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Effects of fermented-chickpea liquor (chickpea yeast) on whole-grain wheat flour bread properties

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RESEARCH ARTICLE

Abstract

Whole-grain wheat flour bread is superior to refined white-flour bread in terms of nutritional quality; yet, it is often time poor in loaf volume, texture, flavour and colour. Being a traditional approach, fermented-chickpea liquor (FCL) or chickpea yeast was claimed to improve sensory properties of bakery products. In this study, effects of FCL on quality characteristics of whole-grain wheat flour bread were investigated. For this purpose, FCL was incorporated into bread formulations at 15, 30 and 45% levels, based on whole-grain wheat flour and substituted with water. Dough mixing, loaf volume, texture, colour and flavour of the control and FCL-containing breads were measured. Additionally, certain microbiological tests and antioxidant activity measurements were carried out. Compared to the control, FCL incorporation into whole-grain wheat flour bread formulations at 30 and 45% levels, respectively, increased loaf volume about 10 and 25%, decreased crumb hardness approximately 10 and 15%, and enhanced antioxidant activity (about 10%) of whole-grain wheat flour breads. However, FCL usage had limited effect on flavour and colour. The best results were obtained when 30 and 45% FCL were used in the breads of whole-grain wheat flour.

Keywords: fermented-chickpea liquor, chickpea yeast, whole-grain wheat flour, bread, quality

1. Introduction

Bread is a staple food for people all around the world and consumers are very sensitive to its colour, flavour and texture. Bread made of whole-grain wheat flour is superior to that made of highly refined white flour in nutritional quality since it contains bran and germ layers of wheat that are rich in vitamins, minerals, dietary fibre and antioxidant phytochemicals (Belobrajdic and Bird, 2013; Dykes and Rooney, 2007; Kumar *et al.*, 2011; Slavin, 2004). However, whole-grain wheat flour bread is poor in sensory properties due to its darker colour, hard and rapidly staling texture and slightly bitter taste (Paşa, 2010; Pyler, 1988). Whole-grain cereals are known to decrease obesity and diabetes due to their high-fibre contents that reduce energy density of diet and rate and extent of starch digestion. Additionally, elevated levels of dietary fibre, ferulic acid, sterols and stanols in whole-grain cereals are determined to reduce blood cholesterol level and prevent from colon cancer and coronary heart disease (Belobrajdic and Bird, 2013; Okarter and Liu, 2010; Slavin, 2004).

Several approaches to overcome the sensory shortcomings of whole-grain wheat flour bread were attempted, including use of hydrocolloids, lignocellulosic enzymes and sour-dough process. Sudha and Rao (2008) incorporated hydroxypropyl methylcellulose into whole-grain wheat flour bread formulation at 0.5 to 1.0% levels to improve dough rheology. Shah *et al.* (2006) used hemicellulase enzyme and improved volume, colour and texture of whole-grain wheat flour bread. In recent years, usage of sour-dough process has become widespread. Sour-dough process is reported to improve loaf volume and texture (Clarke *et al.*, 2002; Corsetti *et al.*, 2000; Crowley *et al.*, 2002), taste and odour (flavour) (Brummer and Lorenz, 1991; Thiele *et al.*, 2002), shelf life (Corsetti *et al.*, 1998a,b; Dal Bello *et al.*, 2007; Lavermicocca *et al.*, 2000) and nutritional quality of bread (Liljeberg and Björck, 1994; Liljeberg *et al.*, 1995). Being quite similar to sour dough and native to certain localities of Turkey, Greece and Macedonia, fermented-chickpea liquor (FCL), also called 'chickpea yeast' or 'sweet yeast' (Çebi, 2009, 2014; Hancıoğlu-Sıkılı, 2003; Hatzikamari *et al.*, 2007a,b; Katsaboxakis and Mallidis, 1996), might help alleviate sensory problems of whole-grain wheat flour bread, especially its poor flavour and texture. FCL was reported to be locally used in many bread and pastry formulations (Baykara, 2006; Çebi, 2009, 2014; Hancıoğlu-Sıkılı, 2003; Narlıoğlu, 2013; Özkaya, 1992).

