



Physico-chemical characterisation of whole meal flours from three wild chickpea varieties and their technological performance in Gluten Free Bread

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ABSTRACT

The present study investigated the physico-chemical characteristics of whole-meal flours from three wild chickpea varieties (white chickpea – WC, red rough chickpea – RRC, red smooth chickpea – RSC) compared to a modern chickpea variety (MC) and their bread-making performances in 30% (w/w flour) substituted GF breads. Wild chickpea flours showed the highest ash, total dietary fiber (TDF), and total antioxidant capacity (6.3%, 13.4%, and 9.5% increase for WC, RRC, and RSC flour compared to MC flour) values compared to MC sample, and red varieties (RRC- and RSC-samples) showed the highest total phenolic content (15.5% and 17.0% increase compared to MC flour). Significant differences were also found in protein content and techno-functional properties. Bread specific volume and crumb hardness were significantly affected by chickpea variety, with red varieties (RRC- and RSC-samples) revealing the lowest impact. ¹H NMR proton molecular mobility significantly changed as a function of chickpea variety, and these differences might be associated to the different macroscopic bread quality. Overall, the tested wild chickpea flours revealed valuable chemical composition, and differed in the techno-functional and bread-making performances, with red varieties showing the most promising results to improve GF breads.

1. Introduction

In recent years, the attention has moved from the technological to the nutritional value of gluten free (GF) breads since they are characterised by high starch, fat and sodium contents, and lower contents in micronutrients, proteins, and dietary fibre comparing to gluten-containing equivalents (Melini et al., 2017; Aguiar et al., 2023). Nutrient deficiencies are a problem in GF diet, which can be associated to the large use of refined flours and starches, especially in GF bakery products and pasta (Saturni et al., 2010). Bread is considered a staple food for consumers; hence, its fortification could considerably affect the nutrient intake of people suffering from gluten related disorders (Kahraman et al., 2022).

In this context, alternative and non-traditional flours, such as flours from minor cereals, pseudocereals and pulses, are gaining increasingly attention since they are characterised by interesting chemical

composition (Gao et al., 2018). Considering pulse flours, several studies reported some significant nutritional and health benefits: they have a low glycaemic index (GI), and they are an important source of nutrients and bioactive compounds (Melini et al., 2017). Among pulses, chickpea is a widely consumed pulse around the world; it is a valuable source of proteins, dietary fibres, minerals, vitamins, and several bioactive components such as phenolic acids and isoflavones (Rachwa-Rosiak et al., 2015; Kaur and Prasad, 2021). Furthermore, chickpea is characterised by a low GI (Rachwa-Rosiak et al., 2015) and its proteins showed good functional properties (Grasso et al., 2022). The genus *Cicer* comprises 44 species, including 35 wild perennials, 8 wild annuals, and the cultivated annual (Sharma et al., 2013). It is a cool season leguminous crop belonging to family Fabaceae and subfamily *Faboideae*, cultivated in 50 countries (Chandora et al., 2020). Actually, *Cicer arietinum* L. is the only cultivated species and *Cicer reticulatum* is the wild annual species, considered as the progenitor of the cultivated species (Kaur and Prasad,

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2021). *Cicer arietinum* is not able to colonize successfully without human intervention, whereas wild *Cicer* species are capable to naturally grow in inhospitable areas (Sharma et al., 2013). Cultivated chickpea gene pool possesses narrow genetic base due to domestication bottleneck, genetic drift, migration, and lesser use of genetic resources in chickpea breeding (Chandora et al., 2020). A key factor contributing to low grain yield is the loss of invaluable genes linked to higher productivity including biotic and abiotic stress tolerance in the cultivated types and a more general narrowing down of their genetic base starting with its domestication (Chandora et al., 2020). Crop Wild Relatives (CWR) provide the broadest range of genetic diversity and represent additional novel gene pool of genetic resources in grain pulses, including chickpea (Singh et al., 2010). This is of considerable significance to improve the chickpea production in the country which can help to underpin future food and nutritional security (Chandora et al., 2020).

Some recent articles reported that wild chickpea varieties are characterised by a valuable nutritional profile (Kaur et al., 2019; Summo et al., 2019; Sharma et al., 2021). Kaur et al. (2019), comparing 15 wild chickpea species to cultivated chickpea genotypes (10 desi and 5 kabuli), found that wild species had lower starch, and phytic acid contents, and higher antioxidant potential. Summo et al. (2019) investigated the nutritional and technological potential of 57 chickpea accessions, including local landraces at risk of genetic erosion compared to modern cultivated chickpeas (desi and kabuli). Results showed that pigmented wild chickpeas were characterized by high dietary fiber content, high amount of bioactive compounds (anthocyanins and carotenoids), and high levels of poly unsaturated fatty acids (PUFA) (Summo et al., 2019). Moreover, brown accessions were characterized by high value of water absorption capacity, which is associated to the suitability for mixing with cereal flours (Summo et al., 2019). Sharma et al. (2021), investigating the genetic variability for protein and mineral concentrations in 41 accessions of cultivated and 8 wild *Cicer* species, identified promising wild chickpea accessions for multiple seed nutrients.

In the literature, the incorporation of chickpea flour in GF breads has been little explored. Miñaro et al. (2012) investigated the possibility of substituting soy protein with other pulse flours, included chickpea (8.3% w/w flour), whereas Aguilar et al. (2015) tested a similar level of chickpea flour incorporation (7.8%, w/w flour) alone or combined with tiger nut flour to replace emulsifiers and/or shortening. Santos et al. (2018, 2020), and Santos et al. (2021) tested higher levels (i.e., 50, 75, and 100% w/w flour) of chickpea flour incorporation into a GF bread. The high levels tested by the authors were achieved thank to the inclusion of 25% whole eggs in GF bread formulation (Santos et al., 2018, 2020, 2021). Kahraman et al. (2022) developed a healthy rice-based GF bread using raw, roasted, or dehulled chickpea flours at 25% w/w flour substitution level and evaluated the technological and nutritional quality of the obtained breads.

Considering the scant information reported in the literature about the use of chickpea flour for GF bread fortification, especially about whole meal chickpea flour from wild species, the aim of the present study was to perform a physico-chemical characterization of whole meal chickpea flours (i.e., 3 wild and 1 modern variety) and to test their bread-making performances in GF bread. The level of bread fortification considered in the experiments (30% w/w flour), corresponded to the highest level mostly reported in the literature for the fortification of wheat-flour bread (Boukid et al., 2019). GF breads were developed using the least amount of additives and additional ingredients in the formulation, in order to improve the nutritional profile of the final product.

2. Materials and methods

2.1. Materials

Commercial gluten free flour for bread-making (Schär, mix B, bread; ingredients: maize starch, rice flour, vegetable fibers (psyllium and

bamboo), whole meal rice flour 3.8%, lentil flour 3.6%, dextrose, HPMC, salt 0.73%), fresh brewer's yeast, and salt were purchased from a local supermarket. Three wild chickpea varieties, (i) White (WC), (ii) Red Rough (RRC) and (iii) Red Smooth (RSC) Chickpeas were provided by Claudio Grossi local farm (Lesignano de' Bagni, Parma, Italy) and one Modern Chickpea (MC), produced by Cooperativa Agricola Valdibella (Palermo, Sicily, Italy), was provided by a local farm (Bergamina, Parma, Italy). Chickpea seeds of the four varieties were milled using a laboratory mill (mod. ZM300, Retsch GmbH, Germany) equipped with a 1000- μ m sieve to obtain whole meal chickpea flours.

2.2. Bread-making process

GF-bread samples were prepared in 1.281 kg batches to obtain approx. 1.100 kg bread. Preliminary trials were performed to optimize the formulation, and the processing conditions of GF-bread (CTR). Bread was fortified with 30% of chickpea flour since this level corresponded to the highest level mostly reported in the literature for the fortification of wheat-flour bread (Boukid et al., 2019). The optimal amount of water was estimated according to the producer indications. The basic formulation was: flour (660 g), water (594 g, 90% of flour weight), brewer's yeast (16.5 g, 2.5% of flour weight), and salt (10.6 g, 1.6% of flour weight). The specific formulation of each sample is reported in Table 1. The level of chickpea flour incorporation was set to 30% (chickpea flour w/w total flour) according to the maximum amount not resulting in a too strong impairment of bread technological quality (Boukid et al., 2019), and in order to maximize the potential differences in the bread-making attitude of chickpea samples.

Dried ingredients were mixed using Kitchen Aid Professional Mixer (KitchenAid 5KSM5, St. Joseph, Michigan, U.S.A) operating with a dough hook (model KSM35CDH) for a total mixing time of 1 min at 110 rpm. The following samples were obtained: (i) GF-sample (100% GF flour), (ii) MC-sample (70% GF flour, 30% MC flour), (iii) WC-sample (70% GF flour, 30% WC flour), RRC-sample (70% GF flour, 30% RRC flour), RSC-sample (70% GF flour, 30% RSC flour).

Bread-making process was performed using a bread-making machine (Unold Backmeister BIG model 68520/68525, Hockenheim, Germany). Fresh brewer's yeast was dissolved in water, then the dried ingredients (mixed flour and salt) were added. A personalized bread making procedure was created and optimized parameters are reported in Table 2.

