LWT - Food Science and Technology 61 (2015) 510–515

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Nutritional characterization and oxidative stability of α-linolenic acid in bread containing roasted ground flaxseed

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ARTICLE INFO

Article history:
Received 7 June 2014
Received in revised form
31 October 2014
Accepted 6 November 2014
Available online 25 November 2014

Keywords:
ALA
Flaxseed
GI
Dietary fiber
SDG

ABSTRACT

Flaxseed (*Linum usitatissimum*) contain three main bioactive components namely α-linolenic acid, dietary fibers and lignan. These are responsible for numerous reports suggesting positive health benefits from flaxseed. Hence different amounts of ground roasted flaxseed flour was incorporated into bread and soluble and insoluble fibers, in-*vitro* protein digestibility, in-*vitro* glycemic index (GI), in vitro antioxidant activity and α-linolenic acid (ALA) content were evaluated. Protein digestibility of flaxseed enriched bread was significantly lower than the control (70.9 versus 78.5 g/100 g). In-*vitro* GI was significantly lower (51.3 compared to 94.6 g/100 g in the control bread) and ALA content of optimized bread (containing 10 g of flaxseed per 100 g), as determined using GC–MS, was about 1.51 g/100 g of bread whereas omega-6 (linoleic acid) content was about 0.15 g/100 g of bread. During 5 days storage period, peroxide value, anisidine value and free fatty acids were increased in flaxseed supplemented bread samples that were stored at 30 °C and at 4 °C.

1. Introduction

Flaxseed (*Linum usitatissimum*) is a food and fibre crop that is grown in cooler regions of the world which is also known as linseed. Flaxseed contains omega fatty acids such as omega-3 and omega-6, linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), (Reddy, Jayathilakan, Pandey, & Radhakrishna, 2012). All three of these fatty acids have been shown to reduce the risk of cardiovascular diseases (Rodriguez-leyva, Bassett, Mccullough, & Pierce, 2010; Pan et al., 2012; Rodriguez-Leyva D et al., 2013. These authors also stated that ALA has been found to be beneficial in the brain development of infants, reduction in blood lipid concentrations and in the prevention of cardiovascular diseases. It also contains elevated amounts of proteins and dietary fibers and lignan such as secoisolariciresinol diglucoside (SDG). Furthermore, flaxseed dietary fibers exhibit positive effects of reducing constipation (Ganorkar & Jain, 2013). SDG have been proved for antioxidant activity and free oxygen radical scavenging activity (Kitts, Yuan, Wijewickreme, & Thompson, 1999) and has shown to possess anticancer properties (Kangas, Saarinen, & Mutanen, 2002). Several studies have revealed that flaxseed components can provide health benefits in human beings.

There are numerous reports available on the addition of flaxseed in various cereal based foods such as breads (steamed and flat), rice paper, cookies and spaghetti as well as other bakery products (Cameron, Du, & Hosseinian, 2013; Hao & Beta, 2012; Manthey, Lee, & Hall, 2002; Marpalle, Sonawane, & Arya, 2014a; Rodrigues, Fanaro, Duarte, Koike, & Anna Lucia, 2012). Due to the nutritional and functional aforementioned reasons anticipating an increased consumption of flaxseed containing foods, it has become necessary to evaluate whether it is feasible in terms of stability, to incorporate flaxseed flour as an ingredient to bread. Recent reports have focussed on the effect of flax addition on the flavor profile of different food products such as muffins, snack bars and bagels since flaxseed contain such a rich source of biologically active compounds that must be ingested in sufficient quantities and this is in direct association with consumer acceptability (Aliani, Ryland & Pierce, 2011; Aliani, Ryland, & Pierce, 2012). The flaxseed flour was roasted and used to improve the taste and to mask the nutty flavour given by the raw flaxseed flour. Our previous work was

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http://dx.doi.org/10.1016/j.lwt.2014.11.018
0023-6438/

reported with the addition of roasted brown flaxseed flour and their effect on sensory parameters, water absorption, colour and stickiness of dough, crust and crumb and bread quality effects (Marpalle, Sonawane, & Arya, 2014b). Hence the objective of this study was to perform a nutritional evaluation of bread by determining soluble and insoluble dietary fibers, in-*vitro* protein digestibility, in-*vitro* glycemic index, in-*vitro* antioxidant activity and fatty acid profile of bread supplemented with roasted flaxseed flour.

