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Biochemical Characterisation and Dietary Fibre Analysis of Sugar Beet Supplemented Cookies

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Abstract. This study was planned to utilize sugar beet powder as a rich source of dietary fibre in cookies. Purposely, five treatments namely T_1, T_2, T_3, T_4 and T_5 with 4%, 8%, 12%, 16% and 20% sugar beet powder addition in wheat flour were chosen to estimate fibre, antioxidant profiling and engineering properties of cookies. Results showed an increased content of all above mentioned parameters. With the increment in sugar beet powder addition in treatments, dietary fibre analysis have shown that total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) have depicted increasing trend with maximum for T_5 for all dietary fibre types. Significant results were obtained for *in vitro* antioxidant studies including total phenolic content (TPC) and DPPH that showed increasing trend with T_1 0.6 mg GAE/g and maximum values for T_5 with 2.0 mg GAE/g for TPC and for DPPH with T_5 being maximum value of 1.7% and minimum for T_1 with 1.3%. T_5 treatment with 20% sugar beet gave best physicochemical results but disturbed sensory properties while T_3 with 12% sugar beet powder showed good physicochemical and sensory characteristics. Therefore, T_3 with 12% level is considered as the best source of dietary fibre in bakery products and can be considered as the prospective choice to address metabolic syndromes.

Keywords: sugar beet, dietary fibre, biochemical characterisation, cookies, sensory analysis

Introduction

Changing dietary patterns have led to preparation of certain health endorsing foods. Amongst, dietary fibre holds paramount importance in addressing various lifestyle related disorders.

In this regard people put their health at stake and adopt such dietary patterns that are health deteriorating leading to various ailments. In this regard they use junk foods that are deficient in dietary fibres. Therefore, to fulfill this need, sugar beet can be used as a good dietary fibre provider.

Sugar beet (*Beta vulgaris*) a good source of energy, nutrition and dietary fibre belongs to the family Chenopodiaceae (Ahmad *et al*., 2012). It has many varieties of different shapes and colours. Sugar beet has a characteristic barn, silage like, musty or strong earthy odour because of two main compounds namely: geosmin (tarns-l,10-dimemyl-trans-(9)-decalol) and 2-methoxy-3-sec-butylpyrazine (Lu *et al*., 2003). It is a potential source of phenolic and potent antioxidant compound with significant amount of various vital phenolic acids (Vulic *et al*., 2012) with chlorogenic, gallic, gentisic, ferulic and coumeric acids (Belal, 2007; Sakac *et al*., 2004; Brand-Williams *et al*., 1995). Ferulic acid, a potent antioxidant is much higher in sugar beet as compared to wheat flour and wheat bran.

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It is a good source of minerals and vitamins especially; vitamin A, C, folate, potassium, sodium, magnesium, calcium, iron, copper and zinc (Skrbic *et al*., 2010). It contains betaine along with folic acid, vitamin B_{12} and vitamin B_6 which helps to reduce higher levels of homocysteine (Schnyder *et al*., 2002). Sugar beet is also high in bio-flavonoids and has half sweetness than sucrose therefore more suitable for hyperglycemic patients (Vulic *et al*., 2012).

Almost 70% total dietary fibre of sugar beet composed of 1/3rd soluble fibre and 2/3rd insoluble fibre (Filipovic *et al*., 2007). Being a good source of dietary fibre it reduces the risk of cancers, obesity, diabetes, gallstone formation and heart diseases; prevents and treats constipation and cures diverticulitis (Ahmad *et al*., 2012). Besides providing health benefits, it also performs certain functional properties in food products i.e., it can act as antioxidant, anticaking, binding, bulking, dispersing, thickening, stabilizing and texturizing agent in various food products like patties, sauces, sausages, snacks, fruit fillings, yogurt, beverages and bakery (Ralet *et al*., 2009; Thibault *et al*., 2001).

