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Glycemic response and antioxidant activity of pumpkin seed powder (*Cucurbita maxima*) blended biscuits

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Abstract
The present study aimed to evaluate glycemic response and antioxidant activity of pumpkin seed powder (*Cucurbita maxima*) blended biscuits. Refined wheat flour was replaced by pumpkin seed powder at 5, 7.5, and 10% for the preparation of biscuits. Nutrient composition and antioxidant activity were analysed for the organoleptically best accepted pumpkin seed powder incorporated biscuits. Glycemic response of best accepted pumpkin powder incorporated biscuits was investigated in normal healthy subjects administering 25g of available carbohydrates and reference food (white bread) with 4 days wash out period between test food and reference food. Serum blood glucose levels were estimated at 0, 30, 60, 90 and 120min respectively. Among different level of pumpkin seed powder incorporated biscuits, 5% incorporation was best accepted by panalists. Antioxidant activity of 5% pumpkin seed powder incorporated biscuits was 27.60 mg ascorbic acid equivalent/100g. The glycemic index of 5% pumpkin seed powder incorporated biscuits was 74.61.

Keywords: glycemic index, antioxidant activity, nutrient composition, pumpkin

Introduction
Pumpkin belongs to the family cucurbitaceae and is grown all over the world. It has been used frequently as functional food and countries like India, Sri Lanka use it mainly as a vegetable. There has been considerable attention toward the pumpkin seeds recently due to its nutritional value and the health benefits. Pumpkin seeds are generally considered as waste product and it is rich in bioactive compounds with nutraceutical properties (Montesano *et al.* 2018) [1]. In vivo experiments have been proved that natural bioactive compounds in pumpkin seeds such as carotenoids, tocopherols and sterols have wide range of biological activity on prevention of hypertension, diabetes and cancers (Yadav, 2010; Gutierrez, 2016; Dyshlyk *et al.* 2017) [2, 3, 4].
Nutritionally pumpkin seeds are rich in protein, fat, fiber and minerals. *Cucurbita maxima* seeds nutrient composition reveals 33.48% protein, 30.66% fat, 3.07% fiber, 3.98% ash and 524.58kcal energy (Habib *et al.* 2015) [5]. Most abundant fatty acid in the *Cucurbita maxima* seed is the linoleic acid. (Karanjal *et al.* 2013) [6].
Glycemic index is concept of glycemic index where it measures the glucose response in the blood after consumption of carbohydrate containing foods (Jenkins *et al.* 1981) [7]. Originally glycemic index was developed as a guide for selection of foods in diabetes subjects (Venn and Green, 2007) [8]. According to the classification, foods are categorized as low GI foods (<55), medium GI foods (55-69) and high GI foods (>70) (Brand-Miller *et al.* 2003b) [9]. This categorization is useful in the ranking of foods based on glycemic index and provides a guide to diabetes subjects to select foods for better management of blood glucose level.
Consumption of functional foods is one way to prevent diabetes. Pumpkin seeds being a nutraceutical, it can be utilized as a food fortificant in many products to enhance the nutritional value. Biscuits are consumed by all aged groups from children to adult and it has been shown that pumpkin seed powder can be successfully incorporated to biscuits (Freital *et al.* 2014; Giami *et al.* 2005) [10, 11], but no studies conducted on glycemic response. Therefore, purpose of this study was to evaluate glycemic response of pumpkin seed powder blended biscuits and its antioxidant activity.

Materials and Methodology
Preparation pumpkin seed powder
Pumpkin (*Cucurbita maxima*) was purchased from local market in Bengaluru, washed, cut and Seeds were removed. Seeds were thoroughly washed to remove yellow fibrous parts attached.

Seeds were then air dried for 30min and dried at 60℃ for 18 hours in a tray dryer. Dried seeds were ground to a fine powder and kept in air tight container at refrigerated condition (8℃) for further use.

