Nutritional and functional characterization of barley flaxseed based functional dry soup mix

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Abstract Barley flaxseed based functional dry soup mix (BFSM) was developed from whole barely flour (46.296%), roasted flaxseed powder (23.148%) and the seasoning (30.555%) comprising several flavoring compounds and anticaking agent, using simple processing technique. Developed BFSM was nutritious. On dry matter basis it contained: protein (14.31%), carbohydrate excluding crude fiber (54.70%), fat (8.70%), ash (17.45%) and crude fiber (4.84%). It was low glycemic soup, free of antinutritional risk and had calorific value of 319.77 kcal/100 g (wet or sample basis, sb) estimated from its composition. 100 g (sb) of BFSM contained 4.36 g β -glucans and 8.08 g total lipid of which 25.6% was ω -3 fatty acids. Different extracts of BFSM revealed the presence of total phenols (0.57–1.86 mg gallic acid equivalent/g, sb) with antioxidants equivalence of DPPH (20.69–39.07%) and FRAP (120–331 μ m Fe (II)/g, sb).

Keywords Whole barley flour · Flaxseed · Soup mix · β -glucans · Glycemic index · ω -3 fatty acid · Antioxidants

Introduction

Functional foods provide physiological benefits like health-promoting or disease-preventing properties beyond the basic function of supplying nutrients (Kaur and Das 2011). In modern era occurrence of diabetes, cardiovascular diseases

and obesity is rising at an alarming rate. Barley (*Hordeum vulgare* L.) has good amount of soluble dietary fiber, mixed linkage (1 $\rightarrow$ 3, 1 $\rightarrow$ 4)- β -D-glucans (Holtekjølén et al. 2006), commonly known as β -glucans. These are linear homopolymers of D-glucopyranosyl residues linked mostly via two or three consecutive β -(1 $\rightarrow$ 4) linkages that are separated by a single β -(1 $\rightarrow$ 3) linkage (Izydorczyk and Dexter 2008). Owing to the soluble fiber, consumption of barley increases viscosity in the intestinal contents, acting as hypoglycemic and hypocholesterolemic agents (McIntosh et al. 1995; Gee et al. 2007). The key mechanism pertaining to such benefits are entrapment of bile acids and reduction in absorption of glucose and lipids from intestinal wall (Newman and Newman 2008; Drozdowski et al. 2010; Lattimer and Haub 2010; Sullivan et al. 2013). According to Talati et al. (2009), barley-derived β -glucans beneficially affect total cholesterol, LDL-cholesterol, and triglycerides. β -glucans improve insulin sensitivity and positively control metabolic syndrome. The increased viscosity extends gastric transition time, prolongs satiety and reduces the hunger pangs thus preventing obesity (Newman and Newman 2008). Barley's potential as a prebiotic has increased significantly as its β -glucans and resistant starch selectively promote the growth of beneficial intestinal microorganisms (Newman and Newman 2008; Sullivan et al. 2013) resulting in the formation of short chain fatty acids, especially butyrate and propionate; benefits of these fatty acids in the large intestine are healthy colonic mucosa, and provision of an energy source for epithelial cells (Newman and Newman 2008). Studies have also commented on positive immunomodulating function of β -glucans (Khouri et al. 2012). Besides these, phenolics and tocopherols (tocotrienols and tocopherols) in barley act as antioxidants and protect against degenerative diseases like heart disease and cancer (Finocchiaro et al. 2005; Dykes and Rooney 2007; Holtekjølén et al. 2011). In addition, bran in whole

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barley offers insoluble fiber necessary for bowel clearance and colon health (Bhatty 1993; Daou and Zhang 2012).

Although barley was used extensively as a food in the past has now been relegated to only about 3% for human food in most countries (Baik and Ullrich 2008). However, recently, the discovery of the health contribution of β -glucans encouraged the use of barley into bread and pasta, but achieved limited success due to its poor baking quality (Erkan et al. 2006; Madhujith and Shahidi 2006; Newton et al. 2011; Gamel and Abdel-Aal 2012). Suggestions are that, barley can be incorporated into yoghurt, beverage, soup, porridge, cookie, noodle, muffin, and extruded cereal (Edney et al. 2002). However the effect of β -glucans depends on its dose, form, solubility, molecular weight and carrier food, and development of foods with sensorial acceptance along with aforementioned physiological benefits is really a challenge (Suortti et al. 2000; Khoury et al. 2012).

