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Analysis of soluble proteins/aggregates derived from gluten-emulsifiers systems

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ABSTRACT

According to their amphiphilic nature, emulsifiers as Sodium Stearoyl Lactylate (SSL) and Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM) favor interactions between dough components, although the mechanisms of action are not fully elucidated. There is quite information about the nature of emulsifier–gluten protein interaction and its consequence in breadmaking quality, but no reports were found about structural changes in gluten proteins produced by SSL and DATEM and their influence in aggregation–disaggregation gluten protein phenomena. For this reason the aim of this work was to investigate changes on gluten protein structure induced by SSL and DATEM; through the analysis of the nature of soluble aggregates by different SDS-PAGE techniques, and gliadins and glutenins extracted from gluten samples by RP-HPLC. At 1% level, SSL allowed the solubilization of a large proportion of high molecular mass aggregates, suggesting that at this high level, this emulsifier cause changes in native gluten protein network. Nevertheless, no significant differences in the protein quantity extracted of the distinct gliadins and glutenins were observed. On the other hand, the emulsifier SSL at 0.5% (w/w) level also allowed the extraction of a high percentage of γ-gliadins and low molecular weight glutenins in comparison with gluten and gluten-DATEM samples. In conclusion distinct quantity and quality of gliadins and glutenins were extracted from gluten samples, depending on the level and the chemical structure of each emulsifier.

1. Introduction

Gluten proteins play a key role in determining the unique baking quality of wheat flour by conferring viscosity and elasticity on dough. These proteins are distinctive in terms of their amino acid composition, which are characterized by high contents of glutamine and proline and by low contents of amino acids with charged side groups (Shewry, 2003). Gluten proteins are divided in two fractions according to their solubility in alcohol-water solutions. These fractions are the soluble gliadins and the insoluble glutenins. Both fractions are important contributors to the rheological properties of dough. Gliadins are mainly monomeric proteins which are responsible for the extensibility of dough and are related to its viscous behavior. According to their electrophoretic mobility, gliadins were divided into α, β, γ and ω fractions (Shewry, 2003). The amino acid composition of gliadins fractions is similar (Tatham, Shewry, & Belton, 1990), altough ω gliadins have few essential aminoacids and virtually have no methionine and cysteine, so they are unable to form disulfide bridges (Wieser, 2007). SDS-PAGE analysis in the presence of 2-mercaptoethanol shows that α, β and γ gliadins have similar molecular masses (33–40 kD) being indistinguishable by this technique. The ω gliadins have a higher molecular mass than the others fractions (50–65 kD) (Kasarda, Autran, Lew, Nimmo, & Shewry, 1983; Žilić, Barać, Pešić, Dodig, & Ignjatović-Micić, 2011).

Glutenins are polymeric proteins which provide strength and tenacity to dough and they are related to its elastic behavior. They have molecular masses greater than 10^6 Da (Payne, Law, & Mudd, 1980; Wieser, Bushuk, & MacRitchie, 2006). Glutenins also have been classified on the basis of their mobilities in SDS-PAGE under reducing conditions into two main groups, the low molecular weight (LMW) and the high molecular weight (HMW) glutenin subunits (Shewry, Tatham, Forde, Kreis, & Miflin, 1986). LMW glutenins and HMW glutenins have molecular mass ranges between 30–40 kD and 70–150 kD, respectively (D'Ovidio & Masci, 2004; Payne et al., 1980).

