European Food Research and Technology (2021) 247:1107–1115
https://doi.org/10.1007/s00217-021-03691-4

ORIGINAL PAPER

New thermal and rheological approaches of chickpea–wheat dough for breadmaking

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Received: 25 September 2020 / Revised: 5 January 2021 / Accepted: 9 January 2021 / Published online: 10 February 2021

Abstract

Chickpea (*Cicer arietinum* L.) is a legume of Fabaceae family whose grains are rich in proteins, total dietary fiber, unsaturated lipids, minerals and bioactive compounds with antioxidant activity. Therefore, chickpea flour can be an attractive high nutritional complement to wheat flour for the formulation of composite breads. In the present study, the thermal and rheological characteristics of the chickpea–wheat dough obtained by replacement of wheat flour with 100 and 200 g/Kg of chickpea flour were assessed by Differential Scanning Calorimetry (DSC), Rapid Visco Analysis (RVA), Dynamic Mechanical Thermal Analysis (DMA) and Texture Profile Analysis (TPA). Higher gelatinization temperatures in coincidence with higher pasting temperatures with lower breakdown and setback of the pastes were obtained for the chickpea–wheat mixtures. Furthermore, the viscoelasticity of the wheat dough changed with the presence of chickpea flour, leading to higher dynamic moduli (E', E'') and lower values of tangent of the phase angle ($\tan \delta = E''/E'$) suggesting the formation of a more elastic matrix. At higher deformations (TPA), higher values of hardness and elasticity were observed. These changes were associated with a marked disruption of the gluten network by the presence of certain chickpea components like proteins, assessed by different microscopic techniques.

Keywords Wheat · Chickpea · Rheology · Microstructure · Thermal properties

Introduction

Bread products based on wheat represent an important part of the western diet due to cultural factors. Bread is also an important part of the diet of developed countries like Spain, which is also at the top ranking per capita of consumption of legumes. That is why wheat breads would be an ideal vehicle for improving nutritional and functional characteristics of the diet with the incorporation of legume flours [1, 2].

Food and Agriculture Organization (FAO) designated 2016 as the "International Year of Legumes" and proposed to announce their nutritional benefits, increase legumes world production, make use of their proteins, and also improve crop rotation [3]. Chickpea (*Cicer arietinum* L.) is among the four most commercialized and consumed legumes worldwide, in addition to beans, peas and lentils [4]. Chickpea composition is characterized by a high content of available carbohydrates-mainly starch (close to 500 g/Kg), proteins (around 200 g/Kg) and lipids (close to 60 g/Kg, of which approximately 850 g/Kg are unsaturated); it also contains significant amounts of dietary fiber (120 g/Kg), minerals (40 g/Kg) and vitamins, whose values vary slightly between different subspecies and crop conditions [5]. Regarding proteins, it is worth highlighting its good amino acid profile, which, like other legumes, has a high lysine content, which is an essential amino acid that is found in very low concentrations in cereals [6]. Due to the quantity and quality of their proteins, the amount of minerals and dietary fiber, chickpea renders a flour that becomes a potential ingredients for the nutritional and functional improvement of cereal-based products, like bread [7].

There is a background in the incorporation of legume flours in bakery mixtures to make use of their proteins and dietary fiber. However, it has been shown that, in general, the partial replacement of wheat flour with legume flour,

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protein isolates or other flours, leads to losses in the unique rheological properties that gluten proteins confer to the dough [8–10]. Specifically, an increase in water absorption and changes in other farinographic parameters (higher development times and increases in weakening of dough) were reported by other authors for chickpea–wheat flour bends [11, 12]. Lower extensibility and resistance and increased adhesiveness have also been described [12, 13], in addition lower volume of the bread pieces and darkening of the crust were detected [13, 14]. However, the improvement in the nutritional profile of the breads can mitigate the less satisfactory perception by consumers about technological quality.

The objective of the present work was to study the influence of chemical components of commercial wheat and chickpea flours on structural, thermal and rheological properties of the composed bread dough, to evaluate actions for improving technological parameters for assuring a high quality for the leavened baked products.

Materials and methods

Materials

Wheat flour (WF) (Molinos Harineros del Sur S.A., Sevilla, Spain), Chickpea flour (CF) (Herba Ricemills S.L.U., Sevilla, Spain) and commercial sea salt (Eliges, Madrid, Spain) were used to obtain dough. Farinographic data of water absorption and development time for WF and WF–CF mixtures were provided by Molinos Harineros del Sur S.A (Sevilla, Spain).