Chickpea (*Cicer arietinum*) is rich in carbohydrates, proteins, vitamins, minerals, dietary fibre and protective phytochemicals, and processed into nutritious foods by various techniques, including steeping, dehulling, fermenting, boiling, steaming, frying or roasting (Desphande and Damodaran, 1990). Production of FCL and its use in the bakery products is yet another use of chickpea. As a traditional practice, FCL production varies extensively. In general, coarsely ground or cracked chickpea is subjected to spontaneous fermentation in three- to five-fold water, which was previously boiled and usually cooled down to about 50 °C, in closed containers at 35-40 °C for 15-20 h. Formation of foam atop the liquor is considered a successful fermentation process (Baykara, 2006; Çebi, 2009; Hatzikamari *et al.*, 2007a,b). Boiled hot water is directly added to the cracked chickpea in Greece (Hatzikamari *et al.*, 2007a,b), whereas boiled and cooled warm water (about 50 °C) is used in Turkey (Baykara, 2006; Çebi, 2009). Once the fermentation is over, FCL is sieved and the fermented liquor plus foam is used in the bakery products either directly or upon creating a preferment dough (Baykara, 2006; Özkaya, 1992).

Some research has been conducted on biochemical and microbiological properties of FCL and its effects on bakery goods. Özkaya (1992) investigated the effects of commercial baker's yeast, sour dough and FCL on dough and bread properties, and found that FCL weakened the dough but improved the taste, odour and crust colour of bread. Tülbek *et al.* (2003) reported that FCL increased the nutritional properties and shelf life of bakery products. Baykara (2006) compared the properties of breads made of baker's yeast, 50:50 mixture of FCL-commercial yeast and FCL alone. The staling rates of the breads were similar, but the sensory properties of the breads containing 50:50 mixture were unsurpassed. Narlıoğlu (2013) used commercial yeast, FCL and their mixtures in 'poğaça' processing. It was found that FCL-containing dough was softer with lower baking loss and that the best sensory quality was achieved by use of FCL-yeast mixture.

Hancıoğlu-Sıkılı (2003) investigated flavour and microbiological properties of FCL. Breads containing FCL had higher levels of propyl and n-butyl aldehydes, ethyl butyl ketone, acetic, propionic and valeric acids than did breads containing baker's yeast and sour dough. These compounds were claimed to be responsible for the characteristic flavour of breads baked with FCL. *Enterococcus mundtii*, *Enterococcus gallinarum*, *Lactobacillus bifermantans*, *Streptococcus thermophilus*, *Enterococcus casseliflavus* and *Saccharomyces cerevisiae* were isolated from FCL, while *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus sanfrancisco*, *E. mundtii*, *E. gallinarum*, *Pediococcus urinae-equi*, *S. cerevisiae*, *Lactobacillus viridencens* and *S. thermophilus* were found in FCL containing bread doughs. Çebi (2014) investigated the effects of *Lactobacillus brevis* FK2, *Lactococcus lactis* FK5 and *L. plantarum* FK25, isolated from FCL and FCL-containing doughs, on volatile compounds, texture and colour of breads. A total of 58 volatile compounds were isolated from the dough, bread crumb and crust. Of those compounds, ethyl acetate, trans-2-heptenal, hexanal and 2,3-dihydro-1H-indole were associated with *Lactobacillus* species. Furthermore, *Lactobacillus* species isolated from the FCL-containing doughs positively influenced crumb texture, cohesiveness, chewiness and lightness (L*) of breads.

Hatzikamari *et al.* (2007a) studied the biochemical changes and microorganisms of FCL. It was found that the activities of cellulase, α-galactosidase, invertase and protease significantly increased during fermentation. Furthermore, *Bacillus* and *Clostridium* species along with free fatty acids, reducing sugars and free amino acids increased especially after 10^{th} hour of fermentation. It was also noticed that *Bacillus* species predominated the fermentation medium in the first 8-10 h, which was later dominated by *Clostridium* species. Hatzikamari *et al.* (2007b) reported that *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus licheniformis*, *Clostridium perfringens* and *Clostridium beijerinckii* were among the most common *Bacillus* and *Clostridium* species found in FCL and FCL-containing doughs. Çebi (2009) isolated a total of 120 lactic acid bacteria (LAB) from FCL and FCL-containing doughs. *L. lactis*, *L. brevis* and *L. plantarum* were common in FCL, whereas *L. lactis*, *L. brevis*, *L. plantarum*, *L. pentosus* and *Weisella confuse* were widespread in FCL-containing doughs. *L. lactis* was determined as the dominant LAB, while *L. brevis* and *W. confuse* were as the gas-producing heterofermentative LAB. The studies conducted on FCL in Turkey (Çebi, 2009; Hancıoğlu-Sıkılı, 2003) reported only LAB in FCL, as opposed to *Bacillus* and *Clostridium* species in Greece (Hatzikamari *et al.*, 2007a,b). Although the underlying mechanism for this difference is unknown, use of warm water (about 50 °C) in Turkey and boiling hot water in Greece at the beginning of fermentation might be a plausible reason.

