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Glycemic Indices of Kale Leaves Based Chakli and Twisters on Normal Human Subjects

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Abstract: ***The aim of present study was to develop kale based food products by utilizing its powder as an ingredient and to determine glycemic index and glycemic load on normal human subjects through IAUC (Incremental Area Under Curve) method. The food products (chakli and twisters) were developed by incorporation of different proportioned at 5 %, 10%, 15% and 20% of kale powder.The products 5% kale chakli and 10% kale twisters were most acceptable by sensory panel while comparing to other variants. Further study was conducted on 30 normal healthy human subjects who were fed best selected test products and anticipated for fasting and post prandial blood glucose level at different intervals (0, 30, 60, 90 and 120 min). The data indicated that blood glucose level of test kale chakli and twisters were significant increased (P ≤0.05) by 14% and 8% respectively at 60 min while these values were significantly decreased by 21% and 7% respectively at 120 min when compared with 60 min. The IAUC value was low in both 5 & 10% kale chakli and twisters (1612.2 and 1544.2 mg.min/100ml) when compared to control (3324.5 mg.min/100ml).The data revealed that GI (46.44) and GL (14.5) were found to be low in 10% kale twisters when compared to 5% kale chakli which belong to low GI and moderate GL category. Thus, kale possesses nutritional and health benefits which could be competently used in the management of diabetes mellitus.***

Keywords: Kale, Glycemic Response, Glycemic Index, Glycemic Load, Incremental Area Under Curves, Diabetes Mellitus

1. Introduction

Functional food which affects beneficially one or more target roles in the body, beyond sufficient nutritional effects, in a way that is relevant to either improved state of health and/or reduction of risk of various diseases [1]. Clinical trials have shown that diets with low Glycemic Index (GI) improve glycemic control in diabetes, increase insulin sensitivity, reduce body weight, influence memory, and may reduce serum cholesterol level. Currently, there is a great deal of interest in the promising health benefits of low GI foods which was originally designed for people with diabetes as a guide to food selection, advice being given to select foods with a low GI [2]. The GI concept has been extended to also take into account the effect of the total amount of carbohydrate consumed glycemic load, a product of and quantity of carbohydrate eaten provides an indication of glucose available for energy or storage following a carbohydrate containing meal. Properly determined GI values for individual foods have been used successfully to predict the glycemic response of a meal [3, 4].

Green leafy vegetables are considered essential for well-balanced diets since they supply vitamins, minerals, dietary fiber and various bioactive components [5].In the daily diet vegetables have been strongly associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer [6].In India, various underutilized leafy vegetables are available seasonally but are not exploit to the extent they should be although their high nutritive value [7].

Kale (*Brassica oleracea*) is underutilized leafy vegetable belonging to the cabbage family *Brassicaceae* that contains a large amount of health-promoting phytochemicals [8]. It is one of the most nutritious vegetables particularly rich in dietary fiber, protein, minerals and antioxidant compounds [9]. Kale is among all leafy vegetables that contain measurable and negligible amounts of oxalates (0.02 mg/100 g)[10].It is a non-starchy vegetable and can be a brilliant addition to a diabetes meal plan, adding fiber and many other nutraceutical benefits [11]. New research findings in this area indicate the potential value of kale has good nutritional and nutraceutical profile but its potential role as low GI food has remained unrealized and unexploited in prevention of metabolic disorders. Thus, the present study has an attempt to investigate the effect of low GI products based on kale powder on blood glucose in healthy human subjects.

2. Methods and Materials

Sample Collection

Kale leaves were collected in month of November-December and identified by the scientist of krishi Vigyan Kendra (KVK) of Banasthali University, Rajasthan, India. Then, leaves were washed and sun shade dried after which they was reduce to fine powder by grinding and packed in air container and stored at refrigeration temperature (5 $^{\circ}$C) for further study .

Product Development and Sensory Evaluation

The products (Chakli and Twisters) were developed with kale leaves powder in different proportioned at 5%, 10%, 15% and 20% levels. The sensory evaluation was carried out by 25 semi-trained panel in order to get their response for all sensory attributes (color, appearance, texture, taste and after taste) of kale based products which were compared with their standard product**.** All sensory characteristics were analyzed by five point composite score whereas overall acceptability of the product was appraised by structured hedonic scale [12, 13]. The best selected products were taken for further investigated for glycemic response on normal human subjects.

Study Subjects

Thirty normal human subjects of the age 20-24 y, height157.62±4.14 cm, weight 55.36±4.72kg and BMI 22.30±1.98 (kg/m^2) were randomly selected from PG hostel of Banasthali University campus. The purpose of the study was explained to each subject and consent to participate in the study was taken. The subjects were given general instructions to avoid any physical exertion, medication, fasts and feasts during the experimental period.

