

Effect of black pepper (*Piper nigrum*) and cinnamon (*Cinnamomum verum*) on properties of reduced-fat milk-based ice cream

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Abstract

The objective of this research was to study the effect of black pepper powder (BP) and cinnamon powder (CP) to be used as ingredients in reduced-fat milk-based ice cream. Total extractable phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric ion reducing antioxidant power (FRAP) activity, and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging of BP and CP were determined and expressed as mg of Trolox equivalent. In addition, the mixed spice (BP and CP) was mixed into reduced-fat ice cream and evaluated physical, chemical, and sensory properties. The results indicated that BP and CP exhibited a high value of TPC (656.91 ± 17.00 and 2391.85 ± 178.90) and showing the potential of antioxidant properties based on DPPH (18.54 ± 7.10 and 87.31 ± 1.50), FRAP (37.45 ± 3.10 and 108.09 ± 4.50), and ABTS (80.37 ± 19.70 and 701.49 ± 9.40), respectively. Moreover, the reduced-fat ice cream mixed with difference amount of mix spice exhibited the significance of physical and chemical properties. The reduced-fat ice cream with 0.1% mixed spice showed the suitable properties of viscosity (452.23 ± 5.10), melting rate (0.18 ± 0.01), overrun (43.46 ± 4.10), and hardness (7.72 ± 0.71). The sensory evaluation of the reduced-fat ice cream without mixed spice and 0.1% mixed spice also provided high sensory rating scores in the range of 6.5–7.7 and 6.3–6.8, respectively with higher TPC and antioxidant activities in the reduced-fat ice cream with 0.1% mixed spice. In conclusive, the BP and CP can be used as flavouring agent in reduced-fat dessert product and can be used as functional ingredients to create functional dessert.

Keywords: Black pepper powder, cinnamon powder, antioxidant, textural properties, functional dessert

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1. Introduction

Milk-based ice cream is a frozen dairy dessert composed of several ingredients such as cream, egg yolk, sugar, milk and other materials (Yeon *et al.*, 2017) which vary popular among all ages of consumers in Thailand. However, this product rich in creamy flavor which generally considered as a high-sugar and high-fat food (Limsuwan *et al.*, 2014) and can cause harmful effects of many diseases such as diabetes or obesity because of the abrupt increase of blood sugar (Her *et al.*, 2005). Many scientific data addressed the formulations of reduced-fat base ice cream can decrease the risk of chronic diseases and had a health benefit (Arora *et al.*, 2001; Limsuwan *et al.*, 2014).

Herbs are known to possess potential as the main source of new pharmaceuticals, health care, nutraceutical including functional products (Salawu *et al.*, 2011). Besides, from the research revealed the data about the functional food market in ASEAN countries exhibited rapid growth especially in Thailand (Kantatasiri, 2012).

Cinnamon (*Cinnamomum verum*) belongs to family of Lauraceae which possesses antiallergic, antiulcerogenic, antipyretic and antioxidant activity (Mathew and Abraham, 2006) while black pepper (*Piper nigrum*), a family Piperaceae was reported to have the activities of antihypertensive, antiplatelet, antioxidant, antitumor, anti-asthmatics, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, antidepressants and immunomodulator (Damanhour and Ahmad, 2014). Moreover, both herbs contain several of antioxidant compounds that can effectively scavenge reactive oxygen species (ROS) (Gülçin, 2005; Mathew and Abraham, 2006; Vidanagamage *et al.*, 2016). The oxidative stress is a condition associated with an increased rate of cellular damage induced by ROS and oxidized active molecules (O_2 , H_2O_2) via the formation of the lipid peroxidation product (Roopha and Padmalatha, 2012) leading to cause of many diseases such as inflammation, diabetes, cardiovascular diseases and cancer (Arulselvan *et al.*, 2016). Therefore, the objective of this research was to study the utilization of BP and CP as ingredients in milk-based ice cream by using reduced-fat base ice cream for a basic formula.