2.3. Physico-chemical characterization of chickpea flour

2.3.1. Proximate composition

The proximate composition analysis of 4 chickpea flours (MC, WS, RRC, SRC) was performed according to the methods of AOAC International for vegetable matrices (AOAC International, 2002). Analyses were performed in duplicate for all parameters except for total (TDF), soluble (SDF), and insoluble dietary fiber (IDF) which were analyzed in one

Table 1
Bread formulation.

Samples	GF flour (g)	Chickpea flour (g)	Water (g)	Yeast (g)	Salt (g)
GF	660	–	594	16.5	10.6
MC	462	198 (Modern Chickpea)	594	16.5	10.6
WC	462	198 (White Chickpea)	594	16.5	10.6
RRC	462	198 (Red Rough Chickpea)	594	16.5	10.6
RSC	462	198 (Red Smooth Chickpea)	594	16.5	10.6

Bread samples: CTR = 100% GF flour; MC = 70% GF flour, 30% modern chickpea flour; WC = 70% GF flour, 30% white chickpea flour; RRC = 70% GF flour, 30% red rough chickpea flour; RSC = 70% GF flour, 30% red smooth chickpea flour.

Table 2
Bread making conditions.

Bread making step	Time (min)
Mixing 1	10
Leavening 1	5
Mixing 2	15
Leavening 2	60
Baking	80
Total bread making time	2h 50 min

replicate. Moisture, crude ash, crude fat, and total nitrogen contents were determined according to AOAC 925.09, AOAC 923.03, AOAC 920.39, and AOAC International 984.13 using a Kjeldhal system, respectively. From the total nitrogen determined, protein percentage of the samples was determined using 6.25 as nitrogen-to-protein conversion factor. TDF, SDF, and IDF were determined by enzymatic-gravimetric official method AOAC 991.43 (AOAC, 2012). Digestible carbohydrates were determined by difference.

2.3.2. Functional properties

Water absorption index (WAI) and water solubility index (WSI) were determined according to the method of Du et al. (2014) with some modifications. Each flour sample (2.50 g) was dissolved in 30 mL of distilled water in a pre-weighed centrifuge tube (50 mL), stirred for 10 min, and heated up to 90 °C in a water bath for 15 min. Then the samples were cooled down to room temperature and centrifuged at 3000 g for 10 min. The solid content of the supernatant was determined by transferring the supernatant into a tared evaporating dish and the sediment was weighed. The supernatant was evaporated at 105 °C overnight to determine the weight of the dry solids. The following equations were utilized to determine WAI and WSI:

$$WAI = \frac{\text{weight of sediment}}{\text{weight of flour sample}}$$

$$WSI = \frac{\text{weight of dissolved solids in supernatant} \times 100}{\text{weight of flour sample}}$$

Water-holding (WHC) and oil-holding capacity (OHC) were determined according to the method described by Gupta et al. (2018). 100 mg of flour sample were added with 1 mL of distilled water or sunflower oil (ratio 1:10 w/v) into pre-weighed 2 mL Eppendorf tubes. The samples were mixed for 30 s with a vortex, stored for 30 min at room temperature, and then centrifuged at 2061 g for 20 min. The supernatant was decanted using a micropipette, and WHC and OHC were calculated as a weight gain of water or oil per gram of dry flour (g/g).

Foaming capacity was measured according to the method described by Carcea Bencini et al. (1986) with some modifications. 1.5 g of chickpea flour samples were added to 50 mL of distilled water (ratio 3 g flour/100 mL distilled water w/v) and were stirred for 30 min using a magnetic stirrer. The mixed samples were transferred to a 100 mL of graduated cylinder to measure the volume before whipping. Then the sample mixtures were homogenized using Ultra Turrax (IKA) at 10,000 rpm speed for 3 min, and the volume was measure immediately after whipping (t = 0 min) and 30 min after whipping (t = 30 min). The volume increase caused by whipping was measured as a percentage. Foaming activity was defined as percentage of volume increase.

The foaming capacity (FC) and foaming stability (FS) of each flour variety were determined using the following equations:

$$FC = \frac{V_2 - V_1}{V_1} \times 100\%$$

$$FS = \frac{V_3}{V_2} \times 100\%$$

where FC is the foaming capacity, FS is the foaming stability, V₁ is the

volume of the suspension (mL) before whipping, V₂ is the volume of the suspension (mL) immediately after whipping (t = 0 min), and V₃ is the volume of the whipped suspension (mL) after 30 min from whipping (t = 30 min).

2.3.3. Extraction of phenolic compounds

The antioxidant compounds of the chickpea flour samples were extracted using methanol-distilled water solution (70:30 v/v) according to Paciulli et al. (2023) with little modifications. Nine g of each chickpea flour were put into 50 mL flask and added with 30 mL of the methanol-distilled water solution. The flasks were stirred at 600 rpm for 120 min at room temperature. Then, the samples in the flasks were transferred into 5 mL Eppendorf and centrifugated at 15312 g for 10 min at room temperature, to separate the liquid phase (containing antioxidants) from the solid phase. Samples were filtered using 0.2 µm filter to remove solid particles in suspension. For each chickpea flour sample the extraction was performed in 3 replicates.

2.3.4. Evaluation of the total phenolic content (TPC)

Total phenolic content (TPC) of chickpea flours was determined by means of the Folin–Ciocalteu assay described by Singleton et al. (1999) according to the small-scale method proposed by Paciulli et al. (2023). In a 4 mL cuvette, 1160 µL of distilled water were mixed with 50 µL of sample extract, and 100 µL Folin–Ciocalteu reagent were added. After that, in the time-range of 2–8 min, 300 µL sodium carbonate (20% w/v) were added. Each cuvette was covered with laboratory film and incubated in the dark at room temperature for 30 min, and then, the absorbance at 760 nm was measured using a spectrophotometer UV-Vis (Thermo Scientific, Waltham, Massachusetts, United States). To prepare the blank sample, the same reagents were used, except the sample extract which was replaced with 50 µL of methanol-distilled water 70:30 (v/v) solution. To quantify the phenolic content of each chickpea flour sample, the external standard method was applied; a calibration curve was obtained using a stock solution of gallic acid (1 mg/mL), and preparing the following dilutions with 70% methanolic solution (v/v) as a solvent: 0.7 mg/mL, 0.6 mg/mL, 0.5 mg/mL, 0.4 mg/mL, 0.3 mg/mL, 0.2 mg/mL, 0.1 mg/mL. TPC were expressed as milliequivalents of gallic acid on g of dry matter (meqGAE/g dm).

For each sample extract the Folin–Ciocalteu assay was performed in two replicates, for a total of 3 extraction replicates x 2 TPC analysis replicates = 6 replicates for each sample.

2.3.5. Evaluation of the total antioxidant capacity (TAC)

The ability of phenolic compounds to reduce DPPH (2, 2-diphenylpicrylhydrazyl) was performed to measure the free radical scavenging activity of the chickpea flour samples following the method described by Paciulli et al. (2023). 1500 µL of a methanolic solution of DPPH prepared at 0.05% (w/v) (0.5 g/L) and 500 µL of the sample extract were added in a 4 mL cuvette. The cuvettes were covered with laboratory film and stored in the dark for 30 min at room temperature. Then, samples were put into a spectrophotometer UV-Vis (Thermo Scientific Waltham, Massachusetts, United States) to measure the absorbance at 517 nm. To prepare the blank sample, the same protocol was applied, and the sample extract was replaced with 500 µL of methanol-distilled water 70:30 (v/v) solution.

To quantify the antioxidant capacity of each sample, the external standard method was applied. A calibration curve was obtained using a stock solution of Trolox (0.1 mg/mL) dissolved in a 70% methanolic solution (v/v). From Trolox stock solution (0.1 mg/mL), the following dilutions were prepared using a methanol-distilled water (70:30 v/v): 0.05 mg/mL, 0.04 mg/mL, 0.03 mg/mL, 0.02 mg/mL, 0.01 mg/mL, and 0.005 mg/mL. The results were expressed as Trolox Equivalents Antioxidant Capacity on g of dry matter (TEAC/g dm).

For each sample extract the DPPH assay was performed in two replicates, for a total of 3 extraction replicates x 2 TAC analysis replicates = 6 replicates for each sample.

2.4. Dough rheological analysis

Rheological measurements were performed at 25.0 ± 0.1 °C using a MCR 102 rheometer (Anton Paar, Gratz, Austria) equipped with 25-mm parallel plate profiled geometry (PP25/P2) and a Peltier temperature control system. Then, the same ingredients used in bread formulation [Table 1](#) (Paragraph 2.2), except fresh yeast, were added to prepare the dough samples using the same conditions applied in the bread-making process. The dough sample to be analyzed was taken out from the central part of the dough. Analyses were performed according to [Tidona et al. \(2021\)](#). Frequency sweep curves were fitted by using power law equations ([Sharma et al., 2016](#)):

$$G'(\omega) = k' \cdot \omega^{n'}$$

$$G''(\omega) = k'' \cdot \omega^{n''}$$

Where k' and k'' coefficients represent the magnitude of storage (G') and loss (G'') moduli (Pa) at a frequency of 1 rad/s, ω is the angular frequency (rad s⁻¹), and n' and n'' values reflect the dependency of viscoelastic properties on the frequency variation. The ratio between the loss and the storage moduli (G''/G' , defined as $\tan \delta$) was also calculated within the frequency range.

