Effect of Solid-State Fermentation on the Nutritional Value of Chickpea Flour and Physicochemical, Antioxidant Activity and Sensory Evaluation of Pan Bread

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is a most important legume that is utilized worldwide and high source of amino acids and protein. The effect of fermentation periods on the nutritional value of chickpea flour and the effect of replacement of wheat flour at 0, 5, 10, 15 and 20% with fermented chickpea flour (FCF) on the physicochemical, sensory properties, and antioxidant activity of pan bread were studied. Fermentation until 3 days resulted in a significant high in crude protein, lipids and crude fiber of fermented chickpea flour, while carbohydrate was reduced when compared to wheat flour. Also, the fermentation process at 1, 2, 3 and 4 days caused a significant decrease in phytic acid of FCF with reduction percent 26.30, 43.49, 53.33 and 66.21% respectively, while free amino acids (FAA) contents of FCF were increased with an increase in the fermentation time compared to native chickpea flour. As the FCF level increased, the bread crust color became darker, for bread crumb color, no significant effect was found in L^* with replacing wheat flour with FCF at different ratios. The addition of FCF at all levels increased the total phenolic compounds and antioxidant activities of bread when compared to control bread. Finally, it could be concluded that incorporating up to 10% of FCF in bread enriched the nutrition value and more prefer by sensory evaluation.

Keywords: fermented chickpea flour, nutrition, antioxidant, sensory, bread.

INTRODUCTION

Recently, the production of the wheat cereals has not been sufficient to cover the increasing demand for bread for human needs. So, many efforts have been done to substitute part of the wheat flour with other cereals or legumes flour. Flours from maize, barley and chickpea are among the most studied for the production of bread flour (Hefnawy *et al*., 2012).

The replacement of wheat flour with chickpea flour improves the protein and nutritional quality of the bread. This high protein content in chickpea replaced bread would be of nutritional importance in most countries, such as Africa and Asia. Chickpea cultivars are Desi and Kabuli. Kabuli seeds are large and light-colored beans, ram-head shape, and have low fibre content (Singh *et al.*, 2004). The Desi seeds are small, wrinkled at the beak, with green, brown or black color.

Chickpea (*Cicer arietinum L.*) is the third most important pulses seeds in the world (Boye *et al*., 2010), and the second most commonly grown legume in the world. Chickpea seeds, which are rich in crude protein (17–22%), are a cheap source of carbohydrates, minerals, and vitamins and are very important legumes in some tropical countries (Rachwa-Rosiak *et al*., 2015). The chickpea protein is rich in arginine but lower in the amount of sulphur amino acids like methionine and cysteine. The percentage of starch in chickpeas is ranging from 40 to 48%. The chickpea contains sufficient vitamins like vitamin A, niacin, thiamine, and folate. (Harsha, 2014). Chickpea seeds are a good source of protein, dietary fiber, vitamins (niacin, thiamine and ascorbic acid), minerals, unsaturated fatty acids, and the essential amino acids which are deficient in wheat (Zafar *et al*., 2015).

Chickpea seeds help in the reduction of blood pressure because it contains β-sitosterol, and linoleic acid and phytosterol. The chickpea seed lipid contains important phytosterols such as sterols, tocopherols, and tocotrienols which explain the anti-bacterial, anti-inflammatory, anti-fungal, and anti-ulcerative properties of chickpea flour which can help in the reduction of cholesterol level, cardiovascular and cancer disease (Moreau *et al*., 2002 and Ziena et al., 2019).

Although the high nutritional values and health benefits of chickpea, it contains anti-nutritional factors including trypsin, chymotrypsin inhibitors, phytates, phytic acid, flavonoids, lectins, α-amylase inhibitors, tannins, saponins, phenolics, and oxalic acid. These anti-nutritional factors reduce protein availability and digestibility by bonding the protein with other minerals (Jukanti *et al*., 2012). So, it is necessary to reduce the anti-nutritional factors levels to improve protein digestibility and availability.

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Fermentation gives a wide range of microbial and enzymatic processing of foods to introduce desirable properties like extended shelf life, safety, good flavor, nutritional improvement, antinutrient elimination, and improvement of health. The microorganism type, the fermentation conditions used, significantly affect the phytate removal during the fermentation process (Olika *et al*., 2019).

Solid-state fermentation (SSF) is an efficient, controlled, and economic method to improve the nutritional value and functional properties of legumes and cereals. Many biochemical and enzymatic changes occur in legumes during the fermentation process. For example, proteins can be hydrolyzed by proteases enzyme, resulting in the production of short chains compounds and low molecular weights. Thus, the digestibility, physicochemical properties, nutritional quality, and bioactivity of resulting substrates are improved (Rhyu and Kim, 2011 and Xiao *et al*., 2018).

This study aims to determine the effect of solid-state fermentation of chickpea flour at different times on the enhancement of functional and nutritional properties of chickpea flour and its effect on physicochemical properties, antioxidant activity and sensory evaluation of pan bread.

MATERIALS AND METHODS

Materials: -

Chickpea (*Cicer arietinum L*.) seeds were purchased from the local market, Qassim Region KSA. Wheat flour (80% extraction rate) was obtained from Saudi Grains Organization (SAGO). All other ingredients such as sugar, shortening, instant active dry yeast, and salt were purchased from the local market in Qassim Region. The chemicals were obtained from Arkan Development Comp. Limited at Qassim Region, KSA.