2. Materials and methods

Refined wheat flour and gluten were gifted by General Mills Pvt. Ltd., Mumbai, India. Branded whole wheat flour (Aashirwad Atta, ITC Foods), shortening (Godrej), table salt (Tata), glycerol mono stearate (GMS), sugar, active dry yeast (Blue Bird Foods) and brown flaxseed were procured from a local market in Mumbai, India. All other chemicals used for the analysis were of analytical grade. Enzyme samples such as α-amylase (26900 U/ml), protease (1, 05,000 HUT/g), amylo-glucosidase (402 GU/g), pancreatin (amylase: 39 USP U/g. lipase: 7.65 USP U/g) and pepsin were gifted by Advance Enzymes, Mumbai, India.

2.1. Preparation of roasted flaxseed flour and bread

Brown flaxseed was roasted on pan about 80–90 °C for 10 min. Flaxseed flour was prepared using laboratory grinder having 0.5 mm particle size (Marpalle et al., 2014b). Bread was prepared using a straight dough method (Amendola, Rees, & Lundberg, 2002). Initially all the ingredients were weighed, mixed into white wheat flour and kneaded uniformly by adding water to form finished bread dough. Dough was kept for bulk fermentation for about 1 h at 30 °C and 80% relative humidity (RH) followed by scaling, intermediate proving, moulding and second proving (for about 1–1.25 h). Finally baking was carried out at 220 °C for 20–25 min in baking oven. After baking, breads were removed from pan and allowed to cool at room temperature.

Ingredients were optimized for the preparation of bread based on sensory overall acceptability score and contained 1 g of salt, 3 g of yeast, 0.5 g of GMS, 6 g of sugar, 2.5 g of shortening, 65 g of water and 10 g of roasted ground flaxseed (Marpalle et al., 2014b).

The control and roasted ground flaxseed bread samples were prepared as described earlier, and were periodically evaluated for peroxide value, FFA and Anisidine value during five days of storage at 30 °C and 4 °C. In the case of 4 °C samples, before analysis, they were brought to room temperature.

2.2. Nutritional characterisation of bread

2.2.1. Dietary fiber

Total, soluble and insoluble dietary fibre contents were determined using an enzymatic method (Furda, 1981). The sum of insoluble dietary fibre and soluble dietary fibre contents were calculated as total dietary fibre. For every sample assayed; a blank was run to measure any error in the reading contributed from reagents. Samples were dehydrated and defatted followed by soxhlet extraction using petroleum ether. In 1 g of sample, 40 ml of MES-TRIS blend buffer solution of pH 8.2 was added and mixed using a magnetic stirrer. While stirring at low speed; heat-stable α-amylase solution (50 μL) was added. Samples were then covered with aluminium foil and incubated for 35 min in a shaking water bath at 95–100 °C. Samples were then removed and 10 ml water was added and cooled to 60 °C. To this solution, 100 μL of protease solution was added and incubated for 30 min at 60 °C. Samples were then removed and pH of solution was adjusted to 4.1–4.8 with 0.561 N HCl and 5(g/100 g) NaOH. Then 200 μL of amylo-glucosidase solution was added and then incubated at 60 °C during 30 min. Then, the digested solution was centrifuged at 5000 × *g* for 5 min and supernatant was separated. The residue was washed twice with hot (70 °C) water (10 ml), and all the supernatants were pooled for determination of soluble dietary fibers (SDF). Insoluble residue was washed with 15 ml 95% ethanol and acetone (twice) centrifuging at 5000 × *g* for 5 min between washes. Then the insoluble residue was oven dried at 100–105 °C. The whole experiment was carried out in duplicate so that one of the portions of residue was used for ash content and the other for protein determination using the Kjeldhal method. Insoluble dietary fiber (IDF) was calculated as = weight of insoluble residues − weight of ash − weight of protein.