Due to its various health benefits there is an increasing demand of dietary fibre in food products. Recommendations for daily dietary fibre (DF) intake vary from country to country with a range 18-38 g/day, of which 38 g/day is for men and 26 g/day is recommended for women (Rodriguez *et al*., 2006). Based on 2010 con-sumption data, sugar beet fibre consumption by humans was about 97522.35 kg of which about 29545 kg approx. was consumed in the form of baked goods such as bread, cakes and cookies while, 59090 kg was consumed in health products in the form of fibre tablets (Galisteo *et al*., 2008).

Fibre from sugar beet can be better utilised in bakery products by making its composite with wheat flour. In bakery products cookies have been recommended as a better utilisation of composite flour than bread because of their ready to eat form, excellent eating quality, and greater consumption and extended shelf life (Okpala and Chinyelu, 2011; Piga *et al*., 2005). Since cookies are usually prepared from wheat flour with about 72% extraction rate i.e., without bran and germ which causes low dietary fibre content (<2.5%), low protein content (7 to 10%) lacking certain essential amino acids (lysine, tryptophan and threonine), low vitamin and mineral content of cookies (McWatters *et al*., 2003).

The main objective to produce composite flour is to acquire a product which is better than its individual components, improved performances and economy. Composite flour knowledge has been used as a tool for extending short supplies of wheat and corn in the formulation of baked products (Okpala and Chinyelu, 2011; Piga *et al*., 2005).

In the present study, sugar beet powder is being used as a fibre source in cookies formulation. For this purpose its powder was replaced by wheat flour at various substitution levels to produce composite flour. The research of Thibault *et al*. (2001) indicated that sugar beet fibre with its odourless and colourless properties has potential to be used as a dietary fibre source in cookies but exceeding its level from 16% disturbed sensory properties of cookies. Various studies indicated that more than 20% addition of sugar beet alters the rheological properties of bakery products. This composite flour is being used in cookies preparation with the objective of better nutrition and a fibre enriched end product with excellent overall acceptability.

Materials and Methods

Procurement of raw material. Commercially available varieties of sugar beet were purchased from Ayub Agricultural Research Institute (AARI), Faisalabad. Chemicals were purchased from Sigma Aldrich and local market.

Preparation of sugar beet powder. Sugar beets were washed to remove adhering contaminants followed by peeling and cossette preparation. The cossettes were dried by dehydrator as described by Filipovic *et al*. (2007) at 30-35 °C for 24 h and coarsely powdered by grinder, sieved and then stored in polythene bags.

Chemical analyses. The commercial white wheat flour and sugar beet powder were tested for their proximate analyses according to AACC (2000) i.e., moisture content with method No. 44-15A, crude protein with method No. 46-10, crude fat 30-10, crude fibre method No. 32-10 and ash content with method No. 08-01.

Dietary fibre analyses. The sugar beet powder and the cookies were analysed for total dietary fibre, soluble dietary fibre and insoluble dietary fibre according to AACC (2000) as mentioned and described below:

Total dietary fibre (TDF). The sugar beet powder and the cookies were analysed for total dietary fibre according to AACC (2000) method No. 32-05. The sample was dispersed in a buffer solution and incubated with heat-stable α-amylase at 95-100 °C for 40 min. After cooling the samples up to 60 °C, these contents were incubated at 60 °C for 30 min with addition of 100 µL protease solutions. Finally contents were incubated with amyloglucosidase enzyme at 60 °C for 30 min. Total dietary fibre was precipitated with the addition of ethyl alcohol in 1:4 ratio. The contents were filtered and washed with ethyl alcohol and acetone. A blank sample was run throughout entire method with samples to determine any contribution from reagents to residue. The TDF was calculated by the following formula:

$$(\%)\ \text{TDF} = \frac{\text{Residue wt - protein - ash - blank}}{\text{Sample weight}} \times 100$$

Soluble dietary fibre (SDF). The samples were investigated for soluble dietary fibre by following the method as given in AACC (2000) method No. 32-07, by utilising Megazyme assay kit (Megazyme, Ireland). The samples were dispersed in buffer solution and incubated with heat stable α–amylase at 95-100 °C for 35 min. After cooling, samples were again incubated with addition of 100 µL protease solution at a temperature of 60 °C for 30 min. Finally, the residue was incubated with amyloglucosidase at a temperature of 60 °C for 30 min. After filtration, residue was washed and rinsed with 10 mL distilled water. The filtrate was weighed and soluble dietary fibre (SDF) was precipitated with four volumes of ethyl-alcohol. The contents were filtered, dried and analysed for protein and ash content. A blank sample was also run through entire protocol along with samples to observe any contribution from reagents to residues. The soluble dietary fibre was calculated with the following expression:

$$(\%)\ \text{SDF} = \frac{\text{Residue wt - protein - ash - blank}}{\text{Sample weight}} \times 100$$

