

## **Development and nutritional evaluation of cereal and pulse based biscuits for diabetic patients**

Abbreviations:

IDF: International Diabetes Federation

T2DM: Type 2 diabetes mellitus

GI: Glycemic index

GL: Glycemic Load

Running title: Development of low glycemic index biscuits for diabetic patients

Ethical Issues: Informed consent was obtained before conducting the experiment before feeding the food items and checking the blood **glucose** of subjects. The privacy rights of human subjects will always be observed.

### **Abstract**

Addition or supplementation of legumes and oilseeds into cereal-based foods has many health benefits.

**Objective:** This study was aimed to analyze the sensory, nutritional and glycemic properties of biscuits from cereal (oats and barley) and legume based (soybean and chickpea) combinations for diabetic patients.

**Design:** Intervention study. Different blends of salty biscuits were prepared using the cereal and pulses above mentioned.

**Subjects:** Ten healthy subjects in the age group of 20-40 years were selected from department of Food and Nutrition, Punjab Agricultural University for examining the glycemic index.

**Results:** Biscuits made from these cereal pulse combinations were highly acceptable and were chosen for nutritional analysis. The results of nutritional analysis showed increased protein (11.72g/100g), crude fiber (1.5g/100g) and ash content (4.68g/100g) and decreased content of carbohydrates (47g/100g) in blend containing refined wheat flour, barley and soy flour (25:50:25). Glycemic index of the acceptable and highly nutritious blend (Refined wheat flour, barley and soy flour 25:50:25) was 38.7, whereas for control salty biscuits, it was 84.

**Conclusion:** The incorporation of barley and soy flour in biscuits in the above ratio lowers the glycemic index of biscuits and it can be recommended to diabetic patients for maintaining blood glucose level.

Key words: Glycemic index, Type 2 diabetes, cereals and pulse

### **Introduction**

Diabetes mellitus is the leading cause of death and disability worldwide. It is the most challenging public health problems of the 21st century. According to International Diabetes Federation (IDF) 2015, one in 11 adults had diabetes in 2015 and the number will be one in 10 of 2040. India is one of the 7 countries of the IDF South East Asia (SEA) region. 415 million people have diabetes in the world and 78 million people in the SEA region; it will rise to 190 million by 2040. India is at the second position after China with 69.1 million cases of Type 2 diabetes mellitus (T2DM)<sup>(1)</sup>. The dietary carbohydrates are the major determinant of postprandial glucose response. Glycemic index (GI) was originally designed as a guide to food selection for diabetic patients, to select foods with a low GI. Glycemic index describes the blood glucose response after consumption of a carbohydrate containing test food relative to a carbohydrate containing reference food, typically glucose or white bread<sup>(2)</sup>. The concept of Glycemic Load (GL) takes account of the GI of a food and the amount eaten<sup>(3)</sup>. Diets rich in carbohydrates based foods, which have low glycemic index, are more slowly digested, absorbed and metabolised and hence have been associated with a reduced risk of T2DM and cardiovascular disease.

Low GI diets also assist in weight management <sup>(4)</sup>. These findings show no negative effects of a low GI diet and suggest that the GI should be an important consideration in the dietary management and prevention of diabetes <sup>(5)</sup>. Several interventional studies have also reported beneficial health effects of consuming low-GI diets <sup>(6)</sup>. Improved glycemic control through diet could decrease the intake of medications, lessen the risk of diabetic complications, improve the quality of life and increase life expectancy <sup>(7)</sup>. Beside drug treatment, healthy lifestyle with appropriate diet plays very important role in the management of diabetes. The dramatic increase in the incidence of diabetes has prompted the researchers to explore the potentiality of cereals and legumes in the management or control of diabetes and its complications.