For determination of SDF, the supernatants were water washed and 4 times volume of 95% ethanol was added and kept for 12 h for precipitation. The supernatant was discarded and the residue was washed twice with 15 ml 95% ethanol and acetone and centrifuged at 5000 g for 5 min. Similar to IDF, residue was then oven dried at 100–105 °C. SDF content was calculated as = weight of insoluble residues − weight of ash − weight of protein.

2.2.2. In vitro protein digestibility

Protein digestibility (in-*vitro*) was assessed employing pepsin and pancreatin following the method of Akeson and Stahmann (1964). Initially 100 mg of dried and defatted sample was incubated with 1.5 mg pepsin in 15 ml of 0.1 N HCl at 37 °C during 3 h. After neutralization with 7.5 ml of 0.2 N sodium hydroxide and addition of 4 mg pancreatin in 7.5 ml of phosphate buffer with pH 8.0, the digestion mixture was incubated for an additional 24 h at 37 °C. Then 5 g of trichloroacetic acid (TCA) was added to the protein digest. Finally digested samples were centrifuged at 5000 × *g* during 10 min. The supernatant was discarded and residue was oven dried at 40–50 °C. The nitrogen contents of the sample and the undigested residue were determined by the micro-Kjeldahl method (AOAC, 1995). The digested protein of the sample was calculated by subtracting residual protein from total protein of the sample according to Goni, Garcia-Alonso, and Saura-Calixto (1997).

$$\text{Protein digestibility (g/100g)} = \frac{\text{Digested protein}}{\text{Total protein}} \times 100$$

2.2.3. In-vitro glycemic index (GI)

In-*vitro* GI of bread samples were determined according to methodology described by Goni et al. (1997). Initially 50 mg of dried and defatted bread samples were dispersed in 10 ml of HCl–KCl buffer of pH 1.5. Gastric phase was developed for 1 h at 40 °C by addition of 0.2 ml of a solution containing 1 g of pepsin in 10 ml HCl–KCl buffer and the volume was adjusted to 25 ml with phosphate buffer pH 6.9. To this 5 ml of α-amylase solution in phosphate buffer containing 2.6 IU were added to each sample. These samples were incubated at 37 °C with moderate agitation. At 30 min intervals during 3 h, 1 ml of sample was taken and the amylase was inactivated immediately by incubating at 100 °C for 5 min with vigorous shaking and refrigerated until the end of incubation time. Then 3 ml of 0.4 M sodium acetate buffer (pH 4.75) was added to each aliquot. The digested starch was completely hydrolysed into glucose by incubating aliquots with 60 μL of amylo-glucosidase at 60 °C for 45 min. Then final volume was adjusted to 10 ml with distilled water. Glucose was determined using 3, 5-dinitrosalicylic acid method. The glucose was converted into starch using multiplying factor 0.9. Commercial white bread was used as a reference product. The graphs were plotted between

starch hydrolysis (%) in function of time. The area under the hydrolysis curve (AUC) was calculated using a trapezoidal formula. The hydrolysis index (HI) was obtained by dividing the area under hydrolysis curve of each sample by the corresponding area of a reference sample (white bread). The expected GI was calculated using the following equation:

$$EGI = 39.71 + (0.549 \times HI)$$

2.2.4. In vitro antioxidant activity

Antioxidant activity of optimized bread was determined using DPPH (1, 1 diphenyl-2-picrylhydrazyl) free radical-scavenging assay. 1,1-diphenyl-2-picryl- hydrazyl (DPPH) is a stable free radical which has an unpaired valence electron in one atom of the nitrogen bridge (Eklund et al., 2005). Initially, extraction of antioxidant compounds was done in methanol using a 1:10 proportion. Then 0.1 ml of extract was mixed with 3.9 ml of 0.2 mM DPPH solution. The absorbance was measured at 517 nm after 90 min of incubation. Similarly a blank was measured at the same wavelength. The standard curve for trolox was plotted between 20 and 200 μg/ml. Results were expressed in μg Trolox equivalents (TE)/g. Inhibition (g/100 g) was determined using equation:

$$\text{Inhibition}\left(\frac{g}{100g}\right) = \frac{(A_0 - A_1) \times 100}{A_0}$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of sample extract.

2.2.5. Calories

The caloric value was calculated using the Atwater conversion factors: 9 kJ/g of lipid, 4 kJ/g of carbohydrate, and 4 kJ/g of protein (Frary & Johnson, 2005).

2.2.6. Fatty acid profile

Fatty acid profile, which included omega 3 and omega 6 content, was determined using gas chromatography mass spectroscopy (Varian 450 GC-Varian 220 MS). Initially fat in the bread was extracted using modified method of Hara and Radin (1978). Approximately 8 g of moisture free ground bread samples were weighed in conical flasks. To this 20 ml of hexane/isopropanol (3:2 v/v) were added. Then cotton-covered flasks were kept on an orbital shaker for 24 h then left to settle at 4 °C in the dark, then decanted into a 50 ml centrifuge tube. The extract was centrifuged at 5000 g for 10 min at 4 °C. The supernatant was then transferred into a 100 ml round bottom flask and placed on a Buchi Rotovapor-R (Postfach, Switzerland) set at 40 °C and evaporated to dryness.

Fatty acids from the extracted fat were made volatile by converting them into methyl esters. The esters were identified and quantified with GC–MS and compared with MS library. Initially 2 drops of fat were taken in amber coloured bottle using a capillary tube and thoroughly mixed with 1 ml of diethyl ether. To this, 1 ml of 0.5 N methanolic KOH was added. Then the content was vigorously shaken for about 10 min. The reaction was stopped using 1 ml of 1 N HCl and 1 ml of petroleum ether was subsequently added, mixed and allowed to settle down to form two layers. The upper pet ether layer was collected and evaporated to dryness on 60 °C water bath to collect methyl ester. Finally this was dissolved in 10 μL of HPLC grade hexane and 2 μL was then injected using micro syringe into the GC–MS that was equipped with flame ionisation detector (FID), using nitrogen as the carrier gas in a CP sil 88 column. The conditions for separations were as follows: Initial temperature 70 °C hold time 1 min, then at 180 °C, rate 5 °C/min, hold time 10 min and at 280 °C, rate 5 °C/min, hold time 5 min.

2.3. Oxidative stability

Oxidative stability of ALA was determined using peroxide value (PV) and p-anisidine value (AV). Peroxide value was determined using iodometric titration. PV was expressed as meq active oxygen/kg sample. Totox number which indicates overall oxidation state was determined by using following equation (AOCS, 1989).

$$TOTOX = AV + (2 \times PV)$$

2.4. Statistical analysis

The data were subjected to analysis of variance (ANOVA test) using SPSS version 19 for windows. Means were separated by Fisher's protected least significant difference (LSD). The significant level was established at $p \leq 0.05$. All treatments were done in triplicate. When standard curves were prepared, Coefficient of Determination (R^2) $\geq$ 0.9 were considered acceptable for the regression line representing the data.

3. Results and discussion

3.1. Nutritional evaluation of bread

Nutritional evaluation was carried out with respect to soluble, insoluble dietary fiber in-*vitro* protein digestibility, in-*vitro* glycemic index in-*vitro* antioxidant activity and fatty acid profile for optimised bread.