Assessment of Glycemic Response, Glycemic Index and Glycemic Load

On the first day of the study, glucose tolerance test (GTT) was conducted on overnight fasted subjects. A 50g glucose dissolved in 200ml water was given to the subjects. The subjects were instructed to finish the glucose solution within 15 min and to avoid physical exertion during the experimental period. The blood glucose level was measured at 0, 30, 60, 90 and120 min with the help of a glucometer using glucostix which is based on the action of glucose oxidase. The tests on the reference food should be repeated two times in order to reduce the variability within the subjects [14]. The food products Chakli (5%) and Twisters (10%) based on kale powder were evaluated in two consecutive days. The subjects were asked to follow the same instructions as for the glucose tolerance test. The incremental area under the blood glucose curve is calculated for each blood glucose response curve geometrically by the trapezoid rule, disregarding the area below baseline using methods described by Wolever and others [15]. Briefly, areas below baseline values were subtracted from total area under the curve. If blood glucose values fell below baseline, IAUC at those data points were also subtracted from the total.

$$\frac{At}{2} + At + \frac{(B-A)t}{2} + Bt\,\frac{(D-C)}{2} + Dt + \frac{(E-D)t}{2} + Et \ldots\ldots\ldots\ldots \text{etc}$$

Where A, B, C, D and E represent positive blood glucose increments; t is the time interval between blood samples. If the blood glucose increments D is positive (i.e. greater than baseline) only the area between D and E above the baseline is included. The Glycemic indexes (GI) of best selected products were determined by feeding the healthy subjects. The GI of each food was expressed as % mean glucose response of the test food divided by the standard food taken by the same subject and was determined by the following formula: GI=IAUC of Food IAUC of Glucose/IAUC of test product ×100 The glycemic load was calculated based on the quantity of the product per serving and the respective available carbohydrate content. GL=Available Carbohydrate (g) × Glycemic Index (GI) x100.

Statistical analysis

All data were triplicate and expressed as Mean ± Standard Error of Mean. The results of the study were statistically analyzed to ascertain its significance. The analytical data obtained for kale leaves powder based products were subjected to paired t-test for significant difference at P≤0.05 level

3. Results and Discussion

Table 1: 5 Point Composite Scores for Various Sensory Attributes of Product 1: Chakli.

Attributes	Standard	VK1	VK2	VK3	VK4
Color	4.7±0.48	4.4±0.70^{ns}	3.2±0.33	3.7±0.83	3.4±0.83
Appearance	4.8±0.70	4.7±0.59 ^{ns}	3.1±0.73	4.4±0.79 ^{ns}	3.0±0.59
Texture	4.6±0.63	4.4±0.73 ^{ns}	3.7±0.06	3.0±0.65	3.1±0.51
Taste	4.5±0.51	4.2±0.35 ^{ns}	4.0±0.61 ^{ns}	2.3±0.81	3.3±0.59
After taste	4.5±0.48	4.2±0.45 ^{ns}	4.0±0.61 ^{ns}	3.0±0.79	3.9±0.67

Table 2: 5 Point Composite Scores for Various Sensory Attributes of Product 2: Twisters.

Attributes	Standard	VK1	VK2	VK3	VK4
Color	4.4±0.48	3.4±0.70	4.2±0.33 ^{ns}	3.4±0.83	2.5±0.83
Appearance	4.8±0.70	3.5±0.59	4.6±0.73 ^{ns}	4.1±0.79	2.0±0.59
Texture	4.9±0.63	4.2±0.73 ^{ns}	4.8±0.06 ^{ns}	4.5±0.65 ^{ns}	2.1±0.51
Taste	4.7±0.51	4.1±0.35	4.2±0.61 ^{ns}	3.9±0.81	2.4±0.59
After taste	4.8±0.48	4.1±0.45	4.3±0.61 ^{ns}	3.5±0.79	2.9±0.67

Data are reported as Mean±SEM group of 15 panel members each. S = Standard; Variants: VK_1, VK_2

VK_3 and VK_4 = Incorporated with 5%, 10%, 15% , 20% kale leaves powder; ns-non significant at P≤ 0.05

The products chakli and twisters were prepared by incorporating kale leaves powder in four different proportioned i.e. VKI (5%), VK2 (10%), VK3 (15%) and VK4 (20%) respectively and these test products were compared with their standard products. The result stated that Mean ± SEM for all the attributes (colour, appearance, texture, taste and after taste). The mean score secured for all the attributes of test chakli were ranged from 2.13±0.06 to 4.8±0.48. The data revealed that 5% of kale incorporated chakli (VK1) were insignificant (P ≤0.05) in all sensory attributes when compared to standard while other variants were not comparable to standard and showed significant value. In other product twisters, the mean score secured were ranged from 2.0±0.59 to 4.9±0.63 for all attributes and found that 10% twisters were highly acceptable when compared with other variants as shown in table 1 and 2.

