.69±0.10$^{e(b)}$	6.57±0.03$^{d(a)}$	0.011±0.001$^{a(a)}$	0.012±0.001$^{a(a)}$	0.011±0.001$^{a(a)}$	0.015±0.002$^{a(b)}$
CS-1	6.35± 0.08$^{bc(b)}$	6.37±0.07$^{bc(b)}$	6.31±0.08$^{bc(b)}$	6.21±0.01$^{b(a)}$	0.015±0.001$^{b(a)}$	0.015±0.002$^{b(a)}$	0.014±0.002$^{b(a)}$	0.017±0.002$^{ab(b)}$
CS-2	6.45±0.06$^{d(c)}$	6.48±0.02$^{d(c)}$	6.38±0.07$^{cd(b)}$	6.27±0.01$^{b(a)}$	0.015±0.001$^{b(a)}$	0.015±0.001$^{b(a)}$	0.015±0.001$^{b(a)}$	0.019±0.04$^{b(b)}$
CS-3	6.51±0.04$^{d(b)}$	6.51±0.05$^{d(b)}$	6.46±0.07$^{d(b)}$	6.37±0.05$^{c(a)}$	0.015±0.001$^{b(a)}$	0.015±0.001$^{b(ab)}$	0.015±0.002$^{b(a)}$	0.018±0.001$^{ab(b)}$
CS-4	6.21±0.06$^{a(b)}$	6.21±0.07$^{a(b)}$	6.15±0.09$^{a(ab)}$	6.08±0.06$^{a(a)}$	0.014±0.001$^{b(a)}$	0.014±0.001$^{b(a)}$	0.015±0.002$^{b(a)}$	0.017±0.001$^{ab(b)}$
CS-5	6.30±0.06$^{b(b)}$	6.30±0.06$^{b(b)}$	6.26±0.09$^{b(b)}$	6.13±0.05$^{a(a)}$	0.014±0.001$^{b(a)}$	0.015±0.001$^{b(a)}$	0.015±0.002$^{b(a)}$	0.019±0.001$^{ab(b)}$
CS-6	6.38±0.05$^{c(b)}$	6.39±0.06$^{c(b)}$	6.33±0.08$^{bc(b)}$	6.23±0.10$^{b(a)}$	0.015±0.001$^{b(a)}$	0.015±0.002$^{b(a)}$	0.014±0.001$^{b(a)}$	0.017±0.001$^{ab(b)}$

[a] Means ± standard deviation in the same column followed by the different letter are significantly different (P<.05)
[(a)] Means ± standard deviation in the same row followed by the different letter are significantly different (P<.05

Table 6. Sensory evaluation of calcium-enriched soymilk

Samples	Mouthfeel	Flavor	Visual appearance	Overall acceptability
CS-0	6.87 ± 1.5^{c}	6.20 ± 1.7^{a}	7.33 ± 1.1^{c}	7.13 ± 1.3^{d}
CS-1	4.40 ± 2.2^{a}	5.93 ± 2.3^{a}	5.13 ± 1.6^{ab}	5.27 ± 1.7^{ab}
CS-2	6.00 ± 1.7^{bc}	5.67 ± 2.1^{a}	5.93 ± 2.0^{b}	6.33 ± 1.8^{bcd}
CS-3	6.80 ± 1.2^{c}	5.80 ± 2.0^{a}	7.47 ± 1.3^{c}	7.13 ± 1.5^{d}
CS-4	4.27 ± 1.5^{a}	5.27 ± 2.0^{a}	4.47 ± 1.7^{a}	4.53 ± 1.5^{a}
CS-5	5.27 ± 1.9^{ab}	6.07 ± 1.8^{a}	6.00 ± 1.7^{b}	5.60 ± 2.2^{abc}
CS-6	6.53 ± 1.2^{c}	5.93 ± 2.0^{a}	5.93 ± 1.8^{b}	6.67 ± 0.8^{cd}

Means ± standard deviation in the same column followed by the different letter are significantly different (P<.05)

4. CONCLUSION

The findings in this study have shown that the addition of ca-lactate able to increases the calcium content in soymilk (120.02 to 153.82 mg/100g) to be equivalent with or even more than cow's milk (120mg/100g). The addition of ca-lactate also affects the physicochemical properties of soymilk but able to be maintained by the appropriate addition of TPC. Adding more calcium increased the particle diameter, sedimentation, and viscosity significantly (P<.05) while increasing TPC shows the opposite results. CS-3 sample showed high stability and had no significant difference (NS) in some properties with the control sample. Storage of calcium-enriched soymilk for 21 days at refrigeration temperature also influences some of the physicochemical properties. From these findings, it is recommended that pasteurized calcium-enriched soymilk should be stored under refrigeration and consume within 14 days of storage to avoid some unacceptable changes, which occurred after 14 days of storage. This study also found that the addition of ca-lactate and TPC influenced the mouthfeel and visual appearance which were detectable by panelists. However, the appropriate addition of ca-lactate and TPC were able to produce calcium-enriched soymilk without changing the panelist's preferences from the control soymilk. Based on all of the results above, it can be concluded that the addition of 0.8% of ca-lactate in combination with 1.4% of TPC (CS-3 sample) was able to produce a stable and acceptable calcium-enriched soymilk with calcium content (120.78 mg/100 g) equivalent to cow's milk.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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