International Journal of Modern Agriculture, Volume 3, No.3, 2014

ISSN: 2305-7246

CHARACTERIZATION AND UTILIZATION OF PSYLLIUM HUSK FOR THE PREPARATION OF DIETETIC COOKIES

Tahira Batool Qaisrani[1], Masood Sadiq Butt[2], Safdar Hussain[1] and Muhammad Ibrahim

[1]The Ghazi University, Dera Ghazi Khan, Pakistan
[2]National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan
*Corresponding author (e-mail: tahira7qais@gmail.com)

Abstract

Present research was carried out to characterize and utilize psyllium husk for preparation of dietetic cookies. Straight grade flour was replaced with psyllium husk in different combinations @ 5, 10, 15, 20 and 25%. Regarding characterization of psyllium husk, mean values obtained for moisture, ash, crude protein, crude fat, crude fiber and nitrogen free extract (NFE) in husk were 6.43±0.05, 3.85±0.04, 2.08±0.06, 0.09±0.01, 3.83±0.02 and 83.72±0.08%, respectively. Moreover, total dietary fiber and arabinoxylan content were 76.63±1.32 and 46.71±2.14%, respectively. Physical characteristics of dietetic cookies *i.e.* diameter and spread ratio were diminished with the addition of husk while thickness was increased. Results regarding storage of the cookies showed increase in diameter and spread ratio whilst thickness depicted decreasing trend. Chemical assay revealed higher crude protein content in control cookies. Whereas, moisture, ash, crude fiber, NFE along with dietary fiber and arabinoxylan contents were higher in psyllium husk based cookies. Softer cookies with low gross energy were obtained with the addition of psyllium husk. Conclusively, psyllium husk based cookies showed gradual enhancement in dietary fiber content as the amount of husk was increased in the recipe. The resultant cookies may have potential to manage the lipid profile and glucose concentrations in human subjects.

Key words: psyllium husk, dietary fiber, arabinoxylan, dietetic cookies, physico-chemical properties.

Introduction

Dietary components and human health have established links reflecting the pivotal role of nutrients in normal body functioning. The health grievances like obesity, hypercholesterolemia, diabetes mellitus and cardiovascular disorders (CVD) are associated with improper nutrient intake and deskbound life style. In this context, functional foods are one of the emerging implementations gaining attention as preventive and curative device against various health infirmities (Urala *et al.*, 2007; Bacha, *et al.*, 2011).

Psyllium (*Plantago ovata*) commonly called Isabgol is an annual herb originated from West Asia. The species is indigenous to Mediterranean region, West Asia and extending up to Sind in Pakistan (Singh and Virmani, 1982). The genus *Plantago* has over 200 species of which two; *P. ovata* and *P. psyllium* are commercially cultivated round the globe. Psyllium was introduced as a medicinal plant by Indian Muslims and seeds were firstly collected from some wild species. In Pakistan, it was firstly cultivated in Lahore and Multan districts and then moved to Bengal, Mysore and Indian Coromandel coast (Husain, 1977). The husk (outer covering of seed) is obtained as milling product of the seed yielding 10-25% of dried seed weight.

Considering chemical composition, the resultant husk is containing 6.83% moisture, 0.94% protein, 4.07% ash and 84.98% of total carbohydrates (Guo *et al.*, 2008). Psyllium husk is used in food, pharmaceutical and cosmetic industry. In foods, it is used in ice cream, instant juices, breakfast cereals and in bakery products like biscuits, cakes, breads and muffins with varying functional and health aspects (Ziai *et al.*, 2005).

Primarily, its imperative use was as a laxative and colon cleansing agent. It is assumed that dietary fiber from psyllium husk may help in weight management and fat loss by acting as bulking agent. In this context, considerable factors are increasing satiety, reducing caloric intake, ingestion rate and fat absorption (Cummings *et al.*, 2004). Like other herbs, psyllium is used traditionally as medicine and its consumption has been expanded due to its recognized nutraceutical potential. It is used in the treatment of constipation, diarrhea and ulcerative colitis as reported by Ramkumar and Rao, (2005). Considering such pharmacological aspects, use of this herb has increased as an anti-obesity, hypoglycemic and hypocholesterolemic agent (Anderson *et al*., 2000; Moreyra *et al*., 2005; Ziai *et al*., 2005; Pal *et al*., 2011).

The health improving properties are attributed to its active fiber fraction the arabinoxylan (AX); a polysaccharide containing heteroxylan, with main monosaccharides; arabinose and xylose, collectively referred as arabinoxylan (Marlett and Fischer, 2003; Fischer *et al*., 2004). This active fraction of psyllium husk, the arabinoxylan is a highly branched polysaccharide constituting more than 60% of the weight of psyllium husk. Exclusively, arabinoxylan from psyllium husk is resistant to fermentation as compared to those extracted from wheat, oat or barley (Van-Craeyveld *et al*., 2008). Psyllium husk may also have a symbiotic relation with medicines being used to reduce the problems related to obesity, dyslipidemia and CVD.

Different cereal and vegetable based soluble and insoluble fibers are in use to overcome the menace of high cholesterol and glycemic disorders. The foremost fiber sources are psyllium husk, oat, guar gum and some other cereals. However, psyllium husk fiber appears one of the effective sources with least adverse effects (Galisteoa *et al*., 2010). It has high water soluble fiber that soothes the lipid profile and glycemic response in individuals with hypercholesterolemia. High fiber diets increase peripheral insulin sensitivity in healthy adults (Karhunen *et al*., 2010).

