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Research Article

Effects of butterfly pea extract and flower petals on sensory, physical, chemical and microbiological characteristics of sugar-free ice cream

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Abstract

The objective of this research was to study the feasibility of using a Thai herb, the butterfly pea, to enrich sugar-free ice cream. Formulations of ice cream containing 0.3% liquid extract from dried butterfly pea flower and 1%, 3% or 5% fresh butterfly pea flower petals were tested in comparison with a basic sugar-free ice cream formula. The results of sensory testing showed that there was no significant difference among the different formulations, except for the smoothness. The ice cream containing 3% butterfly-pea flower petals received the highest overall "liking" score. Consumer acceptance testing showed that the 3% flower petal ice cream was more acceptable than the 5% flower petal formulation and all 100 participating panelists accepted the idea of using Thai herbs as an ingredient in frozen desserts. The ice cream that contained butterfly pea flowers had purple colouration and had higher anthocyanin content, total phenolic compounds and antioxidant activity; as measured using ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picryl-hadrazyl (DPPH) methods than did the basic sugar-free ice cream without butterfly pea flowers. These values increased with increasing butterfly pea flower petal content, to a statistically significant degree ($p<0.05$). The 3% flower petal formula showed 0.585±0.139 mg/100 g fresh weight of anthocyanin content, 0.884±0.017 mgGAE/1 g dry weight of total phenolic compounds, 10.114±0.562 µmol Trolox/1 g dry weight of antioxidant activity as measured by FRAP and 7.579±0.223 µmol Trolox/1 g dry weight as measured by DPPH. The (*in vitro*) glycaemic responses of the sugar-free ice cream formulation was lower than that of the normal ice cream made with sugar and the former showed lower calorie and carbohydrate content. The addition of butterfly pea flowers to the ice cream did not affect the glycaemic index, but increased the fibre content. The sugar-free ice cream made with butterfly pea flower extract and petals showed good potential as a healthy frozen dessert product with natural colouration, antioxidative properties and lower glycaemic response.

Keywords: confectionary, low-calorie, healthy food, herb, additive, butterfly-pea, antioxidant, anthocyanin, total phenolic compounds, *Clitoria ternatea* Linn., Thailand.

Introduction

Ice cream is a very popular frozen dessert among consumers of all ages in many countries, including Thailand, mainly because of its flavour and refreshing coolness. The ice cream consumption in Thailand has grown steadily over the years. Similar trends were also observed in other groups of the population. The follow-up examination of dietary data from 1991 to 2008 in 2,894 American adults found that the consumption of ice cream in that population increased significantly [1, 2]. There are different varieties of ice cream formulations, most are considered as a high-sugar and high-fat food. Research has shown that obese individuals tend to consume more high-sugar and high-fat food than do those with average weight [3]. Additionally, dietary patterns of persons with diabetes, who are not successful at controlling their blood sugar levels, showed that they consume more high-sugar food than do diabetic individuals who can control their blood sugar levels [4]. The development of a low-sugar and a low-fat ice cream could provide a good dietary alternative for such consumers. Several herbs native to Thailand possess anti-oxidant properties and have been traditionally used as food ingredients in Thai cuisine. Butterfly pea (*Clitoria ternatea* L.) is one of the herbs commonly used to flavour and colour Thai desserts and beverages. The flower's purple colour is attributable to anthocyanin, which has proven antioxidant properties [5, 6]. Anthocyanins are absorbed as intact molecules in the stomach [7] and have systemic activity, exerting antineoplastic, anticarcinogenic, antiatherogenic, antiviral and anti-inflammatory effects, as well as decreasing capillary permeability and fragility, inhibiting platelet aggregation and stimulating immune function [8, 9, 10, 11]. Therefore, the current experiment was designed to study the feasibility of using butterfly pea extract and flower petal as an ingredient in sugar-free and low-fat ice cream for natural colouration and antioxidative health benefits. The extract and flower petal effects on sensory, physical, chemical and microbiological characteristics of ice cream were also studied.

Figure 1. Butterfly pea (*Clitoria ternatea* Linn.).

