



## Total Phenolic Compounds, Antioxidant Activity and Nutritional Values of Sugar-free and Reduced-fat Milk-based Ice Cream Enriched with Selected Herb Ingredients

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### Abstract

The objective of this research was to reduce the sugar and fat content in milk-based ice cream and to study the feasibility of using Thai herbs to flavour milk-based ice cream. Test formulations of reduced sugar and reduced-fat ice cream were developed using maltitol in place of sugar and a mixture of whey protein and maltodextrin (1:1) in place of fat. Gac fruit and the mixture of centella extract and green tea were selected to flavour the reduced-sugar and fat milk-based ice cream. Sensory testing was done in the laboratory with 20 untrained tasters. The test recipes were analyzed for nutritional values, total phenolic compounds, antioxidant activity, glycemic response and bacteria count. Data were analyzed using computer software to obtain mean, standard deviation, variance, comparison of means using Bonforoni method, and Friedman test of ranking at 95% confidence for the sensory evaluation scores. Results showed that there was no statistically significant difference between the sensory scores for the control and reduced-sugar ice cream made with maltitol in place of 50%, 75% or 100% of the sugar. The scores for the ice cream made with whey protein and maltodextrin (1:1) in place of 25% and 50% of the whipping cream in sugar-free ice cream were no different from those for the control sugar-free formula, and in fact the overall liking scores for the former 2 formulas were higher than for the control formula. Gac fruit extract and the mixture of centella extract and green tea were the herbal ingredients chosen for adding to sugar-free and 35% reduced fat ice cream. In the sensory evaluation, the overall liking score for the gac fruit formula was higher than for the mixture of centella extract and green tea formula to a statistically significant degree ( $p<0.05$ ), but was not significantly different from the score for the control formula. The total phenolic compounds and antioxidant activity of the mixture of centella extract and green tea formula were higher than those of the gac fruit formula or the control formula, to a statistically significant degree ( $p<0.05$ ). Fat content was reduced to be less than 1% but carbohydrate content by difference method was found to be increased. The glycemic response of the sugar-free formula with or without herb ingredient was lower than the control formula. The total bacteria count of all formulas was within the acceptable standard and neither *E. coli* nor *S. aureus* were detected. The new formulations showed a good potential of health benefit on blood glucose and a lowering risk of chronic diseases and is recommended for the commercial production.

**Keywords:** herbal ice cream, gac fruit, centella, green tea, antioxidant, phenolic compound, glycemic response

## 1. Introduction

Milk-based ice cream products are popular among general consumers because of their rich creamy flavor and refreshing coolness. Because of the ingredients used, ice cream, particularly the milk-based type, is generally considered as a high-sugar and high-fat food. Dietary sugar undisputedly affects blood sugar. A low carbohydrate diet is generally recommended to lose weight. Some low-carb diets also promote health benefits beyond weight loss, such as reducing risk factors associated with heart diseases, diabetes, and metabolic syndrome (1). Diabetic patients who are not successful at controlling their blood sugar have been found to eat more high-sugar foods than people with diabetes who can control their blood sugar (2). The Thai Dietary Guideline recommends avoiding high-sugar foods and eating fats in moderation (3). However, ice cream is such a popular food that it would be difficult to prohibit people of all ages from eating it. Dietary data shows a trend of steadily increasing ice cream consumption in the Thai population (4). A follow-up study of dietary intake in the US population from 1991 to 2008 showed a decrease in total calorie intake and some decrease in high sugar and high fat food intake, but the consumption of ice cream still increased (5, 6). Efforts to reduce the sugar and fat content of ice cream can provide a good alternative product for consumers. Developing low calorie and good tasting foods and beverages can be accomplished with the use of low calorie artificial sweeteners (7) and fat replacers (8). However, a good combination must be selected so the substitution of sugar and fat will not interfere with sensory acceptability of ice cream. The use of the artificial sweeteners, sucralose, maltitol and a combination of sucralose and maltitol in sherbet ice cream showed no significant difference in sensory evaluation tests, and the 100% sucralose formula received the highest overall liking score (9). Besides the

