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EFFECTS OF CALCIUM FORTIFICATION (CALCIUM LACTATE GLUCONATE) ON THE PHYSICOCHEMICAL AND SENSORY PROPERTIES OF SOY-CORN MILK

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Abstract: Osteoporosis is a global public health problem. One of the keys to osteoporosis prevention is to maintain a balanced diet to achieve adequate calcium intake. Soy-corn milk is a nutritious beverage having higher sensory acceptability than soy milk. Its low calcium content (16.97 mg/100 mL) led the idea of calcium fortification using calcium lactate gluconate (CLG), so that it could be a good source of calcium for lacto-vegetarians or lactose-intolerant people.

Purpose and methodology: The research's objectives were to investigate the effects of calcium (CLG) fortification (0.3% to 1.5%) on the physicochemical and sensory properties of soy-corn milk and to determine the optimum fortification level.

Findings: 0.6% CLG exhibited the best result, with pH value of 6.54, calcium content of 85.62 mg/100 mL, viscosity of 53.2 cP colloidal stability of 91.93% and 87.81% on second and third day of storage respectively, and insignificantly (P>0.05) different sensory properties (preferences of consistency and taste) from those of 0% CLG treatment.

Keywords: *Soy-corn Milk; Calcium Fortification; Calcium Lactate Gluconate*

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INTRODUCTION

Soy-corn milk is a nutritious beverage –a blend of soy bean cotyledons and grains of sweet corn. Soy-corn milk had higher sensory acceptability than soy milk (Kolapo and Oladimeji, 2008). Srianta et al. (2010) suggested soybean and sweet corn ratio of 70:30 was the optimum ratio producing soy-corn milk with the best sensory properties. Based on former investigation, soy-corn milk contains 1.82% protein. However, soy-corn milk contains only 16.97 mg/100 mL calcium, which is very low compared to the recommended daily intake for calcium, i.e. 1000 mg/day. Calcium is an essential mineral that is responsible for structural functions and many metabolic functions in human body (Sizer and Whitney, 1997). Inadequate dietary calcium has long been associated with osteoporosis (L'Abee, 2003 in Pathomrungsiyounggul, et al., 2007).

Osteoporosis is a global public health problem which is currently affecting more than 200 million people worldwide (AAOS, 2009). One of the keys to osteoporosis prevention is to maintain a balanced diet to achieve adequate calcium intake. Therefore, calcium fortification in soy-corn milk is needed to enhance its nutritive value and provide calcium, especially for lacto-vegetarians and lactose-intolerant people who cannot fulfill the requirement of calcium due to their dairy-free diet. Henceforth their nutrition and health will be improved through this means of food technology.

Gestner (2002) reported some Ca salts that had been widely used in food industry were calcium carbonate, calcium phosphate, calcium lactate, calcium gluconate, and calcium lactate gluconate. Calcium lactate gluconate (CLG) contains 12,74% calcium, has neutral taste, great bioavailability, and the best solubility characteristic compared to other calcium salts. These properties lead to the utilization of calcium lactate gluconate as a calcium fortifier in soy-corn milk. Calcium fortification is intended to enhance the calcium content in soy-corn milk without adversely affecting its physicochemical and sensory properties. The objectives of this study were to investigate the effects of calcium fortification some physicochemical and sensory properties of soy-corn milk and to investigate the optimum fortification level that produces soy-corn milk based on those properties.

MATERIALS AND METHODS

Materials

Soybean and yellow sweet corn on the cob were obtained from a local supermarket in Surabaya, East Java, Indonesia. Calcium lactate gluconate 13 was obtained from Purac Asia Pacific Ltd. All other chemicals were purchased from local distributor.