2. Materials and Methods

2.1 Materials

All ingredients (fresh milk, whipping cream, sugar, skim milk, guar gum, inulin, black pepper seed and cinnamon bark) of ice cream production were purchased from the local market (Songkhla, Thailand).

2.2 Chemicals

The chemicals used for determination of antioxidant activities were purchased from Sigma–Aldrich, Seelze, Germany. The solvents and others chemical were obtained from Merck, Darmstadt, Germany; Ajax Finechem, Auckland, New Zealand; QRAC, Selangor, Malaysia; Fisher Scientific, Leicestershire, England; and LAB–SCAN, Dublin, Ireland.

2.3 Sample preparation for the analysis of TPC and antioxidant activities

Black pepper seed and cinnamon bark were grounded to be a fine powder with 20–40 mesh while reduced–fat ice cream was melted to be a liquid. The black pepper powder and cinnamon powder were drenched in in 95% ethanol (1:5 w/v) at ambient temperature for 5 d while the reduced–fat ice cream sample was extracted in in 95% ethanol (1:5 w/v) at ambient temperature for 6 h. All mixture was filtered through Whatman No.4 filter paper. The collected solution was stored at 4°C for the determination of TPC and antioxidant activities (Atotaibi, 2016).

2.4 Determination of TPC

The TPC was measured using a modified Folin–Ciocalteu method (Tan and Kassim, 2011). The prepared sample (0.5 mL) was added to the tube. Then, an amount of 0.1 mL of Folin– Ciocalteu reagent (10% v/v) and 0.08 mL of sodium carbonate (7.5% w/v) were added and mixed thoroughly. After incubation for 30 min in the dark at ambient temperature, the absorbance was measured at 765 nm using spectrophotometry. The TPC was expressed as mg of Trolox equivalent (TE)/g sample via the calibration curve.

2.5 Determination of antioxidant activities

2.5.1 DPPH radical scavenging activity

DPPH radical scavenging activity was determined by the DPPH assay using the modified method from Shimada *et al.* (1992). The prepared sample (1.5 mL) was added to 1.5 mL of 0.2 to mM DPPH in 95% ethanol. The mixture was shaken lightly and stand at ambient temperature for 30 min in the dark. The absorbance was determined at 517 nm using spectrophotometry. The activity was calculated from the calibration curve of Trolox and expressed as mg of Trolox equivalent (TE)/g sample.

2.5.2 FRAP activity

FRAP assay was determined by the modified method of Benzie and Strain (1996). The stock solutions included 300 mM acetate buffer [3.1 g sodium acetate trihydrate ($C_2H_3NaO_2 \cdot 3H_2O$) and 16 mL acetic acid ($C_2H_4O_2$)], pH 3.6, 10 mM of 2, 4, 6–tripiryridyl–s–triazine (TPTZ) solution in 40 mM HCl, and 20 mM Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and then warmed up at

37°C before using. The prepared sample (15 mL) was allowed to react with 2.85 mL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were performed at 593 nm using spectrophotometry. The activity was calculated from the calibration curve of Trolox and expressed as mg of Trolox equivalent (TE)/g sample.

2.5.3 ABTS radical scavenging

ABTS assay was determined by the modified method of Arnao *et al.* (2001). The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowed them to react for 12–14 h at room temperature in the dark. The solution was then diluted by mixing 1 mL of ABTS solution with 36 mL of distilled water to obtain an absorbance of 1.1 ± 0.02 units at 734 nm. Fresh ABTS solution was prepared and used within 2 h. The prepared sample (0.15 mL) was mixed with 2.85 mL of ABTS solution and the mixture was kept at room temperature for 2 h in the dark. The absorbance was then measured at 734 nm using spectrophotometry. The activity was calculated from the calibration curve of Trolox and expressed as mg of Trolox equivalent (TE)/g sample.

