



COCONUT OIL BASED COOKIES FORTIFIED WITH BIO-CALCIUM: CHARACTERISTICS AND NUTRITIONAL COMPOSITIONS

Supatra Karnjanapratum¹, Soottawat Benjakul^{2*}

¹Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok 10520, Thailand;

²Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai,

Songkhla 90112, Thailand

*soottawat.b@psu.ac.th

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ABSTRACT

The main objective of this study was to develop coconut oil based cookie fortified with tuna bone bio-calcium (Bio-Ca) as healthy food rich in medium chain fatty acid and calcium. The impacts of Bio-Ca supplementation and replacement of shortening with coconut oil on the quality and sensory properties of cookie were determined. Colour, diameter, thickness as well as hardness of cookies varied with levels of Bio-Ca powder and coconut oil. Bio-Ca powder at 12% could be fortified into cookie using coconut oil as shortening replacer without the adverse effect on sensory properties. Scanning electron microscopy showed that the developed cookies had less porosity with denser structure, compared to that of the control. The developed cookie showed higher saturated fat, ash and protein contents but lower energy value, compared to the control. It was rich in calcium and phosphorus. Thus, it could serve as the mineral supplement, especially for calcium.

1. Introduction

Calcium (Ca) is a structural component of human body combined with phosphorus to comprise the mineral portion of bone and teeth (Weaver and Heaney, 1999). Ca also plays an important role in the treatment and prevention of Ca deficiency in patients. There is a numerous forms of Ca used for supplementation, particularly the purified Ca carbonate with a high Ca content (about 40%) (Hassan, 2015). However, only small portion of Ca can be absorbed, mainly associated with its precipitation in gastro-intestinal track. Food Ca must be dissolved in the acidic environment of the stomach and remained soluble (Gueguen and Pointillart, 2000). From total amount of absorbed Ca, 90% takes place in the intestines, in which the pH is slightly alkaline and

precipitation hinders calcium uptake (Wasserman, 2004). Ca bound with proteins, so called bio-calcium (Bio-Ca), showed a high solubility under the neutral pH with a high Ca bioavailability, compared with inorganic counterpart (Stupar et al., 2009; Jung et al. 2006). Jung et al. (2006) found that the Bio-Ca from fish bone had the increased Ca solubility and bioavailability in ovariectomised rats. Recently, Bio-Ca powder from pre-cooked skipjack tuna bone has been produced and characterized (Benjakul et al., 2017). With the fine particle (17.07-20.29 μm) and negligible amount of volatiles of tuna bone Bio-Ca, it could be beneficially applied in foods, particularly snack products, for calcium fortification.

Cookies are flour-based products, which attract consumers owing to their various tastes, long shelf-life, relatively low cost and

availability for all consumer groups. Due to the increasing demand for health promoted natural products, there are several kinds of cookies fortified with the selected ingredient or supplement such as sunflower oil (Jacob and Leelavathi, 2007), soybean/pea protein (Bashir et al. 2015) and fructooligosaccharide (Handa et al., 2012). Coconut oil is one of the few foods that can be classified as health food from natural source. Coconut oil has been proven to provide a multitude of health benefits such as reducing inflammation and supporting almost all processes of the body (DebMandal and Mandal, 2011). It has a distinctive flavour and is mainly composed of three medium chain fatty acids (MCFAs), including caprylic acid, lauric acid and capric acid. Those MCFAs possess specific functional characteristics unlike long-chain fatty acids (LCFAs) found in other plant based oils (Yong et al., 2009). The fortification of Bio-Ca powder as well as the replacement of shortening with coconut oil could be of choice in production of cookie rich in MCFAs and Ca in bioavailable form. However, the level of both coconut oil and Bio-Ca could affect the quality and acceptability of consumers. This study aimed to develop coconut oil based cookie fortified with Bio-Ca at various levels, in which shortening was replaced by coconut oil at 65 and 100%. Physical, textural, sensory and nutritional characteristics of resulting cookies were evaluated in comparison to the typically made cookie.

