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Changes in durum wheat kernel and pasta proteins induced by toasting and drying processes

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ABSTRACT

Durum wheat kernels were subjected to a toasting process and the proteins characterised by size exclusion-high performance liquid chromatography (SE-HPLC) and sodium dodecyl sulphate–polyacrylamide gel electrophoresis. With this physical process, albumins and globulins, as well as glutenins and gliadins, polymerised as seen by a shift of the SE-HPLC profile to lower elution times. The polymerisation seemed to happen mainly through disulphide bonds, even though the participation of ω-gliadins to the aggregation suggested the involvement of other kinds of interactions. It led to the revelation of a new peak originated by thermal aggregation of small polymeric proteins. The changes in the chromatographic profile were accompanied by increasing amounts of total unextractable polymeric proteins. The replacement of semolina with toasted durum wheat flour (5%, 10%, 15%, 20% and 30%) for the production of pasta in the shape of spaghetti significantly ($p < 0.001$) affected the molecular size distribution of the polymeric proteins, even though the replacement of semolina with 5% and 10% of toasted durum wheat flour (TDWF) did not significantly ($p > 0.05$) change the unextractable polymeric proteins (UPP) when compared with spaghetti made with 100% durum semolina. On the other hand, the replacements of semolina with 15–30% TDWF showed significant ($p < 0.001$) increase in UPP when compared with 100% durum semolina spaghetti.

1. Introduction

Wheat is currently the most important crop in the world, with total annual yields of almost 600 million tonnes. Much of the success of wheat stems from its processing properties and in particular its ability to form cohesive doughs which can be baked into bread or formed into pasta and noodles. These properties derive largely from the gluten proteins, which form a continuous viscoelastic network within the dough. Gluten contains hundreds of proteins components, which are present either as monomers or, linked by interchain disulphide bonds, as oligo- and polymers (Wrigley & Bietz, 1998). They are unique in terms of their amino acids composition, which are characterized by high contents of glutamine and proline and by low contents of amino acids with charged side groups. Traditionally gluten proteins have been divided into gliadins and glutenins, according to their polymerisation properties: gliadins are monomeric proteins that form only intra-molecular disulphide bonds, if present, whereas glutenins are polymeric proteins whose subunits are held together by inter-molecular disulphide bonds, although intra-chain bonds are also present. Among these storage proteins, glutenins (polymeric proteins) have been shown to be extremely important in determining rheological properties. Moreover, a certain amount of these polymers remains unextractable in various extracting systems (e.g. acetic acid solution or SDS–phosphate buffer) and the proportion of this fraction, known as unextractable polymeric proteins (UPP), is reported to be related to the technological response (Gupta, Khan, & MacRitchie, 1993; Jia, Fabre, & Aussenac, 1996).

Gluten proteins are susceptible to heat treatment and their behaviours subjected to relatively high temperatures have been studied by a numbers of workers. It was shown that molecular size of the glutenin aggregates increases and, hence, their extractability decreases (Booth et al., 1980; Schofield, Bottomley, Timms, & Booth, 1983; Weegels, de Groot, Verhoek, & Hamer, 1994b). At 100 °C, gliadins undergo similar changes. The extractability of gliadins from bread by 60% ethanol is much lower than that from flour, and α-and γ-giadins are more affected than ω-giadins (Wieser, 1998). The effects have been ascribed to sulphidryl

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(SH)-disulphide interchange reactions induced by heat, that affect all gluten proteins except the cysteine-free ω-giadins (Booth et al., 1980; Schofield et al., 1983). Morel, Redl, and Guilbert (2002) suggested that below 60 °C no changes in free sulphydryl groups occur. Heating to at least 90 °C leads to disulphide bond linked aggregates and conformational changes affecting mostly gliadins and low molecular weight albumins and globulins (Guerrieri, Alberti, Lavelli, & Cerletti, 1996). Although, Kokini, Cocero, Madeka, and de Graaf (1994) proposed that cross-links among gliadin molecules are formed above 70 °C in the absence of glutenins, others believed that gliadins cross-link only with glutenins (Redl, Morel, Bonicel, Vergnes, & Guilbert, 1999; Singh & MacRitchie, 2004). As a matter of fact, the incorporation of gliadin monomers in the glutenin network leads to a three-dimensional structure (Morel et al., 2002).

Apart from its fundamental interest, the effects of heating on gluten proteins are relevant to practical processes, such as drying of rain-damaged wheat, drying of gluten from starch/gluten manufacturing plants and, in relation to effects on gluten proteins during baking, pasta making and extrusion and the potential for producing modified glutens with unique technological properties. A traditional pasta used by poor Apulian people, which lived in Southern Italy, was home made brown pasta by durum semolina obtained from toasted kernel. The toasted kernel was the product of burning remained straw in the fields; in this way residual kernels were subjected to high temperature treatment. The poor people looked for toasted kernel in the straw ash and picked up it manually. Today pasta from toasted kernel, "Grano arso" called in Italy, is an actual commercial activity in Puglia.

