ผลของการเสริมแคลเซียมต่อสมบัติทางเคมีกายภาพ รีโอโลจีและจุลินทรีย์ในโยเกิร์ตชนิดกวนผสมกล้วยบด

Impact of Calcium Fortification on Physicochemical, Rheological and Microbiological Properties of Stirred Yogurts Blended with Banana Purée

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สาขาวิทยาศาสตร์การอาหารและโภชนาการ คณะเทคโนโลยีและนวัตกรรมผลิตภัณฑ์การเกษตร มหาวิทยาลัยศรีนครินทรวิโรฒ

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Received : 21 October 2017

Accepted : 14 December 2017

Published online : 8 January 2018

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมแคลเซียมแลคเตทที่ความเข้มข้น 400 600 และ 800 มิลลิกรัม / 100 กรัม ต่อสมบัติทางเคมีกายภาพ รีโอโลจีและจุลินทรีย์ในโยเกิร์ตชนิดกวนผสมกล้วยบดในระหว่างเก็บรักษาที่อุณหภูมิ 4°C เป็นเวลา 14 วัน การเสริมแคลเซียมมีผลทำให้ร้อยละของการขับน้ำออก (% syneresis) เพิ่มขึ้น แต่ทำให้ความหนืดของผลิตภัณฑ์ลดลง ค่ามอดูลัสสะสม (G') ของผลิตภัณฑ์ลดลงตามความเข้มข้นของปริมาณแคลเซียมแลคเตท จากผลการศึกษาดังกล่าวสรุปได้ว่าโครงข่ายเคซีนโปรตีนของผลิตภัณฑ์โยเกิร์ตอ่อนแอลงเนื่องจากการเสริมแคลเซียม จำนวนแบคทีเรียที่ผลิตกรดแลคติกลดลงตามระยะเวลาในการเก็บรักษา การศึกษานี้พบว่าการเสริมแคลเซียมแลคเตทที่ความเข้มข้น 400 มิลลิกรัม / 100 กรัม จะส่งผลให้ได้ผลิตภัณฑ์โยเกิร์ตที่มีสมบัติทางเคมีกายภาพและรีโอโลจีใกล้เคียงกับโยเกิร์ตที่ไม่มีการเสริมแคลเซียมมากที่สุด

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Abstract

This study aims at exploring the impact of fortification of stirred yogurts blended with banana purée with calcium lactate at 400, 600 and 800 mg/100 g on their physicochemical, rheological and microbiological properties during storage at 4°C for 14 days. Calcium fortification resulted in increased syneresis and reduced apparent viscosity. Storage modulus (G') of yogurts decreased as the level of calcium fortification increased. These results suggested that the casein network in fortified stirred yogurts was weakened by fortification of calcium salt. Lactic acid bacteria counts decreased during storage. In this study, it was found that fortification of 400 mg calcium lactate/100 g resulted in a product with properties close to the non-fortified yogurt.

Keywords : stirred yogurt, calcium fortification, rheology, banana purée

Introduction

Osteoporosis is a disease, in which bones become fragile because of reduced bone density, and it affects more than 75 million people worldwide (Vavrusova & Skibsted, 2014), especially people from industrialized countries (Cashman, 2002). Calcium has been recognized as a key component in stimulating bone health, thus increase in dietary calcium intake is one of the strategies for preventing osteoporosis. About 90% of calcium absorption occurs in the small intestines, where calcium is either absorbed in the ionized form or bound to soluble organic molecules (Gueguen & Pointillart, 2000). Dairy products, green vegetables and cereals are high in calcium. However, organic compounds, such as phytic acid and oxalate found in some plants, inhibit calcium absorption as they form insoluble complexes with calcium ions resulting in bioavailability reduction of calcium from foods, while calcium from dairy products is more easily absorbed (Heaney, 2000). Calcium recommended daily allowance (RDA) is about 1000 mg/ day for adults, but demand for calcium increases during growth, pregnancy and lactation. In addition, calcium absorption decreases with age (Vavrusova & Skibsted, 2014). Thus, calcium fortification in foods is carried out in various products including dairy products, juices, cereals, etc. to overcome calcium deficiency problem. Calcium fortification in dairy products has been reported to improve functional, technological and nutritional properties of the products (Pirkul *et al.*, 1997). Yogurt is a fermented dairy product widely consumed throughout the world because of its nutritional values and health benefits, which primarily link to live microorganisms. Calcium bioavailability of yogurts becomes higher because calcium is in ionized form in acidic condition of yogurts, thus it is readily absorbed in the small intestines (Unal *et al.*, 2005).