2. Materials and methods

2.1. Materials

Palm oil shortening (50% palm oil, 26% hydrogenated palm kernel olein, 16% hydrogenated palm oil, 7% palm stearin and 0% trans-fat) (OP Kream Brand, Katevanich Industry Co., Ltd, Thailand), coconut oil (Naturel, Lam Soon (Thailand) Public Co., Ltd, Thailand), commercial wheat flour (Kite, United Flour Mill Public Co., Ltd, Thailand), icing sugar, backing powder, salt and peanut butter

were purchased from a local market in Hat Yai, Songkhla, Thailand.

2.2. Preparation of Bio-Ca

Bio-Ca powder was prepared from pre-cooked skipjack tuna bone according to the method described by Benjakul et al. (2017). Briefly, bones of pre-cooked skipjack tuna (Songkla Canning Public Co., Ltd., Songkhla, Thailand) were longitudinally cut and cleaned to remove the remaining meat or other residues. Prepared bones were soaked in 2 M NaOH with a bone/solution ratio of 1:10 (w/v) at 50°C for 30 min to remove non-collagenous proteins. Bones were then dried using a laboratory scale rotary tray dryer at 50°C for 2 h and ground using a crushing mill (YCM-1.1E, Yor Yong Hah Heng, Bangkok, Thailand) to obtain particle sizes of approximately 3-4 mm. Ground bone was further soaked in hexane using a sample/solvent ratio of 1:10 (w/v) at 25°C and continuously stirred for 60 min. After draining, samples were allowed to stand at room temperature until dried and free of hexane odour. The bone sample was then bleached by soaking in 10 volumes of 2.5% (v/v) sodium hypochlorite for 30 min, followed by soaking in 10 volumes of 2.5% (v/v) hydrogen peroxide for 60 min. The sample was washed with running water for 5 min. The sample was dried in a rotary tray dryer at 50 °C for 5 h and ground into the fine particles using a Ball Mill (PM 100, 127 Retsch GmbH, Haan, Germany). The obtained powder was sieved using a sieving machine (Vibratory Sieve Shaker analysette 3 Pro, FRITSCH GmbH, Deutschland, Germany), in which the particles having the sizes less than 75 µm were collected. The powder was referred to as “Bio-Ca powder”.

2.3. Preparation of cookie

The cookie was prepared using the traditional technology and formulation. The batter formulation of control cookie was as follows (based on batter weight): 55.20% wheat flour, 20.7% icing sugar, 20.7% shortening, 2.0% peanut butter, 1.0% salt and 0.4% baking powder. For coconut oil based cookie, the

coconut oil was used to substitute the shortening at 65% and 100%. Bio-Ca powder was added into the batter at 8.5%, 12.0% and 15.5% (w/w). Firstly, all dry ingredients were mixed together in a dough mixer (KitchenAid casserole multifunctional 5k, KitchenAid, Benton Harbor, MC, USA) for 3 min. Thereafter all liquid ingredients including shortening and peanut butter, previously melted at 50 °C for 10 min, were added to the dry mixture and mixed at low speed for 3 min. The resulting cookie batter was sheeted to a thickness of a 2.0 cm and cut with a circular cookie cutter (diameter: 2.4 cm). The shaped cookie batter was baked in an electric oven (Mamaru MR-1214, Mamaru (Thailand) Co., Ltd., Bangkok, Thailand) at 180 °C for 10 min. After baking, cookies were allowed to cool at room temperature for 1 h before analyses. The cookies prepared using the mixed lipid were referred to as “ML cookies”, while those made using 100% coconut oil were termed as “CO cookies”.

2.4. Analyses

2.4.1. Colour

The colour of samples was determined using a colourimeter (ColorFlex, Hunter Lab Reston, VA, USA) and reported in the CIE system, including L*, a* and b*, representing lightness, redness/greenness and yellowness/blueness, respectively. Total difference of colour (ΔE^*) and chroma difference (ΔC^*) were calculated as described by Takeungwongtrakul et al. (2015)

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

$$\Delta C^* = \sqrt{\Delta a^{*2} + \Delta b^{*2}} \quad (2)$$

where ΔL^* , Δa^* and Δb^* are the differences between the corresponding colour parameter of the sample and that of control cookie.

