Effect of bread incorporated with roselle calyces on postprandial glycemic and appetite responses in healthy individuals

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Abstract: Roselle is a medicinal plant and has been used in various animal studies as a blood glucose lowering agent. However, the effect of roselle calyces on blood glucose in human is not yet determined. Therefore, the objective of the current study was to determine the effect of roselle calyces on blood glucose and satiety responses in healthy individuals. In this randomized, controlled, crossover study two types of breads were tested in 20 healthy subjects. The breads included, control bread (CB) prepared from 100% all-purpose wheat flour and roselle bread (RB) incorporated with 4% roselle calyces powder. Blood glucose of the study subjects was tested by glucometer, using finger prick method. Palatability, satiety and gastrointestinal symptoms were determined by using 9-point hedonic scale, visual analogue scale (VAS) and gastrointestinal questionnaire, respectively. The results showed a significant decrease in blood glucose levels of study individuals after taking RB compared to CB at time points 30, 45 and 60 min ($P < 0.001$). Composite appetite score was not significantly affected after consuming any of the two breads. Compared to CB, RB significantly decreased incremental peak glucose (IPG), increased glycaemic profile (GP) and exhibited lower glycemic index (GI). The present study concluded that bread incorporated with roselle calyces powder significantly reduced postprandial level of blood glucose in healthy individuals and hence consumption of RB in place of CB would exert beneficial effect on blood glucose level.

Keywords: appetite, bread, glycemic response, roselle

Introduction

Type II diabetes mellitus (DM) is a disorder of carbohydrates metabolism which can occur due to insufficient insulin secretion or reduced insulin sensitivity [1]. It is estimated that by 2025 number of pre-diabetic and diabetic patients may double in Pakistan, India and Bangladesh [2]. Obesity is a medical condition of excess body fat accumulated in body. Obesity is linked with cardiovascular disease (CVD), DM and other non-communicable diseases (NCDs) [3]. Obesity is increasing at an alarming rate in developing countries in men, women and even children. According to a study, in Pakistan one out of four people were found to be overweight or obese [4].

Roselle (*Hibiscus Sabdariffa* L.), is a medicinal plant that belongs to family malvaceae [5]. Its urdu name is sabdriqa [6]. It's various parts like calyces, leaves and flowers are used for treatment of various illnesses [7]. The effect of roselle calyces on biomarkers of cardiometabolic health have been investigated in various animals and human studies [8]. Roselle calyces extract can increase insulin secretion in healthy and diabetic rats by regenerating islets of langerhans [9]. Roselle calyces have also been shown to improve the kidney function in normal and hypertensive patients[7].

From centuries bread is an integral part of human food [10]. It is an important staple food of our daily diet [11]. Bread is mainly made of refined wheat flour which contains rapidly digestible starch and rise blood glucose level rapidly and hence increases the risk of diabetes. Due to its extensive use bread is the preferred food for fortification and enrichment [12]. In the process of making bread, several chemical and biochemical changes occurs in wheat flour that affects quality of bread [10]. Nutritional quality of bread may be improved by addition of other ingredients as described in different studies [13].

However, no study has yet reported the effect of bread incorporated with roselle calyces on postprandial glycemic and appetite responses. Therefore, the present study was conducted to determine the effect of bread incorporated with roselle calyces on postprandial glycemia and appetite response. The hypothesis of this study was that bread incorporated with roselle calyces would reduce postprandial glycemia and increase postprandial satiety.

Materials and methods

Study site

This study was carried out in the lab. of Human Nutrition, The University of Agriculture Peshawar Pakistan.

Selection of subjects

Subjects for this study were selected by directly personal communication. A consent form was given to the subjects who agreed to take part in this study. Eligibility for the study was checked by filling a health questionnaire from the subjects. Inclusion criteria included, 18-60 years age and having blood glucose level less than 5.6 mmol/l. Exclusion criteria included history of diabetes, CVDs, asthma, gastro intestinal diseases and use of any medications that affect glucose metabolism. The study was approved by the Human Research Ethics Committee of the department of Human Nutrition (HN-HREC/2019-009), The University of Agriculture Peshawar, Pakistan.

Roselle calyces

Roselle calyces were obtained from the department of Horticulture, The University of Agriculture Peshawar, Pakistan. The calyces were dried in an oven at 50 ºC for 24 hours and then grinded using a laboratory grinder.