The husk obtained after milling is white hydrophilic material forming clear and colorless mucilaginous gel after water absorption. Its carboxymethyle derivative is fibrous and mucilaginous in taste (Fischer *et al*., 2004). This fiber has laxative effect with the increment of increased fecal output and stool frequency. It has a wide application in functional foods, thickener in ice cream, application in cosmetics and printing as well as drug in pharmaceutical industry (Singh, 2007). Research indicates that husk is quite safe to use in functional and nutraceutical foods. The FDA has approved the use of food products containing psyllium husk due to its associated health claims (Leeds, 2009). For supplementation of fiber, cookies are appreciated by the consumers due to appealing taste and better storage stability. Bakery products are prepared from different dosage of psyllium husk and previous literature suggested that replacement of psyllium husk up to 50% is possible without detrimental change in quality (Ganji and Kuo, 2008). Psyllium husk is becoming popular as therapeutic agent against various physiological ailments. Therefore, it is desire need to explore the indigenous food sources for the welfare of the society (Sahu, 2011). Keeping in view the above facts, present research was designed to characterize locally available psyllium husk variety namely *Plantago ovata* Forsk followed by the preparation of dietetic cookies.

Materials and Methods

Characterization of husk and product development was carried out in Postgraduate Research Laboratory, National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad.

Procurement of raw materials

Indigenous psyllium husk (*Plantago Ovata* Forsk) was purchased from Qarshi Industries (Pvt.) Ltd. Pakistan. For the preparation of dietetic cookies, non-nutritive sweeteners including aspartame and sorbitol were used. The sweeteners, straight grade flour and shortening were procured from the local market.

Characterization of psyllium husk

The husk was characterized for various aspects like proximate analysis, dietary fiber and arabinoxylan content.

Chemical characteristics: The chemical composition of husk was estimated through moisture, crude fat, crude fiber, crude protein, total ash and nitrogen free extract (NFE) determination.

Moisture content: Psyllium husk was analyzed for moisture content by using air forced draft oven (Model: DO-1-30/02, PCSIR, Pakistan). The sample was dried at 105±5˚C to constant weight and calculations were made (AACC, 2000; Method No. 44-15A).

Crude protein: For determination of crude protein, nitrogen percentage was estimated through Kjeltech Apparatus (Model: D-40599, Behr Labor Technik, GmbH-Germany). The protein was calculated by multiplying percent nitrogen with conversion factor (AACC 2000; Method No. 46-10).

Crude Fat: Crude fat in oven dried sample was estimated by Soxtec System (Model: H-2 1045 Extraction Unit, Hoganas, Sweden). Sample weighing 5g was used for extraction of crude fat with petroleum ether. After extraction, residue was dried till constant weight (AACC, 2000; Method No. 30-10).

Crude fiber: After fat extraction, husk sample was examined for crude fiber through Labconco Fibertech (Labconco Corporation Kansas, USA). Fat free 2g sample was digested firstly with 1.25% H_2SO_4 and finally with 1.25% NaOH. The residue was dried at

130˚C for 2 hours and weighed followed by ignition at 550±15˚C and cooled for further calculations (AACC, 2000; Method No. 32-10).

Total ash: For determination of ash content, sample was taken in pre-weighed crucible and charred on burner till no fumes before incineration in the Muffle Furnace (MF-1/02, PCSIR, Pakistan) to obtain white grayish residue with no other taints obtained (AACC, 2000; Method No. 08-01).

Nitrogen Free Extract (NFE)

NFE was calculated according to following expression;

NFE (%) = 100 – (Crude protein% + Crude fat% + Crude fiber% + Total ash%)

Dietary Fiber: Determination of dietary fiber was done using the method described by Prosky *et al*. (1984). The sample in duplicate was treated with buffers (40mL, pH 8.2, 24°C). Stirring of samples till uniform dispersion of samples was done on magnetic stirrer. Alpha-amylase solution (50µL) was added and sample was heated at 95-100°C for 35 min. After cooling, protease solution (100µL) was added and incubation was done for 30 min at 60°C. Following the same procedure amyloglucosidase solution (200µL) was added and samples were incubated. Precipitation was done with 4 vol. of 95% ethanol and sample was filtered and dried. The obtained residue was divided into two parts which were further used for protein and ash determinations. Total dietary fiber was determined by subtracting the protein and ash contents determined in dried residues. The total dietary fiber was calculated following the formula given in respective method.

Determination of arabinoxylan

Arabinoxylan content of husk sample was isolated followed by derivitization of sample for monosaccharides determination and subsequently calculated the arabinoxylan.

Isolation: In 100g husk sample, arabinoxylan was isolated by using alkali method. The sample was heated in KOH solution for two hrs followed by centrifugation at 6000×*g* for 20 min at 4°C. The supernatant was taken for precipitation and kept the precipitates overnight at refrigeration temp in absolute ethanol (final concentration of 65% v/v) and centrifuged (Cleemput *et al*., 1995). The arabinoxylan fiber thus obtained was finally freeze dried for 4 hrs.

Derivitization of precipitates: Arabinoxylan was derivitized for monosaccharide determination (Vallance *et al*., 1998). Standard gum (1-2mg) and individual monosaccharide standard samples (1mg each) were heated in water bath at 100°C for 30 and 10 min, respectively, with triflouroacetic acid (0.1mL analytical grade, undiluted). Then samples were allowed to cool at room temperature before pyridin (0.2mL, analytical grade) was added to the vials. Hexamethyle dizilazane (0.3mL, derivitization grade) and triflouroacetic acid (5 drops, analytical grade, undiluted) were added to the samples. The samples were shaken vigorously for 30 sec and allowed to stand for 1 hr prior to analysis. Later, 0.1mm samples were removed and placed directly into vials. The material was heated for 30 min at 105°C with triflouroacetic acid (0.1mL, analytical grade, undiluted) in water bath. The samples were shaken vigorously for 30 sec and allowed to stand for 1 hr prior to analysis.