nutritional quality, functional properties have become another focus of food product development today. Cam et al. (10) found that enrichment of ice creams with pomegranate peel resulted in significant changes in the pH, total acidity, color, and sharp improvements in antioxidant and antidiabetic activities as well as the phenolic content of ice cream. Replacement of milk fat by pomegranate seed oil also increased the conjugated fatty acid content. However, perception of oxidized flavor increased with the additional seed oil. Reduced fat and calorie ice cream made with 3% carbohydrate based fat replacer (N-Lite D) and 20% red dragon fruit (*Hylocereus costaricensis*) flesh showed a decrease in fat and calorie content of 49.09% and 25.26%, respectively, and an increase in total polyphenolic compound and antioxidant activity (8). Many herbs are commonly used in Thai foods and many are reported to have antioxidant properties (11). Gac fruit and centella were two types of herb selected for the study. Gac fruit (*Mormordica cochinchinensis* Spreng) or Fak Khao, in Thai is a member of the melon family native to Southeast Asia. The yellowish orange color of the gac fruit extract showed a good potential as a natural food colorant and it is found to contain a high concentration of lycopene and beta carotene (12). Centella (*Centella asiatica* Urban.) or Bai bua bok, in Thai is a small, herbaceous, annual plant. It has been used as a refreshing drink or eat as raw in salad-typed dishes in Thailand. It is widely used as an important medicinal herb in the orient and is becoming popular in the West. The herb is recommended for the treatment of various skin conditions, relieving anxiety and improving cognition (13, 14). Therefore, the present research was designed to study the effect of sugar and fat substitution on sensory, nutritional and functional properties of milk-based ice cream and the feasibility of flavouring and enriching the ice cream with the extract from those two types of herbs selected.

## 2. Materials and Methods

The formula of milk-based ice cream of Kaothien (15) was selected and used as the control; consisting of sugar 10.51%, dextrose 1%, maltodextrin 4.00%, stabilizer 0.40%, milk powder 6.51% skim milk powder 1%, hot water 9.01%, fresh milk 47.55% and whipping cream 20.02%.

**2.1 Substituting sugar in ice cream.** Maltitol (Siam Bakeryland, Thailand) was used in place of 50%, 75% and 100% of the sugar and dextrose in the formula. These low-sugar formulas were subjected to sensory testing by a panel of 20 untrained taste testers in the laboratory, using a 9-Point hedonic scale (1= dislike the most and 9= like the most) to determine which was the most acceptable. The most acceptable low-sugar formulation would be used for further study of reduced fat formulations.

**2.2 Substituting fat in ice cream.** A mixture of whey protein and maltodextrin (1:1) (Siam Bakeryland, Thailand) was used in place of 25%, 50%, 75% and 100% of the whipping cream, and those reduced-fat formulations were then subjected to sensory testing by a panel of 20 untrained taste testers in the laboratory, as previously described.

**2.3 Feasibility study of using the extract from gac fruit and centella in ice cream.** Gac fruit (*Momordica cochinchinensis*) and centella (*Centella asiatica*) leaves were purchased from the local market in Bangkok. Liquid extracts of gac fruit and centella were prepared and substituted in place of water in the most acceptable reduced sugar and reduced fat ice cream formula from the earlier experiment. For gac fruit, the red aril surrounding the seeds was separated from the seeds by filtration and mixed with water 1:3 before adding to the ice cream mix. For centella, leaves were washed and cut, leaving about 3 cm of petiole, and macerated in a blender with water at the ratio of 1:3. After blending, the solution was filtered

through cheesecloth. Green tea powder purchased from the bakery shop was used to mix with the centella extract in order to provide more intense coloring before being added to the ice cream mix. All the ice cream flavoring with gac fruit extract and the mixture of centella extract and green tea were subjected to sensory evaluation.

**2.4 Nutritional values analysis.** The ice cream sample was analyzed for the proximate analysis (16) of moisture, ash, protein, fat, carbohydrate and fiber content. Caloric content was calculated based on the contents of protein, fat and carbohydrate.

**2.5 Antioxidant activity and total phenolic compounds analysis.** Ten grams of ice cream were mixed with 50 ml of absolute ethanol and left to stand at room temperature (approximately 28 °C) for 24 hours, then centrifuged at 8,000 rpm for 15 minutes. The clear supernatant was separated for analysis of total phenolic compounds using the Folin-Ciocalteu colorimetric method (17) and antioxidant activity using the DPPH (2,2- diphenyl-1-picryl-hydrazyl) method using different concentrations of Trolox solution as the standard for comparison (18), and also by the FRAP (Ferric reducing antioxidant power) method, measuring light absorbance at 593 nm and comparing to standard Trolox solution (19).