The literature reviewed thus far indicates that FCL or so-called 'chickpea yeast' might help improve the flavour and texture of whole-grain wheat flour breads, which established the aim of this study.

2. Materials and methods

Materials

Whole-grain wheat flour, vital gluten, fungal α-amylase, ascorbic acid and shortening were provided by Komgıda A.Ş. (Karaman, Turkey). Whole-grain wheat flour, obtained in a short-flow roller-mill designed specifically for whole-grain wheat flour production, had a particle size distribution of 7.6% over 250 μm, 5.9% over 150 μm, 78.3% over 75 μm, 6.2% over 38 μm and 2% below 38 μm. Diacetyl tartaric acid ester of mono and diglycerides was obtained from Kimbiotek A.Ş. (İstanbul, Turkey). Compressed baker's yeast (*S. cerevisiae*), salt, sugar (sucrose) and Kabuli-type chickpea were purchased from local markets (Karaman, Turkey).

Preparation of fermented-chickpea liquor

The FCL was prepared essentially by the method of Çebi (2009) with slight modification. Precleaned chickpea (100 g) was coarsely ground in a heavy-duty Waring blender, sifted through a 0.2-mm screen and the overs were placed in a 1-l glass container. Then 1 g of salt and 350 ml of boiled and cooled water (about 50 °C) were added to the container and its lid was closed. The cracked chickpea was left for spontaneous fermentation at 40 °C for 16 h. Once the fermentation ended and 1-2 cm of foam was formed atop the fermented liquid, the liquid phase plus foam was sieved through a 75-μm screen and used in breadmaking trials.

Characterisation of whole-grain wheat flour

Damaged starch content of whole-grain wheat flour was measured on the SDmatic (Chopin, Villeneuve-la-Garenne, France) by the ICC method 172 (Cauvain and Young, 2009). Falling number (FN) of whole-grain wheat flour, an indicator of α-amylase activity, was measured and adjusted to about 250 seconds on the Perten FN instrument (Perten, Hägersten, Sweden) by the AACC method 56-81B (AACC, 2000). Dough mixing and thermorheological properties of whole-grain wheat flour and FCL-containing flours were measured on the Chopin-mixolab using the 'Chopin+' protocol that was adopted as the ICC method 173 (Cauvain and Young, 2009).

Formulation of whole-grain wheat flour dough and breadmaking

The formulation used in the production of whole-grain wheat flour bread with FCL, which was modified from Paşa (2010), is given in Table 1. The formulation was consisted mainly of whole-grain wheat flour, water, FCL, baker's yeast, salt and other minor ingredients. Vital gluten at 2% level was incorporated into the formulation to increase the rather low protein content (11.0%) of the whole-grain wheat flour, which is a common practice when low-protein whole-grain wheat flours are used in breadmaking (Paşa, 2010). The straight-dough procedure was adopted in breadmaking. In brief, all ingredients were weighed in a laboratory mixer (KM023; Kenwood Ltd, Havant, UK) and mixed for 10 min. A water level of 65%, based on flour weight with 14% moisture basis (mb), was used. This level was adjusted from

Table 1. Bread dough formulation used in production of whole-grain wheat flour bread.

Component	Unit	Control bread	FCL containing bread[1]		
			15%	30%	45%
Whole-grain wheat flour[2]	g	100	100	100	100
Fermented-chickpea liquor (FCL)	ml	–	15	30	45
Water	ml	65 (opt.)[3]	50 (65-15)	35 (65-30)	20 (65-45)
Compressed baker's yeast	g	3	3	3	3
Salt	g	1	1	1	1
Sugar (sucrose)	g	1	1	1	1
Vital gluten	g	2	2	2	2
Fungal α-amylase (falling number)	sec	245	245	245	245
Ascorbic acid	mg	5	5	5	5
Shortening	g	1	1	1	1
DATEM[4]	g	0.5	0.5	0.5	0.5

[1] Based on flour weight (14% mb).
[2] 14% moisture basis.
[3] Mixolab optimum water absorption.
[4] DATEM = diacetyl tartaric acid ester of mono and diglycerides.