Determination of monosaccharides: The arabinoxylan isolated from psyllium husk was fractionated to determine monosaccharide. The analysis was carried out by using gas chromatography (Agilent 6890 GC technology) equipped with Microsoft detector (Agilent 5973 MS Detector) whilst DB-5 column (30m×0.25mm ID and film thickness of 0.25µm, Agilent, Palo Alto, CA, USA) was used with methyl polysiloxane polymer phase. Helium (He) was used as carrier gas at constant flow rate of 1.3mL min^{-1}. Temperatures for injector and MS source were maintained at 280 and 230°C, respectively. The column temperature program consisted of injection at 65°C and hold for 2 min. with temperature increase of 6°C min^{-1} to 300°C for an isothermal hold of 15 min. The MS was operated in the electron impact mode with ionization energy of 70ev. The samples were analyzed in the splitless mode (splitless time: 30 sec). The acquired data was processed with HP-Chemstation Software (Mendeiros and Simoneit, 2007).

Product development

Preliminary trials were carried out for the development of dietetic cookies supplemented with psyllium husk. The rationale was to obtain an acceptable quality product. After fixing the recipe, different treatments of cookies were made and evaluated for various quality traits.

Preparation of dietetic cookies

Six different treatments of cookies were prepared using varying levels of psyllium husk (Table 1) according to the instructions of AACC (2000). The recipe followed for control was: 500g commercial straight grade flour (CSGF), 250mL sorbitol, 0.84g aspartame, 220g vegetable shortening and 7g baking powder.

All the treatments were analyzed fortnightly from 0 day to two months storage for physical, chemical and sensory characteristics with the objective to select the best treatment as functional food against life style-related disorders.

Table 1: Different treatments of dietetic cookies

Treatments	Flour (%)	Psyllium husk (%)
T_0	100	--
T_1	95	5
T_2	90	10
T_3	85	15
T_4	80	20
T_5	75	25

$T_{0\ =}$ control

Physical analyses of cookies

Cookies were analyzed for physical traits like diameter (mm), thickness (mm) and spread factor according to their respective methods in AACC (2000). Diameter (D) of six cookies was measured. The cookies were kept horizontally to take reading. For duplicate reading cookies were rotated at an angle of 90° and repeated the procedure for average calculation.

Likewise, thickness (T) was calculated by measuring the height of six cookies with ruler kept on top of one another. The practice was repeated twice and average value was presented in (mm). Spread factor (SF) was calculated by incorporating the values of D and T in subsequent formula;

SF = $(D/T \times CF) \times 10$

CF= Correction factor

Chemical composition of cookies

The proximate analyses of cookies regarding crude protein, crude fat, crude fiber, total ash and nitrogen free extract (NFE) were estimated on dry weight basis by their respective procedures as mentioned in the preceding section.

Gross energy

Gross energy (Cal/g) was calculated by combusting the sample using the Oxygen Bomb Calorimeter (C-2000, IKA WERKE). Initially, 0.5g of sample was weighed and fed to calorimeter piston, after internal burning at high pressure the energy generated was measured automatically and reading was displayed on the screen of Bomb Calorimeter as described in the method of Krishna and Ranjhan (1981).

Texture analysis

Texture of cookies was measured through texture analyzer (model TA.XT plus, Texture Technologies Corp.) equipped with 3-point bend attachment (TA-92), a heavy-duty platform (TA-90) and stainless steel blade (TA-92). The resistance of the cookies to fracture was measured. Three cookies were selected randomly and applied to the base of analyzer. The internal gap adjustment of the base was set to place the cookie properly. Gap of base and distance of blade were kept constant for all samples. The data was obtained in the form of force-deformation curve indicating force, rupture distance, and gradient. Settings included pretest speed of 5mm/s, test speed of 3mm/s, posttest speed 10mm/s, distance 20mm and trigger force 50g (Piga *et al*., 2005).

Dietary fiber and arabinoxylan determination

Dietary fiber of cookies was determined following the method described by Prosky *et al*. (1984). For determining arabinoxylan content, the isolation of arabinoxylan was accomplished by alkali method (Cleemput *et al.*, 1995) followed by derivitization of isolates (Vallance *et al.* (1998). Finally, monosaccharides (arabinose and xylose) were determined by using GC-MS (Mendeiros and Simoneit, 2007) followed by arabinoxylan calculation as discussed in preceding section for husk sample.

Statistical analysis

The data obtained was subjected to statistical analysis using Cohort version 6.1 (Costat-2003). Level of significance was estimated by using the analysis of variance technique (ANOVA) using two factor factorial under complete randomized design (CRD). Further, Least Significant Difference test (LSD) in cookies were applied for comparing means (Steel *et al*., 1997).

Results and Discussion

Present study was planned to formulate dietetic cookies with psyllium husk having potential to manage lipid profile and other allied parameters in normal and hypercholesterolemic subjects. For the intention, project was contemplated in two phases; firstly, psyllium husk was characterized for its constituents emphasizing on fiber fraction in general and arabinoxylan in particular. Second phase comprised of product development in which dietetic cookies were prepared with varying levels of psyllium husk and tested for different parameters during two months storage.

Characterization of psyllium husk

Compositional estimation of the supplement (husk) is important because addition of husk in cookies may play a mandatory role in the adjustment of physical and chemical characteristics. Chemical analysis is also important to assess the efficacy of the supplement. Thus, husk was examined for its constituents including moisture, ash, protein, crude fat, crude fiber and nitrogen free extract (NFE) along with dietary fiber especially the arabinoxylan content, the physiologically active component against different ailments.

