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# The polypeptide composition, structural properties and antioxidant capacity of gluten proteins of diverse bread and durum wheat varieties, and their relationship to the rheological performance of dough

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**Summary** The aim of this study was to compare five bread and five durum wheat genotypes for gliadins and glutenins profiles, the concentration of free sulphhydryl groups and disulphide bonds, antioxidant capacity of gluten proteins and their bread-making performance. On average, bread wheat had significantly higher concentration of total sulphur-rich (S-rich) and sulphur-poor (S-poor) subunits of gliadins, as well as total low molecular weight (LMW) and high molecular weight (HMW) subunits of glutenins than durum wheat. However, durum wheat had higher concentration of S-rich γ-gliadins and S-poor D-LMW-glutenins, but did not possess S-poor ω-gliadins. The concentration of disulphide bonds and total cysteine was higher in the durum gluten than that in the bread gluten, as well as antioxidant capacity (on average 90.6 vs. 85.9 mmol Trolox Eq $kg^{-1}$, respectively). In contrast to the bread wheat, the concentration of HMW-glutenins was negatively associated with extensibility, as well as resistance to extension in durum wheat flour dough.

**Keywords** Antioxidant capacity, bread and durum wheat, disulphide bonds, free sulphhydryl groups, protein compositions, rheological properties of dough.

## Introduction

Wheat endosperm is not only a source of carbohydrates, but also of proteins, minerals, vitamins and bioactive compounds. The ability of wheat flour to be processed into different foods is largely determined by the proteins. Technologically, glutenins and gliadins are the most important wheat storage proteins. They constitute of up to 85% of the total grain proteins and confer elasticity and extensibility properties that are essential for functionality of wheat flours (Kuktaite, 2004). The functional properties of wheat proteins depend on the composition of their constituent polypeptides, their molecular characteristics and interactions with one another and other flour constituents, such as starch (Song & Zheng, 2007) and nonstarch polysaccharides (Elgadir *et al.*, 2012). Generally, gliadins are responsible for the viscous and extensible properties, while glutenins confer strength and elasticity of dough (Wieser, 2007). It is understood that wheat gluten owes its unique viscoelastic behaviour to an appropriate balance in the amounts of gliadin and glutenin proteins. However, some reports suggest that the overall function of wheat proteins derives mainly from glutenin, and that gliadin is only as diluents (Kieffer, 2006). Glutenins can be broadly classified into two groups, the high molecular weight (HMW) and the low molecular weight (LMW) subunits with the molecular weight (Mw) range of 80–160 kDa and 30–55 kDa, respectively. They link together and form heterogeneous mixtures of polymers by disulphide bonded linkages of polypeptides (Gianibelli *et al.*, 2001). Differences in the glutenin subunits size, polarity and the number of cysteine residues influence the ability to form disulphide bonds necessary for building up the glutenin polymer structure. This variation in glutenin subunits is a critical factor in determining bread dough end-product quality, particularly through its influence on the polymer size distribution (Kasarda, 1999).

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Unlike glutenins, gliadins are a polymorphic mixture of proteins with the Mw range of 30–80 kDa that can be separated into sulphur-rich (S-rich) and sulphur-poor (S-poor) gliadins consisting of α-, β-, γ- and ω-subunits (Žilić *et al*., 2011).

The differences between bread wheat and durum wheat can be largely attributed to their gluten protein properties. Bread wheat gluten proteins upon hydration and mixing, form a strong, cohesive, viscoelastic network that allows wheat flour dough to retain yeast fermentation gases and to produce a light, aerated-baked product (Veraverbeke & Delcour, 2002). Also, the macromolecular network of hydrated gluten and starch is able to form a continuous network of particles together. These two independent networks and their interaction give rise to the rheological properties of doughs (Song & Zheng, 2007). On the other hand, durum wheat normally has weaker and less extensible gluten characteristics than bread wheat. These characteristics are associated not only with pasta cooking quality, particularly with respect to its firmness and increased tolerance to overcooking, but also with inferior bread-making performance as measured in terms of loaf volumes and crumb characteristics (Hareland & Puhr, 1999).

The aim of this study was to compare bread and durum wheat genotypes for: (i) polypeptide composition of gliadins and glutenins; (ii) the concentration of free sulphhydryl groups (-SH), disulphide bonds (-S-S) and total cysteine; and (iii) antioxidant capacity of gluten proteins. Rather similar starch content within the samples of investigated bread and durum wheat flours made it possible to study the effects of bread and durum wheat gliadins and glutenins, as well as their subunits and structural parameters on resistance to extension, extensibility, water absorption and a degree of softening of dough.

## Materials and methods

### Plant material and field trail

Five bread wheat (*Triticum aestivum* L.) and five durum wheat (*Triticum durum* Desf.) genotypes of different origin, pedigrees and a growth type (Table S1) were used. Considerable variation existed among bread (CV = 6.9%) and durum wheat (11.0%) genotypes for the total protein content. However, coefficients of variation for starch content were low for both species (on average CV = 1.1%) (Table S1). Grain samples of bread and durum wheat collected from plants grown in a field-microtrial at the Maize Research Institute Zemun Polje, north Serbia (44°52′N and 20°19′E, 82 m ASL) in 2012 growing season. Standard agronomic practices were used to provide adequate nutrition and protection against pests and diseases. The site is of moderate continental climate, with cold winters and hot and dry summers. Climate variables during the growth cycle of wheat (November–June) were typical for the site (with the average daily temperatures of 9.6 °C and total precipitations of 340 mm). After harvesting, the damaged grains and foreign materials were removed from the samples and purified samples were used for milling.

### Flour samples preparation and gluten isolation

Bread and durum wheat flours (<180 μm) were produced in a milling company (Žitoprodukt d.o.o. Belgrade-Batajnica, Serbia) on the experimental mill (Laboratory mill; Bühler MLU-202, Uzwil, Switzerland) with six grinding passages, three fluted roll break and three smooth roll reduction passages. Before grinding, durum wheat grains were tempered to 16.5% moisture. To determine the concentration of free sulphhydryl groups and disulphide bonds, as well as antioxidant capacity of gliadin and glutenin, wheat gluten proteins were isolated. Gluten was prepared by extensive washing of dough under 2% NaCl solution and afterwards with tap water (AACC, 2000). Isolated gluten was air-dried in a fan-oven at room temperature (max 25 °C) for approximately 10 h. Wet gluten content expressed as % of dry matter (d.m.)