**2.6 Glycemic response analysis.** The sample of ice cream was analyzed for the glycemic response by using the *in vitro* starch digestibility method developed by Ratanakopan (20).

**2.7 Microbiological analysis.** Total number of colony forming units as a total plate count and the number of *Escherichia coli* and *Staphylococcus aureus* were determined by the method of BAM online 2001 (21).

**2.8 Statistical analysis.** The data from chemical testing were analyzed statistically by ANOVA and Bonferroni method to compare differences in means, and sensory evaluation data were analyzed using Friedman Test of Ranking (22), using computer software.

### 3. Results and Discussion

#### 3.1 Substituting sugar in ice cream

In the sensory evaluation, no statistically significant differences were found in appearance, color, odor, taste, smoothness, hardness and overall liking scores between the ice cream formulas using maltitol in place of 50%, 75% or 100% of the sugar and the control. This could be explained by the properties of maltitol. Maltitol has a pleasant sweet taste remarkably similar to sucrose, and is about 90% as sweet as sugar. It exhibits a negligible cooling effect in the mouth compared to most other polyols. It also provides a creamy texture to food; so it may also be used to replace fat (23). Therefore, the 100% maltitol formula was chosen for the next part of the study and was considered as a sugar-free formula.

#### 3.2 Substituting fat in sugar-free ice cream

When the whipping cream in ice cream was substituted with whey protein and maltodextrin mix (1:1) at 25%, 50%, 75% and 100% of the whipping cream, the sensory evaluation showed no statistically significant difference in appearance or color, but for the odor, taste and hardness, the scores for the control formula and the 25% formula were significantly higher than for the other formulas. The difference in odor and taste scoring could be due to the more favorable flavor of the whipping cream over the whey protein and maltodextrin. Besides, according to Liou and Grun 's findings (24), some flavor compounds were perceived differently in full-fat and low-fat ice cream; for example furaneol and ethyl-3-methy-3-phenylglycidate (candy flavor) were perceived more strongly in full-fat ice cream, while cis-3-hexen-1-ol (grassy flavor), alpha-ionone, and gamma-undecalactone were perceived more strongly in low-fat ice cream. For the smoothness, the fat substitute formulas tended to have higher scores than the control formula. For the overall liking score, the 25% substitution formula got the highest mean score of  $7.50 \pm 0.51$  on a

scale of 1 to 9, which was higher than the overall liking scores for the control sugar-free formula, the 75% substitute formula and the 100% substitute formula to a statistically significant degree ( $p < 0.05$ ), but not significantly different from the score for the 50% substitute formula. For that reason, the researchers chose to use the range between 25% and 50%, which is 35% substitution for the next part of the experiment.

#### 3.3 Feasibility study of using gac fruit extract and the mixture of centella extract and green tea to enrich sugar-free and reduced-fat ice cream

When the sugar-free and 35% fat-reduced ice cream formulas enriched with gac fruit extract and with the mixture of centella extract and green tea were subjected to sensory evaluation, there were no statistically significant differences in the mean scores for appearance, color, odor and hardness, but there were statistically significant differences in the mean scores for taste, smoothness and overall liking. The formula enriched with gac fruit extract got significantly higher scores than the control formula with no herbal flavoring and the formula with the mixture of centella extract and green tea flavoring ( $p < 0.05$ ). The gac fruit formula provided more color and better appearance including higher overall liking score.

#### 3.4 Analysis of nutritional values of herb flavored ice cream

The substitution of fat in milk-based ice cream resulted in a significant decrease in fat content, from  $5.55 \pm 0.41$  to less than 1% (Table 4). However, there was no significant difference in calorie content and crude fiber. This could have resulted from a significant increase in carbohydrate content. The increase in carbohydrate content could come from the use of maltitol and maltodextrin in the test formulas. Both maltitol and maltodextrin are basically carbohydrate compounds, similar to sugar. Even the absorption rate of maltitol in the human body is very slow, allowing part of the ingested