Flaxseed (or linseed) (*Linum usitatissimum* L.) is a rich source of α -linolenic acid, soluble and insoluble fiber and lignan, all having several health benefits like prevention of cardiovascular diseases, diabetes mellitus, colon cancer, hypertension, obesity and constipation (Hall III et al. 2006; Maddock et al. 2006; Anonymous 2010). But, flaxseed may contain cyanogenic glycoside (CG), an antinutritional factor, which requires prior removal. Heat treatment, even traditional roasting in iron skillet, may serve this purpose (Wanasundara et al. 1993; Yamashita et al. 2007; Lawley et al. 2008; Kaur and Das 2012). Added advantage in traditional roasting is that it develops attractive nutty flavor (Kaur and Das 2012). According to Alpers and Sawyer-Morse (1996), Aliani et al. (2011) and Hussain et al. (2012) flaxseed has been drawing interest in bakery products including unleavened flat breads. Giada (2010) has published an article on food applications of flaxseed and its components. Recently, Parameshwari and Nazni (2012) observed that chapattis containing traditionally roasted flaxseed powder could be used for blood lipid lowering.

Demand for convenient healthy foods has triggered introduction of several soup mixes in the consumer market with an expected growth rate of more than 25% within few years (Anonymous 2013). Erkan et al. (2006) has studied the physicochemical and sensory properties of barley based thick soup, called Tarhana, fermented to develop a flavor popular over limited region of Southwest Asia, Europe and Africa (İbanoglu et al. 1999). In general, formulation and characterization of barley based soup is very scanty. Report on addition of microencapsulated flaxseed oil in soup formulation as source of ω -3 fatty acid is available in one study by Rubilar et al. (2012). Inglett et al. (2013) has published on water absorption and rheological properties of barley-flaxseed composites developed for future incorporation into food to make it functional. Soup mix based on whole barley flour (WBF) and roasted flaxseed powder (RFP) could be an excellent

possibility to extract maximum benefits of their functional ingredients without any antinutritional risk. However, extensive search reveals non-availability of published literature or patent on soup mix formulation based on WBF and RFP.

The objective of the present study was nutritional and functional characterization of a barley flaxseed based functional dry soup mix (BFSM) developed in this laboratory.

Materials and methods

Materials

WBF (light cream in color, 60 mesh size) of composition as described in Table 1 was obtained from Cardin Health Care Pvt. Ltd. Vadodara, Gujarat, India. Onion and garlic powder were procured from Motherland Marketing, Ahmedabad, Gujarat, India. Tricalcium phosphate, TCP (extra pure E341, Hi-Media Laboratories Pvt. Ltd., Mumbai, India), used as anti-caking agent, was purchased from local dealer. Flaxseed (grain) was purchased from a bulk stock in Goalbazar, the Railway market in Kharagpur, West Bengal, India. The remaining ingredients were from branded flexible pouches: skim milk powder (SMP, Ludhiana Distt. Co-op Milk Producers Union Ltd., Punjab, India), spices, like coriander powder (J. K. Spices & Food Products, Howrah, India), and turmeric and black pepper powder (Sunrise Spices Ltd., Kolkata, India), food grade citric acid (Jason Chemical Works, Kolkata, India), and common salt (Tata Chemicals Ltd., Mumbai, India). All the raw materials were stored in air tight containers at room temperature. Besides raw materials, the chemicals used in experiments for different property measurement were Analar/Extrapure grade. Unless otherwise mentioned the water used in analyses was glass distilled.

Table 1 Proximate composition of whole barely flour (WBF) and roasted flaxseed powder (RFP)

Component	Amount [@] (%)	
	WBF	RFP
Moisture	10.51±0.24 ^S	2.60±0.04
Fat	0.87±0.01	34.74±0.21
Protein	10.21±0.67	18.96±3.10
Crude fiber	1.82±0.03	12.20±0.05
Ash	0.99±0.02	3.71±0.11
Carbohydrate excluding crude fiber	75.61±0.54	27.79±3.22
Carbohydrate including crude fiber [*]	77.42±0.55	39.99±3.17

[@] Mean of 3 replications±SD; ^S Moisture content refers to the WBF as received; ^{*} FAO (1998)

Methods

Preparation of RFP

Flaxseed was manually cleaned (Kaur 2013), sieved through wire mesh and passed through laboratory aspirator (H.T. McGill, Texas, USA). Cleaned flaxseed was roasted at 144–150 °C in an iron skillet with stirring till popping. RFP (vide Table 1 for composition) was obtained by grinding roasted seed in a domestic grinder (Sumeet Research and Holdings Limited, Chennai, India) at medium energy level.

Preparation of BFSM

Since rheology and sensory appeal are the main criteria for soup delicacy, these two properties were selected as the basis for formulation of BFSM. The whole scheme of stage wise development (Kaur 2013) is summarized as follows.