### Extraction of protein fractions and total protein content

Different protein fractions were obtained by successive extractions of defatted wheat flour with a series of solvents (in a ratio 1:4 w/v) according to the Osborne procedure described by Žilić *et al*. (2011). Wheat flour was defatted for 6 h using diethyl ether-based Soxhlet cold extraction procedure. Gliadins were extracted with 70% (v/v) aqueous ethanol and soluble glutenins with 50% (v/v) 1-propanol containing 1% dithiothreitol (DTT). Extraction of gliadins and glutenins was triple repeated by stirring for 30 min at 4 °C, followed by centrifugation 10 min at 15 500 ***g***. Supernatants of each protein fractions were collected and transferred to the volumetric flask, and the corresponding extraction solutions were added up to 2 mL. The extracts were used for SDS-PAGE. Total protein content in wheat flours was determined by the Kjeldal method.

### SDS-PAGE

The extractable protein composition of each protein fraction was detected by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) performed according to Fling & Gregerson (1986), on 12.5% separating gels and 5% stacking gels in vertical electrophoretic unit (LKB, Sweden). Prior to the electrophoresis, extractable proteins have been diluted in

the 1:2 ratio (v/v) with the sample buffer (0.055 M Tris–HCl, pH 6.8, 2% (w/v) sodium dodecyl sulphate (SDS), 20% (v/v) glycerol, 4.3% (v/v) β-mercaptoethanol, 0.0025% (w/v) bromophenol blue), heated at 90 °C for 5 min and cooled at the room temperature. Molecular weights of the polypeptides were estimated by using low molecular weight standards (Amersham Biosciences, Uppsala, Sweden). For visual presenting, the protein profiles of the bread and durum wheat genotypes are shown in Fig. S1a, b and S2a, b. The protein bands on the destained gel were quantified using the SigmaGel software version 1.1 (Jandal, San Rafael, CA, USA) and expressed as percentages of total extractable proteins. The concentrations of total gliadin and glutenin protein fractions, as well as their subunits were calculated as the sum of the concentration of polypeptides that compose them.

### Solid-phase assay for the free and total sulphhydryl (SH) content

Colorimetric reactions were conducted under the conditions described by Žilić *et al.* (2012). For the determination of the free -SH content, the reaction buffer consisting of 8 M urea, 10 m M 5,5′-dithiobis (2-nitrobenzoic acid) (Ellman's reagent-DTNB), 3 m M ethylene-diaminetetraacetic acid (EDTA), 1% sodium dodecyl sulphate (SDS) and 0.2 M Tris–HCl pH 8.0 (Buffer A) and diluting buffer with 0.93 mL of 8 M urea, 1% SDS, 3 m M EDTA and 0.2 M Tris–HCl, pH 8.0 (Buffer B) were used.

To determine the total -SH content (total cysteine), the reaction buffer consisting of 8 M urea, 0.1 M sodium sulphite, 3 m M EDTA, 1% SDS, 0.2 M Tris–HCl, pH 9.5 and 10 m M disodium 2-nitro-5-thiosulphobenzoate ($NTSB^{2-}$) (Buffer A), synthesised from DTNB in the presence of sodium sulphite and $O_2$ and diluting buffer with 2.98 mL of 8 M urea, 1% SDS, 0.1 M sodium sulphite, 3 m M EDTA and 0.2 M Tris–HCl pH 8.0 (Buffer B) were used.

Free -SH and total -SH contents were calculated from the absorption readings using a molar absorption coefficient of 13 600 $M^{-1}$ $cm^{-1}$ at 412 nm. The disulphide content was calculated as half the difference between total -SH and free -SH contents. The concentrations were expressed as nmol per mg of dry matter (d.m.).

### Analysis of total antioxidant capacity

Measuring of the total antioxidant capacity of wheat flour and gluten samples was taken based on QUENCHER method described by Žilić *et al.* (2012) using 7 m M aqueous solution of ABTS (2,2-azino-bis/3-ethil-benothiazoline-6-sulphonic acid) with 2.45 mM $K_2O_8S_2$ as the stock solution. The samples (10 mg) were mixed with 20 ml of $ABTS^{\bullet+}$ working solution, that obtained by diluting the stock solution in water/ethanol (50:50, v/v), and the mixture was shaken for 25 min. After centrifugation, the absorbance measurement was taken at 734 nm. The total antioxidant capacity was expressed as the Trolox equivalent antioxidant capacity (TEAC) in mmol of Trolox per kg of d.m.

### Farinograph and extensograph procedure

The Brabender farinograph and extensograph (Brabender, Duisburg, Germany) were used for measuring the rheological properties of doughs, in particular the water absorption and the degree of softening, as well as resistance to extension and the extensibility, respectively, based on the official procedures (AACC International, 2000). The result of dough softening degree and resistance to extension is given in BU, while water absorption and extensibility of dough expressed in % of the flour and mm, respectively.

### Statistical analysis

All analyses were performed in duplicate per genotype, and the results were statistically analysed using Statistica software version 5.0 (StatSoft Co., Tulsa, OK, USA). The analytical data are reported as mean ± standard deviation. Significance of differences between genotypes and species means was analysed by Tukey (HSD) test and *t*-test, respectively. Differences at $P < 0.05$ were considered significant. Principal component analysis (PCA) was used for studying relationships among rheological properties and protein profiles for each species.

## Results and discussion

### Polypeptide composition of gliadin and glutenin protein fraction

The content of total proteins was significantly higher in durum than bread wheat flours and varied from 10.63 to 13.88% and from 8.91 to 10.45%, respectively (Table S1). The ranges indicate the genotypic variations for protein properties even within such small set of genotypes. However, the variations in the total protein content do not adequately account for established differences in varietal end-use quality characteristics. According to the research of Gafurova *et al.* (2002), the qualitative ratio of wheat protein fractions provided important information to determine the food techno-functionality. In addition, according to the results of Peña *et al.* (1995) gliadin and glutenin subunits composition variation is partly responsible for differences in the bread-making quality among wheat

