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Original Article

Effect of fat content on characteristics of ice cream fortified with calcium and vitamin D3

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ABSTRACT

Calcium and vitamin D play important roles in bone homeostasis. Several studies reported inadequate intakes of these two nutrients in many population groups. Food fortification is one way to solve the problem. The objective of this study was to develop the ice cream as a functional food for resolving calcium and vitamin D inadequate intakes by determining the effects of fortification of ice cream with calcium and vitamin D3. Five ice cream formulations contained different amounts of fat including regular fat (RF), reduced fat (RDF), light (L), low fat (LF), and fat free (FF) were design (10%, 7.5%, 5%, 2.5% and <0.625% fat content, respectively). An inulin was used as a fat replacement in LF and FF formulas. 200 mg of elemental calcium and 200 IU of vitamin D3 per serving were fortified in each formula. Ice creams were stored at −20°C. Physical and microbiological properties were evaluated on day 0, 7, 14, and 28. An overrun of L and FF were higher than RF, RDF and FF ($P < 0.05$). Hardness tended to increase as the lower of fat content of an ice cream and as the longer the products stored. Melting rate inclined with further reduction of fat on day 0, but no significant difference was found among the treatments on day 14 and 28. The ice creams formulated with the inulin had lower viscosity ($P < 0.05$) compared to non-containing inulin formulas. Aerobic plate counts were <100 CFU/ml, and no *Escherichia coli* and coliform found at 1:100 dilutions throughout the study. Calcium was preserved; however, the amount of cholecalciferol was inconsistent. Alternative techniques of vitamin D fortification may be needed to improve the stability.

INTRODUCTION

Calcium and vitamin D play important roles in bone homeostasis. Calcium is a major constituent of bones and a second messenger in cell signaling pathways. Obtaining sufficient calcium is important to decrease the risk of osteoporosis, hypertension, colorectal cancer, gastric cancer, nephrolithiasis, neurogenerative disease, and degenerative joint disease [1-3]. The recommended daily allowance of calcium varies for different age groups and ranges from 700 to 1300 mg/day [4]. Several studies have reported instances of inadequate calcium intake worldwide including in Europe [5], United States [6], and Asia [7,8]. Vitamin D is also essential for bone health. The Institute of Medicine (2012) recommended that vitamin D requirements are 400 IU/day in infants (0-1 year), 400 IU/day in children and adults (1-70 years), and 800 IU/day in elderly (800 IU/day) [4]. Long-term vitamin D deficiency (<30 ng/ml or <75 nmol/L; National Institute of Health 2014) can result in osteoporosis in adults and rickets in children. Many other health problems such as cardiovascular disease, Type 2 diabetes, several cancers, and autoimmune disorders are also related to low vitamin D levels [9,10]. Hypovitaminosis D is a worldwide problem [11-13] including tropical countries such as Thailand [14-17]. Reported that approximately 50% of post-menopausal women had vitamin D insufficiency in Thailand and Malaysia [15,18]. Chailurkit *et al*. stated that vitamin D insufficiency is a common and varies across geographical regions in Thailand including 64.6% of people in the capital city, Bangkok [16]. Rojroongwasinkul *et al*. reported the prevalence of vitamin D insufficiency in Thailand ranged from 27.7% to 45.6% among children aged 0.5-12.9 years [17].

Risk factors of vitamin D deficiency include lack of exposure to sunlight, wearing sunscreen or concealing clothes,

dietary inadequacy, mal-absorption, or use of anticonvulsants [19,20]. Obtaining vitamin D either from food or supplements could be one of the effective ways to improve vitamin D status. However, supplements may depend on individual conditions such as financial status and may not be easily applied to the entire population [21]. Very few foods naturally contain vitamin D [19,22-24], and the main sources are fatty fish and liver, which are not typically consumed every day. Thus, fortifying foods that are commonly consumed by a whole population is important. Black *et al*. showed that vitamin D food fortification is a potentially effective public health strategy that can increase circulating 25-hydroxy vitamin D concentration in community-dwelling adults [25]. Milk is one of the food products commonly fortified with vitamin D. However, other dairy products such as cheese, butter, cream, yogurt, as well as ice cream are usually not fortified with vitamin D [22,24], which leads to public misconceptions that all milk products are a rich source of vitamin D [22,26]. Trang *et al*. and Tripkovic *et al*. also indicated that supplementation with vitamin D3 could raise more efficiently serum calcidiol levels as compared to the effect of vitamin D2 [27,28].