**Table 1** Sensory evaluation of ice cream made with different proportions of maltitol in place of sugar

| Sensory parameter | control     | Maltitol substitution |             |             |
|-------------------|-------------|-----------------------|-------------|-------------|
|                   |             | 50%                   | 75%         | 100%        |
| Appearance        | 7.20 ± 0.69 | 7.20 ± 0.52           | 6.95 ± 0.68 | 7.05 ± 0.60 |
| Color             | 7.00 ± 0.64 | 7.00 ± 0.45           | 7.00 ± 0.32 | 6.80 ± 0.61 |
| Odor              | 6.40 ± 0.88 | 6.30 ± 0.97           | 6.40 ± 0.88 | 6.35 ± 0.98 |
| Taste             | 7.15 ± 0.67 | 7.00 ± 0.56           | 7.05 ± 0.79 | 7.05 ± 0.82 |
| Smoothness        | 7.05 ± 0.68 | 6.90 ± 0.85           | 6.80 ± 0.69 | 6.85 ± 0.75 |
| Hardness          | 7.00 ± 0.91 | 6.80 ± 0.89           | 7.00 ± 0.56 | 7.10 ± 0.71 |
| Overall liking    | 7.30 ± 0.57 | 7.25 ± 0.55           | 7.00 ± 0.45 | 7.15 ± 0.74 |

**Note:** Results of 20 sensory panels. The results did not differ to a statistically significant degree at confidence level 95%

**Table 2** Sensory evaluation of sugar-free ice cream made with different substitution rate of whey protein and maltodextrin (1:1) to substitute for fat

| Parameter      | control sugar-free ice cream | Fat substitution rate   |                         |                         |                         |
|----------------|------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|                |                              | 25%                     | 50%                     | 75%                     | 100%                    |
| Appearance     | 7.15±0.67                    | 7.20±0.67               | 7.20±0.76               | 7.25±0.71               | 7.30±0.65               |
| Color          | 7.20±0.69                    | 7.20±0.69               | 7.30±0.65               | 7.25±0.63               | 7.35±0.67               |
| Odor           | 6.80±0.69 <sup>a</sup>       | 6.75±0.71 <sup>ab</sup> | 6.60±0.82 <sup>b</sup>  | 6.05±0.75 <sup>c</sup>  | 5.90±1.11 <sup>c</sup>  |
| Taste          | 7.45±0.51 <sup>a</sup>       | 7.45±0.51 <sup>a</sup>  | 7.15±0.74 <sup>ab</sup> | 6.85±1.03 <sup>b</sup>  | 6.40±1.18 <sup>c</sup>  |
| Smoothness     | 7.10±0.64 <sup>a</sup>       | 7.15±0.67 <sup>a</sup>  | 7.75±0.44 <sup>b</sup>  | 7.40±0.75 <sup>ab</sup> | 7.25±1.20 <sup>ab</sup> |
| Hardness       | 7.75±0.75 <sup>a</sup>       | 7.70±0.65 <sup>a</sup>  | 7.50±0.88 <sup>b</sup>  | 6.50±1.05 <sup>b</sup>  | 5.75±1.44 <sup>b</sup>  |
| Overall liking | 7.20±0.41 <sup>a</sup>       | 7.50±0.51 <sup>b</sup>  | 7.45±0.68 <sup>ab</sup> | 6.80±0.76 <sup>c</sup>  | 6.40±0.75 <sup>c</sup>  |

**Note:** Results of 20 sensory panels. Mean values with different superscripts in the same row indicate a statistically significant difference ( $p < 0.05$ ).

**Table 3** Sensory evaluation of sugar-free and 35%-reduced fat ice cream made with different herbal flavorings

| Parameter      | Sugar-free and 35% reduced-fat ice cream |                            |                          |
|----------------|--|----------------------------|--------------------------|
|                | No herbal ingredients                    | Centella extract+green tea | Gac fruit extract        |
| Appearance     | 7.30 + 1.21                              | 7.00 + 1.16                | 7.30 + 1.08              |
| Color          | 7.00 + 1.45                              | 7.05 + 1.09                | 7.30 + 1.21              |
| Odor           | 6.15 + 1.59                              | 5.50 + 1.57                | 6.05 + 1.46              |
| Taste          | 6.75 + 1.29 <sup>a</sup>                 | 5.15 + 1.59 <sup>b</sup>   | 7.20 + 1.15 <sup>a</sup> |
| Smoothness     | 7.40 + 0.94 <sup>a</sup>                 | 6.80 + 1.19 <sup>b</sup>   | 7.45 + 0.94 <sup>a</sup> |
| Hardness       | 7.05 + 1.09                              | 6.75 + 1.40                | 7.15 + 1.22              |
| Overall liking | 6.95 + 1.23 <sup>ab</sup>                | 6.35 + 1.34 <sup>a</sup>   | 7.25 + 1.01 <sup>b</sup> |