2.5. Macroscopic bread quality characterization

2.5.1. Bread specific volume and moisture content

The bread volume (L) was measured using the standard rapeseed displacement method (AACC, 10-05.01), 3 replicates for each sample x 3 batches for a total of 9 measurements. Bread specific volume (kg/L) was determined as the ratio between total volume and mass. Crumb and crust moisture contents (MC, g/100 g) were measured by gravimetry at $T = 105$ °C until constant weights were reached, according to standard method (AACC 44-15.02), 3 replicates for each batch.

2.5.2. Texture profile analysis

The texture profile analysis (TPA) of the bread samples was performed by applying a two-bite compression test using a Texture Analyzer (Stable Micro Systems, Goldalming, UK) equipped with a cylindrical acrylic probe (diameter: 35 mm). TPA was performed at the following conditions: 1.0 mm/s as pre-test, test, and post-test speed; 0.049 N as trigger force; 10.0 mm as initial distance; 5.0 s time range between two subsequent measurements. Samples for measurement were taken from the central part of bread loaf and from the central portion of the slice, avoiding the region near the crust. Each bread crumb sample included at least 10 cubes (20 mm height x 20 mm width x 20 mm length). Hardness (N), cohesiveness, and springiness (mm) were measured on at least 10 cubes for each sample x 3 batches for a total of 30 measurements.

2.5.3. Color measurements

The CIE-Lab color parameters L^* , a^* , b^* of bread crust and bread crumb were measured using a colorimeter (CM-36dG, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65 ([Commission Internationale de l'Éclairage CIE, 1978](#)). The Spectramagic Software (version 3.40) was used for data analysis. 10 measurements were performed for each bread sample x 3 batches, for a total of 30 measurements for bread crust, and 30 measurements for bread crumb. The color of the crust was measured on the upper surface of bread sample. Crumb samples were taken out from the center of the bread loaves, and tightly compressed using a press for 30 s to remove any porosity before performing color analysis.

2.6. ¹H molecular mobility and dynamics

Proton molecular mobility and dynamics were investigated with a

low-resolution (20 MHz) ¹H NMR spectrometer (the MiniSpec, Bruker Biospin, Milan, Italy) operating at 25.0 ± 0.1 °C. ¹H free induction decay (FID) and ¹H T₂ Carr-Purcell-Meiboom-Gill (CPMG) experiments were used according to the method reported by [Carini et al. \(2017\)](#) with some modifications as following. FIDs signals were acquired using a single 90° pulse, followed by a dwell time of 7 μs, and a recycle delay of 0.9 s, in a 0.5 ms acquisition window (the experimental window limit for ensuring the homogeneity of the magnetic field), 32 scans and 900 data points.

¹H T₂ (transverse relaxation time) was obtained with CPMG pulse sequence with a recycle delay of 1 s, an interpulse spacing of 0.04 ms, 2500 data points and 32 scans.

Five ¹H FID and five ¹H T₂ curves were acquired for each sample and for each batch, for a total of 5 replicates of analysis x 3 batches = 15 ¹H FID, and 15 ¹H T₂ measurements.

2.7. Data processing

The statistical software SPSS (Version 27.0, SPSS Inc., Chicago, USA) was used to calculate data means and standard deviations. One way ANOVA was performed using of the same software to assess significant differences ($p < 0.05$) among the 4 varieties of chickpeas and to assess significant differences ($p < 0.05$) due to the incorporation of the 30% of chickpea flour in GF-bread. The Tukey HSD test was used as the post-hoc test. Bivariate correlation among response variables were analyzed by Pearson's test to calculate the correlation coefficient (R), and t-test ($p < 0.05$) was performed to assess the significance of the correlation.

3. Results and discussion

3.1. Physico-chemical characterization of chickpea flours

3.1.1. Proximate composition

The average proximate composition of the whole meal chickpea flour samples showed the following values: 24.00 ± 0.93 g/100 g dm protein content, 5.19 ± 0.53 g/100 g dm lipid content, 3.93 ± 0.25 g/100 g dm ash content, 39.63 g/100 g dm available carbohydrates content, and 27.25 g/100 g TDF of which 22.95 g/100 g dm corresponding to IDF and 4.30 g/100 g dm corresponding to SDF. Results were consistent with the ranges of macronutrients reported in the literature for chickpea flours ([Du et al., 2014](#); [Rachwa-Rosiak et al., 2015](#); [Kaur and Prasad, 2021](#)). However, our samples showed a higher content of TDF and ash, and lower content of available carbohydrates compared to the ranges reported in the literature, a result that may be associated both to the refinement degree and to the genetic background of the tested chickpeas varieties ([Singh et al., 2010](#); [Du et al., 2014](#); [Kaur et al., 2019](#); [Summo et al., 2019](#)).

The proximate composition of the four varieties of chickpea flours is reported in [Table 3](#). Chickpea variety had a significant effect on protein, ash, TDF, IDF, and SDF. Conversely, lipid content was not significantly affected by variety showing values around approx. 5 g/100 g dm in all chickpea samples. Considering protein content, it can be observed that RSC showed the highest amount, followed by WC; MC and RRC showed the lowest protein content ([Table 1](#)). This result was consistent with the literature, reporting similar values of protein content in modern and wild chickpea varieties ([Singh et al., 2010](#); [Kaur et al., 2019](#); [Summo et al., 2019](#)). Ash content showed significantly higher values in wild chickpea flours compared to the modern one, suggesting that these flours probably contain higher amounts of minerals and fibers. The wild and modern chickpea varieties analyzed by [Summo et al. \(2019\)](#) did not show significant differences on ash content. Beside the higher ash content found for the wild chickpea flours, samples showed higher TDF compared to the modern flours. In detail, RRC also showed the highest value, followed by SRC and WC; MC was characterized by the lowest value of SDF. Considering the IDF parameter, it can be observed that RRC had the highest value, followed by RSC, and by MC. WC had the lowest IDF among all chickpea varieties. SDF parameter showed the

Table 3
Proximate composition analysis of chickpea whole meal flours.

Sample	Moisture (%)	Ash (g/100 g dm)	Lipid (g/100 g dm)	Protein (g/100 g dm)	TDF ¹ (g/100 g dm)	IDF ¹ (g/100 g dm)	SDF ¹ (g/100 g dm)	Available carbohydrates (g/100 g dm)
MC	8.40 ± 0.07 ^c	3.55 ± 0.03 ^b	5.30 ± 0.01 ^a	23.21 ± 0.16 ^c	25.32	21.19	4.13	42.63
WC	9.39 ± 0.38 ^b	4.15 ± 0.07 ^a	5.23 ± 1.02 ^a	24.27 ± 0.05 ^b	26.02	20.74	5.28	40.33
RRC	10.59 ± 0.19 ^a	4.00 ± 0.02 ^a	5.35 ± 0.81 ^a	23.22 ± 0.07 ^c	30.27	26.40	3.87	37.16
RSC	10.70 ± 0.21 ^a	4.03 ± 0.07 ^a	4.90 ± 0.01 ^a	25.30 ± 0.08 ^a	27.40	23.46	3.94	38.37
<i>P-value</i>	**	**	ns	***	nd	nd	nd	nd

Chickpea whole meal flours: MC = Modern Chickpeas, WC = White Chickpeas, RRC = Red Rough Chickpeas, RSC = Red Smooth Chickpeas. *, **, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. "ns" indicates no significant differences at $p < 0.05$, "nd" indicates not determined, "1" indicates relative standard deviations (RSD) below 5%. Means in a column with different superscripts are significantly different ($p < 0.05$).

opposite trend as compared to IDF, since the highest value was found for WC, followed by MC; red varieties (RRC and RSC) were characterized by the lowest values of this parameter. This result is consistent with Summo et al. (2019), who reported that fiber content was significantly affected by seed color and seed size; indeed, pigmented chickpea varieties showed the highest content of dietary fiber as well as small chickpea seeds were characterized by higher content of dietary fiber.

Concerning the available carbohydrates content, it can be observed that all wild varieties had significant lower available carbohydrates content compared to the modern variety. This result was consistent with Kaur et al. (2019), who reported that wild chickpea species were characterized by the lowest starch content as compared to cultivated chickpea species (desi and kabuli). These data suggests that wild chickpea flours could be associated to lower glycemic index (GI) compared to the modern chickpea flour.

3.1.2. Functional properties

The physical behavior of foods and food ingredients during processing and storage is affected by functional properties (Yegrem et al., 2022). Indeed, functional properties are related to the interactions among the compositions, physico-chemical characteristics, structure, and molecular conformation of food ingredients. Furthermore, since the environmental and processing conditions adopted during the analysis affect the functional properties, it is crucial to standardize the method of analysis (Yegrem et al., 2022). Considering flours, their application as food ingredients mostly depends on the interactions between flours and water molecules (Ladjal Ettoumi and Chibane, 2015).