Insoluble dietary fibre (IDF). Insoluble dietary fibre (IDF) in different samples was estimated by using the method as mentioned in AACC (2000) method No. 32-20. The samples were dispersed in a buffer solution and incubated with heat stable α-amylase at 95-100 °C for 35 min. After cooling, contents were again incubated with addition of 100 µL protease enzyme at 60 °C for 30 min and then the contents were incubated with amyloglucosidase enzyme at 60 °C for 30 min. After filtration, remaining material was washed and rinsed with 10 mL water. The resultant residue was weighed and insoluble dietary fibre was precipitated with four volume of ethyl-alcohol. The contents were filtered, dried and corrected for protein and ash content. A blank was also run through same method to measure any contribution from reagents to residue. The IDF was calculated by the following expression:

$$(\%)\ \text{IDF} = \frac{\text{Residue wt - protein - ash - blank}}{\text{Sample weight}} \times 100$$

Antioxidant potential of sugar beet. Antioxidant potential of sugar beet was determined by following their respective methods. The free phenolic acids in sugar beet powder were extracted using the method described by Sakac *et al*. (2010). Sugar beet powder 10 g was mixed with 100 mL of 96% ethanol. This mixture was shaken at room temperature for 1 h using orbital shaker. Extract was filtered through Whatmann #1 filter paper and then dried. Dried extract was again dissolved in 96% ethanol up to 10 mL final volume using distilled water. Extracted contents were used for the determination of total phenolic content and radical scavenging activity using DPPH method.

Determination of total phenolic content (TPC). The total phenolic compounds in sugar beet powder and cookies were estimated by some modifications in the Folin-Ciocalteu method (FCM) described by Sakac *et al*. (2010) and Ainsworth and Gillespie (2007). From a known concentration of the sample solution, 125 µL sample was taken in test tube. Then 500 µL distilled water was added in it. After that 125 µL of Folin-Ciocalteu reagent was added in it and given a standing time of almost 6 min. Then 1.25 mL of 7% sodium carbonate was added in it. Final volume was made 3 mL by adding 1 mL of distilled water and given 90 min for completion of reaction.

Absorbance of the samples was measured in triplicate at 750 nm using a UV-Vis spectrophotometer (IRMECO Germany). Gallic acid was run as a standard along with the samples and its absorbance was also taken at 750 nm. TPC was calculated by the following formula:

$$C = c \times V/m$$

where:

C = total contents of phenolic compounds (mg/GAE/g)
c = concentration of gallic acid (mg/mL)
V = the volume of the extract
m = weight of extract (g)

Radical scavenging activity by using DPPH method. The antioxidant activity of ethanolic extract of sugar beet powder and cookies was determined based on the radical scavenging ability in reacting with a stable DPPH free radical (Mohdaly *et al*., 2010; Sakac *et al*., 2010). In this method, 4 mg of DPPH was dissolved in 100 mL methanol and 2 mL of this solution was added to 50 µL ethanolic extract. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 60 min. Then the absorbance was measured at 517 nm against blank. The radical scavenging percentage was calculated using the following equation:

$$\text{Reduction of absorbance (\%)} = [(AB - AA) / AB] \times 100$$

where:

AB = absorbance of blank sample (t = 0 min)
AA = absorbance of tested extract solution (t = 15 min)

Cookies preparation. Cookies were prepared according to the treatment plan shown in Table 1 by following the method no. 10-54 as mentioned in AACC (2000) from wheat flour–sugar beet powder composite flour using different percentages as described below:

> T_0 = 100% wheat flour; T_1 = 96% wheat flour + 4% sugar beet powder; T_2 = 92% wheat flour + 8% sugar beet powder; T_3 = 88% wheat flour + 12% sugar beet powder; T_4 = 84% wheat flour + 16% sugar beet powder; T_5 = 80% wheat flour + 20% sugar beet powder

Sensory evaluation of cookies. Cookies were analysed according to the procedure described by Lawless and Heymann (2010).