The use of both calcium and vitamin D supplements were more effective than using calcium alone without vitamin D [3,29]. Dairy products fortified with vitamin D and calcium increased serum calcidiol level in children aged 9-12 years old [30], elderly women [31], and in pre- and post-menopausal women [32], as compared to unfortified milk products. A study by Bonjour *et al*. also revealed that vitamin D and calcium-fortified soft white cheese lowered bone resorption biomarker tartrate-resistant acid phosphatase 5b in post-menopausal women [33]. Calcium-fortified ice cream was an effective system to deliver calcium to the human body since the absorption of calcium from ice cream was reported to be as high as from milk [34]. Due to the fact that ice cream is one of the milk products currently consumed worldwide, the objectives of this study were to determine the effect of calcium and vitamin D fortification on the ice cream and to develop the ice cream as a functional food for resolving inadequate intakes in many regions of the world at every age. Five ice cream formulations including regular fat (RF) (10%), reduced fat (RDF) (7.5%), light fat (5%), low fat (LF) (2.5%), and fat free (FF) (<0.625%) were prepared with addition of 200 mg ionic calcium and 200 IU vitamin D3 per 80 g serving. Physical, microbiological, and vitamin D3 quantification were determined through 28-day storage at −20°C.

MATERIALS AND METHODS

Study Design

Five ice cream formulations including RF (10%; RF), RDF (7.5%; RDF), light (5%; L), LF (2.5%; LF), and FF (<0.625%; FF) ice cream were compared (n = 3/treatment). Each treatment was fortified with 500 mg calcium carbonate (equivalent to 200 mg ionic calcium) and 200 IU vitamin D3 per serving. Ice creams were stored at −20°C throughout the study. A proximate analysis of each sample was measured. Overrun (the increase in volume of ice cream due to air incorporation during initial mixing) was determined on day 0. Other physical parameters including ice cream hardness, melting rate, and viscosity were measured on day 0, 7, 14, and 28. Microbiological properties (aerobic plate counts [APCs] and coliform counts) as well as vitamin D3 retention were also determined on 0, 7, 14, and 28 days of storage.

Ice Cream Production

Composition and caloric energy content of each ice cream formulation are shown in Table 1. All samples consisted of non-fat milk, skim milk powder, fresh whipping cream, sugar, imitation vanilla flavor (Durkee, Iowa, USA) and a mixture of emulsifier, and stabilizer (Palsgaard® extruIce 278, Denmark). An inulin was used as a fat replacement in LF and FF treatments. Ice creams were fortified with 500 mg calcium carbonate (VWR international, Belgium) and 5 μg cholecalciferol (100,000 IU vitamin D3/g; DSM Nutritional Products Inc., Singapore) per serving (serving size = 80 g).

For ice cream production, all ingredients (except cholecalciferol) were mixed together and pasteurized at 68.5°C for 30 min. After cooling to 15°C, cholecalciferol was added into the mix. Next, homogenization was performed with an Ultra-turrax T25 homogenizer (Janke & Kunkel IKA Labortechnik, Germany) at a setting of 20,500/min for 15 min.

Table 1: Ice cream mix formulations and energy content

Ingredient	Ice cream formulation				
	RF	RDF	L	LF	FF
Fat (%)	10.02	7.50	5.00	2.50	0.61
MSNF (%)	11.00	11.00	11.00	11.00	11.00
Sugar (%)	12.00	12.00	12.00	8.00	8.00
Inulin (%)	0.00	0.00	0.00	5.00	5.00
Emulsifying/stabilizing agent (%)	0.50	0.50	0.60	0.60	0.60
Vanilla flavor (%)	2.00	2.00	2.00	2.00	2.00
Ionic calcium (mg/serving)	200.00	200.00	200.00	200.00	200.00
Vitamin D3 (IU/serving)	200.00	200.00	200.00	200.00	200.00
Energy (kcal/serving)	145.00)	128.00	110.00	96.00	82.00

RF: Regular fat, RDF: Reduced fat, L: Light, LF: Low fat, FF: Fat free, MSNF: Milk solids non fat

Ice cream mixes were aged at 4°C for 4 h before processing by a tabletop ice cream maker (Grace KA-608, China). Ice creams were then hardened at −20°C for at least 12 h before the analyses.