Materials and Methods

Materials

The basic and sugar-free and low-fat formula of ice cream based on previous research [12] was used. The basic formula comprised 73.39% water, 0.1% sodium chloride, 0.27% gelatin powder, 0.1% guar gum (KHB1598, Healthy&Tasty), 2.29% powdered lime (Knorr) and 23.85% sugar. The formulation was developed from the basic formula of sherbet ice cream, a type of ice cream formulation which contained higher acid (0.3%) and sugar (25-35%) than do most dairy-based ice creams. Milk solid and fat were also excluded from the formulation. For the sugar-free formula,

3.58% sucralose (D-et®, U-Sing Co., Ltd., Thailand) and 20.27% inulin (Fibruline® Instant, negative inulin, DPO Thailand) were used to substitute sugar.

Ice cream processing

Liquid extract from dried butterfly pea flowers (3%) was added to the basic formula, replacing the normal amount of water during preparation. The ice cream preparation was held at 80°C for at least 1 second before cooling down immediately and storing at 4°C for 24 hours before being transferred to an ice cream maker (Nemox, pro3000). Fresh butterfly pea flower petals were added to the ice cream mix in separate concentrations of 1%, 3% and 5% to create a particular texture. Prior to being used, the fresh butterfly pea flower petals were washed and rinsed. The butterfly pea flower containing sugar-free ice cream was stored at -18°C for future analysis.

Sample analysis

Samples of the basic and sugar-free formula ice cream with and without butterfly-pea flowers were analyzed by the following criteria:

- Physical characteristics: the apparent colour was examined using a HunterLab Color Flex colour tester.
- Chemical characteristics: proximate analysis [13]; anthocyanin content [14]; antioxidant activity by DPPH (2,2-diphenyl-1-picryl-hydrazyl) adapted from Zigoneanu *et al*., [15]; and FRAP (Ferric reducing antioxidant power) using the method developed by Benzie and Strain [16]; total phenolic compound content by using the Folin-Ciocalteu method [17]; and glycaemic response by using the *in vitro* starch digestibility method developed by Ratanakopan [18].
- Sensory characteristics: sensory evaluation by 20 untrained taste panelists giving a score on a 9-point hedonic scale in a laboratory and a consumer acceptance evaluation with a sample of 100 individuals on a central location test.
- Microbiological characteristics: measuring total number of colony forming units as a total plate count (TPC); and the number of *Escherichia coli* and *Staphylococcus aureus.* (BAM online 2001).

Statistical analysis

Data from the physical and chemical testing were analyzed statistically using ANOVA and Bonferroni correction to compare differences in means and sensory evaluation data were analyzed using the Friedman Test of Ranking via computer applications.

Results and Discussion

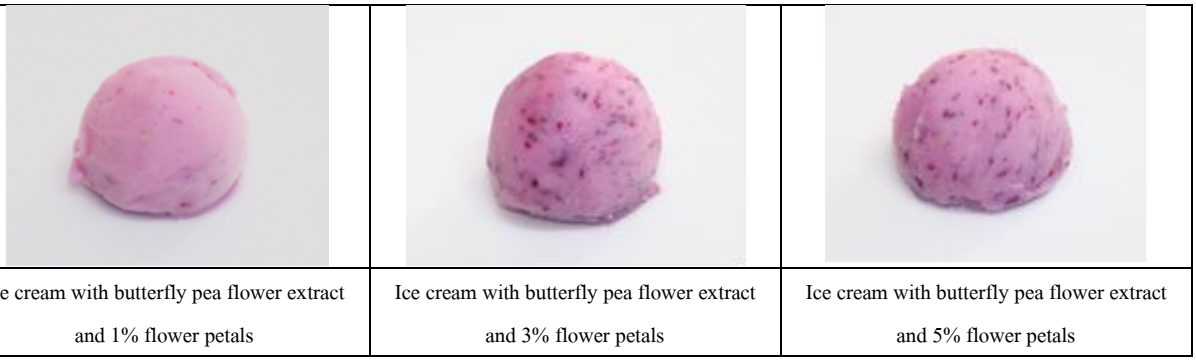
Colour appearance

The addition of butterfly pea flower extract resulted in purple colouration of the ice cream (Fig. 2). With the butterfly pea flower petals added, the appearance of flower petals was seen scattered throughout the ice cream (Fig. 3).

Sugar-free ice cream w/o butterfly pea extract

Sugar-free ice cream with 0.3% butterfly pea flower extract

Figure 2. Sugar-free ice cream with and without 0.3% butterfly pea flower extract.



Ice cream with butterfly pea flower extract and 1% flower petals	Ice cream with butterfly pea flower extract and 3% flower petals	Ice cream with butterfly pea flower extract and 5% flower petals

Figure 3. Sugar-free ice cream with 3% butterflypea flower extract and varying amount of butterfly pea flower petals.