Statistical analysis. The collected data was statistically analysed according to the procedure described by Montogomery *et al*. (2008). The design applied on the data obtained is completely randomized design (CRD).

Table 1. Treatments used for the product development

Treatments	Wheat flour (%)	Sugar beet powder (%)
T_0	100	-
T_1	96	04
T_2	92	08
T_3	88	12
T_4	84	16
T_5	80	20

Results and Discussion

The present project was designed to investigate various physicochemical attributes of sugar beet powder. The main intention of present project was to optimize sugar beet powder quantity as a potential fibre source in cookies production and evaluation of sugar beet fibre cookies for sensory, physical and quality parameters.

Chemical composition of wheat flour. The proximate composition of wheat flour and of sugar beet powder is shown in Table 2. The results pertaining proximate composition of wheat flour revealed that the white flour contained 11% moisture, 1.4% total ash, 10% crude protein, 2.5% crude fat, 0.5% crude fibre and 73.2% NFE content. The results obtained in the present study for the analyses of wheat flour are in close agreement with Ahmad *et al*. (2009), who analysed different wheat varieties and observed 12.5 to 14.6% moisture, 8.23 to 12.71% protein, 1.17 to 1.59% fat, 0.42 to 0.76% crude fibre and 0.42 to 0.66% ash content. Kendler (2006) also analysed wheat varieties for their proximate composition and found that values ranged from 12.49 to 13.27% for moisture, 10.84 to 11.98 % for protein, 0.68 to 0.96 % for crude fibre and 0.40 to 0.58 % for ash contents.

The proximate analysis of sugar beet powder revealed that it contained 5.1% moisture, 5.0% ash, 6.1% crude protein, 0.8% crude fat, 9.0% crude fibre and 74% NFE content. The results obtained in the present study for the proximate analysis of sugar beet powder are in accordance to the findings of McWatters *et al*. (2003) who observed 6.8% crude protein, 0.6% crude fat and 5.5% ash content, with previous study of Westenhoefer (2001) who analysed 4.6% moisture content in sugar beet and also in close agreement with previous study of Belal (2007) who found 12.73 and 18.7% crude fibre in different sugar beet varieties, respectively.

Total dietary fibre, soluble dietary fibre and insoluble dietary fibre content in sugar beet powder was 31%, 10% and 21%, respectively as shown in Table 2. These findings were in consistence with the outcomes of other researchers (Sakac *et al*., 2010; Filipovic *et al*., 2007). Total phenolic content of sugar beet powder was expressed in terms of gallic acid equivalent (GAE) per gram of sugar beet powder from which the extract was obtained and was found to be 1.18 mg GAE/g. The values of total phenolic content in this study are in accordance with the values as observed by Mohdaly *et al*. (2010). DPPH radical scavenging activity of sugar beet powder was expressed in dry weight percentage

Table 2. Chemical composition of commercial white flour and sugar beet powder

Characteristics	Commercial white flour	Sugar beet powder
Moisture (%)	11.0 ± 0.1	5.10 ± 0.1
Crude protein (%)	10.0 ± 0.2	6.10 ± 0.4
Ash (%)	1.40 ± 0.1	5.00 ± 0.6
Crude fibre (%)	0.50 ± 0.1	9.00 ± 0.1
Crude fat (%)	2.50 ± 0.2	0.80 ± 0.1
NFE (%)	73.2 ± 0.3	74.0 ± 0.6
TPC (mgGAE/g)	-	1.18 ± 0.8
DPPH (%)	-	79.1 ± 0.2
TDF (%)	-	31.0 ± 0.1
SDF (%)	-	10.0 ± 0.6
IDF (%)	-	21.0 ± 0.6

NFE = nitrogen free extract; TPC = total phenolic content; TDF = total dietary fibre; SDF = soluble dietary fibre; IDF = insoluble dietary fibre.

of sugar beet powder from which the extract was obtained and was found to be 79.1%. The values of DPPH in this study are in accordance with the previous studies by Sakac *et al*. (2004). These are presented in Table 3 and Fig. 1.