Proximate Analysis

Proximate compositions of ice creams including crude protein, crude fat, and ash were analyzed according to AOAC official methods 930.33, 952.06, and 945.46, respectively [35]. Moisture content was calculated from moisture loss at the oven drying step during total solids examination by gravimetric analysis according to AOAC method 941.08 for ice cream and frozen dessert [35]. Carbohydrate was calculated as described by the Food and Agricultural Organization [36].

Physical Measurements

Overrun

Overrun of the ice creams were calculated using the following equation [37].

$$\text{Overrun} = \frac{\text{Weight of mix} - \text{Weight of ice cream}}{\text{Weight of ice cream}} \times 100$$

Where weight of mix and weight of ice cream are in the same volume. Higher values of overrun indicate greater incorporation of air during ice cream making.

Hardness

Hardness of ice cream samples was determined according to Whelan *et al*., using a TA-XT2i texture analyzer (Stable Micro Systems Ltd., UK) with a P/30C cone Perspex probe. The highest force was recorded as the probe penetrated into the ice cream to a depth of 20 mm at a speed of 2 mm/s [37].

Melting rate

Melting rates of the ice creams were examined according to Whelan *et al*., with slight modification [37]. One cup (80 g) of ice cream was placed on a mesh (12.5 mm × 12.5 mm) at room temperature (21 ± 1°C). Weight of the drip was recorded every 10 min until ice cream entirely melted down. The highest slope from a plot between time and weight of the drip was used to determine the melting rate of the ice cream (R^2 >0.99).

Viscosity

Ice cream viscosity was analyzed according to Aime *et al*. [38]. Samples were brought to 4°C for 4 h before measurements. 8 ml of melted ice cream were transferred to a cup to measure the viscosity using a Bohlin C-VOR 105 viscometer (Worcestershire, UK) at shear rate of 0.01-150/s. The viscosity was read at a shear rate of 11.1/s (T = 25°C).

Vitamin D3 Retention

Retention of cholecalciferol was evaluated by a method described by Kazmi *et al*. [39]. Saponification was performed by mixing 1 g diluted ice cream sample (1:3) with 0.5 ml 60% KOH before extraction by high-performance liquid chromatography (Shimadzu LC-10A, Shimadzu, Kyoto, Japan) using a C18 column (Water Spherisorb ODS2 10 μm 4.6 × 250 mm, Ireland). Methanol:acetonitrile:water (45:45:10) was used as a mobile phase. The sample injection volume was 50 μl. A flow rate of 1.0 ml/min was maintained throughout the test period (5 min, T = 26°C). The elution of vitamin D3 was detected at 254 and 228 nm on an ultraviolet detector at 2.7 min.

Microbiological Analysis

Ice cream samples were quantified for APC and *Escherichia coli coli*/coliform counts using 3M™ Petrifilm (Minnesota, USA) according to AOAC Official Method 989.10 [35].

Statistical Analysis

Statistical Analysis Software version 9.0 (SAS Institute Inc., NC, USA) was used for analysis of variance (ANOVA) to identify differences among ice cream treatments at the 95% confidence level ($P < 0.05$). A completely randomized design with *proc glm* function was used for proximate analysis and overrun experiments. Repeated measures design with *proc mixed* function, using Tukey adjustment to obtain differences of least means squares, was used for other physical analyses, microbial measurements, and vitamin D3 retention of the samples. Mean values and standard error from triplicate analysis were reported.

RESULTS

Five ice cream formulations were analyzed for the amounts of fat, ash, protein, carbohydrate, and moisture as shown in Table 2. Ash contents were not significantly different among the groups but were higher than non-calcium fortified ice cream ($P < 0.05$; average 1.21% for calcium-fortified ice creams versus 0.74% for control). There was a higher protein content in L, LF and FF than RF and RDF ice creams ($P < 0.05$). Calories of each ice cream formulation were also calculated using factors of 4, 4 and 9 calories/g protein, carbohydrate, and fat, respectively. The energy value of 80 g ice cream varied with the ingredients of the mix, and ranged from 82 to 145 kcal/serving (Table 1).