Experimental design

Three formulations with different degrees of replacement of WF with CF were used: WF1000 (without CF addition), CF100 and CF200 (with 100 and 200 g/Kg of WF by CF replacement, respectively). In all cases, 15 g of salt was added per 1 kg of single flour or flour mixture. To obtain the dough, the distilled water content corresponding to the optimal water absorption according to the farinograms was added: 559, 550 and 541 g of water per 1 kg of flour or flour mixture for WF1000, CF100 and CF200 respectively.

Physicochemical characterization of flours and blends

Composition analysis

Moisture, protein, lipids, minerals (ash) and total dietary fiber (TDF) were determined according to AACC methods [15]: 44–19, 46–12, 30–10, 08–01 and 32–05, respectively. Assays were performed by duplicate. Carbohydrates other than fiber were calculated by difference.

Particle size distribution

The particle size distribution of flours (WF and CF) was obtained with Laser Diffraction Analysis using a Malvern Mastersizer 2000 (Malvern lnstruments, UK). The results were expressed as the mean value for five replications.

Differential scanning calorimetry

The starch gelatinization of wheat and chickpea flours was evaluated through differential scanning calorimetry (DSC) in a differential scanning calorimeter equipment (Q100 TA Instruments, USA). Approximately 1 mg of flour was weighed in an aluminum pan, and distilled water was added to reach 100 g/Kg of flour concentration. Samples were heated at 10 °C/min up to 140 °C. As a reference, a sealed empty pan was used. Onset (T_0), peak (T_p) and final (T_f) temperatures were computed from the thermograms by Universal Analysis 2000, Version 4.1D (TA Instruments, USA) Waters LLC.

Viscoamylograph profiles

Flours and mixtures (according to formulations CF100 and CF200) with 15 g/Kg of salt were analyzed using a Rapid Visco Analyser-Super 4 (Perten Instruments, Sweden). Samples were dispersed in distilled water (25 ml) and heated to 50 °C at 160 rpm for 1 min. They were then heated to 95 °C, maintaining that temperature for 2.5 min and finally cooled to 50 °C while stirring for another 2 min. The parameters obtained from the viscosity curves as a function of time were: pasting temperature, related to the beginning of gelatinization for the starch granules; peak viscosity (PV), maximum viscosity that was reached during heating at 95 °C; final viscosity (FV), viscosity that was reached at the end of the test; breakdown (B), viscosity difference between PV and viscosity at the end of the holding period at 95 °C; setback (SB), viscosity difference between FV and viscosity at the end of the maintenance period at 95 °C.

Microstructural characterization of flours

The WF and CF samples were qualitatively analyzed by scanning electron microscopy (SEM). A portion of flour was dispersed on the surface of a specific 10 mm × 10 mm support and covered with silver to be observed in a FEI Quanta 200 microscope (Thermo Fisher Scientific, Waltham, MA, USA) in high vacuum and through detection of secondary electrons.

Chickpea–wheat bread dough

Dough preparation

Flours and salt were premixed for 1 min in a Thermomix TM5 (Vorwerk, Wuppertal, Germany) in "kneading mode". Then, the optimum amount of distilled water was added, according to farinographic water absorption, and was mixed for the corresponding optimum development time (according to farinograms). The dough obtained were allowed to rest for 10 min at room temperature covered with plastic film to avoid dehydration, and then they were manually laminated to 1 cm in thickness and allowed to relax again for 10 min. The dough were prepared in quadruplicate for each formulation.

Dynamic mechanical thermal analysis

Viscoelastic dough characterization tests were performed using small-amplitude oscillatory compression tests with an RSA3 strain-controlled rheometer (TA Instruments, USA) using a parallel plate geometry of 15 mm in diameter with a 1.5 mm gap. For this characterization, the dough prepared according to "Composition analysis" were placed between the plates, using dow corning high vacuum grease to prevent dehydration. Values of the elastic (E') and viscous (E") moduli were recorded, according each test described below, as well as the loss tangent (tan $\delta = E"/E'$).

Strain sweep tests Tests were carried out in triplicate at 25 °C between 0.001 and 2% deformation at a frequency of 1 Hz to estimate the linear viscoelasticity range (LVR). From this test, the critical strain (γ_c) was determined, which is the deformation that marks the limit of the linear viscoelastic interval.