In the present study, results showed that chickpea variety significantly affected the functional properties of the flours. Water holding capacity (WHC) of flours plays a critical role in food preparation process (Du et al., 2014; Ladjal Ettoumi and Chibane, 2015). Data showed a distribution included between 1.75 g water/g flour and 2.10 g water/g flour. The highest WHC value was observed in RRC which resulted significantly different to MC, showing the lowest value of this parameter. RSC and WC showed intermediate values of WHC, with not significant differences compared to both RRC and MC. Similar values of WHC of chickpea flours were reported in the literature (Du et al., 2014; Summo et al., 2019; Yegrem et al., 2022; Herrera and Gonzalez de Mejia, 2021; Stone et al., 2019; Ladjal Ettoumi and Chibane, 2015; Sofi et al., 2023), although our results showed higher mean values of the parameter compared to the above studies. Summo et al. (2019), analyzing 57 chickpea accessions (36 representative of the global chickpea cultivation and 21 accession of Apulian chickpea type), reported similar values of WHC. These authors found a significant effect of the color of seed coat, genetic cluster, and seed size on WHC values (Summo et al., 2019). Considering the color of chickpea seed coat, brown chickpeas had the highest mean value (1.86 g water/g flour), followed by black chickpea (1.60 g water/g flour), and by beige chickpea (1.40 g water/g flour) (Summo et al., 2019). Sofi et al. (2023) reported slight lower WHC values, of 1.6–1.7 g water/g flour in subtropical cultivars from India. Lower values of WHC were reported for cultivated chickpea varieties tested by Stone et al. (2019) (1.02–1.09 g water/g flour), and by Ladjal Ettoumi et al. (2015) in whole meal chickpea flour (1.064 g water/g flour) among legumes tested. It is known that polar amino acid residues of proteins have affinity for water molecules and differences in WHC of different legumes may be associated to the content of these amino acids (Ladjal Ettoumi and Chibane, 2015). Furthermore, carbohydrate composition (fiber and starch) affects the hydration properties of flours since these constituents are characterized by polar or charged side chains (Ladjal Ettoumi and Chibane, 2015). Considering tested whole meal chickpea flours, the fibers content showed higher values (Table 3) as compared to the ranges reported in the literature (Rachwa-Rosiak et al., 2015) and may have affected WHC values. It can be hypothesized that other factors may play a role in determining high WHC values, such as the genetic background of chickpeas (wild chickpea varieties) and flour refinement degree (whole meal chickpea flours).

The OHC of pulse flour is very important for improving the mouth texture and maintaining the flavor of food products (Du et al., 2014). Values of OHC ranged between 1.13 g oil/g flour and 1.26 g oil/g flour; this parameter showed slight but significant differences among chickpea varieties. WC was characterized by the highest value of OHC, whereas RRC showed the lowest value among all chickpea varieties. MC and RSC had intermediate values, not significantly different from both WC and RRC. Similar OHC results were reported in the literature (Du et al., 2014; Summo et al., 2019; Sofi et al., 2023). Slight lower OHC values were found by Herrera and Gonzalez de Mejia (2021) (0.83–0.97 g oil/g flour), whereas higher OHC values were reported by Stone et al. (2019) (1.40–1.53 g oil/g flour). The binding of oils to the flours depends on the surface availability of hydrophobic amino acids and other non-polar side chains such as dietary fiber components (Ladjal Ettoumi and Chibane, 2015). The oil adsorbing mechanism involves capillary interaction, which allows the adsorbed oil to be retained (Du et al., 2014). The OHC values of legume flours are influenced by particle sizes, starch and protein contents, protein types, and non-polar amino-acid side chain ratios on the protein molecule surface (Du et al., 2014). According to Kinsella (1976) and Du et al. (2014), more hydrophobic proteins show superior binding of lipids; hence, the flour having the highest OHC value (MC sample) may be characterized by a higher amount of available non-polar side chains in its molecules compared to the other samples.

The WAI corresponds to the volume occupied by the starch after it swells in hot water, hence it is related to the hydrophilicity and gelation capacity of molecules such as starch and proteins (Kaur and Singh, 2005; Du et al., 2014). Therefore, the parameter reveals the integrity of starch in aqueous dispersions, it is related to starch-water interactions such as starch water absorption and swelling, and it is associated to the gelation capacity of starch (Du et al., 2014; Milán-Noris et al., 2019). However, pulse flours are characterized by significant amounts of other components affecting the starch-water interactions, such as fiber and proteins (Milán-Noris et al., 2019). Therefore, the WAI of legume flours may not completely depend on the water absorption and swelling of the starches (Du et al., 2014). WAI parameter showed values in the range of approx. 4.23–5.11 g/g. Chickpea variety significantly affected WAI values: the highest WAI among all chickpea varieties was observed for RSC. A slight lower value was obtained for RRC which was not significantly different from both RSC and MC. This latter showed a significantly lower value of WAI compared to RSC. The lowest value of the parameter was observed for WC. The range of WAI values obtained in our results is consistent with Du et al. (2014), Summo et al. (2019), and Milán-Noris et al. (2019), whereas Tas et al. (2022) reported lower values of the parameter. In detail, Milán-Noris et al. (2019) analyzing 10 chickpea cultivars differing in seed coat color (black, brown, green, red, and cream), reported a WAI range between 3.94 g/g and 4.74 g/g with significant differences among the tested cultivars. Du et al. (2014), investigating whole pulse flours, showed that WAI ranged between 4.09 g/g and 6.13 g/g, with chickpea flour having the highest value of the parameter. Our data showed significant differences in WAI as a function of chickpea variety highlighting that the tested flours were characterized by different gelatinization capacity and quality of the starch.

WSI is strongly correlated to WAI, and it explains the presence of soluble solids that remain in the aqueous phase after the heating process, hence it indicates the solubility of molecules (Du et al., 2014). Data showed a wider distribution included in the range of approx. 10.83–23.02 g/100 g. WC showed the highest value of the parameter among all chickpea varieties, followed by MC and RSC which showed not significant different values of WSI. The lowest value of WSI was observed in RRC. Similar values of WSI were reported in the literature for chickpea flours (Du et al., 2014; Summo et al., 2019; Milán-Noris et al., 2019). Du et al. (2014) found a WSI value of 24.08 g/100 g for whole meal chickpea flour; Summo et al. (2019) reported a WSI range of approx. 13.8–15.2 g/100 g with no significant differences among the chickpeas tested as a function of the color of seed coat, genetic cluster, and seed size. Milán-Noris et al. (2019) testing different pigmented

chickpea flours, reported higher values of WSI (17.51–28.20 g/100 g). The significant differences obtained in the WSI of the tested chickpea flours can be associated to their different WAI values with a negative correlation between the two parameters ($R^2 = -0.96$; $p = 1.59 \cdot 10^{-6}$). The significant differences among the chickpea varieties highlighted that the samples were characterized by a different gelatinization capacity and quality of the starch.

Foaming capacity and stability generally depend on the interfacial film formed by proteins, which are able to maintain the air bubbles in suspension and slow down the rate of coalescence (Du et al., 2014). Foaming properties are associated to the proteins and some other components, such as carbohydrates, that are present in the flours (Du et al., 2014). However, the proteins play the main role in foaming properties, which are affected by their intrinsic molecular properties (Ladjal Ettoumi and Chibane, 2015). Therefore, foaming behavior in food systems are impacted by protein amino acid sequence and disposition, molecular size, shape, conformation and flexibility, surface polarity, charge, hydrophobicity, etc. (Ladjal Ettoumi and Chibane, 2015). All these characteristics are affected by the processing, and by the physico-chemical environment of the proteins (Kinsella, 1981; Ladjal Ettoumi and Chibane, 2015). Chickpea variety significantly affected FC, whereas it did not impact FS which showed similar values among all chickpea varieties (i.e., approx. in the range of 96.1–96.9%). Considering FC, chickpea varieties were characterized by values in the range of 21.0–26.7%. MC and RSC were characterized by the highest values of FC, whereas WC and RRC showed the lowest values of this parameter. Data were almost in the range reported in the literature, although the use of slightly different procedures, especially flour concentrations, does not allow to perform an appropriate comparison (Du et al., 2014; Yegrem et al., 2022; Herrera and Gonzalez de Mejia, 2021; Ladjal Ettoumi and Chibane, 2015). Yegrem et al. (2022), working with 2% chickpea flour concentration, reported a FC range of 14.54–16.96%, whereas Herrera and Gonzalez de Mejia (2021) using 3% chickpea flour concentration found a FC values of 36.9% in desi and 41.0% in kabuli. Furthermore, Ladjal Ettoumi et al. (2015), using chickpea flour concentration of 4%, reported a FC value of 32.4%. No significant differences were found for FS parameter as a function of chickpea variety, since all the tested chickpea flours were characterized by high FS values, with values ranging between 96.1% and 96.9%. Similar values of FS were found by Stone et al. (2019) who tested different pulse flours and reported values of 83.5% for both desi and kabuli chickpeas. Ladjal Ettoumi et al. (2015) found lower values of FS in whole meal pulse flours (41.3–71.4%), with values of 46.1% for chickpea flour. The high values of FS found in the present results revealed that the native proteins soluble in the continuous phase (water) are particularly surface-active.