**Note:** Results of 20 sensory panels. Mean values with different superscripts in the same row indicate a statistically significant difference ( $p < 0.05$ )

maltitol to be absorbed and leave the rest to reach the large intestine, where metabolism yields fewer calories; 2.1 kcal/g versus 4.0 for sugar (23). Total carbohydrate in this study was calculated by difference (16); the content of other constituents in ice cream (moisture+ash+protein+fat+fiber) were subtracted from the total weight of ice cream. Thus, the content of crude carbohydrate above would cover all carbohydrate based compounds, including maltitol and maltodextrin.

### 3.5 Analysis of total phenolic compound and antioxidant activity

The total phenolic compound and antioxidant activity of the ice cream formulas made with herbal flavorings were significantly higher than those without ( $p<0.05$ ). This shows that addition of herbal ingredients can help increase the health-promoting phenolic compounds and antioxidant activity of ice cream. The total phenolic compound content of the centella and green tea formula was the highest, and it was 3.34 times higher than the control formula and 2.85 times higher than the gac fruit formula. The antioxidant activity of the centella and green tea formula when measured by FRAP was 2.07 times higher than the control formula and 1.46 times higher than the gac fruit formula, and it was 1.63 times higher than the control formula and 1.36 times higher than the gac fruit formula when measured by the DPPH method.

### 3.6 Glycemic response

Glycemic response concept was originally targeted at people with diabetes as a guide to food selection (25, 26). Low glycemic response foods, indicating a lesser effect on blood sugar, are found to be associated with a reduced risk of certain chronic diseases such as diabetes and cardiovascular disease (27). In particular, a study showed improved insulin sensitivity, lipid metabolism and reduced chronic inflammation due to low glycemic response foods in a Dutch population (28). Six formula of

ice cream was prepared to determine the value of glycemic response. Glycemic response of the ice cream samples was analyzed *in vitro*, simulating the digestive process in a test tube, by adding artificial saliva, pepsin, pancreatin and amyloglucosidase to the samples and then measuring the amount of glucose released, and using that value to calculate the glycemic response. Sugar-free ice cream formula made with maltitol substituting at 100% of the sugar yielded a significant lower glycemic response than the control formula and fat-free formula made with whey protein and maltodextrin substituting at 100% of the fat ( $p<0.05$ ). The sugar-free formula had a glycemic response of 0.08%, while that of the control formula was 8.12% and that of the fat-free formula was 7.38%. The sugar-free and fat-free formula had a glycemic response of 0.32%, which was higher than the sugar-free alone ( $p<0.05$ ). This shows that the sugar component of the ice cream has a greater effect on the glycemic response than the fat component. This is probably because when sugar is digested in the body it can be quickly absorbed and converted to glucose in the bloodstream, whereas the sugar substitute used, maltitol, is slowly absorbed. Therefore, when maltitol is used, the rise in blood glucose associated with the ingestion of glucose is significantly reduced. A study in healthy human subjects also showed blood glycemic and insulinemic responses were lower for all the sugar-free desserts containing maltitol (23). Besides, maltitol, like other polyols, is also resistant to metabolism by oral bacteria that break down sugars and starches to release acids that may lead to cavities or erode tooth enamel (23). Thus, the use of maltitol in sugar-free milk-based ice cream besides contributing a lower effect on blood sugar, it possibly also contribute a benefit for oral hygiene. The addition of herbal ingredients to the ice cream did not affect the glycemic response.

