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Changes in wheat kernel proteins induced by microwave treatment

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ABSTRACT

Wheat kernels were subjected to microwave treatment, and the proteins were characterized by size exclusion high-performance liquid chromatography (SE-HPLC) and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). Using this process, the proteins polymerize, forming intermolecular bonds among the same classes of proteins. Furthermore, the polymerization occurs only through disulphide bonds. Although SDS–PAGE did not show any differences for either the number or intensity of protein bands between flour samples before and after microwave treatment, gliadins from treated flours showed significantly reduced cross-reactivity with the R5 antibody. Moreover, the gluten became soluble in an aqueous saline solution, and it was not possible to isolate it using the Glutomatic apparatus. However, the treated flour, in the presence of water, was able to form dough and leaven and produce bread.

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1. Introduction

Gluten is one of the earliest protein fractions described by chemists. It is defined as the "cohesive, visco-elastic proteinaceous material" that remains when wheat dough is washed to remove starch granules and water-soluble constituents (Shewry, 2009). Gluten contains hundreds of proteins, which are present either as monomers or as oligomers and polymers linked by inter-chain disulphide bonds (Shewry & Halford, 2002) and characterized by high contents of glutamine and proline and low contents of charged amino acids.

Traditionally, gluten proteins have been grouped according to their solubility in alcohol-water solutions, such as 60% ethanol, as the soluble gliadins and the insoluble glutenins (Osborne, 1924). The interchain disulphide bonds largely determine these properties, with the glutenin consisting of disulphide-stabilized polymers. Reduction of these inter-chain bonds allows the separation of the glutenin subunits into low molecular weight (LMW) and high molecular weight (HMW) groups. By contrast, the alcohol-soluble gliadin fraction consists mainly of monomeric proteins, which either lack cysteine (ω-gliadins) or contain only intra-chain disulphide bonds (α-type and γ-type gliadins). Gluten proteins are susceptible to heat treatment, and their behaviour under relatively high temperatures has been primarily evaluated in model systems. When glutenin is heated above 55 °C or gliadins are heated above 70 °C, disulphide/sulphydryl (SH) exchange reactions occur (Schofield, Bottomley, Timms, & Booth, 1983). Lavelli, Guerrieri, and Cerletti (1996) showed that 65 °C particularly influenced the S–S structure of HMW albumins and possibly their linkage to glutenin oligomers. Furthermore, LMW albumins and gliadins are affected at higher temperatures. In addition, Singh and MacRitchie (2004) found that glutenins polymerize below 100 °C but that the polymerization of gliadins occurs only at higher temperatures. The molecular size of the glutenin aggregates increases, decreasing their extractability (Lamacchia et al., 2007; Schofield et al., 1983; Weegels, Verhoek, de Groot, & Hamer, 1994a). At 100 °C, gliadins undergo similar changes. The extractability of gliadins from bread, using 70% ethanol, is much lower than that of gliadins from flour, and α- and γ-gliadins are more affected than are ω-gliadins (Wieser, 1998). The effects have been ascribed to sulphydryl (SH)-disulphide interchange reactions induced by heat, which affect all gluten proteins except the cysteine-free ω-gliadins (Schofield et al., 1983). Morel, Redl, and Guilbert (2002) suggested that, at temperatures 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, primarily affecting 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 have hypothesized that gliadins cross-link only with glutenins (Redl, Morel, Bonicel, Vergnes, & Guilbert, 1999; Singh & MacRitchie, 2004) and that the incorporation of gliadin monomers in the glutenin network leads to a three-dimensional structure (Morel et al., 2002). In a study performed on pasta, Lamacchia et al. (2007) showed that gluten proteins undergo changes induced by drying cycles, which

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were consistent with the results found in the model system. Furthermore, the albumins and globulins were completely denatured at 60 °C and incorporated into high-molecular-weight polymers, whereas, because of the conformational structure and low thiol availability, gliadins were only partially denatured at 90 °C and aggregated into polymers of higher molecular weight. In a recent study, Lamacchia, Baiano, Lamparelli, La Notte, and Di Luccia (2010) reported that high temperature applied to durum wheat kernels induced significant changes in proteins but these were different from those reported in a gluten model system and pasta. In particular, proteins were denatured and polymerized, and the albumins and globulins tended to coagulate and interact with gliadins instead of HMW glutenins to form protein aggregates of intermediate molecular weight, revealed as a new intermediate protein peak. The explanation for this phenomenon was that, in the kernel, gluten is not formed and gluten proteins are deposited in different protein bodies (Rubin, Levanony, & Galili, 1992). Tosi, Gritsch, He, and Shewry (2011) confirmed that, in wheat kernels, gluten proteins may form protein bodies by two mechanisms, either transport via the Golgi and Golgi-derived vesicles into the vacuole or by direct accumulation within the lumen of the ER. Additionally, the segregation of protein types into specific populations of protein bodies can occur within the same cell. Differences in the proportions of different types of gluten proteins in protein bodies within the same cell was also demonstrated by immunofluorescence, using double antibody labelling. Therefore, cells of the central starchy endosperm clearly contained protein bodies, which contained HMW subunits of glutenin but little or no gliadin, and this segregation may be maintained, even after protein body fusion and matrix formation.