3.1.3. Total antioxidant capacity (TAC) and total phenolic content (TPC)

Results about TAC and TPC of the 4 chickpea flours showed significant differences as a function of chickpea variety (Fig. 1a).

Considering TAC, it can be observed that values were included in the range of approx. 40.6–44.4 $\mu\text{mol TEAC}/100 \text{ g dm}$. All wild flours showed higher TAC values compared to MC. In detail, RRC and RSC showed the highest TAC values among all chickpea flours tested, significantly different from MC. On the other hand, although WC showed a higher TAC value compared to MC, the difference between these samples was not significant. Finally, MC showed the lowest TAC among all chickpea varieties tested. In the literature there are not many data about TAC values of chickpea flours (Ladjal Ettoumi and Chibane, 2015; Xu et al., 2007; Fernandez-Orozco et al., 2009; Rocchetti et al., 2007; Sofi et al., 2023; Costantini et al., 2021; Mao et al., 2024; Gupta et al., 2017; Summo et al., 2019). Furthermore, in these papers, different methods of analysis as well as different parameters were used to express the antioxidant capacity, factors that make it difficult to compare the results obtained in different articles (Ladjal Ettoumi and Chibane, 2015; Xu et al., 2007; Fernandez-Orozco et al., 2009; Rocchetti et al., 2007; Sofi et al., 2023; Costantini et al., 2021; Mao et al., 2024; Gupta et al., 2017;

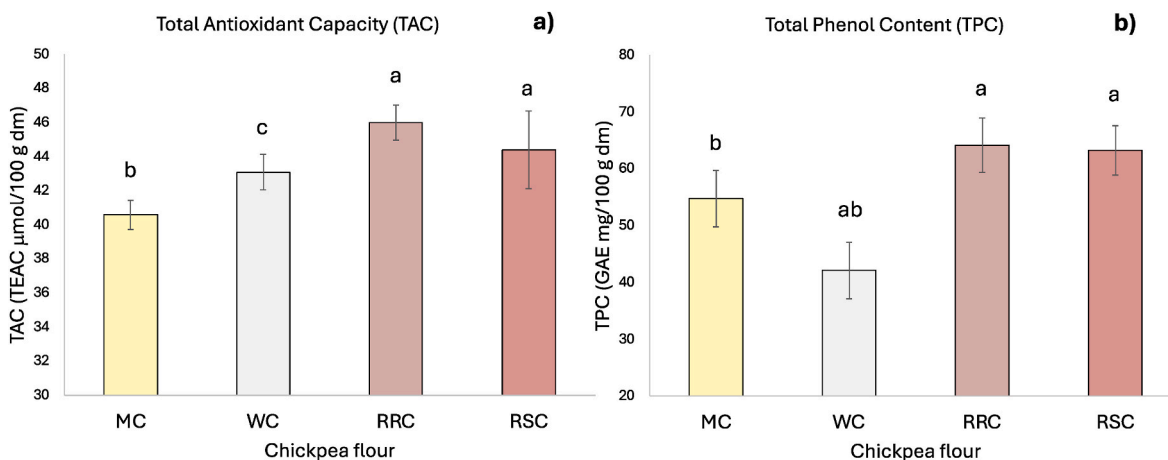


Fig. 1. a) Total Antioxidant Capacity (TAC), and b) Total Phenol Content (TPC) of whole meal chickpea flours. Data are reported as means \pm standard deviations ($n = 6$). Different small letters indicate significant differences among chickpea varieties ($p < 0.05$). Abbreviations of chickpea flour samples: MC = modern chickpea (yellow bars), WC = white chickpea (white bars), RRC = red rough chickpea (light red bars), RSC = red smooth chickpea (red bars).

Summo et al., 2019). However, results obtained in the present study, revealed that the TAC of the tested wild chickpea flours was significantly higher as compared to the modern variety.

The TPC of the chickpea flours showed significant differences as a function of chickpea variety (Fig. 1b). TPC of chickpea samples showed values in the range of 42.1–64.2 mg GAE/100 g dm. Red chickpea samples (RRC and RSC) showed the highest TPC values. Indeed, red varieties were characterized by significantly higher TPC values than MC. A significant lower value of TPC than red varieties was found in MC. However, the lowest TPC value among all chickpea varieties was obtained in WC. TPC values of chickpea samples followed a trend similar to TAC, suggesting that total phenolic content may play a significant role in the total antioxidant capacity of the flours. The only exception was WC, in which compounds other than phenols may contribute to its TAC (Table 4). Similar results were reported in the literature about TPC of pulse flours (Milán-Noris et al., 2019; Fernandez-Orozco et al., 2009; Rocchetti et al., 2017; Gupta et al., 2017). Milán-Noris et al. (2019), testing 10 whole chickpea flours differing in seed coat colour, found a range of TPC of 23.7–44.4 mg GAE/100 g, and Rocchetti et al. (2017) reported that, among the tested gluten free flours, chickpea flour was characterised by TPC value of 45.5 mg GAE/100 g. Gupta et al. (2017), analysing 40 chickpea genotypes including cultivars and advanced lines with different genetic background, reported a TPC range between 4.7 and 35.4 mg/100g. Similar results were also reported by Fernandez-Orozco et al. (2009), who found TPC content in raw chickpea sample of 54 mg catechin/100 g dm, although a different standard was used as reference for quantification. However, other authors reported lower (Sofi et al., 2023; Mao et al., 2024) and higher TPC values (Xu et al., 2007; Ladjal Ettoumi and Chibane, 2015), highlighting that probably differences in the methods of analysis affected the results.

3.2. Dough rheological analysis

Fig. 2 shows the rheological properties of dough samples, which represent a critical factor to determine the bread quality, such as specific volume and textural attributes (Bloksma, 1990). The storage modulus (G') represents the deformation energy stored in the material after removing the oscillation and thus it is indicative of the elastic behavior of the system; the loss modulus (G'') reports the mechanical energy lost by the dough during oscillation and it is an indicator of the material viscous behavior (Sullivan et al., 2011). It can be observed that dough samples were characterized by a higher elastic (G') than viscous modulus (G'') through all the frequency ranges, and that both moduli increased with increasing frequency levels (Fig. 2a). This is the typical behavior observed in dough rheological studies (Mohammed et al., 2012; Miñarro et al., 2012; Aguilar et al., 2015; Maças et al., 2023).

Significant differences were obtained on the parameters of power-law equations describing the dependence of moduli on oscillatory frequency. In detail, GF dough sample significantly affected K' ($p < 0.001$), K'' ($p < 0.001$) and n'' ($p < 0.001$) coefficients, whereas no significant differences were observed on n' coefficient ($p > 0.05$) (Fig. 2b and c). Data showed that the significant effect obtained on K' , K'' and n'' was associated to the incorporation of chickpea flours in GF flour dough, independently of the variety of chickpea used for the substitution. Indeed, all chickpea dough samples (MC, WC, RRC, and RSC dough samples) showed similar values of the above parameters with no significant differences between each other, and they were all significantly different from the CTR sample. The values of rheological parameters of the CTR dough samples vs the mean values obtained for all chickpea substituted samples (MC, WC, RRC, RSC dough samples) were as following: (i) $K' - 10506.13 \pm 1594.36$ Pa vs 7121.31 ± 1231.90 Pa, (ii) $K'' - 4276.56 \pm 854.06$ Pa vs 2473.16 ± 417.01 Pa, (iii) $n'' - 0.11 \pm 0.03$

Table 4
Physico-chemical characterization of whole meal chickpea flours.

Sample	WAI (g/g)	WSI (g/100 g)	WHC (g/g)	OHC (g/g)	FC (%)	FS (%)
MC	4.87 \pm 0.07 ^b	12.44 \pm 0.07 ^b	1.75 \pm 0.14 ^b	1.14 \pm 0.08 ^{ab}	25.38 \pm 1.61 ^a	96.63 \pm 0.66 ^a
WC	4.23 \pm 0.09 ^c	23.02 \pm 0.28 ^a	1.83 \pm 0.06 ^{ab}	1.26 \pm 0.03 ^a	21.84 \pm 1.77 ^b	96.85 \pm 1.77 ^a
RRC	5.06 \pm 0.09 ^{ab}	10.83 \pm 0.39 ^c	2.10 \pm 0.19 ^a	1.13 \pm 0.06 ^b	20.99 \pm 1.83 ^b	96.22 \pm 1.83 ^a
RSC	5.11 \pm 0.10 ^a	11.79 \pm 0.32 ^b	2.04 \pm 0.17 ^{ab}	1.19 \pm 0.10 ^{ab}	26.72 \pm 1.93 ^a	96.08 \pm 1.93 ^a
<i>p-value</i>	***	***	*	*	***	ns

Parameters: Water Absorption Index – WAI; Water Solubility Index – WSI; Water Holding Capacity – WHC; Oil Holding Capacity – OHC; Foaming Capacity – FC; Foaming Stability – FS. Chickpea whole meal flours: MC = Modern Chickpeas, WC = White Chickpeas, RRC = Red Rough Chickpeas, RSC = Red Smooth Chickpeas. *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. “ns” indicates no significant differences at $p < 0.05$. Means in a column with different superscripts are significantly different ($p < 0.05$).