L*, a* and b* values

The addition of butterfly pea flower petals to ice cream significantly decreased the colour lightness, or L* value ($p<0.05$), whereas the a* and b* values increased with increasing concentration of butterfly pea flower petals, also to a statistically significant degree ($p<0.05$) (Fig. 4). This is attributable to the purple colouration due to the anthocyanin in the butterfly pea flowers, which were scattered throughout the ice cream and gave it a more appealing appearance as confirmed from the sensory evaluation (discussed later on).

Sensory evaluation

There was no statistically significant difference in the appearance, colour, odour, taste and overall "liking" scores given by the tasting panels for the sugar-free ice cream formulas with only butterfly pea flower extract added and those with 0, 1, 3 and 5% fresh butterfly pea flower petals. As for the texture smoothness, the mean scores of the 3% and 5% flower petal formulas were lower to a statistically significant degree ($p<0.05$), mainly because the bits of flower petals reduced the smoothness of the ice cream. Nevertheless, the addition of flower petals tended to increase the appearance scores. The panels gave higher mean overall "liking" score to the formulation with 3% butterfly pea flower petals than to other formulations, including the basic formula with no butterfly pea ingredients added. The mean overall "liking" score for the 3% butterfly-pea flower formulation was 7.60 ± 0.99 on a 9-point hedonic scale.

Table 1. Sensory evaluation of herbal sugar-free ice cream.

	Sensory score (9-point hedonic scale) (Mean±S.D.)			
	0%	1%	3%	5%
Appearance	6.80 ± 1.75	7.45 ± 0.99	7.90 ± 0.85	7.55 ± 0.94
Colour	6.95 ± 1.43	7.40 ± 1.23	7.80 ± 0.89	7.70 ± 0.92
Odour	7.05 ± 0.99	7.15 ± 0.93	7.15 ± 0.93	7.40 ± 0.94
Taste	7.55 ± 1.05	7.70 ± 0.92	7.20 ± 0.83	7.10 ± 0.71
Smoothness	7.95 ± 1.05^{a}	7.50 ± 1.39^{ab}	7.25 ± 1.11^{b}	7.20 ± 1.00^{b}
Density	7.45 ± 0.99	7.25 ± 1.20	7.25 ± 1.06	7.25 ± 0.78
Overall-liking	7.40 ± 1.18	7.35 ± 1.36	7.60 ± 0.99	7.35 ± 0.48

Note: Means in the same row with different superscripts signify a statistically significant difference ($p<0.05$)

