



# Glycemic properties of soursop-based ice cream enriched with moringa leaf powder

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## Abstract:

**Introduction.** Diabetes is a common disease all over the world that is often a cause of mortality. Ice cream is popular in many countries. However, sugar and fat in its composition makes ice cream a high-caloric product. Soursop (*Annona muricata* L.) and moringa (*Moringa oleifera* L.), African medicinal plants, contain natural sugars and are rich in phytochemicals. We aimed to produce ice cream with these plants and evaluate its remedial properties.

**Study objects and methods.** The study featured ice cream purchased in a local store (control sample) and soursop ice cream with moringa leaf powder (experimental samples). The experimental ice cream samples included ice cream with soursop, ice cream with soursop and 0.1 g of moringa, and ice cream with soursop and 1 g of moringa. The antioxidant properties, glycemic indices, amylose and amylopectin contents, as well as  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory properties of the samples were determined using the standard methods.

**Results and discussion.** Comparing with the other samples, ice cream with 1 g of moringa showed the highest total phenol and flavonoid contents, ABTS scavenging ability, DPPH radical scavenging ability, hydroxyl scavenging ability, ferric reducing antioxidant properties, and lowest glycemic index. Sensory evaluation revealed a lower overall acceptability of the experimental samples compared to the control ice cream. This could be due a peculiar taste of moringa (the formulation did not include sugar).

**Conclusion.** Ice cream based on soursop and moringa can be a good alternative to sugar-sweetened ice cream due to its antioxidant properties, low glycemic index, and acceptable sensory attributes.

**Keywords:** Ice cream, diabetes, antioxidant properties, glycemic index, phenolic compounds,  $\alpha$ -amylase,  $\alpha$ -glucosidase

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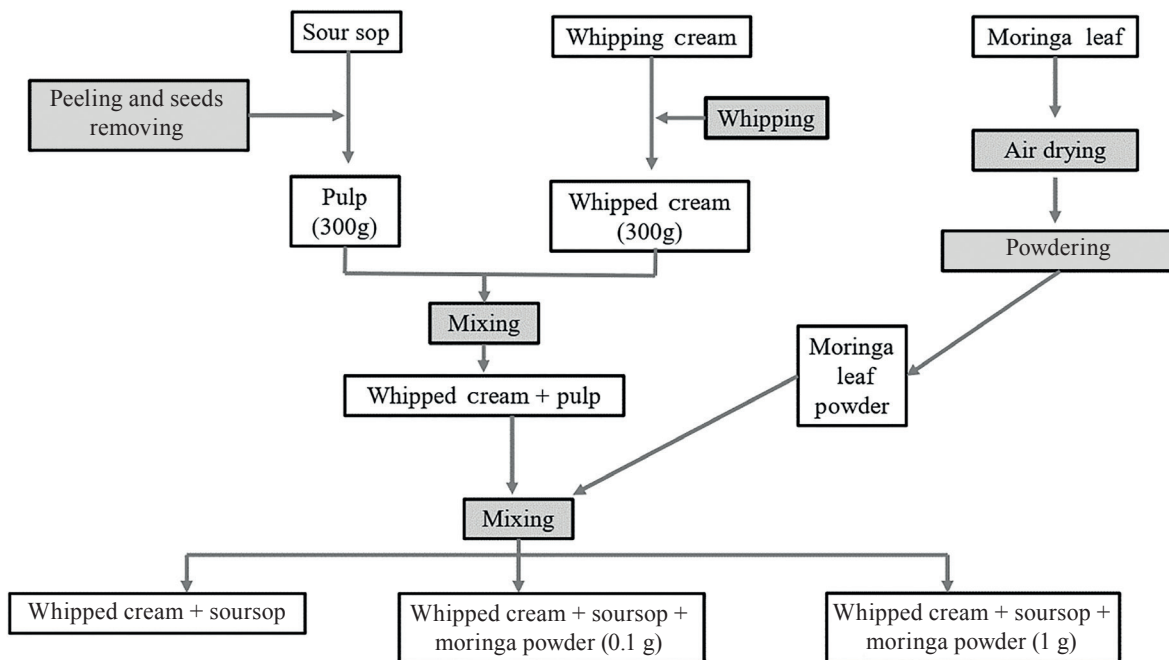
## INTRODUCTION

The World Health Organization reported that by 2035 the number of people with diabetes, a major cause of mortality worldwide, will account for 471 million [1]. Cheap snacks and products with high energy content are risk factors in diabetes development [2, 3]. One of the most popular high energy snacks is ice cream, which mainly contains milk or cream and sugar. Ice cream is a homogenized mixture of milk, flavorings, colorings, and stabilizers frozen at the temperature that is lower than the freezing point to avoid the formation of large ice crystals.

