Comparative study of the effects of fermented and non-fermented chickpea flour addition on quality and antioxidant properties of wheat bread

Yu Xiao, Lu Huang, Yulian Chen, Shanshan Zhang, Xin Rui and Mingsheng Dong

College of Food Science and Technology, Nanjing Agricultural University, Nanjing, P. R. China

ABSTRACT

The main aim of the present study was to compare the effects of Cordyceps militaris-fermented chickpea (CFC) and non-fermented chickpea (NFC) flour addition on quality (including proximate composition, specific volume, crust and crumb colour, molecular mobility, crumb texture, sensory evaluation) and antioxidant properties of wheat bread, and also compare with these properties on the wheat-only bread. The wheat flour was partially substituted by NFC and CFC at a level of 50 g/Kg flour basis, respectively. Addition of chickpea flour (both CFC and NFC) to wheat bread showed higher protein, fat content, yellowness index values as well as enhanced the phenolics content and antioxidant activity whereas lower lightness values were observed, and CFC–wheat bread showed higher phenolics and antioxidant activity than NFC–wheat bread. Furthermore, bread incorporated with CFC exhibited higher specific volume and lower crumb hardness than the wheat-only bread but addition of NFC resulted in a negative effect on specific volume and crumb firmness. Sensory evaluations revealed that breads substituted with CFC got the highest scores in terms of appearance, texture, colour and overall acceptability. Results suggested that bread fortified with CFC improved quality (specific volume, texture, colour, sensory) and antioxidant properties, and thus was consider of great potential in the production of high consumer acceptable as well as quality and antioxidant properties improved bread.

Abbreviations: NFC: non-fermented chickpeas; CFC: Cordyceps militaris-fermented chickpeas; TCD*: total colour difference; LF-NMR: low field nuclear magnetic resonance; DPPH: 2,2-diphenyl-1-picryl-hydrazyl; ABTS: 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt; TPC: total phenolics content; Vitamin C, ascorbic acid.

1. Introduction

Bread is one of the most popular and widespread baked products consumed all over the world. They are traditionally made by adding basic ingredients, such as wheat flour, yeast, sugar, milk powder, water, improver, shortening and salt (Koletta, Irakli, Papageorgiou, & Skendli, 2014). In recent years, bread received a growing attention as a potential functional food in light of its great diffusion and consumption (Ho, Aziz, & Azahari, 2013; Irakli, Katsantonis, & Kleisiaris, 2015). The ever increasing demand for high quality and healthy foods by consumer is a challenge for the baking industry, which requires the development of breads with improved physico-chemical, sensory and nutritional properties.
properties (Mariotti et al., 2014). The quality of bread depends on several physico-chemical characteristics (e.g. texture, specific volume and colour) which could be affected by many factors, such as flour type, additives and other ingredients (Dall’Asta et al., 2013). Thus, industries and researchers are involved in optimizing bread-making technology to improve the quality, taste, variety and availability of bioactive compounds, adding some of components with nutraceutical and functional properties for the final aim to formulate a product with physiological effectiveness encountering consumers’ acceptance in terms of appearance, taste and texture (Dzik, Różylo, Gawlik-Dzik, & Świeca, 2014; Marpalle, Sonawane, & Arya, 2014).

Considerable interest has been generated in fortifying wheat flour with high protein, high lysine and health-stimulating bioactive substances in recent years (Irakli et al., 2015; Villarino et al., 2015). Since legume proteins have adequate amounts of lysine and are deficient in sulphur amino acids, whereas cereal proteins are deficient in lysine, but are rich in sulphur amino acids, the combination of grain and legume proteins would provide better balance of overall essential amino acid, which would contribute to combat world protein calorie malnutrition (Angioloni & Collar, 2012; Hallén, Ibanoglu, & Ainsworth, 2004; Villarino et al., 2015). Chickpea (Cicer arietinum L.), the third most important grain legume in the world, is rich in proteins, essential amino acids (e.g. Leu, Lys and Ile), glutathione, vitamins, minerals and dietary fibre (Reyes-Moreno, Cuevas-Rodríguez, Milán-Carrillo, Cárdenas-Valenzuela, & Barrón-Hoyos, 2004; Xiao et al., 2015; Yamsaengsung, Schoenlechner, & Berghofer, 2010). Furthermore, chickpea is a good source of health-promoting bioactive substances such as phenolic compounds (Xiao et al., 2014).

Therefore, it is very attractive for using chickpea to be supplemented into bread to obtain a product with health promotion effects. Although there are a few studies have been conducted on the inclusion of chickpea flour for bread making (Angioloni & Collar, 2012; Collar, Conte, Fadda, & Piga, 2015; Man, Păucean, Muste, & Pop, 2015; Mohammed, Ahmed, & Sengea, 2012), some negative effects were correspondingly observed on bread quality for chickpea flour incorporation. The negative effects of the application of chickpea flour in bakery products were, similar to other flours which derived from plant materials, the problems which related to losses in the baked volume and hard (poor) texture (Man et al., 2015; Ragaee, Guzar, Dhull, & Seetharaman, 2011; Yamsaengsung et al., 2010). Consequently, the use of chickpeas as a supplemented material to bread baking is limited and has not yet been widely applied. In order to maintain the quality properties of substituted breads, some attempts have been tried by several researchers. In recent years, some additives such as enzymes, hydrocolloids and emulsifiers are applied in bread baking to improve the quality of bread (Goesaert, Slade, Levine, & Delcour, 2009). According to the literatures, fungal enzymes can improve the crumb structure, porosity and gas retention, which resulted in an increment in bread specific volume, and softening of the bread crumb with finer pore structure (Goesaert et al., 2009; Kim, Maeda, & Morita, 2006).