Test breads

Two types of breads were used.

1. Control bread prepared from all-purpose wheat flour.
2. Treatment bread prepared from all-purpose wheat flour incorporated with 4% roselle calyces.

Ingredients for bread preparation and composition of nutrients for the breads are given in the Table 1. Total weight obtained by adding weight of bread and water was 350 gm. Available carbohydrates in both control and roselle bread were 50 gm.

Table 1. Ingredients and composition of nutrients of test breads

Ingredients	CB	RB
Water (gm)	247	245
Bread (gm)	103	105
Total wt. (gm)	350	350
Energy (Cal)	291.66	275.02
Available CHO (gm)	50	50
Fats (gm)	5.38	4.46
Proteins (gm)	10.81	8.72
Dietary fiber (gm)	1.54	7.35

CB: control bread; RB: roselle bread.

Bread preparation

The method suggested by Marpalle, Pandurang for bread preparation was followed with some changes [14]. Bread was prepared in the lab of Human Nutrition AUP. Control bread was prepared from 100g all-purpose wheat flour per 100 gm bread and treatment bread was prepared from 4 gm roselle added to 96 gm of all-purpose flour per 100 gm bread. 6 gm sugar, 1 gm salt, 5 gm oil and 2 gm yeast were added per 100 gm bread. Water (65 ml) per 100 gm was added for mixing the ingredients and then all were kneaded together. The dough was left for 30-40 minutes in sunlight for fermentation and then it was baked in oven for 30 minutes at 220 °C. Bread after baking was cooled down and then wrapped in sheet to be stored in refrigerator till usage.

Study design and protocol

This study was designed as randomized, controlled crossover trial. Study protocol was explained to the subjects and they were asked to fill consent form and health questionnaire. There were two sessions for each subject with 1-2 weeks gap as a washout period. One type of bread i.e. control or treatment was consumed by each subject, in each session. Any strenuous activity was forbidden before the session day and they were requested to take the same type of diet before both sessions. Subjects were asked to come on fasting for session at 8 am and they were asked to stop eating from 10 pm a day before the session, giving them a total of 10-12 hours fasting time, however they could take up to 2 glasses of water before sleep. On reaching to lab, after 5 minutes of relaxation time their baseline measurements were taken which included weight, height, waist circumference and blood pressure. Then their fasting blood glucose level was checked using prick method and subjects were asked to fill gastro intestinal wellbeing questionnaire and satiety questionnaire (Visual Analogue Scales for Appetite Measurement**)** at 0 min. Then they were given bread containing 50 g of available carbohydrates with water and asked to finish it within 10 min. After finishing bread, they were asked to fill 9-point hedonic scale questionnaire for palatability. When subject started to eat bread, time was noted and exactly after 15 min blood glucose level was checked and then satiety questionnaire was filled by them. Glucose level was checked every 15 min during first hour of session i.e., at 0, 15, 30, 45 and 60 min, and every 30 min (i.e., at 90 and 120 min) in second hour of session and satiety questionnaire was filled at same time as checking of glucose level. Gastro intestinal wellbeing questionnaire was filled at 0, 30, 60 and 120 min. The session was of two hours and any physical activity, drinking or eating was prohibited during this time.

Baseline measurements

Baseline measurements include weight, height, waist circumference and blood pressure. Weight was measured by digital laboratory scale. Subjects were asked to stand straight on the scale and remove any heavy items and shoes before weight measurement. Height was measured using a stadiometer. The B.P was measured using sphygmomanometer. Waist circumference was measured by inch tape between last rib and above belly button.

Blood glucose analysis

The glucose level of subjects was checked by using prick method. Finger of the subject was first cleaned by alcohol swab, then by lancet gun finger was pricked. First drop of blood was cleaned by cotton and second drop was put on strip for measurement. Glucometer was used to check blood glucose. Blood glucose sheet was used for recording of blood glucose level.

Satiety measurement

Satiety was measured by visual analogue scale (VAS) [15]. VAS has four questions and for each question there is a 100 mm line to which the subjects responded according to their level of agreement by placing a vertical line on this 100 mm line. This questionnaire was filled at 0, 15, 30, 45, 60, 90 and 120 min, time interval.

