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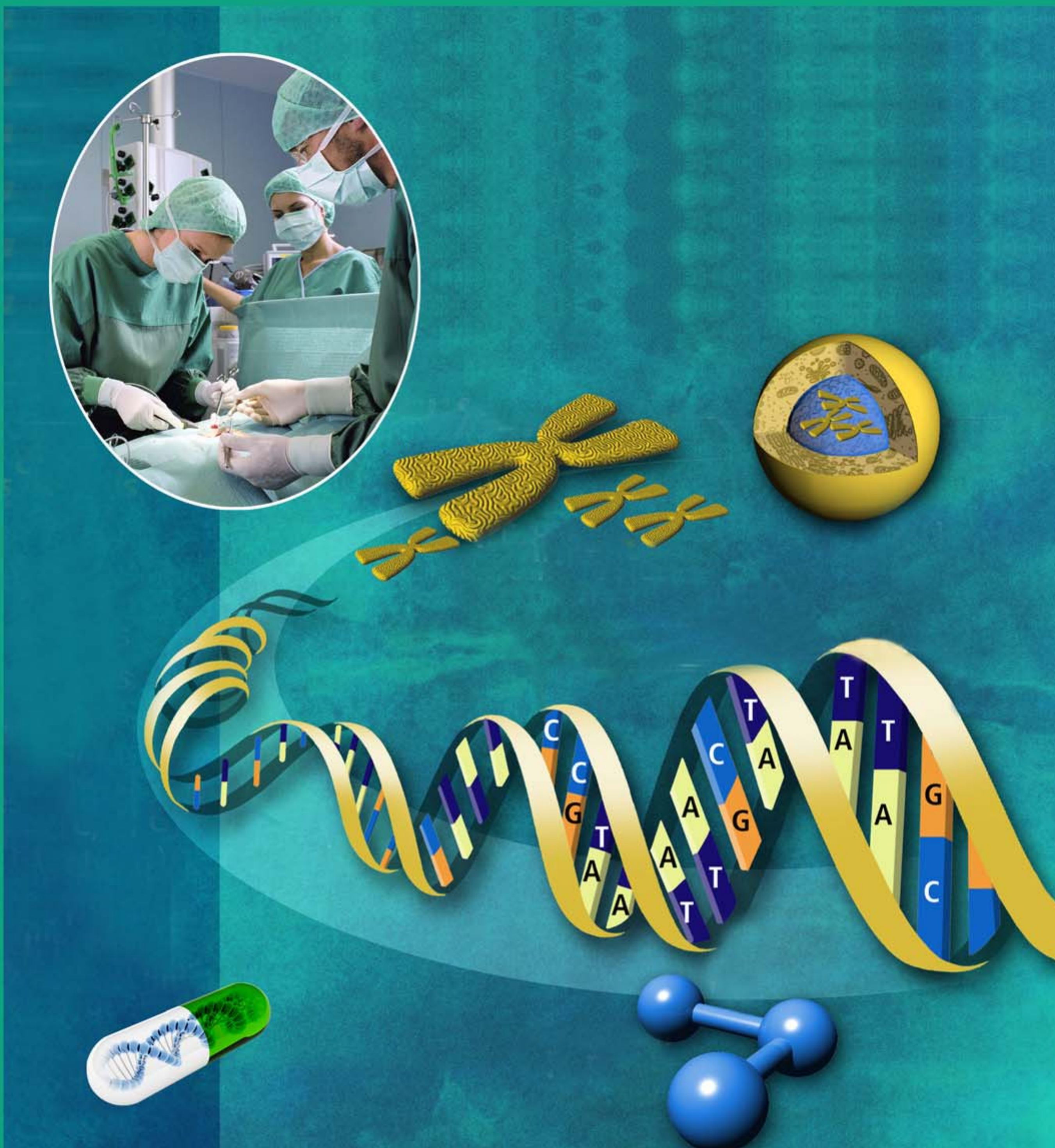

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Research Paper

COMPARATIVE STUDY OF PROCESSED AMARANTH GRAINS ON GLYCEMIC INDICES IN NIDDM SUBJECTS

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The glycemic response of processed amaranth grains were assessed using fifty Non Insulin Dependent Diabetes Mellitus (NIDDM) subjects. The post-prandial serum glucose concentration over a period of 2 h were determined half hourly, after the ingestion of the experimental diets. Blood glucose curves were constructed to calculate the glycemic index of the test foods. The results revealed that the Glycemic Indices (GI) of Roasted Amaranth Grains Flour Chapatti (RAGFC), Boiled Amaranth Grains Flour Chapatti (BAGFC), Popped Amaranth Grains Flour Chapatti (PAGFC) and Raw Amaranth Grains Flour Chapatti (RaAGFC) did not showed any differences at ($p < 0.05$). However, popped amaranth grains flour Chapatti gave the lowest glycemic index and the value was significantly lower than the other test foods. The popped amaranth grains flour elicit low postprandial rise of blood glucose and can be recommended for use in the diet of diabetic Indians.