Calcium salts commonly used in the fortified foods include calcium carbonate, calcium citrate, calcium gluconate, and calcium chloride. Ranjan *et al.* (2005) added calcium lactate and calcium gluconate to buffalo milk, and found that absorption and retention of calcium increased with fortification of calcium lactate and calcium gluconate when compared to unfortified milk. Calcium lactate, however, is higher in solubility (0.307 mol/l) compared to calcium gluconate (0.080 mol/l), and has neutral taste (Vavrusova & Skibsted, 2014). Fortification of calcium did not affect microbiological properties of yogurts. Pirkul *et al*. (1997) reported approximately identical numbers of both *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *Bulgaricus* for both calcium fortified and control yogurts during storage.

Researches on the effect of calcium fortification on properties of set-typed yogurt include studies from Singh & Muthukumarappan (2008), Santillán-Urquiza *et al*. (2017) and Pirkul *et al*. (1997), but so far no such study on stirred-type yogurt has been done. Incorporation of fruits into yogurts is a common practice in flavor enhancement. Banana is an important fruit cultivated throughout tropical and subtropical regions, and it is rich in minerals, vitamins, carotene and phenols with characteristic flavor (Xu *et al*., 2016). It is also a natural source of prebiotics, i.e. oligosaccharides and polysaccharides, which help stimulate growth of probiotic bacteria (Srisuvor *et al*., 2013).

The objective of this study is to determine the effect of calcium fortification at different levels on properties of stirred yogurts. Stirred yogurts blended with banana purée were fortified with calcium lactate at 400, 600 and 800 mg/100 g. Physicochemical properties (pH, viscosity and syneresis), rheological and microbiological properties were assessed at day 1, 7 and 14 during storage at 4°C.

Methods

1. Preparation of banana purée

Fresh bananas (*Musa cavendishii* L.) were purchased from the local market in Bangkok, Thailand. Bananas with total soluble solids content approximately 20° Brix were selected. They were peeled and blanched in boiled water for 7 min to inactivate enzymes. Samples were then longitudinally cut in halves, and seed portions were removed. Banana flesh was blended with a combination of 0.2% (w/w) citric acid and 0.05% (w/w) ascorbic acid (Srisuvor *et al*., 2013) with the ratio of banana flesh to acid solution of 9:1. Purée was packed in a vacuum bag and stored at -18°C until use.

2. Preparation of culture

Yogurt culture YF-L812 (Chr.Hansen, Hørsholm, Denmark), a mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*, was used as a starter culture. The stock culture was prepared by inoculating 130 mg of freeze-dried culture to 1000 g of autoclaved skim milk and incubating at 43°C overnight. Stock cultures were then stored at -80°C. Working cultures were prepared by thawing frozen stock cultures, and 0.8 ml thawed culture was inoculated to 80 g autoclaved skim milk. Milk was then incubated at 43°C overnight before use.

3. Preparation of fortified yogurts blended with banana purée

Sucrose (5% w/w) and corn starch (0.75% w/w) were added to pasteurized skim milk acquired from a local supermarket. Milk was heated to 85°C for 30 min, cooled to 43°C, and fortified with calcium lactate pentahydrate (Sigma Aldrich, MO, USA) at 400 (400-Ca), 600 (600-Ca) and 800 (800-Ca) mg/100 g. Non-fortified sample was used as a control sample. Fortified milks were subsequently inoculated with 2% (w/w) inoculation rate of working culture and incubated at 43°C for 6 h. Changes in pH levels during fermentation were monitored every 10 min using a pH meter Model Eutech pH 700 (Eutech Instrument, IL, USA). Yogurt gels were stirred using an overhead stirrer (HS-50A, Daihan, Korea) at 500 rpm for 30 s, and 10% (w/w) of thawed banana purée were then added. The mixtures were blended for another 30 s, and stored at 4°C for 14 days. Changes in pH level were investigated at day 1, 7 and 14 of storage.

4. Syneresis

Thirty grams of yogurts were transferred to a 50-ml conical centrifuge tube, and stored at 4°C before analyses at day 1, 7 and 14. Syneresis was defined as weight percentage of whey released after centrifugation of yogurts at 680 ×*g* at 4°C for 10 min (Vivar-Quintana *et al*., 2006) using Sorvall Legend XTR Centrifuge (Thermo Scientific, Waltham, MA, USA).