Table 2: The proximal composition of ice creams

Component	RF	RDF	L	LF	FF
protein (%)	2.79±0.04^a	2.84±0.02^a	2.98±0.06^b	2.92±0.03^b	2.90±0.05^b
Fat (%)	9.78±0.48^e	7.08±0.28^d	4.72±0.17^c	2.44±0.10^b	0.61±0.03^a
Ash (%)	1.20±0.14^a	1.21±0.04^a	1.20±0.12^a	1.22±0.08^a	1.25±0.07^a
Moisture (%)	65.53±0.28^a	67.77±0.34^b	70.27±0.10^c	71.78±0.36^d	73.73±0.19^e

Means not sharing a common superscript letter within the same row (group) are significantly different (P<0.05). RF: Regular fat, RDF: Reduced fat, L: Light, LF: Low fat, FF: Fat free

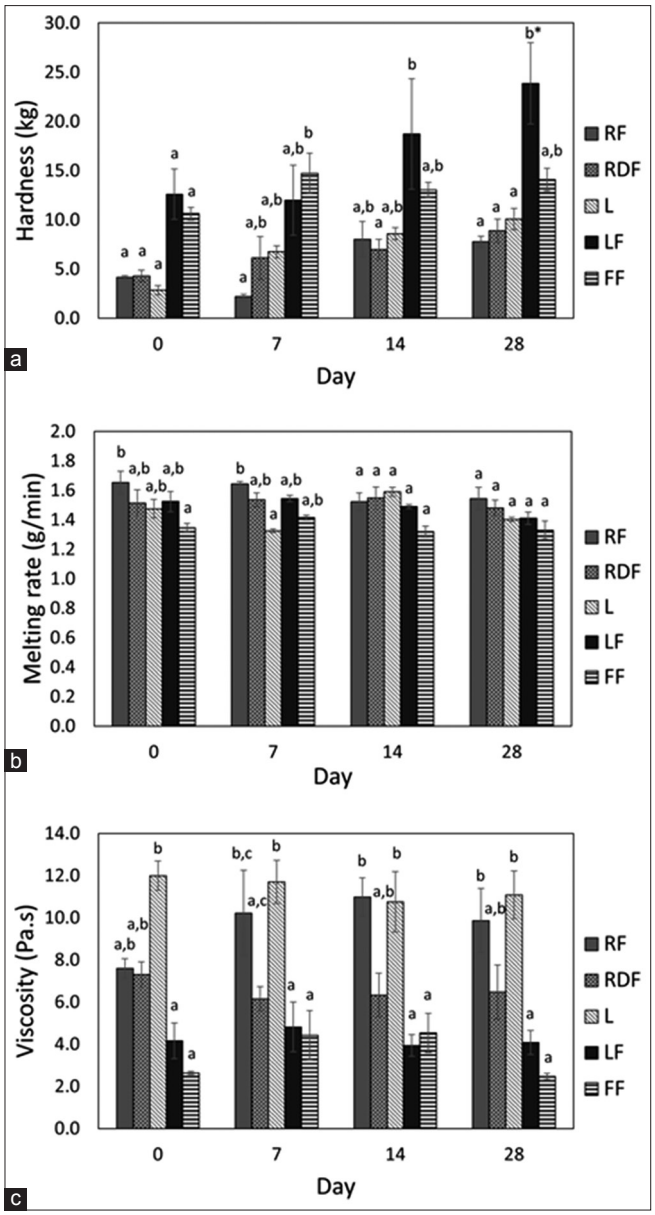

Figure 1: Hardness (a), melting rate (b), viscosity (c) of ice creams on 0, 7, 14 and 28 days of storage at −20°C. RF: Regular fat (10%), RDF: Reduced fat (7.5%), L: Light (5%), LF: Low fat (2.5%), FF: Fat free (<0.625%). Data are shown as mean ± standard error of mean. Means not sharing a common superscript letter within the same day are significantly different ($P < 0.05$). An asterisk (*) indicates a significant difference from day 0