Frequency sweep tests The mechanical spectra were performed in triplicate at 25 °C with a constant deformation, within the LVR, between 0.05 and 80 Hz. The values of the elastic modulus and the loss tangent at 1 Hz (E'_1 and tan δ_1) were selected for comparison.

Thermal analysis To characterize dough behavior during baking, the latter was simulated with a thermal process in the rheometer by heating the samples from 25 °C to 100 °C at a speed of 5 °C/min at a frequency of 1 Hz. Subsequently, the samples were cooled again to 25 °C at 10 °C/min at the same frequency. These tests were performed in triplicate for each dough. On "baked" samples, strain sweep and frequency sweep tests were performed as described above.

Texture profile analysis (TPA)

Fresh dough discs of 30 mm in diameter and 10 mm thickness were analyzed by TPA tests on a Texture Analyzer TA.XT2i (Stable Micro Systems, Godalming, UK) with a flat probe (SMSP/75) 75 mm in diameter. The test speed was 0.5 mm/s and the sample compression was 40% of its original height. Twelve repetitions of the test were performed for each analyzed dough. The following parameters were calculated from the graphs of force as a function of time: hardness (N), maximum force reached during the first compression; springiness, distance corresponding to the second compression divided by the distance of the first compression; resilience, quotient between the area corresponding to the retraction of the first peak and the total area of the first peak; cohesiveness, quotient between the area of the second peak and the area of the first peak; and adhesiveness (N·s), corresponding to the area of the negative force peak.

Microstructural characterization of dough

Fresh dough prepared as described in "Dough preparation" were qualitatively analyzed by two types of microscopy.

Environmental scanning electron microscopy (ESEM) Dough portions of approximately 0.1 g were placed on specific supports for observation in a JEOL 6460LV microscope (JEOL Ltd., Akishima, Japan) under environmental mode at 10 °C and 40% relative humidity. Images of various fields and different magnifications were obtained.

Confocal scanning laser microscopy (CSLM) Dough portions of approximately 0.5 g were spread over slides and stained with a 0.01 g/l solution of Rhodamine B. The samples were incubated in the dark for 60 min at room temperature, the excess of fluorophore was washed with a small portion of distilled water and observations were made in a LSM 7 DUO confocal laser microscope (Carl Zeiss, Oberkochen, Germany). The excitation wavelength was 561 nm, while filters for emission were set between 566 and 703 nm.

Statistical analysis

The Statgraphics Centurion XV v15.2.06 software (StatPoint Inc.) was used for the statistical analysis. ANOVA was performed to determine significant effects of treatments when necessary, and the least significant difference (LSD) test was applied to compare mean values at a confidence level of 95%.

Results and discussion

Flour characteristics

Both flours presented a fine particle size, allowing an adequate preparation of the mixtures. While WF showed a characteristic whitish color, CF exhibited yellowish coloration. As presumed, CF respect to WF, contained significantly higher ($p < 0.05$) amounts of proteins (219 ± 4^b vs. 114 ± 2^a), minerals (30.2 ± 0.2^b vs. 5.8 ± 0.3^a), lipids (58.8 ± 0.6^b vs. 10.9 ± 0.8^a) and total dietary fiber (150 ± 3^b vs. 32.7 ± 0.7^a), with a lower moisture content (80.6 ± 0.5^a vs. 128.2 ± 0.9^b), all values expressed in g per Kg of flour in wet basis. The carbohydrate content (different from TDF) estimated by difference was markedly higher for WF (70.84%) compared to CF (46.14%). These values are in accordance with those mentioned by other authors for CF [9, 16].

The micrographs obtained by SEM for WF and CF are presented in Fig. 1a, b, respectively. WF shows a much more diverse distribution of particle sizes than CF, where it was possible to clearly observe the presence of a large number of small particles, accompanied by a smaller number of larger particles. These observations were corroborated by size distribution measurements (Fig. 1c). For WF, approximately 99% of the volume of flour was represented by particles with a size range between 10 and 200 µm. On the other hand, there were two predominant particle size ranges for CF between 5 and 50 µm (corresponding to 40% of the total volume of flour) and between 100 and 400 µm (representing 44% of the total volume). Differences in the particle size of flours would influence the hydration properties and therefore the physicochemistry of dough.