Palatability of the bread

Palatability of bread was tested by the method of Meilgaard *et al.* (1999)[16] using 9 point hedonic scale questionnaire. The questionnaire has 9 scales ranging from "dislike extremely" to "like extremely". Subject rated colour, texture, flavor and overall acceptance of the bread on this scale.

Gastrointestinal symptoms

It was measured using the questionnaire developed by Bovenschen *et al*., (2006)[17]. It includes four of the gastrointestinal symptoms that are abdominal pain, nausea, vomiting and heart burn. Subject rated them on a scale of 7 points ranging from none to unbearable given in questionnaire. It was filled at time interval 0, 30, 60 and 120 min.

Calculations

Incremental blood glucose and satiety values were calculated to generate response curves of glucose and satiety. To calculate this incremental value of blood glucose and satiety, 0 min (fasting) value of each subject was subtracted from after meal value of the subject for control and roselle breads. Trapezoidal rule was applied to determine the iAUCs (Incremental area under the curves) of blood glucose and satiety while overlooking area underneath zero.
Peak time of the control and treatment bread was the value at the time point when blood glucose level was the highest. IPG (Incremental peak glucose) was measured as the maximum value of all the values of blood glucose at different time points. The time in min at which concentration of blood glucose was higher than baseline concentration was divided by IPG value to obtain glycemic profile (GP). iAUC of roselle bread was divided by iAUC of control bread and then multiplied with 100 for satiety and blood glucose to get satiety index (SI) and GI.

Statistical analysis

SPSS Inc., Chicago, IL is a statistical software which was used for statistical analysis of this study. ANOVA (two way repeated) was used to measure the time by treatment, treatment and time effect on responses of blood glucose and satiety, and gastrointestinal related symptoms. The adjustment of multiple comparison was done by Bonferroni adjustment. Paired t test was used for above parameters if significant effect was observed for treatment or time-by-treatment interaction, in order to find out the mean difference at each point of time and time-by-treatments. To compare the effect of treatment on satiety and blood glucose, palatability, blood iAUC, satiety iAUC, peak time, GP, GI, SI and peak glucose, paired sample t test was used. Significance level of $p \leq 0.05$ was used.

Results

Baseline characteristics

Baseline characteristics of healthy individuals are given in Table 2. All the subjects completed two sessions of the study. BMI of healthy subjects was in normal range. Waist circumference healthy individuals was also in normal range (88-102 cm). Fasting blood sugar level for healthy subjects was 89.30 ± 1.248 which falls in normal range that is less than 100 mg/dl. Systolic and diastolic blood pressure of healthy subjects was within normal range.

Table 2. Subjects' baseline characteristic (n = 20)*

Variable	Values
M/F	10/10
Age (yrs)	21.55 ± 0.276
Height (cm)	162.64 ± 2.033
Weight (kg)	61.68 ± 2.832
BMI (kg/m^2)	23.28 ± 0.945
Waist circumference (cm)	76.96 ± 2.256
Fasting plasma glucose (mg/dl)	89.30 ± 1.248
Systolic blood pressure (mm Hg)	108.50 ± 2.979
Diastolic blood pressure (mm Hg)	73.00 ± 3.007

*Values are means ± SEM. BMI: body mass index.

Acceptance of test breads

Acceptance of both control and roselle calyces containing breads was assessed by 9-point hedonic scale. Acceptance features of the test breads (CB and RB) are given in Table 3. All parameters of palatability for both the breads scored more than like slightly (6) on 9-point hedonic scale. Colour, texture, flavors and overall acceptance between the control and roselle calyces containing breads was observed to be non-significant. P values of bread for colour (P =0.702), texture (P =0.225), flavour (P =0.320) and overall acceptability (P =0.218) were observed.