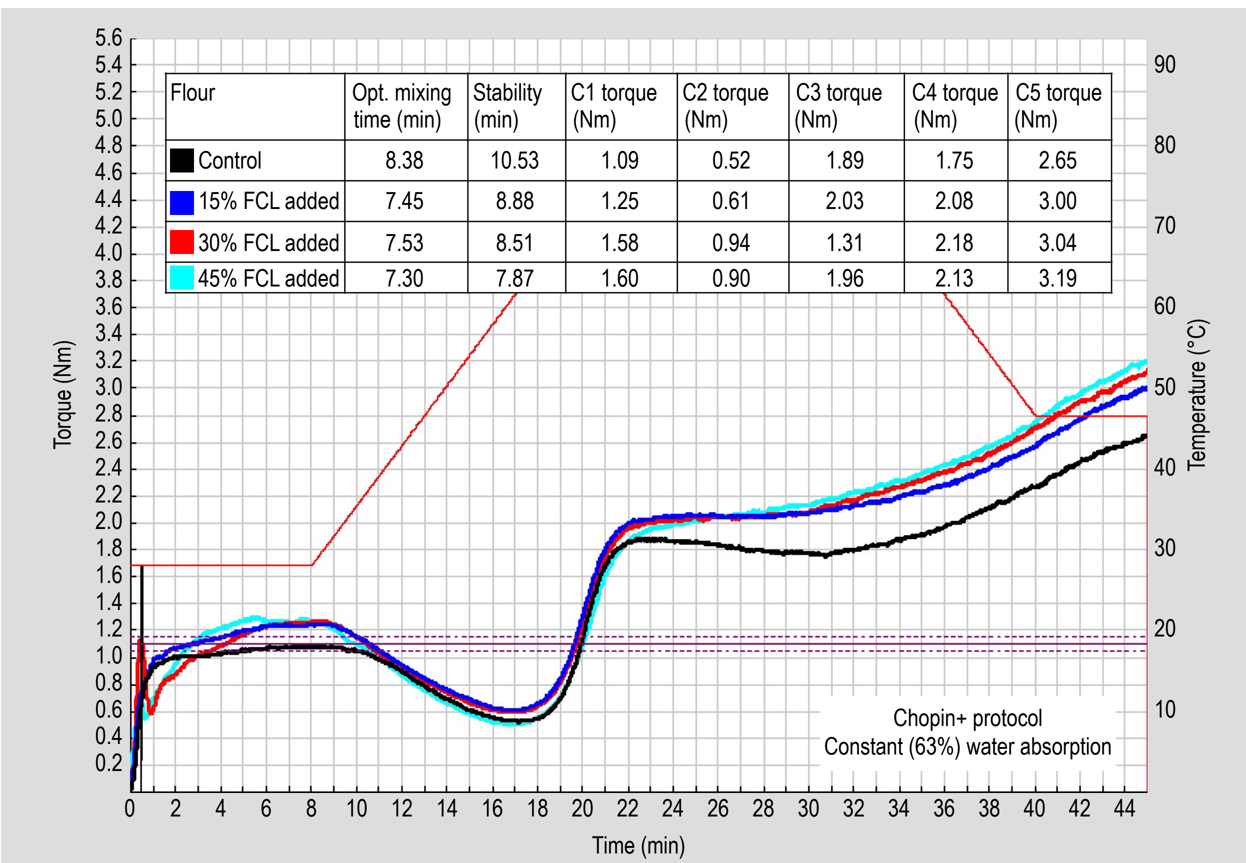
the mixolab (Chopin) optimum water absorption level of 63% for whole-grain wheat flour (Figure 1). FCL was added to the formulation at 15, 30 and 45% levels based on flour weight (14% mb) and substracted from optimum water requirement as seen in Table 1. The mixed dough was first rested at room temperature for 30 min, then divided and rounded for intermediate proofing at 30 °C for 30 min, and finally shaped and placed in baking pan for final proofing in vapour-saturated cabinet at 30 °C for 55 min. The fermented dough was baked in a convection oven at 200 °C for 20 min. The bread was cooled at room temperature for 1 h and quality-associated measurements were performed.

Analysis of fermented-chickpea liquor and FCL-containing dough

The pH of FCL was directly measured. For pH measurement of FCL-containing dough, 10 g of dough was first homogenised in 90 ml of distilled water for 1 min and then pH was measured. For microbiological analyses, FCL was directly sampled and inoculated for microbial counts. In the case of FCL-containing dough, however, the dough (25 g) was first homogenised in 225 ml of physiological saline water for 1 min and then proper dilutions were prepared for inoculations. Total yeast and mould counts in FCL or FCL-containing doughs were carried out on potato dextrose agar, *Enterobacteriaceae* family counts on violet red bile dextrose agar, total aerobic mesophilic bacteria counts on plate count agar (CM325; Oxoid, Basingstoke, UK) and LAB counts on De Man, Rogosa and Sharpe agar as described by Çebi (2009).