Earlier oat and barley were used for malting and brewing purposes but these days they are being used as functional food ingredients. They contain soluble dietary fiber,  $\beta$ -glucan and various minerals. Barley has also the highest amount of vitamin E among other cereals <sup>(8)</sup>. It has the preventive role in the development of cardiovascular diseases, T2DM and cancer <sup>(9)</sup>. Legumes are important source of dietary proteins. They provide well-balanced essential amino acid profile when consumed with cereals. Besides this legume proteins also possess functional properties including solubility, water and fat binding ability. Legumes, when blended with cereals, may provide the promising alternative source of nutritional and functional proteins <sup>(10)</sup>. Soybean is also a rich source of protein and bioactive compounds, isoflavones, which have the preventive role in various cancers and improved bone health <sup>(11-15)</sup>. Combinations of wheat flour with legumes have been used in the production of baked products <sup>(16)</sup>. But meager work is conducted on assessing GI of cereal and legume-based combinations so, keeping all the aspects in view, the present study is designed to incorporate barley and oat flour along with legume flour (soybean and chickpea flour) into the refined wheat flour to improve the functional properties of biscuits, which would lead to improvement in the glycemic control in patients with diabetes mellitus.

**Key words:** Glycemic index, glycemic load, oat flour, barley flour, type 2 diabetes mellitus

## **Materials and methods**

### *Materials*

The raw material including refined wheat flour and functional food ingredients such as oat flour, barley flour, soy flour and chickpea flour were collected at one lot and stored in airtight plastic containers and used for entire study.

### *Designing of blends/ Sample preparation*

Biscuits were prepared from cereals and legumes based blends. Ten different blends were prepared using the Oats, barley, soybean and chickpea flours in different proportions by incorporating in refined wheat flour. These were stored in airtight container. The proportion of different ingredients used in each blend to prepare biscuits is given in Table 1.

### *Method used for product preparation*

The control sample was prepared from 62.11 % refined wheat flour, 29.81% butter, 29.81% salt, 2.48 %, baking powder 0.62% and water 4.96 %. Butter was mixed until creamy. The dried ingredients were sifted twice and were put into the butter. They were uniformly mixed to obtain consistent dough using water. The dough was rolled out and cut into square pieces. The biscuits were baked at 150° C for 15 minutes in preheated 4 Pan Electric Convection Oven (Empire Products ©, Model No. EKFA 412D Country: United States), cooled at room temperature and sealed in airtight plastic container for further analysis.

List of ingredients in given in Table 2. Blends 1-10 (Table 1) were used to prepare test samples.

### *Sensory analysis*

The developed biscuits were evaluated organoleptically by a panel of 15 subjects comprising of students and faculty of department of Food and Nutrition, PAU, Ludhiana. Each product was prepared and tested thrice. The samples were coded to avoid any bias. The panelists were asked to score the samples for color, appearance, flavor, texture, taste and overall acceptability by using a scorecard of 9-point Hedonic Rating Scale. The highly acceptable products and the control samples were weighed, homogenized and oven dried at 60°C. Dried samples were stored in airtight plastic bags for further analysis.

#### *Nutritional analysis*

Moisture, total ash, crude protein, crude fiber and crude fat were assessed using standard methods<sup>(17)</sup>. The content of carbohydrates was calculated by subtracting the sum of moisture, protein, ash, fat and crude fibre from 100. The energy content was calculated by factorial method.

$$\text{Energy (Kcal)} = (4 \times \text{protein}) + (9 \times \text{fat}) + (4 \times \text{carbohydrate})$$

#### *Assessment of glycemic index*

Glycemic index of biscuits was estimated, through a scientific approach of determining the glucose response in healthy subjects through meal tolerance test. The subjects for the present work were selected from department of Food and Nutrition, Punjab Agricultural University, Ludhiana, in the age group of 20-40 years (n=10). The glycemic response was analyzed by taking one drop of blood on glucose test strip using Glucometer (PRODIGY® Pocket).