2.6 Preparation of reduced-fat ice cream mixed with BP and CP

Five formulations of ice cream were manufactured by using the reduced-fat milk-based ice cream formula for production. The mixed spice of BP and CP in the ratio of 1:3.75 was prepared and added in ice cream sample: 0.0% (control), 0.1% (M0.1), 0.3% (M0.3), 0.5% (M0.5), and 0.7% (M0.7) as shown in Table 1. All ingredients were homogenized for 2 min and the mixture was heated at 80°C for 2 min and cooled down to 4°C before ripened at 4°C for 18–24 h. After ripening, the ice cream mix was produced and then frozen in an ice cream maker for 20 min. The ice cream was stored at minus 20°C before analysis of physical, chemical and sensory properties.

Table 1 Reduced-fat ice cream formulation

Ingredients (%w/w)	Sample				
	Control	M0.1	M0.3	M0.5	M0.7
Fresh milk	51.69	51.63	51.57	51.50	51.40
Whipping cream	19.01	18.99	18.96	18.94	18.92
Sugar	7.76	7.75	7.74	7.73	7.72
Skim milk	19.05	19.02	19.00	18.97	18.94
Guar gum	0.25	0.25	0.25	0.25	0.25
Inulin	2.19	2.18	2.18	2.18	2.17
Mixed BP and CP	-	0.10	0.30	0.50	0.70
Total	100.00	100.00	100.00	100.00	100.00

2.7 Physical properties analysis

2.7.1 Color measurement

Color values were reported in the Complete International Commission on Illumination (CIE) system color profile by using a Hunter LabMiniscan colorimeter (VA, USA). The L^* represents light/dark while, $a^*/-a^*$ represents red/green and $b^*/-b^*$ represents blue/yellow.

2.7.2 Viscosity test

The viscosity of reduced-fat ice cream was measured using Brookfield Viscometer with spindle LV-3 (63) at 60 rpm. The ice cream sample (400 mL) analyzed under $14 \pm 1^\circ\text{C}$ and kept in the cold water at $14 \pm 1^\circ\text{C}$ to prevent the temperature fluctuation during the analysis.

2.7.3 Melting test

Melting rates of reduced-fat ice cream samples were measured by the modified method of Rosalina and Richard (2004) in a controlled temperature (25°C) chamber. The samples were tempered in a freezer at -20°C for 24 h before analysis. For melting rate, 30 g of sample was placed on a 2-mm stainless-steel screen with a funnel and graduated cylinder beneath to collect the melt. Volumes were recorded once every 10 min until the sample completely melting.

2.7.4 Overrun test

The percentage of overrun was measured by comparing the weight of melted ice cream in a fixed volume container with the weight of frozen ice cream (Arbuckle, 1986). Percentage of overrun was calculated as a followed equation;

$$\% \text{ Overrun} = \frac{(\text{Weight of melted ice cream} - \text{Weight of frozen ice cream})}{\text{Weight of frozen ice cream}} \times 100$$

2.7.5 Hardness test

The hardness of reduced-fat ice cream was measured using a texture analyzer (TA-XT2, Texture Analyser, Stable Micro System Ltd., UK) equipped with a 6 mm diameter stainless steel cylindrical probe (modified method of Bolliger *et al.*, 2000). The sample was stored at $-22 \pm 1^\circ\text{C}$ for 24 h before analysis. The conditions for analysis were as follows: penetration distance was 15 mm, the force was 5.0 g, probe speed during penetration was 2.0 mm/s, probe speed pre- and post-penetration was 1.0 mm/s. Hardness was measured as the peak compression force (g) during the penetration of the sample, and adhesiveness as the negative peak force during withdrawal.