Chemical analysis

Moisture contents were determined by the AACC oven-drying (135 °C) method 44-15A (AACC, 2000). Protein contents were measured by the AACC Dumas method 46-30 (NDA 701; Velp Scientifica, Bohemia, NY, USA) and nitrogen-to-protein conversion factors of 5.70 or 6.25 were used (AACC, 2000). Crude oil contents were quantified by Soxhlet extraction (Gerhardt Soxtherm, Königswinter, Germany) according to AACC method 30-25 (AACC, 2000). Ash contents were analysed using ashing furnace (MF106; Nüve, Ankara, Turkey) at 900 °C until reaching to a constant weight as described by the AACC method 08-01 (AACC, 2000). Total carbohydrate contents were calculated by mass difference of proximate compositions.



Flour	Opt. mixing time (min)	Stability (min)	C1 torque (Nm)	C2 torque (Nm)	C3 torque (Nm)	C4 torque (Nm)	C5 torque (Nm)
Control	8.38	10.53	1.09	0.52	1.89	1.75	2.65
15% FCL added	7.45	8.88	1.25	0.61	2.03	2.08	3.00
30% FCL added	7.53	8.51	1.58	0.94	1.31	2.18	3.04
45% FCL added	7.30	7.87	1.60	0.90	1.96	2.13	3.19

Figure 1. Mixolab dough mixing and thermorheological properties of whole-grain wheat flours containing fermented-chickpea liquor (FCL).

Bread analysis

Weight, volume and baking losses of breads were determined by Elgün *et al.* (2002). Moisture contents were determined by the AACC method 44-15A (AACC, 2000). The pH of bread was measured by homogenising 10 g of bread in 90 ml of distilled water. Crumb hardness of breads was measured using texture analyser (TA.TX2; Stable Micro Systems, Godalming, UK) at 2nd, 24th and 72nd hours of storage by the AACC method 74-09.01 (AACC, 2000). For this purpose, bread slices of 25 mm in thickness were used and compressed at 25% with a 36-mm diameter probe. Crust and crumb colours of breads were measured using HunterLab colorimeter (Color Flex, Reston, VA, USA). Sensory evaluation of breads was performed using a 10-point hedonic scale by semi-trained panellists. Breads were scored from 1 to 10 by shape and symmetry, cell structure and homogeneity, chewing texture, taste and odour (flavour) and overall sensory properties (Elgün *et al.*, 2002).

Total phenolic content and DPPH radical scavenging activity analysis

Total phenolic contents of breads were determined according to Singleton *et al.* (1999). In brief, slices of breads were first freeze-dried, milled in the Waring blender to pass through 375-μm screen, extracted with ten-fold acidified methanol (HCl/methanol/water, 1/80/10, w/w) at room temperature for 2 h by shaking at 200 rpm and clarified by centrifugation (1000×*g*, 10 min). The clarified extract (250 μl), 2N Folin-Ciocalteu reagent (250 μl) and distilled water (5.75 ml) was combined, mixed and rested at room temperature for 8 min. Then, sodium carbonate solution (7.0%, 2.5 ml) and distilled water (5.0 ml) were added, mixed and rested for another 2 h. Finally, absorbance readings at 750 nm were performed on both bread extracts and gallic acid standard solutions to figure out total phenolic contents of breads.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of breads was determined by the method of Beta *et al.* (2005). The bread extracts prepared for total phenolic assays were used. In short, bread extract (100 μl) was mixed with DPPH solution (3.9 ml, 6×10^{-5} mol/l) and rested in dark at room temperature for 30 min. Finally, absorbance readings at 515 nm were performed on bread extracts, acidified methanol and DPPH solution to figure out antioxidant activity of breads.

Experimental design and statistical analysis

A completely randomised experimental design with two replications and triplicate measurements were used in the study. The data were subjected to analysis of variance (ANOVA) and the means were compared by the Duncan's multiple comparison test.

3. Results and discussion

Properties of whole-grain wheat flour used in production of breads containing fermented-chickpea liquor

Chemical composition of whole-grain wheat flour, used as a base or control flour in FCL-added breadmaking, is given in Table 2. The composition was typical of a whole-grain wheat flour (Gordon and Wrigley, 2004). It contained moderate level (5.2%) of damaged starch. Dough mixing and thermorheological characteristics of the control and FCL-containing whole-grain wheat flours, as measured by the Mixolab device, are shown in Figure 1. Optimum water absorption, mixing time and stability of the control flour were respectively 63.0% (14% mb), 8.38 min and 10.53 min. When FCL was added at 15, 30 and 45% based on flour weight, water absorption levels slightly increased, while mixing times and stabilities somewhat decreased depending on the FCL addition level. The increase in water absorption was most likely a result of dry matter provided by the FCL, which was 4.1% in average. The weakening effect of FCL is consistent with the previously published farinograph and extensograph studies (Narlıoğlu, 2013; Özkaya, 1992). However, Saad *et al.* (2015) added dried FCL to bread formulation at 1.5, 3.0 and 4.5% levels, and found that dried FCL addition slightly increased water absorption, mixing time, stability and loaf volume.