Figure 2 shows the typical amylograms for WF, CF and mixtures with 100 and 200 g/Kg of replacement of WF with CF (CF100 and CF200). Comparing the pasting behavior of WF with that of CF, it can be observed that the rapid and high increase up to PV is not evident in CF. CF suspensions exhibited lower viscosities in all the assayed period, although it was resistant to shear-heating treatment as demonstrated by the absence of breakdown.

The profiles obtained for WF–CF mixtures were similar to that of WF, indicating that pasting behavior is governed by wheat components, particularly starch, although the attained viscosity values were lower. Peak viscosity for WF was 1.25 Pa·s and it decreased with the addition of 100 and 200 g/Kg of CF to 1.09 and 0.97 Pa·s, respectively. Pasting temperature was higher for CF (80 °C) respect to that observed for WF (65 °C) and WF–CF blends (between 66 and 68 °C). WF–CF samples had lower breakdown values (0.39 and 0.31 for mixtures with 100 and 200 g/Kg of flour replacement, respectively) when compared with WF (0.46), indicating that the addition of CF to the samples conferred them some resistance to the swelling of starch granules. Final viscosity and setback are parameters related to the capacity to form gels and retrogradation, which showed much lower values for CF (FV = 0.61 Pa·s, SB = 0.11 Pa·s) than for WF (FV = 1.48 Pa·s, SB = 0.69 Pa·s). As expected, mixtures with CF showed lower values than WF, being lower for mixtures with 200 g/Kg of replacement (FV = 1.23 Pa·s,

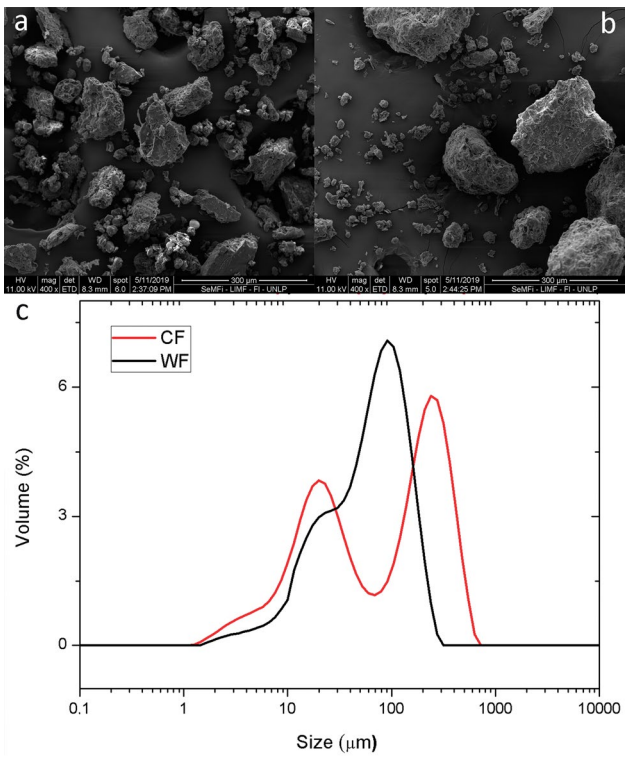

Fig. 1 Micrographs for wheat (**a**) and chickpea (**b**) flours obtained by SEM, and of particle size distribution for chickpea (CF) and wheat (WF) flours (**c**)

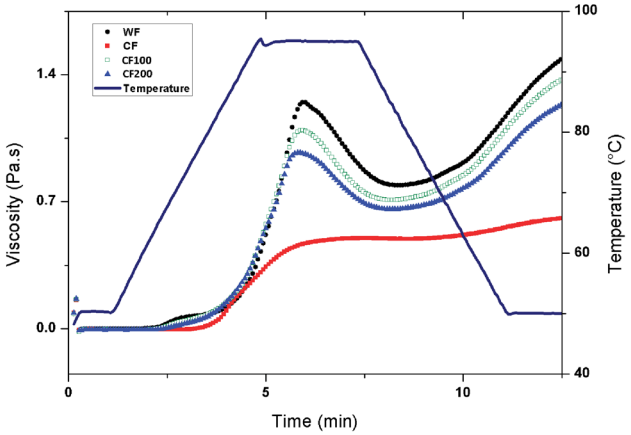

Fig. 2 Amylograms obtained for wheat (WF) and chickpea (CF) flours and mixtures with 100 and 200 g/Kg of replacement of wheat flour with chickpea flour (CF100 and CF200, respectively).