Soy-corn milk preparation

The cleaned soybeans were soaked in water for 24h at 25°C and boiled for minutes. The corn was shucked, steamed for 30 minutes, and then decobbed. The boiled soybeans and corn kernels were blended using automatic soybean milk maker (Akebonno) with the ratio of wet soybeans and corn kernels to water of 1:5 at 95°C for 20 minutes. The hot soy-corn milk was filtered through cheesecloth and 4.8 L of filtrate was collected. 10% sugar and 0,02% xanthan gum were added to soy-corn milk, heated until 70°C, and stirred for 10 minutes. Calcium lactate gluconate (Purac) was then added when the temperature reached 50°C and stirred for 10 minutes. Preparation of samples was carried out in triplicate.

Analysis of Samples

Calcium Content

A flame photometer (BWB XP) was used to measure the calcium content of soy-corn milk according to the method of BWB (2009). Preparation of samples was done by wet ashing. 5 mL samples of soy-corn milk in flasks were heated and oxidized by 5 mL HNO_3 and 1 mL $HClO_4$. 10 mL solution of HCl:deionized water (1:1) was added into the flask to make aqueous solution of minerals, and filtered through Whatman filter paper 44 when cool. 2 mL 10% lanthanum chloride was then added to the filtrate and made up to 50 mL with deionized water. The Ca content in aqueous samples was determined using calibrated flame photometer BWB XP by aspirating the samples and reading the results from the display.

pH

A pH meter (Hanna Instrument) was used to measure pH of samples at 25°C

Viscosity

A viscometer (Brookfield DV-E) was used. A 250 ml sample of soy-corn milk in beaker glass was measured at 25°C using spindle 1, speed of 30 rpm, within 30 seconds of measuring time.

Colloidal Stability

Colloidal stability was measured according to Srianta et al. (2010) with modification. 10 ml samples of soy-corn milk were placed in graduated tubes held in racks in the refrigerator at 5°C undisturbed for 3x24 hours. Changes in colloidal stability were indicated by the separation of two layers. Level of visible and line demarcation between the settled and remaining portion of the soy-corn milk was measured.

Sensory Evaluation

The sensory evaluation of soy-corn milk was done by 50 panelists who were familiar with soy-corn milk. Hedonic method was used with the scores ranging from 1 to 7, with 1 represents dislike extremely and 7 represents like extremely. The panelists were requested to evaluate consistency and taste of soy-corn milk. The test was conducted in a sensory evaluation room.

Statistical Analysis

Statistical analysis of data for effects of CLG on the physicochemical and sensory properties of soy-corn milk was performed by one-way ANOVA and SPSS 17.0 for Windows. Mean differences were analyzed using Duncan's multiple range test at $P \leq 0,05$. Optimum fortification level was determined by additive weighting method (DeGarmo et al., 1993). The analyzed parameters were calcium content, colloidal stability, and sensory properties.

RESULTS AND DISCUSSION

Effect of CLG on Calcium content

Calcium content is proportional to the incremental calcium

Calcium lactate gluconate (%)	Calcium Content (mg/100 mL)
0%	16.97[a]
0.3%	49.99[b]
0.6%	85.62[c]
0.9%	113.92[d]
1.2%	150.99[e]
1.5%	197.02[f]

Table 1:
Effect of CLG on Calcium Content

lactate gluconate concentration. Calcium content in the calcium lactate gluconate is high enough (12,74%) to increase the calcium content in soy-corn milk significantly (P <0.05) within a range of 49,99 to 197,92 mg/100 mL.

Effect of CLG on pH

Table 2 summarizes changes in pH for the 6 treatments. All Ca-fortified soy-corn milk had lower pH lower than soy-corn milk with no added CLG. A higher Ca addition caused a significant (P <0,05) pH decrease in soy-corn milk, from 7.10 (0% CLG) to 6.50 (1,5% CLG).