**Table 1** The gliadin polypeptides composition identified in bread and durum wheat flours by SDS-PAGE

| Subunits | Groups | Polypeptides MW (kDa) | Bread flour | | | | | CV (%) | Durum flour | | | | | CV (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | BW1 | BW2 | BW3 | BW4 | BW5 | | DW1 | DW2 | DW3 | DW4 | DW5 | |
| | | 102.1 | 2.0 | 3.1 | 2.1 | 2.2 | 2.3 | | n.d. | n.d. | n.d. | n.d. | n.d. | |
| | | 96.1 | 1.9 | 3.3 | 2.9 | 1.6 | 4.0 | | 2.1 | 1.4 | 1.1 | 0.5 | 0.7 | |
| | | 87.6 | 3.0 | 2.3 | 3.2 | 1.8 | 3.3 | | n.d. | n.d. | n.d. | n.d. | n.d. | |
| S-poor | ω – Gli | 76.2 | n.d. | n.d. | n.d. | 3.0 | 2.4 | | n.d. | n.d. | n.d. | n.d. | n.d. | |
| | | 65.7 | 3.1 | 2.7 | 3.8 | 1.8 | 1.6 | | n.d. | n.d. | n.d. | n.d. | n.d. | |
| | Sum ω – Gli | | $3.1^{c}$ | $2.7^{c}$ | $3.8^{b}$ | $4.8^{a}$ | $4.0^{b}$ | 21.5 | – | – | – | – | – | 0 |
| S-rich | γ – Gli | 50.8 | 3.5 | 6.1 | 4.2 | 4.9 | 4.2 | | 9.6 | 10.6 | 8.5 | 11.4 | 8.9 | |
| | | 47.1–44.8 | 15.0 | 15.5 | 16.2 | 6.1 | 6.7 | | 7.4 | 7.8 | 6.0 | 7.3 | 7.2 | |
| | | 44.1 | n.d. | n.d. | n.d. | 5.3 | 5.4 | | 7.4 | 7.1 | 7.0 | 7.1 | 7.3 | |
| | Sum γ – Gli | | $18.5^{cd}$ | $21.6^{b}$ | $20.4^{bc}$ | $16.3^{d}$ | $16.3^{d}$ | 17.7 | $24.4^{a}$ | $25.5^{a}$ | $21.4^{b}$ | $25.8^{a}$ | $23.4^{ab}$ | 7.4 |
| | α/β-Gli | 41.7–34.5 | 53.6 | 44.5 | 46.3 | 48.8 | 45.2 | | 40.5 | 37.3 | 35.0 | 41.0 | 37.1 | |
| | | 32.0 | 5.6 | 3.1 | 3.9 | 2.8 | 4.0 | | 3.7 | 4.7 | 4.3 | 0.0 | 4.3 | |
| | Sum α/β – Gli | | $59.2^{a}$ | $47.6^{bc}$ | $50.2^{b}$ | $51.6^{b}$ | $49.2^{bc}$ | 8.7 | $44.2^{cd}$ | $42.0^{d}$ | $39.3^{d}$ | $41.0^{d}$ | $41.4^{d}$ | 4.2 |
| | | 30.1 | n.d. | n.d. | n.d. | n.d. | n.d. | | 4.96 | 5.0 | 5.3 | 4.2 | 5.1 | |
| | | 28.9 | n.d. | n.d. | n.d. | n.d. | n.d. | | 5.1 | 4.4 | 5.2 | 3.3 | 4.5 | |
| | | 28.3 | 5.7 | 3.3 | 4.4 | 4.5 | 2.5 | | n.d. | n.d. | n.d. | n.d. | n.d. | |
| | | 27.6 | n.d. | n.d. | n.d. | n.d. | n.d. | | 3.8 | 5.4 | 7.2 | 6.2 | 6.6 | |
| | | 24.1 | n.d. | n.d. | n.d. | n.d. | n.d. | | 1.5 | 2.3 | 2.5 | 0.0 | 2.4 | |
| | | 23.1 | 5.9 | 3.7 | 3.0 | 2.8 | 3.5 | | n.d. | n.d. | n.d. | n.d. | n.d. | |
| | | 21.4 | n.d. | n.d. | n.d. | n.d. | n.d. | | 4.4 | 4.1 | 4.8 | 6.5 | 5.3 | |
| | | 17.9–7.1 | 0.7 | 12.5 | 9.9 | 14.5 | 14.7 | | 8.8 | 10.0 | 13.1 | 12.5 | 10.6 | |
| Total per genotype | | | | | | | | | | | | | | |
| S-rich subunit (γ + α/β- Gli) | | | $77.7^{a}$ | $69.2^{c}$ | $70.6^{bc}$ | $67.9^{cd}$ | $65.5^{cd}$ | | $68.6^{c}$ | $67.5^{cd}$ | $60.7^{d}$ | $66.8^{cd}$ | $64.8^{cd}$ | |
| S-poor + S-rich | | | $80.8^{a}$ | $71.9^{b}$ | $74.4^{a}$ | $72.7^{a}$ | $69.5^{b}$ | | $68.6^{bc}$ | $67.5^{bc}$ | $60.7^{c}$ | $66.8^{bc}$ | $64.8^{bc}$ | |
| S-poor/S-rich ratio | | | 0.04 | 0.04 | 0.05 | 0.07 | 0.06 | | – | – | – | – | – | |
| Mean per species | | | | | | | | | | | | | | |
| S-poor subunits (ω – Gli) | | | $3.7^{A}$ | | | | | | – | | | | | |
| Total S-rich subunits | | | $70.2^{A}$ | | | | | | $65.7^{A}$ | | | | | |
| S-rich γ-Gli | | | $18.6^{A}$ | | | | | | $24.1^{B}$ | | | | | |
| S rich α/β-Gli | | | $51.5^{A}$ | | | | | | $41.6^{B}$ | | | | | |
| S-poor + S-rich | | | $73.9^{A}$ | | | | | | $65.7^{B}$ | | | | | |
| S-poor/S-rich ratio | | | $0.05^{A}$ | | | | | | – | | | | | |

n.d., not detected; MW-molecular weight. Means followed by the same letter (lower case) within the same row are not significantly different ($P > 0.05$). Letters correspond to ranking of groups after Tukey test. Means followed by the same letter (upper case) within the same row are not significantly different ($P > 0.05$). Letters correspond to ranking of groups after *t*-test.