To increase our insight into the behaviour of gluten proteins during heat treatment, the size exclusion-high performance liquid chromatography (SE-HPLC) was used to examine polymerisation of the different protein fractions of durum wheat kernels subjected to a toasting process and to evaluate their changes in molecular size distribution. In addition, the effect of the partial replacement of semolina with toasted durum wheat flour (5%, 10%, 15%, 20% and 30%) was studied to improve and optimize the process of traditional pasta production from "Grano arso". To reach this objective, SE-HPLC profiles and the UPP of the composite pasta were evaluated thus to gain a better understanding of size distribution and possible interactions at the molecular level.

2. Experimental

2.1. Raw materials

Semolina and toasted durum wheat flour (TDWF) derived from the same commercial blend of Italian durum wheats (Simeto, Ciccio, Arcangelo) were supplied by Molino Daddario Antonia (Cerignola, Foggia, Italy). The toasted meal was obtained by the milling of heat treated caryopses previously harvested and threshed. In particular, cleaned durum wheat grains were arranged on steel bearing placed into direct contact with a wood-fire for 120 s until kernels browned; then, grains were cleaned again and dampened; and after eight hours the grains were subjected to grinding using a roller mill. The particle size of the TDWF used was in the range of 200–355 μm.

2.2. Pasta production

Pasta was produced in a 2-kg pilot plant (NAMAD, Rome, Italy) consisting of a mixer, an extruder and a dryer. The flour was mixed with tap water (for 10 min) to obtain a dough water content of 44–45%. The extrusion conditions were as follows: temperature = 50 °C (the extrusion temperature varied between 45 °C and 55 °C); kneading time = 15 min, pressure = 60–125 atm as a function of the specific formulation and vacuum degree = 700 mm Hg. A Teflon die-plate of 1.70 mm diameter was used. Pasta in the shape of spaghetti was dried at 50 °C for 16 h. The diameter of the obtained spaghetti was about 1.70 ± 0.03 mm. Six kinds of spaghetti were produced: a control, made of 100% durum wheat semolina and pasta in which 5%, 10%, 15%, 20% and 30% of semolina was replaced with TDWF (named respectively as 5% TDWF-, 10% TDWF-, 15% TDWF-, 20% TDWF-, and 30% TDWF-wheat spaghetti).

2.3. Proteins extraction for SE-HPLC

Proteins from semolina, toasted durum wheat flour and milled spaghetti samples were extracted following the method of Gupta et al. (1993). Samples (10 mg) were suspended in 1 mL 0.5% sodium-dodecyl-sulphate (SDS)–phosphate buffer (pH 6.9) and mixed initially in a vortex-mixer and later kept at room temperature for 30 min. The suspensions were then centrifuged for 10 min at 17,000 g to obtain supernatant ("extractable" or "SDS-soluble" proteins).

The resulting residues were extracted with 0.9 ml 0.5% SDS–phosphate buffer (pH 6.9) by sonication for 15 s using a Microson Ultrasonic (Falc Instrument, Bergamo, Italy) cell distributor, ensuring that the samples were completely dispersed within the first 5 s and then heating to 35 °C for 30 min. The supernatants from centrifugation for 10 min at 17,000*g* were termed "unextractable" proteins. All extracts were filtered through a 0.45 μm PVDF filter prior to SE-HPLC analysis.

2.4. SE-HPLC analysis

Polymeric proteins from the semolina, toasted durum wheat flour and milled spaghetti samples were fractionated through size exclusion-high performance liquid chromatography (SE-HPLC) using a Phenomenex Biosep TM SEC 4000 column (Phenomenex) (Kuktaite, Johansson, & Juodeikiene, 2000; Kuktaite, Larsson, & Johansson, 2003). Proteins from semolina and milled spaghetti were extracted with a two step extraction procedure (Gupta et al., 1993). The first step in this method extracts the SDS-extractable proteins (soluble in diluted SDS), whilst the second extract contains the SDS-unextractable proteins (proteins soluble only after sonication). The extracted proteins were separated on SE-HPLC according to Tosi et al. (2005) by loading 20 μl of sample into an eluant of 50% (v/v) acetonitrile and water containing 0.05% (v/v) trifluoracetic acid (TFA) at a flow rate of 0.7 mL/min for 30 min. Proteins were detected at a wavelength of 214 nm. Three replicates of each samples were used for the investigation of protein composition. The percentage of unextractable polymeric protein (UPP) was calculated as described by Gupta et al. (1993). Briefly, the percentage of total UPP was calculated as [peak 1 + 2 area (unextractable)/peak 1 + 2 area (total)] × 100. Peak 1 + 2 (total) refers to the total of peak 1 + 2 (extractable) and peak 1 + 2 (unextractable) (Johansson, Prieto-Linde, & Jonsson, 2001; Kuktaite et al., 2000, 2003).

The SE-HPLC column was calibrated using protein standards with a range of molecular weights (KDa) as follows: ribonuclease A (13.7), chymotripsinogen (25.0), ovalbumin (43.0), bovine serum albumin (67.0), aldolase (158), catalase (232), ferritin (440) and thyroglobulin (669).

The molecular weights were calculated from the line of best fit through the calibration points, given by: log molecular weight = 8.1448–0.1995 × elution time (min).

2.5. SDS–PAGE (sodium dodecyl sulphate–polyacrilamide gel electrophoresis) analyses

One hundred gram were sequentially extracted by the Osborne procedure (Osborne, 1907) with modifications. Albumin and globulins