Initially, soup premixes were prepared (D-optimal mixture design, ‘Design-expert’ software ver. 7.0.0, Stat-Ease Int. Co., Minneapolis, USA) containing variable amounts WBF and RFP, and 2.470 g of flavoring (selected from preliminary sensory trials) that consisted of salt and SMP (1 g each), garlic powder (0.15 g), coriander powder and onion powder (0.1 g each), turmeric, black pepper, and citric acid (0.04 g each). If not indicated specifically, mass of the materials quoted throughout the text is on wet or sample basis (sb). The limits of variables were: $4.00 \text{ g} \leq \text{WBF} \leq 5.00 \text{ g}$, and $1.00 \text{ g} \leq \text{RFP} \leq 2.00 \text{ g}$, where $\text{WBF} + \text{RFP} = 6.00 \text{ g}$. Soup premix (8.47 g) was added to 100 ml potable water (Aquaguard cooler cum purifier, Uttarakhand, India) followed by cooking on heater with continuous stirring so as to mimic normal cooking process. The ultimate concentration of the cooked soup premixes, CSPs, was found to be at 8.47% (g/100 ml). The limit of WBF and RFP in the formulation was selected from their viscous behavior (Kaur 2013; Kaur and Das 2014), flavor of RFP (excessive amount imparted bitter taste) and the knowledge of viscosity of some commercially available popular soup mixes. Dependent parameters measured of the CSPs were: apparent viscosity using Brookfield digital viscometer, model: RVT-DV-1 (Brookfield Engineering Laboratory, Stoughton, USA), and sensory qualities (appearance, aroma, taste, consistency and overall acceptability) by subjective method (9-point hedonic scale). Optimization of the amount WBF and RFP in CSP was done on the basis to maximize the dependent parameters. The optimized formulation of soup premix was referred ‘Optimized soup premix, OSP’.

The next step was estimation of the amount of TCP to be added to OSP, using one factor Response surface methodology provided by the software ‘Design-expert’. Design of experiment was done with TCP ($\leq 2 \text{ g/100 g OSP}$) as

independent parameter and caking behavior of the resultant mixes, namely, hygroscopicity, degree of caking, flowability (time required for flowing), and angle of repose, as the responses. The optimum value of TCP for minimizing the caking behavior of the mix was determined following numerical optimization.

BFSM was finally prepared by mixing OSP and optimum amount of TCP. Composition of BFSM is shown in Table 2, where the term seasoning refers to flavoring together with TCP. These ingredients were mixed by tumbling (20–30 min) in a jar followed by mixing (20–30 min) with wire whipper (Anjali Marketing & Research Centre, Mumbai, India). BFSM was then packed in different lots (each of 500 g) in self-sealing (zip lock) pouches with expelling air maximally possible and stored in ambient condition. The pouches were shaken well before withdrawal of the sample for analyses. For each replication, sample was taken from randomly selected lot.

Nutritional characterization of BFSM

Proximate composition

Moisture, protein, fat, ash, crude fiber and carbohydrate content of BFSM were determined following the standard methodology (Ranganna 1987; Cardoso-Santiago and Areas 2001; AOAC 2012).

Elemental composition

Elements like Ca, Mg, Fe, Na, K, Zn, Cu and Mn were estimated (Ranganna 1987) by atomic absorption spectrophotometer (AAS, Perkin Elmer, AAnalyst 700, USA) using standard flame operating conditions. Phosphorus was

Table 2 Amount of ingredients required for BFSM formulation

Ingredient	Percentage (g/100 g of BFSM))
WBF	46.296
RFP	23.148
Seasoning	30.555
Salt	11.574
SMP	11.574
Garlic powder	1.736
Coriander powder	1.157
Onion powder	1.157
Turmeric	0.463
Black pepper	0.463
Citric acid	0.463
TCP	1.968

estimated with UV Vis spectrophotometer (Cary-50, Varian Australia Pvt. Ltd., Australia) at 400 nm (AOAC 2012). Millipore water was used in estimation of elements.

Cyanogenic glycoside (CG)

The amount of CG (in the form of hydrogen cyanide, HCN) was determined by steam distillation of sample, absorbing the distillate in alkaline medium (2.5% NaOH) and then titrating with 0.02 N AgNO₃ solution till the end point recognized by appearance of faint but permanent turbidity against black background (AOAC 2012). Blank was run using distilled water instead of sample. Content of HCN was calculated according to the equation 1 given below.

$$\text{HCN}(\text{mg}) = \text{ml of 0.02 N AgNO}_3\text{ solution used} \times 1.08 \quad (1)$$

To estimate the amount of CG in the procured flaxseed and the effectiveness of roasting on its removal, the amount was also determined in the powder made from raw as well as roasted flaxseed.

Calorific value (CV)

The calorific value (heat of combustion) was determined by calculation from food components following the principle of Nutrition Labeling and Education Act (NLEA) of 1990 (Painter 2006) and bomb calorimeter.