Methods: -

Solid-state fermentation of chickpeas flour:

Solid-state fermentation of chickpea flour was carried out according to the method described by Xiao *et al*. (2015) with some modifications including the culture added. The chickpea seeds were ground using a hammer mill to 1 mm mesh and sterilized at 121 °C for 25 min followed by cooling to room temperature (23±2 °C). Then, it was inoculated with 1.4% of active dry yeast extract (*saccharomyces cerevisiae*) with 4% sucrose. Spraying with distilled water was applied to keep moisture content around 20%. Chickpea flour with a thin layer (0.5 cm) over the tray was incubated at 30±1 °C and 85% relative humidity for 1, 2, 3 and 4 days. After incubation, the residual yeast was inactivated and fermented chickpea was dried in a hot air oven at 52 °C for 12 h. Dried of finally fermented chickpea flour (FCF) was milled in a laboratory mill, sieved (mesh size 180 μm), and stored in air-tight containers before further analysis.

Determination of phytic acid:

Phytic acid concentration of chickpea flour before and after fermentation at different periods was measured following the method described in AOAC (2005). Phytic acid content was expressed in equivalent phytic acid (mg/g).

Determination of free amino acids:

The concentration of free amino acids in chickpea flour before and after fermentation at different periods was determined by the Cd-ninhydrin method as illustrated by Folkertsma and Fox (1992). Free amino acids content was expressed as an equivalent of leucine in the sample.

Determination of gluten parameters:

According to standard AACC method 38-12 (2010)**,** wet, dry gluten and gluten index were determined using (Glutomatic perten instruments AB type 2200, Huddinge, Sweden).

Preparation of pan bread samples:

Pan bread samples were prepared using the AACC approved straight-dough method 10–10.03 (AACC International, 2010). The bread was baked at 230 °C for 15 min at 80% relative humidity in a conventional oven. Immediately after baking, the pan bread were cooled to 30 °C within 60 min and then packed in plastic bags until the analysis. To study the effect of FCF on the quality of bread, wheat flour was replaced at 0, 5, 10, 15 and 20% with fermented chickpea which added to flour according to the results of primary investigations.

Proximate chemical composition:

Moisture, ash, fat, protein, and total dietary fiber content of wheat, chickpea flours and FCF at different fermentation periods and bread samples were determined according to the methods shown in AOAC (2005). Total carbohydrate was calculated by difference. In addition, the chemical composition of pan bread and samples containing various levels of FCF were determined.

Physical properties of pan bread:

Weight (g) and baking loss (%) of pan bread were determined after baking and cooling. The volume (cm^3) of different prepared pan bread was determined according to the method mentioned by the AACC-approved method 10–05.01 (AACC International, 2010). Specific volume (cm^3/g) was calculated by dividing the volume (cm^3) by their weight (g).

Color attributes of pan bread:

The color parameters L* (100 = white; 0 = black), a* (+, red; -, green) and b* (+, yellow; -, blue) values of wheat pan bread and bread samples containing different

FCF levels were determined using a Hunter Lab Color QUEST II Minolta CR-400 (Minolta Camera, Co., Ltd., Osaka, Japan) according to the method described in Francis (1983).

Determination of phenolic content and antioxidant activity (DPPH):

The extracted phenolic compounds from bread sample were prepared as described by Bloor (2001). Total phenolic content was measured by the Folin–Ciocalteu assay along with a spectrometer at 765 nm as described by Singleton et al. (1999). Gallic acid was applied as a standard, and the results were expressed as mg galic acid equ/g.

The ability of samples extracts to scavenge free radicals was determined by the method described by Blois (1958). The scavenging effect was calculated from the reduction of absorbance at 517 nm against (DPPH radical solution in methanol) using the following equation: Scavenging activity (%) = [(Abs. control – Abs. sample)/Abs. control] x 100

Measurement of pan breadcrumb hardness:

The hardness of breadcrumb samples was determined according to the AACC approved method 74–09.01 (AACC International, 2010). The bread slices (1.25 cm thick) from the center of the pan bread were compressed to 50% of their initial thickness at a test speed of 1 mm s^{-1}. The value of the maximum force during the first cycle of compression (F2) was recorded as the firmness or hardness.

Sensory evaluation of pan bread samples:

Substituted pan bread samples with different levels of FCF were introduced to sensory evaluation by 20 semi-trained panelists of food science and human nutrition department staff. The panelists were asked to evaluate each loaf for appearance, crumb color, crumb texture, odor, taste, and overall acceptability. A 10-point scale was used where 10″excellent and 1″ extremely unsatisfactory according to the method described by AACC (2010).

Statistical analysis:

The statistical analysis was carried out with 3 replicates of the experiments, except for the sensory evaluation which was 10 replicates. Data were means ± standard errors. Statistical analysis was conducted with the SAS program (2004) using one-way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test with $p \leq 0.05$ being considered statistically significant.