2.4.2. Measurement of hardness

Hardness of cookies was determined by a texture analyser (Stable Micro Systems, Godalming, Surrey, UK) using a test speed of 10 mm/s required to cut the cookies with a load cell of 50 kg. A special pasta blade and plate (probe TA 47, 60 mm x 20 mm) were used to imitate

the biting action of a tooth. The maximum force required to break cookies was calculated for each sample. Ten measurements were made for each sample.

2.4.3. Physical measurement of cookies

Cookies were evaluated for thickness (cm), diameter (cm) and spread ratio. Five cookies were used for the evaluation of each sample. The spread ratios were determined according to Chang et al. (2014). Spread ratio was calculated by dividing the diameter by thickness of cookies.

2.4.4. Sensory evaluation

Sensory evaluation was performed by 50 untrained panellists, who were familiar with the consumption of cookies. Panellists were asked to evaluate for appearance, colour, odour, texture, taste and overall likeness using a nine-point hedonic scale, in which a score of 1 = not like very much, 5 = neither like nor dislike and 9 = like extremely. The samples were labelled with random three-digit codes. Panellists were instructed to rinse their mouth with water after each sample evaluation. The order of presentation of the samples was randomised according to “balance order and carry-over effects design” (Meilgaard et al., 2006).

2.5. Determination of chemical composition and energy value

The sample was analysed for moisture, protein, fat and ash contents using analytical method No. of 925.45(A), 981.10, 948.15 and 923.03, respectively (AOAC, 2002). Water activity (a_w) was measured using a water activity meter (4TEV, Aqualab, Pullman, WA, USA). The energy, total carbohydrate, total sugar, saturated fatty acid, polyunsaturated fatty acid, monounsaturated fatty acid and trans-fat contents were estimated using compendium of methods for food analysis (Department of Medical Sciences and National Bureau of Agricultural Commodity and Food Standards, 2003).

2.6. Determination of mineral

Inductively coupled plasma optical emission spectrometer (ICP-OES) (Optima 8000, Perkin Elmer Instrument, Waltham, MA, USA) was used for determination of Ca and P in samples as per the method of Feist and Mikula (2014). The wavelengths used for Ca and P detection were 317.933 and 213.617 nm, respectively.

2.7. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to examine the microstructure of cookies. The samples were mounted on individual bronze stubs and sputter-coated with gold layer. The specimens were visualised with a scanning electron microscope (LEO 440i, Oxford, UK) at an acceleration voltage of 15 kV and 5-10 Pa pressure.

2.8. Statistic analysis

Experiments were run in triplicate using three lots of samples. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by the Duncan's multiple range tests (Steel and Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Physical characteristics and hardness of cookies

3.1.1. Colour

Surface colour of cookie containing coconut oil at 65% substitution of shortening (ML cookie) or 100% coconut oil (CO cookie) incorporated with tuna bone Bio-Ca powder at various levels in comparison with the control cookie is shown in Table 1. For ML cookies, surface had the lower L* value as the level of tuna bone Bio-Ca powder was above 8.5% ($P < 0.05$), compared with that of control (shortening based cookie without Ca fortification). Furthermore, all CO cookies showed the darker surface ($P < 0.05$) than the control as indicated by lower L* values. It was noted that CO cookie with 15.5% Bio-Ca powder showed the lowest L* value ($P < 0.05$).

For a* value, the lowest value was obtained for the control ($P < 0.05$). For both ML and CO cookies, a* values increased with increasing level of Bio-Ca powder added ($P < 0.05$). The similar a* value was found between ML and CO cookies when Bio-Ca powder at the same level was incorporated ($P > 0.05$). The increase in b* value of ML cookie was observed as the Bio-Ca level increased ($P < 0.05$). There was no difference in b* value of CO cookies in all Bio-Ca levels used ($P < 0.05$). Overall, the cookie surface turned to be browner in colour, when Bio-Ca powder and coconut oil were used at higher proportion. The increases in ΔE^* and ΔC^* values of both cookies were also found as the amount of Bio-Ca powder increased, especially for ML cookies ($P < 0.05$). Generally, CO cookies had the higher ΔE^* and ΔC^* values, compared with ML cookies when the same Bio-Ca powder was added ($P < 0.05$). Tuna bone Bio-Ca powder used in the present study was creamy whitish in colour (Benjakul et al., 2017). The shortening and coconut oil used plausibly exhibited the different physical property, governed by solid fat content (SFC), which represented the amount (%) of solid fat present in the oil at any given temperature. SFC was influenced by types of triacylglycerols in the oil (Liang et al., 2014). At room temperature (25-28 °C), shortening was in solid state with high plasticity, while coconut oil was in liquid form with low proportion of crystal phase. SFC can influence appearance, flavour release, melt rate, shelf-life and stability of fat based food products (Liang et al., 2014). Solid state of shortening might be associated with the light scattering of products as indicated by the higher lightness. Although coconut oil was liquid in nature at room temperatures, the consistent batter could be formed after the addition of Bio-Ca powder. This helped in molding the cookies prior to baking. For Bio-Ca powder, the proteins in powder might undergo Maillard reaction during baking at high temperature. This could lead to the increases in a*- and b*-values.