#### *Glucose tolerance test*

After overnight fasting of the selected subjects, blood glucose test were performed and 50 gram carbohydrate in the form of glucose and on subsequent day salty biscuits were provided containing 50 gram available carbohydrate were given to the subjects. Fasting blood glucose was checked. The volunteers were asked to consume test product within 10-12 minutes. The blood samples were drawn and checked after every half an hour interval for two hours for the postprandial level. The blood glucose response curves were plotted for both oral glucose tolerance test and test product.

The glycemic index was calculated using the formula given by Wolever and Jenkins<sup>(18)</sup>.

$$\text{Glycemic index} = \frac{\text{Area under glucose curve after test meal}}{\text{Area under glucose curve after reference meal}} \times 100$$

The Glycemic load (GL) was calculated based on the quantity of the recipe per serving and the respective available carbohydrate content<sup>(19)</sup>. The following formula was used:

$$\text{Glycemic load} = \text{Available carbohydrates (g)} \times \text{GI} / 100$$

#### *Statistical analysis*

The results of organoleptic scores, nutritional analysis and glycemic index were statistically analyzed using analysis of variance technique with the aid of Microsoft statistical analysis tool pack. The limit of probability fixed for the test of significance ( $p \leq 0.05$ ) and least significance difference was calculated.

#### *Ethical Issues*

Informed consent was obtained before conducting the experiment before feeding food items and checking the blood glucose of human subjects. The privacy rights of human subjects will always be observed.

## **Results and discussion**

#### *Sensory evaluation of biscuits*

The mean scores of acceptability trials of salty biscuits are presented in Table 3. The mean sensory scores of color, appearance, flavor, texture, taste and overall acceptability ranged from 6.7 to 7.75, 7.0 to 7.6, 6.6 to 7.75, 6.6 to 7.8, 6.3 to 7.7 and 6.64 to 7.68 respectively. (Table 3)

With respect to color, it was found that biscuits made from blend 1,2,3,4,5,9 and 10(6.7-7.5) were on par with control (7.03) and blend 6,7 and 8 (7.6, 7.7, 7.75 respectively) had significantly higher color values as compared with control. There was no significant difference in appearance of different flour combinations (7.0- 7.6) compared to control (7.1) biscuits. The scores for flavor of blend 8 (7.75) was significantly higher than control (7.13) while scores of other flour combinations (6.6-7.4) were in par with control. In case of texture feel of biscuits blend 8 and 10 had significantly higher scores (7.8 and 7.7 respectively) compared with control (7.13), while other blends had scores (6.6-7.5) in accordance with control. When the taste scores were compared with control biscuits (7.2), all flour combinations had taste score (6.6-7.7) similar to control while blend 1 had significantly lower taste scores (6.3). Overall acceptability of blend 8(7.68) was found to be significantly higher when compared with control (7.13), rest of the other blends were equally preferred by the panelists (6.64-7.52). All the blends, which had overall acceptability higher than the control sample were chosen for nutritional analysis.