Calculation method (Painter 2006) involved multiplication of percent fat, protein and carbohydrates (excluding dietary fiber) by their physiological energy change coefficients (as full energy of combustion is not available in human body), i.e., 9.0, 4.0 and 4.0 kcal/g, respectively, followed by their subsequent addition (FAO 2003) (equation 2), where H_a is calorific value (kcal/100 g) by calculation.

$$H_a = \% \text{Fat} \times 9.0 + \% \text{Protein} \times 4.0 + \% \text{Carbohydrate (excluding crude fiber)} \times 4.0 \quad (2)$$

The total or gross heat value of BFSM was determined experimentally by using digital bomb calorimeter (Toshiba, Delhi, India). About 1 g BFSM was burnt in compressed oxygen (25 kg/cm²) in the calorimetric bomb immersed in water (ISO-1928 2009). The gross heat value was calculated as given in equation 3

$$H_c = \{ [W_c \Delta T - (C_t L_t + C_w L_w)] m_s \} \times 0.1 \quad (3)$$

where H_c is gross heat value of the sample (kcal/100 g), W_c is heat capacity of the calorimeter (2370 cal/°C), ΔT is rise in temperature (°C), m_s is mass of the sample (g), C_t and C_w are calorific value of thread (2.1 cal/cm) and wire (2.33 cal/cm), and L_t and L_w are the length of thread (10 cm) and wire (7 cm).

Functional characterization of BFSM

Total phenolics and antioxidant capacity

BFSM (10 g) was extracted by shaking continuously with 100 ml of different solvent(s) (distilled water, absolute ethanol, 80% ethanol, absolute methanol, 80% methanol, absolute acetone, and 80% acetone) in a water bath at 25±1 °C for 90 min followed by centrifugation (REMI Instruments Ltd., Mumbai, India) at 950×g for 15 min, as described by Atala et al. (2009). The extract was used for the assessment of total phenolics content (TPC) using a modified Folin-Ciocalteu assay by Slinkard and Singleton (1977) methodology. Antioxidant capacities of the extracts were determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and ferric reducing antioxidant property (FRAP) following the method of Brand-Williams et al. (1995) and Benzie and Strain (1999), respectively.

ω-3 fatty acid content

Estimation of ω-3 fatty acid involved extraction of total lipids from BFSM (Bligh and Dyer 1959) with sequential use of different solvent systems, preparation and purification of methyl esters of the fatty acids (Mangold 1969; Christie 1982) and identification of the fatty acids by gas chromatography (GC 1000 EPC Gas Chromatograph, Chemito Instruments Pvt. Ltd, Mumbai, India) using BPX-70 megabore capillary column (SGE, Australia). The whole procedure was carried out in Drug Research & Development Centre, Jessore Road, Kolkata, India.

β-glucans content

β-glucans content was determined following the standard enzymatic method (AOAC 2012); prior to enzymatic treatment, the sample was desugared. The whole procedure was carried out in an accredited food hygiene and health laboratory (certified by Bureau of Indian Standards), National Accreditation Board for Testing and Calibration Laboratories (NABL), Pune, India.

Glycemic index (GI)

GI of BFSM was assessed by in vitro as well as in vivo studies.

In vitro analysis was done following the method of Goñi et al. (1997). It involved the treatment of BFSM (50 mg) with pepsin, α -amylase and amyloglucosidase to convert starch to glucose. Same procedure was followed for reference food, that is, commercially available white bread of popular brand, WB (freshly collected from a retail outlet). Area below the hydrolysis curve (AHC, indicating glucose digestion) for BFSM and WB was then calculated at regular time intervals. Hydrolysis Index (HI) of BFSM was calculated as the ratio between the AHC of the BFSM and the AHC of the WB, and expressed in percentage. The GI was calculated according to equation 4.

$$GI = 39.71 + 0.549 \times HI \quad (4)$$

In vivo analysis was carried out to investigate the effect of consumption of cooked BFSM, i.e., in soup form on blood glucose in human subjects, as compared with that of freshly collected white bread (WB) as a reference food (Foster-Powell et al. 2002; Chlup et al. 2004; Shobana et al. 2007; Atkinson and Brand-Miller 2011). The test was carried out after getting the ethical clearance from Institute Ethical Committee, Indian Institute of Technology (IIT), Kharagpur, West Bengal, India.

The study was conducted on the healthy students (four male and six female) of the Department of Agricultural and Food Engineering, IIT, Kharagpur during October, 2012. Selected with their consent through the proforma in three languages, i.e., English, Hindi (national language of India) and Bengali (regional language of West Bengal, India), they were requested to fast overnight and maintain their usual activity schedule throughout the study period. The volunteers, i.e., students were also informed that they could quit the experiment at any time, without feeling forced to give any explanation. The age, sex, height of the volunteers was noted and is described in Table 3. Their basal metabolic rate (BMR) was measured.

BFSM at the rate of 8.64 g (8.47 g OSP +2% TCP (optimum value) of 8.47 g) per 100 ml potable water was cooked as mentioned above. The cooked soup (250 ml, i.e., 21.60 g BFSM) was served in bowls to the volunteers. A portion of WB, providing equivalent amount of available carbohydrate (composition of WB was analyzed in the laboratory) present in served soup, was given to each volunteer. The blood

glucose level after overnight fasting and after every 30 min interval up to 120 min following consumption of the food was recorded according to internationally accepted protocol of finger-prick method. All the subjects consumed WB and BFSM on two different occasions with an interval of about 5–7 days; both the tests were performed at room temperature.