3.1.2. Diameter, thickness and spread ratio

Diameter, thickness and spread ratio of ML and CO cookies containing Bio-Ca powder at different levels are presented in Table 2. All cookies showed the lower diameter (2.44-2.71 cm) than the control (2.78 cm), except that of ML cookies fortified with 8.5% Bio-Ca powder ($P>0.05$) which had similar diameter to that of control. The diameter of cookie decreased as the level of Bio-Ca powder used increased ($P<0.05$). On the other hand, the increases in thickness of cookies were noticeable as the levels of Bio-Ca added increased ($P<0.05$). ML cookies showed the lower diameter with the higher thickness, compared to CO cookies at the same level of Bio-Ca powder used ($P<0.05$). The result suggested that the different proportions of coconut oil used, the presence of shortening as well as the level of Bio-Ca powder had the influence on diameter and thickness of resulting cookies. Those factors caused the varying of spread ratio of the resulting cookies. Spread ratio value decreased with increasing level of Bio-Ca powder ($P<0.05$) for both ML and CO cookies. CO cookies generally showed the higher spread ratio than ML cookies, when the same level of Bio-Ca was added ($P<0.05$). Hassan (2015) used the chicken eggshell powder for Ca fortification in biscuits and found that the thickness increased with the decreased spread ratio as the level of eggshell powder increased. Jacob and Leelavathi (2007) reported that the cookies containing sunflower oil had relatively higher spread value, compared with those used margarine and hydrogenated fat. Coconut oil with the liquid state showed the flow behaviour. Nevertheless, Bio-Ca powder could increase consistency of the resulting mixture, in which the cookies could be moulded before baking. During heating, the oil could flow to some extent as indicated by higher spread ratio. The result indicated that fat/oil type and level of Bio-Ca powder directly determined the physical characteristics, including diameter, thickness and spread ratio of cookies.

3.1.3. Hardness

Hardness of ML and CO cookies added with tuna bone Bio-Ca powder at various levels are shown in Table 2. Increases in hardness were found as the Bio-Ca level increased for both cookies ($P<0.05$). Hardness of the control was 30.54 N. The cookies added with Bio-Ca powder at the highest level (15.5%) showed the highest hardness ($P<0.05$). At the same level of Bio-Ca used, ML cookies showed the lower hardness than CO cookies ($P<0.05$). Due to the fine particle of tuna bone Bio-Ca powder (17.07-20.29 μm) (Benjakul et al., 2017), it could fill the void of cookie's structure, thus providing the denser and harder texture. Hassan (2015) found that the addition of Ca from different sources led to smaller size pores or air cells in the puffed crisp biscuit. This was related with the harder texture. The oil used for preparing cookie also influenced the texture of final product (Mamat and Hill, 2014). Jacob and Leelavathi (2007) studied the effect of fat types on cookie dough and cookie quality. Cookies containing liquid oil had a relatively harder texture, compared to bakery and hydrogenated fat. The ratio of the solid phase to the liquid phase or SFC is an important factor determining the functionality of the fat/shortening in the dough. Cookies made from sunflower oil had the hardest texture, whilst those containing bakery fat, margarine and hydrogenated fat were softer. Soft fat lowered the breaking strength of the cookies (Jacob and Leelavathi, 2007). In the present study, the lower hardness of ML cookies containing 35% shortening was attained, compared to CO cookies. In addition, the liquid oil could be dispersed upon mixing throughout the cookie batter in the form of globules that are less effective in their shortening and aerating action (Hartnett and Thalheimer, 1979). ML cookies might have the lower incorporated air. As a result, more compact structure was formed, leading to the increased hardness of CO cookies. Bio-Ca powder at higher levels caused the increased hardness of cookies. Those particles might construct the stronger matrix as well as strengthen the cookies via filler effect.