Formulation of biscuits

Biscuits were prepared substituting refined wheat flour at 5, 7.5 and 10% with pumpkin seed powder which is presented in table 1. All the ingredients were mixed together and kneaded for 15 min till it became a soft dough. Dough was then fermented for 3 hours, made into thin sheets (0.2cm) cut into round shape biscuits (6cm diameter) using a biscuit cutter and baked at 160℃ for 15min. Biscuits were then cooled and packed in air tight containers for further analysis.

Table 1: Formulation of biscuits

Ingredients (g)	Control	Treatment 1	Treatment II	Treatment III
Refined wheat flour (g)	100	95	92.5	90
Pumpkin seed powder (g)	-	5	7.5	10
Vegetable oil (g)	24	24	24	24
Yeast (g)	1	1	1	1
Salt (g)	1.5	1.5	1.5	1.5
Sugar (g)	1	1	1	1
Water (ml)	36	36	36	36

Treatment I- 5% pumpkin seed powder blended biscuits
Treatment II- 7.5% pumpkin seed powder blended biscuits
Treatment III- 10% pumpkin seed powder blended biscuits

Sensory evaluation

Organoleptic evaluation was carried out for biscuits by using semi trained members (n=20) from the Department of Food Science and Nutrition, University of Agricultural Sciences, GKVK, Bangalore, on 9-point hedonic scale (9- like extremely, 8- like very much, 7- like moderately, 6- like slightly, 5- neither like nor dislike, 4- dislike slightly, 3- dislike moderately, 2- dislike very much, 1-dislike extremely) for appearance, colour, aroma, taste, texture and overall acceptability.

Proximate analysis

Proximate analysis was carried out for the pumpkin seed powder and best accepted biscuits by sensory evaluation among the different variations of pumpkin seed powder blended biscuits. Moisture and ash content were analysed using AOAC (1980) [12] procedures. Protein content of samples was analysed by using the Micro- Kjeldahal method. Digestion and distillation were carried out by using Gerhardt Turbotherm digestion unit and Gerhardt distillation unit (Model: Gerhardt, VAP-200, Germany). Total fat content of the samples was determined by Socxhelt apparatus (Model: SOCS Plus, T43-51-40-2L) using petroleum ether as a solvent (boiling point- 60℃-80℃). Crude fibre content of the samples were estimated with fat and moisture free samples. Fiber content was estimated using acid (H_2SO_4) and subsequent alkali (NaOH) digestion by boiling in the Gerhardt fiber bag system. Carbohydrates was calculated by using difference method. Beta-carotene was analysed by method described by Ranganna (2002) [13] and absorbance was measured using UV visible spectrophotometer at 450nm (Model: V-5000, China). Iron and zinc (mineral solutions) were estimated by using atomic absorption spectrophotometer (Model: PerkinElmer-700, Japan). All chemicals used in the study were of analytical grade and all proximate analysis was performed in triplicates.

Antioxidant activity assay (DPPH)

Antioxidant activity was determined for the best accepted biscuit by sensory evaluation.

Antioxidant activity was determined by using 2.2′-difenil-1-picrilhidrazil radical (DPPH) method (Kang and Saltviet, 2002) [14]. Ascorbic acid was used to prepare the standard curve. 0.02Mm DPPH solution was prepared and absorbance was measured at 517nm. 100µg/100ml ascorbic acid (working solution) was prepared and 0.1ml, 0.15ml, 0.2ml, 0.25ml, 0.30ml were pipetted out to test tubes with concentration of 10, 15, 20, 25, 30µg/ml. Each test tubes were added 0.3ml acetate buffer (pH=5.5), 2.5ml of 0.02Mm DPPH and total volume was taken to 3.8ml by adding 80% ethanol with required volume. Test tubes were incubated in the dark for 30min and absorbance was measures at 517nm using spectrophotometer. Each absorbance which were taken from the ascorbic acid concentrations, was deducted by 0.02Mm DPPH solution's absorbance and standard curve was plotted between concentration verses absorbance.

