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Fate of SDS-insoluble glutenin polymers from semolina to dry pasta

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ABSTRACT

Pasta cooking quality is well known to be related to semolina protein content and composition, however impact of the unextractable polymeric protein content (%UPP) remains disputed. In this work different semolina samples, of variable protein contents (10.5–14.2%) and %UPP (20.2–46.3%) are studied. The changes in %UPP induced by the successive pasta processing steps (mixing, extrusion, drying) but also those occurring during resting periods at 35 °C, applied in-between them, were investigated. Effect of a resting period was moderate after mixing, but pronounced after extrusion. Resting of extruded pasta at 35 °C significantly increased %UPP, which can even grow beyond that of the semolina. No relationship was found between pasta viscoelastic index (VI) and semolina %UPP or protein content. However, cooked pasta VI was found related to the calculated %UPP of rested fresh pasta.

1. Introduction

Durum wheat semolina is the acknowledged raw material to make pasta, through hydration and mixing, sheeting or extruding and drying steps (Antognelli, 1980; Sicignano, Di Monaco, Masi, & Cavella, 2015). Purpose of semolina hydration and mixing is to produce wet agglomerates of few hundred micro-meters showing good flowing ability (Cuq, Mandato, Jeantet, Saleh, & Ruiz, 2013) in order to feed the extruder. Alternatively, the agglomerates are passed several times between rollers until forming a cohesive sheet. Then drying transforms fresh pasta into a convenient and long shelf-life food product (Mercier & Cantarelli, 1986). Cooking, the ultimate processing step, is left to the consumer discretion.

Pasta firmness and tolerance to over-cooking is strongly linked to the semolina protein content, and especially to the gluten proteins. Gluten forms a viscoelastic network that embeds the starch granules and limit their swelling during pasta cooking (Dexter & Matsuo, 1977a; D'Egidio, Mariani, Nardi, Novaro, & Cubadda, 1990; Wasik, 1978).

Gluten is composed of alcohol-soluble monomeric gliadins (α-β-, γ- and ω-) and polymeric glutenins (formed by the disulphide concatenation of high and low molecular weight glutenin subunits) (Shewry & Halford, 2002). Gluten refers to the assembly of gliadin and glutenin polymers into a viscoelastic cohesive and hydrated mass stabilized by hydrogen, ionic and hydrophobic bonds. In semolina, glutenin polymers exist as both SDS-soluble and SDS-insoluble fractions, like in common wheat flour (Žilić, Barać, Pešić, Dodig, & Ignjatović-Micić, 2011). The percentage of SDS-insoluble polymers over total glutenin polymers is referred as percent of unextractable polymeric protein (%UPP).

In the 1990's, bread quality was found strongly related to wheat flour %UPP (Gupta, Khan, & MacRitchie, 1993). During dough mixing, %UPP decreases until becoming very low, while subsequent resting allows for its recovery. Weegels, van de Pijpekamp, Graveland, Hamer, and Schofield (1996) showed that the amount of %UPP measured after 45 min of dough resting, better predicted flour breadmaking quality than does the initial flour %UPP. Indeed, extent of %UPP recovery was found related to dough relaxation time, *i.e.* to dough elasticity (Weegels et al., 1996). The mechanical energy delivered during mixing determines the rate and extent of % UPP recovery during subsequent dough resting. Over-mixing jeopardizes %UPP recovery and so negatively impact dough elastic properties. In the case of pasta making, the fate of SDS-insoluble glutenin polymers through processing steps is still poorly documented. Especially, the ability of %UPP to recover after the mixing and extrusion steps has never been examined despite the role of this polymeric protein fraction in dough elasticity. Indeed, the semolina %UPP content was first associated with pasta dough mixing strength (Edwards et al., 2007; Sissons, Ames, Hare, & Clarke, 2005), a feature related to pasta cooking quality (Dexter & Matsuo, 1980); but the robustness of this relationship remains disputed (Sissons, Soh, & Turner, 2007).