cultivars. In our study, the polypeptide composition of the individual soluble fractions (gliadin and glutenin) was analysed by SDS-PAGE (Fig. S1a, b and S2a, b) and quantified by densitrometric analyse (Tables 1 and 2). The bread and durum flour polypeptides with the Mw ranging between 32 and 41.7 kDa and between 44.1 and 50.8 kDa belonged to sulphur-rich α/β- and γ-subunits of gliadins, respectively (Fig. S1a and b, Table 1). The S-rich subunit was the most abundant gliadin subunit in both species. Its concentration in bread wheat genotypes ranged from 65.5% (BW5-Zemunska rosa) to 77.7% (BW1-ZP 87/I) of total extractable proteins, while among the tested durum wheat genotypes, the lowest and the highest S-rich subunits concentration of 60.7 and 68.6% of total extractable proteins was detected in DW3 (37EDUYT no. 7817) and DW1 (ZP 120/I), respectively. The average value of durum wheat genotypes for the S-rich subunits concentration was for about 6% lower than that of bread wheat. Within this subunit, the α/β-subunit was more abundant than γ-subunit in both species. However, the bread wheat genotypes had a much stronger band in α/β-region with molecular weight of about 32–41.7 kDa. Given that α/β-gliadin subunits play the largest positive role in increasing loaf volume (Khatkar *et al.*, 2002), these subunits could be the target for breeding programs improving durum wheat for bread-making quality. Among bread wheat genotypes, considering the concentration of its α/β-gliadin subunits, advanced line BW1 (ZP 87/I) can be used such variety improver in the breeding program. On the other hand, strong polypeptide with molecular weight of 44.1 kDa in γ-region that appeared in all durum wheat genotypes was absent in the most of bread

**Table 2** The glutenin polypeptide composition identified in bread and durum wheat flours by SDS-PAGE

| Subunits | Groups | Polypeptides MW (kDa) | Bread flour | | | | | | Durum flour | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | BW1 | BW2 | BW3 | BW4 | BW5 | CV (%) | DW1 | DW2 | DW3 | DW4 | DW5 | CV (%) |
| HMW | A – Glu | 110.0 | n.d. | n.d. | n.d. | n.d. | n.d. | | 2.1 | 1.3 | 1.7 | 2.2 | 1.4 | |
| | | 108.7 | 1.1 | 4.1 | 3.9 | 0.4 | 0.5 | | n.d. | n.d. | n.d. | n.d. | n.d. | |
| | | 102.8 | 4.0 | 6.0 | 4.4 | 1.5 | 4.3 | | 3.7 | 1.4 | 1.1 | 1.4 | 3.1 | |
| | | 96.2 | 2.6 | 7.6 | 3.7 | 0.4 | 2.7 | | 5.2 | 4.3 | 2.7 | 3.6 | 1.7 | |
| | | 87.8 | 4.4 | 8.0 | 5.9 | 2.0 | 5.5 | | 4.4 | 2.0 | 1.8 | 3.2 | 2.6 | |
| | | 80.6 | n.d. | n.d. | n.d. | n.d. | n.d. | | 2.4 | 2.6 | 2.4 | 2.6 | 2.1 | |
| | Sum A – Glu | | $12.1^{c}$ | $25.6^{a}$ | $17.9^{b}$ | $4.4^{e}$ | $12.9^{c}$ | 53.6 | $17.8^{b}$ | $11.7^{c}$ | $9.7^{d}$ | $13.0^{c}$ | $11.0^{c}$ | 24.9 |
| LMW | D – Glu | 74.8 | n.d. | n.d. | n.d. | n.d. | n.d. | | 12.8 | 5.6 | n.d. | 13.0 | 12.6 | |
| | | 71.1–65.0 | 7.8 | 24.0 | 14.0 | 16.2 | 7.2 | | n.d. | n.d. | n.d. | n.d. | n.d. | |
| | | 60.2 | n.d. | n.d. | n.d. | n.d. | n.d. | | 4.3 | 2.1 | 13.2 | n.d. | n.d. | |
| | | 52.6 | n.d. | n.d. | n.d. | n.d. | n.d. | | 22.1 | 10.5 | 8.8 | 7.7 | 9.4 | |
| | Sum D – Glu | | $7.8^{g}$ | $24.0^{b}$ | $14.0^{f}$ | $16.2^{ef}$ | $7.2^{g}$ | 50.0 | $39.2^{a}$ | $18.2^{de}$ | $21.9^{bc}$ | $20.7^{cd}$ | $22.0^{b}$ | 34.4 |
| | B -Glu | 50.3–43.0 | 28.4 | 19.9 | 44.9 | 42.2 | 38.1 | | n.d. | n.d. | n.d. | n.d. | n.d. | |
| | | 47.2–43.5 | n.d. | n.d. | n.d. | n.d. | n.d. | | 24.9 | 13.2 | 13.0 | 12.5 | 12.7 | |
| | Sum B – Glu | | $28.4^{c}$ | $19.9^{e}$ | $44.9^{a}$ | $42.2^{a}$ | $38.1^{b}$ | 30.0 | $24.9^{d}$ | $13.2^{f}$ | $13.0^{f}$ | $12.5^{f}$ | $12.7^{f}$ | 35.5 |
| | C – Glu | 41.5 | n.d. | n.d. | n.d. | n.d. | n.d. | | 3.9 | 5.1 | 4.9 | 4.5 | 4.8 | |
| | | 40.9–35.0 | 48.1 | 30.5 | 23.2 | 37.2 | 41.8 | | 8.0 | 30.1 | 30.4 | 34.1 | 31.3 | |
| | | 30.7 | n.d. | n.d. | n.d. | n.d. | n.d. | | n.d. | 4.1 | 4.8 | 4.1 | 4.3 | |
| | SumC – Glu | | $48.1^{a}$ | $30.5^{d}$ | $23.2^{e}$ | $37.2^{c}$ | $41.8^{b}$ | 26.8 | $11.9^{f}$ | $39.3^{bc}$ | $40.1^{bc}$ | $42.6^{b}$ | $40.4^{bc}$ | 37.0 |
| | | 29.5 | n.d. | n.d. | n.d. | n.d. | n.d. | | n.d. | 4.1 | 4.2 | 1.8 | 3.8 | |
| | | 28.0 | n.d. | n.d. | n.d. | n.d. | n.d. | | 2.6 | 5.7 | 5.9 | 5.9 | 4.3 | |
| | | 21.9 | n.d. | n.d. | n.d. | n.d. | n.d. | | 1.9 | 1.1 | 2.4 | 1.4 | 3.3 | |
| | | 17.8 | n.d. | n.d. | n.d. | n.d. | n.d. | | 1.6 | 1.9 | 2.2 | 2.4 | 2.6 | |
| | | 16.2 | 3.8 | n.d. | n.d. | n.d. | n.d. | | n.d. | n.d. | n.d. | n.d. | n.d. | |
| Total per genotype | | | | | | | | | | | | | | |
| LMW (D + B + C-Glu) | | | $84.3^{b}$ | $74.4^{cd}$ | $82.1^{bc}$ | $95.6^{a}$ | $87.1^{b}$ | | $76.0^{c}$ | $70.7^{d}$ | $75.0^{cd}$ | $75.8^{cd}$ | $75.1^{cd}$ | |
| S-rich LMW (B+C-Glu) | | | $76.5^{a}$ | $50.4^{c}$ | $68.1^{b}$ | $79.4^{a}$ | $79.9^{a}$ | | $36.8^{d}$ | $52.4^{c}$ | $53.1^{c}$ | $55.1^{c}$ | $53.1^{c}$ | |
| HMW + LMW | | | $96.4^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | | $93.8^{ab}$ | $82.3^{b}$ | $84.6^{b}$ | $88.8^{ab}$ | $86.1^{b}$ | |
| Mean per species | | | | | | | | | | | | | | |
| HMW (A-Glu) | | | $14.6^{A}$ | | | | | | $12.7^{A}$ | | | | | |
| LMW (D + B + C – Glu) | | | $84.7^{A}$ | | | | | | $74.5^{B}$ | | | | | |
| S-poor LMW (D -Glu) | | | $13.8^{A}$ | | | | | | $24.4^{A}$ | | | | | |
| Total S-rich LMW (B+C-Glu) | | | $70.8^{A}$ | | | | | | $50.1^{B}$ | | | | | |
| S-rich LMW (B-Glu) | | | $21.1^{A}$ | | | | | | $15.2^{B}$ | | | | | |
| S-rich LMW (C -Glu) | | | $36.1^{A}$ | | | | | | $34.9^{A}$ | | | | | |
| HMW + LMW | | | $99.2^{A}$ | | | | | | $87.2^{B}$ | | | | | |
| S-poor/S-rich LMW | | | $0.2^{A}$ | | | | | | $0.48^{A}$ | | | | | |
| HMW/LMW | | | $0.2^{A}$ | | | | | | $0.17^{A}$ | | | | | |