The incremental area under the blood glucose response curve (IAUC) for the test and reference samples was calculated geometrically using the standard method (Wolever and Jenkins 1986), for each volunteer. Areas where the curve dropped below the baseline were excluded. GI and GL (glycemic load) were calculated as given in equations 5 and 6.

$$GI = (IAUC \text{ for served soup} / IAUC \text{ for WB}) \times 100 \quad (5)$$

$$GL = (GI \times \text{available carbohydrates (g) in one serving, 250 ml}) / 100 \quad (6)$$

Statistical analysis

Except the few properties investigated with the help of outside laboratories, all the experiments following objective methodology were replicated thrice (sample replicate). For in vivo GI, the replication was 10. The mean and standard deviation (SD) was evaluated. The effect of parametric variation on the mean, e. g., extraction medium on total phenolics and its antioxidant capacity, and time and nature of food on blood glucose level was investigated by one way and two way analysis of variance (anova, F-test). Calculations were done by Minitab (ver. 15.1.20.0., Minitab, Inc., Pennsylvania, PA, USA).

Results and discussion

Nutritional characterization of BFSM

Proximate composition

Proximate composition of the BFSM is given in Table 4 that gives the idea of its nutritive value. BFSM contains protein (14.31%), carbohydrate excluding crude fiber (54.70%), fat (8.70%), ash (17.45%) and crude fiber (4.84%), on dry basis (db). It may be worth comparing this composition with available information on different soups. Rokhsana et al. (2007) reported the proximate composition (% db) of rice, corn and wheat based soups as: 20.12, 20.43 and 20.86% for protein; 69.72, 71.36, 71.89% for carbohydrate; 1.50, 1.35, 1.34% for fat; 6.20, 4.84, 5.18% for ash; and 2.45, 2.01, 0.72% for crude fiber, in that order. Thus, compared to these, BFSM possesses less protein and carbohydrate, whereas it is superior in fat, ash

Table 3 Particulars of the volunteers of the in vivo study

Particular	Description
Age	20–30 years
Weight	Normal (excluding overweight and obese)
Medication	Not on any type of prescribed medication
Smoking	Non-smoker
Blood glucose	Normoglycemic

Table 4 Proximate and elemental composition of BFSM

Component	BFSM (sb) [§]	BFSM (db) [#]
Proximate composition (%)		
Moisture	9.77±0.58	–
Fat	7.85±0.10	8.70
Protein	12.91±1.21	14.31
Crude fiber	4.36±0.24	4.84
Ash	15.74±0.19	17.45
Carbohydrate excluding crude fiber	49.36±1.09	54.70
Carbohydrate including crude fiber*	53.73±0.87	59.54
Elemental composition (mg/100 g)		
Ca	873.49±0.44	968.07
Mg	125.86±0.73	139.49
Fe	20.67±0.20	22.91
Na	5726.17±24.55	6346.19
P	583.29±0.45	646.45
K	200.97±0.05	222.73
Zn	4.32±0.01	4.79
Cu	0.66±0.00	0.73
Mn	3.86±4.98	4.28

[§] Mean of 3 replications±SD; [#] Dry basis value was obtained by dividing the

mean of sb by the solid content (1–0.0977)

and crude fiber content. Erkan et al. (2006) reported the percentage (db) for protein, fat, and ash ranging within 10.10–15.90, 3.39–4.65, and 1.98–2.48, respectively, for three types of Tarhana prepared from hulled and hull-less barley. In the present study, BFSM contains comparable protein but higher fat and ash content. This is probably due to the presence of RFP in mix. On comparison with a market available soup of popular brand (MAS) that contains protein (6%) and carbohydrate (68%), as mentioned on its package, BFSM has more protein (12.91%) and less carbohydrate (53.73%). Prevalence of less carbohydrate may indicate suitability of BFSM for diabetic personnel (Singh et al. 2003).

Elemental composition

Elements, in macro and micro amount, are necessary for biological processes and perform a vital role in metabolic functions, normal growth and development (Melø et al. 2008). Physiologically, the most important macro minerals include calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na) and potassium (K). They help in maintenance of pH, osmotic pressure, nerve conductance, muscle contraction, energy production and almost all other aspects of biological life (Chekri et al. 2012). The elements that are required in micro amount include iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn). Elemental composition of BFSM is included in Table 4. It is evident from the table that BFSM contains

all these elements. Scanty information is available for elemental composition of other soups. Erbaş et al. (2005) reported elemental content (mg/100 g, db) of wheat-yogurt based Tarhana that include Ca (267.90), Mn (3.23), Zn (4.40), Fe (9.70), Na (2149.20), K (594.80), Mg (158.20), and Cu (1.00). Comparing with this report, it is observed that BFSM is a better source of Ca, Na, Fe, Zn and Mn. Moreover, the value of Na and K of BFSM is found to be comparable with the Na (4911 mg/100 g) and K (211 mg/100 g) of MAS as reported on its package.