Several studies reported that grains (e.g. wheat and chickpea) fermented by fungus contain sufficient fungal enzymes such as protease and amylase (Bhanja, Kumari, & Banerjee, 2009). Xiao et al. (2014, 2015) and Reyes-Moreno et al. (2004) reported that the physico-chemical and functional properties of chickpea flour were improved (due to the action of fungus enzymes) by solid state fermentation with Cordyceps militaris or Rhizopus oligosporus. In addition, the phenolic compounds and antioxidant activity of chickpea were also found to be greatly enhanced by fermentation with C. militaris (Xiao et al., 2014). Therefore, C. militaris-fermented chickpeas can serve as excellent nutraceuticals and functional ingredients with health promotion effects, as well as use fermented chickpea flour as a functional agent in fabricated foods. In addition, C. militaris-fermented chickpea flour contained sufficient fungal enzymes which would promote its application in food industry. To the best of our knowledge, the application of C. militaris-fermented chickpea flour in food industries especially in bakery products has not been investigated. Therefore, it is our specific aim to evaluate the effects of C. militaris-fermented chickpea flour addition on quality and antioxidant properties of wheat bread, and also compared with inclusion of non-fermented chickpea (NFC) flour bread as well as wheat-only bread counterparts. It is expected that composite flour produced from fermented chickpeas will have the advantage of improving overall quality and nutrition properties of bread.

2. Materials and methods

2.1. Materials

The ingredients used in the formula of bread were high-gluten wheat flour (Dachengliangyou Co., LTD, Shanghai, China), sugar, salt and eggs (fresh) were purchased from the Suguo supermarket of Nanjing, Jiangsu Province, China. The yeast was purchased from Cereals, Oil and Food Co., LTD (Suzhou, China). Other compounds were the same as previously described (Xiao et al., 2014, 2015). Chickpeas were purchased from the supermarket of Xinjiang Uygur autonomous region, China. C. militaris SN-18 were stored and activated for solid state fermentation as detail described in Xiao et al. (2014). The spore suspension (approximately 10^8–10^9 spores/ml) was prepared by washing the potato dextrose agar (PDA) plate with sterile water and then filtered with sterilized cheesecloth.

2.2. Preparation of fermented and non-fermented chickpea flour

The preparation of fermented and NFC flour was according to the method of Xiao et al. (2014). Briefly, the chickpea seeds were first thoroughly washed under tap water and then were soaked with distilled water overnight. After decanting the water, the chickpeas were sterilized at 121°C for 25 min. After cooling down to room temperature, 0.5% (v/w) of lactic acid was added into the sterilized chickpeas and the C. militaris seed culture was inoculated (2.5 ml of spore suspension per 100 g wet chickpea) and incubated at 25°C for 8 days (CFC, C. militaris-fermented chickpeas). The non-inoculated chickpeas were used as NFC. For further analysis, the CFC and NFC were lyophilized and then grounded using a food grinder; the flours were passed through a 0.2 mm sieve and then refrigerated at 4°C.

The protease activity and amylase activity of CFC and NFC were determined by the method of Xiao et al. (2015) and Bhanja et al. (2009), respectively. One unit (U) of protease activity was defined as the amount of enzyme that releases 1 μg of tyrosine per minute under the assay conditions. One unit (U) of amylase activity was defined as the amount of enzyme that liberates 1 μg of reducing sugar (glucose) per
minute under the assay conditions. Results were expressed as U/g of dry chickpea substrate (U/g).

2.3. Bread preparation

Three types of bread samples (wheat-only bread, NFC–wheat bread, CFC–wheat bread) were prepared in this study. The dough prepared in this study was sweet dough. The basic dough recipe (provided by a bread company) contained 500 g of wheat flour, 40 g of egg, 100 g of sugar, 5 g of salt, 3 g of yeast and 200 ml of water (wheat-only bread, control bread). Dough was prepared according to the following method: first of all, yeast was dissolved in water at 28°C and was mixed with sugar, salt and egg in a mixer (B25, HENGLIAN Mechanical Equipments, Guangdong, China) for 2 min, the rest of ingredients were then put in and mixed for 50 min. After complete mixing of the dough, the dough was divided into pieces of 60 g. The dough was then moulded and rounded manually and placed into an aluminium pan and proofed for 2 h at 32°C and 82% relative humidity. The proofed dough was then baked in an automatic thermostatic oven (YXD-60, XINNANG Electric Equipments, Guangzhou, China) at a top temperature of 190°C and a bottom temperature of 200°C for 12 min. The bread was turned out of the oven and placed in ambient air to cool down to room temperature.

In this study, the NFC–wheat bread and CFC–wheat bread were prepared following the control bread preparation steps, but the wheat flour was partially substituted by NFC and CFC at a level of 50 g/Kg wheat flour, respectively. Our preliminary experiments indicated that 50 g/Kg (based on flour basis) showed the highest sensory evaluation scores and specific volume among the different levels of chickpea flour (50, 100, 150, 200 and 300 g/Kg) incorporated wheat bread. Furthermore, according to the study performed by Sulieman, Sinada and Ali (2013) who also demonstrated that bread incorporated with chickpea flour at 50 g/Kg level was found to be more acceptable in sensory evaluation compared with other levels of chickpea flour substituted wheat bread. Therefore, in this study, bread incorporated with chickpea flour at 50 g/Kg level for the preparation of NFC-wheat and CFC–wheat bread, and then to compare the subsequent effect on the quality and antioxidant properties of wheat bread. Determination of the quality properties of the control (wheat-only), NFC-wheat and CFC–wheat breads were performed 12 h after baking. Meanwhile, the breads were kept at room temperature (25°C) in polyethylene packages before the analysis to prevent moisture losses. Each sample treatment was processed in triplicate.

2.4. Proximate analysis

The proximate composition of breads, including moisture, ash, crude fat and crude protein, was determined according to the methods of AOAC 14.091, 14.103, 14.093 and 14.108, respectively (AOAC, 1990). Crude protein was determined according to the micro-Kjeldahl method, crude fat were estimated by using the Soxhlet apparatus method. Ash content was determined by igniting the samples up to 550°C in an electric furnace (Thermolyne Sybran, Model 6000, U.S.A.), the carbohydrate content was calculated by subtracting the contents of crude fat, crude protein and ash of dry matter.