Keywords: Amaranth grains, Chapattis, Processing, Glycemic indices

INTRODUCTION

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate (Huizinga and Rothman, 2006). Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people. It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe (Wild *et al*., 2004) The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025 (Sicree *et al*., 2006). The countries with the largest number of diabetic people will be India, China and USA by 2030. It is estimated that every fifth person with diabetes will be an Indian. The type-II diabetes is non-insulin dependent and is the most common form of diabetes and patients retain endogenous insulin production but exhibit excessive adiposity and resistance to peripheral action of insulin

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(ICMR Bulletin, 1987). The development and progression of micro-vascular complications is associated closely with chronic hyperglycaemia. Therefore, tight glycemic control is by far the most effective approach in the prevention of diabetic vascular complications (The diabetes control and complications trial research group, 1993). Although euglycaemia can be achieved in diabetes patients by conventional insulin and oral hypoglycaemic drug treatment, microvascular and neurological complications cannot be prevented by this (Pirart, 1978). Insulin treatment has also been reported to increase cholesterol synthesis (Bhathena *et al*., 1974) and secretion of very low density lipoproteins (Revan and Bernstein, 1978). Adherence to long term carbohydrate restricted diet also leads to the development of insulin resistance and thus serum cholesterol levels are raised in diabetic patients (Pedersen *et al*., 1982). Diet has been recognized as a corner stone in the management of diabetes mellitus. Persons with diabetes may get substantial benefits by increasing their intake of dietary fiber (ICMR Bulletin, 1987).

Amaranth grains are lentil-shaped and compared to cereal grains, they are very small (approximately 1 mm diameter) and light (Belton and Taylor, 2002). These grains have high nutritional and functional values which are associated with the quality and quantity of their proteins, fats and antioxidant potentia (Gorinstein *et al*., 2002; Gorinstein *et al*., 2007; and Pasko *et al.,* 2007). Nutritionally, amaranth grains have higher protein content, higher digestibility, higher protein utilization, and a higher protein efficiency ratio than traditional cereals such as corn and wheat (SalcedoChavez *et al*., 2002). The grain of amaranth is excellent in the pursuit of weight loss. This protein is not accompanied with fat or cholesterol. The high-fiber content of the food makes it a low glycemic index food, and suited to people who eat a balanced diet to control their glucose levels (www.diethealthclub.com).

The propensity of individuals to develop diabetes and obesity is due to the increased consumption of carbohydrate rich foods with a high Glycemic index (Willett and Leibel, 2002). The glycemic index (GI) is a property of carbohydrate-containing foods that provides a basis for predicting their postprandial blood glucose response (Jenkins *et al*., 1981). The glycemic load (GL) is the product of a food's GI and carbohydrate content divided by 100 (Foster Powell *et al*., 2002). Since food processing is one of the intrinsic factors that determine starch digestibility, amaranth's nutritional features are likely to be affected by the type of processing it undergoes. In the present study, an attempt has been made to determine the glycaemic indices of four types of processed amaranth grains also to correlate the values of glycaemic index and its usefulness in the management of NIDDM.

MATERIALS AND METHODS

Collection of food samples

Amaranth grains (*Amaranthus cruentus*) used for this investigation were procured from the Sree Krishna agro foods store, wheat flour and salt were purchased from Kannan departmental store in Salem, Tamil Nadu, India. The amaranth grains were cleaned thoroughly to remove adhering of dust and foreign matters. The grains were soaked in water to remove the small stones and washed with tap water to remove dirt and sun dried for 12 h. The dried grains were used for processing.

Preparation of processed samples

Roasted Amaranth Grains Flour (RAGF)

The selected amaranth grains were placed on a hot plate at 120^{0}C for about 5 min to prepare roasted grains, stirring and checking was done often to prevent from charring. Grains were transferred from hot plate when they are lightly browned and crispy. After cooling it made into flour.