3.1.1. Effect of flaxseed level on soluble, insoluble and total dietary fiber of bread

The results for TDF, SDF and IDF in bread samples are given in Table 1. Control bread with refined wheat flour contained 1.33, 0.65, and 0.68 (g/100 g) TDF, SDF and IDF, respectively. All the dietary fibre components increased significantly ($p < 0.05$) when refined wheat flour were increasingly supplemented with roasted ground flaxseed. When wheat flour was supplemented with 5 g/100 g with roasted ground flaxseed there was increase of 2.55, 1.27 and 1.28 (g/100 g) TDF, SDF and IDF, respectively. At 10 and 15 g/100 g supplementation, there was an increase in total (3- and 4- fold), soluble (2.9- and 3.9- fold), and insoluble (3- and 4.4- fold) dietary fiber contents as compared with control bread which could be attributed to high dietary content in flaxseed (Carter, 1993).

3.1.2. Effect of flaxseed level on in vitro protein digestibility of bread

Table 2 shows the results of effect of flaxseed supplementation on in vitro protein digestibility of bread. Control bread (refined wheat flour bread without flaxseed) had 78.45 (g/100 g) in *vitro* protein digestibilities. Upon blending refined wheat flour with roasted ground flaxseed; protein digestibility decreased significantly ($p < 0.05$) up to 70.93(g/100 g). This decrease could be due to lignan (phenolic compounds) content in flaxseed (Thompson, Robb, Serraino, & Cheung, 1991). Polyphenols are also known to be

Table 1
Effect of flaxseed level on soluble, insoluble and total dietary fiber of bread.[a]

(g/100 g) Flaxseed	Soluble dietary fiber (SDF)	Insoluble dietary fiber (IDF)	Total dietary fiber (TDF)
0	0.65a ± 0.02	0.68a ± 0.03	1.33a ± 0.05
5	1.27b ± 0.32	1.28b ± 0.25	2.55b ± 0.30
10	1.92c ± 0.23	2.16c ± 0.30	4.08c ± 0.24
15	2.54d ± 0.40	3.02d ± 0.35	5.56d ± 0.40

[a] Means in the same column followed by different letters differ significantly $p \leq 0.05$.

Table 2
Nutritional characterization of optimized bread.[a]

	In-*vitro* protein digestibility (g/100 g)	In-*vitro* glycemic index (GI)	Inhibition (g/100 g)	In-*vitro* antioxidant activity (TE μg/g)	Calories (kcal)/100 g
Control (0)	78.45a ± 0.43	94.61a ± 4.56	–	–	253.41a ± 0.30
Optimized bread	70.93b ± 0.40	51.26b ± 2.50	63.02 ± 2.78	1489.59 ± 65.84	261.15b ± 0.35

[a] Means in the same column followed by different letters differ significantly $p \leq 0.05$s.

associated with proteins to form insoluble complexes, thus affecting the in vitro digestibility of proteins (Feng, Chen, Kramer, & Reeck, 1991). In addition it has been suggested that plant polyphenols may be oxidised to quinones at neutral to alkaline pH that may then go on to form peroxides that are highly reactive oxidising agents and could bring about oxidation of several amino acid residues and polymerisation of proteins (Damodaran, 1996). This could be a mechanism by which flavonoids and phenolic acids hinder protein digestion in flaxseed bread. A highly significant ($p < 0.05$) and negative correlation was obtained between polyphenols concentrations and protein digestibility.

3.1.3. *In vitro glycemic index (GI)*

In *vitro* starch hydrolysis was carried out in this study to simulate the in *vivo* situation of carbohydrate digestion characteristics and to estimate the metabolic glycemic response of food (Goni et al., 1997).

The kinetics of in *vitro* starch hydrolysis for white bread (reference) and bread substituted with 10 g/100 g roasted ground flaxseed are summarised in Table 3. The profile of in *vitro* starch hydrolysis (Table 3) exhibited a gradual increment in all tested bread samples as time increased. White bread underwent a higher starch hydrolysis during 30–120 min, as compared to optimized bread (bread with 10 g/100 g flaxseed). This increased GI could be due to the fact that the control bread exhibits a sponge structure which is highly accessible to α-amylase and tends to elicit high glycemic responses (Cavallero, Empilli, Brighenti, & Stanca, 2002).