Table 3. Parameters of acceptability of the test breads*

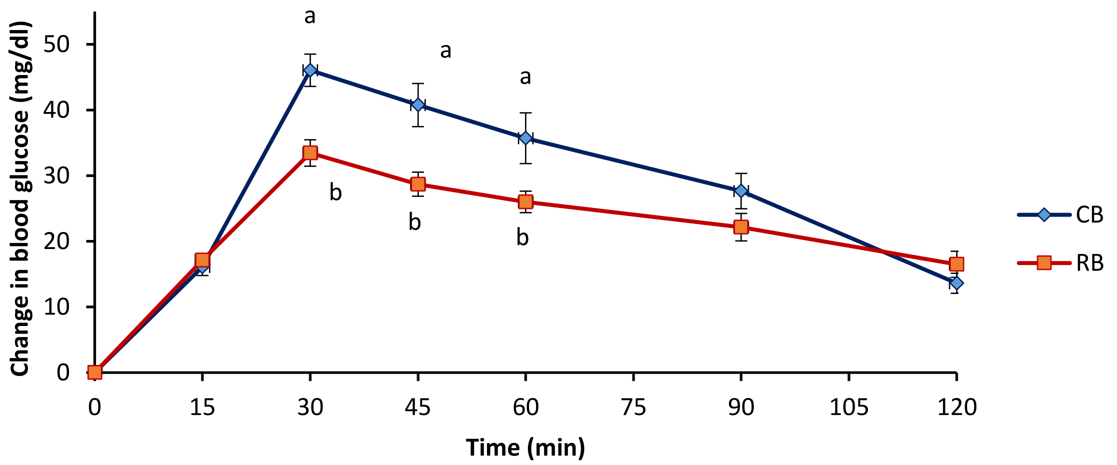
Test food	Colour	Texture	Flavor	Overall acceptability
CB	6.20 ± 0.40	6.45 ± 0.41	6.35 ± 0.47	6.75 ± 0.40
RB	6.05 ± 0.23	5.90 ± 0.27	5.85 ± 0.23	6.20 ± 0.20

*Values are means ± SEM. Values for acceptability parameters are not significantly different. $P < 0.05$ (paired t-test). CB: control bread; RB: roselle bread.

Blood glucose response

Figure 1 (A, B) shows the responses of postprandial glucose and their iAUCs in healthy subjects. The effect of time × treatment (P <0.001), treatment (P <0.001) and time (P <0.001) was significant on the postprandial levels of blood glucose in healthy subjects. Compared to control bread, roselle calyces-containing bread lowered the postprandial level of blood glucose significantly (P <0.001) shown by post hoc pair wise comparison. Blood glucose levels of healthy subjects after taking roselle calyces containing bread, was found to be significantly low at time point of 30 min (P <0.001), 45 min (P =0.001) and 60 min (P=0.011), by further analyzing the results with paired t-test. Furthermore, iAUCs (P=0.009) for blood glucose was significantly lowered with roselle calyces containing bread (containing 4% powder of roselle calyces) as compared to control bread.

A



B

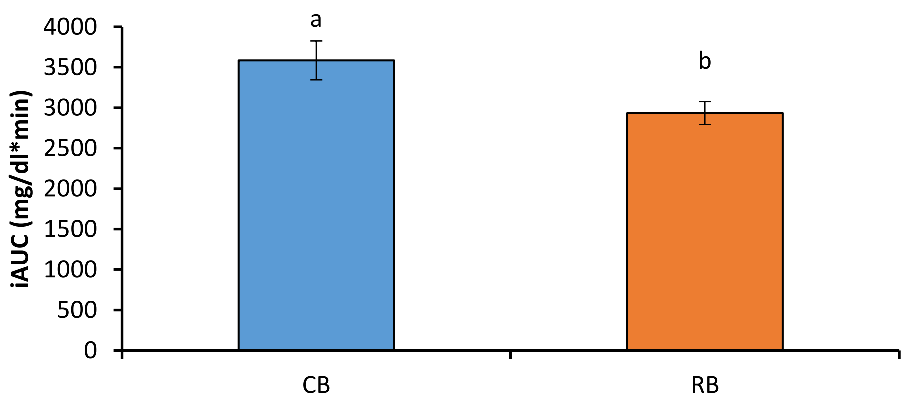
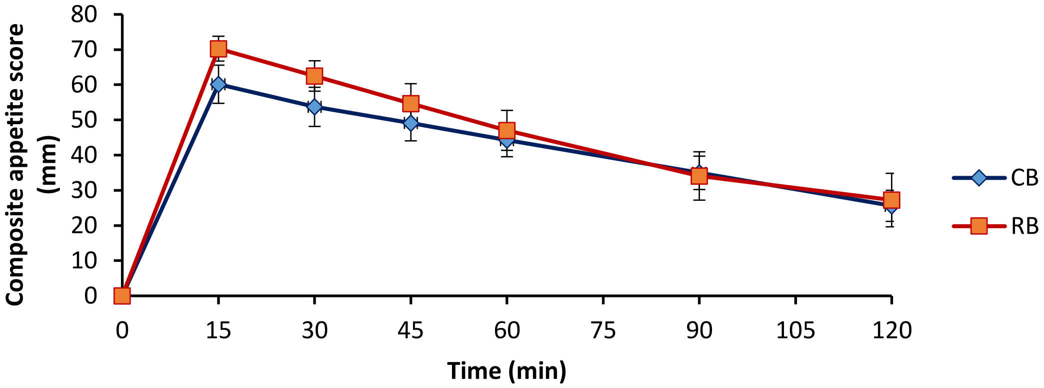