Overrun of L and FF ice creams was greater than other formulations ($P < 0.05$; Table 3). Hardness, melting rate and viscosity of the samples were illustrated in Figure 1a-c. There was no significant difference in hardness between the groups on day 0. However, FF and LF ice creams had the highest hardness on day 7 and day 28, respectively (Figure 1a; $P < 0.05$). Statistics showed that only the hardness of LF treatment changed over time. Hardness of LF ice cream on day 28 was significantly higher than day 0 and 7. Melting rate of RF was faster than FF and L samples on day 0 and 7, respectively (Figure 1b; $P < 0.05$). No significant difference

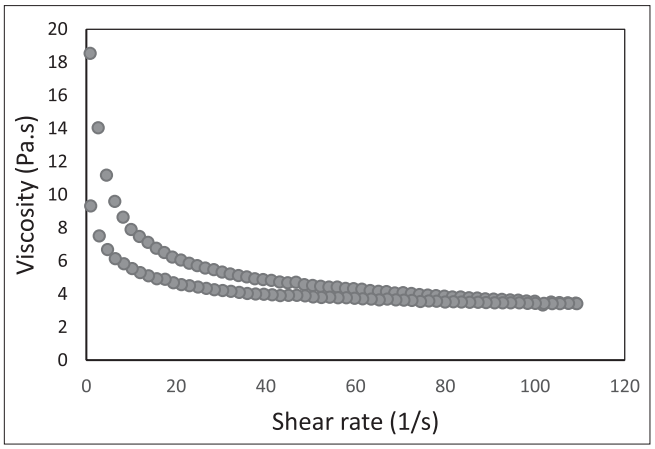

Figure 2: Effect of the shear rate on the viscosity of regular fat formula on day 0

was found between the treatments on day 14 and 28, and the melting rates of all ice creams were not changed by storage time ($P > 0.05$). The viscosity of each ice cream treatment was not changed over time. Nevertheless, the results showed that LF and FF ice cream tended to have a lower viscosity than other groups through 28-day storage (Figure 1c). All ice cream formulas exhibited pseudoplastic behavior as shown in Figure 2.

Retention of cholecalciferol in the ice creams is presented in Figure 3. Vitamin D3 in RF and LF samples was found to be lower on day 7, compared to day 0 ($P < 0.05$). However, it was elevated again on day 14. Vitamin D3 content in RDF and FF ice creams tended to go down during storage, but the change was not statistically significant. L sample showed the highest cholecalciferol level on day 7.

Microbial examination of the ice creams is shown in Figure 4. APCs were smaller than 40 CFU/g throughout 28 days for every group. APC values were expected to be low because the ice cream mix was pasteurized before proceeding with ice cream processing. There was no *E. coli* or coliform found in any samples.

DISCUSSION

Ice cream can be characterized as a multiphase food product consisting of air bubbles and ice crystals surrounded with the aqueous phase of the mix (sugar, protein, salts, polysaccharides, and water), intermixed with a network of fat in a partial coalescence state [40,41]. The study of Biasutti *et al*. indicated that factors affecting the overrun (air incorporation) of ice cream were ingredients of the mix and the manufacturing process including extrusion and homogenization steps. Conventional homogenization was found to make high overrun ice cream while high pressure homogenization produced the lower overrun [40]. The results from this study showed that overrun of FF and L ice creams were higher than RF, RDF and LF groups ($P < 0.05$), which could be explained by the lower ratio of non-fat dry milk/milk fat in RF and RDF formulas. Table 1 shows the same level of milk solids non-fat in all treatments, which was 11% in all formulations. It seems that higher fat levels

Table 3: Overrun (measure of air incorporation) of ice creams

Parameter	RF	RDF	Light	LF	FF
Overrun (%)	32.70±1.62[a]	30.49±2.93[a]	55.73±1.02[b]	23.61±3.28[a]	49.18±1.87[b]

Data are shown as mean±SEM. Means not sharing a common superscript letter within the same row (group) are significantly different ($P<0.05$). RF: Regular fat (10%), RDF: Reduced fat (7.5%), L: Light (5%), LF: Low fat (2.5%), FF: Fat free (<0.625%), SEM: Standard error of mean