5g of sample was homogenized with 10ml 80% methanol (10ml×4 time). All extracted samples were pooled together, filtered and marked up to 50ml using 80% methanol. 0.2ml of extracted sample was taken to test tube and 0.3ml of acetate buffer, 0.8ml of 80% methanol and 2.5ml of DPPH were added to it and mixed vigorously. Simultaneously, blank sample was prepared using 0.3ml of acetate buffer, 1ml of 80% methanol and 2.5ml of 0.02Mm DPPH. All test tubes were incubated in dark place for 30min and absorbance was measured at 517nm. Antioxidant activity was calculated using standard curve and expressed as ascorbic acid equivalents (AAE).

Antioxidant activity (mg AAE/100g)

$$= \frac{(AB - AS) \times \text{standard value (µg)} \times \text{total volume extracted} \times 100}{\text{Assay volume} \times \text{weight of sample (g)} \times 1000}$$

Where,
AB- Absorbance of blank sample
AS- Absorbance of testing sample

Evaluation of glycemic index of biscuits

Subjects

Subjects were selected from the University of Agricultural Sciences, GKVK, Bengaluru, who were healthy and age between 20-40 years (8 females). Participant weight, height and initial fasting blood glucose level were assessed prior to the experiment. Subjects who had diabetes, cardiovascular disease or any other illness revealed on past clinical examination, were excluded. Participants were instructed to

avoid any medication and physical exertion during study period.

Preparation of test foods

The study included one test meal (5% pumpkin seed powder blended biscuits) which was best accepted by panalists through sensory evaluation and white bread as reference food. Both test food and the reference food had 25g of available carbohydrate. To get the 25g of available carbohydrates from foods, weight of test food and reference food were 42.80g and 33.50g respectively.

Experimental procedure

FAO (1998) [15] protocol was followed for an evaluation of glycemic index. Subjects were studied on separate days in the morning after 10-12 hours over night fasting and initial fasting blood glucose level was tested. Reference food was then administered to each subjects and serum blood glucose level was measured at 30 60, 90 and 120min using 28 G pricking lancets and blood glucose was estimated using in vitro diagnostic kit of the Abbott Diabetes Care Ltd, Oxon, UK. After 4 days wash out period, test food was administered to each subjects and same procedure was carried out as previous test. Blood glucose levels were plotted against time for both test food and reference food. Incremental area under the curve was calculated by using trapezoid method and Glycemic index was calculated as the area under curve of test food over that of reference food multiplying by 100.

Ethical consideration

The purpose and protocol of the study were explained to the subjects and written consent was obtained. Procedures followed in the study were in accordance with the Helsinki Declaration of 1975, as revised in 2008.

Statistical analysis

Statistical analysis was performed through the statistical software SPSS 16.0. ANOVA (Analysis of Variance) was carried out to test the significant difference between means for sensory characters where appropriate. The 't' test was used to test the significance of mean differences between the intervals of biochemical parameters. All parametric variables were expressed as mean± SD (standard deviation). $P< 0.05$ was considered as statistical significance.

Results and Discussion

Proximate composition of pumpkin seed powder the 5% pumpkin seed powder blended biscuits