Boiled Amaranth Grains Flour (BAGF)

Poured three cups water, one cup of amaranth grains and ½ tsp. of salt in the saucepan and mixed them together and the mixture heated up to the boiling point (100 °C). Once the boiling point is reached, the stove turned into 'low'. The mixture further heated for 25 minutes with lid placed on the saucepan. Turned off the stove and allow saucepan sitting for 10 more minutes. Dry the cooked grains in the sun light using a white muslin cloth for four hours and made into flour.

Popped Amaranth Grains Flour (PAGF)

Amaranth grains were heated on a hot plate at 180 ^{0}C for about 10 seconds until they popped. Keep stirring the grains with a spoon to prevent burning. As soon as popping stopped, empty the pan, cool it and made into flour.

Raw Amaranth Grains Flour (RaAGF)

Raw grains were cleaned and washed with distilled water and dried under sunlight. After drying the grains were made into flour.

Preparation of experimental diets

All the processed grains were floured in a flour mill, passed through 105 μ mesh sieve and packed in polyethylene pouches and stored in a refrigerator (<10° C) for food preparation. About 20g of (optimized from our previous study) each processed grain flour was incorporated into four types of chapatti, namely Roasted Amaranth Grains Flour Chapatti (RAGFC), Boiled Amaranth Grains Flour Chapatti (BAGFC), Popped Amaranth Grains Flour Chapatti (PAGFC) and Raw Amaranth Grains Flour Chapatti (RaAGFC) which were formulated in the proportion of 80:20 (wheat flour: each processed Amaranth grains flour. All the four types of chapatti were analyzed for proximate composition of moisture, ash, crude fat, crude fibre and protein (AOAC, 1995). Carbohydrate was determined by difference. 50g of available carbohydrate for each test food sample was calculated from the results of the proximate analysis and the measured portion of the food was served to the subjects.

Experimental design

Fifty Non Insulin Dependent Diabetes Mellitus (NIDDM) subjects diagnosed since two to ten years, of either sex aged between 40 to 60 years were included in the study. Diabetes was diagnosed on the basis of WHO criteria (2006). All the patients were on oral hypoglycaemic drugs. The study was conducted with the approval of the institutional ethical committee. Patients diagnosed as diabetes but suffering from gastro intestinal disorder were excluded from the study. They were offered a single meal of one of the four test foods on different days. The other 10 persons were administered 50 g glucose in 300 ml distilled water. The serving size was determined by calculating the quantity of the test food that will give 50 gm carbohydrate when eaten. Blood samples were collected before feeding (0 min) and at 30, 60, 90 and 120 min after the test meal was given. The subjects were not allowed to perform strenuous activities on the day of GI determination.

Anthropometric measurements

Body weight was measured (to the nearest 0.5 kg) with the subject standing motion less on the bathroom weighing scale. The weighing scale was standardized every day with a weight of 50 kg. Height was measured (to the nearest 0.1 cm) with the subject standing in an erect position against a vertical scale of portable stadiometer and with the head positioned so that the top of the external auditory meatus was in level with the inferior margin of the bony orbit. BMI was calculated as weight in kilograms divided by squared height in meter.

Determination of blood glucose

All subjects for the investigation fasted overnight. Their blood samples were collected through finger prick using a hypodermic needle or lancets. Each blood sample was placed on a test strip which was inserted into a calibrated glucometer (Accu-Check/One touch) which gave direct readings after 45 seconds based on glucose oxidase assay method. The determination of glucose level was done at intervals, i.e., 0 (fasting level), 30 mins, 60 mins, 90 mins and 120 mins.

Determination of Glycemic Index (GI)

GI was calculated from the blood glucose response curve. The incremental area under the curve (IAUC) for each test meal for each subject was calculated as the sum of the surface triangle and trapezoids of the blood glucose curve and the horizontal baseline running in parallel to the time axis from the beginning of the curve to the point at 120 min. This reflects the total rise in blood glucose concentration after eating the test food. The IAUC for test and control (50 g of pure glucose - IAUCS) was obtained in a similar way.

GI for each food was calculated from the formulae:

$$GI = \frac{\text{Incremental area under curve of (IAUC)}}{\text{Incremental area under curve of sample (IAUCS)}} \times 100$$

The average of the each (0, 30, 60, 90 and 120 min) measures for each subject was taken as the GI for that test food for the subject. The GI for each food was finally calculated as the mean of the average of the GIs in six subjects in the group.