Chemical composition

Mean values for moisture, ash, crude protein, crude fat, crude fiber and NFE in husk were 6.43±0.05, 3.85±0.04, 2.08±0.06, 0.09±0.01, 3.83±0.02 and 83.72±0.08%, respectively (Table 2). The results are similar to the findings of Marlett and Fischer (2003) as they reported values for protein, ash, and total carbohydrates as 35.0, 33.5 and 902.4mg/g, respectively in husk. Present results are also in corroborated with the finding of Guo *et al.* (2008), they found moisture, ash, protein, fat and NFE values in psyllium husk as 6.83±0.04, 4.07±0.02, 0.94±0.00, 0.04±0.11 and 84.98±4.26%, respectively.

Table 2: Compositional analysis of psyllium husk

Constituents	Quantity (%)
1. Moisture	6.43±0.05
2. Ash	3.85±0.04
3. Crude protein	2.08±0.06
4. Crude fat	0.09±0.01
5. Crude fiber	3.83±0.02
6. Nitrogen free extract (NFE)	83.72±0.08
6.1. Dietary fibers	76.63±1.32
6.1.1. Arabinoxylan	46.71±2.14

Dietary fiber and arabinoxylan

In psyllium husk, total dietary fiber and arabinoxylan contents were 76.63±1.32 and 46.71±2.14%, respectively (Table 2). Earlier, Guo *et al.* (2008) explored the chemistry of psyllium husk and noted total carbohydrates up to 84.98% considering it as dietary fiber. In the current study, value for dietary fibers showed variation with the work of Guo *et al.* (2008) might be due to varietal differences and estimation methods.

Earlier studies supported the current results that arabinoxylan content in husk is ranging from 45 to 60% further indicated that the major fractions are arabinose and xylose whilst minor fractions include some other sugars and uronic acid. Considering psyllium husk as source of dietary fiber some researchers inferred that arabinoxylan is the active fraction helpful to manage various physiological ailments (Fischer *et al.*, 2004; Saghir *et al.*, 2008).

Product development

The control and psyllium husk containing cookies were analyzed for physical and chemical traits on fortnightly basis during two months storage. Likewise, gross energy and texture were estimated during the entire study on respective intervals.

Physical characteristics of dietetic cookies

It is the prime objective of the bakers and consumers that product should be of acceptable quality in the first look. For the reason being, the control and psyllium husk supplemented cookies were evaluated for physical characteristics like diameter, thickness and spread ratio.

Diameter: Means for the effect of treatments on diameter of the cookies presented a decreasing trend (Table 3). The diameter of control cookies (T_0) was 44.94±0.08mm that decreased with increase of psyllium husk and minimum value 40.86±0.08mm was recorded in cookies containing 25% psyllium husk (T_5). During two months storage, means for diameter ranged from 41.95±0.06 to 42.20±0.06mm while maximum value appraised at 60 days showing increasing trend in diameter as function of storage (Table 4).

The significant effect of treatment on diameter of cookies may be due to addition of fiber that alters dough rheology and allied characteristics in baked products. During storage, fiber addition may also be a factor for deterioration having potential to absorb moisture from the surroundings. Similarly, significant differences due to treatments and storage were also observed by Hussain *et al.* 2006. They also found decreasing trend in cookies diameter due to addition of fiber content.

Thickness: Thickness increased gradually due to treatments with increasing level of psyllium husk as indicated in Table 3. Means for thickness were estimated as 10.24±0.06mm in control cookies whereas maximum value 11.02±0.08mm in T_5. Results for storage period explicated in Table 4 showed reduction in thickness as means determined at zero days was 10.85±0.02 that decreased to 10.05±0.02mm at 60 days.

Regarding present study, in different treatments of cookies sugar was replaced with other sweeteners hence no reduction in thickness was observed. Another possible justification for variation in thickness within treatments is that flour was replaced with husk thereby decreased starch and protein contents resulting gradual condensation of dough that consequently increased thickness. Findings of Sharif *et al.* (2005) are analogous to the instant results as they reported significant increase in thickness of bakery products considering fiber the principal factor for this change, they further recommended that up to 20% replacement of flour with fiber is acceptable in bakery products.

Spread ratio: Means for spread ratio in Table 3 indicating diminution trend due to treatments as values ranged from 42.63±0.14 to 37.09±0.16 in T_0 to T_5, respectively. Whereas, results regarding the effect of storage presented in Table 4 revealed that spread ratio increased during two months as minimum value was observed at the initiation of the study (38.69±0.07) while maximum (39.67±0.07) perceived at the termination stage.

Highly significant results in spread ratio due to treatments and storage are function of variations in diameter and thickness. Both of these parameters varied with addition of psyllium husk and absence of sugar, eventually affected the spread ratio. Previous studies of Hussain *et al.* (2006) and Sharif *et al.* (2009) strengthened the current results as they reported significant effect of fiber addition on spread ratio of cookies. They further elucidated that spread ratio decreased as function of fiber and the possible reason may be the absorption of moisture that increased the diameter and accordingly affected spread ratio.