### *Nutritional analysis*

Moisture content of control sample (4.75%) was significantly high compared to all the test blends (0.9-1.56%). Total ash content of blend 3,4,6 and 7 (3.1, 4.68, 3.00, and 2.61% respectively) was significantly high as compared to other samples and control as well. All the test blends had significantly higher crude fiber(1.2-3.57%) and highest in blend 6 (3.78%) followed by blend 7(3.57%) and blend 9 (2.77%)when compared with control (0.49%). Blend 3 had significantly high level of crude fat (35.88%). Rest of the samples (31.19-33.45%) had fat content similar to control (31.73%). In case of crude protein blend 3 and 4 had significantly high levels (14.18 and 11.72% respectively) and blend 10 had significantly less percentage of crude protein (8.21%) as compared to control (9.24%). Other studies observed that the crude protein and ash content of 30% cassava pigeon pea biscuit was significantly higher than other biscuit samples<sup>(20)</sup> The carbohydrate content of blend 8 and 10 was significantly high (54.00 and 53.87 % respectively) and blend 3, 4 and 5 had carbohydrate content (44.07, 47.33 and 51.07 % respectively) significantly lower as compared to control biscuit samples (51.78%). Energy content was significantly high and maximum in blend 3(555.92kcal) followed by blend 9 and 10 (545.09 and 545.68 kcal respectively) compared to control (529.65 kcal) and energy content was non significantly different in blend 4, 6, 7 and 8 (536.71, 526.13, 528.58 and 534.83 kcal respectively). Blend 4 (refined flour, barley and soybean flour 25:50:25) was chosen for assessment of glycemic index because it had significantly high overall acceptability (7.22), significantly low carbohydrate content (47.33%) and energy (536.71 kcal) was in accordance with control(Table 4). The findings concluded that supplementation of refined wheat flour with barley, oat and soy flour significantly increased ash, fiber, and protein contents, showed improved nutritional composition and these blends had higher acceptability as compared to control so blend 4 was chosen for assessment of glycemic index.

### *Glycemic index*

Blend 4 comprising of refined wheat flour, barley flour and soy flour (25:50:25) was chosen for assessment of glycemic index because of its high overall acceptability and nutritional composition. The glycemic index of control and test samples is presented in Table 5. The fasting blood glucose of the selected subjects for the study ranged from 67 to 112. The rise in blood glucose after half an hour, 1 hour, 1 hour 30 minutes and 2 hours ranged from 90 to 162, 96 to 142, 96 to 127 and 83 to 119 in case of reference glucose (Fig 1). For control biscuit sample, the rise in blood glucose after half an hour, 1 hour, 1 hour 30 minutes and 2 hour ranged from 91 to 111, 87 to 123, 95 to 127 and 78 to 124 mg/dl and for test biscuits prepared from blend 4, the range was 76 to 106, 85 to 110, 88 to 102, 73 to 100mg/dl (Fig 1). The glycemic index came out to be 38.68, which was lower than the control (83.99).

The lowering of glycemic index in biscuits can be attributed to the addition of legumes which contains 5-10% more amylose compared to cereal grains and this amylose is more resistant to digestion. With the incorporation of legumes, the protein content had increased and higher amount of proteins may physically encapsulate starch, preventing the enzyme access<sup>(21)</sup>. Apart from proteins and amylose content the crude fiber had also increased in all the enriched products. Dietary fiber also inhibits starch digestibility by increasing the viscosity of intestinal contents and thereby slowing the absorption of carbohydrates from the food. The effect of consumption of crackers and cookies made from

barley flour enriched with  $\beta$ -glucan in comparison with similar products made from wheat flour on fasting and postprandial glucose, found glycemic index values as 78, 81, 49 and 34 for whole wheat crackers, whole wheat cookies, barley crackers and barley cookies, respectively<sup>(22,23)</sup>.

Table 6 displays that the mean GI and GL of the supplemented product were significantly lower as compared to the control samples. Anything with GI value of 70 or more is a high GI food, moderate GI foods ranged from 56 to 69 and low GI foods have scores from 0 to 55<sup>(24)</sup>. According to this classification of **World Health Organization** (WHO), biscuits (refined flour, barley flour and soybean flour; 25:50:25) with 38.68 GI fall under the low GI products with a decrease of 117% when compared with control. Increase in protein and crude fiber and decrease in carbohydrates were responsible for lowering the glycemic value of the developed product.