Dietary fibre content in wheat-sugar beet powder cookies. Total, soluble and insoluble dietary fibre content is presented in Table 4 and graphically expressed in Fig. 2. The values for total dietary fibre content of the cookies prepared from wheat-sugar beet powder composite flour (4-20%) were ranged from 0.33-6.27%. Insoluble and soluble dietary fibre content were ranged from 0.20-4.07 and 0.13-2.20%, respectively. The highest values were shown by T_5 at 20% level and lowest by T_1 at 4% level. The values for total, soluble and insoluble dietary fibre content in different types of wheat-sugar beet powder cookies were close to the findings of Rodriguez *et al*. (2006).

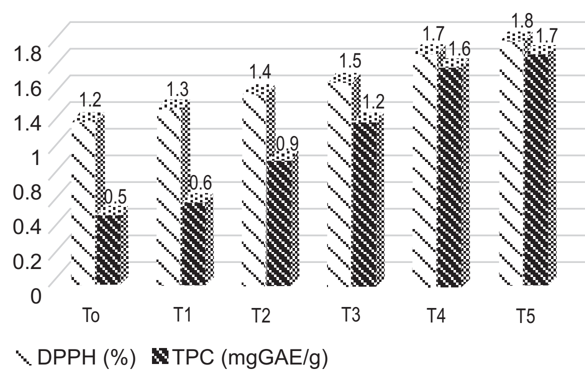

Fig. 1. Antioxidant profiling of sugar beet supplemented cookies.

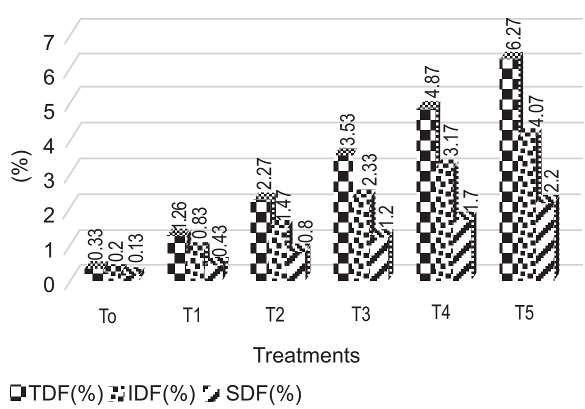

Fig. 2. Dietary fibre analysis of sugar beet supplemented cookies.

Total phenolic content and DPPH in wheat-sugar beet powder cookies. Total phenolic content and DPPH radical scavenging effect of sugar beet powder is shown in Table 3.

The values for TPC (mg GAE/g) and DPPH (%) of the cookies prepared from wheat-sugar beet powder composite flour (4-20%) were ranged from 0.6-2 mg GAE/g and 1.3-1.7%, respectively. The highest value of TPC and DPPH was shown by T_5 with a mean of 2 mg GAE/g and 1.7% at 20% level and lowest by T_1 with a mean of 0.6 mg GAE/g and 1.3% at 4% level, respectively. The values for TPC (mg GAE/g) and DPPH (%) in different types of wheat-sugar beet powder cookies are in accordance with previous study (Sakac *et al*., 2004).

Sensory evaluation of cookies. Sensory evaluation of cookies was conducted for different sensory attributes like colour, crispiness, flavour, taste, texture and overall acceptability. The sensory scores for all the above mentioned sensory attributes are shown in Table 5 and graphically expressed in Fig. 3. The scores were decreased highly significantly with increased substitution level of sugar beet powder in wheat-sugar beet powder composite flour cookies. The values of each parameter for sensorial characteristics were highest for T_3 (12%) treatment and