5. Apparent viscosity

Apparent viscosity was determined using the method reported by Senaka Ranadheera *et al*. (2012) with a slight modification. A Brookfield Viscometer Model DV-3T and spindle number LV2 (Brookfield Engineering Laboratories, MA, USA) were operated at ~25°C with a rotational speed of 0.5 rpm. Apparent viscosity of samples was read at the 15 second during the measurement.

6. Rheological properties

Dynamic oscillatory measurements were performed with a HAAKE RheoStress 1 Rheometer (Thermo Sciencetific, Waltham, MA, USA) using a plate and cone geometry with 1 mm gap at 4°C. Amplitude sweeps were carried out with strain ranging from 0.05 to 100% and frequency of 1 Hz. Storage modulus (G’) and tan delta (tanδ) were obtained.

7. Calcium contents

Fortified yogurt samples were analyzed for their total calcium contents by the inductive coupled plasma emission spectroscopy (ICP/AES, Ultima2, Horiba) as described in AOAC (2016).

8. Microbiological analyses

The number of lactic acid bacteria in fortified yogurts was determined at day 1, 7 and 14 during storage at 4°C following ISO 15214:1998 method of enumeration (1998). The dilution factors 10^{-5}, 10^{-6} and 10^{-7} were spread-plated on MRS agar.

9. Statistical analysis

Analysis of variance was carried out using SigmaPlot 11.0 (Systat Software, CA, USA). The level of significant difference was determined at $p < 0.05$. The differences between means were analyzed using Duncan's multiple range tests.

Results and Discussion

Changes in pH of fortified yogurts during storage

The fermentation of all yogurt samples was stopped at 6 h when the pH of the control, 400-Ca, 600-Ca and 800-Ca samples reached 4.5, 4.7, 4.9 and 4.9, respectively. All samples were then blended with banana purée and stored overnight in a refrigerator. Changes in pH levels of samples were monitored on day 1, 7 and 14 as shown in figure 1. The pH levels of samples made with fortification of calcium lactate were significantly higher than the control yogurt for the entire storage period. In addition, the pH level increased with an increase in concentration of calcium lactate. These results are in agreement with studies conducted by Pirkul *et al*. (1997) and Yazici *et al*. (1997). The higher pH levels of fortified yogurts might result from an increase in buffering capacity when calcium salts were added to milk prior to fermentation. It was also reported that buffering intensity of dairy products proportionally increased with the concentration of added lactate ions (Salaün *et al*., 2005). The taste of yogurts, especially sourness is directly influenced by its pH and acidity. Thus, calcium fortification could affect the perceived flavor of yogurt samples through differences in pH and acidity. There were also significant decreases in pH from day 1 to day 7 for all samples, but after day 7, the pH levels remained stable or slightly increased. The pH decreases were due to post-acidification during storage by lactic acid bacteria presenting in yogurts (Ramirez-Santiago *et al*., 2010; Santillán-Urquiza *et al*., 2017).

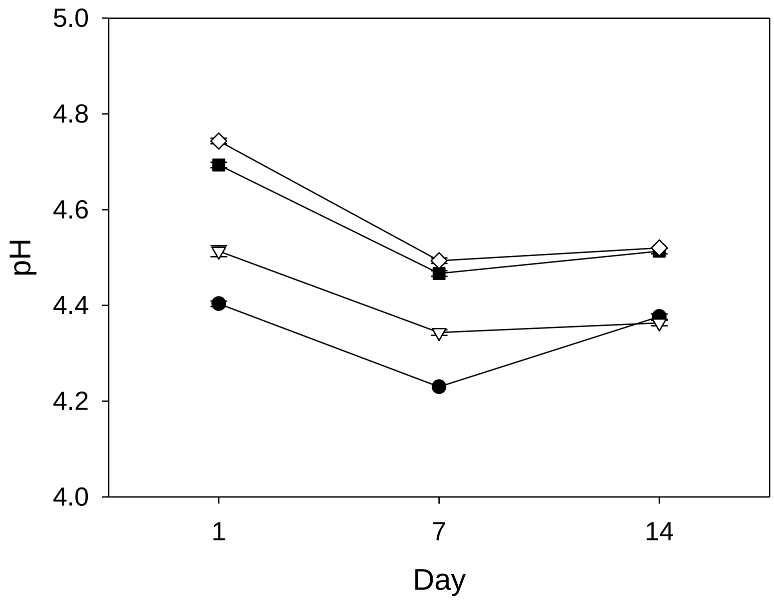

Figure 1 Changes in pH of control yogurt (●), 400-Ca (▽), 600-Ca (■), and 800-Ca (◇) fortified stirred yogurt during storage at 4°C.