**Table 4** Nutritional values of sugar-free and 35%-reduced fat herbal ice cream in comparison to the control formula

| Nutrition values<br>per 100 g | Control formula                | Sugar-free and 35%-reduced fat herbal ice cream |                                |                                |
|-------------------------------|--------------------------------|---|--------------------------------|--------------------------------|
|                               |                                | No herbal<br>flavoring                          | Centella+green tea             | Gac fruit                      |
| Energy (kcal)                 | 158.71 $\pm$ 3.81 <sup>a</sup> | 156.79 $\pm$ 2.65 <sup>a</sup>                  | 153.35 $\pm$ 2.90 <sup>a</sup> | 156.29 $\pm$ 2.10 <sup>a</sup> |
| Moisture (g.)                 | 65.73 $\pm$ 0.45 <sup>a</sup>  | 60.33 $\pm$ 0.56 <sup>b</sup>                   | 61.15 $\pm$ 0.77 <sup>b</sup>  | 60.22 $\pm$ 0.41 <sup>b</sup>  |
| Ash (g.)                      | 0.78 $\pm$ 0.11 <sup>a</sup>   | 0.89 $\pm$ 0.05 <sup>a</sup>                    | 0.97 $\pm$ 0.04 <sup>a</sup>   | 0.90 $\pm$ 0.05 <sup>a</sup>   |
| Protein (g.)                  | 4.42 $\pm$ 0.47 <sup>a</sup>   | 5.40 $\pm$ 0.22 <sup>b</sup>                    | 5.28 $\pm$ 0.20 <sup>bc</sup>  | 5.70 $\pm$ 0.41 <sup>d</sup>   |
| Carbohydrate (g.)             | 22.77 $\pm$ 0.54 <sup>a</sup>  | 31.75 $\pm$ 0.71 <sup>b</sup>                   | 30.93 $\pm$ 0.68 <sup>b</sup>  | 31.46 $\pm$ 0.56 <sup>b</sup>  |
| Fat (g.)                      | 5.55 $\pm$ 0.41 <sup>a</sup>   | 0.91 $\pm$ 0.1 <sup>b</sup>                     | 0.95 $\pm$ 0.04 <sup>b</sup>   | 0.85 $\pm$ 0.18 <sup>c</sup>   |
| Crude fiber (g.)              | 0.75 $\pm$ 0.13 <sup>a</sup>   | 0.72 $\pm$ 0.06 <sup>a</sup>                    | 0.72 $\pm$ 0.05 <sup>a</sup>   | 0.87 $\pm$ 0.04 <sup>a</sup>   |

**Note:** Mean Values of three replications. Values with different superscripts in the same row indicate a statistically significant difference ( $p < 0.05$ ).



No herbal flavoring



Gac fruit formula



Centella+green tea formula

**Figure 1.** Colors of sugar-free and reduced-fat ice creams with herbal flavorings**Table 5** Total phenolic compound and antioxidant activity of different ice cream formulas

| Sugar-free ice cream                              | Total phenolic compound           | Antioxidant activity                   |  |
|---|-----------------------------------|--|--|
|   |                                   | FRAP method                            | DPPH method                            |
|   | (mgGAE/100g fresh weight)         | $\mu$ mol Trolox/100 g<br>fresh weight | $\mu$ mol Trolox/100 g<br>fresh weight |
| 35% fat substitute                                | 3.5723 $\pm$ 0.0856 <sup>a</sup>  | 169.558 $\pm$ 1.5957 <sup>a</sup>      | 41.3504 $\pm$ 0.7548 <sup>a</sup>      |
| 35% fat substitute<br>+centella extract+green tea | 12.1190 $\pm$ 0.0701 <sup>b</sup> | 350.4613 $\pm$ 1.7802 <sup>b</sup>     | 67.5536 $\pm$ 0.7919 <sup>b</sup>      |
| 35% fat substitute<br>+gac fruit extract          | 4.2470 $\pm$ 0.0182 <sup>c</sup>  | 240.1073 $\pm$ 1.0736 <sup>c</sup>     | 49.7758 $\pm$ 0.7575 <sup>c</sup>      |

**Note:** Mean Values of three replications. Values with different superscripts in the same column indicate a statistically significant difference ( $p < 0.05$ ).