2.5. Specific volume and density of breads

The specific volume and density of the bread samples were determined using the detail method of Ho et al. (2013). The specific volume was calculated as follows: Specific volume \((\text{cm}^3/\text{g}) = \frac{\text{Loaf volume of bread}}{\text{Weight of bread}}\). The bread density was determined using the following equation: Density \((g/cm^3) = \frac{\text{Weight of bread}}{\text{Loaf volume of bread}}\).

2.6. Bread texture profile analysis (TPA)

Texture profile analyser TA.XT Plus (Stable Micro Systems, Surrey, England) was used to measure the crumb texture of bread. A cylindrical probe of 50 mm diameter (P50) was selected to conduct the experiment. Measurements were carried out on three slices (2 cm thickness) taken from the centre of each loaf for bread type. Two crumb samples (2 cm × 2 cm × 2 cm) were extracted from the centre of each slice. A 2 cm × 2 cm × 2 cm bread crumb cube was placed carefully on the centre of the probe. The sample bread was then compressed by the probe at the rate of 1 mm/s to 50% of the original height. The evaluated parameters were hardness, resilience, cohesiveness, springiness and chewiness.

2.7. Colour determination

A Chroma Meter (Konica Minolta, Osaka, Japan) was employed to determine the colour of crust and crumb. Crust colour was determined on nine preselected locations on the crust of each loaf, while crumb colour was determined on three points on the three central slices of each sample for bread type (Dall’Asta et al., 2013). Chroma and hue angle were calculated using the following equation: chroma = \((a^2 + b^2)^{1/2}\) and hue angle = arctangent \(b^*/a^*\), respectively. The total colour difference (TCD*), which is the parameter of the overall colour difference evaluation between the control bread and test breads substituted with NFC or CFC, was calculated using the following equation: \(\text{TCD}^* = \sqrt{[(\text{difference of } L^* \text{ value})^2 + (\text{difference of } a^* \text{ value})^2 + (\text{difference of } b^* \text{ value})^2]/2}\) (Ho et al., 2013).

2.8. Molecular mobility determined by low-field proton nuclear magnetic resonance \(^1\text{H NMR}\)

Protons distributions in bread crumb were determined by a low-field \(^1\text{H NMR} \) (21.3 MHz) spectrometer (Niumag Co., Ltd., Shanghai, China) with a magnetic field strength of 0.5 T. Approximately 2 g of bread crumb were prepared in the NMR tubes (25 mm inner diameter) and compressed with a glass plunger. The NMR tubes were sealed with parafilm to prevent moisture loss and placed at the incubator of 25°C. The bread crumb was measured with \(^1\text{H NMR}\). Proton relaxation times were obtained from the experimental T2 curves using a UPEN software.

\[ T_{2b} = \frac{3}{4} T_{2a} \]

\[ T_{2} = \frac{1}{2} \left( T_{2a} + T_{2b} \right) \]
2.9. Sensory evaluation
The sensory evaluation was evaluated by 12 trained panelists (six males and six females), using a questionnaire of organoleptic acceptance. All the instructions were provided to panelists before evaluation. Different types of bread samples were presented in random order coded with random three-digit numbers on white plastic dishes, and served at the day of manufacture as well as under normal illumination (daylight). Water was provided to the panellists in order to cleanse their palates in between every sample. Breads were evaluated for texture, appearance, flavour, colour, taste and overall acceptance on a 9-point hedonic scale (1 – extremely dislike, 2 – dislike moderately, 4 – neither like nor dislike, 6 – like slightly, 7 – like moderately, 8 – like very much, 9 – extremely like) (Marpalle et al., 2014). The obtained average scores in the results were based on a single evaluation of each of the 12 individuals.

2.10. Evaluation of antioxidant property

2.10.1. Preparation of solvent extracts
The solvent extracts of bread samples were prepared based on the method of Xiao et al. (2014). Briefly, the bread crumb samples were firstly lyophilized and grounded using a food grinder, and then the flours were passed through a 0.2 mm. The ground powder of the bread samples was extracted by shaking with 40-fold solvents of variant polarities (80% methanol, 80% ethanol, 80% acetone and deionized water) at 50°C for 4 h. The extracts were then centrifuged at 15,000 g for 15 min at 4°C before the supernatants were collected. The residues were again re-extracted twice under the same conditions. The combined supernatants were evaporated to dryness under a reduced pressure at 50°C, and then redissolved in a known volume (25 ml) of the respective solvent system, and stored in the dark at 4°C for the determination of antioxidant property. The determination of antioxidant property of the extract was completed within 5 days.

2.10.2. Determination of total phenolics content (TPC)
The TPC was determined using the Folin–Ciocalteu’s method (Ho et al., 2013). A calibration curve was obtained using gallic acid as a standard. The samples were independently analysed in triplicate, and results were presented as micrograms of gallic acid equivalents per gram of dry weight sample (µg GAE/g d.w).

2.10.3. Measurements of antioxidant activities

2.10.3.1. Determination of DPPH radical-scavenging activity. The DPPH radical-scavenging activity of the test samples was performed according to the detail method of Xiao et al. (2014). The ascorbic acid (vitamin C) calibration curve was plotted as a function of the percentage of DPPH radical-scavenging activity. The final results were expressed as micrograms of vitamin C equivalents (VCE) per gram of dry weight sample (µg VCE/g d.w).

2.10.3.2. Evaluation of ABTS radical cation (ABTS·+) scavenging activity. The ABTS·+-scavenging activity of the bread samples was analysed by using the method of Bhanja et al. (2009). A standard curve was plotted by using different concentrations of vitamin C similar to DPPH radical assay. ABTS·+-scavenging activity was expressed as µg VCE/g d.w.

2.10.3.3. Assay of ferric reducing antioxidant power. Ferric reducing antioxidant power of the samples was carried out according to the method of Irakli et al. (2015). The measurement was compared to a calibration curve of ferrous sulfate solution, and the results were expressed as micromoles of Fe (II) equivalents per gram of dry weight sample (µmole Fe (II)/g d.w).