Syneresis

Syneresis of fortified yogurts during storage was shown in figure 2. All yogurt samples exhibited significant increases in % syneresis on day 14. The control sample showed lower % syneresis compared with fortified yogurts on day 1 and 7, but there was no significant difference among fortified samples. The increase in % syneresis during storage is in agreement with other studies (Ramirez-Santiago *et al*., 2010; Chen *et al*., 2012; Lobato-Calleros *et al*., 2014). Stirred yogurt is a result of structural breakdown of casein gel formed by aggregation of casein particles (Lee & Lucey, 2006). These casein particles undergo rearrangements during storage, and syneresis of yogurts is related to an unstable casein network (Lobato-Calleros *et al*., 2014). The higher degree of syneresis from fortified stirred yogurts compared with the control yogurt might result from higher pH level of fortified samples (figure 1). It was reported that acid milk gel with pH close to isoelectric point at pH around 4.6 exhibited smaller level of wheying-off as a result of greater attractive forces (Lucey, 2001). In addition, calcium salt might lead to higher ionic strength of fortified yogurt, which could reduce electrostatic interactions between casein particles, and led to a weaker protein matrix (Yazici *et al*., 1997). Singh & Muthukumarappan (2008) reported higher water holding capacity of set-typed yogurt fortified with calcium lactate compared to non-fortified samples. The higher water holding capacity indicated lower degree of syneresis, and the authors concluded that calcium fortification increased colloidal calcium phosphate (CCP) linkage between casein micelles, hence more intense network was formed.

However, it must be noted that there was no difference in acidity between fortified and non-fortified yogurts in Singh & Muthukumarappan (2008) as acidity of samples had an important role on the yogurt structural properties.

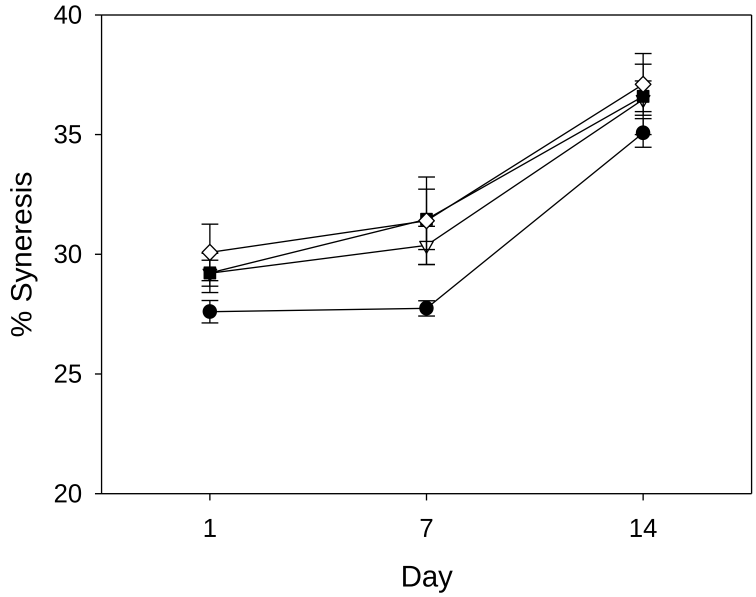

Figure 2 % Syneresis of control yogurt (●), 400-Ca (▽), 600-Ca (■), and 800-Ca (◇) fortified stirred yogurt during storage at 4°C.

Apparent viscosity and rheological properties

Apparent viscosity of yogurts was shown in figure 3. Viscosity of samples ranged from 21.26 to 39.98 Pa.s. The control and the sample fortified with 400 mg calcium lactate/100 g had significant higher viscosity, while the sample fortified with 800 mg calcium lactate/100 g showed the lowest viscosity value of 21.26 Pa.s. Thus, fortification of calcium lactate decreased apparent viscosity of the samples. The decrease in viscosity was possibly due to a weaker protein matrix.