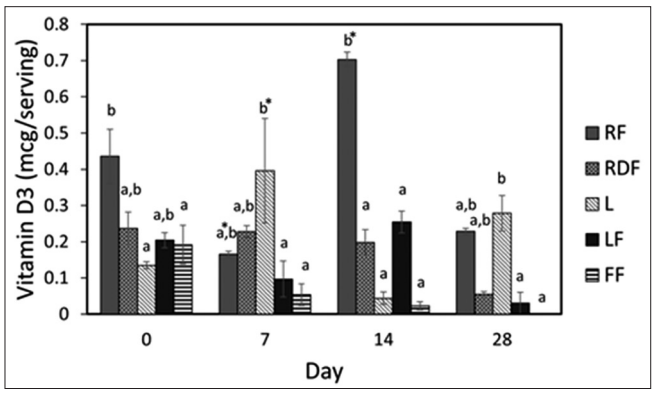

Figure 3: Vitamin D3 retention in ice creams on 0, 7, 14 and 28 days of storage at −20°C. RF: Regular fat (10%), RDF: Reduced fat (7.5%), L: Light (5%), LF: Low fat (2.5%), FF: Fat free (<0.625%). Data are shown as mean ± standard error mean. Means not sharing a common superscript letter within the same day are significantly different ($P < 0.05$). An asterisk (*) indicates a significant difference from day 0

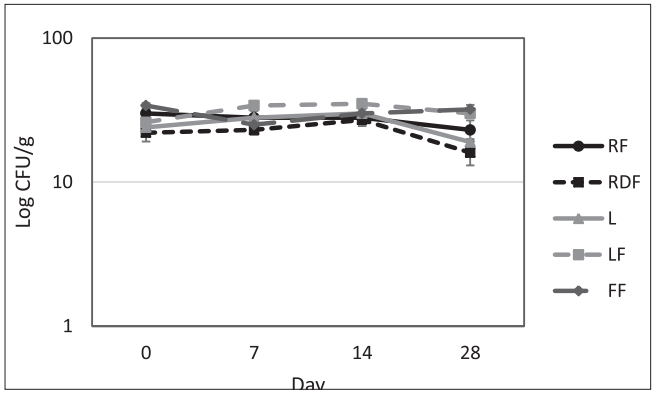

Figure 4: Aerobic plate counts of ice creams on 0, 7, 14 and 28 days of storage at −20°C. RF: Regular fat (10%), RDF: Reduced fat (7.5%), L: Light (5%), LF: Low fat (2.5%), FF: Fat free (<0.625%). Error bars represent standard error of the mean

decreased air entrapment. Air bubbles and fat droplets will both be coated and emulsified with milk proteins after mixing. If there is high fat, then some protein will emulsify the fat, and there will be less protein to stabilize the air bubbles. Thus, there will be fewer stable air bubbles in high-fat samples.

All ice cream formulations presented pseudoplastic behaviors, which were similar to the previous study on rheological properties of ice creams by Aime *et al*. [38]. Their study revealed that ice cream viscosity decreased as shear rate increased. The viscosity of ice cream can be influenced by the mixing process, homogenization pressure, aging time, and ice cream composition [41]. Higher levels of stabilizer, protein, corn syrup solid, fat, and total solid can increase ice cream viscosity as well [41]. The results from this study showed that RF and RDF treatments, which tended to have a higher viscosity than FF, had significantly lower overrun than the FF ice cream ($P < 0.05$). In this study, the inulin was added to LF and FF formulas to increase the viscosity of ice cream that contained less fat. Inulin is a carbohydrate-based fat replacer that functions as a bulking agent. It is resistant to hydrolysis in the stomach and intestine and can be used as a prebiotic [42]. Inulin makes ice cream chewy and protects ice cream against heat shock [43]. Inulin interacts with the aqueous phase of the ice cream and thus lowers the concentration of free water, therefore, thickening the mix [44] and slowing the melting rate of ice cream [45]. High viscosity ice cream can incorporate less air than samples with less viscosity [40], resulting in low overrun [41].