There are many varieties of ice cream, but generally ice cream contains 10% of milk fat, less than 10% of non-milk fat (caseins, whey proteins, lactose), 13–20% of sweeteners, 0.1–0.7% of stabilizers and emulsifiers, and about 64% of water [4]. Ice cream has become a popular product due to its cooling

properties and the enormous amount of energy it supplies. However, a high amount of carbohydrates fats in ice cream can increase bad cholesterol deposition around the belly and have become one of the leading causes of obesity and such diseases as diabetes, atherosclerosis, and hypertension [5]. All these diseases caused by ice cream consumption have been found to result from excess energy deposition, which is a central factor to hyperglycemia. Despite a high demand for ice cream, there has been little effort to improve its nutritional and medicinal properties. Hence, there is a need to develop functional ice cream without the mentioned disadvantages which would treat a wide array of metabolic diseases.

Herbs are widely available, effective, safe, and acceptable raw materials which can be used as functional plants in the food industry [6]. Various types of plants that have been used in the treatment of heart-



**Figure 1** Production process for soursop based ice cream enriched with moringa leaf powder

related diseases have shown promising therapeutic potential. Soursop (*Annona muricata* L.) is a tropical plant popular in ethnomedicine due to its antioxidant properties [7]. Soursop is rich in phytochemicals such as flavonoids, phenolic acids, phytosterols, saponins, and cardiac glycosides [7, 8]. *Moringa oleifera* L. (*Moringaceae* family) is a fast growing plant of economic and medical importance widely distributed in Africa, America, and Asia [9–11]. Some of the phytochemicals present in moringa leaf, which have medicinal potential, are mainly natural antioxidants such as flavonoids, carotenoids, vitamins, and phenolic acids [12–17].

Therefore, this study aimed to produce soursop-based ice cream enriched with moringa leaf powder and then assess its antioxidant properties, glycemic index, amylose and amylopectin contents, as well as  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory properties.

### STUDY OBJECTS AND METHODS

Soursop (*Annona muricata* L.) and moringa (*Moringa oleifera* L.) leaves were collected from the botanical garden at the Federal University of Technology, Akure. The moringa leaves were washed, air dried, and finely powdered using a stainless steel blender. The powdered samples were kept dry at room temperature for further analysis.

The soursop was peeled and seeds were separated from the pulp. Whipping cream (600 g) was stirred for 15 min using a mixer. Thereafter, 600 g of the soursop pulp was mixed together with the whipping cream for another 15 min. The mixture was divided into three parts and frozen (Fig. 1). This produced three

experimental samples of soursop-based ice cream: with no moringa, with 0.1 g of moringa, and with 1 g of moringa. Ice cream purchased at a local store served as control.

Sensory analyses were conducted in well illuminated odorless laboratory booths. Water was provided for mouth rinsing in between successive evaluation. Sample attributes (color, texture, taste, aroma, etc.) were rated from 1 to 7, where 1 = very poor and 7 = excellent. Panelists made their responses on score sheets which were designed in line with the test procedures [18].

The total phenol content was determined according to the method reported by Singleton *et al.* and calculated as gallic acid equivalent (GAE) [19].

The total flavonoid content was determined using a slightly modified method reported by Meda *et al.* [20]. The absorbance of the reaction mixture was subsequently measured at 415 nm, and the total flavonoid content was subsequently calculated.

DPPH free radical scavenging ability was evaluated as described by Gyamfi *et al.* [21]. Ice cream samples (0.05 mL) were incubated in the dark for 30 min with 1 mL of 0.4 mM DPPH after thorough mixing. The absorbance was measured at 516 nm, and the radical scavenging ability was subsequently calculated as percentage of the control.