RESULTS AND DISCUSSION

Chemical composition of wheat flour and FCF:

The proximate chemical composition of wheat, chickpea flour and fermented chickpea flour (FCF) at 30 ºC for 1, 2, 3 and 4 days was determined and the results are found in table (1). It could be noticed that wheat flour has a higher moisture content (11.46%) with a significant ($p \leq 0.05$) high than other samples. FCF for one day fermentation didn't appear significant ($p \geq 0.05$) difference with raw chickpea flour in moisture content being 5.60 and 6.11%, respectively. The same trend was found between FCF at 2 and 3 days, while fermentation for 4 days resulted in a significant ($p \leq 0.05$) decrease in moisture content (2.76%) compared to all previous samples. Chickpea flour and FCF at different periods were significantly ($p \leq 0.05$) higher in ash (3.43%) than wheat flour (0.64%), and fermentation period didn’t significant ($p \geq 0.05$) effect in ash content which ranged from 3.03 to 3.57%. As it is known, chickpea flour contains a higher concentration of protein (17.61%) than wheat flour 10.40%. A gradually significant ($p \leq 0.05$) increase was found in the protein of chickpea flour when fermented for up to 3 days, while FCF at 4 days exhibited a decrease in protein content of (16.62%) may be due to the high hydrolytic analysis of resulted enzymes during fermentation times. The same results also were observed for the lipids content of FCF compared to wheat flour. A high significant ($p \geq 0.05$) increase was recorded in crude fiber for chickpea flour and FCF at different fermentation periods ranging from 6.68 to 9.09% when compared to 0.65% for wheat flour. These results are in harmony with those obtained by Ali *et al.* (2021) who reported that the proximate composition of chickpea flour contained 11.08% moisture, 19.0% crude protein, 6.65% crude fat, 3.67% ash and 6.06% crude fiber.

Table 1. Chemical composition of wheat flour and fermented chickpea flour (on a dry weight) at 30 ºC for different periods

Treatments	Chemical composition (%)					
	Moisture	Ash	Crude protein	Lipids	Crude fiber	Carbohydrates*
Wheat flour	11.46 ± 0.09[a]	0.64 ± 0.07[b]	10.40 ± 0.09[e]	1.86 ± 0.05[d]	0.65 ± 0.06[c]	86.45 ± 0.12[a]
Chickpea flour	6.11 ± 0.26[b]	3.43 ± 0.05[a]	17.61 ± 0.66[cd]	4.96 ± 0.21[c]	8.67 ± 0.33[a]	65.32 ± 1.02[c]
FCF at 1 day	5.60 ± 0.65[b]	3.18 ± 0.38[a]	18.40 ± 0.37[bc]	5.65 ± 0.14[bc]	8.41 ± 0.27[a]	64.36 ± 0.22[c]
FCF at 2 days	3.47 ± 0.58[c]	3.03 ± 0.18[a]	19.12 ± 0.32[b]	5.89 ± 0.26[b]	8.36 ± 0.56[a]	63.61 ± 0.72[c]
FCF at 3 days	3.20 ± 0.15[c]	3.53 ± 0.21[a]	20.72 ± 0.37[a]	7.99 ± 0.38[a]	9.09 ± 0.19[a]	58.67 ± 0.33[d]
FCF at 4 days	2.76 ± 0.25[d]	3.57 ± 0.13[a]	16.62 ± 0.44[d]	5.79 ± 0.22[b]	6.68 ± 0.37[b]	67.35 ± 0.26[b]

FCF: Fermented Chickpea Flour, Carbohydrates*: calculated by difference. Data are the mean ± SE, n=3, Values followed by the same letters in the same column are not significantly different (p≤0.05).

Effect of fermentation on phytic acid and free amino acids contents:

The changes in phytic acid and free amino acids (FAA) contents of chickpea flour which effected by fermentation time were studied and the data are illustrated in Figure (1). As expected, the fermentation process resulted in a decrease in phytic acid contents of FCF after 1, 2, 3 and 4 days being 5.51, 4.22, 3.49 and 2.52 mg/g, as well as reduction percent 26.30, 43.49, 53.33 and 66.21% respectively compared to raw chickpea flour (7.47 mg/g).

The reduced phytic acid content with the fermentation time extent could attributed to the acidic 3-phytase from *S. cerevisiae* with optimum pH of 2.5–6.0 (Greiner *et al*., 2001).

During the yeast fermentation, increased phytase and phosphatase activities capable of hydrolyzing the phytates to orthophosphate and inositol resulted in a significant reduction in phytic acid content as described by Bilgiçli and Elgün (2005).

On the other hand, FAA contents of FCF increased with increase in the fermentation time compared to native chickpea flour (21.3 mg/g). FAA gradually increased in FCF at 1, 2, 3, and 4 day of fermentation, recording 31.23, 36.61, 47.16 and 55.04 mg/g causing increasing percentage 46.74, 71.87, 121.49 and 158.51%, respectively. The hydrolysis of chickpea flour proteins with yeast enzymes may be lead to an improved availability of protein digestibility and amino acids. Shrivastava and Chakraborty (2018) reported that the lower phytic acid content with the higher free amino acids was obtained when the chickpea flour was fermented with yeast extract (1.4%) for 83 hr.

As previous results of the effect of fermentation times on the improvement of chickpea flour, the best treatment is found to be 3 days of fermentation compared to other period treatments.