SB = 0.57 Pa·s) than for mixtures with 100 g/Kg of replacement (FV = 1.37 Pa·s, SB = 0.66 Pa·s).

These results are in agreement with previous studies [12], where the decreased in viscosity of WF–CF mixtures was attributed to the lipids and proteins present in CF. These authors also found higher values of pasting temperature that they related to a higher content of protein and higher resistance to swelling and rupture of chickpea starch. The presence of fiber was related to decreased pasting ability, since fiber can strongly interact with water [17]. In this sense, Collar et al. [18] reported that the presence of fiber can strongly influence the pasting properties of starch in blends of wheat flour with different types of fiber. These authors attributed the increase of pasting temperatures to the competence for water between starch and fiber that restricts the starch granule swelling, consequently decreasing the setback values. The lower values obtained for final viscosity in samples with CF can also be influenced by the presence of fiber due to the capacity of insoluble fiber to interfere with the formation of amylose gels during cooling, leading to a weaker matrix.

The starch gelatinization process for WF and CF was also studied by DSC. Wheat flour showed significantly ($p < 0.05$) lower onset (55.2 ± 0.3 °C), peak (61.7 ± 0.3 °C) and final (71.7 ± 0.5 °C) temperatures associated to the starch gelatinization peak with respect to that observed for chickpea flour (63.6 ± 0.3 °C, 71.9 ± 0.7 °C and 79.9 ± 0.8 °C, respectively). As expected, these results are in agreement with the increase in pasting temperatures observed for CF and CF–WF blends in RVA assays. These difference in almost 10 °C of gelatinization temperature could be due to the different type of starch present in CF jointly with the contribution of some globular chickpea proteins that would be denatured around 70 °C.

Rheological behavior

Values of critical strain (γ_c) in a limited range (0.023–0.038%) (Table 1) was obtained for the three dough analyzed (WF1000, CF100 and CF200), indicating similarity between them with respect to the deformation tolerance without breaking the internal structure of the material. Figure 3a shows the typical mechanical spectra (*E*' and *E*" as a function of frequency) obtained for the dough with 0, 100 and 200 g/Kg of replacement of WF with CF. In all systems a viscoelastic behavior was observed with values of *E*' greater than *E*" in the frequency range evaluated, with a strong dependence of both moduli on frequency. The replacement of 10% of WF with CF led to a slight increase in the elastic component (*E*') of the matrix, while the increment to 20% decreased both moduli, although with a significant decrease in tan δ value evaluated at 1 Hz of frequency (Table 1), suggesting the formation of a dough with

Table 1 Rheological and textural parameters of doughs

	γ_c (%)	E'_1 (Pa)	tan δ_1 (−)	Hardness (*N*)	Springiness (−)	Resilience (−)	Cohesiveness (−)	Gumminess (*N*)	Adhesiveness (*N*. s)
WF1000	0.023^a	$5.7E5^a$	0.38^b	2.8^a	0.94^a	0.087^b	0.66^a	1.8^a	5.2^a
CF100	0.038^b	$6.8E5^b$	0.38^b	3.1^b	0.94^a	0.087^b	0.68^b	2.1^c	6.3^c
CF200	0.022^a	$6.6E5^b$	0.32^a	3.0^b	0.96^b	0.083^a	0.66^a	2.0^b	5.6^b
PSD	0.009	0.001	0.06	0.3	0.03	0.007	0.03	0.2	0.9

WF1000, CF100, and CF200: formulations with 0, 100, 200 g of chickpea flour/1 kg of total flour. Mean values from at least triplicates for measurements from rheometer (made at 1 Hz) and twelve replicates for textural measurements are reported. Letters within the same column indicate significant differences by LSD test ($p < 0.05$)