FCL has been used in the form of a prefermented dough (sponge) in almost all previous studies (Baykara, 2006; Çebi, 2009, 2014; Hancıoğlu-Sıkılı, 2003; Narlıoğlu, 2013; Özkaya, 1992). In this study, however, FCL was directly used without preparing a preferment. Furthermore, to the best of our knowledge, this is the first study focusing on the effects of FCL on whole-grain wheat flour bread rather than white-flour bread.

Table 2. Chemical composition of whole-grain wheat flour used in breadmaking.

Component	Content (%)[1]
Moisture (wet basis)	12.2
Protein (N×5.7)	11.0
Crude oil	1.69
Ash	1.33
Total carbohydrate	72.0
Damaged starch	5.2

[1] 14% moisture basis.

Properties of breads containing fermented-chickpea liquor

The pH, baking loss, moisture content and loaf volume of breads containing different levels of FCL are listed in Table 3. As expected, pH of breads decreased by FCL level. Moisture contents (42.0-42.5%) and baking losses (10.8-13.3%) of breads were similar. In terms of loaf volume, however, FCL additions, especially at 30 and 45% levels, significantly increased loaf volumes of whole-grain wheat flour breads. Given the fact that loaf volume is the chief quality parameter of breads and that whole-grain wheat flour breads are usually poor in volume, texture, colour and flavour (Paşa, 2010; Pyler, 1988) as opposed their superior nutritional qualities (Belobrajdic and Bird, 2013; Dykes and Rooney, 2007; Hemdane *et al.* 2016; Kumar *et al.*, 2011; Okarter and Liu, 2010; Slavin, 2004), the loaf volume increase in whole-grain wheat flour breads stemming from FCL usage is of foremost importance. The underlying mechanism for loaf volume increase by usage of FCL is not clear, yet FCL might have acted in a similar manner to sour-dough and improved loaf volume. It was reported that sour-dough (Katina *et al.*, 2006) and FCL (Hatzikamari *et al.*, 2007a) both raised proteolytic enzyme activity, leading to favourable changes in gluten structure, bread texture and loaf volume (Katina *et al.*, 2006).

Colour characteristics of whole-grain wheat flour breads produced using FCL are given in Table 4. Although FCL addition did not have any significant effect on crumb colour, it significantly reduced crust lightness (L*) and yellowness (b*). Bread crust colour is mostly provided by caramelisation and Maillard reactions, where reducing sugars and free amino acids play pivotal roles (Pyler, 1988). Since FCL used in the study contained 4.1% dry matter, most of which was reportedly sugars and free amino acids (Hatzikamari *et al.*, 2007a), it is plausible that the increase in crust colour was a result of FCL.

Crumb hardness and crumb cell structures of FCL-containing whole-grain wheat flour breads are shown in Figure 2 and Figure 3, respectively. In general, addition of FCL at 30 and 45% levels reduced the rate and extent of crumb hardness as compared to the control bread. However, utilisation of FCL at 15% level caused unexpectedly an increase in crumb hardness. The results demonstrate that at higher levels (30 or 45%), FCL can reduce crumb hardness and staling rate of whole-grain wheat flour bread. Baykara (2006) found that FCL had no effect on staling rate of bread, whereas Tülbek *et al.* (2003) claimed that FCL extended shelf life of bread. Çebi (2009) reported that *L. brevis* FK2, *L. lactis* FK5 and *L. plantarum* FK25, all isolated from FCL, improved crumb structure, cohesiveness and chewiness of

Table 3. Baking properties of whole-grain wheat flour breads containing fermented-chickpea liquor (FCL).[1]

Bread	pH	Baking loss (%)	Moisture content (%)	Loaf volume (ml)
Control	5.85 a	10.8 ns[2]	42.1 ns	415.4 c
15% FCL added	5.75 b	12.1	42.4	421.5 c
30% FCL added	5.70 c	13.3	42.0	454.9 b
45% FCL added	5.65 d	12.3	42.5	523.8 a

[1] Different letters in the same column indicate significant difference (P<0.05).
[2] No significant difference (P>0.05).