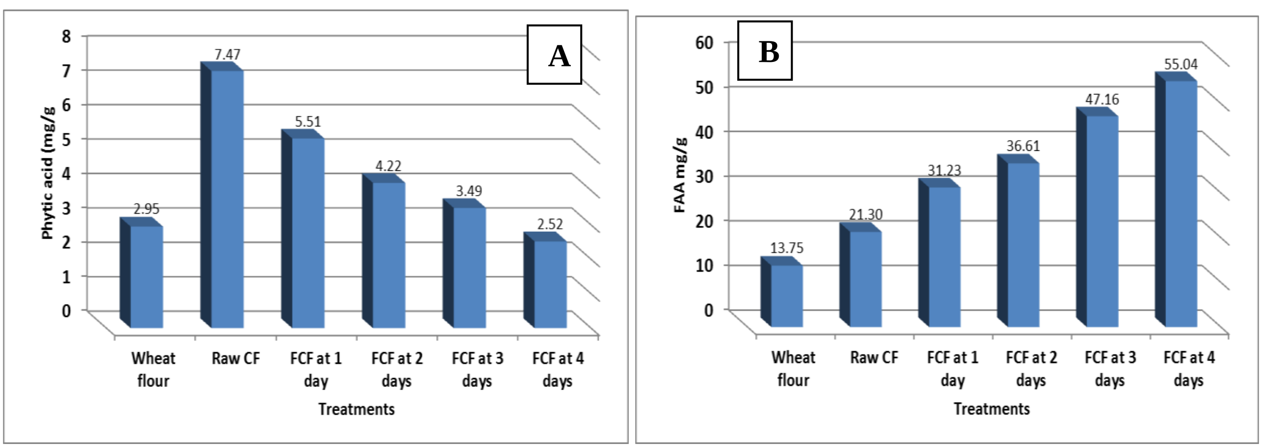

Fig. 1. phytic acid (A) and free amino acids (B) of chickpea flour as affected by fermentation times

Gluten parameters of dough containing FCF:

Gluten matrix is a very important criterian in bread making, so the effect of incorporated FCF at 0, 5, 10, 15 and 20% in wheat flour on gluten parameters were investigated and the data are tabulated in table (2). Results revealed that a gradually significant decrease in wet dough gluten was appeared with increased FCF levels. Wet gluten was 27.96% for wheat flour with a significant effect followed by 25.39, 23.81, 21.20 and 18.17% recorded by 5, 10, 15 and 20% of FCF, respectively. The same trend was found in the gluten index which is the related to formation of gluten net in bread dough and the ability of retention of yeast gas. As expected, the high significant ($p \leq 0.05$) value of gluten index (89.29) was given by wheat flour. Adding FCF at different ratios resulted in a significant ($p \leq 0.05$) decrease in gluten index from 86.52 for 5% FCF to 75.40 for 20% FCF dough. These results may be because chickpea doesn't contain gluten and its addition caused decrease of gluten in wheat flour composition. Hung *et al.* (2007) reported that the existence of a fiber-rich source interfered with the optimal gluten matrix formation and diluted the protein in bread dough mixing.

Chemical composition of pan bread:

Bread samples prepared from wheat flour and substitution by 5, 10, 15 and 20% with FCF at 3 days were analyzed for proximate composition and the results are shown in table (3). It could be observed that control wheat bread and bread flour with 5% FCF had the highest moisture content 38.54 and 36.66%, respectively. While a significant ($p \leq 0.05$) decrease was found in bread moisture when 10, 15 and 20% of FCF were added being 33.32, 27.64 and 24.98%, respectively. These results attributed to the low moisture content of FCF (3.20%) compared with wheat flour (11.46%) (Table 1). Incorporating FCF in bread resulted in a significant ($p \leq 0.05$) increase in ash when compared to control bread (1.38%). Ash of FCF bread ranged between 1.57 for 5% of FCF to 2.87% for 20% of FCF. Also, the clear reason for the results is the high content of ash in used FCF (Table 1). The same trend was found in crude protein, where significant ($p \leq 0.05$) high protein in bread was recorded by incorporating FCF at different levels. The bread protein was 14.40, 16.38, 18.18 and 19.29% for bread samples containing 5, 10, 15 and 20% of FCF, respectively compared with control sample (12.78%).

Also, the same results were recorded in the case of lipids and crude fiber of FCF bread, where a significant ($p \geq 0.05$) increase in bread fiber was given when replacing wheat flour at 5, 10, 15 and 20% with FCF which is 3.73, 4.85, 5.78 and 6.91%, respectively as compared to 1.72% for control bread.

On the other side, the carbohydrate and energy of bread samples were significantly ($p \leq 0.05$) and low when adding FCF at various levels as compared to 81.61 (400 kcal/g), respectively which were recorded by control bread.

Table 2. Effect of fermented chickpea on gluten parameters of wheat flour dough composite

Treatments	Wet gluten (%)	Dry gluten (%)	Gluten index
Wheat flour	27.96 ± 0.45^{a}	8.66 ± 0.08^{a}	89.29 ± 0.40^{a}
Wheat flour with 5% FCF	25.39 ± 0.34^{b}	6.09 ± 0.14^{b}	86.52 ± 0.47^{b}
Wheat flour with 10% FCF	23.81 ± 0.19^{c}	5.19 ± 0.11^{c}	84.14 ± 0.27^{c}
Wheat flour with 15% FCF	21.20 ± 0.39^{d}	4.64 ± 0.22^{d}	81.19 ± 0.41^{d}
Wheat flour with 20% FCF	18.17 ± 0.15^{e}	4.13 ± 0.06^{d}	75.40 ± 0.76^{e}

FCF: Fermented Chickpea Flour, Data are the mean ± SE, n = 3, Values followed by the same letters in the same column are not significantly different ($p \leq 0.05$).