Cyanogenic glycoside (CG)

The raw flaxseed (as procured) contains 156.15±3.40 mg HCN/kg which lies within the allowable limit quoted in literature (Daun et al. 2003), and thus ensures safety of the raw material used. On roasting of whole seed followed by grinding, the amount reduced to 17.55±1.35 mg HCN/kg (RFP contains 2.60% moisture, vide Table 1), indicating about 88.76% reduction. Further, for preparing in the soup form, BFSM powder was cooked. Perhaps these two stages of heat treatment completely destroyed the CG as it was not detected in the cooked soup (Daun et al. 2003; Feng et al. 2003; Yang et al. 2004; Imran et al. 2013).

Calorific value

The calorific value of BFSM as estimated by calculation and measured by Bomb calorimeter is found to be 319.77±2.80 and 467.03±10.22 kcal/100 g. The coefficients used in Eq. 2 for calculation of the calorific value do not take care of the portion of energy that is lost due to incomplete digestion and absorption, faeces, and other physiological processes (FAO 2003). Therefore, the value calculated by general factor system is less than the value obtained from bomb calorimeter that indicates the total combustible energy of food. The value obtained for BFSM by calculation using the physiological energy change coefficients is found to be in the same order in relation to several soups based on rice, wheat and corn having 347, 350 and 353 kcal/100 g, respectively (Rokhsana et al. 2007), and MAS with 340 kcal/100 g as reported on its package.

Functional characterization of BFSM

Total phenolics and antioxidant capacity

A wide range of phenolic antioxidant compounds, in bound and free forms, has been found in barley (Holtekjølén et al. 2006; Gamel and Abdel-Aal 2012). A high percentage of the free phenolics are proanthocyanidins or flavonoids, while the bound compounds are mainly phenolic acids and often covalently linked to macromolecules such as polysaccharides

(Bonoli et al. 2004; Holtekjølén et al. 2011). Bonoli et al. (2004) also noted that free phenolic compounds, depending on nature, can be selectively extracted from barley flour by simple solid–liquid extraction using different extraction solvent/solvent mixtures, while bound phenolic compounds can only be recovered by either acidic or alkaline hydrolytic digestion. Polyphenols in flaxseed include lignans (mainly secoisolariciresinol diglucoside, SDG), phenolic acids and flavonoids (Kasote 2013). SDG has been found to be soluble in water and alcohol (Tourè and Xueming 2010). The value of TPC of different extracts of BFSM, probably consisting of free soluble phenolics, along with its antioxidant activity is given in Table 5. One way anova indicated that the value changes significantly ($p < 0.01$) with nature of solvent, i.e., extraction medium. It is seen from the table that TPC of different medium lies in the range of 0.57 to 1.86 mg gallic acid equivalent (GAE)/g of BFSM. Thus, for BFSM, extraction capacity of the solvent follows the order: aqueous >80% acetone >absolute ethanol >80% ethanol >80% methanol >absolute acetone >absolute methanol. Dill leaf aqueous extract, used as condiment in several food products, is a rich source of antioxidants amounting 65.05 mg GAE/g dried plant (Isbilir and Sagiroglu 2011). Rekha et al. (2010) prepared a soup mix containing dill leaf and reported that TPC of its aqueous extract was 1.70 mg GAE/g mix. Tom-Yum, a popular commercially available Thai soup, containing several herbs and spices which contribute to its antioxidants was found to indicate TPC (in 30% v/v methanolic extract) as 1.50 mg GAE/g of dry mix (Siripongvutikorn et al. 2009). Thus, the soup mix in the present study is at par with these reported values.

The primary mode of action of an antioxidant is scavenging free radicals by transfer of either hydrogen atom or single electron. The activity of several antioxidants has been

measured using DPPH, a stable free radical. For different solvent extracts of BFSM, the DPPH scavenging capacity decreased in this order: 80% acetone >80% ethanol >80% methanol >absolute methanol >absolute ethanol >aqueous >absolute acetone (Table 5). Isbilir and Sagiroglu (2011) reported the scavenging effect of water and ethanol extracts of dill leaves, both having concentration of 800 µg/ml, as 79.73 and 87.22%. Depending on the type of cultivar, mung bean extract indicated the radical scavenging activity within 42.1–47.2% (Li et al. 2012).