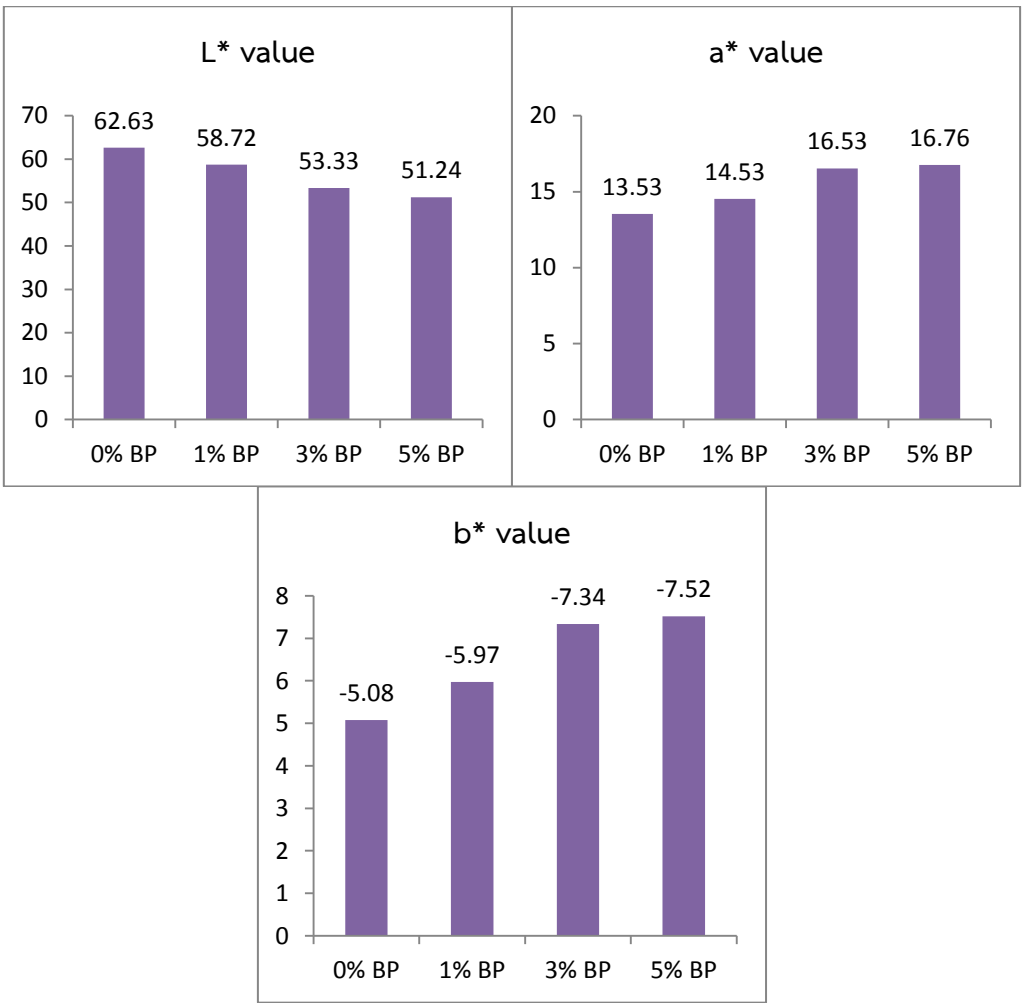


Figure 4. Colour parameters (L*, a*, and b* values) of sugar-free ice cream formulas with 0%, 1%, 3% or 5% BP (butterfly pea flower petals).

Consumer acceptance

The sugar-free ice cream formulations with 3% and 5% butterfly pea flower petals added were assessed by consumer acceptance test, as scored by 100 individuals at the Kasetsart University campus in Bangkok; more than half (60%) of the individuals were women. The ages of the individuals ranged from under 20 to over 41, but the largest number (41%) were in the 21-25 age group. Slightly more than half (56%) had bachelor's degrees and 25% had postgraduate degrees. The mean consumer acceptance scores for the sugar-free butterfly pea ice cream formulations containing 3% and 5% butterfly pea flower petals were not significantly different, but the mean score for the 3% formulation was higher, which was consistent with the results of the earlier sensory evaluation testing. The overall likability scores for the 3% and 5% formulations were 7.39±1.19 and 7.18±1.47 points, respectively, on a 9-point hedonic scale. Ninety two percent of the consumers agreed to buy the ice cream if it was to be sold and all of them accepted the concept of using herbs as an ingredient in ice cream products.

Analysis of anthocyanin content, total phenolic compound and antioxidant activity

Sugar-free ice cream formulations with butterfly pea flower extract and butterfly pea flower petals showed significantly higher anthocyanin, total phenolic compounds and antioxidant content, as measured using FRAP and DPPH, than did the basic formula without butterfly pea extract and flower petals ($p<0.05$) (Fig. 5). These values increased with increasing butterfly pea flower petal content, owing to the phenolic content and antioxidant activity of butterfly pea flowers [5]. Anthocyanin was undetectable in the basic formulation with no butterfly pea extract and flower petal and the formulation's antioxidant activity – as analyzed by either FRAP or DPPH - was 7.97-12.45 times lower than that of the other formulations with butterfly pea flower extract and flower petals. The maximum antioxidant activity was 12.459±0.738 µmol Trolox/g dry weight, as

estimated by FRAP and 8.078±0.112 µmol Trolox/g dry weight, as measured by DPPH for the formulation with 5% butterfly pea flower petals. This indicates that the use of herbal ingredients not only gives an appealing colour to the ice cream, but also makes the formulation healthier by virtue of the antioxidative compounds.