ABTS radical scavenging ability was determined according to the method described by Re *et al.* [22]. The radicals were generated by adding 7 mmol/L ABTS aqueous solution to a reaction mixture containing 2.45 mmol/L  $K_2S_2O_8$ , keeping in the dark for 16 h, and adjusting the absorbance to 0.700 with ethanol at 734 nm. 0.2 mL of appropriate dilution of the ice

cream samples was added to 2.0 mL of ABTS solution and absorbance was measured at 734 nm after 15 min. The radical scavenging ability and Trolox equivalent antioxidant capacity were subsequently calculated.

Ferric reducing antioxidant property of the samples was determined by assessing its ability to reduce  $\text{FeCl}_3$  solution as described by Pulido *et al.* [23]. The reducing property was subsequently calculated using ascorbic acid equivalent.

Hydroxyl radical scavenging ability was determined using the method of Halliwell and Gutteridge [24]. The reaction mixture contained 1–100  $\mu\text{L}$  of the ice cream samples, 400  $\mu\text{L}$  of 0.1 M phosphate buffer, 120  $\mu\text{L}$  of 20 mM deoxyribose, 40  $\mu\text{L}$  of 20 mM hydrogen, and 40  $\mu\text{L}$  of 500 M  $\text{FeSO}_4$ . The mixture was incubated at 37°C for 30 min. Thereafter, 0.5 mL of 2.8% TCA (trichloroacetic acid) and 0.4 mL of 0.6% TBA (thiobarbituric acid) solution were added. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm using a spectrophotometer.

**$\alpha$ -amylase activity assay.** The reaction mixture contained the sample dilution (500  $\mu\text{L}$ ) and 0.5 mg/mL of  $\alpha$ -amylase in 500  $\mu\text{L}$  of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was incubated for 10 min at 25°C. 500  $\mu\text{L}$  of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was then added to the reaction mixture and incubated for another 10 min at 25°C. Dinitrosalicylic acid (DNSA) was used to stop the reaction before incubating for 5 min at room temperature. Absorbance was measured at 540 nm, and the percentage enzyme inhibitory was calculated [25].

The  $\alpha$ -glucosidase inhibitory activity was determined by the method of Apostolidis *et al.* [26]. The reaction mixture contained 100  $\mu\text{L}$  of  $\alpha$ -glucosidase solution (EC 3.2.1.20; 1.0 U/mL) in 0.1 M phosphate buffer (pH 6.9). Ice cream samples (50  $\mu\text{L}$  each) were put in the mixture and incubated at 25°C for 10 min. 50  $\mu\text{L}$  of 5 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside solution was added, and the reaction mixture was incubated for 5 min at 25°C. The absorbance was read at 405 nm.

Glycemic index and starch hydrolysis rate *in vitro* were determined according to the method of Goni *et al.* [27]. Each ice cream sample (50 mg) was incubated with pepsin (1 mg) in 10 mL of HCl-KCl buffer (pH 1.5) at 40°C for 60 min. 2.5 mL of phosphate buffer

(pH 6.9) and 5 mL of  $\alpha$ -amylase solution were added to the reaction mixture. The mixture was incubated at 37°C in a shaking water bath. To activate the enzyme, we were taking 0.1 mL of the mixtures every 30 min during three hours and boiled. The residual starch was digested to glucose by the addition of 3 mL of  $\alpha$ -glucosidase and incubated at 60°C for 45 min. The glucose concentration was assayed by the addition of 200 mL of DNSA. After stopping the reaction by boiling, 5 mL of distilled water was added and absorbance read at 540 nm.

To determine amylose-amylopectin content, 1 mL of 95% ethanol and 9 mL of 1 N NaOH were added to in volumetric flasks containing 100 mg of each ice cream sample. Thereafter, the reaction mixture was heated in boiling water for 10 min. 1 mL of 1 N acetic acid and 2 mL of iodine solution were added to 5 mL portion of the solution. After thorough shaking, the absorbance was measured at 620 nm. Amylopectin content was derived from the difference between the starch and amylose contents [28, 29].

**Statistical analysis.** The results were expressed as mean  $\pm$  standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyze the results followed by Turkey's post hoc test, with levels of significance accepted at  $P < 0.05$ .