Several studies reported that ice cream with high viscosity had slow melting rate [40,41,44]. Granger *et al*. found that melting time is related to ice cream ingredients, especially type of emulsifier [46]. Other factors influencing ice cream melting behavior are the interactions among the ingredients such as fat, air, protein, and the polymer network [46]. Meltdown characteristics are also associated with fat and non-fat milk solids content [44]. Nevertheless, the melting rate and viscosity of all ice cream formulations in this study were stable through 28 days storage at −20°C.

All of the ice cream formulations in this study did not show significant differences in hardness, except the LF formula. Hardness of LF treatment on day 28 was significantly higher than day 0 and 7 ($P < 0.05$). Moreover, hardness of LF and FF tended to be higher than RF and RDF ice creams on day 0. This finding is in agreement with the report by El-Nagar *et al*. (2002) that reviewed the inverse relationship between hardness and freezing point, as well as sugar content and total solids in the mix, overrun, and the type and quantity of stabilizer added into the ice cream [44].

Amounts of $CaCO_3$ and cholecalciferol in the ice cream were 500 mg and 5 μg per 80 g serving, respectively, which equals 200 mg ionic calcium and 200 IU vitamin D3 per serving (80 g). Proximate analysis was performed to confirm the retention of calcium in the products after 28-day storage. Ash was greater in all fortified ice cream treatments than in the control (unfortified ice cream; $P < 0.05$) (Data not shown).

Cholecalciferol is a fat-soluble vitamin that is not stable in light, heat and air. In this study, cholecalciferol was added into the mix after pasteurization but before homogenization, when the temperature was 15°C to minimize heat degradation. However, the amount of vitamin D3 in ice cream on day 0, 7, 14 and 28 were inconsistent and did not show any predictive trend. In contrast to the study by Kazmi *et al*., which reported that cholecalciferol in butter

oil was stable through 28-day storage [39], vitamin D3 in FF ice cream was not detected on day 28 of this study. It appears that cholecalciferol was lost in the LF system in this study. The results could be influenced by the crystallization of milk fat, which is a vehicle for fat-soluble vitamins that could affect the solubility of cholecalciferol [47]. The melting temperature of milk fat is in the range between −40 and 40°C. Wiking *et al*. found that anhydrous milk fat could crystallize at temperatures below 20°C [48]. Another factor that probably affected the solubility of cholecalciferol was non-uniform dispersion of vitamin D3 in the cold mix. Thus, fortification method may also impact the retention of vitamin D3 in ice cream.

Type of calcium salt affects levels of calcium element, which could be influenced the bioavailability of calcium from the food product. Food amounts, calcium solubility, and enhancers were also described calcium absorption factors [49]. Calcium carbonate contained 40% elemental calcium, absorbed by passive diffusion, was used in this study. It was reported to have an effect on the mouth feel [50]. For vitamin D, although there are several researches revealed that dietary vitamin D could help against vitamin D deficiency, there is very little data on factors that affect absorption of vitamin D. Borel *et al*. reviewed that vitamin D3 is apparently absorbed with a similar efficiency to vitamin D2, and the food matrix has slight effect on bioavailability of vitamin D [51]. Henceforward, further studies are needed to better understand about the bioavailability and consumer acceptance of this product.

Every ice cream product in our research met the standard in microbiological testing, which indicated that the manufacturing process was clean and no contamination occurred during processing and storage. No *E. coli* were detected, and APCs were <40 CFU/g throughout the study. APC is an indicator of the bacterial level in the sample and processing environment, while coliform count indicates the contamination after pasteurization or efficacy of the pasteurization process. Goff and Hartel suggested that APCs and coliform counts should not be >20,000 and 10 CFU/g, respectively [41]. According to the regulation for ice cream by the ministry of public health, Thailand (regulation no. 222, 2002), APC must not be greater than 600,000 CFU/g and a 0.01 g ice cream sample must not contain any *E. coli*.

CONCLUSIONS

Ice cream physical properties including overrun, viscosity, hardness, and melting rate were slightly different among the treatments, but microbiological counts were not affected by fat content or fortification during 28-day storage at −20°C. Ice cream of all fat levels (<0.625 - 10% fat) could be fortified with calcium without calcium loss during 28-day frozen storage. However, unconventional methods of vitamin D fortification such as encapsulation pre-treatment may be needed to improve vitamin D dispersion in ice cream, particularly in low-fat and fat-free formulas.

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