PAPER

QUALITY AND ANTIOXIDANT ACTIVITY OF BREAD FORTIFIED WITH FLAXSEED

R. MERAL* and I. SAIT DOGAN
Yüzüncü Yıl University, Faculty of Engineering-Architecture, Department of Food Engineering, 65080 Van, Turkey
*Corresponding author: Tel. +90 432 2251024, Fax +90 432 2251104,
email: raciyemeral@yyu.edu.tr

ABSTRACT

The objective of this study was to determine the effects of flaxseed on the rheological and bread-making properties and antioxidant activity of bread. Wheat flour was replaced with flaxseed at the level of 2, 4, and 8%. The water absorption, dough development time, stability and mixing tolerance index of the dough did not significantly change with the addition of flaxseed. The test baking results showed that the addition of %2 flaxseed yielded the highest loaf volume. The incorporation of flaxseed increased the antioxidant properties of the bread when compared to the control bread.

- Keywords: antioxidant activity, bread, DPPH, dough rheologhy, flaxseed, phenolic compounds, TEAC -

INTRODUCTION

Bread is one of the major components of the human diet in most parts of the world. For several thousands of years, people have used wheat to produce bread. In the past few decades, researchers have worked on fortifying bread with natural compounds because of demands to natural and healthier food. Thus, whole grains and other seeds have become popular in the production of bread. Many researchers have studied the fortification of bread, and looked at, for example dietary fibre (WANG *et al.*, 2002; GOMEZ *et al.*, 2003; KAACK *et al.*, 2006) and antioxidants (HOLTEKJOLEN *et al.*, 2008; PENG *et al.*, 2010). Although the impact of various ingredients on the antioxidant properties of bread has been widely studied, the impact of flaxseed on the antioxidant activity of bread is still fairly unknown.

Flaxseed (*Linum usitatissimum*) is a readily available oilseed and a major dietary source of α-linolenic acid, dietary fibres and lignans (UDENIGWE and ALUKO, 2010). Flaxseed contains 30-40% fat, 20-25% protein, 20-28% dietary fibre, 4-8% ash and the oils contains vitamins A, B, D and E, minerals and amino acids (MENTES *et al.*, 2008). Flaxseed is also a rich source of an oligomeric complex containing the lignan secoisolariciresiniol (SDG). SDG is a precursor of mammalian lignans which are recognized as protecting against hormonal-dependent breast cancer. Other phenolic compounds in the complex are two hydroxycinnamic acid derivates, *p*-coumaric acid-4-*O*-glucoside and ferulic acid-4-*O*-glucoside (STRANDAS *et al.*, 2008). The mentioned flaxseed components have been reported to possess various physiological activities relevant to human health sustenance, especially in the prevention of cardiovascular disease, cancer and diabetes. Flaxseed consumption has been demonstrated to exhibit potential health benefits, including decreased tumor growth, reduced serum cholesterol levels, and a decreased in the formation of breast, prostate, and colon cancers (HOSSEINIAN *et al.*, 2010).

The objectives of this research were to observe the influence of flaxseed on bread-making quality and the antioxidant properties of breads.

MATERIALS AND METHOD

Materials

The experimental wheat flour was obtained from a local miller (Van, Turkey). The salt and instant active dry yeast were purchased from a commercial local food ingredient company. The flour treatment agent (Pantera) which included emulsifier, alpha amylase, ascorbic acid and citric acid, was obtained from Puratos, Istanbul, Turkey. The flaxseed was also purchased from a local market. The flaxseed was ground with a laboratory mill (Perten LM 120, Sweden) just before use. After the flaxseed was ground (particle size<0.5 mm) wheat flour was replaced with ground flaxseed at the levels of 0, 2, 4, and 8%.

Chemicals

Gallic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate ($K_2S_2O_8$), Folin-Ciocalteu reagent, sodium carbonate (Na_2SO_3), sodium chloride (NaCl) were obtained from Sigma-Aldrich Company (St. Lois, MO, USA). Methanol for extraction was analytical grade and purchased from Merck (Darmastadt, Germany).

The rheological characteristics of dough

The effects of the flaxseed on dough rheology during mixing were determined by using Dough Test Instrument (Yücebaş Machine Company, İzmir, Turkey), that followed the AACC method (1983). The parameters obtained were water absorption (WA), dough development time (DDT), dough stability and mixing tolerance index (MTI).