## RESULTS AND DISCUSSION

The results of the sensory evaluation of the control (commercial ice cream) and experimental (soursop-based ice cream enriched with moringa leaf powder) samples are presented in Table 1. The control ice cream had higher overall acceptability compared to the soursop-based ice cream samples. The experimental samples had no significant differences in their overall acceptability.

Aroma, taste, color, flavor, texture, and general acceptability of food have a significant effect on its sensory quality, which is one of the major criteria in food selection by consumers [30]. The overall acceptability and aroma of the soursop-based ice cream was not significantly different. However, moringa leaf powder reduced such attributes as texture, taste, and color. The ice cream samples with moringa demonstrated reduced acceptability, which could be due to a peculiar taste of moringa leaf powder (no sugar in the formulation).

The soursop-based ice cream had a high amount of phenolic and flavonoid content compared to the control

**Table 1** Sensory attributes of soursop-based ice cream enriched with moringa leaf powder

Ice cream	Texture	Taste	Color	Aroma	Overall acceptability
Commercial ice cream (control)	6.09 $\pm$ 0.04 <sup>a</sup>	6.11 $\pm$ 0.03 <sup>a</sup>	6.12 $\pm$ 0.07 <sup>a</sup>	6.21 $\pm$ 0.04 <sup>a</sup>	6.25 $\pm$ 0.05 <sup>a</sup>
SS	5.37 $\pm$ 0.08 <sup>b</sup>	5.89 $\pm$ 0.04 <sup>b</sup>	5.92 $\pm$ 0.11 <sup>a</sup>	5.91 $\pm$ 0.04 <sup>b</sup>	5.82 $\pm$ 0.03 <sup>b</sup>
SS + MLP (0.1 g)	5.11 $\pm$ 0.03 <sup>c</sup>	5.71 $\pm$ 0.03 <sup>c</sup>	5.71 $\pm$ 0.06 <sup>b</sup>	5.82 $\pm$ 0.03 <sup>b</sup>	5.78 $\pm$ 0.05 <sup>b</sup>
SS + MLP (1g)	4.95 $\pm$ 0.04 <sup>d</sup>	5.28 $\pm$ 0.04 <sup>d</sup>	5.05 $\pm$ 0.07 <sup>c</sup>	5.79 $\pm$ 0.06 <sup>b</sup>	5.79 $\pm$ 0.04 <sup>b</sup>

SS – soursop

MLP – moringa leaf powder

**Table 2** Total phenol and flavonoid contents in soursop-based ice cream enriched with moringa leaf powder

Ice cream	Total phenols (mg GAE/g)	Total flavonoids (mg QE/g)
Commercial ice cream (control)	4.13 ± 0.02 <sup>d</sup>	0.98 ± 0.04 <sup>d</sup>
SS	16.24 ± 0.04 <sup>c</sup>	9.13 ± 0.05 <sup>c</sup>
SS + MLP (0.1 g)	22.27 ± 0.05 <sup>b</sup>	12.21 ± 0.04 <sup>b</sup>
SS + MLP (1 g)	30.25 ± 0.05 <sup>a</sup>	19.11 ± 0.03 <sup>a</sup>

Values represent mean ± SD, n = 3

SS – soursop

MLP – moringa leaf powder

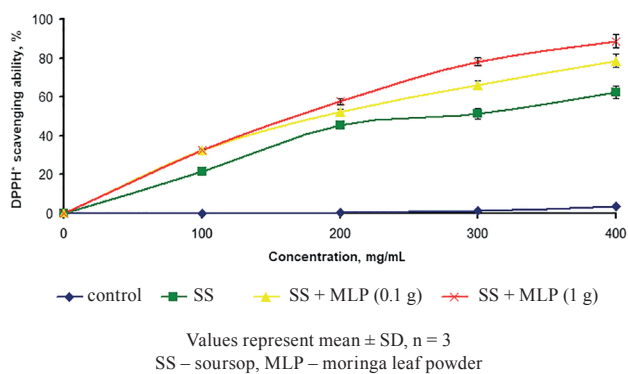
sample (Table 2). Our results consistent with the data by Tungmunnithum *et al.* who studied phenolics and flavonoids in medical plants [31]. The authors found that these compounds are responsible for the biological activity of the plants. Phenolic compounds, especially flavonoids, are remarkable antioxidants which have been widely researched for their medicinal properties against various diseases. Phenolic compounds are good iron chelators which scavenge free radicals, preventing oxidative stress [32]. In this study, the sample with moringa leaf powder (1 g) showed the highest total phenol and flavonoid content compared to the other samples.