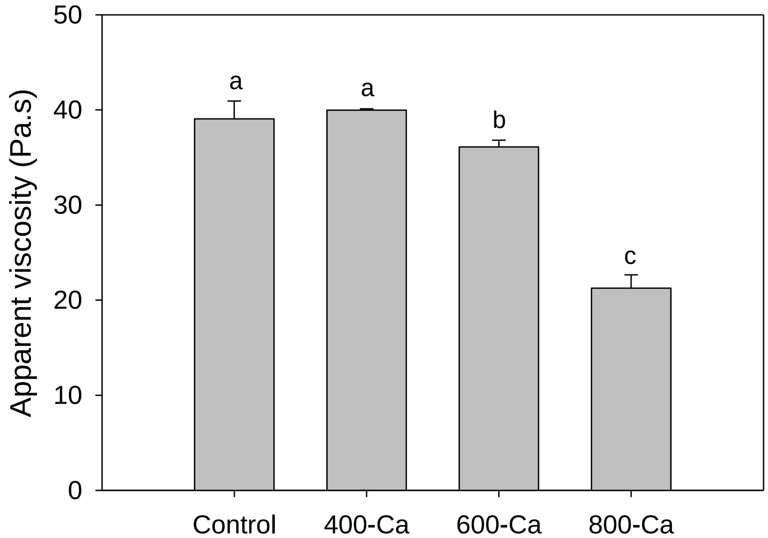

Figure 3 Apparent viscosity of yogurts determined at a rotational speed of 0.5 rpm at ~25°C. Values with different letters are significantly different (P < 0.05).

Rheological properties of fortified stirred yogurts were characterized by three important parameters, storage modulus (G'), loss modulus (G'') and loss tangent (LT). The G' values indicate stiffness or solid-like property, and G'' values determines liquid-like property of the samples. The LT values are a ratio of liquid-like to solid-like properties (G''/G'), so LT helps to indicate the viscoelastic nature of samples i.e. more liquid-like or solid-like (Rao, 1999). The G' and LT values as a function of applied strain (%) were shown in figure 4. All samples exhibited linear viscoelastic behavior at small deformation. However, the samples showed a decrease in G' as the strain increased, whereas the LT values increased as a degree of deformation increased. These results indicated that the samples exhibited viscoelastic nature at small strain, but as strain increased structural breakdown occurred, thus samples began to show viscous behavior. These characteristics indicated that all stirred yogurt samples had weak gel-like structure (Ramirez-Santiago *et al*., 2010).

Fortification of calcium salt led to a significant reduction in G' values, and an increase in LT values compared to the control sample. These results suggested that the casein network in fortified stirred yogurts was weaken by fortification of calcium salt. These rheological properties were in agreement with the apparent viscosity values (figure 3) in which calcium addition might interfere with the protein-protein interactions because of higher ionic strength in calcium fortified yogurts (Yazici *et al*., 1997), and also conformed with the degree of syneresis (figure 2) as expression of whey from networks under pressure is a measure of network rigidity (Lucey, 2001). Thus, calcium fortification resulted in more liquid-like nature of the samples.

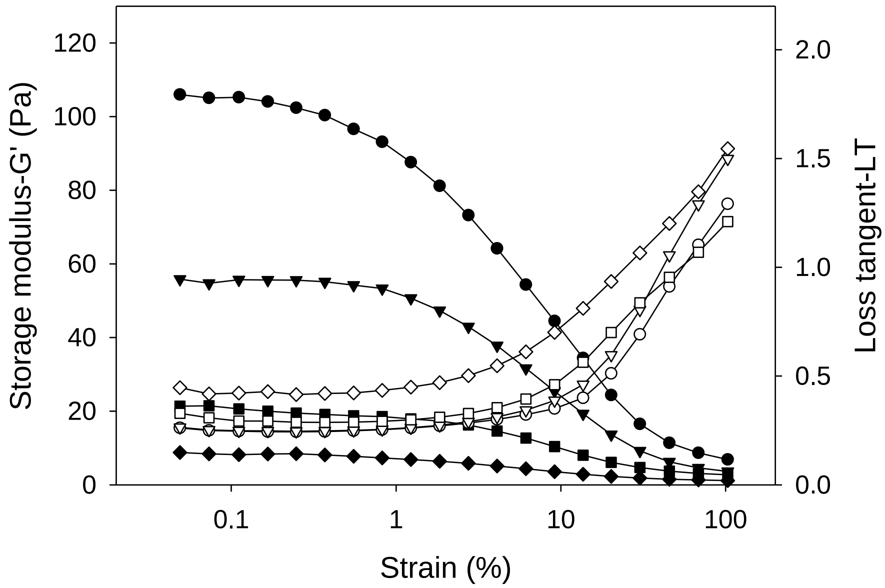

Figure 4 Storage modulus of control yogurt (●), 400-Ca (▼), 600-Ca (■) and 800-Ca (◆) fortified stirred yogurt, and loss tangent of control yogurt (○), 400-Ca (▽), 600-Ca (□) and 800-Ca (◇) fortified stirred yogurt as a function of strain determined on the first day of storage at 4°C.