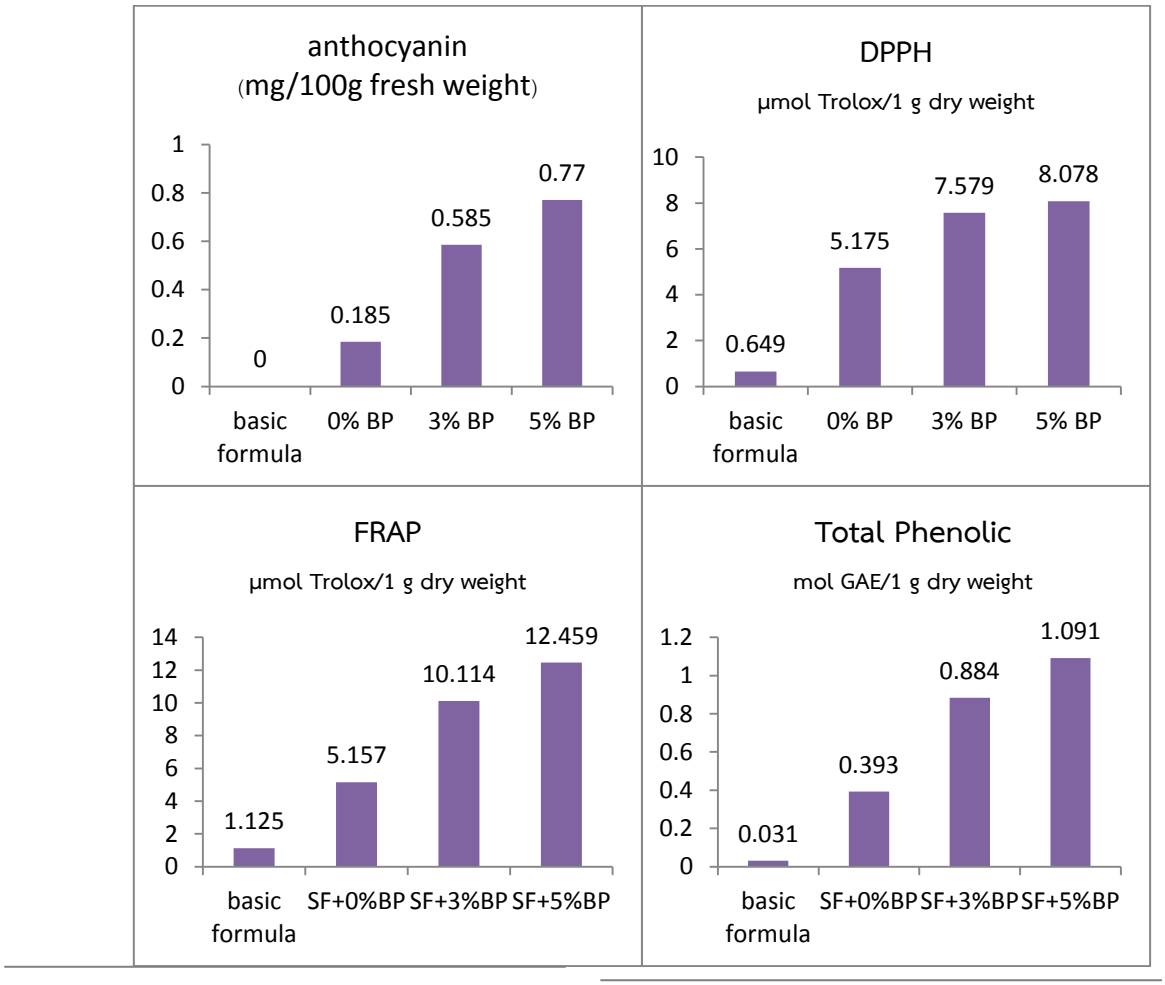


Figure 5. Anthocyanin content and antioxidant activity of sugar-free ice cream formulations without (basic formula) and with 0%, 3%, and 5% BP (butterfly pea flower petals).

Glycaemic response analysis

Glycaemic response concept was originally introduced to classify different sources of carbohydrate-rich food for their effect on post-meal blood glucose level and it was originally targeted at people with diabetes as a guide to food selection [19, 20]. Low glycaemic response food is associated with a reduced risk of certain chronic diseases such as diabetes and cardiovascular disease [21]; in particular, a study showed improved insulin sensitivity, lipid metabolism and reduced chronic inflammation due to low glycaemic response food in a Dutch population [22]. Glycemic response was analyzed *in vitro*, simulating the digestive process in a test tube, by adding artificial saliva, pepsin, pancreatin and amyloglucosidase to the ice cream samples and then measuring the amount of glucose released. The rapidly available glucose, slowly available glucose and total glucose values from sugar-free ice cream samples were significantly lower than those of the basic ice cream formulation (with sugar), and the glycaemic response of the sugar-free ice cream formulations was lower than that of the basic formula to a statistically significant degree ($p<0.05$). This could be attributable to the zero-sugar content of the sugar-free ice cream and the sugar substitutes (sucralose and inulin) are not digested by normal enzymes in the human gut. Sucralose is a modified sucrose molecule that cannot be broken down and absorbed by the human digestive system but has a sweet taste very similar to sugar. Its sweetness is 400-800 times that of sugar but it has no calories and does not cause dental cavities [23, 24]. Inulin is a fructan polymer composed of fructose molecules