The viscoelastic characteristics of dough

The viscoelastic behaviour of the dough was determined by Texture Analyser, using SMS/Kieffer dough and gluten extensibility rig. The dough samples prepared as above were placed in a Teflon mould. The whole mould was placed in a chamber at 30° and 85% RH for 90 min to allow stress relaxation. The extension test of a strip of dough was evaluated using SMS/Kieffer Dough and Gluten Extensibility Rig with a 5 kg load cell. For each kind of dough sample, four measurements were performed (WANG *et al.*, 2003).

Bread-making and evaluation

Bread samples were prepared using a straight dough method with slight modifications. The basic dough formula for 500 g flour consisted of salt (7.5 g), instant active dry yeast (5 g), a flour treatment agent (4 g) and water, which needed to have a consistency of 500 BUE. After all the ingredients were added the dough was kneaded for the DDT. After mixing, the dough was placed in the fermentation cabin (Özköseoğlu, İstanbul, Turkey) at a temperature of 30°C and 85% RH for 45 min. The fermented dough was divided into three 200 g pieces, moulded using a dough forming device (Şimşek Laborteknik, Ankara, Turkey) and put

into pans (Teksan Machine Company, Bursa, Turkey) for the final proofing. The dough samples were fermented until they reached 2 cm above the rim and then baked at 218°C for 21 min. Following baking, the bread loaves were cooled at room temperature. The loaf weights and volumes were measured 1 h after removal from the oven. The loaf volumes were measured using the rapeseed displacement method (PLESSAS *et al.*, 2003) and specific volumes were calculated. Bread firmness was determined using TA.XT plus Texture analyser (TA.TX2. Stable Micro Systems Ltd. Godalming Surrey, UK) equipped with 5 kg load cell and 36 mm cylinder aluminum probe (P36/R) after 3 and 72 h of baking. The firmness was expressed as the force required to compress 25% of the bread.

Extraction of phenolics from bread

The bread (control and flaxseed-enhanced bread) was dried at 40°C. The bread was ground into fine powder using a laboratory mill (Perten LM 120, Sweden). Samples from each type of bread of approximately 10 g were mixed with 30 mL methanol and stirred for 22 h in the dark and at 35°C using a shaking incubator (Heidolph Unimax 1010, Germany). After this, the homogenates in the tube were centrifuged at 12,000 *g* 15 min at 20°C and the supernatant was transferred into an amber volumetric flask. The precipitate was extracted again using the same solvent and in the same conditions and made up to a final volume of 100 mL. The extracts were stored at -20°C for four weeks (IACOPINI *et al.*, 2008; YEMIS *et al.*, 2008).

Determination of total phenolic content

The concentration of total phenolics (TPC) in the methanol extract of bread samples was determined using the Folin-Ciocalteau colorimetric method (YEMIS *et al.*, 2008). Briefly, a 150 µL sample extract and 3.0 mL of 2% sodium carbonate (w/v in water) were transferred into a test tube. After about two minutes 150 µL Folin-Ciocalteu's reagent (1:1, v/v in water) was added and mixed throughly. The mixture was left to stand for 45 min at room temperature in the dark. The absorbance of the reaction mixture was measured at 765 nm in a spectrophotometer (PG Instrument T80 UV/VIS Spectrophotometer, UK). All the spectrometric measurements were carried out in triplicate**.** The calibration curve was performed using gallic acid and the results were expressed as mg of gallic acid equivalents 100 g of sample (mg GAE/100 g of sample).

Determination of total flavonoid content

The concentration of total flavonoids (TF) in the methanol extract of the bread samples was determined using the colorimetric method (RAMOMOORTHY and BONO, 2007). 1 mL of each bread extract was mixed with 1 mL aluminium trichloride in methanol. The mixture was left to stand for 10 min at room temperature in the dark. The absorbance of the reaction mixture was measured at 510 nm in the spectrophotometer. The calibration curve was performed using quercetin, and the results were expressed as mg of quercetin equivalents for 100 g of the samples (mg QE/100 g of sample).

Free radical scavenging activity

The free radical scavenging activity of the extract was measured using DPPH· free radical scavenging method (BRAND-WILLIAMS *et al.*, 1995) with some modifications. One mL of the sample extract was added to 3 mL DPPH· solution (4 mg L^{-1} in methanol). The mixture was shaken vigorously and allowed to stand for 30 min at room temperature in the dark. Then the absorbance was measured at 517 nm. Methanol was used instead of the sample for the control measurements. The inhibition of DPPH· was determined according to the following equation.

$$\% \, Inhibition = \left[\frac{(A \; control - A \; sample)}{A \; control} \right] * 100$$

A control: Absorbance of control at 517 nm
A sample: Absorbance of sample at 517 nm