Table 4. Colour properties of whole-grain wheat flour breads containing fermented-chickpea liquor (FCL).[1,2]

Bread	Crust colour			Crumb colour		
	L*	a*	b*	L*	a*	b*
Control	41.5 a	11.6 ns[3]	16.8 a	49.9 ns	4.9 ns	14.3 ns
15% FCL added	40.4 a	10.4	15.2 b	49.0	4.6	14.2
30% FCL added	37.3 b	11.6	14.9 b	49.5	4.4	14.1
45% FCL added	33.6 c	11.5	12.9 c	48.1	5.4	14.2

[1] Different letters in the same column indicate significant difference (P<0.05).
[2] L* = lightness; a* = redness; b* = yellowness.
[3] No significant difference (P>0.05).

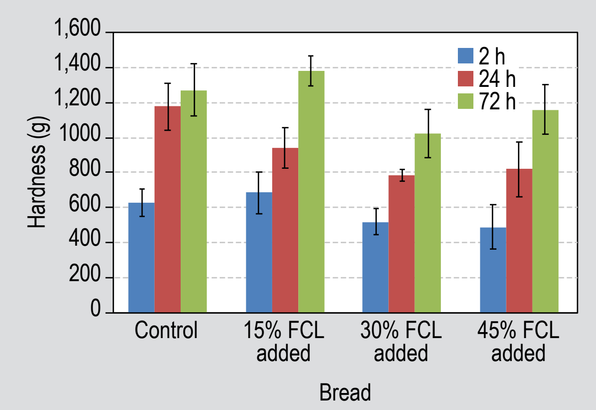


Figure 2. Crumb hardness and staling rates of whole-grain wheat flour breads containing fermented-chickpea liquor (FCL).

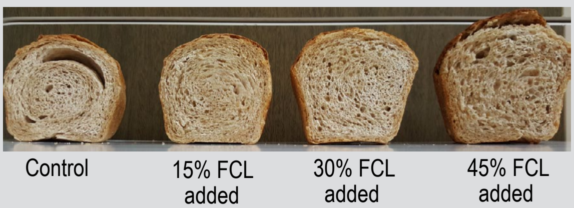


Figure 3. Crumb cell structures of whole-grain wheat flour breads containing fermented-chickpea liquor (FCL).

bread. Similar to FCL in principal, sour-dough process was proved to reduce crumb hardness and staling rate of bread through starch hydrolysis to dextrins due to acidification and microbial enzyme activity (Clarke *et al.*, 2002; Corsetti *et al.*, 1998a,b, 2000; Crowley *et al.*, 2002; Dal Bello *et al.*, 2007; Lavermicocca *et al.*, 2000). Additionally, proteolytic activity in sour dough was reported to change gluten structure and thus bread texture (Katina *et al.*, 2006). FCL was also determined to have elevated levels of hydrolytic enzymes and hydrolysis products (Hatzikamari *et al.*, 2007a). The results obtained in this study confirm that FCL is promising in improvement of whole-grain wheat flour bread texture, which is a major shortcoming of whole-grain wheat flour breads (Hebeda *et al.*, 1990).

Sensory properties of FCL-containing whole-grain wheat flour breads are listed in Table 5. As compared to the control bread, FCL addition did not cause any significant modification on chewing texture, flavour and overall sensory quality of breads; however, bread symmetry and cell structure were slightly modified. As can be seen in Figure 3, incorporation of FCL at 15 and 30% levels improved cell structure and uniformity of whole-wheat flour breads; however, further addition (45%) of FCL led to an open cell structure with deteriorated uniformity. Previous studies claimed that FCL improved crust colour, flavour and other sensory properties of bakery products (Baykara, 2006; Narlıoğlu, 2013; Özkaya, 1992). Indeed, FCL and FCL-containing doughs had an immense and somewhat irritating putrid odour; yet, this flavour was lost during baking and could not be detected in the breads by the sensory panellists.

When all bread quality parameters discussed thus far were taken into account together, use of 30 and 45% FCL in whole-grain wheat flour bread formulation appears to be the best levels.