2.10.3.4. Measurement of reducing power. The reducing power of the bread samples was determined according to the method of Xiao et al. (2014). The vitamin C calibration curve was prepared as a function of the reducing power. The final results were expressed as µg VCE/g d.w.

2.11. Statistical analysis
All experiments were conducted independently in triplicate, and the results were represented as the mean values of three individual replicates ± the standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan’s multiple range tests were conducted to determine significant differences (p < 0.05) between the means by SPSS 17.0 software package.

3. Results and discussion

3.1. Proximate composition
The results of the proximate composition carried out on the bread samples are presented in Table 1. Different proximate compositions were found between breads substituted with chickpea flour (NFC and CFC) and the wheat-only bread, whereas no significant different (p > 0.05) was observed between NFC-wheat and CFC–wheat breads. As shown in Table 1, the incorporation of chickpea flour yielded significantly (p < 0.05) higher protein and fat contents than the wheat-only bread, possibly due to the relatively high level of chickpea flour. These results are consistent with Marpalle et al. (2014) and Man et al. (2015) in incorporation of flaxseed and chickpea flours to bread, respectively. The inclusion of chickpea flour to the formulation decreased the ash and carbohydrate contents with regard to the control sample.

Table 1. Proximate composition of bread samples.

| Parameter       | Wheat-only | NFC–wheat | CFC–wheat |
|-----------------|------------|-----------|-----------|
| Moisture (g/kg) | 287.67 ± 5.08a | 286.64 ± 4.89a | 288.69 ± 2.31a |
| Dry matter (g/kg)| 712.33 ± 5.08a | 713.36 ± 4.89a | 711.31 ± 2.31a |
| Crude fat (g/kg)| 24.11 ± 3.35b | 29.92 ± 1.36b | 31.72 ± 2.63b |
| Crude protein (g/kg)| 123.66 ± 2.51bc | 132.73 ± 3.42a | 136.40 ± 3.38a |
| Ash (g/kg)     | 15.68 ± 0.13a | 14.37 ± 0.93a | 13.99 ± 0.32b |
| Carbohydrate (g/kg)| 836.55 ± 5.96a | 822.98 ± 5.57b | 817.89 ± 6.12b |

*a, b: Different letter in a row are significantly different (p < 0.05).

*b: Moisture and dry matter of breads are presented based on fresh bread weight and air-dried weight, respectively, others are presented on dry weight basis.

Each value is expressed as the mean ± standard deviation (n = 3). Means with different letters within a row are significantly different (p < 0.05).
Besides, moisture and dry matter of the three variant breads were not varied remarkably.

### 3.2. Specific volume and density of bread samples

The specific volume and density of breads are presented in Table 2. Significant differences in specific volume and density were found among the three different breads. The incorporation of NFC decreased the specific volume and increased the density of the bread. Ragae et al. (2011), Yamsaengsung et al. (2010) and Ho et al. (2013) also reported that partial substitution of wheat flour with barley, oat, rye, chickpea and banana pseudo-stem flour resulted in reduction of specific volume in bread. Ho et al. (2013) and Man et al. (2015) demonstrated that the protein content was diluted when partially substituted banana pseudo-stem or chickpea flours for wheat flour, and interfered with the optimal gluten matrix formation during dough mixing, fermentation and baking process. Therefore, the significant reduction in specific volume of NFC–wheat bread is primarily due to the dilution of gluten in the flour blends. However, it was noted in Table 2 that the specific volume of the bread was significantly (p < 0.05) improved with the addition of CFC. Addition of fermented flour increased the specific volume of bread than inclusion of non-fermented flour was also reported in previous investigations (Hallén et al., 2004). Kim et al. (2006) reported that bread substituted with polished flours which contained fungal amylase enhanced the specific volume. They stated that the inclusion of amylase was related with the improvement of gas retention during the fermentation process. The damaged and gelatinized starch could be hydrolysed by amylase, resulting in the formation of low-molecular mass dextrans which served as fermentable sugar for gas production (Poutanen, 1997). According to the results of enzymes activity determination, the fungal protease activity and amylase activity (which produced by C. militaris during fermentation) of CFC were 28.86 ± 1.49 and 11.72 ± 0.35 U/g, respectively, whereas NFC showed no protease and amylase activity. Thus, the improvement specific volume of CFC–wheat bread could be probably due to the effect of fungal amylase in the CFC.

### 3.3. Colour properties

The crust and crumb colour parameters of the bread samples are summarized in Table 2. The results indicated that the chickpea flour addition had statistically significant (p < 0.05) differences from control bread on both the bread crumb and crust colour. The L* values of the crust breads substituted with chickpea flour (NFC and CFC) were significantly lower than the control (wheat-only), probably due to partial whitening of white colour by substituted with chickpea flour. According to the report of Ho et al. (2013), the crumb colour is mainly influenced by the colour of the substituted flour. In addition, colour analysis of the crust indicated that both breads with the addition of NFC and CFC flour had a darker crust (lower L* values) (Table 2). The result is similar to Dall’Asta et al. (2013), Marpalle et al. (2014) and Yamsaengsung et al. (2010) who demonstrated that the incorporation of chestnut, flaxseed or chickpea flour also resulted in a lower L* value of bread crust. The darkness of the supplemented bread crust may be ascribed to the darkening effects of chickpea flour itself as well as the higher protein, as the wheat flour was partially substituted with chickpea flour. According to Koletta et al. (2014) and Ho et al. (2013), the crust colour was mainly related with the caramelization and Maillard reactions. The higher contents of protein and lysine in the substituted breads than wheat-only bread contributed to an increase in the Maillard reaction during the baking process. In addition, the CFC–wheat bread had a darker crust than NFC–wheat bread (Table 2) probably due to the higher reducing sugar content (produced during fermentation) of CFC, which can enhance the Maillard and caramelization browning (Dall’Asta et al., 2013).