2.8 Sensory evaluation

Ice cream samples (45 g) were coded randomly with three-digit codes and served randomly one by one of each sample to panelists and during testing samples, the panelists were served some water to drink. The sensory quality of ice cream product was determined by 100 panelists with 9-point hedonic scale as 1 (Dislike extremely); 2 (Dislike very much); 3 (Dislike moderately); 4 (Dislike slightly); 5 (Neither like nor dislike); 6 (Like slightly); 7 (Like moderately); 8 (Like very much) and 9 (Like extremely). The preference rating score of reduced-fat ice cream product was evaluated in terms of appearance, color, aroma, flavor, texture, sweetness, oiliness, and overall acceptability (Meilgaard *et al.*, 2007).

2.9 Statistical analysis

The experiment was designed as a completely randomized design (CRD) and a randomized complete block design (RCBD). Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range tests. Significance was declared at $P < 0.05$ using the statistical software.

3. Results and Discussion

3.1 TPC and antioxidant activities from BP and CP extracts

The results of the BP and CP extracts on TPC and antioxidant activities (DPPH, ABTS, and FRAP) were presented in Table 2. The CP extract expressed the higher value of TPC and antioxidant activities than BP. Moreover, the antioxidant activities in both extracts exhibited the highest value of FRAP assay followed by ABTS and DPPH assay, respectively. As known that using an aqueous solvent (95% ethanol) for extraction in both herbs provide more high polarity antioxidant compounds which are easily determined by FRAP and/or ABTS assays while DPPH assay has high of affinity to lipophilic antioxidants (Martysiak-Żurowska and Went, 2012; Berker *et al.*, 2013). Similar to the finding of Shahid *et al.* (2018) who reported the antioxidant activity of CP extract had high in FRAP than DPPH assay. In addition, the

difference of TPC including the antioxidant activity depends on many variable factors such as plant type, plant location, extract preparation including a bioactive compound in the plant. It has been established that cinnamaldehyde and piperine are the major compounds of CP and BP, respectively. Cinnamaldehyde including its derivatives such as cinnamic acid, methyl cinnamate, and cinnamyl alcohol was reported as a strong antioxidant (Woehrlin *et al.*, 2010; Rao and Gan, 2014; Suryanti *et al.*, 2017). While, piperine was reported to provide the antioxidant activity and antimicrobial (Butt *et al.*, 2013; Zou *et al.*, 2015).

Table 2 TPC and antioxidant activities from BP and CP extracts

Antioxidant activities (mg TE/g sample)	BP	CP
TPC	656.91 ± 17.00 ^b	2391.85 ± 178.90 ^a
DPPH	18.54 ± 7.10 ^b	87.31 ± 1.50 ^a
ABTS	37.45 ± 3.10 ^b	108.09 ± 4.50 ^a
FRAP	80.37 ± 19.70 ^b	701.49 ± 9.40 ^a

Remark: TPC means total extractable phenolic content; TE mean Trolox equivalent.

^{a-b} mean within a row with different letters are significantly different ($P < 0.05$), Values are represented as mean ± standard deviation (n=3)

3.2 Physical properties of reduced-fat ice cream mixed with BP and CP

3.2.1 Color value of reduced-fat ice cream mixed with BP and CP

Color value of reduced-fat ice cream mixed with a variation of BP and CP as showed in Table 3. The detail was expressed in L^* , a^* and b^* values. It was found that all samples showed the lower value in lightness (L^*) and higher value in redness (a^*) compared with control. However, the result showed that the yellowness (b^*) value was not changed significantly compared with control except in the M0.7 sample. The decrease of L^* and increased in a^* of sample related to the decreasing of whiteness. This result may depend on the increasing of mixed BP and CP affected the color tone of ice cream. Similar to the finding of Yeon *et al.* (2017) who mentioned the decreasing of color value in ice cream contain with fermented pepper powder, L^* decreased while a^* and b^* increased. It suggested that BP and CP can added that shade of light brown which caused higher redness and yellowness.