Trolox equivalent antioxidant capacity (TEAC)

The TEAC value was determined using YEMIS *et al.* (2008) with slight modifications. 7 mM ABTS salt solution was reacted with 2.45 mM potassium persulphate solution and the reaction mixture was allowed to stand in the dark for 16 h at room temperature and used in 2 days. Then the radical solution was diluted with a potassium phosphate-saline buffer (pH 7.4) to an absorbance of 0.7±0.005 at 734 nm. 50 µL of the sample extract or standard (different concentrations of trolox) and 3 mL of diluted ABTS· solution were added to the macrocuvvete and absorbance was taken as being at 734 nm on a spectrophotometer after 6-min incubation. The results were expressed as TEAC values (µmol trolox/g sample). Triplicate analyses were performed.

Statistical methods

The data were analysed using the StatGraphics Centrium 15.1 (STATGRAPHICS, 2006) for one-way ANOVA. The Student Newman Keuls (SNK) procedure was used to identify any significant differences (p<0.05) between the samples.

Table 1 - Influence of flaxseed on dough properties.

Substitute level (%)	Water absorption (%)	DDT (min)	Stability (min)	MTI (BUE*)
0	57.7±0.42^{a}	5.2±0.14^{ab}	6.4±0.14^{b}	41.1±9.54^{b}
2	58.6±0.35^{a}	6.5±0.42^{a}	5.9±0.28^{b}	63.7±0.07^{a}
4	58.7±0.35^{a}	5.8±0.07^{ab}	6.1±0.07^{b}	54.6±0.14^{b}
8	58.9±0.14^{a}	5.7±0.21^{ab}	7.5±0.42^{a}	38.6±3.25^{b}

*BUE: brabender units equivalent. The values are given as mean±SD (n=2). Values followed by different superscripts within the same column are significantly different (p<0.05).

RESULTS AND DISCUSSION

Influence of flaxseed on dough properties

The effects of the flaxseed on dough rheology are given in Table 1. The addition of flaxseed did not alter the WA and DDT. The stability value and MTI are indicators of the flour strength, with higher stability and lower MTI values suggesting a stronger dough. Flaxseed did not change the stability up to the addition level of 8%. While the MTI increased at the level of 2%, further increases did not significantly change it. A possible reason is the dilution of gluten by the added non-gluten proteins. Similar findings were observed with the addition of triticale flour to wheat flour (COSKUNER and KARABABA, 2005).

Influence of flaxseed on the viscoelastic characteristics of dough

Two parameters were used to characterize the extensigram: extensibility at maximum resistance to extension (E at R_{max}) and maximum resistance to extension (R_{max}). Extensibility represents the deformation of the dough before it ruptures. The larger the extensibility, the further the dough can be extended before it ruptures (WANG *et al.* 2003). The effects of flaxseed on viscoelastic characteristics of dough are shown in Table 2. The extensibility of the dough including flaxseed was significantly higher (p<0.05) than that of the control dough. The addition of flaxseed led to a decrease in the R_{max} value. High extensibility and low R_{max} indicates lower elasticity. A possible reason for this is the dilution of gluten by the added non-gluten proteins. Kieffer extensibility analysis gives information about the viscoelastic behaviour of a dough and measures dough extensibility and resistance to extension (WANG *et al.*, 2003). A combination of good resistance and good extensibility results in desirable dough properties (KARAOGLU, 2006).

Table 2 - Influence of flaxseed on viscoelastic properties of dough.

Substitute level (%)	E at R_{max} (mm)	R_{max} (g)
0	43.2±1.3^{d}	30.5±0.7^{a}
2	70.2±0.9^{a}	26.8±0.3^{b}
4	67.6±3.0^{b}	19.2±0.4^{c}
8	63.8±2.8^{c}	14.2±1.0^{d}

The values are given as mean±SD (n=4). Values followed by different superscripts within the same column are significantly different (p<0.05).

Influence of flaxseed on bread quality evaluation

The effect of flaxseed substitution on bread quality characteristics are summarized in Table 3.

The specific volume value of bread containing 0, 2, 4 and 8% flaxseed were 3.9, 5.5, 5.4 and 5.0 cm^3/g, respectively. The addition of flaxseed increased the specific volume. The baking test results showed that the addition of flaxseed at the 2% level gave the highest bread volume. The specific volume increased by 30, 28 and 23%, respectively. MENTES *et al.* (2008), who observed the effects of flaxseed on bread properties, determined that there was a significant increase in the specific volume value of bread with the addition of flaxseed. The effect of flaxseed on the spe-

Table 3 - Influence of flaxseed on bread quality.