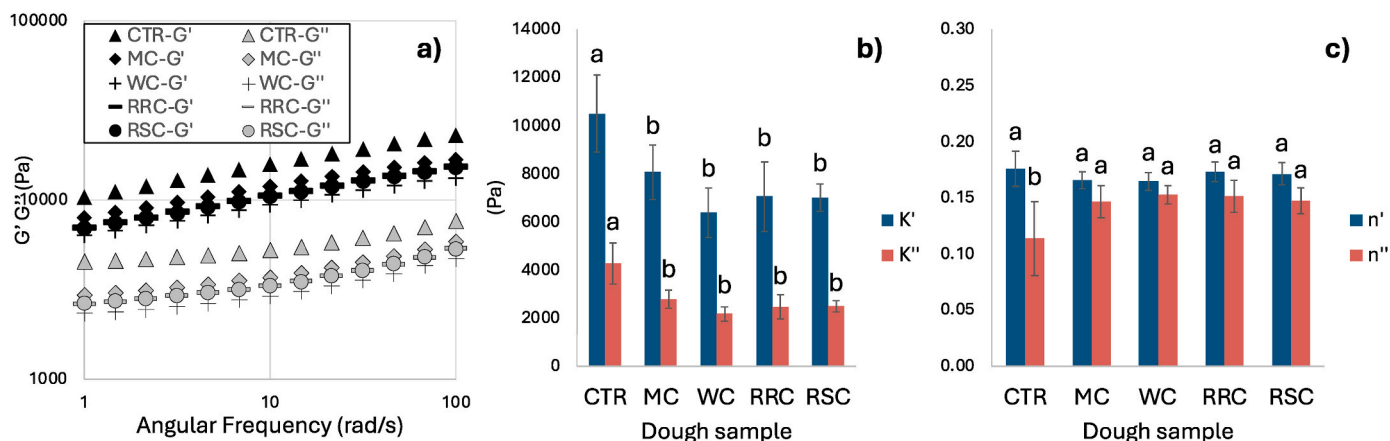


Fig. 2. a) Frequency sweep curves of GF dough samples, b) K' (blue bars) and K'' (orange bars) and c) n' (blue bars) and n'' (orange bars) coefficients of GF dough samples. Data are reported as means \pm standard deviations ($n = 10$). Different small letters indicate significant differences among chickpea flours ($p < 0.05$). Abbreviations of GF dough samples: CTR = 100% GF flour; MC = 70% GF flour, 30% modern chickpea flour; WC = 70% GF flour, 30% white chickpea flour; RRC = 70% GF flour, 30% red rough chickpea flour; RSC = 70% GF flour, 30% red smooth chickpea flour.

vs 0.15 ± 0.01 , respectively. Overall, the data showed that CTR dough sample was characterized by significant higher values of K' and K'' , and by a significant lower value of n'' as compared to chickpea substituted samples (MC, WC, RRC, and RSC dough samples). In the literature Aguilar et al. (2015) and Maças et al. (2023) reported an opposite effect compared to the results obtained in the present study, which was a significant increase of G' in GF dough samples substituted with chickpea flours. This different result could be associated to the significant lower levels of pulse flour substitution used in the above studies (7.8% chickpea flour, and 15% pea flour w/w flour), to the different source and chemical composition of the pulse flours as well as to the composition of the GF flour used as base to produce the dough blends.

3.3. Macroscopic bread quality characterization

3.3.1. Bread specific volume and moisture content

Results related to macroscopic bread quality characterization are reported in Table 5 and bread images are shown in Fig. 3. Considering bread specific volume, results showed that the substitution of GF flour with chickpea flour significantly affected the parameter, and significant differences were obtained as a function of chickpea variety (Table 5). The highest value of bread specific volume was obtained for CTR (2.75 ± 0.05 L/kg), whereas chickpea breads had values in the range of 1.81–2.15 L/kg. The lowest value of bread specific volume was observed for WC, whereas no significant differences were found among MC, RRC, and RSC (2.15 – 2.30 L/kg). Scant information is reported in the literature

about the effect of GF flour substitution with chickpea flour in GF breads (Kahraman et al., 2022; Burešová et al., 2017; Miñarro et al., 2012; Aguilar et al., 2015), and no information were found about the effect of different chickpea varieties on GF bread. Kahraman et al. (2022) reported that GF bread substituted with 25% (w/w flour) chickpea flour was characterized by a slight lower bread specific volume as compared to the control sample, but the difference was not significant. A similar result was observed by Aguilar et al. (2015), also when a smaller level of chickpea flour substitution in GF bread (7.8% w/flour w) was tested. Conversely, Miñarro et al. (2012) found that 8.3% of chickpea flour in GF bread significantly increased the specific volume as compared to the GF bread formulated with soy flour. Differences among the above articles could be assigned to the different composition of GF flour used for the experimental trials, the different bread formulations, and processing conditions, as well as the different levels and type of chickpea flours tested. Beside the significant reduction of bread specific volume observed at 30% (w/w flour) chickpea flour substitution level, the results of the present study outlined that the three wild chickpea varieties had a different impact on the parameter, in comparison with MC.

Considering the moisture content (MC) of bread samples, measured on the crust and crumb, no significant differences were found among all tested samples and this result was consistent with what reported by Kahraman et al. (2022).

3.3.2. Texture analysis

Concerning texture parameters of bread crumb, results showed that

Table 5
Macroscopic bread quality characterization.

Bread sample	Specific volume (L/kg)	Hardness (N)	Cohesiveness	Springiness (%)	L* crust	a* crust	b* crust	L* crumb	a* crumb	b* crumb	Crust MC (%)	Crumb MC (%)
CTR	2.75 ± 0.05^a	2.34 ± 0.19^{bc}	0.534 ± 0.020^a	89.87 ± 0.98^a	86.92 ± 0.80^a	0.43 ± 0.08^b	12.19 ± 0.33^b	79.03 ± 0.70^a	-1.89 ± 0.06^c	13.70 ± 0.96^c	28.84 ± 0.78^a	47.08 ± 0.09^a
MC	2.15 ± 0.12^b	4.20 ± 1.01^b	0.436 ± 0.008^b	76.03 ± 2.72^b	68.90 ± 0.31^b	6.20 ± 1.27^a	30.39 ± 0.19^b	74.57 ± 0.23^b	-0.36 ± 0.50^a	26.12 ± 0.50^a	29.64 ± 0.74^a	46.85 ± 0.38^a
WC	1.81 ± 0.06^c	9.95 ± 0.73^a	0.451 ± 0.044^b	75.82 ± 5.48^b	68.48 ± 1.24^b	6.48 ± 1.57^a	32.59 ± 3.09^a	75.68 ± 0.79^b	-0.73 ± 0.26^b	23.85 ± 0.65^b	28.88 ± 1.09^a	47.06 ± 0.08^a
RRC	2.30 ± 0.08^b	2.23 ± 0.31^c	0.449 ± 0.039^b	75.49 ± 3.53^b	64.95 ± 1.40^c	7.50 ± 1.40^a	31.07 ± 2.29^a	59.99 ± 0.39^c	2.62 ± 0.23^a	15.61 ± 0.18^d	29.33 ± 0.94^a	47.11 ± 0.36^a
RSC	2.17 ± 0.16^b	4.18 ± 0.93^b	0.417 ± 0.012^b	77.82 ± 0.78^b	61.58 ± 1.17^d	7.22 ± 1.14^a	31.00 ± 1.87^a	60.73 ± 0.14^c	2.43 ± 0.14^a	19.84 ± 0.33^c	29.43 ± 0.89^a	47.04 ± 0.19^a
p-value	***	***	**	**	***	***	***	***	***	***	ns	ns

Bread samples: CTR = 100% GF flour; MC = 70% GF flour, 30% modern chickpea flour; WC = 70% GF flour, 30% white chickpea flour; RRC = 70% GF flour, 30% red rough chickpea flour; RSC = 70% GF flour, 30% red smooth chickpea flour. *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. “ns” indicates no significant differences at $p < 0.05$. Means in a column with different superscripts are significantly different ($p < 0.05$).