Abbreviations: OCT, optimum cooking time; SME, specific mechanical energy; UPP, unextractable polymeric protein (%UPPs: %UPP in semolina, %UPPEx: %UPP reached at maximum polymerisation after resting, %UPPFP: % of UPP just after pasta extrusion); SDS, sodium dodecyl sulphate; VI, viscoelastic index.

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Pasta processing steps also influence glutenin polymer solubility in SDS. Similarly, to what observed with wheat bread dough (Don, Lichtendonk, Plijter, & Hamer, 2003; Don, Lichtendonk, Plijter, van Vliet, & Hamer, 2005; Weegels, Hamer, & Schofield, 1997), mixing of semolina/water blends results in a decrease in %UPP (Icard-Vernière & Feillet, 1999; Kratzer, 2007). This change, which was associated to an increase of SDS-soluble protein, was attributed to the dissociation and de-polymerization of the largest glutenin polymers (the SDS-insoluble ones). Bruneel, Buggenhout, Lagrain, Brijs, and Delcour (2016) observed no impact of pasta dough sheeting on protein solubility while Kim et al. (2008) reported a drop of %UPP with the number of sheeting passes. The impact of extrusion on the protein assembly remains less documented even if a slight loss of protein extractability in dilute acetic acid was reported by Dexter and Matsuo (1977b) and Kratzer (2007).

Pasta drying definitively increases the amount of SDS-insoluble protein (De Stefanis & Sgrulletta, 1990; Dexter, Matsuo, & Morgon, 1981; Petitot et al., 2009) and %UPP was found to be 15–20% beyond that of the semolina in pasta dried at low temperature (Lamacchia et al., 2007). However, the calculation of %UPP (percentage of SDS-insoluble polymers over total glutenin polymers) in dried pasta appears inappropriate since gliadin partly form SDS-insoluble disulfide cross-linked aggregates and could be therefore count as total glutenin polymers (Bruneel et al., 2016; Dexter et al., 1981).

The aim of this study was to investigate the effect of the pasta making steps (mixing, extrusion and drying) on the amount of %UPP, in relation to the viscoelastic properties of the cooked pasta. The possible impact of %UPP recovery after extrusion is discussed in relation to the final pasta viscoelastic behavior.

2. Materials and methods

2.1. Semolina samples

Semolina samples (S1–S7) were prepared from 7 durum wheat grains batches grown in 2015, in the south-eastern (Aix-en-Provence, S1–S3) and south-western (Castelnaudary, S4–S7) France. Durum wheat cultivars were *Claudio* (S1–S3), *Dakter* (S4), *Miradoux* (S5 and S6) and *Pescadou* (S7), all genotypes showing the same high- (7 + 8) and low-molecular-weight (LMW-2) glutenin subunits composition. Grains were milled on the semi-industrial semolina pilot mill (150 kg/h) installed in 1974 by Bühler at INRA-Montpellier, France (Lempereur, Chaurand, Abecassis, & Autran, 1997), after cleaning and moisturizing in two steps to successively reach grain moisture of 15% and 17%. Semolina were kept at 4 °C until pasta making. Semolina moisture and ash content were determined respectively with AACC International Method 44-15.02 and AACC International Method 08-12.01. Semolina particle size distribution was analyzed with laser granulometry in ethanol (Mastersizer2000, Malvern Instrument, England). Median diameter D50 (value of the particle diameter at 50% in the cumulative distribution) was given.