Table 3. Antioxidant profiling of sugar beet supplemented cookies

Treatments	DPPH (%)	TPC (mgGAE/g)
T_0	$1.2^{f} \pm 0.01$	$0.5^{f} \pm 0.1$
T_1	$1.3^{e} \pm 0.01$	$0.6^{e} \pm 0.1$
T_2	$1.4^{d} \pm 0.01$	$0.9^{d} \pm 0.1$
T_3	$1.5^{c} \pm 0.01$	$1.2^{c} \pm 0.1$
T_4	$1.6^{b} \pm 0.01$	$1.6^{b} \pm 0.1$
T_5	$1.7^{a} \pm 0.01$	$2.0^{a} \pm 0.1$

Table 4. Dietary fibre analysis of sugar beet supplemented cookies

Treatments	TDF	IDF	SDF
		(%)	
T_0	$0.33^{f} \pm 0.2$	$0.20^{f} \pm 0.1$	$0.13^{f} \pm 0.1$
T_1	$1.26^{e} \pm 0.1$	$0.83^{e} \pm 0.1$	$0.43^{e} \pm 0.1$
T_2	$2.27^{d} \pm 0.1$	$1.47^{d} \pm 0.1$	$0.80^{d} \pm 0.1$
T_3	$3.53^{c} \pm 0.1$	$2.33^{c} \pm 0.1$	$1.20^{c} \pm 0.1$
T_4	$4.87^{b} \pm 0.1$	$3.17^{b} \pm 0.1$	$1.70^{b} \pm 0.1$
T_5	$6.27^{a} \pm 0.1$	$4.07^{a} \pm 0.1$	$2.20^{a} \pm 0.1$

lowest for T_1 (4%) treatment as compared to control. Therefore, on sensory evaluation basis it was suggested that replacement of wheat flour with less than or equal to 12% sugar beet powder for cookies preparation gave the best overall acceptability of cookies and results were according to the findings of McWatters *et al*. (2003). Similarly, regarding particle size, colourless and odourless properties of these fibres have revealed better sensory characteristics of cookies (Sakac *et al*., 2010).

Table 5. Sensory evaluation of sugar beet supplemented cookies

Treat-ments	Colour	Cris-piness	Flavour	Taste	Texture	Overall accept-ability
T_0	7.2^{ab}	8.0^{a}	8.0^{a}	7.0^{ab}	8.0^{a}	7.2^{b}
T_1	7.2^{ab}	7.0^{ab}	7.0^{ab}	7.2^{a}	6.2^{bc}	7.2^{b}
T_2	6.5^{bc}	6.2^{bc}	6.2^{bc}	6.2^{b}	6.0^{bcd}	6.5^{b}
T_3	8.0^{a}	7.2^{ab}	7.8^{ab}	7.8^{a}	7.0^{ab}	8.2^{a}
T_4	5.5^{cd}	5.2^{cd}	5.2^{cd}	5.2^{c}	5.2^{cd}	5.0^{c}
T_5	4.5^{d}	4.5^{d}	4.2^{d}	4.2^{d}	4.5^{d}	4.0^{d}

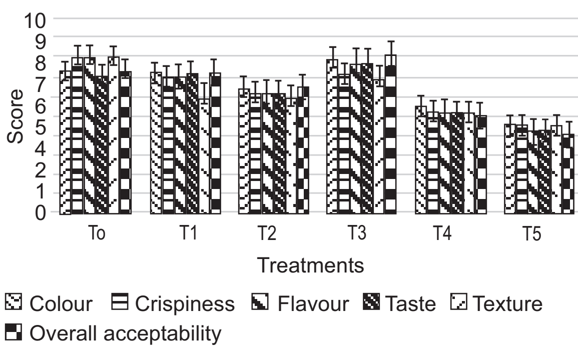

Fig. 3. Sensory evaluation of sugar beet supplemented cookies.

Conclusion

In a nutshell, sugar beet a vital source of dietary fibre provides many potent benefits. Current research has revealed that sugar beet in cookies behaves as a remarkable source of dietary fibre. The chemical composition, dietary fibre estimation both individually as well as in composite form have shown significant results and can be a good source in bakery products as cookies. Exhibits a good *in vitro* antioxidant potential including total phenolic content and DPPH by its addition in cookies. It can be recommended to be used in our routine diet in order to improve fibre intake.

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