Table 3 Color values of reduced-fat ice cream mixed with BP and CP

Sample	L^*	a^*	b^*
Control	89.79 ± 0.45 ^a	1.09 ± 0.12 ^d	16.09 ± 0.31 ^b
M0.1	87.01 ± 0.09 ^b	1.27 ± 0.05 ^c	16.13 ± 0.11 ^b
M0.3	80.34 ± 0.45 ^c	2.91 ± 0.11 ^b	16.37 ± 0.10 ^b
M0.5	76.76 ± 0.52 ^d	3.88 ± 0.22 ^a	16.37 ± 0.32 ^b
M0.7	77.47 ± 0.24 ^d	3.96 ± 0.33 ^a	19.13 ± 0.09 ^a

Remark: ^{a-d} mean within a column with different letters are significantly different ($P < 0.05$). Values are represented as mean ± standard deviation ($n=3$)

3.2.2 Textural properties of reduced-fat ice cream mixed with BP and CP

Textural properties of reduced-fat ice cream mixed with BP and CP were presented in Table 4. The results showed all parameters that consist of viscosity, melting rate, overrun and hardness from sample products significantly different from control. The viscosity of the ice cream sample M0.7 showed significantly highest value compared with other samples. This finding result happened because of the mucilage production of BP and CP during ripening and the sample becomes more sticky and higher of viscosity. Shaheen *et al.* (2015) addressed that mucilage had presented in CP while Yeon *et al.* (2017) found that the ice cream contained fermented pepper powder showed higher viscosity than control. In addition, the presence of the melting rate is related to air content and fat network in ice cream (Muse and Hartel, 2004) and may correlate to other physical properties including viscosity, hardness, and overrun (Prapasuwannakul *et al.*, 2014). Aranda-Gonzalez *et al.* (2016) reported that higher viscosity ice cream melted more slowly while Sofjan and Hartel (2004) stated that the lower overrun effect to the higher melting rate of ice cream. Moreover, Muse and Hartel (2004) reported that ice cream with higher overrun tends to have softer. This effect may have been related to the air cell size in each ice cream during freezing. The increase in overrun promoted the break-up of larger air cells into smaller ones during freezing and led to higher apparent viscosity and softness (Sofjan and Hartel, 2004). Moreover, in this study, the high viscosity of sample M0.3 and control showed a slow of melted rate compared with other samples. However, there are many variable factors effect on the physical properties of ice cream such as fat network, air content, total solid, ice crystal content and ice crystal size (Sakurai *et al.*, 1996; Muse and Hartel, 2004; Sonthisawate and Chantarapanont, 2015; Yeon *et al.*, 2017).

Table 4 Textural properties of reduced-fat ice cream mixed with BP and CP

Sample	Viscosity (cP)	Melting rate (g/min)	Overrun (%)	Hardness (g.force × 10 ³)
Control	507.97 ± 3.57 ^c	0.33 ± 0.01 ^a	81.51 ± 9.02 ^a	1.18 ± 0.06 ^d
M0.1	452.23 ± 5.10 ^d	0.27 ± 0.03 ^b	43.46 ± 4.10 ^b	7.72 ± 0.71 ^a
M0.3	620.00 ± 3.30 ^b	0.34 ± 0.03 ^a	39.77 ± 4.00 ^b	7.18 ± 0.90 ^a
M0.5	422.23 ± 5.10 ^d	0.18 ± 0.01 ^c	64.32 ± 5.70 ^a	3.79 ± 0.38 ^b
M0.7	1883.00 ± 3.00 ^a	0.22 ± 0.02 ^c	33.68 ± 7.50 ^b	1.87 ± 0.39 ^c

Remark: ^{a-d} mean within a column with different letters are significantly different ($P < 0.05$). Values are represented as mean ± standard deviation ($n=3$)

3.3 Sensory evaluation of reduced-fat ice cream mixed with BP and CP

The sensory evaluation of reduced-fat ice cream mixed with BP and CP were evaluated in terms of appearance, color, aroma, flavor, texture, sweetness, oiliness and overall acceptability in Fig 1 and the appearance of the product was shown in Fig 2. The results showed that the increasing of mixed spice can significantly decreased the sensory rating score. The decreasing of rating score in sweets product can happen due to the increasing of pungency from spice that affected the sensory evaluation rating score to be decrease (Yang and Lee, 2019). However, the amount of mixed spice (0.1–0.7%) can maintain the rating score of consumer toward ice cream product in the range of neither like nor dislike to like extremely (5.0–7.7). From the result of all sensory evaluation, sample with 0.1 mixed spice (M0.1) was accepted and showed the difference was not significant ($P \geq 0.05$) from control. Therefore, in sensory evaluation, the ice cream with 0.1% mixed spice demonstrated similarity to the control sample.