**Table 6** Glycemic response of different ice cream formulas

| Ice cream                   | Glucose content (%)     |                         |                         | Glycemic Response (%)  |
|-----------------------------|-------------------------|-------------------------|-------------------------|------------------------|
|                             | RAG                     | SAG                     | TG                      |                        |
| Control formula             | 20.23±0.97 <sup>a</sup> | 5.14±0.48 <sup>b</sup>  | 30.60±1.26 <sup>a</sup> | 8.12±0.33 <sup>a</sup> |
| Sugar free                  | 0.19±0.02 <sup>b</sup>  | 4.51±0.43 <sup>c</sup>  | 15.94±0.18 <sup>b</sup> | 0.08±0.01 <sup>c</sup> |
| Fat free                    | 18.28±0.89 <sup>a</sup> | 6.86±0.81 <sup>ab</sup> | 29.33±0.85 <sup>a</sup> | 7.38±0.88 <sup>a</sup> |
| Sugar-free and fat-free     | 0.80±0.09 <sup>b</sup>  | 4.36±0.86 <sup>c</sup>  | 14.49±0.64 <sup>b</sup> | 0.32±0.05 <sup>b</sup> |
| +gac fruit extract          | 0.97±0.09 <sup>b</sup>  | 7.80±0.56 <sup>a</sup>  | 14.76±1.00 <sup>b</sup> | 0.38±0.07 <sup>b</sup> |
| +centella extract+green tea | 1.06±0.21 <sup>b</sup>  | 5.28±1.00 <sup>b</sup>  | 15.10±0.37 <sup>b</sup> | 0.42±0.05 <sup>b</sup> |

**Note:** RAG = rapidly available glucose, SAG = slowly available glucose, and TG = total glucose

Sugar free formula = control formula with 100%subtition of sugar with maltitol

Fat free formula = control formula with 100% substitution of whipping cream with whey protein and maltodextrin, 1:1

**Mean** values of three replications. Values with different superscripts in the same column indicate a statistically significant difference ( $p < 0.05$ ).

**Table 7** Bacteria count in different ice cream formulas

| Ice cream   | TPC                   | <i>E. coli</i> | <i>S. aureus</i> |
|---|-----------------------|----------------|------------------|
|   | CFU/g                 | MPN/g          | CFU/g            |
| control formula   | 1.1 x 10 <sup>2</sup> | ND             | ND               |
| Sugar-free  | 2.1 x 10 <sup>2</sup> | ND             | ND               |
| Sugar-free and 35% fat substitute   | 5.2 x 10 <sup>2</sup> | ND             | ND               |
| Sugar-free and 35% fat substitute with gac fruit flavoring                  | 1.4 x 10 <sup>2</sup> | ND             | ND               |
| Sugar-free and 35% fat substitute with centella extract+green tea flavoring | 2.0 x 10 <sup>2</sup> | ND             | ND               |

Note: TPC= total plate count, CFU = colony forming unit, MPN = Most probable number and ND = non detected



### 3.7 Microbiological analysis

The total plate count was within the safe level and neither *E. coli* nor *S. aureus* were detected. The Ministry of Public Health's standard in Announcement 222 (29) states that ice cream is a specially controlled product and the total plate count for an ice cream product must not be over 600,000 colonies per gram and it must not contain any *E. coli* or other disease-causing bacteria. Ice cream is a product that is not normally heat-processed, so the production process must meet the Good Manufacturing Practice standards. According to the recommendation, the ice cream mix should be heated to at least 68.5 °C and kept at that temperature for at least 30 minutes, or if heated to 80 °C or more, it should be kept at that temperature for at least 25 seconds, and be immediately chilled to 4 °C and kept at that temperature for 24 hours before becoming frozen (29, 30).

## 4. Conclusion

A sugar-free milk-based ice cream was developed with the use of maltitol in place of 100% of the sugar in the mixture. However, to reduce fat content, substitution of a mixture of whey protein and maltodextrin (1:1) for 50% or more of the whipping cream was not as acceptable to taste panels, so for the final stage of testing, a formula with the mixture of whey protein and maltodextrin (1:1) substituting for 35% of the whipping cream was selected, and a reduction of more than 82.88% of the fat content was achieved. Even though the calorie and carbohydrate contents were not found to be significantly lower than the standard ice cream by the proximate analysis, the glycemic response was significantly lower than the control formula. Enrichment of the ice cream with liquid extract from gac fruit or a mixture of centella extract and green tea both imparted a more appealing color and showed a higher content of total phenolic compounds and antioxidant activity as measured by FRAP and DPPH methods. The

formulations with the herb ingredients shows good potential as a healthy frozen dessert and could add a unique characteristic to Thai healthy ice cream.

## 5. Acknowledgement

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## 6. References

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