Pathomrungsiyounggul et al. (2007) suggested that calcium ions competed with hydrogen ions for the same binding sites on soy protein molecule and hydrogen ions were released from protein when Ca was added to soy-corn milk. Calcium is located at the end of Hofmeister's series, which arranges ions according to the lyotropic effect, thus promotes protein salting in and destabilization of the native structure (Damodaran et al., 1972 in Scilingo and Anon, 2004). Scilingo and Anon (2004) found that large amounts of calcium addition (5.0-9.73 mg Ca/g protein) induced selective insolubilization of glycinin fraction, suggesting a specific interaction of calcium ions and glycinin (11s).

Calcium lactate gluconate (%)	pH
0%	7.10[e]
0.3%	6.68[d]
0.6%	6.57[c]
0.9%	6.54[b]
1.2%	6.51[a]
1.5%	6.50[a]

Table 2:
Effect of CLG on pH

Kroll (1984) reported the position of protein molecules bound by Ca ions were the side-chain carboxyl groups of aspartic and glutamic acid residues and the side-chain imidazole group of histidine residues. Calcium could also bind to phosphate group of phytate in the product and hydrogen ions were released. Phytate has a strong chelating ability with multivalent ions, especially Zn, Ca, and Fe, which showed better ability in alkaline condition (Hui, 2003).

This result is in accordance with other research results on calcium-fortified soy-based beverage. Shun Tang et al. (1993) reported pH of soy-milk decreased from 6,7 to 5,8 owing to the addition of 14 mM or 0.45% calcium chloride. Yazici et al. (1997) found that pH of soy-milk which had been adjusted to 8 fell to 7.3 after the addition of 1,55% calcium lacto gluconate. Agustinus (2010) also reported that Ca addition (0.5% to 2%) caused a pH decrease from 6.96 to 6.43 in red rice-soy milk.

Effect of CLG on Viscosity

Table 3 shows that calcium fortification caused an increase in the viscosity for all treatments. Kohyama et al. (1995) and Liu (1997) in Pathomrungsiyounggul et al. (2007) reported that viscosity increase in calcium-fortified soy milk was affected

Calcium lactate gluconate (%)	Viscosity (cP)
0%	39.8[a]
0.3%	49.7[b]
0.6%	53.2[c]
0.9%	58.5[d]
1.2%	63.0[e]
1.5%	69.5[f]

Table 3: Effect of CLG on Viscosity

by the interaction of calcium with glycinin fraction of soy protein, according to two possible mechanisms. Adding Ca ions to soy-corn milk neutralizes the net charge of the protein and hydrophobic interactions between the neutralized protein molecules lead to formation of aggregates which in turn affected viscosity. Another mechanism was suggested by Scilingo and Anon (2004), that addition of Ca lead to insolubilization of the soybean proteins, mainy glycinin fraction because of specific calcium-glycinin interaction. The viscosity is positively-correlated to the calcium content in soy-corn milk. Each concentration increment of 0.3%, resulted in significant ($P < 0.05$) viscosity increase. The more calcium dispersed in the system will lead to formation of more aggregates, thus affects viscosity. The viscosity increase (from 39.8 to 69.5 cP) is also affected by the particles of calcium lactate gluconate which has a high molecular mass (324 g/mol) because the calcium salt is made of calcium lactate and calcium gluconate. Akalin and Erisir (2008) stated that addition of substances with high molecular mass will cause an increase in viscosity.

Effect of CLG on Colloidal Stability

Table 4 shows that colloidal stability of soy-corn milk was significantly ($P < 0.05$) affected by the addition of calcium lactate

CLG (%)	Colloidal Stability		
	Day 1	Day 2	Day 3
0%	100.00%	100.00% [e]	100.00% [d]
0.3%	100.00%	97.90% [d]	94.12% [c]
0.6%	100.00%	91.93% [c]	87.81% [b]
0.9%	100.00%	88.86% [b]	86.10% [b]
1.2%	100.00%	84.63% [a]	82.57% [a]
1.5%	100.00%	83.53% [a]	80.80% [a]