Determination of glycemic load (GL)

Glycemic load represents both the quality and quantity of CHO in a food. Glycemic load values were calculated by multiplying the total amount of CHO (gm) ingested by the glycemic index value of each of food and dividing by 100.

$$GL = \frac{\text{Grams of Carbohydrate}}{100} \times GI$$

Statistical analysis

Statistical analysis was done by SPSS 15 Statistical programme. Comparisons between test foods: Roasted Amaranth Grains Flour Chapatti (RAGFC), Boiled Amaranth Grains Flour Chapatti (BAGFC), Popped Amaranth Grains Flour Chapatti (PAGFC) and Raw Amaranth Grains Flour Chapatti (RaAGFC) were done by the student's t-test. ANOVA and Duncan multiple range tests were used to measure significant difference among the GI of tests foods. Statistical significant was set at $p < 0.05$.

RESULTS

The proximate analysis of the processed amaranth grains flour incorporated chapattis were represented in Table 1. The percentage carbohydrate contents ranged from 69% in RaAGFC to 70.6% in RAGFC. BPp had the lowest carbohydrate content while Fp had the highest value. All the chapattis had the similar lipid value of 2.6%, while fibre content had the highest of 3.45% in PAGFC. Processing affected the proximate contents. Percentage lipid was highest in the fried plantain meal. However, moisture was reduced in roasted and popped amaranth grain flour chapattis.

Table 1: Proximate Analysis of the Processed Amaranth Grains Flour Incorporated Chapattis on Dry Weight Percent

Nutrients	RaAGFC	RAGFC	BAGFC	PAGFC
Moisture	11.23±.02646[d]	10.37±.02082[b]	10.77±.01528[c]	10.63±.42319[a]
Carbohydrate(g)	69.83±.76376[a]	70.60±.10000[a]	70.52±.02082[a]	70.25±.03055[a]
Protein(g)	12.36 ±.32146[a]	12.34±.01528[a]	12.33±.01000[a]	12.54±.01528[a]
Fat(g)	2.63 ±.02646[a]	2.68±.01000[b]	2.66±.03215[ab]	2.66±.01528[ab]
Fiber(g)	2.94±.01528[a]	3.22±.19079[b]	3.37±.07000[bc]	3.45±.01000[c]

Note: Values are the means ± standard errors of means (SEM) of four (3) determinants. Means with same superscript are not significantly different using Duncan's Multiple Range Test ($P < 0.05$).

Table 3: Available Carbohydrate in 100 g of the Processed Chapatti and Serving Sizes Used for Glycemic Index Determination

Experimental diets	Available Carbohydrate in 100 g of processed food (g)	Serving size of processed sample (g)
Roasted Amaranth Grains Flour Chapatti (RAGFC)	67.22	117.5
Boiled Amaranth Grains Flour Chapatti (BAGFC)	66.9	118
Popped Amaranth Grains Flour Chapatti (PAGFC)	66.18	116.34
Raw Amaranth Grains Flour Chapatti (RaAGFC)	67.58	121.39

The anthropometry of control and test subjects was represented in Table 2. The volunteers were aged between 43.7±5 to 53.5±4.63. Their estimated Body Mass Index (BMI) was 24.33±2.94 to 26.66±3.50 kg/m^2.

Table 3 represents the available carbohydrate content in 100 g of the processed meals and the serving sizes containing 50 g available carbohydrate. Raw Amaranth Grains Flour Chapatti had the highest available carbohydrate of the processed foods while Popped Amaranth Grains

Table 4: Blood Glucose in NIDDM Subjects with Glucose and Experimental Diets

Control and Experimental diets	Time Interval in minutes with blood glucose values (mg/dl)				
	0 mint	30 min	60 min	90 min	120 min
Glucose	132.50±19.76a	194.83 ±39.36a	181.33±13.75b	177.50±12.91c	159.833±16.15c
RAGFC	139.50±29.24a	173.50±39.97a	163.83±23.63ab	154.16±18.88bc	135.50±15.97bc
BAGFC	135.16±36.83a	200.00±29.95a	175.83±32.17ab	166.166±32.96bc	146.00±28.28c
PAGFC	114.66±9.02a	147.33±28.69a	135.66±22.92a	117.50±7.63a	110.00±12.88a
RaAGFC	122±14.16a	167.83±43.22a	157.66±51.02ab	141.50±43.19ab	116.00±21.49ab

Note: Values are the means + standard errors of means (SEM) of ten individuals per group. Means with same superscript are not significantly different using Duncan's Multiple Range Test ($P < 0.05$).