Table 3. Effect of treatments on physical characteristics of dietetic cookies

Treatments	Diameter (mm)	Thickness (mm)	Spread ratio
T_0 = control	44.94±0.08a	10.24±0.06c	42.63±0.14a
T_1 = 5% of psyllium husk	42.60±0.07b	10.39±0.07b	40.24±0.16b
T_2= 10% of psyllium husk	41.85±0.07c	10.65±0.07b	39.29±0.16c
T_3= 15% of psyllium husk	41.22±0.07c	10.83±0.06a	38.06±0.14d
T_4= 20% of psyllium husk	40.95±0.08d	10.93±0.08a	37.46±0.17e
T_5 = 25% of psyllium husk	40.86±0.08d	11.02±0.08a	37.09±0.16f

Mean values carrying same letters in a column are not significantly different

Table 4. Effect of storage on physical characteristics of dietetic cookies

Storage (Days)	Diameter (mm)	Thickness (mm)	Spread ratio
0	41.95±0.06b	10.85±0.02a	38.69±0.07c
15	41.99±0.05b	10.84±0.02a	38.76±0.07c
30	42.07±0.06a	10.26±0.02b	39.15±0.07b
45	42.13±0.06a	10.11±0.02b	39.38±0.07b
60	42.20±0.06a	10.05±0.02b	39.67±0.07a

Mean values carrying same letters in a column are not significantly different

Proximate composition

Moisture: Means for moisture in different treatments ranged from 3.07±0.07 to 3.35±0.07% (Table 5) showing progressive increase in moisture level with the addition of psyllium husk in cookies. The data presented in Table 6 explicated that in fresh cookies moisture content was 3.03±0.07% that increased to 3.34±0.01% at 60 days indicating uplift in this trait with the passage of time.

Increase in moisture of cookies is due to increased psyllium husk level that has tendency to absorb water because of hydrophilic nature. Moreover, cookies were packed in bioriented polyvinylchloride (PVC) wraps and water absorption from the surrounding may also be a factor for increased moisture level during storage and this opinion is strengthened by work of Piga *et al.* (2005). The present results are in confirmatory with the outcomes of Uysal *et al.*

(2007) who reported significant effect on moisture by addition of fiber in oven-baked cookies. They concluded that in cookies, fiber improves the water holding capacity as compared to wheat flour resultantly increasing moisture level. Similarly, Pasha *et al.* (2002) also reported increase in moisture level in the dietetic cookies during storage.

Table 5. Effect of treatments on proximate composition (%) of dietetic cookies

Treatments	Moisture	Ash	Crude Protein	Crude fat	Crude fiber	NFE
T_0 = control	3.07±0.07c	1.17±0.02f	8.04±0.07a	17.62±0.14a	0.39±0.012a	69.91±0.01a
T_1 = 5% of psyllium husk	3.21±0.06b	1.27±0.01e	7.74±0.06b	17.60±0.13a	0.52±0.016b	70.11±0.03a
T_2= 10% of psyllium husk	3.22±0.07ab	1.38±0.02d	7.37±0.08c	17.60±0.11a	0.65±0.017c	70.36±0.03a
T_3= 15% of psyllium husk	3.24±0.07a	1.49±0.02c	7.05±0.06d	17.56±0.13a	0.76±0.047d	70.52±0.01a
T_4= 20% of psyllium husk	3.25±0.06a	1.61±0.01b	6.65±0.06e	17.24±0.13b	0.90±0.016e	70.35±0.04a
T_5 = 25% of psyllium husk	3.35±0.07a	1.70±0.02a	6.27±0.06f	17.04±0.13b	1.03±0.019f	70.61±0.17a

Mean values carrying same letters in a column are not significantly different

Table 6. Effect of storage on proximate composition (%) of dietetic cookies

Storage (Days)	Moisture	Ash	Crude Protein	Crude fat	Crude fiber	NFE
0	3.03±0.07e	1.44±0.02a	7.27±0.04a	17.92±0.01a	0.71±0.02a	69.18±0.03a
15	3.17±0.08d	1.42±0.01a	7.25±0.03a	17.75±0.02b	0.71±0.02a	69.33±0.04a
30	3.21±0.01c	1.44±0.02a	7.18±0.04ab	17.52±0.01b	0.71±0.02a	69.41±0.02a
45	3.27±0.01b	1.43±0.02a	7.14±0.04b	17.37±0.01c	0.71±0.02a	69.62±0.08a
60	3.34±0.01a	1.43±0.02a	7.01±0.03b	17.21±0.01c	0.70±0.02a	70.31±0.03a

Mean values carrying same letters in a column are not significantly different

Ash: Total ash increased gradually in the treatments from control (T_0) to cookies containing 25% psyllium husk (T_5) as depicted in Table 5. Means for ash in T_0 was 1.17±0.01% compared to 1.70±0.02% in T_5 indicating increase in ash percentage with incremental increase in psyllium husk. Regarding the effect of storage on ash content, data indicated non-significant differences (Table 6). The momentous increase of ash in various treatments is attributed to increased psyllium husk level as fiber provides sufficient amount of ash to the recipe, being a compositional constituent (Table 1).

Findings of Hussein *et al.* (2011) delineated a similar increasing trend in ash content due to fiber addition. Furthermore, Pasha *et al.* (2011) also reported increased mineral profile in the baked products attributed to high ash content of the composite flour.

Crude protein: Decrease in protein content was observed by the addition of psyllium husk among different treatments of cookies (Table 5). Highest mean value 8.04±0.07% was reported in T_0 (control) that declined to 6.27±0.06% in T_5 (cookies containing 25% psyllium husk). Similarly, decreasing trend in protein was observed during storage varying from 7.27±0.04% in fresh cookies to 7.01±0.03% after two months (Table 6).

White flour is the main source of protein in cookies and replacement of flour with psyllium husk resulted decrease in protein content. Another reason for reduction in protein content may be owing to increased moisture content of the cookies that changed the overall chemistry of end product. Possible complex formation between husk and protein moiety may also be a factor for reduced estimation of protein and this opinion is supported by earlier work of Bilgicli *et al.* (2007), they reported decreased protein digestibility due to fiber content. Moreover, exploration of Uysal *et al.* (2007) conferred that adding up of fiber from fruit sources decreases protein content in cookies.