3.2. Sensory properties

The effect of addition of tuna bone Bio-Ca powder at various levels on sensory properties of ML and CO cookies is presented in Table 3. There was no difference in appearance, flavour, texture and mouth feel likeness score between control, ML and CO cookies ($P < 0.05$), except for CO cookies added with 15.5% Bio-Ca powder, which showed the lowest score ($P < 0.05$). Moreover, the incorporation of Bio-Ca powder at the highest level (15.5%) reduced the likeness score for colour, taste and overall attributes of both cookies. This was related with the darker colour (Table 1) and increased hardness (Table 2) of cookie, especially for CO cookies. It was noted that the maximal level of

Bio-Ca powder, which showed no detrimental effects on sensory properties of resulting cookies was found at 12% for CO cookies. Likeness scores for all attributes tested of CO cookies containing 12% Bio-Ca were comparable to those of the control ($P > 0.05$). It was reported that the Bio-Ca powder could be used as Ca dietary supplement in bread, pizza, spaghetti or biscuit with small changes in physical and sensory characteristics (Brun et al., 2013; Hassan, 2015). The use of coconut oil as shortening replacer could be of health benefit. CO cookie added with 12% Bio-Ca powder was then selected to study its chemical characteristics and nutritional value in comparison with the control.

Table 1. Color of cookies prepared using mixed lipids or coconut oil with addition of Bio-Ca powder at various levels

Characteristics	Samples						
	Control	ML-8.5*	ML-12	ML-15.5	CO-8.5	CO-12	CO-15.5
Color							
L*	76.28±0.69a	76.47±0.28a	73.34±0.33b	72.31±0.50c	71.87±0.42c	72.04±0.26c	69.19±0.77d
a*	4.52±0.07d	5.18±0.16c	5.93±0.27b	6.76±0.23a	5.35±0.14c	6.12±0.29b	7.45±0.33a
b*	23.92±0.39d	25.94±0.13c	27.79±0.53b	28.80±0.35a	28.94±0.49a	28.67±0.31a	30.93±0.36a
ΔE*	-	1.88±0.04d	2.18±0.30c	2.83±0.21b	2.62±0.29b	2.42±0.15b	3.65±0.22a
ΔC*	-	1.64±0.13e	3.49±0.45d	4.81±0.51b	4.84±0.31cd	5.11±0.49bc	8.23±0.76a

Control: shortening (100%).

ML: mixed lipids (35% shortening+65% coconut oil).

CO: coconut oil (100%).

*Numbers denote the level of Bio-Ca powder (% of batter weight).

Different lowercase letters in the same row indicate significant difference ($P < 0.05$).

Table 2. Characteristics and hardness of cookies prepared using mixed lipids or coconut oil with addition of Bio-Ca powder at various levels

Samples	Diameter (cm)	Thickness (cm)	Spread ratio	Hardness (N)
Control	2.78±0.05a	1.51±0.01d	1.83±0.02c	30.54±2.38b
ML-8.5*	2.78±0.07a	1.44±0.03e	1.93±0.07b	15.85±1.09d
ML-12	2.46±0.04d	1.52±0.01d	1.60±0.02e	21.29±1.71c
ML-15.5	2.44±0.04d	1.64±0.01a	1.48±0.02f	34.28±1.79a
CO-8.5	2.71±0.06b	1.29±0.02f	2.10±0.05a	22.08±1.53c
CO-12	2.67±0.02b	1.57±0.02c	1.72±0.03d	30.64±1.92b
CO-15.5	2.61±0.03c	1.60±0.01b	1.64±0.03e	37.92±1.08a

Control: shortening (100%).

ML: mixed lipids (35% shortening+65% coconut oil).

CO: coconut oil (100%).

*Numbers denote the level of Bio-Ca powder (% of batter weight).

Different lowercase letters in the same column indicate significant difference ($P < 0.05$).