Flour Chapatti (PAGFC) had the lowest serving size.

Blood glucose in NIDDM subjects with glucose and experimental diets were tabulated as follows in Table 4.The mean blood glucose values were relatively lower with different control and experimental diets when compared to blood glucose levels after glucose administration at all the time intervals of blood glucose estimation.

In order to assess the glycaemic responses of various carbohydrate loads, blood glucose area under the curve is calculated by using the formula of Wolever and Jenkins. The mean and standard deviation values were calculated. The data is statistically analyzed by using student 't'- test. The mean AUC values of all the experimental diets are lower than AUC of glucose, and this difference is statistically not significant but popped amaranth grains flour chapatti had a significantly lower AUC value when compared to other processed forms of amaranth grains as depicted in Table 5.

The Glycemic Index (GI) and Glycemic Load (GL) of the four processed amaranth grain chapattis was shown in Table 6 and Figure 5, GI ranged from 44±25.08 to 111.83±75 and the GL ranged from 21.8±12 to 55.7±37.8. The highest GI (111.83) was obtained for boiled amaranth grains flour chapatti however the lowest GI (44) was obtained by popped amaranth grains flour chapatti. The highest GL (55.7) was observed in boiled amaranth grains flour chapatti, the lowest

Table 5: Comparison of Mean SD of Area Under Curve (AUC) (mg/dl/min) of Glucose (Control) with Experimental Diets

Control and Experimental diets	Mean	Std.Deviation	Significance	P Value
Glucose	846.0000	79.09488	0.768	–
RAGFC	767.0000	80.35670	0.997	–
BAGFC	823.1667	140.77843	0.020*	<0.05
PAGFC	620.1667	59.82447	0.260	–
RaAGFC	705.6667	179.67267	0.918	–

Note: * The mean difference is significant at the 0.05 level.

Table 6: Glycemic Indices and Load of the Processed Amaranth Grains Flour incorporated chapattis

Food Samples	GI	GL
Roasted Chapatti	84.83±50a	42±25a
Boiled Chapatti	111.83±75a	55.7±37.8a
Popped Chapatti	44±25.08a	21.8±12a
Raw Chapatti	102.3±76.4a	50.83±38a

Note: Values are the means + standard errors of means (SEM) of ten individuals per group. Means with same superscript are not significantly different (P < 0.05).

GI (21.8) was observed in the popped amaranth grains flour chapatti. The popped grains flour incorporated chapatti had very least GI (44±25.08) and GL (21.8±12) among the variations. The processed amaranth grains incorporated chapattis GI and GL did not differ significantly but Popped Amaranth Grains Flour Chapatti (PAGFC) had a significantly lower GL and GI when compared to other processed forms of amaranth grain flour chapattis.

Figures 1-4 shows the blood glucose response curves for the processed amaranth grains flour chapattis Glycemic responses after ingestion of the experimental diets varied from each other. A small peak of blood glucose at 30 min was observed for all the test foods

Figure 1: Graphical Representation Showing the Glucose Response Area for Roasted Amaranth Grains Flour Chapatti (RAGFC) and Control (Glucose D)

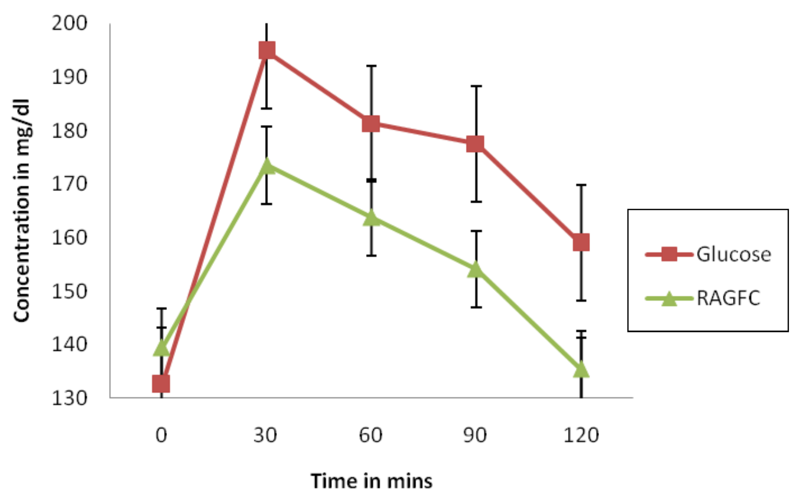


Figure 2: Graphical Representation Showing the Glucose Response Area for Boiled Amaranth Grains Flour Chapatti (BAGFC) and Control (Glucose D)