Crude fat: Treatments showed slightly decreasing trend on fat percentage of cookies. The maximum mean value was recorded as 17.62±0.14% in control that declined up to 17.04±0.13% in T_5 (Table 5). Similar diminishing trend in fat level was observed during two months storage. The means (Table 5) for fat at zero days were 17.92±0.01% that gradually decreased to 17.21±0.02% at 60 days.

Treatments exerted slight decline in fat percentage might be due to increased fiber and moisture contents. Accordingly, Uysal *et al.* (2007) described reduction in fat percentage of cookies due to addition of wheat fiber. Present results are also in harmony with the findings of Pasha *et al.* (2002) that in bakery products increased moisture content may be one of the factors for declining trend in fat during storage.

Crude fiber: Means for crude fiber in different treatments are presented in Table 5. Minimum crude fiber was observed in T_0 (0.39±0.01) nevertheless by adding up of psyllium husk it increased to 1.03±0.01% in T_5 (cookies containing 25% psyllium husk). Storage exhibited non-momentous effect on this attribute (Table 6).

Regarding crude fiber in cookies, data exposed an increasing trend possibly due to adding up of psyllium husk as dietary fiber contributes in its inclination. The similar pattern was observed by Hussein *et al.* (2011) that supplementation of germinated fenugreek seeds flour resultantly enhanced crude fiber content in biscuits. Currently, the investigation of Pasha *et al.* (2011) elucidated that addition of fiber enriched mungbean flour resulted increased crude fiber content in bakery products.

Nitrogen free extracts (NFE): Psyllium husk addition to dietetic cookies explicated non-significant differences in NFE (Table 5). The data indicated that means for NFE ranged from 69.91±0.01 to 70.61±0.17% in T_0 and T_5, respectively. Similarly, storage exhibited non-significant differences; NFE varied from 69.18±0.03 to 70.31±0.03% at 0 and 60 days, respectively (Table 6).

Dietary fiber (DF): The means for total dietary fiber ranged from 0.96±0.02 to 8.09±0.05% in T_0 and T_5, respectively depicting a defined increasing trend with progressive increment of psyllium husk (Table 7). As compared to T_0, the dietary fiber content in T_1 was 2.19±0.07% that gradually increased to 8.09±0.05% in T_5. Means recorded for dietary fiber varied from 4.45±0.08 to 4.40±0.08% revealing statistically non-significant differences as function of storage.

Enhanced dietary fiber in different treatments owes to supplementation of cookies with psyllium husk containing high fiber contents. Similar results are assessed by other researchers that incorporation of fiber enriched sources boost the dietary fiber in resultant bakery products. The current results are supported by the work of Vega-Lopez *et al.* (2001) indicating significant increase for this trait in cookies supplemented with fiber. The results by Nassar *et al.* (2008) showed 14.71 and 15.31% dietary fiber content in biscuits supplemented with 25% pulp and citrus peel, respectively. Whereas, Sharif *et al.* (2009) reported increase in dietary fiber ranging from 7.63 to 11.01% in cookies containing 30 and 50% rice bran, respectively.

Table 7. Effect of treatments on dietary fiber, arabinoxylan, gross energy and hardness of dietetic cookies

Treatments	DF (%)	AX (%)	Gross energy (kcal/g)	Hardness (g)
T_0 = control	0.96±0.02f	0.07±0.01f	4.83±0.29a	178±0.47a
T_1 = 5% of psyllium husk	2.19±0.07e	1.06±0.04e	4.78±0.21b	159±0.58b
T_2= 10% of psyllium husk	3.59±0.06d	1.63±0.04d	4.71±0.20c	143±0.60c
T_3= 15% of psyllium husk	5.12±0.03c	2.87±0.05c	4.68±0.20d	135±0.65d
T_4= 20% of psyllium husk	6.45±0.10b	3.23±0.08b	4.65±0.24e	113±0.78e
T_5 = 25% of psyllium husk	8.09±0.05a	3.41±0.08a	4.62±0.22f	110±0.60f

Mean values carrying same letters in a column are not significantly different

Arabinoxylan (AX): It has been assessed that AX percentage increased with the gradual increase of psyllium husk in various sample of cookies (Table 7). The value for this parameter in control was 0.07±0.01% that significantly increased to 3.41±0.08% in T_5. During storage, AX level remained in the range of 2.09±0.06 to 2.02±0.06% from zero to 60th day, respectively exhibited non-significant decreasing trend (Table 8).

Treatments containing psyllium husk possessed high arabinoxylan content as compared to control certainly due to ample amount of this fraction in husk. Earlier studies by Van-Craeyveld *et al.* (2009) and Saghir *et al.* (2008) supported the current results inferring that psyllium husk comprised of 45-60% of arabinoxylan. Analysis of dietetic cookies as therapeutic food for vulnerable segment showed that psyllium husk has pronounced effect on rheology and composition of product. Pysllium husk based cookies due to high dietary fiber and arabinoxylan contents confirm as suitable dietary intervention against life style-related disorders.

Table 8. Effect of storage on dietary fiber, arabinoxylan, gross energy and hardness of dietetic cookies

Storage (Days)	DF (%)	AX (%)	Gross energy (kcal/g)	Hardness (g)
0	4.45±0.08a	2.09±0.06a	4.72±0.27a	148±0.26a
15	4.43±0.08a	2.06±0.06a	4.72±0.27a	143±0.27b
30	4.40±0.08a	2.04±0.06a	4.71±0.29a	139±0.27c
45	4.37±0.08a	2.02±0.06a	4.71±0.28a	135±0.28d
60	4.35±0.08a	2.00 ±0.06a	4.70±0.28a	133±0.28e

Mean values carrying same letters in a column are not significantly different

Gross energy

Respective means in Table 7 indicated highest value in control (4.83±0.29kcal/g) that reduced gradually with progressive increase of psyllium husk and the lowest value was recorded in T_5 (4.62±0.22kcal/g). However, the effect of storage on gross energy differed non-significantly during two months (Table 8).