In vitro starch hydrolysis at 180 min for white bread and flaxseed bread showed significant differences ($p < 0.05$) where the optimized flaxseed bread showed improved starch hydrolysis. In *vitro* GI of white bread and optimized bread are given in Table 2. GI obtained of optimized bread was about 51.26 and which is significantly different ($p < 0.05$) from the control white bread. This could be due to the SDF present in flaxseed (Carter, 1993). SDF and IDF reduce dietary carbohydrate absorption rate by forming a viscous gel in small intestine, hence reducing the postprandial blood glucose response. However, Wolever (1990) suggested that encapsulation of nutrients within plant cell walls in IDF inhibits starch digestion, which is more important in the further decrease of the GI. Hussain, Anjum, and Alamri (2011) showed that soluble fiber (mucilage) in flaxseed helps in decreasing cholesterol and optimizing blood glucose levels. Low GI foods containing soluble fiber not only prevent certain metabolic ramifications of insulin resistance but also reduce insulin resistance (Reaven, Brand, Chen, Mathur, & Goldfine, 1993).

Table 3
Enzymatic kinetics for in-*vitro* starch hydrolysis of white bread and optimized bread (10 g/100 g flaxseed level).

Bread sample	Degree of hydrolysis (g/100 g)					
	30 min	60 min	90 min	120 min	150 min	180 min
White bread reference	20.70	26	33.35	44.50	45.60	45.60
Optimized bread (10 g/100 g flaxseed level)	5.46	5.90	6.09	6.86	9.34	13.89

3.1.4. *In vitro antioxidant activity and calories*

Radical scavenging ability of an extract of optimized bread (containing 10 g/100 g roasted ground flaxseed) was about 63.02 g/100 g and antioxidant activity by DPPH method was about 1489.59 μg Trolox Equivalent (TE)/g of bread (Table 2). The extent of decrease in the absorbance of DPPH in the presence of antioxidants correlates with the free radical scavenging potential of the antioxidant. These scavenging activities might be due to the presence of different phenolic compounds in flaxseed such as lignan. The most common lignan present in flaxseed is secoisolariciresinol diglucoside (SDG) (Bambagiotti-alberti, Coran, Ghiara, Moneti, & Raffaelli, 1994).

The calorie content in optimized bread (with 10 g/100 g flaxseed) was about 261.15 kcal whereas in control bread with refined flour was about 253.41 kcal. There is significance difference among these two that may be due to the high fat content in flaxseed.

3.1.5. *Fatty acid profile of optimized bread*

Fatty acid distributions of lipid extracted from optimized bread with 10 g/100 g roasted ground flaxseed are presented in Table 4. It has been found that omega 3 content i.e. (ALA) in flaxseed bread was about 26.6 (g/100 g) of total fatty acids. This amounts about 1.5 gm per 100 gm of bread. Omega 6 i.e. linoleic acid content was about 2.65(g/100 g) of total fatty acids i.e. 0.15 gm per 100 gm of bread. This alpha-linolenic acid in bread was due to flaxseed supplementation which contains 23 g per 100 g of flaxseed. Oleic acid and myristic acid content in bread were about 1.33 and 0.42 g per 100 g of bread (Table 4).

3.2. *Oxidative stability of bread*

To evaluate oxidative stability, peroxide value, free fatty acid value and anisidine values were determined and are shown in Table 5. There were no significant increases ($p > 0.05$) in peroxide value both at 30 °C and 4 °C during 2 days storage of breads. After 2 days, there was significant and gradual increase in peroxide value. After the 3rd day of storage, there was an increase in free fatty acid content of bread stored at 30 °C as well as 4 °C. Anisidine value also followed the similar trend, only increasing after 3 days of storage. Totox number at 30 °C and 4 °C after 1 day storage were about 24 and 21.13 respectively, whereas after 5 days storage it has increased up to 39.73 and 37.53 respectively.