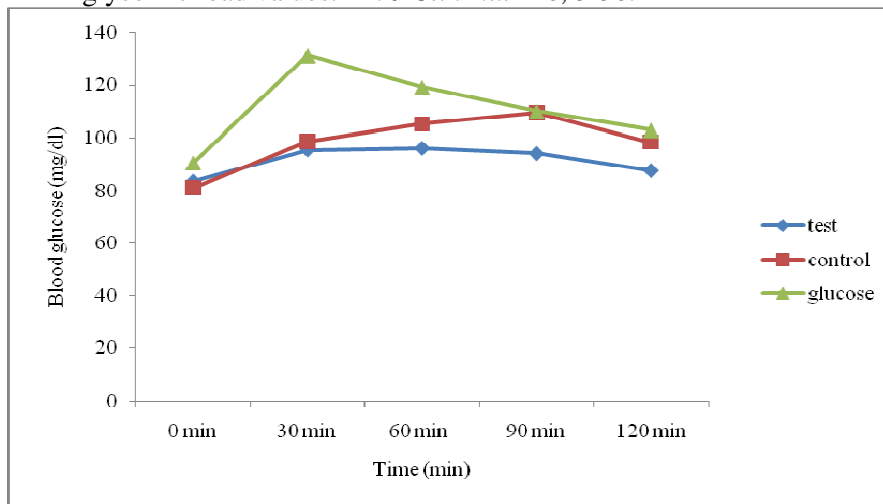
## Conclusions

The study concluded that blends composed of cereal and legume based mixtures, were highly acceptable, when compared with control and chosen for the nutritional analysis. In some of the blends significantly reduced carbohydrates and increased ash, fiber, fat and protein contents were found. Glycemic index and glycemic load of the blend 4 (refined wheat flour, barley and soy flour; 25:50:25) was assessed. The mean GI and GL of the biscuits were significantly lower (GI 38.68) as compared to the control. Biscuits from blend four fall under the category of low GI foods, when compared with control. Increase in protein and crude fiber and decrease in carbohydrates were responsible for lowering the glycemic value of the developed biscuits. Moreover blend four had higher acceptability; hence it can prove to be suitable for diabetics. The developed biscuits can be a good substitute for regular biscuits for diabetic patients for the management of diabetes and to avoid further secondary complications.

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**Fig. 1.** Mean blood glucose curves after consumption of glucose, test salty biscuits and control salty biscuits containing 50g carbohydrates

**Table 1:** Combinations of flours.

	Refined flour (g/100g)	Oat flour (g/100g)	Soy flour (g/100g)
Control	100	-	-
Blend 1	25	50	25
Blend 2	50	25	25
Blend 3	75	-	25
		Barley flour (g/100g)	
Blend 4	25	50	25
Blend 5	50	25	25
		Oat flour (g/100g)	Chickpea flour (g/100g)
Blend 6	25	50	25
Blend 7	50	25	25
Blend 8	75	-	25
		Barley flour (g/100g)	
Blend 9	25	50	25
Blend 10	50	25	25

**Table 2** List of ingredients used in making salty biscuits

Ingredients	Amount	Percentage of ingredients
Refined wheat flour	125g	62.11
Fat	60g	29.81
Salt	5g	2.48
Baking powder	1.25g	0.62
Water	5-10 ml	4.96