held together by β-D (2-1) bonds, with or without a glucose molecule at one end. Because of the β-D (2-1) bonds, inulin cannot be digested by normal enzymes in the human body. It is also considered as a dietary fibre that can be utilized by the bifidobacteria in the large intestine, making inulin a functional prebiotic [25]. The addition of butterfly pea flowers did not significantly affect the glycaemic response of the sugar-free ice cream. These results indicate that the sugar-free ice cream containing butterfly pea flower petals provides lower glycaemic response response than does the basic ice cream formulation (0.16-0.17 of the sugar-free compared with 4.52 of the basic) Fig. 6.

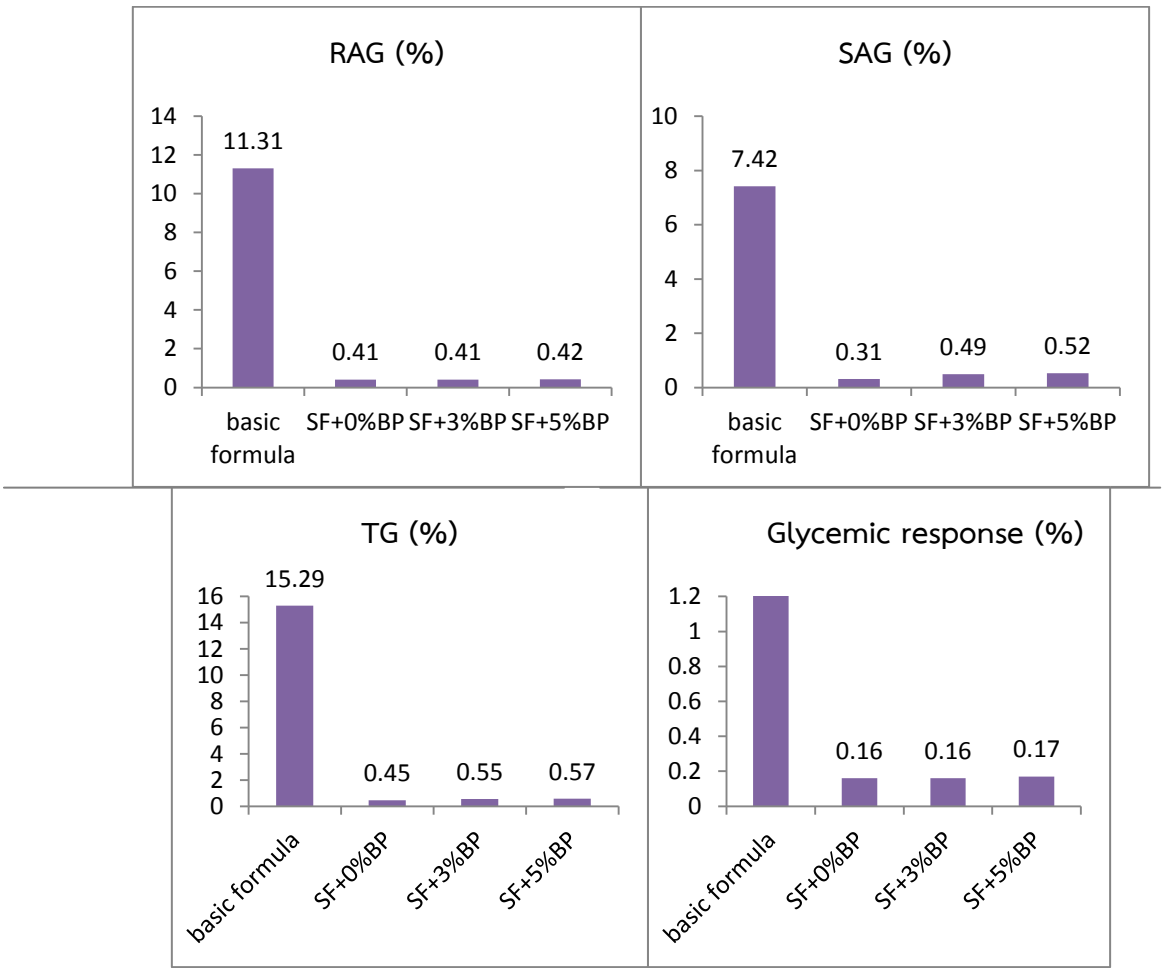


Figure 6. Rapidly available glucose (RAG), slowly available glucose (SAG), total glucose (TG) and glycemic response of basic ice cream formula (with sugar) ; sugar-free ice cream with 0%, 3% and 5% butterfly pea flower petals added.

Nutritional value

The sugar-free ice cream contained 15-18% less kilocalories and 20-26% less carbohydrate content than did the basic formulation with sugar ($p<0.05$). The addition of butterfly pea flower petals increased significantly the crude fibre content by 2-8 times than that of the basic ice cream ($p<0.05$). However, the fibre analyzed was represented as crude fibre content and not all of the indigestible fibre. This could have also influenced the estimated carbohydrate and energy contents, not to be as low as expected.

Table 2. Nutritional values of sugar-free ice cream formulations with butterfly pea ingredients.

Nutritional value per 100g	Basic ice cream formula	Sugar-free ice cream formula with butterfly pea flower petals added		
		0%	3%	5%
Energy, kcal	105.16 ± 2.08[a]	85.63 ± 3.33[b]	88.94 ± 1.75[b]	86.36 ± 3.09[b]
Moisture, g	73.57 ± 0.48[a]	78.19 ± 0.64[b]	77.53 ± 0.34[b]	77.51 ± 0.49[b]
Ash, g	0.01 ± 0.00[a]	0.11 ± 0.03[b]	0.14 ± 0.03[b]	0.18 ± 0.04[b]
Protein, g	1.40 ± 0.26[a]	1.38 ± 0.35[a]	1.47 ± 0.02[a]	1.90 ± 0.20[a]
Fat, g	0.04 ± 0.01[a]	0.19 ± 0.02[b]	0.61 ± 0.02[c]	0.64 ± 0.07[c]
Carbohydrate, g	24.80 ± 0.30[a]	19.60 ± 0.58[b]	19.39 ± 0.37[b]	18.25 ± 0.70[b]