n.d., not detected; MW, molecular weight. Means followed by the same letter (lower case) within the same row are not significantly different ($P > 0.05$). Letters correspond to ranking of groups after Tukey test. Means followed by the same letter (upper case) within the same row are not significantly different ($P > 0.05$). Letters correspond to ranking of groups after *t*-test.

wheat genotypes. The absence of some polypeptide chains which belong to the γ-gliadin region of common and spelt wheat originating from USDA, Canadian and Swiss collection was confirmed by the results of Abdel-Aal *et al.* (1996). In addition, Žilić *et al.* (2011) found that polypeptide chain of 55.7 kDa was not appeared in the γ-gliadin region of the most Serbian bread wheat genotypes. According to the results of Sapirstein *et al.* (2007), γ-gliadins 45 and 42 are useful markers for good and poor pasta quality of durum wheat, respectively. However, it should be noted that our results indicate low heterogeneity of durum genotypes when referring on this feature (CV = 7.4%). The bread wheat flour samples were characterised by the presence of weak intensity ω-gliadin bands. This S-poor subunit of gliadins consisted of one to two polypeptides with the Mw of 65.7–76.2 kDa depending of a bread wheat genotype. According to our study, in durum wheat flour samples, the polypeptides were not detected in region of this molecular weight. However,

Nizar (2002) separated the polypeptides of this subunit from durum wheat using system of polyacrylamide gel electrophoresis under acidic conditions (A-PAGE).

Due to their insoluble nature and extreme size, glutenin polymers are difficult to quantify. For glutenin dissolving, 1-propanol containing 1% dithiothreitol was used in this study. It has enabled the creation of artificial glutenin polymers, in which the subunits composition is identified. Significant differences between two wheat species for low molecular weight glutenin subunit (LMW-GS) (30.7–74.8 kDa) concentrations were determined (Fig. S2a and b, Table 2). The average value of bread wheat flours for the total glutenin subunits (LMW + HMW) concentration was 99.2% of total extractable proteins, which was for about 13% higher than that of durum wheat flours (Table 2). In both species, LMW-GS was the most abundant glutenin subunit. The average value of the total concentration of LMW-GS (B+C+D-groups) was for about 5.8-fold higher than the concentration of HMW-GS, that is a group of glutenin proteins, in both species. According to Gianibelli *et al.* (2001), the LMW-GS are present in gluten at about three times the amount of the HMW-GS, but their size distribution means that they are difficult to study, being mixed with many other polypeptides in the SDS-PAGE pattern of flour. Although S-poor gliadin polypeptides were not detected in durum wheat flours, out result indicate that, on average, durum wheat flour contain by 43% higher concentration of S-poor LMW glutenin polypeptides (D group of proteins) than bread wheat flour. However, due to the very high variation for D-LMW glutenins among bread wheat genotypes (about 50%), this difference was not statistically significant. Masci *et al.* (1999) showed that the D glutenin group is actually composed of modified ω-gliadin components, which have acquired a cysteine residue. The highest concentration of D-LMW glutenins, as well as the lowest concentration of S-rich LMW glutenin polypeptides (B + C groups of proteins) was detected in flour of DW1 (ZP 120/I) durum wheat genotype. Overall, durum wheat flour contained about 70% as much the S-rich LMW glutenins as the bread wheat flour. However, considerable higher variation for B and C groups of proteins was found in durum (35.5 and 37.0%) than in bread wheat flour (30.0 and 26.8%) (Table 2). As shown by research of Payne *et al.* (1987), the variation of specific HMW glutenin subunit has been strongly correlated with differences in bread-making quality between genotypes of European wheat. Their results indicated that variation in HMW glutenin subunits account for between about 47 and 60% of the variation in bread-making performance within 84 British-grown wheats. Based on our research, considerable variation for the HMW glutenin subunit (80.6–110 kDa) concentration was found among bread wheat samples (53.6%). The Serbian bread wheat genotype BW2 (ZP 7/I) showed the greatest potential as sources of HMW glutenins (25.6% of total extractable proteins). In contrast, very low concentration of HMW polypeptides (4.4% of total extractable proteins) was detected for the Mexican origin genotype BW4 (15HRWYT no. 226) (Table 2). Coefficient of variation for HMW-GS among durum wheat flours was two times lower than among bread wheat samples.