Pumpkin seed powder had 4.40% moisture, 3.65% ash and high protein, fat and crude fiber content i.e. 28.93, 23.61 and 21.29% respectively. Carbohydrates content of seed powder was 37.16% whereas high iron and zinc content were observed i.e. 7.26 and 6.88mg/100g respectively. Proximate composition of the pumpkin seed powder is presented in table 2. Habib *et al*. (2015) [5] reported the proximate composition of the pumpkin seed powder, was moisture 4.06%, ash 3.80%, crude fibre 2.91%, total lipid 36.70%, total protein 34.56%. Iron and zinc content were 290 and zinc 39.9 ppm respectively. Crude fiber content was higher in the present study as whole seeds were used in the experiment. According to the Kindki (2017) [16] who evaluated the nutrient composition of *Cucurbita pepo* whole seed powder, found 18.54% crude fiber. Beta-carotene content of pumpkin seed powder was found to be 5.67mg /100g. However, Murkovic *et al.* (2002) [17] reported that beta-carotene content of different varieties of fresh *Cucurbita maxima* seeds ranged from 1.4 to 7.4mg/100g. Kim *et al*. (2012) [18] reported that pumpkin seeds of *Cucurbita moschata* variety contained 7.15 mg/kg β-carotene.

5% pumpkin seed powder blended biscuits had moisture 3.23%, ash 1.97%, protein 13.89%, crude fiber 1.40%, fat 21.02% and carbohydrates 59.89%. Kanwal *et al.* (2015) [19] evaluated 20% pumpkin seed powder supplemented cookies and nutrient composition was moisture 1.55%, protein 12.30%, fat 28.29%, ash 4.13% and fiber 1.60%. Beta-carotene content of 5% pumpkin seed supplemented biscuits was 0.51mg/100g. Ranhotra (1995) [20] reported that 30g of crackers formulated with 10%, 22% and 30% carotene sources had 0.39mg of beta-carotene and that contribution would allow labelling the product as a "good source" of vitamin A. Iron and zinc content of 5% pumpkin seed powder blended biscuits were 3.53 and 1.46mg/100g respectively.

Table 2: Proximate composition of pumpkin seed powder and the 5% pumpkin seed powder blended biscuits (100g)

Parameters	Pumpkin Seed powder	5% PSB
Moisture (g)	4.40±0.8	3.23±0.08
Ash (g)	3.65±0.09	1.97±0.05
Protein (g)	28.93±0.63	13.89±0.00
Crude fiber (g)	21.29±1.58	1.40±0.74
Fat (g)	23.61±1.14	21.02±0.50
Carbohydrate (g)	37.16±0.66	59.89±0.49
β carotene (mg)	5.67±0.04	0.51±0.005
Zinc (mg)	6.88±0.11	1.46±0.18
Iron (mg)	7.26±0.36	3.53±1.01

PSB- Pumpkin seed powder biscuits

Sensory evaluation of biscuits

Table 3 depicts the mean sensory scores for formulated biscuits with different percentage of pumpkin seed powder. Except taste, other sensory attributes, colour, appearance, aroma texture and overall acceptability were not significantly different among varied level of pumpkin seed powder incorporated biscuits. Taste was significantly different ($p<0.05$) in 5% pumpkin seed powder incorporated biscuits compared to other substitutions. 5% pumpkin seed powder incorporated biscuits scored maximum for appearance (8.31), colour (8.46), aroma (8.26), taste (8.39), texture (8.38) and overall acceptability (8.31) compared to other formulated biscuits and it was selected as the best accepted product among panalists. Beyond the 5% incorporation of pumpkin seed powder, had gradual reduction of acceptance for all the sensory attributes. Freitas *et al*. (2014) [10] reported that 48% tasters scored above 5 for the flavour of the cookies which were blended with 10% pumpkin seed powder. In another study, pumpkin seed flour containing up to 15% was accepted and low overall acceptability of cookies was found when more than the 15% was substituted Giami *et al*. (2005) [11].

Table 3: Mean sensory scores for formulated biscuits with pumpkin seed powder (n=20) PSB- Pumpkin seed powder biscuits, Different superscript letters in the same column indicates significant difference ($p<0.05$).