Figure 1. (A, B). Mean (± SEM) changes from baseline in blood glucose and incremental areas under the curves (iAUCs) in healthy subjects (n = 20) after consumption of test breads (CB: control bread; RB: roselle bread). Values with different superscript letters are significantly different at each time point: two-way repeated measure ANOVA, followed by paired t-test, Bonferroni adjustment ($P < 0.05$). Vertical bars with different letters are significantly different, $P< 0.05$ (paired t-test, Bonferroni adjustment).

Subjective appetite response

Figure 2 (A, B) shows the postprandial appetite responses measured in term of composite appetite score along with the corresponding iAUCs**.** A significant variation was observed in composite appetite score over time (P<0.001) but there was no effect of treatment (P=0.330) and time × treatment (P=0.177). Non-significant difference (P=0.330) was found by post hoc pair wise comparison between control bread and roselle calyces bread having roselle calyces containing. iAUCs for composite appetite score were also found to be non-significant (P=0.369) among roselle calyces containing bread and control bread.

A



B

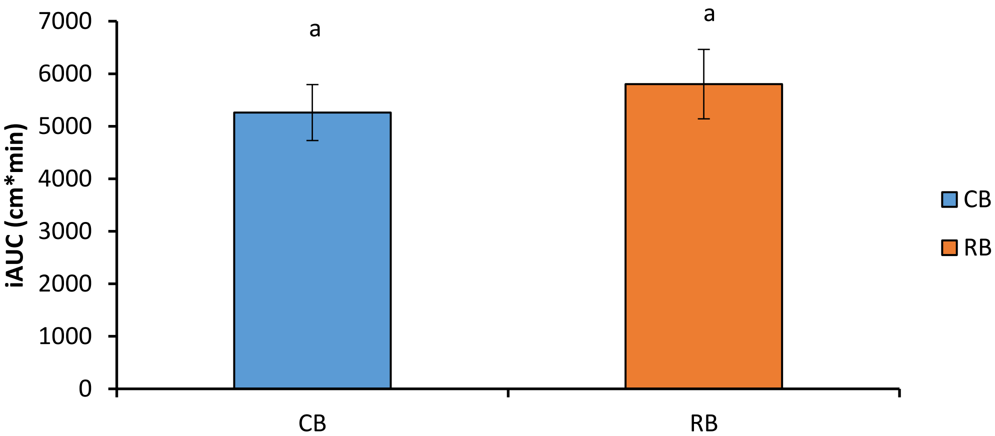


Figure 2. (A, B). Mean (± SEM) changes from baseline in satiety response and incremental areas under the curves (iAUCs) in healthy subjects (n = 20) after consumption of test meals (CB: control bread; RB: roselle bread). Values with different superscript letters are significantly different at each time point: two-way repeated measure ANOVA, followed by paired t-test, Bonferroni adjustment ($P < 0.05$). Vertical bars with different letters are significantly different, $P< 0.05$ (paired t-test, Bonferroni adjustment).

Gastrointestinal related symptoms

The study showed that bread added with roselle calyces containing bread does not cause any gastrointestinal discomfort like abdominal pain, heartburn, vomiting and nausea in individuals (data not shown).

Peak time, peak glucose, glycemic profile, glycemic index and satiety index

Peak time, peak incremental glucose, GP, GI and SI of the test breads are shown in Table 4. There was non-significant difference in peak time (P =0.470) between control bread and bread added with roselle calyces. A significant difference was found in IPG (P =0.043), GP (P =0.025) and GI for bread scale and glucose scale (P =0.019) between roselle calyces-containing bread and control bread. The addition of roselle calyces significantly decreased bread GI from 100 to 81.84 on bread scale and from 71 to 58.10 on glucose scale. There was no significant difference in SI (P=0.109) of control bread and roselle calyces containing bread.