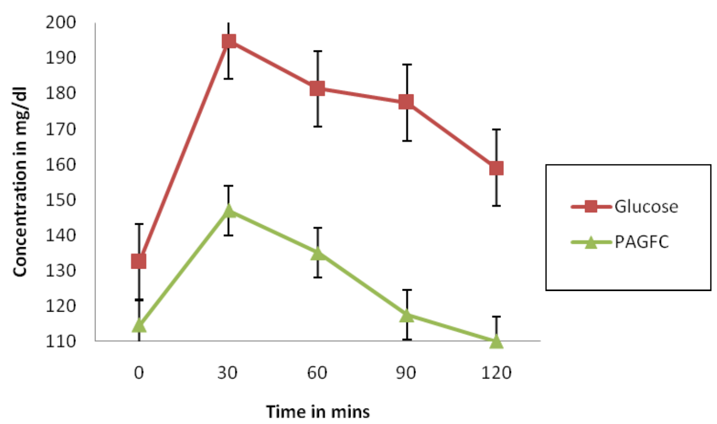


Figure 3: Graphical Representation Showing the Glucose Response Area for Popped Amaranth Grains Flour Chapatti (PAGFC) and Control (Glucose D)

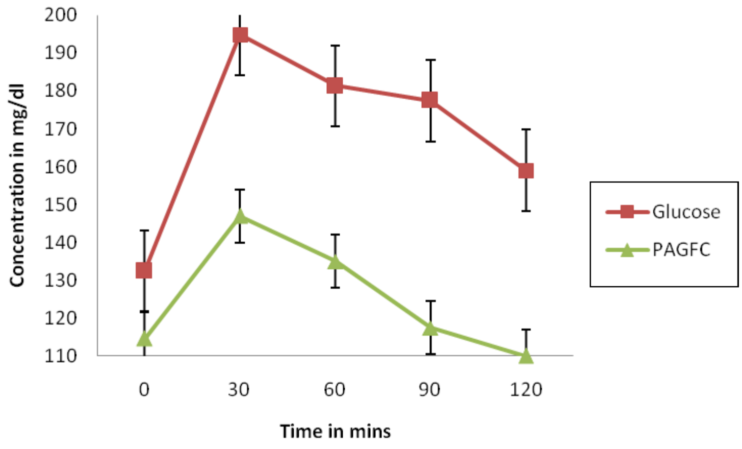


Figure 4: Graphical Representation Showing the Glucose Response Area for Popped Amaranth Grains Flour Chapatti (PAGFC) and Control (Glucose D)

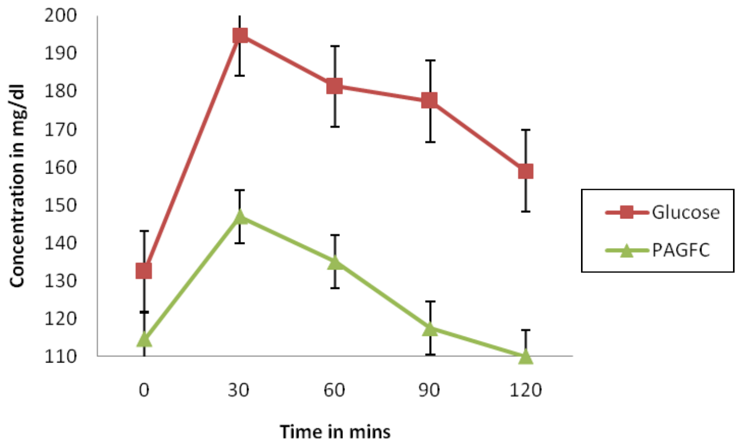
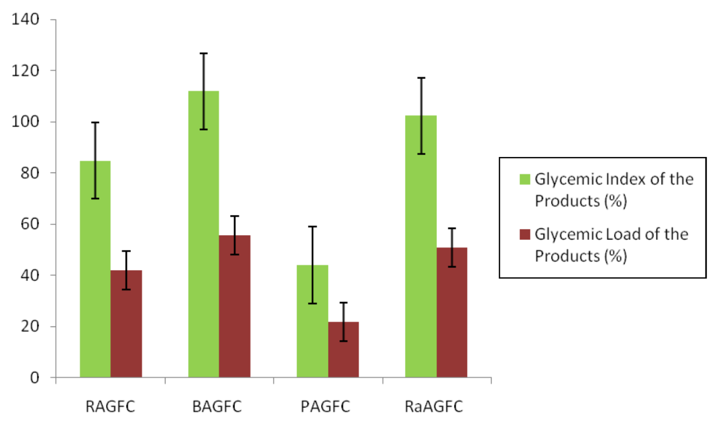