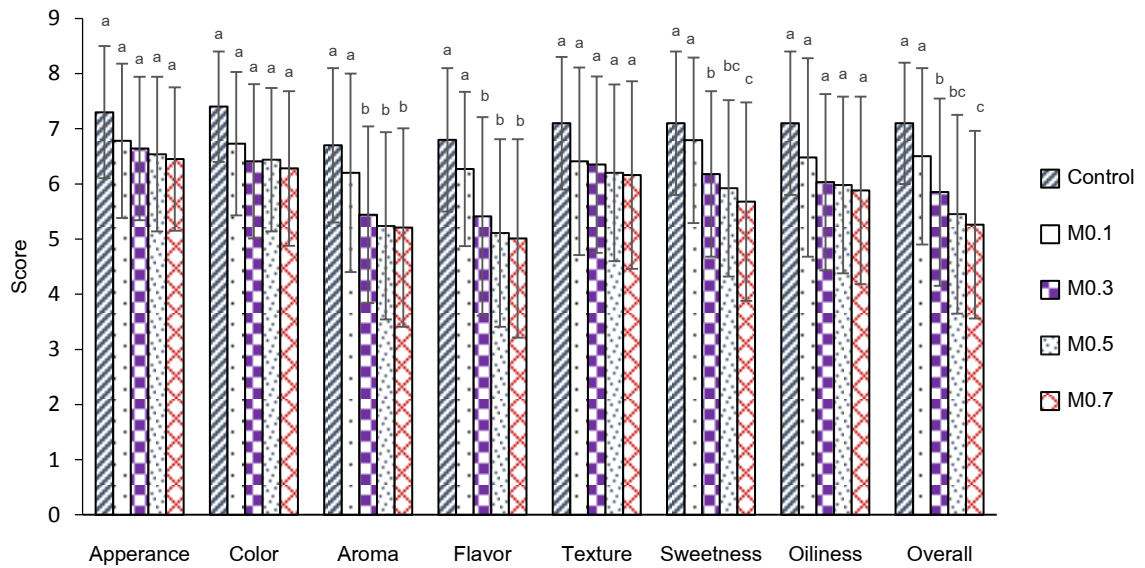


Fig 1 Sensory evaluation of reduced-fat ice cream mixed with BP and CP.

^{a-c} mean different letters are significantly different ($P < 0.05$). Values are represented as mean \pm standard deviation ($n=100$)