Products	Appearance	Colour	Aroma	Taste	Texture	Overall Acceptability
Control	7.82±1.01^a	7.94±1.02^a	7.90±0.91^a	7.53±118^a	7.76±1.30^a	7.88±0.70^a
5% PSB	8.31±0.63^a	8.46±0.52^a	8.26±0.56^a	8.39±0.51^b	8.38±0.65^a	8.31±0.30^a

7.5% PSB	8.31±0.63[a]	8.15±0.69[a]	8.05±0.59[a]	8.23±0.59[a]	8.30±0.48[a]	8.15±0.55[a]
10% PSB	8.00±0.57[a]	7.92±0.64[a]	7.82±0.74[a]	7.92±0.64[a]	8.00±0.58[a]	7.84±0.69[a]

Antioxidant activity of pumpkin seed powder blended biscuits

Antioxidant activity of 5% pumpkin seed powder blended biscuits and control biscuits were 27.60 and 11.82mg/100g ascorbic acid equivalents respectively. Antioxidant activity was increased in 5% pumpkin seed powder incorporated biscuits compared to control. Phytesterol and tocopherols are the active biological compounds found in pumpkin seeds in large quantities (Nawirska-Olszańska *et al.* 2013) [21]. Another compound which is largely contributed to the antioxidant activity of pumpkin seed is polyphenolic compounds (Parry *et al.* 2008) [22]. Nyam *et al.* (2013) [23] found that bread supplemented with 5% pumpkin had a 37.99% increase in DPPH radical scavenging activity in pumpkin seed bread as compared to control bread.

Glycemic index of pumpkin seed powder blended biscuits

Physical and clinical parameters of subjects are presented in table 4. Mean age, weight, height, and body mass index of subjects were 26.5years, 54.39kg, 160.21cm and 21.27kg/m^2 respectively. Mean fasting blood glucose level of the subjects was 91.25mg/dl.

Table 4: Physical and clinical parameters of the subjects

Subject parameters	Mean value ± SD
Age (years)	26.50±3.67
Weight (kg)	54.39±3.79
Height (cm)	160.21±4.16
Body mass index (BMI)	21.27±2.56
Fasting blood glucose (mg/dl)	91.25±3.25

SD- Standard deviation

The mean blood glucose response, mean area calculated under the blood glucose response curve and calculated glycemic index for white bread and 5% pumpkin seed powder biscuits are given in table 5. Blood glucose response of white bread and 5% pumpkin seed powder biscuits was not significantly different at 0, 30, 60, 90, 120min, but at 30min, peak blood glucose response in 5% pumpkin seed powder biscuits was reduced compared to peak glucose response in white bread. Mean area under the blood glucose level in white bread and 5% pumpkin seed powder biscuits were 918.29 and 685.14mg.min/dl respectively and no significant difference was found. But 5% pumpkin seed powder biscuits showed lower mean area than the white bread (Figure 1). Glycemic index of white bread was 100 and 5% pumpkin seed powder incorporated biscuits had 74.61, but still in the high GI range (<55-low GI, 55-69- medium GI and >70-high GI) (Venn and Green, 2007). [25]

Table 5: Mean blood glucose level, mean area under the curve and glycemic index (GI) of reference and test food. PSB- pumpkin seed powder biscuits, IAUC- Incremental area under the curve, GI- Glycemic index, Same superscript letters in the same column indicates no significant difference (p>0.05).

	Blood glucose (mg/dl)					iAUC	
Time (minutes)	0min	30min	60min	90min	120min		GI
White bread	90.71±3.68[a]	115.71±12.00[a]	96.85±11.88[a]	87.14±5.61[a]	85.14±4.45[a]	918.29±305.03[a]	100
5% PSB	91.57±6.24[a]	107.86±9.90[a]	98.85±8.11[a]	86.14±6.12[a]	87.85±6.74[a]	685.14±461.90[a]	74.61

PSB- pumpkin seed powder biscuits, IAUC- Incremental area under the curve, GI- Glycemic index, Same superscript letters in the same column indicates no significant difference (p>0.05).