Table 3. Chemical composition of wheat bread incorporated with different levels of FCF (on a dry weight basis)

Treatments	Chemical composition (%)						
	Moisture	Ash	Crude protein	Lipids	Crude fiber	Carbohydrates**	Energy(kcal / g)
Control bread	38.54±0.80^{a}	1.38±0.03^{d}	12.78±0.28^{e}	2.51±0.13^{e}	1.72±0.14^{e}	81.61±2.39^{a}	400.19±5.38^{a}
Bread with 5% FCF	36.66±0.68^{a}	1.57±0.05^{d}	14.40±0.32^{d}	3.36±0.16^{d}	3.73±0.15^{d}	76.94±1.57^{b}	395.57±3.46^{b}
Bread with 10% FCF	33.32±0.52^{b}	1.88±0.03^{c}	16.38±0.22^{c}	4.98±0.16^{c}	4.85±0.17^{c}	71.91±0.28^{c}	397.95±6.25^{ab}
Bread with 15% FCF	27.64±0.66^{c}	2.24±0.13^{b}	18.18±0.26^{b}	5.76±0.38^{b}	5.78±0.24^{b}	68.04±0.56^{d}	396.76±4.79^{b}
Bread with 20% FCF	24.98±0.34^{d}	2.87±0.09^{a}	19.29±0.16^{a}	6.84±0.32^{a}	6.91±0.33^{a}	64.09±0.83^{e}	395.11±3.19^{b}

FCF: Fermented Chickpea Flour, Data are the mean ± SE, n = 3, Values followed by the same letters in the same column are not significantly different ($p \leq 0.05$). **Carbohydrates****: calculated by difference.

Physical properties of FCF pan bread:

The impact of FCF flour addition on pan bread quality (weight, volume, specific volume and baking loss) are represented in table (4). Regarding to the pan bread loaf weight with FCF, a proportional increment between the bread weight and the FCF levels incorporated was found. The bread weight ranged from 172.34 g for the control bread to 174.75, 179.40, 180.47 and 181.76 g for bread samples containing 5, 10, 15 and 20% FCF, respectively. The high protein and fiber content of the FCF, compared with the wheat flour contribute to higher water absorption in the bread dough and consequently increase the pan bread weight. The higher water absorption capacity of FCF might be attributed to the presence of greater amounts of hydrophilic polysaccharides and proteins (Simona *et al*., 2015). A gradually significant ($p \leq 0.05$) decrease in bread volume was found with increasing the FCF ratio in bread. The highest volume (630.67 cm^3) was recorded to the control bread. While the volume of bread samples containing FCF ranged from 580.56 cm^3 for 5% until 461.64 cm^3 for 20%. The decrease in the pan bread volume is resulting by reduced gluten content in the bread dough and a reduced capacity of the dough to hold the fermentation gases (Simona *et al*., 2015).

The same trend was found in specific volume, whereas, incorporating FCF at different levels caused a significant ($p \leq 0.05$) decrease in specific volume which ranged from 3.66 cm^3/g to control bread to 2.54 cm^3/g in bread with 20% of FCF. According to the literature, the addition of various flours high in fiber, up to 7%, produce a decrease in volume which is proportional to the reduction of gluten content in the blend (Table 3). Brenan and Cleary (2007) stated that valuable amounts of water could be binding with the added fibers during bread making, so less water was available for the development of the gluten matrix, causing reduced bread volume. Mohammed *et al.* (2012) hypothesized that chickpea flour suppresses the steam generated, because of its high water absorption capacity, leading to greater crumb firmness and reduced loaf volume.

Color parameters of FCF pan bread crust and crumb

Color attributes Lab of crust and crumb of the bread samples as affected by containing FCF at different levels were measured and obtained data are presented in table (5). In general, as the FCF level increased, the bread crust color became darker. The control bread crust was significantly ($p \leq 0.05$) lighter (37.36) compared to other treatments. The darkness of bread containing FCF with higher lysine content could be due to an increasing Maillard reaction taking place during baking. No significant difference was appeared in crust redness (a) between control bread and all bread samples containing FCF which ranged from 12.37 to 11.31. Data also revealed that when the concentration of FCF increased, the yellowness of the bread crust significantly ($p \leq 0.05$) decreased from 16.55 for control pan bread to 9.63 for bread containing 20% of FCF.

Regarding breadcrumb color, no significant effect was found in lightness L^* with replacing wheat flour with FCF at different ratios. The lightness L^* of bread crumb ranged between 64.90 given by the control sample to 63.61 recorded by 20% of FCF. On the other hand, as the level of FCF increased in bread making, the redness a* and yellowness b* values increased significantly from 18.11 for control bread to 20.38 for 20% of FCF, indicating that a redder and more yellow bread crumb was obtained as a result of FCF substitution. Similar findings were obtained by Fenn, *et al*. (2010), they stated an increase in redness and yellowness values, and a reduction in the lightness value in pan bread with addition of chickpea flour. Mohammed *et al*. (2012) reported that the wheat bread color was light brown increased significantly with increasing the level of chickpea flour. bread is obtained with the replacement of wheat flour for chickpea flour at 10%.