### Concentration of total cysteine, disulphide bonds and free sulphhydryl groups

The presence of cysteine and disulphide bonds has primarily a structural role in proteins and mainly determines the technological properties of wheat flour. Formation of the gluten network during mixing is enabled through inter- and intramolecular disulphide bonds (-S-S) crosslinking within monomeric gliadin fractions and within and between glutenin polymers, formed as a consequence of sulphhydryl (SH) oxidation and -SH-SS interchange (Johansson *et al.*, 2013). In this study, the concentration of total cysteine, -S-S and free sulphhydryl groups (-SH) is presented in Table 3. As expected, on average, gluten consisted about 4.3-fold higher concentration of total cysteine and -S-S bonds than flour of both species. Thus, in bread wheat gluten samples, the concentration of total cysteine ranged from 42.2 nmol $mg^{-1}$ (BW4-15HRWYT no. 226) to 61.9 nmol $mg^{-1}$ (BW1-ZP 87/I), while in durum wheat gluten samples, it ranged from 50.8 nmol $mg^{-1}$ (DW2-37EDUYT no. 7879) to 59.6 nmol $mg^{-1}$ (DW1-ZP 120/I). However, our results for -S-S bond and total cysteine of durum gluten are lower than those previously reported by Žilić *et al.* (2012) for one durum gluten sample from MRIZP breeding line ZP DSP/01 whose values amounted to 45.4 and 93.9 nmol $mg^{-1}$, respectively. By application of liquid chromatography–mass spectrometry technique, Lutz *et al.* (2012) identified 14 cysteine peptides in wheat gluten. Our result indicates that there are statistically significant differences for mean concentration of total cysteine and -S-S bond between bread and durum wheat gluten. Their higher concentration in durum wheat gluten can be a consequence of a large number of low molecular weight polypeptides (16–30 kDa) extracted in alcohol (Tables 1 and 2) that may contain a cysteine residues. The concentration of free -SH groups are available in a very low concentration in bread and durum flours, as well as gluten samples. According to Rakita *et al.* (2014), on average the concentration of free -SH groups of gluten samples amounted 2.10 μmol $g^{-1}$ protein. In addition, Antes & Wieser (2000) reported the variation of free -SH groups of bread wheat flour in the range of 1.0–1.5 μmol $g^{-1}$, where approximately 30% of them could be assigned to the glutenins. A

**Table 3** The concentration of cysteine, free sulphhydryl group and disulphide bond, as well as antioxidant capacity of bread and durum wheat flours and gluten

| Genotype | Cysteine (nmol/mg d.m.) | Free -SH group (nmol/mg d.m.) | -S-S bond (nmol/mg d.m.) | Antioxidant capacity (mmol TroloxEq/kg d.m) |
|---|---|---|---|---|
| | Flour | | | |
| Bread wheat | | | | |
| BW1 | $13.5 \pm 1.79^{ab}$ | $0.7 \pm 0.05^{ab}$ | $6.4 \pm 0.91^{ab}$ | $23.0 \pm 0.43^{e}$ |
| BW2 | $10.5 \pm 0.66^{ab}$ | $0.5 \pm 0.03^{abc}$ | $5.0 \pm 0.36^{ab}$ | $23.7 \pm 2.17^{de}$ |
| BW3 | $10.0 \pm 0.77^{b}$ | $0.5 \pm 0.04^{bc}$ | $4.8 \pm 0.41^{b}$ | $26.3 \pm 1.40^{bcde}$ |
| BW4 | $14.0 \pm 1.17^{a}$ | $0.4 \pm 0.03^{c}$ | $6.8 \pm 0.60^{a}$ | $25.9 \pm 1.59^{cde}$ |
| BW5 | $13.2 \pm 0.27^{ab}$ | $0.5 \pm 0.05^{abc}$ | $6.3 \pm 0.16^{ab}$ | $25.7 \pm 1.75^{cde}$ |
| CV (%) | 15.0 | 17.5 | 15.6 | 5.9 |
| Durum wheat | | | | |
| DW1 | $10.6 \pm 0.32^{ab}$ | $0.7 \pm 0.06^{ab}$ | $5.0 \pm 0.13^{ab}$ | $28.2 \pm 0.54^{abcd}$ |
| DW2 | $13.9 \pm 0.82^{a}$ | $0.6 \pm 0.03^{ab}$ | $6.7 \pm 0.43^{ab}$ | $31.9 \pm 0.13^{a}$ |
| DW3 | $12.9 \pm 1.23^{ab}$ | $0.7 \pm 0.05^{a}$ | $6.2 \pm 0.65^{ab}$ | $30.2 \pm 0.11^{abc}$ |
| DW4 | $13.4 \pm 0.31^{ab}$ | $0.6 \pm 0.03^{ab}$ | $6.4 \pm 0.17^{ab}$ | $30.8 \pm 1.16^{ab}$ |
| DW5 | $14.1 \pm 0.82^{a}$ | $0.7 \pm 0.01^{ab}$ | $6.7 \pm 0.41^{a}$ | $28.5 \pm 0.32^{abc}$ |
| CV (%) | 10.9 | 5.4 | 11.5 | 5.3 |
| | Gluten | | | |
| Bread wheat | | | | |
| BW1 | $61.9 \pm 2.42^{A}$ | $0.9 \pm 0.09^{B}$ | $30.5 \pm 1.26^{A}$ | $83.4 \pm 2.11^{CD}$ |
| BW2 | $57.8 \pm 4.41^{A}$ | $1.0 \pm 0.01^{AB}$ | $28.4 \pm 2.21^{A}$ | $84.3 \pm 3.62^{CD}$ |
| BW3 | $44.2 \pm 2.23^{B}$ | $1.0 \pm 0.02^{AB}$ | $21.6 \pm 1.13^{B}$ | $85.9 \pm 2.29^{BCD}$ |
| BW4 | $42.2 \pm 4.48^{B}$ | $1.0 \pm 0.05^{AB}$ | $20.6 \pm 2.26^{B}$ | $93.9 \pm 1.43^{AB}$ |
| BW5 | $58.2 \pm 2.21^{A}$ | $1.1 \pm 0.07^{A}$ | $28.6 \pm 1.14^{A}$ | $82.0 \pm 0.01^{D}$ |
| CV (%) | 17.0 | 8.0 | 17.3 | 5.5 |
| Durum wheat | | | | |
| DW1 | $59.6 \pm 2.74^{A}$ | $0.9 \pm 0.01^{B}$ | $29.3 \pm 1.37^{A}$ | $91.8 \pm 1.17^{ABC}$ |
| DW2 | $50.8 \pm 2.19^{AB}$ | $0.9 \pm 0.01^{B}$ | $24.9 \pm 1.09^{AB}$ | $95.3 \pm 4.78^{A}$ |
| DW3 | $58.8 \pm 2.48^{A}$ | $1.0 \pm 0.01^{AB}$ | $28.9 \pm 1.24^{A}$ | $92.3 \pm 1.44^{ABC}$ |
| DW4 | $53.9 \pm 3.87^{AB}$ | $0.9 \pm 0.06^{AB}$ | $26.5 \pm 1.96^{AB}$ | $81.8 \pm 1.69^{D}$ |
| DW5 | $58.4 \pm 3.37^{A}$ | $0.9 \pm 0.06^{B}$ | $28.7 \pm 1.72^{A}$ | $91.8 \pm 0.20^{ABC}$ |
| CV (%) | 6.7 | 5.0 | 6.8 | 5.7 |
| | Mean per species | | | |
| Bread wheat flour | $12.2^{1}$ | $0.5^{1}$ | $5.9^{1}$ | $24.9^{1}$ |
| Durum wheat flour | $13.0^{1}$ | $0.7^{2}$ | $6.2^{1}$ | $29.9^{2}$ |
| Bread wheat gluten | $52.9^{I}$ | $1.0^{I}$ | $25.9^{I}$ | $85.9^{I}$ |
| Durum wheat gluten | $56.3^{II}$ | $0.9^{II}$ | $27.7^{II}$ | $90.6^{II}$ |