In addition, a* and b* values of substituted bread crumbs were significantly greater than the control (a* = −4.39 and b* = 20.98, respectively), similar observations were also reported by Koletta et al. (2014) and Mariotti et al. (2014) for the crumb of breads substituted with barley, rye and oat flour. The higher b* values of substituted bread crumbs may be due to the yellow pigment of the chickpea flour used. However, no significant difference was observed between NFC–wheat and CFC–wheat breads regard to the b* values from the bread crumb (Table 2).

A chroma value near 0 is an indicative of subdued colours, while high chroma values represent vibrant colours (Ho et al., 2013). The crumb colour of the breads containing chickpea flour presented significantly (p < 0.05) greater chroma values than the wheat-only, probably due to the higher b* values (Conejo & Rosell, 2015). This result was expected due to the more intense yellow colour of the chickpea flour. The hue angle was reduced in the bread crumb containing chickpea flour compared with the control. However, an increased trend in the crumb hue angle was observed in the bread containing chickpea flour. Similar observation was also found by the report of Ho et al. (2013). Furthermore, it was noted that the addition of chickpea flour into the bread formulation showed significant difference (p < 0.05) on the TCD* value of both the bread crumb and crust. For the bread crumb or crust colour, the TCD* value between NFC–wheat and CFC–wheat breads also exhibited significant difference.

### 3.4. Texture of breads

The TPA results of the variant breads are shown in Table 3. The hardness or firmness of bread is one of the most

| Table 2. The specific volume, density and colour properties of bread samples. |  |
|---|---|---|
| Bread | Wheat-only | NFC–wheat | CFC–wheat |
| Specific volume (cm³/g) | 3.71 ± 0.24* | 3.22 ± 0.14* | 4.14 ± 0.13* |
| Density (g/cm³) | 0.27 ± 0.02b | 0.31 ± 0.01a | 0.24 ± 0.01a |
| Colour properties | Crumb | | |
| L* | 80.57 ± 0.44a | 79.28 ± 0.66b | 78.01 ± 0.71c |
| a* | −4.39 ± 0.09b | −4.15 ± 0.10a | −3.79 ± 0.16a |
| b* | 20.98 ± 0.61b | 21.26 ± 0.39b | 22.85 ± 0.92a |
| Chroma | 21.44 ± 0.58a | 22.55 ± 0.38a | 23.17 ± 0.89a |
| Hue angle (°) | −78.56 ± 0.55a | −79.39 ± 0.34b | −80.57 ± 0.62b |
| TCD* | 0.5 ± 0.9b | 1.77 ± 0.9b | 3.30 ± 0.9b |
| Crust | | | |
| L* | 52.16 ± 0.66a | 49.88 ± 0.72b | 47.92 ± 1.42c |
| a* | 15.24 ± 0.86a | 14.63 ± 0.30a | 15.21 ± 0.40a |
| b* | 18.73 ± 1.03a | 21.76 ± 0.28b | 24.44 ± 1.65a |
| Chroma | 24.15 ± 1.17a | 26.22 ± 0.28b | 28.80 ± 1.53a |
| Hue angle (°) | 50.86 ± 1.54a | 56.10 ± 0.65b | 58.06 ± 1.47b |
| TCD* | 0.5 ± 0.9b | 4.02 ± 0.95b | 7.33 ± 1.39b |

Each value is expressed as the mean ± standard deviation (n = 3). Mean with different letters within a column are significantly different (p < 0.05).

### Notes

- The variability of the results is due to the use of different samples and different testing conditions.
- The data were analyzed using ANOVA and Tukey’s HSD test for multiple comparisons.
- The results indicate that there is a significant difference among the three bread samples in terms of specific volume, density, and colour properties.
- The addition of chickpea flour resulted in an improvement in the specific volume of bread, indicating a potential for increased nutritional benefits.
- The crumb colour of the breads containing chickpea flour showed significant differences compared to the control, indicating potential benefits for sensory attributes.
- The hardness of the breads was significantly influenced by the type of flour used, with NFC–wheat breads being the hardest.
- The addition of NFC and CFC resulted in a darker crust colour, which is likely due to the higher protein content of these flours.
- Future research could investigate the potential health benefits associated with the consumption of these breads.
Table 3. Texture parameters measured by texture profile analysis (TPA) of bread samples.

| Bread                | Wheat-only | NFC-wheat | CFC-wheat |
|----------------------|------------|-----------|-----------|
| Hardness (N)         | 4.377 ± 0.091<sup>1</sup> | 4.954 ± 0.143<sup>1</sup> | 2.893 ± 0.190<sup>1</sup> |
| Springiness          | 0.776 ± 0.002<sup>2</sup> | 0.764 ± 0.011<sup>2</sup> | 0.803 ± 0.004<sup>2</sup> |
| Cohesiveness         | 0.598 ± 0.006<sup>3</sup> | 0.583 ± 0.003<sup>3</sup> | 0.659 ± 0.008<sup>3</sup> |
| Chewiness (N)        | 2.091 ± 0.060<sup>4</sup> | 2.320 ± 0.117<sup>4</sup> | 1.433 ± 0.086<sup>4</sup> |
| Resilience           | 0.193 ± 0.003<sup>5</sup> | 0.186 ± 0.003<sup>5</sup> | 0.218 ± 0.001<sup>5</sup> |

<sup>1</sup>Each value is expressed as the mean ± standard deviation (n = 3). Different letters in the same row indicate significant differences (p < 0.05) between bread products.

<sup>2</sup>Cada valor se expresa como promedio ± desviación estándar (n = 3). Las distintas letras en la misma fila indican diferencias significativas (p < 0.05) entre los productos de pan.

important texture parameters of bread (Kim et al., 2006; Yamsaengsung et al., 2010). It was found that addition of NFC resulted in a negative effect on crumb texture of bread. As shown in Table 3, hardness of NFC–wheat bread was significant higher than wheat-only bread. Demirkesen, Mert, Sumnu and Sahin (2010), Yamsaengsung et al. (2010) and Tuncel, Yilmaz, Kocabiyik, and Uygur (2014) also found an increase of crumb hardness when chestnut flour, chickpea flour or rice bran was added in bread. Demirkesen et al. (2010) demonstrated that the increase of crumb hardness was associated with amount of fibre when chestnut flour was added in bread. Dall’Asta et al. (2013) stated that the increase of bread hardness probably due to the thickening of the walls which surrounding the air bubbles in the crumb.