Figure 5: Glycemic indices of Amaranth Grain Chapattis



accompanied by a gradual decline in plasma glucose. The control group showed that blood glucose concentration rose to a peak at 30 min and declined rapidly at 60 min until 120 min. In comparison, the subjects who ate the popped amaranth grains flour meal showed that the blood glucose values rose to a small peak after 2 h for all the processed foods with a more gradual decline in blood glucose.

DISCUSSION

Different factors can influence blood glucose response. These include the physical form of the food, degree and type of processing, e.g., cooking method and time, amount of heat or moisture used (Pi-Sunyer, 2002), type of starch (that is, amylose versus amylopectin), and Co-ingestion of protein and fat (Collier *et al*., 1984) with test foods. The effect of moist heat treatment showed that faster rates of digestion were achieved with boiled than roasted amaranth grains flour chapattis. Raw and Roasted amaranth grains flour chapattis allowed the starch granules to swell gelatinize and increase the availability to amylase digestion and thereby increasing starch digestibility (Bahado-Sigh *et al*., 2006).

The greater the changes of the physical form of the meal, the higher the glycemic response (Wolever *et al*., 1986). Processing of amaranth grains through popping, roasting and boiling did not significantly alter their protein, fat and fiber levels. However, these treatments slightly decreased carbohydrate to increase in volume of up to 1,050 % and give the grains a gritty flavour (Saunders and Becker, 1984). The increased volume makes milling easier.

Heat treatment resulted in an increase in fibre content and decrease in nitrogen-free extracts, which is in accordance with the data reported (Hoover and Vasanthan, 1994; and Varo *et al*., 1983). Heat treatment induces the inactivation of antinutritional factors but on the other hand, it might cause amino acid degradation, formation of intramolecular bonds and Millard reactions, which impairs digestibility of nutrients (Hurrell *et al*., 1976; and Nestares *et al*., 1993). The amount of carbohydrate as well as the type of carbohydrate in a food will influence its effect on blood glucose level (Enser *et al*., 2000). The specific type of carbohydrate present in a particular food does not always accurately predict its effect on blood glucose (Wolever *et al*., 2008). During boiling the starch content was significantly increased in amaranth grains, perhaps because of partial loss of soluble materials during boiling (Tamer H Gamel *et al*., 2005). Swelling of the thermal treatment had been increased than the crude grains. The reason was the presence of denatured protein and gelatinized starch, which are able to bind more water (Dengate, 1984). Cooking and popping decreased the water-soluble fraction (albumins+globulins) and alcohol-soluble fraction (prolamins) while glutelins were increased by cooking and decreased by popping in Amaranth species (Tamer H Gamel *et al*., 2005; and (Bahado-Singh *et al*., 2006). The results revealed no significant difference ($P < 0.05$) in the GI among the test foods studied except Rp which was significantly lower in comparison to other processed meal. Roasted and baked foods have a higher GI than fried/boiled meals (www.diethealthclub.com/herbs-and-natural-cures/amaranth.html). However this present study results revealed the same that Boiled and Roasted had the highes GI value than the Popped amaranth grains.

CONCLUSION

The Popped Amaranth Grains Flour Chapattis (PAGFC) had the lowest Glycemic index of all the processed test meal, due to disruption of the fat components, qualitative changes of insoluble fiber component after heat treatment and formation of indigestible complex fiber components with protein and aminoacids and lower retrogradation of starch in popping grains. The knowledge of an effective processing method for dietary staples to control and reduce hyperglycemia is essential in the treatment of diabetes. This is because diet management is crucial to control spikes in blood glucose levels. The findings of this study were useful for health care providers and nutritionists in Diabetes Education.

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