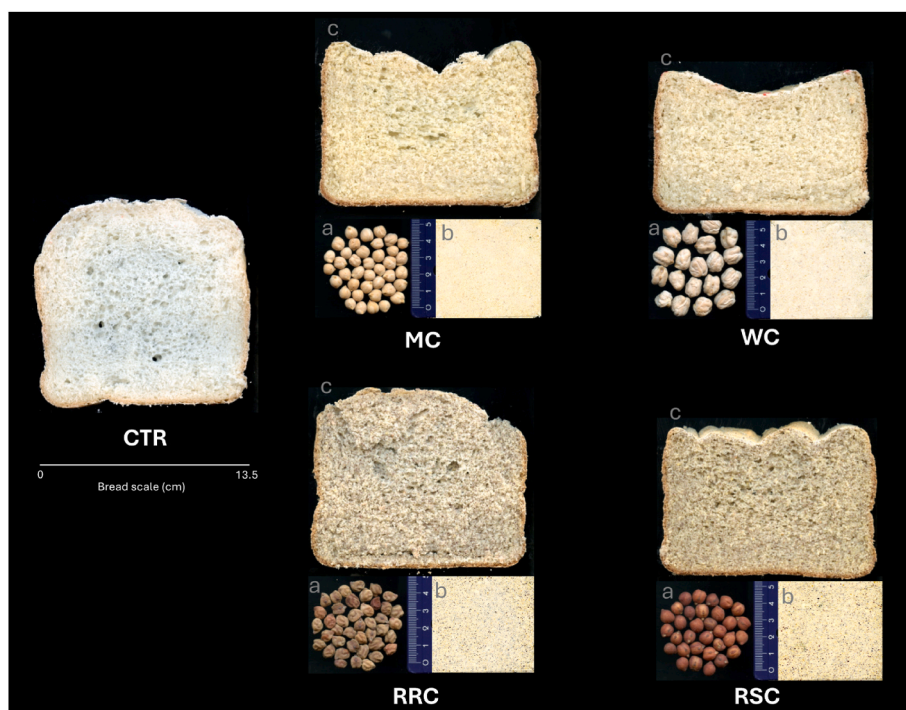


Fig. 3. Images of chickpea seeds (a), chickpea whole meal flours (b), and crumb slices of chickpea GF breads (c) (70% GF flour and 30% chickpea flour). Abbreviations: MC = modern chickpea, WC = white chickpea, RRC = red rough chickpea, RSC = red smooth chickpea; CTR = control sample (100% GF flour).

chickpea variety significantly impacted the texture of bread. Hardness showed values in the range of 2.23–9.95 N (Table 5). The highest value of hardness was found in WC, whereas MC and RSC showed significant lower hardness than WC (4.20–4.18 N). The lowest values of the parameter were observed in RRC. It is interesting to note that CTR was not significantly different from MC, RSC, and RRC. Therefore, the incorporation of 30% chickpea flour of the varieties MC, RSC and RRC, did not change the crumb hardness. A similar result was reported by Aguilar et al. (2015), when replacing GF flour with 7.8% (w/flour w) chickpea flour. Conversely, Kahraman et al. (2022), Burešová et al. (2017), and Minarro et al. (2012) found a significant increase in the hardness of bread crumb when substituting GF flour with chickpea flour in levels of 25%, 30%, and 8.3% (w/flour w), respectively. Regarding cohesiveness and springiness, the data showed values between 0.417 and 0.534, and between 75.5 % and 89.9 %, respectively. CTR was characterized by the highest value of cohesiveness and springiness, whereas chickpea bread samples showed significantly lower values of both parameters, and their values were not significantly different from each other. Hence, independently of chickpea variety, these results outlined the effect of the weakening of GF bread structure by the substitution with chickpea flour. A similar result on cohesiveness was observed by Kahraman et al. (2022), who reported that GF bread substituted with 25% of chickpea flour was characterized by a lower cohesiveness and springiness compared to the control GF bread. On the other hand, Burešová et al. (2017) found the opposite result on crumb cohesiveness, which was higher in bread substituted with 30% chickpea flour, and no significant differences were found on crumb springiness between 30% chickpea bread and control GF bread.

3.3.3. Color measurements

Crust and crumb color parameters showed significant differences among bread samples. Crust a^* and b^* values were significantly affected by the chickpea flour, independently of the variety. Indeed, all chickpea-bread showed similar values of crust a^* and b^* parameters, which were significantly higher compared to CTR, revealing that chickpea flour significantly increased the red and yellow indexes of bread crust. Crust

L^* parameter showed a different trend: the highest value was found in CTR, whereas it significantly decreased in all chickpea-breads with significant differences as a function of chickpea flour. In detail, L^* values measured on the crust of MC and WC were significantly higher compared to RRC and RSC. Therefore, the substitution of GF flour with chickpea flour significantly decreased crust lightness, with the highest impact observed for red varieties, with RSC showing the lowest lightness value. These results were consistent with Kahraman et al. (2022) and Aguilar et al. (2015). Indeed, both Kahraman et al. (2022) and Aguilar et al. (2015) found a significant decrease of crust L^* when substituting GF bread with 25% and 7.8% of chickpea flour, respectively. Furthermore, Kahraman et al. (2022) also reported a significant increase of crust a^* and b^* parameters in substituted bread compared to the control sample.

All crumb color parameters were significantly affected by chickpea variety. Crumb a^* parameter showed the highest values in RSC and RRC, followed by MC and WC. The lowest crumb a^* value was observed in CTR. Therefore, for crumb a^* , a significant effect of chickpea flour can be observed: all chickpea-bread showed a significantly higher red index compared to CTR, with red varieties showing the highest red index among all the chickpea flours tested. Considering crumb b^* parameter, results showed that all samples were significantly different from each other. The lowest value of the parameter was observed for CTR. The yellow index significantly increased in substituted breads as a function of chickpea variety, according to the following increasing trend: RRC, RSC, WC, and MC. A similar result was reported by Kahraman et al. (2022), who observed a significant increase of crumb b^* parameter in GF bread substituted with 25% chickpea flour (w/w flour) compared to the control GF bread, while the authors found a lower crumb a^* value in substituted breads compared to the control sample. Considering crumb L^* parameter, the highest value was observed for CTR. Indeed, the lightness significantly decreased according to the chickpea varieties: a similar significant L^* crumb decrease was observed for MC and WC, whereas RRC and RSC showed the lowest L^* value. A different result was reported by Aguilar et al. (2015), who did not find significant differences in crumb L^* parameter. This effect might be associated to the lower level

of chickpea flour substitution in GF bread compared to the present study (7.8% vs 30% w/flour w) which can explain the small impact of the flour substitution on bread crumb L^* (Aguilar et al., 2015). However, also Kahraman et al. (2022) comparing 25% chickpea bread and control GF bread did not find significant differences in L^* parameter. The differences observed when comparing the results of this study with the literature (Kahraman et al., 2022; Aguilar et al., 2015) can be interpreted as the effect of using different chickpea varieties, as well as the different levels of chickpea flour substitution and GF flours.

3.4. ^1H molecular mobility and dynamics

The FID experiment revealed the presence of two populations which were named A (the less mobile one) and B (the more mobile one), relaxing in the range of 19.0–21.0 μs and 0.35–0.37 ms, respectively. The ^1H T_2 distributions of the relaxation times showed the presence of four different proton populations, defined as popC, popD, popE, and popF, from the least to the most mobile proton population, respectively. The ^1H relaxation time were in the range of 0.39–0.59 ms, 3.37–5.03 ms, 16.22–18.25 ms, 62.42–198.53 ms, for populations C, D, E, and F, respectively. The dominant FID population was population A, which represented 54.76–60.10% of the total detectable protons (pop B represented 39.90–45.24% of the total protons). In the ^1H T_2 time frame window, the dominant population was population E, representing 77.03–82.39% of the total detectable protons, followed by population D (10.35–14.20%), population C (6.81–7.53%), and population F (0.03–2.13%). Since the relaxation times of population B and C overlapped, these proton populations were considered to represent the same protons and therefore, only population C was discussed as belonging to the better resolved CPMG experiment signal. As further confirmation of this hypothesis, the relaxation time of population B and C showed the same results in function of the tested independent variable.

Scant information is given in the literature about the molecular mobility and dynamics in GF breads (Carini et al., 2017; Hager et al., 2014; Rondeau-Mouro et al., 2019; Róžańska et al., 2023), and no information were found in GF bread partially substituted with chickpea flour.

However, our data were consistent with the results reported in the literature (Carini et al., 2017; Hager et al., 2014; Rondeau-Mouro et al., 2019; Róžańska et al., 2023) which showed the presence of one FID and four CPMG proton populations in GF breads. According to the relaxation times of the proton populations detected in our samples (Table 6) and to the results reported in the literature (Bosmans and Delcour, 2016; Carini et al., 2017; Hager et al., 2014; Rondeau-Mouro et al., 2019), the observed proton populations can be assigned to specific physico-chemical domains of bread matrix. The most rigid and abundant

FID population (popA) was assigned to protons of starch crystals and amorphous starch and proteins not in contact with water in both wheat-flour (Bosmans and Delcour, 2016), and GF breads (Carini et al., 2017; Hager et al., 2014). PopE, the dominant population in the CPMG proton distribution, was associated with mobile exchanging protons of water, starch, and gluten in the formed gel network (Bosmans and Delcour, 2016). PopD, the second most abundant CPMG proton population, was associated to OH protons of intragranular water and starch, but also to some CH protons of gluten and exchanging protons of confined water, starch, and gluten (Bosmans and Delcour, 2016; Curti et al., 2014; Hager et al., 2014) and to the presence of fiber (Curti et al., 2013, 2015, 2016) in wheat-flour bread. PopC was attributed in both wheat-flour and GF bread to both rigid and CH protons of amorphous starch and proteins in little contact with water (Bosmans and Delcour, 2016; Curti et al., 2014), whereas in GF bread corresponded mainly to water absorbed in starch granules (intragranular water) (Hager et al., 2014; Carini et al., 2017). Finally, popF was associated to lipid protons in both wheat-flour (Bosmans and Delcour, 2016) and in GF breads (Hager et al., 2014; Carini et al., 2017).