However, addition of CFC significantly (p < 0.05) decreased the hardness of bread (33.90%) compared with the control (Table 3). Kim et al. (2006) demonstrated that whole grain flour supplemented bread contained fungal amylase which could increase the specific volume and decrease the firmness of crumbs due to the large amounts of gas generated in the fermentation process. The gelatinized and damaged starch could be hydrolysed by amylase, resulting in the generation of low molecular weight dextrins which served as fermentable sugar for the production of gas (Poutanen, 1997). In addition, amylase in the sample might hydrolyse a part of starch molecules into monosaccharide and dextrin, consequently reducing the long chain and molecular size of starch. Thus, the starch networks (especially the recrystallized amylopectin network) in bread was weakened and then making soft bread crumb (Goesaert et al., 2009; Hug-Iten, Escher, & Conde-Pettit, 2003). Furthermore, the produced monosaccharides by the action of amylase could be utilized by yeasts for growth, thus greatly producing gas during fermentation process. Besides, along with the amylase, protease could also increase the specific volume and soften bread crumb (Poutanen, 1997). As CFC contained sufficient fungal protease and amylase, the improvement bread crumb texture (lower hardness) of CFC–wheat bread might ascribe to some fungal enzymes presented in the fermented chickpea flour, which can soften the bread crumb and increase specific volume.

3.5. Molecular mobility determined by 1H NMR

NMR measurement is a useful method to reveal proton exchange in bread and also reported in many previous studies (Curti, Carini, Tribuzio, & Vittadini, 2014). Molecular mobility characterization was conducted with 1H NMR experiments which covered a large range of molecular relaxation events. The relaxing proton fractions were characterized in light of 1H T2 relaxation times distribution. The 1H NMR T2 relaxation time observed by CPMG pulse sequence was a good representative of proton mobility (Choi & Kerr, 2003). A longer T2 relaxation time represents a more freedom kind of proton. Three 1H T2 proton populations were observed in all samples by CPMG pulse sequence and designated as A, B and C (starting from the shortest to the longest relaxation time), respectively (Figure 1). The presence of three peaks indicated the existence of three different degrees of proton mobility. Population A represented protons relaxing at ~ 0.4 ms, whereas population B and C indicated protons relaxing at 4 – 6 ms and ~ 57 – 65 ms, respectively (Figure 1 and Table 4). In addition, it was found that the peak area of population B in the bread samples was much higher than the other two populations (A and C), accounting for ~ 87% of the total protons. Similar result was also observed in previous studies (Bosmans, Lagrain, Ooms, Fieren, & Delcour, 2013; Curti et al., 2014). No significant changes in mobility of populations A and C were detected in all bread samples. However, population B shifted towards shorter relaxation times when the wheat bread substituted with NFC (Table 4). Furthermore, the peak area of population B changed significantly when substituted with chickpea flour. Inclusion of NFC decreased the peak area of population B whereas substituted with CFC remarkably increased the peak area of population B (Table 4 and Figure 1).

The presence of multiple proton populations (by low field NMR spectrometers) has been previously demonstrated in bread products (Curti et al., 2014). More recently, Bosmans et al. (2013) and Curti et al. (2014) demonstrated that the least mobile 1H T2 populations (population A) which had a very short T2 relaxation time could be considered as rigid protons belonging to gluten and amorphous starch in little contact with water molecules, and to exchange protons of confined starch, gluten and water. Protons of population B were attributed to the more mobile and exchanging C–H protons of gluten, water and starch in the gel network of bread. The population C was attributed to the mobile protons of lipids. In light of the presented NMR data, the peak area of population B decreased and the peak time of population B dramatically reduced substituted with NFC when compared with wheat-only bread (Table 4 and Figure 1), which suggested that the fraction of mobile and exchanging protons of water, starch and gluten reduced, and water became more immobile. However, the increased peak area of population B (peak time of population B not changed) observed for CFC–wheat bread indicated the presence of stronger interactions in the starch network, suggesting that a possible redistribution of water among bread network and a higher proton exchange between the rigid and exchanging protons of starch and gluten interacting with water molecules. Consequently, a larger amount of water might be available to plasticize the crumb structure of bread, and then result in a softer product. These differences were possible ascribe to the presence of amylases in CFC. The amylases in bread can limit the formation and strength of the permanent amylopectin network, and the water immobilization (Goesaert et al., 2009). As changes in proton mobility has been related to macroscopic and mesoscopic, such as water migration and crumb firming (Bosmans et al., 2013). Therefore, the above result obtained might explain the lower hardness of CFC–wheat bread.
Furthermore, the correlation analysis revealed in Table 5 also supported that the peak area of population B and hardness were highly negative correlated ($r = -0.954$, $p < 0.01$). Accordingly, higher peak area of population B of CFC–wheat bread was remarkably associated with the lower hardness of the bread. Further research will be conducted on the relationship between textural property and molecular mobility in our group. In addition, the population C areas of CFC–wheat and NFC–wheat were significantly higher than wheat-only bread (Table 4) probably due to the higher fat content of the substituted bread samples as shown in Table 1.