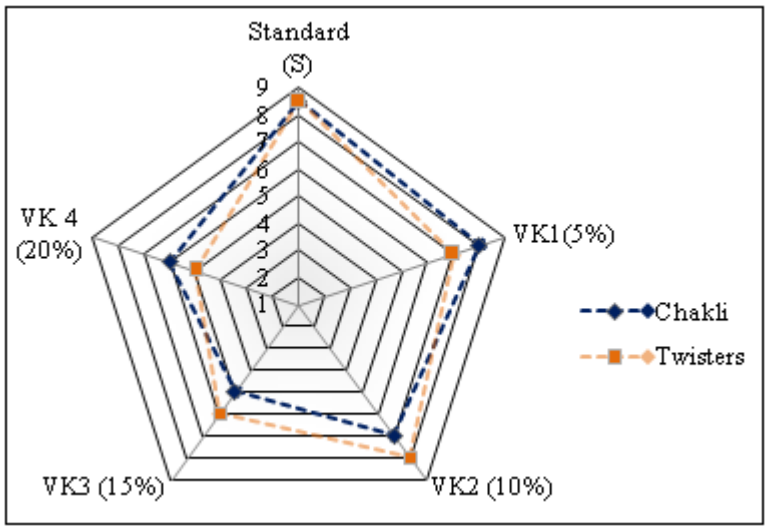

Figure 1: Overall Acceptability Evaluation of Chakli and Twisters Based on Kale Leaves Powder

Overall acceptability assessment of test and standard chakli and twisters were summarized in fig 1. Although food products scored higher than 8 (like very much) for the product expected for VK3 and VK4 (15% and 20% kale) and sensory analysis revealed that products VK1 (5% chakli) and VK2 (10% twisters) incorporated kale powder based products were comparable to corresponding standard product. Among all products, chakli 5% and Twisters 10% incorporated kale leaves powder were most acceptable by the panel. It can be visualized from figure 1 that VK3and VK4 had lower value for overall acceptability which indicates that they were not comparable to standard. The data also depicted that VK2 twisters with 10% incorporation of kale leaves was most acceptable, had higher value i.e. 8.0 ± 0.47.

4. Glycemic response of kale based products

Sample Selection

This study was conducted on 30 normal female subjects of age group 22-23 years recruited from hostel of Banasthali University. The Mean ± SD of weight, height and BMI of the subjects were (55.36±4.72) kg, (157.62±4.14) cm and (22.30±1.98) kg/m^2 respectively and other parameters were taken care that are listed in the table 3. All subjects for the investigation fasted overnight. Their blood samples were collected through finger prick using a hypodermic needle. 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 10 seconds. The determination of blood glucose level was done at intervals i.e. 0 (fasting level), 30min, 60min, 90min, 120min.

Table 3: Demographic Profile of Selected Healthy Normal Subjects

Parameter	Mean±SD	Reference range
N	30	-
Age (years)	22-23	20-30
Weight (kg)	55.36±4.72	<57
Height (cm)	157.62±4.14	-
BMI (kg/m^2)	22.30±1.98	<25
Pulse rate (min^{-1})	76.4±3.16	60-90
Blood Pressure Systolic (mmHg)	121.4±3.28	<130
Blood Pressure Diastolic (mmHg)	74.1±2.21	<85
HbA1c (%)	5.5±0.07	4.4-6.0

Table 4: Blood Glucose Level of Control, Chakli and Twisters Based on Kale Leaves Powder

Groups	0 min	30 min	60 min	90 min	120 min	IUAC (mg.min/100ml)	GI	GL
Control (Glucose)	98±0.89	113±0.23	158±0.56*	130±0.45	115±0.43*	3324.5		
Chakli (5% kale)	99±0.54	110±0.12	127±0.78*	116±0.55	105±0.59*	1544.2	48.86	16.9
Twisters (10% kale)	97±0.23	101±0.26	105±0.67*	100±0.98	98±0.76*	1612.2	46.44	14.5

*significant difference on 0 min v/s 60 min and 60 min v/s 120 min at p<0.05 level
GI: Glycemic index; GL: Glycemic load; IAUC: Increment Area Under Curve