Table 3. Sensory properties of cookies prepared using mixed lipids or coconut oil with addition of Bio-Ca powder at various levels

Samples	Likeness score						
	Appearance	Color	Flavor	Texture	Mouth feel	Taste	Overall
Control	7.75±0.74a	7.79±0.72a	7.75±0.85a	7.88±1.03a	7.88±0.80a	7.88±0.99a	7.58±0.83ab
ML8.5*	7.62±0.65ab	7.50±0.59ab	7.25±1.03ab	7.42±0.97ab	7.29±1.00ab	7.33±1.17abc	7.79±0.66a
ML-12	7.46±0.83ab	7.58±0.78ab	7.17±1.09ab	7.58±1.02ab	7.50±1.06ab	7.62±1.01abc	7.17±0.87bc
ML-15.5	7.63±0.71ab	7.25±0.94b	7.62±0.92ab	7.38±0.97ab	7.33±1.13ab	7.08±0.97bc	7.04±0.95c
CO-8.5	7.88±0.68a	7.79±0.78a	7.50±1.10ab	7.67±0.76ab	7.50±0.78ab	7.50±0.93abc	7.71±0.69a
CO-12	7.50±0.78ab	7.42±0.83ab	7.25±0.90ab	7.50±0.93ab	7.67±0.76ab	7.71±0.81ab	7.33±1.05abc
CO-15.5	7.21±0.72b	7.12±0.85b	7.08±1.10b	7.12±0.99b	7.08±1.06b	7.04±1.20c	7.00±0.88c

Control: shortening (100%).

ML: mixed lipids (35% shortening+65% coconut oil).

CO: coconut oil (100%).

*Numbers denote the level of Bio-Ca powder (% of batter weight).

Different lowercase letters in the same column indicate significant difference ($P < 0.05$).

Table 4. Chemical composition and energy value of the control cookie and the developed cookie

Compositions/energy value	Samples	
	Control	CO-12
Moisture (g/100g)	3.36±0.31a	2.71±0.36b
Protein (g/100g)**	7.60±0.09b	9.95±0.67a
Total fat (g/100g)	24.33±1.26a	23.97±1.08a
Saturated fat (g/100g)	12.44±0.97b	16.81±0.54a
Monounsaturated fat (g/100g)	8.05±0.22a	3.50±0.74b
Polyunsaturated fat (g/100g)	2.63±0.08a	1.46±0.27b
Total carbohydrate (g/100g)	64.02±1.53a	52.55±1.69b
Total sugar (g/100g)	21.77±1.41a	13.62±1.03b
Ash (g/100g)	1.34±0.53b	10.17±0.94a
Calcium (g/100g)	0.04±0.00b	2.94±0.02a
Phosphorous (g/100g)	0.11±0.00b	1.18±0.01a
Sodium (g/100g)	0.37±0.00b	0.39±0.00a
Trans-fat (mg/100 g)	nd	nd
Energy value (kcal/100 g)	505.45±25.48a	465.73±15.67b

** The conversion factor is 6.25.

Control: shortening (100%) without Bio-Ca powder.

CO-12: coconut oil (100%) with 12% Bio-Ca powder.

nd: not detected.

Different lowercase letters in the same row indicate significant difference ($P < 0.05$).

3.3. Chemical characteristics and nutritional value of cookies

The chemical compositions of the developed cookies (CO cookies fortified with 12.0% Bio-Ca powder) in comparison with the control (cookie prepared using shortening) are shown in Table 4. The major component of both samples

was carbohydrate (52.22-64.02%). However, the lower carbohydrate (52.55%) and sugar (13.62%) contents were found in the developed cookie, compared with those of the control ($P < 0.05$). It was noted that the developed cookie had the lower moisture content (2.71 %), compared with that of the control (3.36%). In

addition, the significant increases in protein and ash contents were observed in the developed cookie. Tuna bone Bio-Ca powder consisted of 24.26% protein and 72.20% ash (Benjakul et al., 2017). With addition of Bio-Ca powder, these components in the final product were increased. Benjakul et al. (2017) reported that pre-cooked tuna bone Bio-Ca powder was a good source of Ca and phosphorous (P), present as hydroxyapatite along with collagenous proteins. The marked increases in Ca and P contents were attained in the developed cookie. This result suggested that tuna bone Bio-Ca powder could be effectively used for Ca fortification in cookie.