### 3.6. Sensory evaluation

The sensory evaluation scores are presented in Figure 2. Panellists judged the wheat-only, NFC–wheat and CFC–wheat breads were all acceptable as they received scores greater than 5 of overall acceptance. According to the performed statistical analysis, the substitution of chickpea flour significantly affected appearance, texture and overall acceptance. Inclusion of chickpea flour (NFC and CFC) improved the appearance of bread. However, the formulation of bread substituted with NFC exhibited the lowest texture and overall acceptance scores of 3.17 and 5.58, respectively, among the three variant breads. This result may be attributed to the compactness and hardness of the bread crumb, which resulted from the low specific volume and high density obtained from the NFC–wheat bread, as mentioned earlier (Tables 2 and 3). This interpretation could further be supported by the results obtained from the correlation analysis. As shown in Table 5, texture and overall acceptance positively correlated with specific volume ($r = 0.877$ and $r = 0.813$, respectively) whereas negatively correlated with density ($r = -0.833$ and $r = -0.789$, respectively). In literature, there is agreement that sensory attributes in terms of texture and overall acceptance decreased when addition of chickpea flour in bread (Man et al., 2015). However, the current study revealed that substitution of CFC got high scores of appearance (7.83), texture (7.08) and colour (8.25), which were significantly ($p < 0.05$) higher in comparison to wheat-only bread probably due to the higher specific volume and lower density of CFC–wheat bread.

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**Table 4.** $^1$H NMR $T_2$ relaxation times and areas of protons distributions of different bread samples.$^{ab}$

| Bread         | Population A | Population B | Population C |
|---------------|--------------|--------------|--------------|
|               | $T_2$ (ms)   | Area (au)    | $T_2$ (ms)   | Area (au)    | $T_2$ (ms)   | Area (au)    |
| Wheat-only    | 0.35 ± 0.00* | 1283.05 ± 129.99$^{ab}$ | 5.59 ± 0.00* | 10498.69 ± 58.12$^{b}$ | 64.28 ± 0.00* | 247.42 ± 23.25$^{b}$ |
| NFC–wheat     | 0.39 ± 0.04* | 1170.28 ± 49.22$^{a}$ | 4.75 ± 0.00$^{b}$ | 10198.16 ± 154.08$^{a}$ | 57.84 ± 5.58$^{a}$ | 325.34 ± 17.76$^{a}$ |
| CFC–wheat     | 0.37 ± 0.04* | 1404.36 ± 12.32$^{a}$ | 5.59 ± 0.00$^{a}$ | 11043.14 ± 71.65$^{a}$ | 61.06 ± 5.58$^{a}$ | 333.67 ± 3.50$^{a}$ |

$^{a,b}$au: arbitrary units.

$^{ab}$Results are presented as the mean ± standard deviation ($n = 3$). Lowercase letters in the same column indicate significant differences ($p < 0.05$).

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**Figure 1.** $^1$H NMR $T_2$ relaxation curves of three types of bread samples. (a) Wheat-only breads; (b) NFC–wheat breads and (c) CFC–wheat bread.

**Figura 1.** Curvas de relajación $^1$H NMR $T_2$ de tres tipos de muestras de pan. (a) Panes únicamente de trigo; (b) panes de trigo con NFC; (c) pan de trigo con CFC.
addition of plant materials (Dziki et al., 2014; Ho et al., 2013; Irakli et al., 2015). In addition, it was found that CFC–wheat bread showed higher TPC than NFC–wheat bread mainly due to the higher phenolic compounds in CFC (Xiao et al., 2014). Furthermore, Figure 3(a) also demonstrated that various solvent mixtures had significantly different extraction abilities for the phenolic compounds of bread samples, and acetone may be a suitable solvent for extracting phenolic compounds in the bread samples for the highest TPC. Our results are in agreement with Zhao et al. (2006) who also reported that acetone extract of the grain samples contained the highest amount of phenolic compounds. The various solvent mixtures employed in the current study possessed different polarities, and these solvents could affect the solubility of the phenolics (depending on their chemical structures and polarities) in the samples and the extraction yields (Zhao et al., 2006). Such interactions possibly resulted in the variations of TPC noted across the variant solvent extracts investigated.

### 3.7. Antioxidant property

#### 3.7.1. Total phenolics content (TPC)

The TPC of three bread samples extracted with different polarity solvents are shown in Figure 3(a). It was noticed that chickpea flour addition enhanced the TPC of wheat bread. For example, TPC of 80% methanol extract of wheat-only, NFC–wheat and CFC–wheat were 943.59, 1026.92 and 1078.21 µg GAE/g d.w, respectively. In accordance with our results, many previous studies also reported that the enhancement of TPC of wheat bread by the

*Figure 2. Spider diagram of sensory evaluation of wheat-only breads (control), NFC–wheat bread and CFC–wheat breads.

*Figure 2. Diagrama de araña de la evaluación sensorial de panes únicamente de trigo (control), pan de trigo con NFC y panes de trigo con CFC.

appearance, texture and colour of the bread is an important sensory characteristic for consumers (Ho et al., 2013), and consumers may prefer bread with the incorporation of CFC for the highest scores of colour, appearance and texture. Hence, according to the obtained results, it may be suggested that CFC–wheat bread, among the evaluated bread types, is the best option in terms of consumers’ behaviour.

#### 3.7.2. Antioxidant activity

The antioxidant activity of different bread samples was evaluated based on the four most commonly antioxidant activity assays, and the results are depicted in Figure 3(b–e). Similar to TPC, it was noted that wheat bread substituted with chickpea flour significantly increased the DPPH radical-scavenging activity, ABTS radical cation-scavenging activity, ferric reducing antioxidant power and reducing power. For instance, the reducing power of 80% methanol, 80% ethanol, 80% acetone and water extracts of CFC–wheat bread was 287.50, 275.93, 250.46 and 326.85 µg VCE/g d.w, respectively. However, for wheat-only bread, the reducing power of 80% methanol, 80% ethanol, 80% acetone and water extracts was 205.32, 190.28, 192.59 and 264.35 µg VCE/g d.w, respectively. Previous studies also reported that wheat bread substituted with rice bran, quinoa, amaranth and lupin flour enhanced antioxidant activities (Dziki et al., 2014; Irakli et al., 2015; Villarino et al., 2015). They demonstrated that plant materials addition into the bread enhanced phenolic

### Table 5. Correlation coefficients among volumen especifico, densidad, propiedades texturales (TPA), movilidad molecular (población B) y evaluación sensorial.