Flaxseed oil (FSO) contains about 53.3% of α-linolenic acid (ALA) and 12.7% of linoleic acid (LA), yielding the highest n 3/n-6 fatty acid ratio amongst plant sources (Reddy et al., 2012). FSO is the richest source of valuable omega-3 essential fatty acid (EFA). These oils could be at least partly responsible for the peroxide levels, antioxidant activities and oxidative stability of breads made with

Table 4
Fatty acid profile of optimized bread with 10 (g/100 g) roasted ground flaxseed level.

Fatty acid	Retention time (min)	% Total fatty acids	g/100 gm of product
Linolenic acid (omega 3) C18:3	28.11	26.60	1.51
Linoleic acid (omega 6) C18:2	27.103	2.65	0.150
Oleic acid C18:1	26.787& 26.678	23.40	1.33
Myristic acid	27.384	7.37	0.42

Table 5
Effect of storage on oxidative stability of ALA from bread with 10 g/100 g roasted ground flaxseed.[a]

Days	Peroxide value		FFA		Anisidine value	
	30 °C	4 °C	30 °C	4 °C	30 °C	4 °C
1	6.66 ± 0.57	5.33 ± 0.57	1.16 ± 0.25	1.10 ± 0.10	10.66 ± 0.20	10.46 ± 0.05
2	6.66 ± 0.57	6.33 ± 0.57	1.53 ± 0.15	1.33 ± 0.05	11.26 ± 0.11	10.93 ± 0.15
3	8.66 ± 0.57	7.33 ± 1.14	1.83 ± 0.15	1.43 ± 0.05	11.46 ± 0.15	11.33 ± 0.15
4	10.66 ± 0.57	9.33 ± 0.57	2.26 ± 0.15	2.16 ± 0.05	12.56 ± 0.15	12.03 ± 0.20
5	13 ± 1.0	12.33 ± 0.57	2.7 ± 0.10	2.46 ± 0.05	13.73 ± 0.15	12.86 ± 0.20

[a] Mean ± SD three determinations.

flaxseed blends. Human trials would be necessary to evaluate if the antioxidant levels of the breads are sufficient to exert a biological function on the consumers.

4. Conclusion

The present study showed that soluble, insoluble and TDF increased with the addition of roasted flaxseed in breads. In vitro protein digestibility decreased when 10 g/100 g roasted flaxseed was added to bread due to phenolic content in flaxseed. In *vitro* GI of roasted flaxseed containing bread was about 51.26 which are considered a low GI category. In *vitro* antioxidant activity of optimized bread was increased as was the calorie content in function of supplementation with increasing roasted flaxseed content omega-3 content in optimized bread was about 1.51 gm per 100 gm of bread whereas omega-6 content in bread was 0.15 gm per 100 gm. Peroxide value, anisidine value and free fatty acid get increased with storage period. The results of this study clearly show that the addition of roasted flaxseed, with an optimum addition content of 10 (g/100 g), can improve the nutritional and functional properties of traditional wheat based bread. Previous sensorial evaluations showed roasting flaxseed improved the sensorial acceptability of breads containing these seeds (Marpalle et al., 2014b) which is in accordance with other reports that have focussed on the effect of flax addition on the flavor profile of different food association with consumer acceptability (Aliani et al.,2011; Aliani et al., 2012). The use of roasted flaxseed which improved the nutritional and potential functional properties should be accepted by consumers based on these previous results; however, the biological effect of these breads or other food products need to be tested in well-designed clinical human trials.

Acknowledgement

The authors wish to thank General Mills India, Mumbai for providing raw material during this research work. The authors also thank Department of Biotechnology (DBT), Government of India (2741/DBT/FBT) for financial support to carry out this work.

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