Apart from fundamental interests, the effects of heating gluten proteins are relevant to practical processes, such as the drying of rain-damaged wheat, drying of gluten from starch/gluten manufacturing plants, and in relationship to effects on gluten proteins during baking and extrusion.

Because Lamacchia et al. (2010) showed, for the first time, that the application of high temperature to wheat grains generates protein polymerization different from that reported in a gluten model system or in bread or dried pasta, the effects of heating gluten proteins in wheat grain have potential for producing modified gluten with unique properties.

In the present study, we examined the behaviour of gluten proteins in wheat kernels subjected to high temperature for a short time, using a high percentage of seed moisture and microwave energy (Italian Patented Method N. 0001414717, 2015; Patent Cooperation Treaty n. PCT/IB2013/000797, 2013). The polymerization of the different protein classes was ascertained, as were the changes in the glutenin and gliadin fractions separated by SDS–PAGE. In addition, the effects of changes in the protein fractions induced by the microwave treatment on both the binding ability of the R5 antibody and the bread-making properties were also evaluated.

2. Materials and methods

2.1. Raw materials and microwave treatment

The wheat kernels (Blasco, Adamello, Ofanto, and Simeto varieties and mixtures of soft and durum wheat Canadian grains) used in this study were supplied by CRA (Foggia, Italy) and by the Casillo group S.p.a (Corato, Italy), respectively. Treated wheat flour (TWF) was obtained by milling the microwave-treated caryopses (Italian Patented Method N. 0001414717, 2015; Patent Cooperation Treaty n. PCT/IB2013/000797, 2013) that were previously harvested and threshed. In particular, 100 g of cleaned wheat grains were dampened for at least two hours, until reaching 18–20% humidity, and then measured with a halogen thermal balance (Mettler Toledo, HB43-S, Swiss). These were then drained and subjected to 1000 watt power for 2 min in a microwave apparatus (Delonghi, Italy) to reach a temperature of approximately 110–120 °C and then measured with a thermal camera (FLUKE i 20 model, Italy).

These parameters were set on the basis of the work of Lamacchia et al. (2010), who induced polymer protein changes but eliminated the negative effects due to the burning of the caryopses. After microwave treatment, the wheat kernels were cooled and dried at room temperature (24 °C) for 12–24 h and then ground, using a roller mill. The particle size of the TWF used was in the range of 100–200 μm. Experiments on TWF were performed with the flour obtained from wheat kernels before the microwave treatment as the control wheat flour (CWF). For each grain sample, (Adamello, Blasco, Ofanto, Simeto, mixture of Canadian durum wheat grains and mixtures of Canadian soft wheat grains), three independent experiments were performed.

2.2. Proteins extraction for SE-HPLC

Proteins from the CWF and TWF samples were extracted, using the method of Gupta, Khan, and MacRitchie (1993). Soluble proteins from 10 mg of samples were extracted with 1 ml of 0.5% SDS–phosphate buffer (pH 6.9). The suspension was shaken for 30 min and the solubilized protein (“soluble” or “extractable” protein) was recovered by centrifugation for 10 min.

The resulting residues were extracted with 1 ml of 0.5% SDS-phosphate buffer (pH 6.9) by sonication for 15 s (Microson Ultrasonic cell distributor), ensuring that the samples were completely dispersed within the first 5 s, and then heated to 35 °C for 30 min. The supernatants after centrifugation (10 min at 17,000g) were named “unextractable” proteins or “insoluble” proteins.

Total proteins (10 mg) were extracted in 1 ml of the same buffer, vortexed, and sonicated for 30 s, and the supernatants (“total” protein) were recovered for SE-HPLC analysis.

All extracts were filtered through a 0.45-μm PVDF filter prior to injection on the column.

2.3. SE-HPLC analysis

SE-HPLC was performed, using an LC Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) and Phenomenex Biosep SEC S-4000 column (300 × 7.8 mm, Phenomenex, Torrence, CA, USA).

Each sample (20 μl) was injected onto the column, and the eluted proteins were monitored at 214 nm. Three replicates of each sample were used to investigate the protein composition. The mobile phase was 50% acetonitrile containing 0.05% trifluoroacetic acid with a flow rate of 0.7 ml/min. The SE-HPLC column was calibrated, using protein standards with a range of molecular weights (kDa) as follows: ribonuclease A (13.7), chymotrypsinogen (25.0), ovalbumin (43.0), bovine serum albumin (67.0), aldolase (158), catalase (232), ferritin (440) and thyroglobulin (669).

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 LPP (Large Polymeric Proteins) + SPP (Small Polymeric Proteins) area (unextractable)/peak LPP + SPP area (total)] × 100. Peak LPP + SPP area (total) refers to the total of peak LPP + SPP (extractable) and peak LPP + SPP (unextractable) (Kuktaite, Larsson, & Johansson, 2003).

2.4. Determination of SH and S–S groups

The protein disulphide and sulphydryl contents in the CWF and TWF samples were estimated by colorimetric determination of the

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