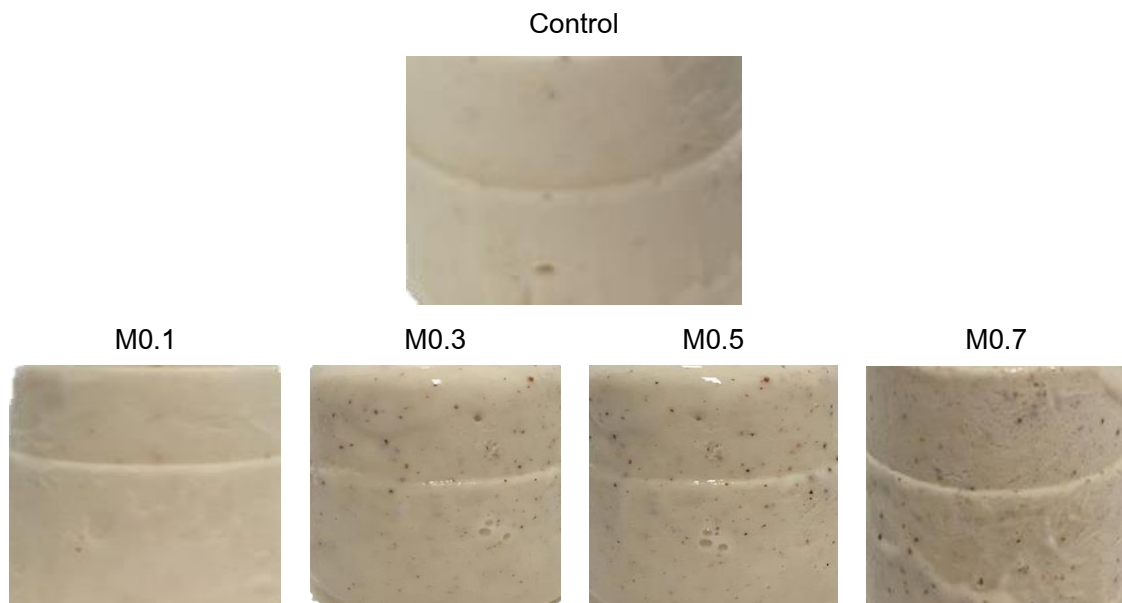


Fig 2 The presence of reduced-fat ice cream mixed with BP and CP at different level; Control (0%), M0.1 (0.1%), M0.3 (0.3%), M0.5 (0.5%), and M0.7 (0.7%)

3.4 TPC and antioxidant activities of the selected reduced-fat ice cream mixed with BP and CP

TPC and antioxidant activities of the selected formula of reduced-fat ice cream mixed with BP and CP at 0.1% was significantly higher than control ($P < 0.05$). This result suggested that ice cream mixed with both herbs had a high antioxidant activity.

Table 5 TPC and antioxidant activities of selected ice cream compared with control

Ice cream Sample	DPPH (mg TE/ 100 g sample)	FRAP (mg TE/ 100 g sample)	ABTS (mg TE/100 g sample)	TPC (mg TE/100 g sample)
Control	73.99 ± 10.29 ^b	28.10 ± 5.58 ^b	0.24 ± 0.07 ^b	95.47 ± 36.23 ^b
M0.1	129.40 ± 9.14 ^a	96.09 ± 41.46 ^a	0.87 ± 0.05 ^a	295.77 ± 11.55 ^a

Remark: TPC means total extractable phenolic content; TE means Trolox equivalent.

^{a-b} mean different letters are significantly different ($P < 0.05$). Values are represented as mean ± standard deviation (n=3)

4. Conclusion

The finding from this research can indicate that BP and CP extracts provided high value of TPC (656.91 ± 17.00 and 2391.85 ± 178.90 mg TE/g sample), DPPH (18.54 ± 7.10 and 87.31 ± 1.50 mg TE/g sample), FRAP (80.37 ± 19.70 and 701.49 ± 9.40 mg TE/g sample), and ABTS (37.45 ± 3.10 and 108.09 ± 4.50 mg TE/g sample) respectively. The BP and CP can also mix to create preference flavor to dessert product such as ice cream and reduce fat ice cream. All ice cream sample with mixed spice showed significant difference of physical and chemical properties compared with ice cream without mixed spice. The reduced-fat ice cream with 0.1% mixed spice (M0.1) exhibited a high value of TPC and high antioxidant activities with high sensory rating score in all sensory attributes. The reduced-fat ice cream with 0.1% mixed spice also showed the suitable properties for mixed spice ice cream. Additionally, the sensory evaluation of the reduced-fat ice cream 0.1% mixed spice also provided high sensory rating scores in the range of 6.3–6.8 with higher TPC and antioxidant activities. In conclude, the BP and CP can be used as flavouring agent in reduced-fat dessert product and can be used as functional ingredients to create functional dessert.

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