Table 4: Effect of CLG on Colloidal Stability during 3 Days of Storage

gluconate on the second and third day of storage. The decrease in colloidal stability was caused by the interaction of calcium ions and soy protein, mainly glycinin. Kohyama et al. (1995) reported that hydrophobic interaction between calcium ions and soy protein caused dehydration, leading to separation of the solution. The specific interaction also promotes the formation of insoluble calcium-glycinin complex, which would affect the stability of the system (Scilingo and Anon, 2004; Speroni et al., 2010). Additionally, fat globules are involved in aggregation. At first, protein coats the fat globules and the coated globules aggregate with each other, leading to formation of big particles (Ono, 2003; Pathomrungsiyounggul et al., 2007). Table 4 also shows that colloidal stability of calcium-fortified soy-corn milk decreases from day 1 to day 3. Calcium-fortified soy-corn milk had a great stability after 24h of storage due to the addition of 0.02% xanthan gum in the preparation. However, 0.02% xanthan gum could not maintain the stability of calcium-fortitifed soy-corn milk.

Effect of CLG on Sensory Properties

The results of sensory evaluation which was carried out using hedonic testing method, involving 50 untrained panelists are summarized in Table 5 and Table 6.

The variation of calcium lactate gluconate concentration did not significantly ($P > 0.05$) affect the parameter of consistency

CLG (%)	Preference Score
0%	4.84
0.3%	4.68
0.6%	4.95
0.9%	4.65
1.2%	4.80
1.5%	4.60

Table 5:
Effect of CLG on Consistency

CLG (%)	Preference Score
0%	5.28[c]
0.3%	5.26[c]
0.6%	4.96b[c]
0.9%	4.70[b]
1.2%	4.04[a]
1.5%	3.86[a]

Table 6:
Effect of CLG on Taste

(Table 5). In contrast it significantly ($P < 0.05$) affects the perception of taste (Table 6). Calcium lactate gluconate provides a neutral taste, but unusual/negative taste characteristic still can be detected when the amount of total calcium reaches a level of 120 mg/100 mL in beverages (Gestner, 2002). Table 6 shows that panelists would prefer soy-corn milk with minimum calcium fortification, but the preference score of soy-corn milk with 0,6% calcium lactate gluconate was insignificantly different ($P > 0.05$)from that of 0% treatment.

CONCLUSION

Incremental concentration of calcium lactate gluconate led to a significant increase in calcium content and viscosity, a significant decrease in pH, colloidal stability, and preference of taste. Based on the physicochemical and sensory properties, CLG concentration of 0,6% gave the best result, with pH of 6,54, calcium content of 85,62 mg/100 mL, viscosity of 53,2 cP, and colloidal stability of 91,93% on second day and 87,81% on third day of storage. The mean value of preference of taste was 4,96, being insignificantly ($P > 0.05$) different to that of 0% CLG treatment (5,28). Further research is needed to investigate the protein digestibility and calcium bioavailability of calcium (CLG)-fortified soy-corn milk.

BIOGRAPHY

Mardon Elian is an alumnus of Food Technology Department, Faculty of Agricultural Technology, Widya Mandala Surabaya Catholic University. He was an assistant lecturer in Laboratory Work of Food Chemistry, Food Biochemistry, Inorganic Chemistry, Analytical Chemistry, and General Microbiology since 2010 until 2011, as well as a research assistant in Center for Food and Nutrition Research in 2011. His main interest lies in the field of functional food.

Ignatius Srianta is the Head of Center for Food and Nutrition Research, Widya Mandala Surabaya Catholic University from 2002 until now. He is a lecturer at Department of Food Technology, Faculty of Agricultural Technology, Widya Mandala Surabaya Catholic University from 2000 until now. He has working experience as a researcher at Technical Development Center of PT Ajinomoto Indonesia in 1999-2000. He is a member of Indonesian Association of Food Technologists. He has teaching experiences on beverage processing technology for several years, as well training in the field of food processing and food analysis in Indonesia and United States of America. He presented/published his researches in national/international journals/seminars.

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