The replacement of shortening by coconut oil in cookie showed the noticeable effect on chemical composition of resulting cookie, especially fat compositions. CO cookie fortified with Bio-Ca powder had the similar total fat content (23.37%) to the control (24.33%). Nevertheless, the higher content of saturated fat with lower mono- and poly-unsaturated fats was obtained in the former. Normally, coconut oil is abundant in medium chain saturated fatty acids (MCFAs) (DebMandal and Mandal, 2011). Patil et al. (2016) found that virgin coconut oil extracted from different maturity stages showed the similar fatty acid profile, in which lauric acid (C12:0) was a major fatty acid (49.74-51.18 g/100g). The coconut oil used in the present study contained saturated fat more than 90%. The shortening used was produced from palm oil, which was high in long chain fatty acid content, mainly palmitic (C-16:0) acid and oleic acid (C-18:1) (Lida et al., 2002). Palm oil consists of trisaturated (S3) (mainly PPP), disaturated (S2U) (mainly POP), and monosaturated (U2S) (mainly POO) triacylglycerides, where P is palmitic acid and O is oleic acid (Timms 1985). The shortening used also contained hydrogenated palm kernel olein (26%) and hydrogenated palm oil (16%). During shortening process, hydrogenation more likely reduced the degree of unsaturation to some degree (O'Brien, 2004). These results indicated that the fat composition of cookie was governed by the fat type used for cookie preparation.

The trans-fat content of both control and CO cookie fortified with Bio-Ca powder was also investigated. It was noted that trans-fat was not detected in both samples tested (Table 4). This result suggested that trans-fat was not formed, especially during baking at 180 °C for 10 min. The energy values of cookie (Table 4) were calculated using the Atwater factor of 4, 9 and 4 kcal/g for calculation from protein, fat and carbohydrate, respectively (Prokopov et al., 2015). The energy value of the developed cookie was lower than that of the control ($P < 0.05$). Since carbohydrate was the major component of cookie, the lower carbohydrate content of the developed cookie yielded the lower energy, compared with that of the control. The results suggested that the use of tuna bone bio-calcium powder for Ca fortification as well as the replacement of shortening with coconut oil in cookie rendered the final product with the improved nutritional value and functional aspect.

3.4. Structure of cookies

Scanning electron microscopic images of the internal cross-sectional area of cookies are shown in Figure 1. The control cookie had an open structure with air cells distributed throughout the cookie matrix. This led to the rough crumb and porous structure. The distinctive difference in the internal structure of the developed cookie was observed, compared to that of the control. The less air cell with the higher uniformity was found in the developed cookie. This dense and smooth matrix was related with lower diameter, higher thickness as well as lower spread ratio (Table 2), compared with those of control. The Bio-Ca powder added could fill the void or air cell in the cookie crumb and provided the denser structure. Moreover, the distribution of Bio-Ca powder throughout the batter matrix might interrupt the aeration property of cookie batter. Coconut oil used was the liquid, which could not entrap and retain the considerable volume of air. Conversely, the shortening used in the control cookie had more solid fat content with high plasticity, which