**Table 3:** Sensory scores of developed blends of salty biscuits

Blends	Flour combinations	Amount (g/100g)	Colour	Appearance	Flavor	Texture feel	Taste	Overall acceptability
Blend 1	Refined flour+Oat+Soybean	25+50+25	6.7±0.82 <sup>a</sup>	7.0±0.82 <sup>a</sup>	6.6±1.35 <sup>a</sup>	6.6±1.07 <sup>a</sup>	6.3±1.49 <sup>a</sup>	6.64±1.04 <sup>a</sup>
Blend 2	Refined flour+Oat+Soybean	50+25+25	6.9±0.99 <sup>a</sup>	7.0±1.05 <sup>a</sup>	6.8±1.23 <sup>a</sup>	6.9±1.10 <sup>a</sup>	6.6±1.33 <sup>a</sup>	6.84±1.11 <sup>a</sup>
Blend 3	Refined flour+Soybean	75+25	7.5±0.75 <sup>a</sup>	7.4±0.81 <sup>a</sup>	7.2±0.94 <sup>a</sup>	7.3±0.96 <sup>a</sup>	7.2±1.01 <sup>a</sup>	7.32±0.83 <sup>a</sup>
Blend 4	Refined flour+Barley+Soybean	25+50+25	7.3±1.06 <sup>a</sup>	7.2±1.14 <sup>a</sup>	7.0±1.33 <sup>a</sup>	7.3±1.49 <sup>a</sup>	7.3±1.49 <sup>a</sup>	7.22±1.26 <sup>a</sup>
Blend 5	Refined flour+Barley+Soybean	50+25+25	7.1±1.37 <sup>a</sup>	7.1±1.37 <sup>a</sup>	6.9±1.37 <sup>a</sup>	7.1±1.37 <sup>a</sup>	7.2±1.22 <sup>a</sup>	7.06±1.35 <sup>a</sup>
Blend 6	Refined flour+Oat+Chickpea	25+50+25	7.6±0.52 <sup>b</sup>	7.6±0.52 <sup>a</sup>	7.0±0.67 <sup>a</sup>	7.3±0.48 <sup>a</sup>	7.1±0.32 <sup>a</sup>	7.32±0.41 <sup>a</sup>
Blend 7	Refined flour+Oat+Chickpea	50+25+25	7.7±0.48 <sup>b</sup>	7.6±0.52 <sup>a</sup>	7.4±0.70 <sup>a</sup>	7.5±0.71 <sup>a</sup>	7.4±0.70 <sup>a</sup>	7.52±0.46 <sup>a</sup>
Blend 8	Refined flour+Chickpea	75+25	7.75±0.71 <sup>a</sup>	7.4±0.76 <sup>a</sup>	7.75±0.92 <sup>a</sup>	7.8±0.82 <sup>b</sup>	7.7±0.99 <sup>a</sup>	7.68±0.74 <sup>b</sup>
Blend 9	Refined flour+Barley+Chickpea	25+50+25	7.2±0.79 <sup>a</sup>	7.5±0.71 <sup>a</sup>	7.3±0.82 <sup>a</sup>	7.3±0.67 <sup>a</sup>	7.6±0.52 <sup>a</sup>	7.4±0.52 <sup>a</sup>
Blend 10	Refined flour+Barley+Chickpea	50+25+25	7.3±1.06 <sup>a</sup>	7.5±0.97 <sup>a</sup>	7.3±0.82 <sup>a</sup>	7.7±0.82 <sup>b</sup>	7.6±0.70 <sup>a</sup>	7.48±0.80 <sup>a</sup>
Control	Refined flour	100	7.03±0.66 <sup>a</sup>	7.1±0.50 <sup>a</sup>	7.13±0.69 <sup>a</sup>	7.13±0.79 <sup>a</sup>	7.2±0.79 <sup>a</sup>	7.13±0.67 <sup>a</sup>
<b>LSD*</b>			<b>0.49</b>	<b>0.50</b>	<b>0.58</b>	<b>0.56</b>	<b>0.60</b>	<b>0.51</b>

Values are presented as Mean± SD

**Key to scores:** 9= Like extremely, 8= Like very much, 7= Like moderately, 6= Like slightly, 5= Neither like or nor dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much, 1= Dislike extremely

**\*Least significance difference at 5% level of significance**

Superscripts with same alphabets imply non-significant difference with control at 5% level of significance.

Superscripts with different alphabets imply significant difference from control at 5% level of significance.