PSD pooled standard deviation

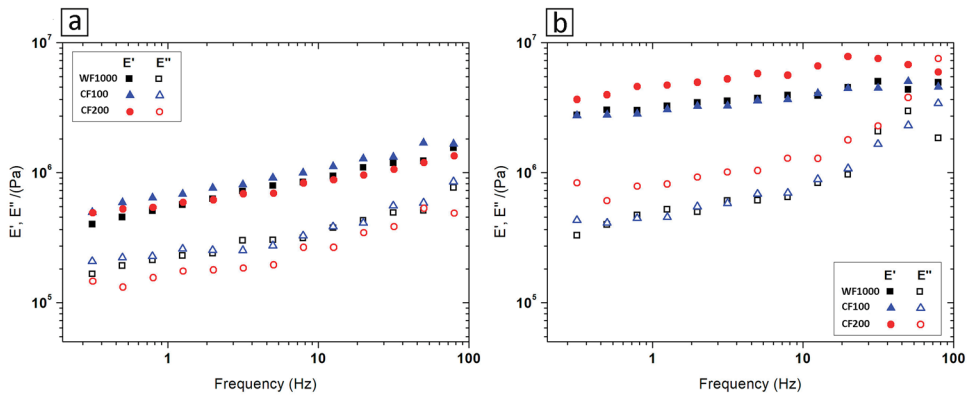

Fig. 3 Evolution of *E*' and *E*" as a function of frequency for (**a**) fresh and (**b**) thermally treated 25–100–25 °C dough with 0, 100 and 200 g/Kg of replacement of WF with CF.

a bit more elastic network. Similar results in the increase of dynamic moduli with partial replacement of wheat flour with mesquite flour (another legume flour) in bread dough were previously reported [19]. In this work, the rheological behavior was associated with the presence of legume flour components (proteins and dietary fiber) capable of competing with gluten proteins for water, leading to the development of a dissimilar gluten matrix.

Figure 4 shows the evolution of the elastic *E*' and viscous moduli *E*'' and tan δ during heating (25–100°C) (Fig. 4a) and cooling (100–25°C) (Fig. 4b) for the wheat dough (WF1000) and the dough with 20% of CF (CF200). During the first stage of heating, the systems increased their viscous character (with a decrease in the elastic modulus leading to an increase in tan δ) up to approximately 50 °C. This fact can be justified as an increase in the mobility of water in the system, mainly due to the weakening of the hydrogen bonds of the solvent with the rest of the components in the system (proteins, carbohydrates and dietary fiber) due to the effect of temperature. Above 60 °C, the rheological properties of the system changed markedly due to the beginning of the gelatinization process of starches, which increased the elastic character of the dough, evidenced by the increase in the dynamic moduli and the decrease in tan δ. The elastic modulus reached its maximum at 78.2 ± 0.8 °C for the WF1000 sample, while that value was significantly higher ($p < 0.05$) for the CF200 dough (80.2 ± 0.9 °C). This shift at higher temperatures of the process associated with gelatinization due to the presence of CF was in agreement to the higher pasting and gelatinization temperatures observed in the RVA and DSC tests, respectively. This shift can be attributed to the influence of the chickpea starch gelatinization and the denaturation of their globular proteins. When heating progressed, the *E*' and *E*'' moduli decreased for both formulations, which can be associated to the partial rupture of the starch granules due to the mechanical force applied to the system at so high temperatures (> 80ºC). On the other hand, the decrease of tan δ was slighter from 80 °C, suggesting a stabilization of the structure once the gelatinization had finished.

After the heating step, during cooling (Fig. 4b), the dynamic viscoelastic moduli increased for both dough in a similar way, reaching values of *E*' significantly higher than those registered before the gelatinization of starches. At this stage, the values of tan δ remained practically stable, indicating a fixation of the system structure. These results showed a significant change in the systems matrix with the thermal process due to the gelatinization of starches, denaturation of wheat [20] and chickpea proteins [21], and the gelation of amylose and globular chickpea proteins during cooling. All these process influenced the rheological properties of dough. In any case, the differences between the final viscoelastic behavior can be observed in Fig. 3b, where, although the systems exhibit the same evolution, the CF200 system presented higher viscoelastic moduli values.

Texture of dough

The mean values obtained for the different textural parameters are summarized in Table 1. Very slight increases in hardness, springiness, gumminess and adhesiveness and a reduction in dough resilience were observed by partially replacing WF with CF, although such changes were not significant in all cases ($p > 0.05$). This indicates that dough of the three formulations presented similar textural properties, allowing to predict a gluten network development, which determines the rheological properties of the WF-based dough, even when replacing 200 g/Kg of WF with CF. Regarding adhesiveness, the significant increase observed when replacing WF with CF could be considered the most significant change in the textural properties of the dough, since excessive adhesiveness can hinder dough handling during the preparation of the bakery products. This property is directly related to the interaction between water and the rest of the components in the dough. When the water retention capacity of the matrix is lower, water can migrate to the surface of the dough, thus increasing the adhesiveness of the system. Other authors have described similar situations when replacing wheat flour with chickpea flour [12, 22] and soy flour [10], associating this effect with the modification