Experimental data showing all ^1H NMR parameters are reported in Table 6. Results showed that chickpea variety significantly affected ^1H NMR parameters.

Considering the relative abundance of proton populations, popD and popF significantly increased with the substitution of 30% GF flour (w/flour w) with chickpea flour. Moreover, popD showed a significant effect of chickpea variety: the highest values of the parameter were found in RRC and RSC, followed by WC and MC showing intermediate popD values, and finally by CTR which was characterized by the lowest popD value.

For popF, CTR showed the lowest value of popF, whereas all chickpea GF breads were characterized by similar values of popF, which were not significantly different from each other. PopE showed an opposite trend compared to popD and popF, since this parameter significantly decreased with the substitution of 30% GF flour (w/w flour) with chickpea flour. Furthermore, chickpea variety showed a significant effect on popE. The highest value of the parameter was obtained in CTR, followed by MC, then by WC and RRC, which were not significantly different from both RSC and MC, and RSC had the lowest value. PopA and popC showed no significant differences among all tested bread samples.

Considering the relaxation time of proton populations, T_A and T_{2F} showed a significant decrease of their values with the substitution of 30% GF flour (w/w flour) with chickpea flour. In detail, T_A had the highest value in CTR, and the lowest value in RRC. The other chickpea breads (MC, WC, and RSC) showed no significant differences compared to CTR and RRC. T_{2F} parameter had a significant reduction in chickpea

Table 6
 ^1H NMR results of bread crumb.

Bread sample	Parameter					Relaxation time				
	Relative abundance									
	PopA (%)	PopC (%)	PopD (%)	PopE (%)	PopF (%)	T_A (μs)	T_{2C} (ms)	T_{2D} (ms)	T_{2E} (ms)	T_{2F} (ms)
CTR-bread	56.04 \pm 1.11 ^a	7.19 \pm 0.18 ^a	10.74 \pm 0.35 ^c	81.97 \pm 0.37 ^a	0.10 \pm 0.12 ^b	20.66 \pm 0.34 ^a	0.44 \pm 0.05 ^a	3.79 \pm 0.46 ^b	17.31 \pm 0.52 ^{ab}	169.25 \pm 38.43 ^a
	58.58 \pm 1.35 ^a	7.14 \pm 0.15 ^a	12.29 \pm 0.36 ^b	79.30 \pm 0.41 ^b	1.27 \pm 0.23 ^a	19.77 \pm 0.16 ^{ab}	0.50 \pm 0.06 ^a	4.75 \pm 0.35 ^a	17.54 \pm 0.71 ^a	69.13 \pm 4.62 ^b
WC-bread	57.36 \pm 1.55 ^a	7.24 \pm 0.30 ^a	12.31 \pm 0.09 ^b	78.66 \pm 0.53 ^{bc}	1.79 \pm 0.54 ^a	19.93 \pm 0.58 ^{ab}	0.53 \pm 0.05 ^a	4.73 \pm 0.14 ^a	16.45 \pm 0.25 ^b	66.87 \pm 5.35 ^b
	58.65 \pm 0.99 ^a	6.93 \pm 0.11 ^a	13.25 \pm 0.38 ^a	78.66 \pm 0.92 ^{bc}	1.16 \pm 0.44 ^a	19.26 \pm 0.24 ^b	0.48 \pm 0.02 ^a	4.40 \pm 0.15 ^{ab}	16.62 \pm 0.06 ^{ab}	72.07 \pm 12.84 ^b
RRC-bread	57.78 \pm 0.85 ^a	7.08 \pm 0.04 ^a	13.90 \pm 0.27 ^a	77.44 \pm 0.51 ^c	1.58 \pm 0.32 ^a	19.73 \pm 0.35 ^{ab}	0.52 \pm 0.01 ^a	4.72 \pm 0.16 ^a	16.41 \pm 0.13 ^b	65.18 \pm 2.82 ^b
	p-value	ns	ns	***	***	**	*	ns	**	*

Bread samples: CTR = 100% GF flour; MC = 70% GF flour, 30% modern chickpea flour; WC = 70% GF flour, 30% white chickpea flour; RRC = 70% GF flour, 30% red rough chickpea flour; RSC = 70% GF flour, 30% red smooth chickpea flour. *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. "ns" indicates no significant differences at $p < 0.05$. Means in a row with different superscripts are significantly different ($p < 0.05$).

bread samples as compared to CTR, but it was not significantly affected by chickpea variety. T_{2D} showed the opposite trend compared to T_A and T_{2F} since this parameter significantly increased with the substitution of chickpea flour. However, similarly to T_{2F} , the chickpea variety almost did not impact the parameter. In detail, the highest value of the parameter was found in CTR, while all chickpea breads were characterized by similar and significant lower values of T_{2D} . RRC was the only chickpea bread sample showing T_{2D} not significantly different from both CTR and the other chickpea bread samples. T_{2C} was not significantly affected by the substitution of GF flour with chickpea flour, showing not significant differences among all tested bread samples.

The substitution of GF flour with 30% (w/w flour) chickpea flours changed the chemical composition of chickpea breads, resulting in a theoretical increase of fiber, protein, and lipid contents, and in a decrease of available carbohydrates, and significant differences were obtained as a function of chickpea varieties. Except popA, popC and T_{2C} , all the other 1H NMR parameters were significantly affected by the substitution of GF flour with chickpea flour. A similar increase of popF and T_{2D} , and a similar decrease of T_{2F} were found in chickpea breads as compared to CTR, showing that these parameters were significantly affected by the substitution of chickpea flour independently of chickpea variety. The negligible value of popF observed in CTR can be associated to the absence of lipids in the GF bread formulation. Indeed, in formulations including lipids a higher value of popF was reported by Carini et al. (2017). The significant increase of popF caused by the substitution of GF flour with chickpea flour could be explained considering their lipid content (4.90–5.35 g/100 g dm, Table 3). Indeed, the inclusion of chickpea flours in GF breads enhanced the lipid amount which can be associated to the increase of popF (Hager et al., 2014; Carini et al., 2017). Chickpea breads also showed a significant and similar decrease of T_{2F} , showing that in chickpea breads protons of popF were more abundant and less mobile as compared to CTR. On the other hand, the significant increase of T_{2D} may be associated to the different carbohydrate composition of chickpea breads, such as the higher fiber content (Curti et al., 2013, 2015, 2016).

Compared to CTR, chickpea breads showed a significant higher value of popD, and lower values of popE and T_A , which showed significant differences as a function of chickpea variety. The above differences could be associated to the different physico-chemical characteristics of chickpea flours, which caused a different and stronger interaction between flour biopolymers and water molecules in the bread system. Furthermore, these results may be the molecular insight of the macroscopic differences observed among bread samples.

4. Conclusions

In the literature, there is scant information about the physico-chemical properties of wild chickpea flours and their performance in GF bread-making. The results of the present research revealed that the tested wild chickpea flours, especially red varieties, had interesting physico-chemical characteristics which made them valuable raw materials to improve the nutritional profile of GF breads. Wild chickpea flours showed higher ash, fiber, and TAC as compared to the modern variety, and red varieties (RRC and RSC) revealed the highest TPC among tested samples. Significant differences were found in protein contents and techno-functional properties as a function of chickpea varieties, suggesting that they had different bread-making performance. Furthermore, red chickpea varieties (RRC and RSC) showed the lowest impact on bread quality. The significant differences on proton mobilities and dynamics may be the molecular insight associated to the different bread-making performances of the chickpea samples.

Overall, the present study showed the potentiality of using wild chickpeas flours as valuable raw materials to improve GF bread nutritional composition while preserving the technological and physico-chemical quality. Moreover, the incorporation of wild chickpea flours could be interesting to improve the nutritional profile of food products

other than GF breads. To further explore both above research issues, additional studies are necessary to investigate the link among the genetic profile of wild chickpeas, the agronomical conditions, the physico-chemical characteristics of chickpea flours, as well as the effect of (bio) technological approaches on the flour techno-functional and chemical properties which might also support in obtaining specific flour technological properties able to meet different food destination uses. Exploring the above issues could aid in identifying the most promising chickpea genotypes considering the biodiversity, sustainability, technological and nutritional requirements.

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CRediT authorship contribution statement

Ottavia Parenti: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Neamtallah Assaf:** Formal analysis, Investigation, Writing – review & editing. **Marcello Alinovi:** Conceptualization, Data curation, Writing – review & editing. **Massimiliano Rinaldi:** Conceptualization, Data curation, Writing – review & editing, Supervision. **Augusta Caligianni:** Data curation, Writing – review & editing. **Emma Chiavaro:** Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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