Calcium contents of yogurts

Calcium contents of control yogurt, yogurts fortified with 400, 600 and 800 mg calcium lactate/ 100 g were 117.19, 166.08, 186.01 and 204.96 mg/100 g, respectively as shown in Table. 1. Fortification of calcium lactate resulted in 42, 59 and 75% increase in calcium contents for samples fortified with 400, 600 and 800 mg calcium lactate/100 g respectively, compared to the control sample (Table. 1). One gram of calcium lactate theoretically provides 130 mg calcium (Pirkul *et al*., 1997). The calcium contents found in this study, therefore, were slightly less than theoretical values. However, Gerstner (2002) reported that high concentration of calcium salts may impart bitterness to the dairy products. Singh & Muthukumarappan (2008) reported that saltiness was perceived at fortification level of 75 mg / 100 ml of yogurt mix, thus they suggested addition of 50 mg calcium/ 100 ml.

Table 1 Calcium contents and percent increase in calcium of calcium fortified yogurts.

Sample	Calcium content (mg/100g)	% Ca increase compared to control
Control	117.19 ± 2.85 [d]	-
400-Ca	166.08 ± 2.55 [c]	42
600-Ca	186.01 ± 2.85 [b]	59
800-Ca	204.96 ± 4.46 [a]	75

Values are mean ± S.E.

[a-d] Different superscripts in the same column were significantly different ($p < 0.05$).

Microbiological analyses

Microbiological analyses of fortified yogurts during storage were shown in table 2. The population of lactic acid bacteria of all samples significantly decreased with storage time. The decrease in lactic acid bacteria count as a function of storage time was also reported in other studies (Akgun *et al*., 2016; Serra *et al*., 2008; Lanciotti *et al*., 2004). The lower number of lactic acid bacterial cells might be attributed to post acidification of yogurts during storage (Kailasapathy, 2006). There was no significant difference of lactic acid bacteria counts between the control yogurt and calcium fortified yogurts on day 1 and 7. However, the viability of lactic acid bacteria on the 14th day of storage was slightly different between various groups. Kaushik & Arora (2017) reported significant difference in *S. thermophilus* and *L. bulgaricus* counts of yogurts fortified with calcium phosphate compared to the control sample, but fortification with calcium citrate did not affect these microbiological properties. Thus, the viability of starter cultures may be affected by different types of calcium salts. In addition, incorporation of banana purée to the yogurts could possibly help the survival of bacterial culture because banana contains sugars, i.e. sucrose, glucose and fructose, which are fermentable by the starter cultures (Adão & Glória, 2005). Oligosaccharides and polysaccharides are also found in banana (Srisuvor *et al*., 2013; Pereira *et al*., in press), thus addition of banana purée could add prebiotic potential to the products.

Table 2 Lactic acid bacteria counts in control and fortified yogurt during storage at 4°C.

Sample	Lactic acid bacteria (log CFU/g)		
	Day 1	Day 7	Day 14
Control	8.24 ± 0.03^{aA}	8.25 ± 0.00^{aA}	7.26 ± 0.00^{bcB}
400-Ca	8.26 ± 0.02^{aA}	8.10 ± 0.03^{aB}	7.51 ± 0.05^{aC}
600-Ca	8.26 ± 0.02^{aA}	8.09 ± 0.10^{aA}	7.13 ± 0.02^{cB}
800-Ca	8.23 ± 0.00^{aA}	8.10 ± 0.02^{aA}	7.42 ± 0.16^{abB}

Values are mean ± S.E.

$^{a-d}$ Different superscripts in the same column were significantly different ($p < 0.05$).

$^{A-C}$ Different superscripts in the same row were significantly different ($p < 0.05$).

Conclusions

In conclusion, fortification of calcium lactate to stirred yogurts resulted in reduction of viscosity and storage modulus, which determined the viscoelastic property of yogurts, and an increase in syneresis, which is a defect in yogurt quality. Thus, fortification of high concentration of calcium is to be avoided. In this study, it was found that fortification of 400 mg calcium lactate led to a product with similar property to the non-fortified yogurt.

Acknowledgements

This work was supported by Strategic Wisdom and Research Institute of Srinakharinwirot University for the funding of this project. The authors would like to thank Dr. Duangratana Shuwisitkul from faculty of pharmacy for her help on rheological measurements.

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