Table 4. Physical properties of pan bread samples prepared from wheat flour and different levels of fermented chickpea flour

Treatments	Weight (g)	Volume (cm^3)	Specific volume (cm^3/g)	baking loss (%)
Control bread	172.34 ± 0.44^{c}	630.67 ± 1.20^{a}	3.66 ± 0.02^{a}	13.83 ± 0.22^{a}
Bread with 5% FCF	174.75 ± 1.52^{bc}	580.56 ± 5.21^{b}	3.32 ± 0.03^{b}	12.63 ± 0.76^{ab}
Bread with 10% FCF	179.40 ± 2.63^{ab}	511.27 ± 7.26^{c}	2.85 ± 0.09^{c}	10.30 ± 0.76^{bc}
Bread with 15% FCF	180.47 ± 0.92^{a}	487.58 ± 1.45^{d}	2.70 ± 0.46^{d}	9.77 ± 0.45^{c}
Bread with 20% FCF	181.76 ± 2.86^{a}	461.64 ± 1.66^{e}	2.54 ± 0.05^{e}	9.12 ± 1.43^{c}

FCF: Fermented Chickpea Flour, Data are the mean ± SE, n = 3, Values followed by the same letters in the same column are not significantly different ($p \leq 0.05$).

Table 5. Color attributes of pan bread samples prepared from wheat flour and different levels of fermented chickpea flour

Treatments	Bread crust color			Bread crumb color		
	L*	a*	b*	L*	a*	b*
Control bread	37.36 ± 0.04[a]	11.94 ± 0.40[a]	16.55 ± 0.13[a]	64.90 ± 0.42[a]	-0.54 ± 0.05[e]	18.11 ± 0.04[d]
Bread with 5% FCF	35.60 ±0.32[ab]	11.33 ± 0.14[a]	14.60 ±0.72[bc]	64.84 ± 0.21[a]	-0.32 ± 0.04[d]	18.69± 0.02[cd]
Bread with 10% FCF	34.47 ± 0.86[b]	12.37 ± 0.03[a]	15.29 ±0.52[ab]	65.48 ± 0.32[a]	0.08 ± 0.03[c]	19.24± 0.33[bc]
Bread with 15% FCF	29.64 ± 0.59[c]	11.83 ± 0.46[a]	13.34 ± 0.78[c]	64.52 ± 0.43[a]	0.32 ± 0.04[b]	19.75± 0.27[ab]
Bread with 20% FCF	25.94 ± 0.85[d]	11.31 ± 0.70[a]	9.63 ± 0.52[d]	63.61 ± 1.09[a]	0.48 ± 0.02[a]	20.38 ± 0.53[a]

FCF: Fermented Chickpea Flour, L*: lightness; a*: redness; b*: yellowness, Data are the mean ± SE, n = 3, Values followed by the same letters in the same column are not significantly different (p≤0.05).

Total polyphenols and antioxidant activity (DPPH) of pan bread:

The total phenolic contents and DPPH scavenging activity of breads FCF-containing are illustrated in Figure (2). The addition of FCF at 5, 10, 15 and 20% highly increased total phenolic compounds being 0.59, 0.67, 0.89 and 1.01 mg GA equ/g, respectively and antioxidant activities being 28.74, 31.11, 34.25 and 39.29%, respectively of bread samples when compared to control bread. As a fermentation product, FCF is a good source and enhance in phenolic compounds, resulting in an increase in the bread. Phenolic compounds found in fermented chickpeas have already been shown to contribute to healthy nutrition (Kumar *et al.*, 2011 and Fosschia *et al.*, 2016). The concentration and composition of bioactive compounds of FCF are developed during fermentation (Dordevic *et al.*, 2010). The obtained results of this study were agreement with those obtained by Sayaslan and Şahin (2018) who reported that fermented chickpeas can contribute to the nutritional quality of wheat flour bread in addition to its contribution to bread volume and texture.

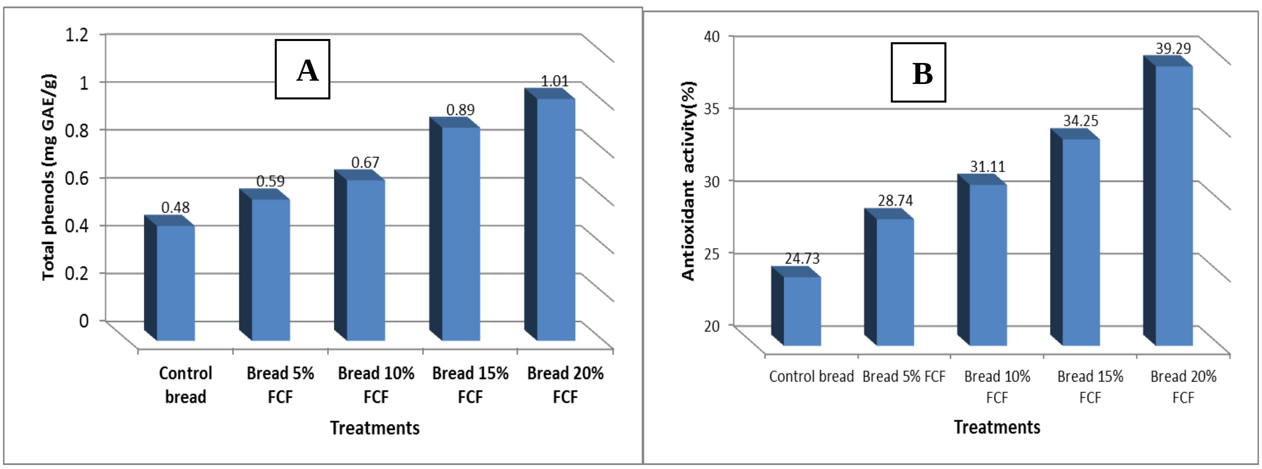

Fig. 2. Total polyphenols (A) and antioxidant activity (DPPH) (B) of bread samples containing different levels of fermented chickpea flour