2.2. Pasta making

Semolina was transformed into pasta using a laboratory pasta extruder (Afrem, Lyon, France, Abecassis, Abbou, Chaurand, Morel, & Vernoux, 1994). Samples were first hydrated and mixed into wet agglomerates, which were then extruded into spaghetti and dried in a temperature and relative-humidity controlled drier (Afrem, Lyon, France). For each test, 7 kg of semolina was hydrated to 47% (d.b.) with tap water at 40 °C and mixed for 5 min at 120 rpm and then 15 min at 60 rpm. The temperature of the mixer was maintained at 40 °C. At the end of the mixing step, the semolina agglomerates were immediately sampled into hermetic bags that were frozen using liquid nitrogen (HP0) or rested at 35 °C for 30, 60 or 120 min (HP30, HP60, HP120) before freezing. Size distribution of the agglomerates was measured in replicate for each sample by manually sieving the product (100 g) during 2.5 min on sieves of 0.16, 0.25, 0.31, 0.4, 0.5, 0.63, 0.71, 0.8, 0.9, 1.25 and 2 mm.

The agglomerates were extruded at 35 rpm and at a temperature of 35 °C, under vacuum (450 mmHg). Fresh pasta was sampled after extrusion into hermetic bags and immediately frozen using liquid nitrogen (FP0) or after resting at 35 °C for 10, 20, 30, 45, 60, 90 or 120 min (FP10, FP20, FP30, FP45, FP60, FP90, FP120). Pasta collection and calculation of the specific mechanical energy (SME: product of torque by extrusion speed over mass flow rate) were performed as soon as extrusion pressure was stable. Spaghetti samples were dried using a low-temperature drying cycle at 55 °C for 17 h The final diameter of dried spaghetti was 1.5 mm. All frozen samples were then freeze-dried (48 h), milled and kept under argon.

2.3. Protein size distribution

SDS-soluble and SDS-insoluble proteins were extracted and analyzed by size exclusion high performance liquid chromatography (SE-HPLC) according to Morel, Dehlon, Autran, Leygue, and Bar-L'Helgouac'h (2000). Dithioerytritol (20 mM) was used to extract the SDS-insoluble protein of dried pasta, in addition to standard sonication. Chromatogram of SDS-soluble proteins (as shown in Fig. 1A for one of the sample) recorded at 214 nm was divided in 5 fractions. The first fraction (F1) corresponds to the largest SDS-soluble glutenin polymers (from the exclusion limit to 680 000 g/mol). Fraction F2 corresponds to the medium range glutenin polymers (from 680,000 to 120,000 g/mol). Fractions F3 and F4 respectively correspond to ω-gliadins and α-, β- and γ-gliadins. Fraction F5 corresponds to albumin/globulins. The percentage of unextractable polymeric protein (%UPP) was determined as the ratio of the total area of the SDS-insoluble protein fraction (as shown in Fig. 1B for one of the sample) over the sum of the polymeric proteins (sum of F1 and F2 areas plus total area of the SDS-insoluble protein fraction). %UPP was determined on the semolina samples (%UPPs) and dried pasta, as well as on the intermediate processing products (wet mixed semolina and fresh pasta) which were sampled at different times after being obtained. The rate of %UPP recovery during resting was fitted according to a first-order reaction law using the following equation:

$$\%\mathrm{UPPEx} - (\%\mathrm{UPPEx} - \%\mathrm{UPPFP})^{*}\exp(-\mathrm{k}^{*}time)$$

where %UPPEx is the %UPP reached at maximum polymerization after resting, %UPPFP is the % of UPP of pasta sampled just after extrusion and k is the %UPP recovery rate constant (in % per min).

2.4. Pasta cooking and viscoelastic behavior assessment

Dried spaghettis (8 g) were cooked in 250 ml of boiling water (salt: 7 g/L) until the optimum cooking time. Tap water was used but demineralized water was added to adjust its hardness to 15f° ± 1. During cooking, spaghettis were subsample every 15 s and then squished between two glass plates to determine the optimum cooking time (OCT); the time for which the continuous white line visible at the middle of pasta strand disappears. Pasta were cooked at 1× OCT and overcooked at 2× OCT. Spaghetti samples were drained and rinsed with 2 × 500 ml of water at room temperature to stop cooking. Pasta strands were kept on a humid sponge under a Petri box at room temperature for 15 min before analysis.