Means followed by the same letter (lower case/upper case) within the same column are not significantly different ($P > 0.05$). Letters correspond to ranking of groups after Tukey test. Means followed by the same numeral (arabic/roman) within the same column are not significantly different ($P > 0.05$). Numerals correspond to ranking of groups after *t*-test.

lower concentration of free -SH groups in investigated bread and durum flours (ranged from 0.4 to 0.7 nmol $mg^{-1}$ and from 0.6 to 0.7 nmol $mg^{-1}$, respectively) than those aforementioned might be explained either by the differences originating from varieties and/or by different employed growing conditions of varieties.

## Antioxidant capacity of wheat gluten

The grain fractions have different antioxidant capacities depending on the content and distribution of phenolic compounds and carotenoids. Therefore, the aleurone layer is the fraction with the highest antioxidant activity, followed by the bran fraction, whole grain and flour. However, in our study the direct measurement procedure was used to show that isolated bread and durum wheat gluten quenches free radicals generated by 2,2-azino-bis/3-ethil-benothiazoline-6-sulphonic acid (ABTS). Wheat gluten, as a highly complex protein, had about 3-fold higher total antioxidant capacity than bread and durum wheat flour samples (Table 3). This fact suggests that wheat gluten, among other, has the potential for use as an excellent dietary additive for oxidative stability of food, as well as health promotion. The values of the antioxidant capacity for gluten of both species were overlapped and ranged from 82.0 to 93.9 mmol

**Table 4** The values of the rheological characteristics of bread and durum wheat flour dough

| Genotype | Resistance to extension (BU) | Extensibility (mm) | Degree of softening (BU) | Water absorption (%) |
|---|---|---|---|---|
| Bread wheat | | | | |
| BW1 | 235.0 ± 7.07$^{a}$ | 91.0 ± 1.41$^{a}$ | 115.0 ± 0.00$^{e}$ | 72.8 ± 0.21$^{g}$ |
| BW2 | 230.0 ± 7.07$^{a}$ | 79.5 ± 0.71$^{b}$ | 150.0 ± 7.07$^{d}$ | 73.0 ± 0.07$^{g}$ |
| BW3 | 172.5 ± 3.54$^{b}$ | 90.0 ± 2.12$^{a}$ | 205.0 ± 7.07$^{a}$ | 73.8 ± 0.14$^{f}$ |
| BW4 | 102.5 ± 3.54$^{c}$ | 80.0 ± 0.71$^{b}$ | 85.0 ± 0.00$^{f}$ | 75.5 ± 0.07$^{e}$ |
| BW5 | 230.0 ± 7.07$^{a}$ | 73.0 ± 1.41$^{c}$ | 115.0 ± 7.07$^{e}$ | 72.7 ± 0.35$^{g}$ |
| CV (%) | 29.5 | 9.4 | 34.2 | 1.6 |
| Durum wheat | | | | |
| DW1 | 0 | 39.3 ± 1.77$^{f}$ | 197.5 ± 3.54$^{ab}$ | 76.7 ± 0.07$^{d}$ |
| DW2 | 115.0 ± 14.14$^{c}$ | 61.5 ± 1.41$^{d}$ | 180.0 ± 0.00$^{bc}$ | 89.7 ± 0.21$^{a}$ |
| DW3 | 40.0 ± 0.00$^{d}$ | 54.0 ± 0.00$^{e}$ | 192.5 ± 3.54$^{ab}$ | 88.7 ± 0.14$^{b}$ |
| DW4 | 0 | 40.0 ± 1.41$^{f}$ | 167.5 ± 10.61$^{cd}$ | 77.2 ± 0.21$^{d}$ |
| DW5 | 102.5 ± 3.54$^{c}$ | 79.0 ± 0.00$^{b}$ | 152.5 ± 3.54$^{d}$ | 83.2 ± 0.14$^{c}$ |
| CV (%) | 104.9 | 29.7 | 9.6 | 6.9 |
| Mean per species | | | | |
| Bread wheat flour | | 193.0$^{A}$ | 82.4$^{A}$ | 134.0$^{A}$ |
| Durum wheat flour | | 51.5$^{B}$ | 54.6$^{B}$ | 178.0$^{B}$ |

Means followed by the same letter (lower case) within the same column are not significantly different ($P > 0.05$). Letters correspond to ranking of groups after Tukey test. Means followed by the same letter (upper case) within the same column are not significantly different ($P > 0.05$). Letters correspond to ranking of groups after *t*-test.