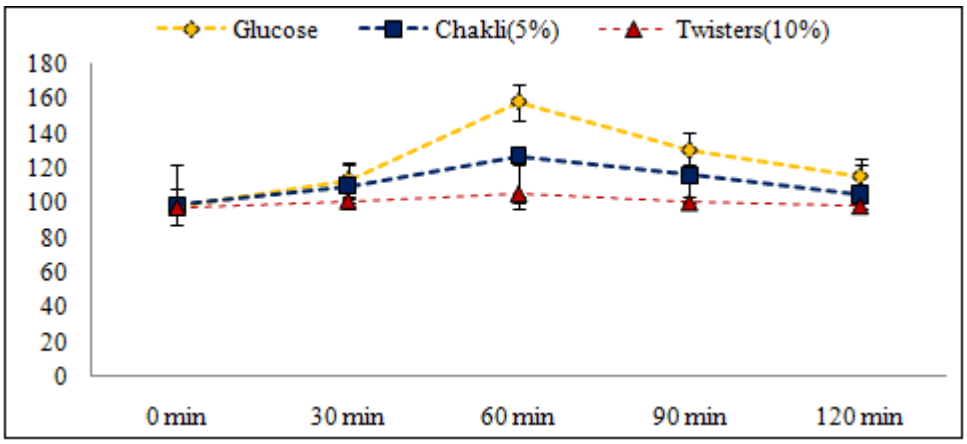

Figure 2: Mean Blood-Glucose Curves (Glucometer Optium) For Control (Glucose), Chakli (5%) and Twisters (10%) Based on Kale Leaves Powder on Healthy Normal Subjects

Multitude of empirical investigation has proved that different carbohydrates exert different effects of glucose absorption and metabolism. Absorption pattern of carbohydrate is reflected by difference in glycemic response. GI of a food is considered a useful indicator for its suitability in diabetic diet and is helpful in lowering the fat and increasing the fiber content of the diet [16]. Comparisons of postprandial glucose responses for test products; 5% chakli and 10% twisters with control (50 g glucose powder dissolve in 200ml water) are summarized in Table 4 and Fig 2. The results revealed that there was a significant peak rise in the blood glucose level at 60 min after consumption of test products chakli (127±0.78 mg/100ml) by 14% and twisters (105±0.67 mg/100 ml) by 8 % when compared with their fasting blood glucose 99±0.54 and 97±0.23 mg/100ml respectively whereas the values were significantly declined P≤0.05 level by 21% and 7% of both test products at 120 min when compared to 60 min value. Similar data was observed by Chaturvedi and others that dal samose, and bati incorporated with 10% leaves and 5% bark of *Ficus religiosa* were found to be lower GI and GL for dal samose (35 & 13) when compared to bati (53 &20) [17].

The IUAC for control (glucose), 5% chakli and 10% twisters were 3324.5, 1612.2 and 1544.2 mg.min/100ml respectively. The data depicts that the both test chakli and twisters had lower IAUC values than control. The GI of twisters and Chakli were 46.44 and 48.86 which falls under the low GI category, whereas the GL were 14.5 and 16.9 which falls under the medium category as represented in fig 3 The data also depicts that 10% twisters have lower GI and GL than 5% chakli which implies that additional kale powder fortification may lower the GI and GL values. The following cut-off limits between low, middle and high GI have been proposed: <55% (low), 55-70% (medium), >70% (high). There is a scale to classify glycemic load (GL) similar to that of GI that are Low≤10, medium 11-19 and high ≥20 [18].

Thus, the present study demonstrated that recipe were low GI and moderate GL nutritious and acceptable food product prepared from kale leaves powder used as effective supportive therapy in the treatment of diabetes mellitus.

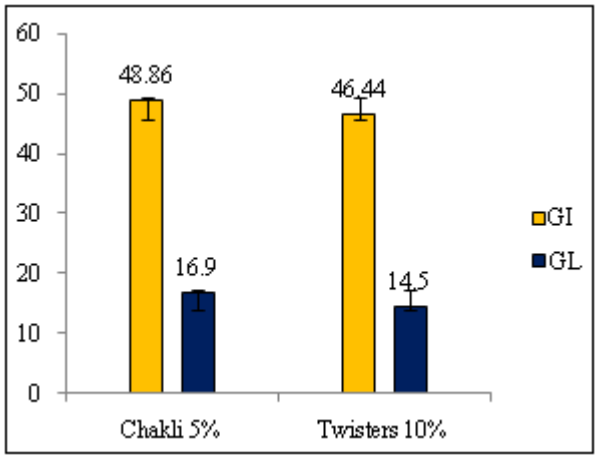

Figure 3: GI and GL of Chakli and Twisters Incorporated by Kale Leaves Powder.

5. Conclusion

Kale leaves can be used as a functional ingredient to produce low glycemic food products with favorable sensory characteristics. The results data support the use of kale powder as a tool for healthy and nutritious products which had low glycemic index and medium glycemic load that help in prevention and management of Type II Diabetes. In view of the present study, more research work could be done on human subjects so that green leafy vegetable with multifarious effects would be available in the future market. It is recommended that the kale leaves can be successfully utilized for the cure of diabetes and related diseases due to their hypoglycemic action

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