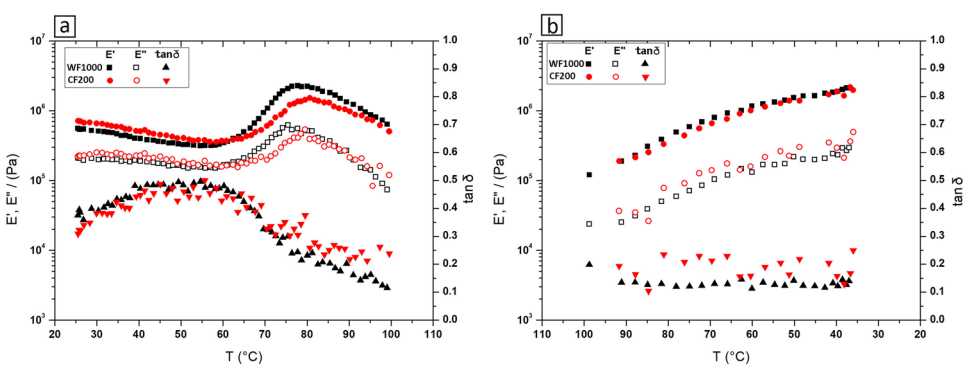

Fig. 4 Curves of *E*', *E*'' and tan δ obtained by (**a**) heating from 25 °C to 100 °C at 5 °C/min and (**b**) cooling from 100 °C to 25 °C at − 10 °C/min for dough with 0 and 200 g/Kg of replacement of WF with CF

in the interaction of water with the new components added to the dough.

Microstructure of dough

Figure 5 shows the micrographs obtained by ESEM (a, b and c) and CSLM (d, e and f) for dough containing 0, 100 and 200 g/Kg of CF (WF1000, CF100 and CF200). In the three micrographs obtained by ESEM, the protein matrix of gluten interpenetrated by starch granules from WF and CF can be observed. By this microscopic method, no substantial differences were perceived on the microstructure of the dough with the partial replacement of WF with CF. The analysis of the micrographs obtained by CSLM using Rhodamine B, a fluorophore that has a great affinity for hydrophobic areas of proteins [23], showed differences in the gluten matrix between dough. The structures observed for the WF1000 (Fig. 5d) and CF100 (Fig. 5e) dough were similar, with a high degree of cross-linking between the thin gluten filaments and a remarkable formation of gluten sheets. On the other hand, the protein structure observed for the CF200 dough evidenced the formation of a less organized and less filamentous gluten network, with the presence of thicker gluten fibers and the absence of intimate cross-linking between thin fibers, which favored the formation of the gluten sheets. This kind of modification of the gluten matrix by incorporation of other kind of proteins has been described in previous works [24, 25]. The presence of non-gluten forming