Sodium Stearoyl Lactylate (SSL) and Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM) are anionic emulsifiers that are widely used in breadmaking (Aamodt, Magnus, Hollung, Uhlen, & Færgestad, 2005; Gómez et al., 2004; Koocheki, Mortazavi, Mahalati, & Karimi, 2009; Ribotta, Perez, León, & Añón, 2004; Selomulyo & Zhou, 2007). Due to their high hydrophilic–lypophilic balance (HLB) related to hydrophilic/hydrophobic character, these additives exhibit dough strengthening effects; although they can also promote emulsification, air incorporation and crumb softness (Stauffer, 1990). Sodium Stearoyl Lactylate (SSL) presents a high HLB value (HLB = 21) (Armero & Collar, 1996; Indrani & Rao, 2003; Köhler, 2001; Stauffer, 1990). SSL molecule has a hydrophilic fraction (polar moiety) and a long hydrophobic chain (non polar portion). The hydrophilic polar

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chain (lactylate) allows SSL to interact with charged amino acid residues of gluten proteins through ionic bonds (Boutte & Skogerson, 2004). In contrast, due to its lower HLB balance (HLB = 9.2), DATEM is a molecule that mainly interacts with the hydrophobic domains of gluten proteins (Armero & Collar, 1996; Köhler, 2001; Shiau, 2004). Hydrophobic interactions between the lypophilic moiety of the emulsifiers and hydrophobic domains of gluten proteins allow the incorporation of the negative charge to gluten matrix. This leads to neutralization of surface charge of proteins and the formation of protein aggregates that impart strength to the dough (Orthoefer, 1997; Stauffer, 1990).

Structure of gluten proteins is straightforward related to the kind of monomers and polymers that conforms the network. Wrigley and Bekes (2002) listed several methods for identifying proteins and consequently wheat varieties. The reversed-phase high-performance liquid chromatography (RP-HPLC) is the most commonly technique used to analyze gluten proteins due to its high resolving power. In RP-HPLC, proteins are separated mainly according to their surface hydrophobicity (Lookhart, Bean, & Bietz, 2003). This technique is complemented by gel electrophoresis; which is based on the separation of proteins by size or charge (Bietz, 1983; Lookhart & Albers, 1988; Marchetti, Cardós, Campaña, & Ferrero, 2011). SDS-PAGE is one of the mostly used methods for identifying high and low molecular weight glutenins. A technique, none widely used, is Multi-Stacking electrophoresis (MS-SDS-PAGE) that allows fractionating glutenin protein fraction into different molecular mass polymers (Huang & Khan, 1997).

There is slight information about the nature of emulsifier-gluten protein interaction in dough and breadmaking quality, but no reports were found about the aggregation–disaggregation phenomena of gluten structure produced by SSL, DATEM or the blend of both emulsifiers. Therefore, the objective of this research was to investigate changes on gluten matrix induced by SSL and DATEM analyzing the nature of soluble protein aggregates and the percentage of gliadins and glutenins extracted from the different emulsifier-gluten systems.

2. Materials and methods

2.1. Materials

Commercial *Triticum aestivum* wheat flour with 13.2% moisture, 10.7% protein, 0.70% ash, 29.8% wet gluten and 9.8% dry gluten was provided by Molino Campodónico Ltda. mill (La Plata, Argentina). All the composition values, except moisture content, are expressed on dry matter basis.

Emulsifiers Sodium Stearoyl Lactylate (SSL) and Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM) were provided by DANISCO A/S (Copenhagen, Denmark).

2.2. Preparation of gluten samples

Gluten samples were prepared from wheat flour (control sample) or a blend composed by flour and emulsifier: SSL, DATEM or SSL + DATEM (1:1); at 0.25, 0.5 and 1.0% (w/w, flour basis) levels. Samples were codified as: G for native gluten, GS for SSL-gluten, GD for DATEM-gluten and GSD for SSL + DATEM-gluten. Levels of emulsifiers were written after the codified letters. Wheat gluten samples were prepared in the Glutomatic equipment (AACC International, 2000). Distilled water (4.9 mL) was incorporated to 10 g of flour or flour-emulsifier blend and mixed during 1 min. Dough was then placed onto a steel mesh and washed with distilled water until obtaining gluten. Emulsifier levels higher than 1.0% did not allow gluten formation. Gluten samples, prepared in duplicate, were freeze dried, milled and stored at 4 °C until analysis.