Figure 2 demonstrates that the ice cream with moringa leaf powder (1 g) had the highest DPPH scavenging ability at all the concentrations (100–400 mg/mL) among all the samples. Also, this ice cream sample showed the highest ABTS scavenging ability compared to the other samples (Fig. 3). The control ice cream sample had the lowest both DHHP and ABST scavenging activities.

The highest ferric reducing antioxidant properties and hydroxyl radical scavenging ability belonged to the experimental sample with moringa leaf powder in the amount of 1 g (Figs. 4 and 5). Among the other samples, these parameters decreased from ice cream with moringa leaf powder (0.1 g) to the control sample (without soursop and moringa powder).

Reducing property of the samples was assessed based on their ability to reduce  $Fe^{3+}$  to  $Fe^{2+}$ . The results revealed that the control ice cream had significantly lower reducing property compared to the soursop-based samples. Similarly, the ice cream with 1 g of moringa exhibited the highest hydroxyl radical scavenging ability compared to the other soursop-based samples, while the hydroxyl radical scavenging ability of the control ice cream was comparably low.

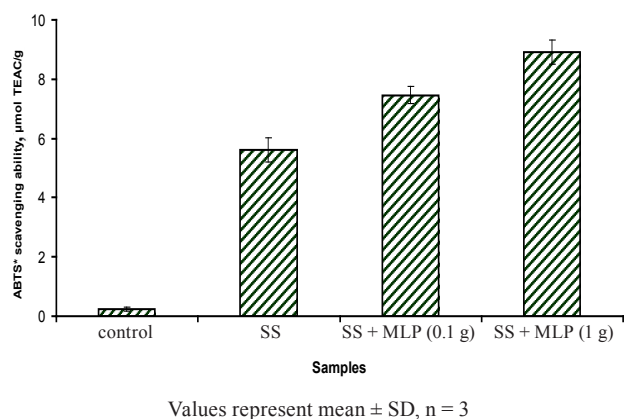
The antioxidant properties of the sour-sop based ice cream samples was directly proportional to increasing moringa leaf powder proportion (Figs. 2–5). Therefore, the antioxidant properties can be linked to phenolic compounds that majorly present in the moringa and the soursop. Furthermore, the ability of the samples to scavenge DPPH radical could be due to the presence of multiple hydroxyl groups in phenolic compounds, which

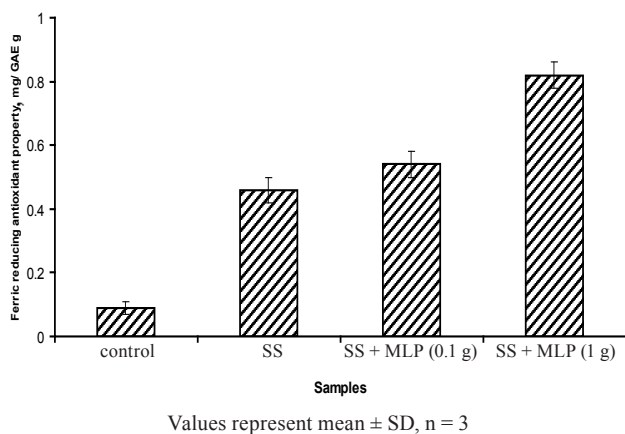
**Figure 2** DPPH radical scavenging ability of soursop-based ice cream enriched with moringa leaf powder

are able to donate their protons to finally break the chain reaction of the free radicals [32].

ABTS is a water soluble free radical initiator that is oxidized to form a stable green radical ABTS<sup>+</sup> in the presence of reactive oxygen [33]. All the soursop-based ice cream samples exhibited a remarkable ABTS radical scavenging ability, with the highest radical scavenging ability in the sample containing 1 g of moringa leaf powder. This could also be explained by synergistic effects of phenolic compounds present in moringa and soursop [34, 35]. These results prove that moringa and soursop increased the antioxidant properties of the ice cream samples due to phenolic compounds in their compositions. The correlation between antioxidant properties and phenolic content has been established for many food products [36].