Table 4. Peak time, IPG, GP, GI and SI of test breads*

Test meal	Peak time	IPG	GP	GI (bread scale)	GI (glucose scale)	SI
CB	47.25 ± 2.49	51.80 ± 2.85^{a}	2.36 ± 0.10^{b}	100.00 ± 0.0^{a}	71.00 ± 0.0^{a}	100.00 ± 0.0
RB	51.00 ± 3.83	43.30 ± 2.75^{b}	2.96 ± 0.21^{a}	81.84 ± 5.37^{b}	58.10 ± 3.81^{b}	124.14 ± 14.34

CB: control bread; RB: roselle bread; IPG: incremental peak glucose; GP: glycemic profile: GI: glycemic index: SI: satiety index. *All values are means ± SEMs (n = 20). Values in the same column with different superscript letters are significantly different from each other, $P < 0.05$ (paired t-test).

Discussion

The purpose of this study was to reduce the glycaemic index and increase satiety index of bread by addition of 4% of roselle calyces powder. As hypothesized the roselle added bread decreased post prandial blood glucose level of healthy subjects. This bread was acceptable and no significant difference was found between control and roselle bread. Similarly GI symptoms also were not affected by addition of roselle bread.

In the present study glucose level of the subjects were reduced after consumption of roselle calyces containing bread as compared to control bread. This reduction in glucose level may be because of increased insulin level. A previous study conducted on rats has shown an increase in the insulin level of rats after they were treated with roselle extract [9]. The most definite cause of increased blood glucose level is considered to be the low production of insulin by beta cells or decreased response to insulin [18]. Different studies have been conducted to progress beta cells activity or increase its production and response in order to reduce blood glucose levels [19]. Roselle aqueous extract when administrated orally had shown a significant effect on fasting blood glucose level. Alpha glucosidase enzyme which acts on starch and disaccharides to break it down to glucose, roselle inhibits this enzyme, which can subsequently reduce blood glucose level [20]. A previous study has concluded that addition of roselle in milk reduces HbA1c by 31.71%. In the same study roselle extract addition in milk also reduced glycogen level in blood [21]. Roselle can enhance the glucose ingestion and as a result it decreases blood glucose level, thus preventing diabetes [22]. Administration of roselle extract in a reasonable amount decreases glucose level and as a result may protect against diabetes [23].

The present study showed no significant effect of roselle calyces containing bread on appetite, while a previous study has shown a positive effect of roselle on reducing appetite and increasing satiety of the subjects. After consumption of a supplement containing roselle, feeling of hunger was reduced in the intervention and satiety was increased in this group [24]. A study of, soybean meal substituted with roselle seed performed on catfish has revealed that by substituting soybean with roselle can reduce appetite. This reduction in appetite has been attributed to high level of dietary fiber in roselle seed meal, and increased amount of anti-nutrients which could have decreased appetite, resulting in weight reduction in catfish [25].

There are different glycemic responses of different carbohydrate containing foods [26]. Different studies have proven that foods with low GI are healthier nutritionally than high GI foods [27]. Low GI foods are linked with control and avoidance of CVD and metabolic diseases such as diabetes and myocardial infarction [28]. Wheat products such as bread and biscuits with a low GI and normal taste and texture may be of high interest specifically for diabetic patients [29]. Alpha amylase enzyme which breaks down the starches into simple glucose is secreted by pancreas. Inhibition of this enzyme may cause reduction in the GI [30]. Bread incorporated with some product that can inhibit alpha amylase enzyme may result in reduction of GI of the bread. In an earlier study white kidney bean extract was incorporated into bread and results showed a decrease in the GI of the bread [31]. In the present study roselle addition to the bread has shown a significant decrease in the GI of the bread.

Conclusion

This study concluded that addition of 4% of roselle calyces powder to bread significantly reduces postprandial glucose level of healthy subjects, however it did not significantly cause any changes in composite appetite score. Long term studies explaining the mechanism of roselle calyces on how it affects blood glucose are recommended and in future bread incorporation with roselle calyces may be used as preventive measure by healthy individuals, to control their blood glucose. The treatment bread was acceptable to subjects in term of appearance, texture, flavour and overall acceptance and it did not cause any gastro intestinal symptoms in study subjects.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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