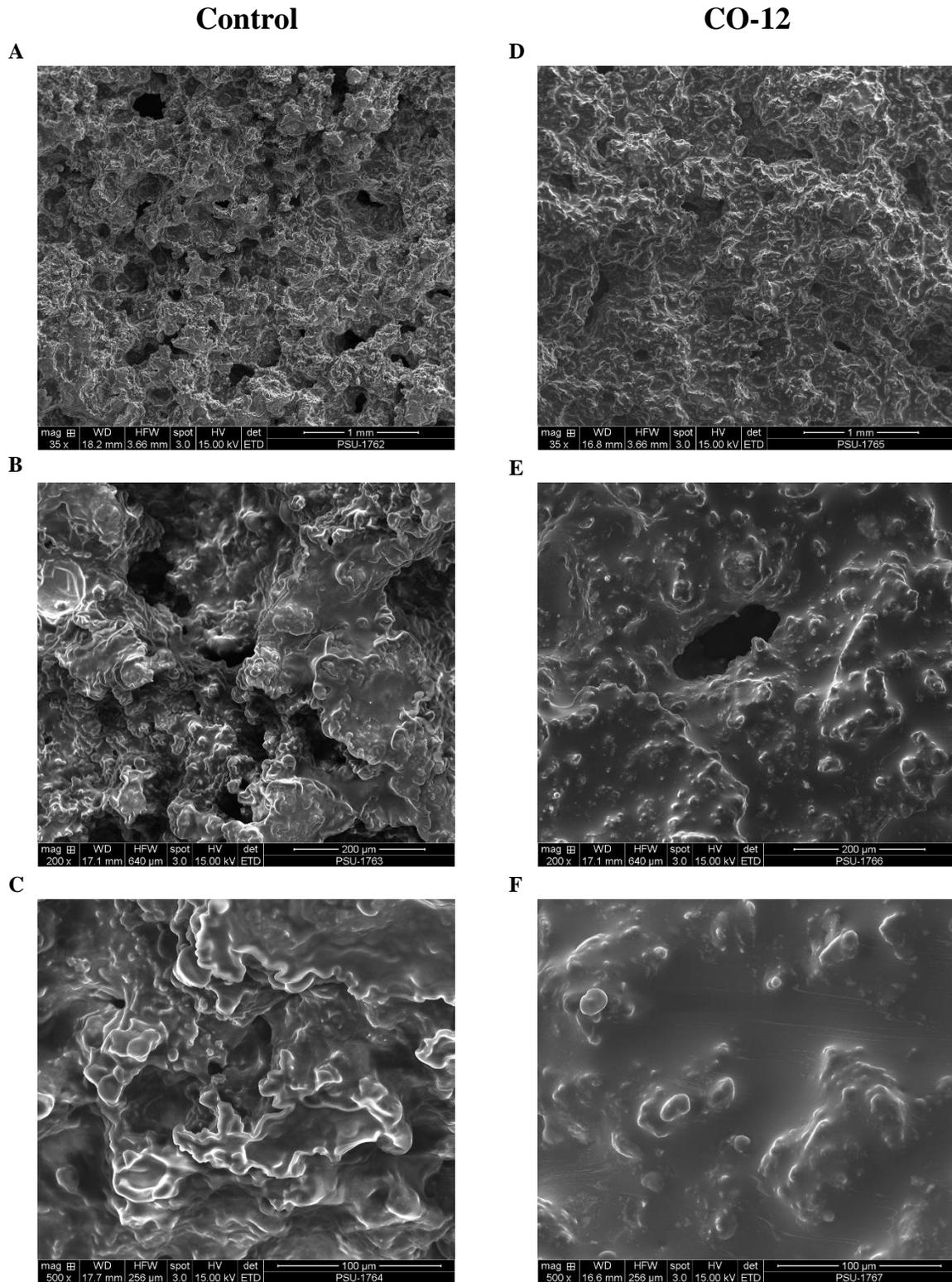


Figure 1. Scanning electron microscopic photographs of internal structure of the control cookie (A, B, C) and the developed cookie. Control: 100% shortening used for preparing cookie without bio-calcium fortification. CO-12 cookie: 100% replacement of shortening with coconut oil and fortification of 12% bio-calcium powder. A and D: 35x magnification, B and E: 200x magnification, C and F: 500x magnification.

could trap and hold air bubbles into the cookie batter throughout mixing and baking processes. The fat crystals in semi-solid fat could be enveloped in a protein membrane, which allowed the fat crystals attach to air bubbles. These fat crystals were melted during baking and the protein membrane was incorporated into the surface of air bubbles as they expanded, resulting in the rupture resistance of air cells (Manley, 2000). These results suggested that use of Bio-Ca powder for fortification and the replacement of shortening with coconut oil in cookie had the impact on internal structure of resulting cookie, which was associated with characteristics of cookie.

4. Conclusions

Incorporation of Bio-Ca powder and replacement of shortening with coconut oil had the impact on the cookie quality and sensory properties. Bio-Ca powder at 12% could be fortified into cookie prepared using coconut oil instead of shortening without the adverse effect on sensory properties. Therefore, cookies with health benefit could be prepared and served as an alternative for health conscious consumers.

5. References

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