Gradual reduction in gross energy among the treatments might be due to replacement of flour with that of psyllium husk resulting in reduced starch and protein levels in composite blends. Similar trend was presented by Uysal *et al.* (2007) revealing that fiber addition regardless of source resulted decrease in calorific value of the cookies. Likewise, Bilgicli *et al.* (2007) indicated lower energy in the cookies by adding up of fiber.

Hardness

Means for hardness were the highest in control (178±0.47g) whereas in supplemented cookies the values decreased gradually ranging from 159±0.58 to 110±0.60g in T_1 and T_5, respectively (Table 7). Means depicted in Table 4.10 for hardness of cookies during storage explicated the highest value 148±0.26g at beginning of study that reduced subsequently during storage and the lowest value 133±0.28g was recorded at 60th day.

Significant differences in hardness were observed in treated and control cookies nevertheless with increasing level of psyllium husk the hardness decreased simultaneously. Hardness in control treatment compared to other treatments is attributed to starch content of white flour and also the binding of starch with protein during baking whilst in treated cookies flour was replaced with psyllium husk that imparts lesser amount of starch and protein eventually producing softer product. Moreover, husk from psyllium seed is potentially hydrophilic in nature thereby absorbed excessive moisture and affected the hardness. Psyllium husk is an excellent mucilloid therefore bound moisture even during baking consequently imparts considerable soft texture to the cookies. Current results are comparable with the work of Uysal *et al.* (2007) and Toma *et al.* (2009) illuminated that fiber addition reduces hardness in cookies. Likewise, Piga *et al.* (2005) elucidated that moisture retention in cookies during storage imparts less resistance to the applied force thus softer product is attained.

References

AACC. American Association of Cereal Chemists. 2000. Approved Methods of American Association of Cereal Chemists. The Am. Assoc. Cereal Chem. Inc., St. Paul. Minnesota.

Anderson, J.W., T.J. Hanna, X. Peng, and R.J. Kryscio (2000). Whole grain foods and heart disease risk. J. Am. Coll. Nutr. 19: 291-299.

Bacha. U. M. Nasir, A. Khalique, A.A. Anjum and M.A. Jabbar. (2011). Comparative assessment of various agro-industrial wastes for saccharomyces cerevisiae biomass production and its quality evaluation as single cell protein. J Anim. Plant Sci.21(4): 844-849

Bilgicli, N., S. Ibanoglu, and E.N. Herkin (2007). Effect of dietary fiber addition on the selected nutritional properties of cookies. J. Food Engg. 78: 86-89.

Cleemput, G., M.V. Oort, M. Hessing, M.E.F. Bergmansl, H. Gruppen, P.J. Grobet, and J.A. Delcour (1995). Variation in degree of D-xylose substitute in arabinoxylans extracted from European wheat flour. J. Cereal. Sci. 22: 73-84.

CoStat Statistical Software, 2003. CoHort v. 6.2, Monterey, CA.

Cummings, J.H., L.M. Edmond, and E.A. Magee (2004). Dietary carbohydrates and health: Do we still need the fiber concept? Clinc. Nutr. Suppl. 1: 5-17.

Fischer, M.H., N.X. Yu, G.R. Gray, J. Ralph, L. Anderson and J.A. Marlett (2004). The gel-forming polysaccharide of psyllium husk (*Plantago ovata* Forsk). Carb. Res. 339: 2009–2017.

Galisteoa, M., R. Morona, L. Riveraa, R. Romeroa, A. Anguerab, and A. Zarzueloa (2010). *Plantago ovata* husks-supplemented diet ameliorates metabolic alterations in obese Zucker rats through activation of AMP-activated protein kinase. Comparative study with other dietary fibers. Clin. Nutri. 29: 261-267.

Ganji, V., and J. Kuo (2008). Serum lipid responses to psyllium fiber: differences between pre and post-menopausal, hypercholesterolemic women. Nutr. J. 7: 22-27.

Guo, Q., S.W. Cui, Q. Wang, and J.C. Young (2008). Fractionation and physicochemical characterization of psyllium gum. Carbohyd. Polym. 73: 35-43.

Husain, A. (1977). Achievements in the research on medicinal plants, their present and future value in India. In: Proceedings of fourth symposium of pharmacognosy and chemistry of natural products. Leiden: Gorlaeus laboratories.

Hussain, S., F.M. Anjum, M.S. Butt, M.I. Khan, and A. Asghar (2006). Physical and sensoric attributes of Flaxseed flour supplemented cookies. Turk. J. Biol. 30:87-92.

Hussein, A.M.S., S. Amal, A. El-Azeem and Amany. 2011. Physiochemical, sensory and nutritional properties of corn-fenugreek flour composite biscuits. Aust. J. Basic Appl. Sci. 5:84-95.

Karhunen, L.J., K.R. Juvonen, S.M. Flander, K.H. Liukkonen, L. Lahteenmaki, M. Siloaho, D.E. Laaksonen, K.H. Herzig, M.I. Uusitupaand and K.S. Poutanen. 2010. A psyllium fiber-enriched meal strongly attenuates postprandial gastrointestinal peptide release in healthy young adults. J. Nutr. 140:4737-4744.

Krishna, G., and S.K. Ranjhan (1981). Gross energy value of herbage, urine, milk and silage. Laboratory Manual for Nutrition Research. Vikas Publishing House (Pvt) Ltd, Delhi.

Leeds, A.R. (2009). Dietary Fiber; Role in Nutrition Management of Disease. In: Guide to Nutritional Supplements. Caballero. B. (ed.). Academic press. USA.