The effect of moringa and soursop on the  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity of the ice cream samples are presented in Figs. 6 and 7. The sample with 1 g of moringa leaf powder showed the strongest inhibition of  $\alpha$ -amylase activity at the concentrations tested (50–200 mg/mL) and the highest  $\alpha$ -glucosidase inhibitory ability compared to the other soursop-based samples. The control sample demonstrated the lowest  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities.

**Figure 3** ABTS scavenging ability of soursop-based ice cream enriched with moringa leaf powder

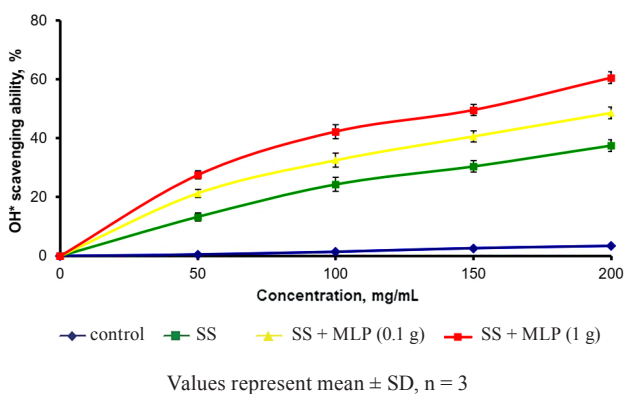


**Figure 4** Ferric reducing antioxidant properties of soursop-based ice cream enriched with moringa leaf powder

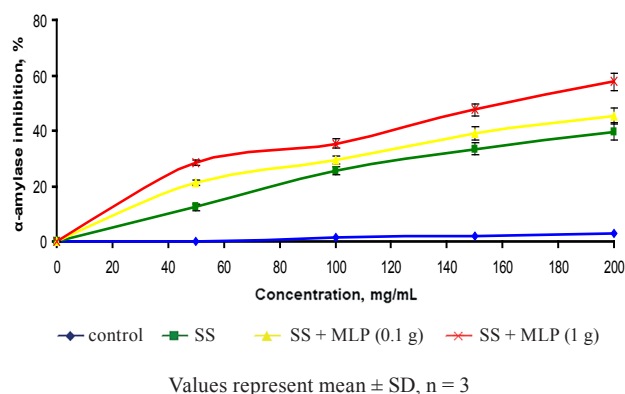
*In vitro* estimated glycemc indices of the samples are presented in Fig. 8. The results revealed that the control ice cream had the highest glycemc index (61.24) compared to the other samples (27.14–28.61). Figures 6 and 7 revealed that the sour-sop based ice cream samples inhibited carbohydrate hydrolyzing enzymes.

The control ice cream had the lowest amylose content (14.32%) compared to the soursop-based ice cream (32.35–35.34%) (Table 3). There was no significant difference in the amylopectin content of the samples with soursop and moringa leaf powder (64.66–67.65%), while the control ice cream had the highest amylopectin content (85.68%).

A therapeutic and practical way to control postprandial rise of glucose level in blood is the control of carbohydrate hydrolyzing enzymes [37]. Starch is converted to disaccharides and oligosaccharides by pancreatic  $\alpha$ -amylase, before further conversion to glucose is catalyzed by intestinal  $\alpha$ -glucosidase [38, 39]. Therefore, inhibition of both  $\alpha$ -amylase and  $\alpha$ -glucosidase activities would result in a reduction of glucose absorbed into the blood. The ability of the sour-sop based ice creams to inhibit the enzymes could be of therapeutic benefit in the management of hyperglycemia.