| SpecVol | Den | Har | Spri | Coh | Che | Res | T_B | PopB | App | Tas | Fla | Col | Ove Acc |
|---------|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|
| SpeVol  | 1   | -0.994 | -0.891 | 0.830 | 0.894 | 0.857 | 0.885 | 0.827 | 0.899 | 0.435 | 0.877 | 0.801 | 0.581 |
| Den     | -0.969 | 1   | -0.800 | -0.862 | 0.830 | -0.866 | -0.850 | -0.883 | -0.370 | -0.833 | -0.779 | -0.604 | 0.619 |
| Har     | -0.948 | -0.964 | 0.935 | 0.705 | 0.859 | 0.734 | 0.935 | 0.504 | 0.417 | 0.851 | 0.917 | 0.786 |
| Spri    | 0.908 | -0.964 | 0.935 | 0.648 | 0.962 | 0.737 | 0.951 | 0.685 | 0.540 | 0.892 | 0.788 | 0.816 |
| Coh     | 1    | -0.968 | 0.977 | 0.648 | 0.962 | 0.737 | 0.951 | 0.685 | 0.540 | 0.892 | 0.788 | 0.816 |
| Che     | 1    | -0.981 | 0.663 | 0.938 | 0.727 | 0.922 | 0.577 | 0.511 | 0.846 | 0.796 | 0.809 |
| Res     | 1    | 0.750 | 0.101 | 0.637 | 0.696 | 0.664 | 0.406 | 0.784 |
| T_B     | 1    | 0.634 | 0.899 | 0.691 | 0.742 | 0.771 | 0.809 |
| PopB    | 1    | 0.777 | 0.120 | 0.197 | 0.844 | 0.605 |
| App     | 1    | 0.629 | 0.441 | 0.910 | 0.896 |
| Tas     | 1    | 0.466 | 0.557 | 0.500 |
| Fla     | 1    | 0.199 | 0.520 |
| Col     | 1    | 0.709 |
| Ove Acc | 1    | 1    |

*Correlation was significant at the 0.05 level (two-tailed).
*a Correlation was significant at the 0.01 level (two-tailed).

*SpesVol, volumen especifico; Den, densidad; Har, hardesa; Spri, springiness; Coh, cohesiveness; Che, chewiness; Res, resilience; T_B, T_B, relaxation time of population B; PopB, the peak area of population B; App, Appearance; Tex, texture; Tas, taste; Fla, flavour; Col, colour; Ove Acc, overall acceptance.

La correlación fue significativa a nivel 0.05 (dos colas).
*La correlación fue significativa a nivel 0.01 (dos colas).
compounds which are responsible for their higher antioxidant capacity (Dziki et al., 2014; Villarino et al., 2015). Therefore, the enhanced antioxidant activities of bread samples substituted with chickpea flour may be related to the higher TPC present in it (Figure 3(a)). Besides, as shown in Figure 3(b-e), it was also noted that CFC–wheat bread exhibited higher antioxidant capacity than NFC–wheat bread regardless of different solvent employed, probably due to CFC showed higher phenolics and antioxidant activities than NFC in previous studies (Xiao et al., 2014).

Figure 3(b–e) also illustrates that various solvent extracts of wheat-only, NFC–wheat and CFC–wheat breads showed significant different antioxidant activities, suggesting that extraction solvent remarkably affected antioxidant activities. A different solvent system might have selected efficacy for extracting individual antioxidant compounds. Zhao et al. (2006) reported that the changes of solvent polarity would alter the ability to dissolve a selected group of antioxidant substances and influence the evaluation of antioxidant activity. The different solvent extracts of bread samples showed significantly (p < 0.05) different antioxidant activities probably due to the variant phenolic compounds content in the extracts. Several previous studies have confirmed that the phenolic compounds in the samples are responsible for their higher antioxidant capacity (Dziki et al., 2014; Villarino et al., 2015). Therefore, the result observed was probably due to the fact that phenolic compounds in different solvent extracts of bread samples had different contributions to antioxidant activity. In addition, the characteristics of phenolics, which can act synergistically, additively or antagonistically, should also be considered (Jacobo-Velázquez & Cisneros-Zevallos, 2009; López, Rico, Rivero, & de Tangil, 2011).

However, regardless of the extraction solvents employed in the current investigation, the antioxidant activity of bread was significantly (p < 0.05) enhanced with addition of CFC. These results clearly demonstrate that CFC addition enhanced antioxidant activities compared to wheat-only bread. Although α-tocopherol and BHA were good antioxidants, they are additives and used in milligram levels in foods. However, CFC enhanced wheat bread could be consumed in gram levels as food. Therefore, CFC enhanced wheat bread could be developed as a functional food with more effective antioxidant properties.

4. Conclusions

The present study demonstrated that substitution of CFC with wheat flour in the bread formula significantly improved the quality and antioxidant properties compared to the wheat-only bread. Addition of CFC to bread improved the specific volume, density, texture and sensory properties. However, these improvements were not observed in bread with the inclusion of NFC. 1H NMR
mobility was different in the NFC–wheat and CFC–wheat breads as compared with the wheat-only bread, which may have affected the development of the gluten network resulting in different textural properties. Breads containing CFC and NFC both resulted in a darker crust and crumb appearance, as well as enhanced TPC and antioxidant activity than the wheat-only bread. The sensory profile results suggested that NFC–wheat and CFC–wheat breads were both acceptable but CFC supplemented bread showed the highest scores regard to appearance, texture, colour and overall acceptance among the three variant breads. Therefore, the results demonstrated that addition of CFC flour into bread not only improved quality properties, but also enhanced antioxidant properties of wheat bread. These results indicate that consumers may willingly choose CFC–wheat bread. Consequently, CFC can be used, as a natural nutraceutical-rich material, in the production of higher consumer-acceptable as well as quality and antioxidant properties improved bread. In addition, further research is needed to optimize the processing and formulation factors in CFC–wheat bread manufacture to maximize the level of CFC incorporation while maintaining improved quality properties and high consumer acceptability, to give a product with maximum potential nutritional and health benefits.

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