Fibre, g	0.18±0.02[a]	0.53±0.02[b]	0.86±0.02[c]	1.52±0.02[d]

Note: Means in the same row with different superscripts signify a statistically significant difference ($p<0.05$).

Microbiological testing

The total plate count of the sugar-free formulations was within the safe levels and neither *E. coli* nor *S. aureus* were detected. Thailand's Ministry of Public Health Standard [26] states that the total plate count for an ice cream product must not be over 600,000 colonies per gram and that it must not contain any *E. coli* or other pathogenic bacteria. Ice cream is a product that is not normally heat-processed, thereby increasing the risk of microbiological contamination. The production process must also meet the GMP (Good Manufacturing Practice) standards and provide appropriate pasteurization conditions for the ice cream mix before being frozen. Holding the ice cream mix to at least 68.5°C for at least 30 seconds, or 80°C or more, for at least 25 seconds before being frozen is usually recommended, including an automatic thermometer to record the actual temperature and length of time [26, 27].

Table 3. Microbiological qualities of sugar-free ice cream with butterfly pea flower ingredients.

Sugar-free ice cream	Total plate count	*Escherichia coli*	*Staphylococcus aureus*
	CFU/g	MPN/g	CFU/g
Regular ice cream	20	ND	ND
0% Butterfly Pea Flower	10	ND	ND
3% Butterfly Pea Flower	10	ND	ND
5% Butterfly Pea Flower	20	ND	ND

Note: ND = not detected

Conclusion

To summarize, sugar-free ice cream products containing dried butterfly pea flower extract and fresh butterfly pea flower petals offered health benefits and were accepted by consumers. The addition of the butterfly pea flower extract and flower petals gave the ice cream a more appealing colour, along with increasing the anthocyanin and total phenolic content and antioxidant activity of the ice cream. Sugar-free ice cream made with sucralose and inulin instead of sugar showed a lower glycaemic response than did the basic ice cream formulation. These results showed that sugar-free ice cream made with butterfly pea flower extract and petals had good potential as a healthy frozen dessert product.

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