Microbiology of doughs containing fermented-chickpea liquor (FCL)

Since the best baking results were obtained when FCL was used at 30% level, microbiological studies were conducted only on the doughs containing 30% FCL. As can be seen in Table 6, FCL and 30% FCL-containing dough had significantly higher counts of LAB, total aerobic mesophilic bacteria and total yeast and mould. Çebi (2009) also isolated a total of 120 LAB from FCL and FCL-containing dough. LAB are mostly probiotics and can contribute to health. Although, total yeast and mould count increased in FCL and FCL-containing dough, no fungi growth was observed upon five days of inoculation. This can be due to increased number of LAB, which was known to control moulding via bacteriocin production (Dalie *et al.*, 2010; De Vuyst and Vandamme, 1994; Schnürer and Magnusson, 2005; Şengün, 2011). On the other hand, *Enterobacteriaceae* family was not detected neither in the control dough nor in FCL and FCL-containing dough probably because of

Table 5. Sensory properties of whole-grain wheat flour breads containing fermented-chickpea liquor (FCL).[1,2]

Bread	Shape and symmetry	Cell structure and homogeneity	Chewing texture	Taste and odour (flavour)	Overall
Control	7.9 b	8.2 a	8.2 ns[3]	8.2 ns	8.1 ns
15% FCL added	8.0 b	8.0 a	8.4	8.2	8.3
30% FCL added	7.6 c	7.4 b	8.2	8.2	8.1
45% FCL added	8.5 a	8.0 a	8.5	8.4	8.4

[1] Different letters in the same column indicate significant difference (P<0.05).
[2] All properties on a 1 to 10 scale: 1 = worst, 10 = best.
[3] No significant difference (P>0.05).

their vulnerability to lower pH values (Çebi, 2009). The pH values of FCL ranged from 4.6 to 4.8 throughout the study.

Total phenolic content and DPPH radical scavenging activity of fermented-chickpea liquor incorporated breads

Total phenolic contents and DPPH radical scavenging activities of FCL-containing whole-grain wheat flour breads are listed in Table 7. FCL additions at 30 and 45% significantly increased total phenolics and DPPH radical scavenging activities of breads. Phenolics found in whole-grain wheat flour and chickpea have already been shown to contribute to healthy nutrition (Dykes and Rooney, 2007; Fosschia *et al.*, 2016; Kumar *et al.*, 2011; Okarter and Liu, 2010;). It was reported that the amount and composition of bioactive compounds are modified during fermentation (Dordevic *et al.*, 2010). Additionally, phenolics of chickpea were transferred to the liquid phase during fermentation and cooking (Clemente *et al.*, 1998). Being a spontaneous fermentation product, FCL is likely to be enhanced in phenolics, leading to an increase in the breads. Similar in principle to FCL, sour dough was reported to modify and stabilise certain bioactive compounds (Banu *et al.*, 2010; De Angelis *et al.*, 2007). In this respect, rye-based sour dough, rich in LAB, was found to increase total phenolics and antioxidant capacities of breads (Banu *et al.*, 2010). The result of this study proved that FCL can also contribute to the nutritional quality of whole-grain wheat flour bread in addition to its contribution to bread volume and texture.

4. Conclusions

The study focused on direct utilisation of FCL in whole-grain wheat flour bread production based on the hypothesis that FCL might contribute to poor flavour and texture of whole-grain wheat flour breads. For this purpose, FCL was incorporated into whole-grain wheat flour bread formulation at 15, 30 and 45%, based on flour weight. Effects of FCL on dough mixing properties, breadmaking qualities and antioxidant activities were studied. The results indicate that FCL can improve loaf volume, retard staling and enrich nutritional quality of whole-grain wheat flour breads. However, FCL has rather limited effect on flavour and colour of breads. Further studies are required to better understand and make effective use of FCL in bakery products.

Table 6. Microbiological properties of whole-grain wheat flour breads containing fermented-chickpea liquor (FCL).[1]

Dough or FCL	Unit	Lactic acid bacteria count	*Enterobacteriaceae* family count	Total aerobic mesophilic bacteria count	Total yeast and mould count
Control dough	log cfu/g	7.82 b	nd[2]	7.84 c	7.84 c
FCL	log cfu/g	7.84 b	nd	8.08 b	7.97 b
30% FCL-added dough	log cfu/g	8.15 a	nd	8.14 a	8.11 a

[1] Different letters in the same column indicate significant difference (P<0.05).
[2] Not detected.

Table 7. Total phenolic contents and DPPH radical scavenging activities of whole-grain wheat flour breads containing fermented-chickpea liquor (FCL).[1]

Bread	Total phenolic content (mg/100 g)[2]	DPPH radical scavenging activity (%)[2]
Control	0.44 b	27.3 b
15% FCL added	0.44 b	26.8 b
30% FCL added	0.63 a	30.5 a
45% FCL added	0.64 a	29.7 a

[1] Different letters in the same column indicate significant difference (P<0.05).
[2] Dry-matter basis.

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