TroloxEq kg$^{-1}$ and from 81.8 to 95.3 mmol TroloxEq kg$^{-1}$ in bread and durum wheat samples, respectively. Gluten isolated from CIMMYT wheat lines showed the highest ABTS radical scavenging activity among the bread and durum wheat samples. Generally, the increased concentration of total cysteine and -S-S bonds in glutens contributed to the improvement of its antioxidant potential. The cysteine residues represent facile targets for oxidation thus affecting the antioxidant capacity of proteins. However, microenvironment in protein and proximity to the oxidant source cause the reactivity of an individual cysteine. Žilić *et al.* (2012) emphasised that the structural molecular integrity is the most important issue for proteins if they work as antioxidants. According to results of this study, durum wheat gluten had the highest antioxidant capacity (74.39 mmol Trolox kg$^{-1}$) compared with the other tested proteins and protein hydrolysates.

### Technological properties of dough and its relation to wheat proteins

In this study, resistance to extension, extensibility, degree of softening and water absorption were determined and the results are shown in Table 4. As expected, on average, bread wheat flour dough had by about 3.7- and 1.5-fold higher values for resistance to extension and extensibility than durum wheat flour dough, respectively (Table 4). Dough of durum wheat genotypes DW1 (ZP 120/I) and DW4 (ZP 34/I) has not expressed resistance to extension, and it had the lowest extensibility. Considerable variation for resistance to extension among genotypes of bread (29.5%) and particulary durum wheat (104.9%) was found. Generally, dough rheology depend on interplay of flour ingredients, such as wheat proteins, starch, water, nonstarch polysaccharides, in particular arabinoxylans and lipids, as well as phenolic compounds. The irreplaceable contribution of starch, as the major constituent of wheat flour, in dough behaviour is related to its water absorption, gelatinisation and retrogradation properties (Goesaert *et al.*, 2005). However, the most of the differences in doughs are usually attributed to the gluten proteins. From that reason, and bearing in mind the rather similar starch content within the samples of the bread and durum wheat flours, only proteins effect on flour dough behaviour were investigated. To visualise similarities–dissimilarities of the interrelationships among the rheological properties of dough and the proteins subunits, as well as its structural characteristics in bread and durum wheat, genotype by trait biplots were prepared (Fig. S3). The analysis showed high positive association of HMW-GS with resistance to extension in bread wheat, as shown by acute angles between their vectors (Fig. S3a). Our results are in well accordance with data of Payne *et al.* (1988). Among others, the spiral structure formed by the repetitive central domain is the one of the features of HMW subunit may be related to their role in determining gluten elasticity. It should be noted that the bread wheat genotype BW2 (ZP 7/I) with the highest HMW-GS concentration, and resistance to extension

of 230 BU is characterised by high strength gluten. According to our results, the concentrations of S-rich subunits of gliadins were positively associated with extensibility of bread wheat flour dough (Fig. S3a). Lonkhuijsen *et al.* (1992) and Fido *et al.* (1997) found that presence of γ-gliadins in high concentration has positive effect on increased loaf volume and dough extensibility, respectively. In contrast to the bread wheat, the concentration of total HMW-GS was negatively associated with extensibility, as well as resistance to extension (indicated by obtuse angles between vectors for those proteins and dough characteristics) in durum wheat flour dough (Fig. S3b). In the presence of an oxidising agent during dough mixing, sulphhydryl groups can be oxidised to disulphide bands resulting in the strengthening of dough. On the other hand, reducing agents, as well as proteolytic enzymes of the wheat grains can cause the production of sulphhydryl groups with consequent softening of the dough (Aja *et al.*, 2004). Our results confirm that degree of softening of dough was positively associated with the concentration of free -SH groups in bread wheat species (Fig. S3c). For analysed genotypes of durum wheat, flour water absorption was positively associated with concentration of free -SH groups (Fig. S3d). In previous investigations of Koppel & Ingver (2010), strong correlation between water absorption and protein content was established. In accordance with aforementioned investigations, bread wheat genotype BW1 (ZP 87/I) and durum wheat genotype DW5 (DSP-MD-01 no. 66) showed the lowest and the highest protein content (Table S1), respectively, that resulted in the lowest and the highest water absorption, respectively. On average, durum wheat flour dough had higher both, the degree of softening and the water absorption (178.0 BU and 83.2%, respectively) than bread wheat flour dough (134.0 BU and 73.6%, respectively) (Table 4). Although durum flours usually produce a smaller loaf volume than those of bread wheat, due to the high water absorption capacity, the durum bread has more prolonged shelf life that is an important demand of costumers for specialty breads (Liu *et al.*, 1996). Generally, high protein quantity provides both, high water absorption and good baking performance.

## Conclusion

The results of SDS-PAGE showed that concentration of α/β-, γ-gliadins and ω-gliadins, as well as the concentration of LMW-GS and HMW-GS was significantly different between the genotypes, as well as bread and durum species. Generally, durum wheat had higher concentration of S-rich γ-gliadins and S-poor D-LMW-glutenins, but did not possess S-poor ω-gliadins. The bread wheat genotypes had a much stronger band in α/β-gliadin subunits, which play the largest positive role in increasing loaf volume. On the other hand, strong polypeptide in γ-gliadin region that appeared in all durum wheat genotypes and can be useful markers for pasta quality was absent in the most of bread wheat genotypes. The average value of bread wheat flours for the total glutenin subunits was for about 13% higher than that of durum wheat flours.

The flour doughs showed a clearly different behaviour regarding the effect of protein composition and its structural parameters. Based on the results of principal component analysis the concentration of free -SH groups was positively associated with flour water absorption and degree of softening of dough in durum and bread wheat species, respectively. In contrast to the bread wheat, the concentration of HMW-glutenins was negatively associated with extensibility, as well as resistance to extension of durum wheat flour dough. In addition, the observed differences in rheological performance of flour doughs are due to the differences in total and gluten proteins content between bread and durum wheat varieties. Although durum wheat varieties had poor rheological properties of dough, due to the high antioxidant capacity of their gluten proteins, as well as a high content of essential sulphur-rich amino acids, durum wheat could be used as functional ingredient for bread-making. Although the starch contents in the analysed bread and durum wheat flour samples were similar, this study recommend investigating in detail the effects of starch properties on dough behaviour.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1**. SDS-PAGE patterns of gliadin proteins from bread and durum wheat flours.

**Figure S2**. SDS-PAGE patterns of glutenin proteins from bread and durum wheat flour.

**Figure S3**. Principal component analysis of interrelationship among rheological properties of dough and protein profiles, as well as its structural characteristics. (a,b) Bread wheat and (c,d) Durum wheat.

**Table S1.** Names, origin, type and pedigree information of tested genotypes of bread and durum wheat with different content of total proteins and wet gluten.