**Table 4:** Nutritional composition of most acceptable blends of salty biscuits (g/100g on dry weight basis).

Blends	Flour combinations	Amount (g/100g)	Moisture	Total Ash	Crude Fiber	Crude Fat	Crude Protein	Carbohydrate	Energy (kcal)
Blend 3	Refined wheat flour+Soybean	75+25	1.56±0.08 <sup>b</sup>	3.1±0.02 <sup>b</sup>	1.2±0.02 <sup>b</sup>	35.88±1.04 <sup>b</sup>	14.18±0.70 <sup>b</sup>	44.07±1.703 <sup>b</sup>	555.92±5.36 <sup>b</sup>
Blend 4	Refinedwheat flour+Barley+Soybean	25+50+25	1.38±0.08 <sup>b</sup>	4.68±0.01 <sup>b</sup>	1.5±0.10 <sup>b</sup>	33.39±3.48 <sup>a</sup>	11.72±0.06 <sup>b</sup>	47.33±3.37 <sup>b</sup>	536.71±18.10 <sup>a</sup>
Blend 6	Refined wheat flour+Oat+Chickpea	25+50+25	0.9±0.01 <sup>b</sup>	3.00±0.03 <sup>b</sup>	3.78±0.12 <sup>b</sup>	31.49±1.00 <sup>a</sup>	9.61±0.17 <sup>a</sup>	51.07±1.31 <sup>b</sup>	526.13±4.46 <sup>a</sup>
Blend 7	Refined wheat flour+Oat+Chickpea	50+25+25	0.31±0.07 <sup>b</sup>	2.61±0.01 <sup>b</sup>	3.57±0.08 <sup>b</sup>	31.38±1.16 <sup>a</sup>	9.50±0.28 <sup>a</sup>	52.04±1.02 <sup>a</sup>	528.58±5.26 <sup>a</sup>
Blend 8	Refined wheat flour+Chickpea	75+25	0.54±0.06 <sup>b</sup>	2.22±0.02 <sup>a</sup>	2.41±0.14 <sup>b</sup>	31.19±0.19 <sup>a</sup>	9.33±0.28 <sup>a</sup>	54.20±0.00 <sup>b</sup>	534.83±0.57 <sup>a</sup>
Blend 9	Refined wheat flour+Barley+Chickpea	25+50+25	0.29±0.23 <sup>b</sup>	1.99±0.44 <sup>a</sup>	2.77±0.14 <sup>b</sup>	33.45±0.16 <sup>a</sup>	8.92±0.12 <sup>a</sup>	52.09±0.63 <sup>a</sup>	545.09±0.60 <sup>b</sup>
Blend 10	Refined wheat flour+Barley+Chickpea	50+25+25	1.00±0.46 <sup>b</sup>	1.53±0.99 <sup>a</sup>	2.19±0.17 <sup>b</sup>	33.04±1.04 <sup>a</sup>	8.21±0.44 <sup>b</sup>	53.87±0.96 <sup>b</sup>	545.68±3.78 <sup>b</sup>
Control	Refined wheat flour	100	4.75±0.00 <sup>a</sup>	2.01±0.03 <sup>a</sup>	0.49±0.03 <sup>a</sup>	31.73±0.29 <sup>a</sup>	9.24±0.09 <sup>a</sup>	51.78±0.263 <sup>a</sup>	529.65±1.22 <sup>a</sup>
<b>LSD*</b>			<b>0.29</b>	<b>0.59</b>	<b>0.17</b>	<b>2.22</b>	<b>0.51</b>	<b>0.32</b>	<b>11.09</b>

Values are presented as Mean±SD.

**\*Least significance difference at 5% level of significance**

Superscript with same alphabets imply non-significant difference with control at 5% level of significance.

Superscripts with different alphabets imply significant difference with control at 5% level of significance.

**Table 5: Glycemic index of control and test biscuits**

Product	Quantity administered (grams)	GI	GI Category
Biscuit (control)	94	84	High
Biscuit (test)	108.5	38.68	Low

**Table 6: Glycemic load of biscuits**

Product	GI	Normal Serving size (g)	Available carbohydrate(g)	Glycemic load (GL)
Biscuit (control)	84	20	11	9.24
Biscuit (test)	38.68	20	9	3.48