Marlett, J.A., and M.H. Fischer (2003). The active fraction of psyllium seed husk. Proceedings of the Nutrition Society. 62: 207-209.

Mendeiros, P.M., and B.R.T. Simoneit (2007). Analysis of sugars in environmental samples by gas chromatodraphy-mass spectrometry. J. Chromatogr. A. 1141: 271-278.

Moreyra, A.E., A.C. Wilson, and A. Koraym (2005). Effect of combining psyllium fiber with simvastatin in lowering cholesterol. Arch. Intl. Med. 165: 1161-1166.

Nassar, A.G., A.A. AbdEl-Hamied, and E.A. El-Naggar (2008). Effect of citrus by-products flour incorporation on chemical, rheological and organoleptic characteristics of biscuits. World J. Agric. Sci. 4: 612-616.

Pal, S., A. Khossousi, C. Binns, S. Dhaliwal, and S. Radavelli-Bagatini (2011). The effects of 12-week psyllium fiber supplementation or healthy diet on blood pressure and arterial stiffness in overweight and obese individuals. Br. J. Nutr. 26: 1-10.

Pasha, I., M.S. Butt, F.M. Anjum, and N. Shehzadi (2002). Effect of dietetic sweeteners on the quality of cookies. Int. J. Agri. Biol. 4: 245-248.

Pasha, I., S. Rashid, F.M. Anjum, M.T. Sultan, M.M.N. Qayyum, and F.Saeed (2011). Quality evaluation of wheat-mungbean flour blends and their utilization in baked products. Pak. J. Nutr.10: 388-392.

Piga, A., P. Catzeddu, S. Farris, T. Roggio, A. Sanguinetti, and E. Scano (2005). Texture evolution of “Amaretti” cookies during storage. Eur. Food. Res. Technol. 221: 387-391.

Prosky, L.P., N.G. Asp, I. Furda, J.W. DeVries, T.F. Schweizer, and B.F. Harland (1984). Determination of total dietary fiber in foods, food products and total diets: Interlaboratory study. J. AOAC. Int. 67: 1044-52.

Ramkumar, D., and S.S. Rao (2005). Efficacy and safety of traditional medical therapies for chronic constipation: systematic review. Am. J. Gastroenterol. 100: 936-971.

Saghir, S., M.S. Iqbal, M.A.Hussain, A. Koschella, and T. Heinze (2008). Structure characterization and carboxymethylation of arabinoxylan isolated from Ispaghula (Plantago ovata) seed husk. Carbohyd. Poly. 74: 309-317.

Sahu, S.K. (2011). Localized food systems: the way towards sustainable livelihoods and ecological security –a review. J. Anim. Plant Sci. 21(2 Suppl.): 388-395.

Sharif, K., M.S. Butt, and N. Huma (2005). Oil extraction from rice industrial waste and its effect on physico-chemical characteristics of cookies. Nutr. Food Sci., 35: 416-427.

Sharif, M..K., M.S. Butt, F.M. Anjum, and H. Nawaz (2009). Preparation of fiber and mineral enriched defatted rice bran supplemented cookies. Pak. J. Nutri. 8: 571-577.

Singh, A.K., and O.P. Virmani (1982). Cultivation and utilization of Isabgol (*Plantago ovata*

Fersk) a review. Current Research in Medicinal and Aromatic Plants. 4: 109-120.

Singh, B. (2007). Psyllium as therapeutic and drug delivery agent. Int. J. Pharmaceutics. 334: 1-14.

Steel, R.G.D., J.H. Torrie, and D. Dickey (1997). Principles and procedures of statistics: A biometrical approach. 3rd Ed. McGraw Hill Book Co. Inc., New York, USA.

Toma, A., M.B. Omary, L.F. Marquart, E.A. Arndt, K.A. Rosentrater, B. Burns-Whitmore, L. Kessler, K. Hwan, A. Sandoval, and A. Sung (2009). Children’s acceptance, nutritional and instrumental evaluations of whole grain and soluble fiber enriched foods. J. Food Sci. 74: 139-146.

Urala, N., and L. Lähteenmäki (2007). Consumers' changing attitudes towards functional foods. Food Qual. Prefer. 18: 1-12.

Uysal, H., N. Bilgiçli, A. Elgün, E. NurHerken, and M.K. Demir (2007). Effect of dietary fiber and xylanase enzyme addition on the selected properties of wire-cut cookies. J. Food Eng. 78: 1074-1078.

Vallance, S.L., B.W. Singer, S.M. Hitchen, and J.H. Toe (1998).The development and initial application of chromatographic method for the character media. JAIC. 37: 294-311.

Van-Craeyveld, V.V., J.A. Delcour, and C.M. Courtin (2008). Extractability and chemical and enzymic degradation of psyllium (Plantago ovata Forsk) seed husk arabinoxylans. Food Chem. 112: 812-819.

Van-Craeyveld, V.V., U. Holopainen, E. Selinheimo, K. Poutanen, J.A. Delcour, and C.M. Courtin (2009). Extensive dry ball milling of wheat and rye bran leads to in situ production of arabinoxylan oligosaccharides through nanoscale fragmentation. J. A. Food Chem. 57: 8467-8473.

Vega-Lopez, S., R.L. Vidal-Quintanar, and M.L. Fernandez (2001). Sex and hormonal status influence plasma lipid responses to psyllium. Am. J. Clin. Nutr. 74: 435–441.

Ziai, S.A., B. Larijani, S. Akhoondzadeh, H. Fakhrzadeh, A. Dastpak, F. Bandarian, A. Rezai, H.N. Badi, and T. Emami (2005). Psyllium decreased serum glucose and glycosylated hemoglobin significantly in diabetic outpatients. J. Ethnopharmacol.102: 202-207.