Crumb hardness (staling rate) of pan bread containing FCF flour:

The effect of incorporated FCF at 5, 10, 15 and 20% in bread on crumb hardness (g) during storage for 5 days at room temperature (23±2ºC) was investigated and the data are illustrated in Figure (3). On the first day, fairly difference was found in hardness between control bread, 5 and 10% of FCF reaching gave 829.13, 846.60 and 902.10 g, respectively. While the addition of 15 and 20% of FCF caused high value breadcrumb hardness (1223.58 and 1399.23g), respectively when compared to the control sample. The same trend occurred on the third and fifth day of bread storage, whereas replacing wheat flour with FCF at all selected ratios resulted increase in bread hardness. Bread samples containing 5 and 10% are still more soft and acceptable to consume until the fifth day of storage. These results may be attributed to FCF helping in keeping the moisture inside the gluten network thus showing a clear negative effect on crumb hardness. Our results are in accordance with Shrivastava and Chakraborty (2018), who reported that increasing the levels of FCF led to an increase in crumb hardness. The hardness is the formation of the cross-links between gluten proteins and starch in which moisture acts as a plasticizer (Mohammadi, *et al*., 2014). At a lower concentration of water, the formation of crosslinks between protein accelerates and starch, thus producing the firm bread. Eliasson (2006) explained that starch can be made retrogradation, where the amylose and amylopectin molecules are re-associated, forming crystalline with consequent expulsion of water molecules and hardening of the bread structure.

Sensory evaluation of pan bread containing FCF:

Pan bread samples partially replaced with different levels of 0, 5, 10, 15 and 20% with FCF were subjected to sensory evaluation and the mean values of panelists are presented in table (6). It is clear that the addition of FCF in bread caused a significant ($p \leq 0.05$) decrease in all properties compared to control bread. No significant difference was found in appearance and crumb color between bread containing 5 and 10% of FCF, which recorded 8.7, 8.1 and 9.0, 8.3, respectively. For crumb texture, bread with 5% of FCF had the same acceptability as panelists compared with control bread. Also bread sample containing 10% was preferable to 15 and 20% of FCF. Regarding to odor and taste, the same trend was found. Whereas, up to 15% of FCF in bread samples didn't occur an undesirable taste or odor when compared with control bread. Finally, it could be noticed that no significant ($p \geq 0.05$) difference was found in overall acceptability between pan bread with 5 and 10% of FCF being 8.6 and 8.3, respectively. While incorporated 15 and 20% of FCF are not well preferred and caused a significant decrease in the overall acceptability of bread by panelists as compared to the control sample. Da Costa *et al*. (2020) found no difference in sensory evaluation of the bread elaborated with different concentrations of whole chickpea flour, and all samples presented good acceptance. Belobrajdic and Bird (2013) reported that fermented chickpeas improved the crust color, flavor and other sensory properties of bakery products. The incorporation of fermented chickpeas at 15 and 30% levels improved the cell structure and uniformity of whole-wheat flour bread reported by Sayaslan and Şahin (2018).

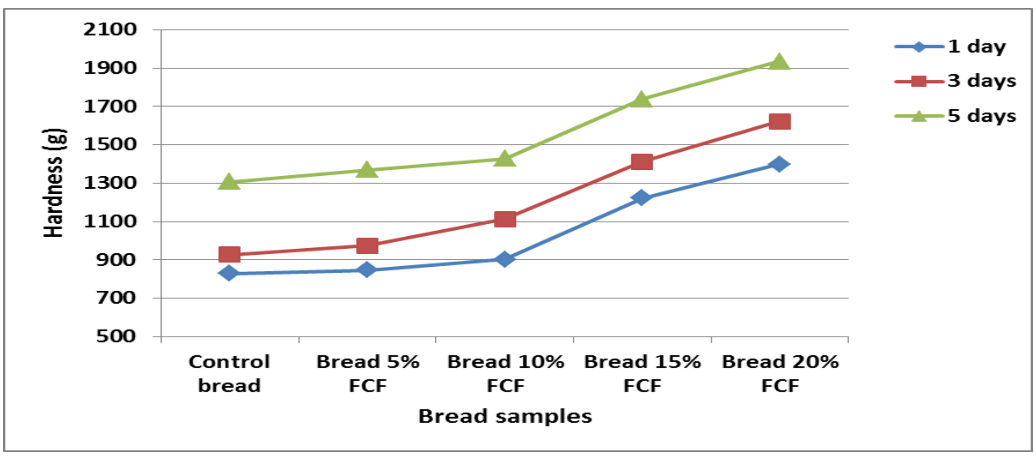

Fig. 3. Hardness (staling rate) of pan bread samples with different levels of FCF during 5 days of storage at room temperature

Table 6. Sensory evaluation of bread samples incorporated with different levels of FCF