2.3. Identification of total gluten proteins by SDS-PAGE

2.3.1. Protein extraction

Gluten proteins were extracted with 0.0625 M Trisma base-pH 10 buffer. Samples were centrifuged at 9350 x g during 15 min at 4 °C. Supernatant was mixed with an equal volume of 0.5 mol/L Trisma base-0.4% w/v SDS-0.01% w/v bromophenol blue-50% v/v glycerol-pH 6.8 buffer. Protein content of extracts was determined by Bradford method (Bradford, 1976). Protein extracts were analyzed by monodimensional 1D (30 mg/mL) and bi-dimensional 2D (50 mg/mL) SDS-PAGE.

2.3.2. 1D- and 2D-SDS-PAGE

One (1D) and two (2D) dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on gluten extracts according to the known Laemmli method. A continuous 12% separating gel and a 4% stacking gel were prepared. A continuous dissociating buffer system containing 0.375 mol/L Tris–HCl, pH 8.8, 0.1% SDS for the separating gel, and 0.025 mol/L Tris–HCl, 0.192 mol/L glycine, 0.1% SDS, pH 8.3 for the running buffer was used. Protein extracts were assayed by 1D-SDS-PAGE, without staining. Each first-dimension slab gel portion was treated with 10 volumes of SDS buffer (62.5 mmol/L Tris–HCl pH 6.8–1% SDS-0.2 mol/L β-mercaptoethanol (β-ME)-20% sucrose) for 30 min at 55 °C with two changes of solution. Treated gels were placed on the top of the second-dimension SDS-slab gel (Puppo, Calvelo, & Añón, 2005). The electrophoresis was carried out at a constant voltage of 200 V. Gels were fixed and stained with 0.1% R-250 Coomasie Brilliant Blue in water/methanol/acetic acid solution (5:5:20) for 12 h and were discolored with water/methanol/acetic acid (65:25:10). Low molecular weight (LMW) markers (Pharmacia calibration kit) of phosphorylase b (94 kD), albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20.1 kD), α-lactalbumin (14.4 kD) were used. Molecular weight of proteins was determined using the Sigma-Gel Software (Jandel Scientific- Version 1.0-1994-1995). Calibration with LMW markers was preformed and the molecular mass of proteins samples was determined by extrapolation from the calibration curve.

2.4. Identification of gluten aggregates by multi-stacking SDS-PAGE

2.4.1. Protein extraction

Protein aggregates present in gluten samples were analyzed by multi-stacking (MS) SDS-PAGE. Proteins were extracted (30 mg/mL) with a 0.125 mol/L Tris-HC-2% w/v SDS-10% v/v glycerol-0.01% w/v bromophenol blue-pH 6.8 buffer, for 2.5 h at 25 °C. Dispersions were heated in boiling water for 3 min (Ribotta, León, & Añón, 2001) and centrifuged at 9350 × *g* for 15 min at 4 °C.

2.4.2. MS-SDS-PAGE

Proteins were separated in a preparative gel (1 mm thickness) composed by several stacking portions of different acryl amide percentages: 4, 6, 8, 10 and 12%; and a continuous gel (14%) (Huang & Khan, 1997). Each gel portion was cut and placed in a test tube. Proteins were eluted for 48 h at 25 °C, using the same buffer described above containing 5% v/v β-mercaptoethanol (β-ME). Gels were heated in boiling water for 10 min. Reduced proteins were analyzed in a 12% continuous gel with a 4% stacking gel (Puppo et al., 2005). The amount of extract loaded in each lane was the same for all samples. The same LMW protein standards described for 1D and 2D-SDS-PAGE electrophoresis were used.

2.5. Identification of gliadins and glutenins

2.5.1. Gliadins

Enriched gliadins fractions were extracted from 100 mg of gluten or gluten-emulsifier system with 1 mL of 50% 1-propanol solution. Dispersions were first stirred during 5 min and then centrifuged at