Glycemic index may be influenced by several factors such as chewing of food by individuals, digestion and absorption, composition (fiber, fat and resistant starch), physical properties of the food etc… (Suzuki *et al.* 2005) [24]. Reduction of GI in pumpkin seed powder biscuits can be explained by fiber content of the biscuits. 5% pumpkin seed powder biscuits had 1.40 per cent of crude fiber so it contributes crude fiber on pumpkin seed powder biscuits. Fat and protein content of the pumpkin seed biscuits was 21.03 and 13.89% respectively (table 2). High fat content delays the gastric emptying hence reduces the digestion rate and absorption. Further, high protein content could stimulate the insulin secretion so that the glucose level in the blood will not increase rapidly (Jenkins, 2007) [25]. Susiloningsih and Nilasari (2018) [26] reported glycemic index of biscuits formulated with pedada flour (*Onneraia caseolaris*) with tuber starch based on rat model. Glycemic index of biscuits was ranged from 48.83 to 63.42. It was reported that biscuits with highest fat (15.55g) and protein (2.58g) content (pedada flour-80g and taro starch- 20g) had lowest GI (48.83) compared to other formulations. Similarly, Venn and Green (2007) [8] reported that incorporation of protein into carbohydrates can slow down the peak glucose response. Jerkins (2007) [25] reported that food high in fat and protein tend to show low GI compared to similar food with low fat and protein. Fabusiwa *et al.* (2018) [27] evaluated glycemic index of pigeon pea (PP) blended biscuits with wheat flour (WF). Glycemic index of 100g PP, 75g PP, 50g PP, 25g PP and 100g WF was 48.63, 51.67, 53.12, 54.47 and 67.01 respectively. It was observed that when pigeon pea proportion was increased, glycemic index was reduced in biscuits due to increase of fiber content.

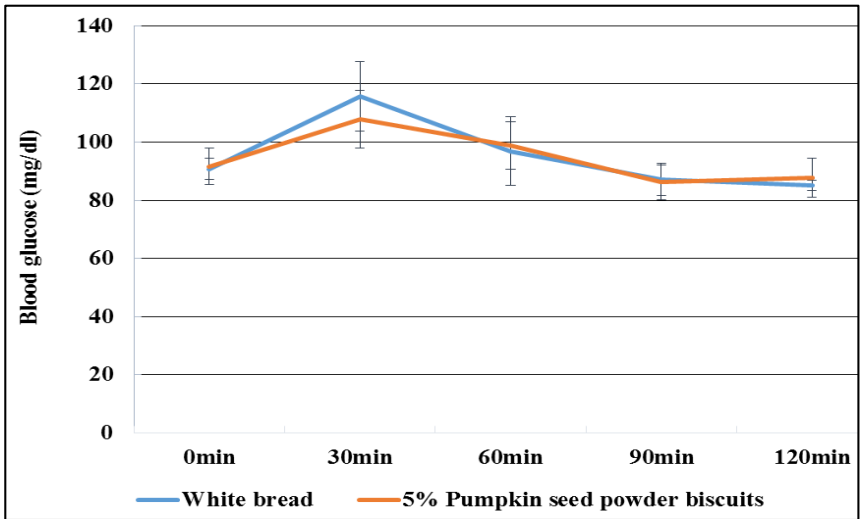

Fig 1: Mean area under the blood glucose response curve for white bread and 5% pumpkin seed powder biscuits

Conclusion

From above results, it was observed that pumpkin seed powder was rich in protein, fat, fiber, bet-carotene, iron and zinc. Supplementation of pumpkin seed powder up to 5% was acceptable and biscuits made with 5% pumpkin seed powder exhibited increased antioxidant activity compared to control. Peak glucose response of 5% pumpkin seed powder blended biscuits was reduced compared to white bread peak glucose response. However, further research into higher incorporation levels are recommended. Thus, it can be concluded that pumpkin seed power supplemented products should be promoted among individuals due its nutritional and health benefits.

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