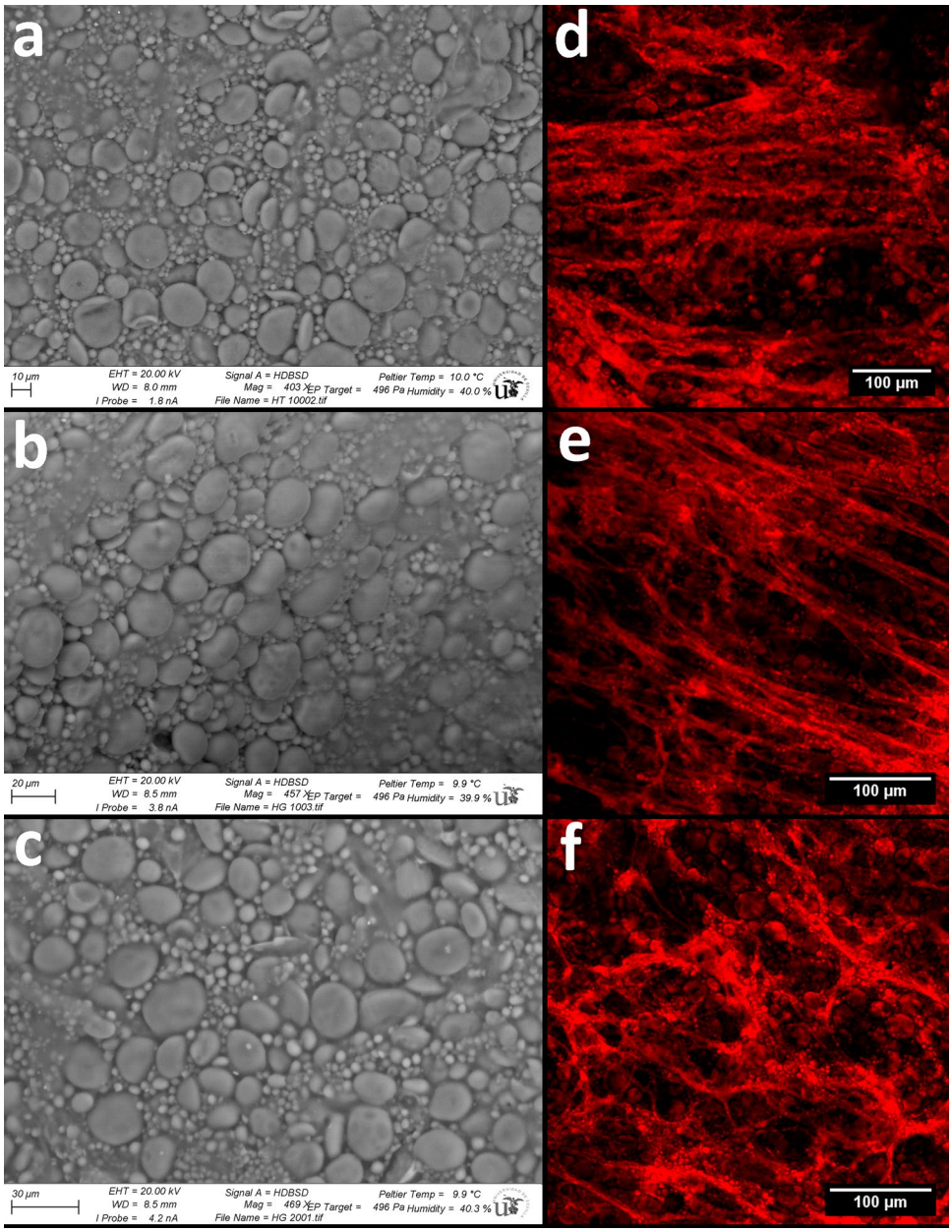

Fig. 5 Micrographs obtained by environmental scanning electron microscopy and confocal laser scanning. Microscopy for fresh doughs with 0 (**a** and **d**), 100 (**b** and **e**) and 200 g/Kg (**c** and **f**) of replacement of wheat flour with chickpea flour.

components conduct consequently to "dilution" of glutenins and gliadins (which form the network); in addition, insoluble components such as certain molecules of dietary fiber, may contribute to network disruption during dough processing. As a consequence of these changes in the gluten matrix, the rheological properties of the dough were modified, which explains the differences observed in the oscillatory tests. This type of changes in the dough would led to partial loss of the dough capacity of retaining the gas generated during the fermentation process and would also influence the technological quality of the final product [26].

Conclusions

The incorporation of chickpea flour (as partial replacement of wheat flour) in bread dough led to significant changes in the rheological, thermal and microstructural properties, especially when the replacement was 200 g/Kg. The presence of protein, starch and dietary fiber from chickpea flour would be responsible for the slight increases in the dynamic moduli and texture of the dough, mainly due to the modifications in the gluten network, evidenced by CSLM. The competition for available water and a probable disruptive effect of insoluble components would explain these modifications. The competence for water and consequent restriction of the water available in the system would also be responsible for the increment in temperatures associated with the gelatinization of starches, also globular proteins of chickpea would contribute to gelation during thermal process. These modifications allow us to predict a slight loss of technological quality of the final leavened product, although with an improvement of the nutritional quality of the bread. Nevertheless, the improvement in nutritional quality of breads without significant changes in their physical properties should be confirmed by experimental assays.

Acknowledgements The authors acknowledge the Asociación Universitaria Iberoamericana de Postgrado (AUIP) for conceding the funds for the stay that allowed carrying out this study. The authors also kindly thank Herba Ricemills S.L.U. for providing the chickpea flour used in this study and the Microscopy services (CITIUS—Universidad de Sevilla) for providing full access and assistance to confocal and scanning electron microscopes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements The article does not contain any studies with human participants or animal subjects.

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