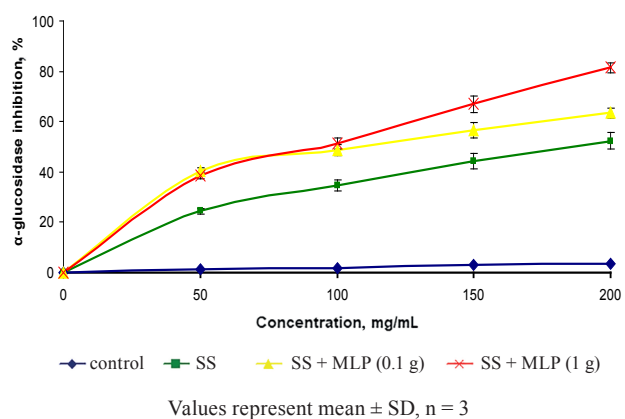
**Figure 5** OH• scavenging ability of soursop-based ice cream enriched with moringa leaf powder



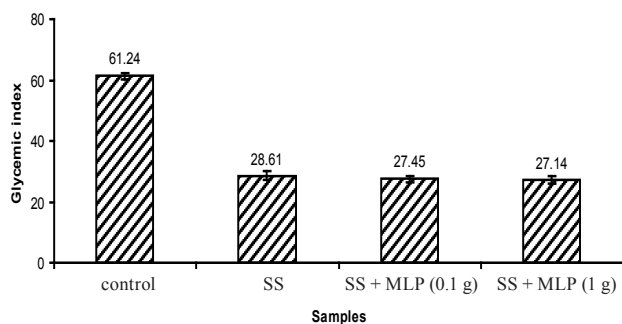
**Figure 6**  $\alpha$ -amylase inhibitory ability of soursop-based ice cream enriched with moringa leaf powder

Interestingly, this tendency for enzyme inhibition by the samples was similar to the tendency for total phenolic and flavonoid contents [40]. In addition, the synergistic contribution of phenolic compounds in soursop and moringa leaves can make ice cream a potent inhibitor of  $\alpha$ -amylase and  $\alpha$ -glucosidase activities. Our previous studies showed the presence of phenolic compounds, such as gallic acid, elagic acid, rutin, quercetin, kaempferol, epicatechin and chlorogenic acid, in soursop and moringa leaves [34, 35, 41].

The soursop-based ice cream samples had low glycemc indices (Fig. 8) which can be attributed to a number of factors. First, phenolic compounds in soursop and moringa leaves are potent inhibitors of  $\alpha$ -amylase and  $\alpha$ -glucosidase activities, which results in a slower breakdown of starch into glucose [42]. This is further evidenced by the fact that moringa powder increased phenolic content and reduced glycemc indices. Second, an amylose and amylopectin ratio in food products have a significant effect on postprandial glucose response [43]. Starchy products with a high amylopectin to amylose ratio often digest faster and are absorbed quicker than those with a low amylose to amylopectin ratio and, consequently, produce a high postprandial glucose and insulin response [34]. The control ice cream



**Figure 7**  $\alpha$ -glucosidase inhibitory ability of soursop-based ice cream enriched with moringa leaf powder



**Figure 8** Glycemic indices of soursop-based ice cream enriched with moringa leaf powder

used in this study possessed a low amylose content and high amylopectin content and, thus, the highest glycemic index compared to the experimental ice cream samples, which had a low amylopectin content and a high amylose content.

### CONCLUSION

Moringa leaf powder added into soursop-based ice cream improved the antioxidant properties of the final product, reduced its glycemic index, and enhanced inhibition of carbohydrate hydrolyzing enzymes.

**Table 3** Amylose and amylopectin contents of soursop-based ice cream enriched with moringa leaf powder

Ice cream	Amylose, %	Amylopectin, %
Commercial ice cream (control)	14.32 ± 0.11 <sup>b</sup>	85.68 ± 0.04 <sup>b</sup>
SS	34.36 ± 0.12 <sup>a</sup>	65.64 ± 0.16 <sup>a</sup>
SS + MLP (0.1 g)	32.35 ± 0.12 <sup>a</sup>	67.65 ± 0.14 <sup>a</sup>
SS + MLP (1 g)	35.34 ± 0.09 <sup>a</sup>	64.66 ± 0.11 <sup>a</sup>

Values represent mean ± SD, n = 3

SS – soursop

MLP – moringa leaf powder

Soursop-based ice cream with moringa leaf powder can be used to control postprandial hyperglycemia and oxidative stress. The results revealed that moringa-enriched soursop-based ice cream could be an alternative to the sugar-sweetened ice-cream. However, further *in vivo* experiments and clinical trials are recommended.

### CONFLICT OF INTEREST

The author declares no conflict of interest regarding the publication of this article.

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