Substitute level (%)	Specific volume (cm^3/g)	Crumb firmness (g) (3 h)	Crumb firmness (g) (72 h)
0	3.9±0.07^{c}	426±14.14^{a}	1423±11.31^{a}
2	5.5±0.14^{a}	227±18.38^{b}	747±4.24^{b}
4	5.4±0.21^{a}	192±4.24^{c}	730±0.70^{c}
8	5.0±0.00^{b}	153±9.19^{d}	600±13.43^{d}

The values are given as mean±SD (n=3). Values followed by different superscripts within the same column are significantly different (p<0.05).

Table 4 - TPC, TF content and antioxidant activity of breads containing flaxseed.

Substitute level (%)	TPC (mg GAE/100 g)	TF (mg QE/100 g)	DPPH inhibition (%)	TEAC (µmol Trolox)
0	0.37±0.14^{d}	0.12±0.05^{d}	29±0.0^{b}	1.10±0.21^{d}
2	0.49±0.70^{c}	0.50±0.02^{c}	35±0.0^{a}	1.21±0.17^{c}
4	0.53±0.14^{b}	0.58±0.01^{b}	35±0.7^{a}	1.41±0.11^{b}
8	0.55±0.01^{a}	0.67±0.00^{a}	37±2.8^{a}	1.93±0.16^{a}

The values are given as mean±SD (n=3). Values followed by different superscripts within the same column are significantly different (p<0.05).

cific volume value might be due to fat and protein existing in the flaxseed. When fat is used in the bread formula, it contributes to a homogeneous mix. Fat wraps around the gas cells and prevents gas release from the dough. Thus, a higher volume is obtained. Fat also protects the freshness of the bread and retards staling (ELGUN and ERTUGAY, 1997).

The texture analysis revealed that the addition of flaxseed decreased the initial crumb firmness. The firmness values of bread including 2, 4 and 8% flaxseed were lower than that of the control bread (p<0.05). Similar findings were obtained by MENTES *et al.* (2008). Flaxseed also reduced the firmness of bread during storage. The firmness of the bread containing flaxseed after 72 h of storage was lower than that of the control bread. Among the evaluated breads, the lowest firmness value was observed in bread containing 8% flaxseed. SABANIS and TZIA (2009) reported that a larger loaf size produced softer bread. Another reason might be the higher protein content of flaxseed, which contributes to the reduction of the firming rate during staling (KIM and D'APPOLONIA, 1977) and its higher water-binding capacity.

Influence of flaxseed on the TPC, TF and antioxidant activity of breads

The TPC, TF and antioxidant activities of breads are given in Table 4. The TPC values of bread ranged from 0.37-0.55 mg GAE/100 g. The TPC increased significantly with increasing flaxseed levels in the bread and the highest TPC was found in bread containing 8% flaxseed. Many studies have shown that phenolic compounds have antioxidant activity. There is a positive correlation between the TPC and antioxidant activity. The TF value of bread made with flaxseed ranged from 0.12-0.67 mg QE/100 g. The TF increased significantly with increasing flaxseed levels in bread. There is a positive correlation between the TF and antioxidant activity (SANTAS *et al.*, 2010).

The antioxidant activities of bread prepared with different levels of flaxseed substituted for wheat flour were analysed using the DPPH radical scavenging activity and TEAC assays and the results are shown in Table 4. The antioxidant activities of the bread increased significantly as the level of flaxseed substitution increased. Bread containing flaxseed (0, 2, 4, and 8%) showed 29-37% DPPH inhibition. DPPH inhibition increased by 17-22% with the addition of flaxseed. The TEAC values of the bread containing flaxseed ranged from 1.10-1.93 µmol Trolox. The TEAC values of bread significantly increased. The results showed that the addition of flaxseed greatly enhanced the antioxidant activity of bread. AMAROWICZ *et al.* (1997) found that the hydrophobic parts of flaxseed contain a higher proportion of phenolic compounds and are a more powerful antioxidant than the hydrophilic parts. Compared to other oilseeds, flaxseed contains a low amount of phenolic acids. Therefore, the antioxidant activity of flaxseed flours and extracts might be due to the presence of other types of phenolic compounds (PHILIPH, 1974).

CONCLUSION

Flaxseed improved the rheological properties and extensibility of dough. The specific volume value increased by 30, 28, and 23%. Flaxseed also reduced the firmness of the bread crust and the bread stayed fresh during storage. The addition of flaxseed improved the antioxidant activity of the bread. Overall, flaxseed could be included in the formula to improve the functionality of bread.

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