Treatments	Appearance (10)	Crumb Color (10)	Crumb Texture(10)	Odor (10)	Taste (10)	Overall acceptability (10)
Control bread	9.4 ± 0.16[a]	9.3 ± 0.21[a]	9.2 ± 0.25[a]	9.6 ± 0.22[a]	9.4 ± 0.21[a]	9.6 ± 0.16[a]
Bread with 5% FCF	8.7 ± 0.21[b]	9.0 ± 0.33[ab]	8.7 ± 0.21[a]	9.3 ± 0.42[a]	9.1 ± 0.26[a]	8.6 ± 0.35[b]
Bread with 10% FCF	8.1 ± 0.23[b]	8.3 ± 0.31[bc]	7.9 ± 0.24[b]	8.3 ± 0.46[b]	8.1 ± 0.23[b]	8.3 ± 0.21[b]
Bread with 15% FCF	6.8 ± 0.25[c]	7.5 ± 0.34[c]	6.2 ± 0.29[c]	8.2 ± 0.25[b]	7.5 ± 0.31[b]	7.4 ± 0.27[c]
Bread with 20% FCF	5.9 ± 0.28[d]	6.6 ± 0.31[d]	5.8 ± 0.20[c]	7.9 ± 0.28[b]	6.5 ± 0.37[c]	6.1 ± 0.24[d]

FCF: Fermented Chickpea Flour, Data are the mean ± SE, n = 10, Values followed by the same letters in the same column are not significantly different ($p \leq 0.05$).

CONCLUSION

It could be concluded that the solid-state fermentation of chickpea flour (FCF) using *saccharomyces cerevisiae* for up to 3 days caused a significant increase of crude protein and lipids. while it didn't effect ash and dietary fiber of FCF. The fermentation process resulted in a significant decrease of phytic acid and an increase in FAA contents of FCF, which are very important nutrition factors in legumes compared to raw chickpea flour. Also, wet and dry gluten and gluten index of wheat flour dough incorporated with different ratios of FCF were negatively affected because of the dilution of gluten in wheat chickpea flour composite. Many advantages were obtained in bread chemical composition when partially replaced by FCF until 20%. Whereas, ash, protein, lipids, and crude fiber contents of pan bread were significantly high as compared with control pan bread. Data also revealed that adding FCF at different levels caused a significant low in specific volume which ranged from 3.66 cm^3/g by control bread to 2.54 cm^3/g by bread with 20% of FCF because the reduction of gluten content in the mixture dough. As a fermentation process, FCF considers a good source of bioactive compounds, resulting in an increase in the bread. Bread containing 5 and 10% of FCF still has more softness and less hardness during 5 days of storage at room temperature. Finally, replacing wheat flour at 10% with FCF in bread resulted in more preferable sensory properties and nutritional enhancement of pan bread.

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الملخص العربي

تأثير التخمير بالحالة الصلبة على القيمة الغذائية لدقيق الحمص والصفات الفيزيوكيماوية، النشاط المضاد للأكسدة والتقييم الحسي لخبز القوالب

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يعتبر الحمص من أهم البقوليات يتم استهلاكها في جميع أنحاء العالم الى جانب أنه ومصدرًا عاليًا للأحماض الأمينية والبروتينات. تمت دراسة تأثير فترات التخمير على القيمة الغذائية لدقيق الحمص وتأثير استبدال دقيق القمح بدقيق الحمص المتخمر FCF بنسبة صفر ، ٥ ، ١٠ ، ١٥ ، ٢٠٪ على الخصائص الفيزيائية والكيميائية والحسية والنشاط المضاد للأكسدة لخبز القوالب. نتج عن التخمير لمدة تصل إلى ٣ أيام ارتفاع معنوي في البروتين الخام والدهون والألياف الخام لدقيق الحمص المتخمر، بينما انخفض الكربوهيدرات مقارنة بدقيق القمح. أيضاً، أدت عملية التخمير لمدة ١ و ٢ و ٣ و ٤ أيام الى انخفاض معنوي في حمض الفيتيك في FCFمما يعادل نسبة ٢٦,٣٠ و ٤٣,٤٩ و ٥٣,٣٣ و ٦٦,٢١٪ على التوالي، بينما زادت محتويات الأحماض الأمينية الحرة في دقيق الحمص المتخمر FCF بشكل ملحوظ وهذا مع زيادة وقت التخمير مقارنة مع دقيق الحمص العادي. لوحظ انخفاض كبير في مؤشر الجلوتين الرطب والجاف، والذي يرتبط بتكوين شبكة الجلوتين في العجين والقدرة على الاحتفاظ بالغاز، وهذا عند زيادة مستوى دقيق الحمص المتخمر. أدى إضافة FCF في الخبز إلى زيادة معنوية في الرماد) تراوحت بين ١,٥٧ وهذا بالنسبة للخبز المحتوي على ٥٪ من FCF حتى ٢,٨٧٪ عند اضافة ٢٠٪ FCF (مقارنةً بخبز المقارنة (١,٣٨٪). تم تسجيل نفس النتائج في حالة البروتين والدهون والألياف الخام للخبز. مع زيادة مستوىFCF، أصبح لون قشرة خبز القوالب أغمق، وبالنسبة للون لبابة الخبز، لم يظهر تأثير معنوي في L * عند استبدال دقيق القمح بـ FCF بنسب مختلفة. من ناحية أخرى، مع زيادة مستوى FCF في الخبز، زادت قيم الاحمرار a والإصفرار b بشكل ملحوظ مما يشير إلى زيادة احمرار لبابة الخبز نتيجة الاستبدال الذي تم اضافة FCF على جميع المستويات إلى زيادة معنوية في إجمالي المركبات الفينولية والنشاط المضادات للأكسدة في الخبز عند مقارنتها بخبز دقيق القمح فقط. أخيرًا، يمكن الاستنتاج أن إضافة ما يصل إلى ١٠ ٪ من دقيق FCF في الخبز يثري القيمة الغذائية وكان أكثر تفضيلاً عن طريق التقييم الحسي.