Ferric reducing ability measured for a sample may indirectly reflect its total antioxidant power. Here, FRAP value of several extracts varies within 120–331.33 µm Fe (II)/g BFSM (Table 5). Thus, it has more FRAP value when compared with high antioxidant foods like banana, orange, red plum and strawberry, having 1.64, 11.81, 20.57 and 33.52 µm Fe (II) /g, respectively (Apak et al. 2007). Li et al. (2012) reported FRAP value of several mung bean soup mixes as 206–217 µm Fe (II) /g. Thus, from these values it appears that antioxidants present in BFSM has considerable ferric reducing capacity.

It may be noted from Table 5 that amount of total phenolics is not proportional with DPPH scavenging activity, whereas a positive correlation (Pearson's correlation = 0.918, plot not shown) is observed with FRAP value. It is worth mentioning that antioxidant compounds such as polyphenols may be more efficient reducing agents for ferric iron but some may not scavenge DPPH free radicals as efficiently due to steric hindrance (Wong et al. 2006; Fidrianny et al. 2014). It has also been reported that structural variation of polyphenolic compounds as well as nature of reaction medium show different behaviour towards DPPH free radical scavenging effect, while FRAP value is linearly related to the molar concentration of the antioxidant(s) present (Katalinic et al. 2006; Liu et al. 2007;

Table 5 Total phenolics and antioxidant capacity of different extract of BFSM

Extraction medium*	TPC [#] (mg GAE/g BFSM)	DPPH ^S (%)	FRAP [#] (µm Fe (II) /g BFSM)
Aqueous (water)	1.86±0.07	23.09±0.50	331.33±3.06
80 % acetone	1.39±0.05	39.07±0.38	311.33±3.06
80 % ethanol	0.88±0.06	30.52±0.14	120.00±5.29
80 % methanol	0.79±0.02	28.95±0.31	121.33±4.16
Absolute acetone	0.74±0.05	20.69±0.33	186.67±8.33
Absolute ethanol	1.37±0.06	25.49±0.14	272.67±5.03
Absolute methanol	0.57±0.01	28.38±0.25	126.00±2.00

* Effect of extraction medium (F-test) on TPC, DPPH and FRAP is positive ($p < 0.01$);[#] sb;^S

Mean of 3 replications±SD

Villãno et al. 2007; Bhoyar et al. 2011; de Oliveira et al. 2012; Terpinc et al. 2012; Fidrianny et al. 2013).

ω-3 fatty acid content

The total lipid content of BFSM is found to be 8.08%, and its fatty acid profile is presented in Table 6. It is apparent from the table that ω-3 fatty acid, an important factor for reducing the risk of cardiovascular diseases, constitutes 25.6% of the total fatty acids. Presence of several ω-3 fatty acids in fish meal soup was reported by Stevanato et al. (2007). Rubilar et al. (2012) reported the development of ω-3 fatty acid enriched soup powder incorporating microencapsulated flaxseed oil, where microencapsulation was done separately by spray drying of the extracted oil. The content of ω-3 and ω-6 fatty acids of few edible oils are compared with that of BFSM in Table 7, which shows superiority of the latter with respect to ω-3 fatty acid. It may be worth mentioning that for maintenance of sound health, the ratio of ω-6 to ω-3 fatty acids should be around 1 whereas, the food habit of current lifestyle leads the ratio to be 10:1 to 20:1 (Simopoulos 1991; Singh et al. 2012). Thus, to maintain the required ratio, BFSM may be a good source of ω-3 fatty acid contributing to the functionality requirements of consumers.

β-glucans content

The amount of β-glucans is found to be 4.36 g per 100 g of BFSM. One serving (250 ml, 8.64 g per 100 ml) of BFSM thus provides 0.9418 g of β-glucans. According to the United States Food and Drug Administration (USFDA 2008; Thondre and Henry 2009), if a food product contains at least 0.75 g of β-glucans per serving it is eligible for the ‘health claim’ tag with respect to the cholesterol-lowering effect of β-glucans and thereby can reduce risk of coronary heart disease. On the basis of this statement, BFSM in the present study may

be categorized as ‘health food’. Erkan et al. (2006) reported the β-glucans content in three type of barley based Tarhana to be 2.40, 2.90 and 3.55% (db). It is evident from these values that BFSM has higher β-glucans content in comparison to barley Tarhana.

Glycemic index (GI)

Foods can be classified into three groups on the basis of GI scale that ranges from 1 to 100 as: high GI foods (GI 70+, rapid rise in blood-glucose levels), intermediate GI foods (GI 56–69, medium rise in blood-glucose levels), and low GI foods (GI 55 or less, slower rise in blood-glucose levels) (Howlader and Biswas 2009). This classification is based on the rate at which 50 g of available carbohydrate affects the blood glucose level. In vitro analysis of BFSM indicated that having the GI of 52.89 it falls under the low-glycemic foods when compared with the high glycemic food, i.e., white bread whose GI is 80.89.

For in vivo analysis, the average age, height, weight and BMR of the volunteers were 25.7 ± 2.00 year, 163.43 ± 10.50 cm, 57.85 ± 10.00 kg, and 1454.39 ± 199.09 , respectively. A number of factors, viz., rate of gastric emptying, insulin response, and amylase in gut influence the glucose metabolism (Shobana et al. 2007), and therefore, a person to person variation in blood glucose level is observed even if fed with same amount of available carbohydrate. The mean value of blood glucose level of the volunteers, for BFSM and WB at different time interval, is represented in Fig. 1. When analyzed by two way anova, it is observed that blood glucose level is controlled ($p < 0.01$) by the nature of food as well as time interval. Referring to Fig. 1, in the first 30 min, blood glucose level increases similarly for both WB and BFSM. After this, BFSM displays continuous decreasing tendency whereas for WB, a steady level is maintained within 30–60 min, following

Table 6 Fatty acid composition of total lipid in BFSM

Fatty acid ^a	Amount (%, w/w of total fatty acids)	Fatty acid ^a	Amount (%, w/w of total fatty acids)	Total fatty acid	Amount (%, w/w of total fatty acids)
14:0	1.0	20:0	0.2	*Total -ω3	25.6
15:0	1.4	21:0	0.2	*Total -ω6	12.0
16:0	11.0	22:0	0.4	Total saturates	24.2
17:0	1.2	22:1	0.1	Total monoenes	38.2
18:0	6.5	23:0	0.1		
18:1ω9	36.7	23:1	0.1		
18:2ω6	11.9	24:0	0.4		
18:3ω6	0.1	25:0	0.2		
18:3ω3	25.6	26:0	0.1		

^a Carbon chain length: number of double bonds; * ‘ω’ refers to the position of double bond in relation to methyl group on the end of the fatty acid

Table 7 Amount of ω -3 and ω -6 fatty acids in BFSM and different oils*

Fatty acid	%, w/w of total fatty acids					
	BFSM	Cod liver oil	Almond	Olive	Sunflower	Walnut
ω -3	25.6	—	—	1	1	5
ω -6	12.0	5	17	10	68	51

(Source: Zamora 2012); * ' ω ' refers to the position of double bond in relation to methyl group on the end of the fatty acid

a decreasing trend thereafter. In the period following 30 min, WB maintains higher blood glucose level compared to that of BFSM. Based on the area generated under these curves, GI of BFSM is found to be 55.457 ± 6.30 , categorizing it as low glycemic food; corresponding GL is calculated to be 5.37 ± 1.89 .

Molecular weight and solubility of β -glucans control the viscosity inside the gastrointestinal tract and, therefore the health benefits derived from it (Suortti et al. 2000). Also as reported by Lattimer and Haub (2010), besides the nature of food source molecular weight of β -glucans depends on the former's processing history. They also quoted extrusion, baking, and hot water extraction process decreased molecular weight, while Naumann et al. (2006) and Theuwissen and Meinsk (2007) obtained β -glucans through dry milling process in which it was not significantly degraded. Moreover, Liu (2010) reported that rheological properties of β -glucans may be affected by its interactions with starch and protein in integrated food system. A significant negative relationship was also seen between fat and protein content of the foods and its glycemic index (Jenkins et al. 1981). On background of these possibilities, ability of β -glucans in presence

of other constituents of BFSM in making the cooked soup to act as low glycemic food appears quite promising for functional food development.

However, in addition to reduction in absorption of glucose and lipids from intestinal walls caused by β -glucans as mentioned above, studies indicated that diet with high soluble fiber may also impair protein digestibility (Anonymous 1984; Kaneko et al. 1986). Regarding minerals, it was reported (Wisker et al. 1991; Shah et al. 2009) that consumption of barley fiber did not affect absorption of calcium but lowered slightly for magnesium. Dietary fiber may also reduce rates of polyphenols absorption mainly by physically trapping the antioxidants within the fiber matrix, as stated by Palafox-Carlos et al. (2011). However, these were some scattered isolated observations. The actual effects incurred will be determined by the nature of whole food, its intake and the subject consuming it. Aqueous extract of BFSM shows considerable phenolics content and good antioxidant capacity, reasoning their easy bioavailability. Palafox-Carlos et al. (2011) opined that only the compounds released from the food matrix and/or absorbed in the small intestine are potentially bioavailable and in a condition to exert their beneficial effects.

Fig. 1 Variation of average blood glucose value with time for WB and BFSM

Conclusion

Barley flaxseed based functional dry soup mix (BFSM) is nutritious with reasonable calorific value. It is free of antinutritional risk. Low glycemic index of BFSM additionally favors its use by diabetic. BFSM containing 0.9418 g β -glucans per serving of 250 ml (8.64 g/100 ml) can be called as 'health food'. Ingredients in BFSM highly favor its use as functional soup containing good source of phenolics with adequate antioxidant activity, and ω -3 fatty acid.

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