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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

15700×g for 5 min at 25 °C. Precipitate was reserved for glutenins extraction and supernatant was kept for further RP-HPLC analysis of gliadins.

2.5.2. Glutenins

Enriched glutenins fractions were extracted from the previous precipitate with 1 mL of 50% 1-propanol-1% DTT solution. Dispersions were stirred in the same conditions used for gliadins and centrifuged at 15700×g for 8 min at 25 °C. Supernatant was analyzed by RP-HPLC analysis (Lookhart et al., 2003).

2.5.3. Protein fractionation by RP-HPLC

Enriched protein extracts (gliadins and glutenins) were analyzed by RP-HPLC according to Lookhart et al. (2003) procedure. A Partisil-5 (type C18, 250 x i.d. 4.6 mm) column from Whatman (Whatman Inc., Clifton, NJ, USA) was used. Proteins were eluted at 70 °C with 1 mL/min of a gradient water-acetonitrile 75–25% solution, for 90 min. Proteins were detected at 210 nm with a photodiode UV detector (WATERS model 996, Millipore Corp., Milford, MA, USA). All samples were analyzed at least in duplicate. Peaks were divided in three groups (I, II, III) for gliadins and in two groups (I, II) for glutenins. The area percentage of each group was calculated from the chromatogram.

2.6. Statistical analysis

Results were subjected to a one-way analysis of variance according to the general linear model procedure with least-square means effects. A multiple range test was applied to determine which means were significantly different ($p<0.05$) according to Fisher's Least Significant Differences (LSD). Statistical analysis was carried out using Statgraphics Plus 5.1 software (Statpoint Technologies, Inc., Virginia, USA).

3. Results and discussion

3.1. Gluten protein aggregates

Analysis of the total protein extract was performed by SDS-PAGE. Proteins were extracted with a 0.0625 M Tris-base buffer at pH 10 without denaturing agents, like urea or SDS, to prevent the disruption of emulsifier–protein interaction during extraction. The SSL emulsifier favored the formation of soluble aggregates of high molecular mass (>100 kD) (Fig. 1, arrow). Low proportion of these aggregates in GD and GSD samples was observed.

All profiles also showed the presence of protein bands corresponding to α, β, and γ-gliadins (33–40 kD) and low molecular weight glutenins (LMW-glutenins, 30–40 kD). Proteins in the range of ω-gliadins (ω-gliadins, 50–65 kD) were also observed. Gliadins (α, β and γ) and LMW-glutenins were present in high proportion in GS samples, mainly in GS1 that presented high contents of 52 and 37.5 kD proteins (Fig. 1).

Total protein content (μg/mL) of extracts were 150.3 ± 0.8, 530.1 ± 3.0, 514.2 ± 0.7, 503.2 ± 2.6 for G, GS1, GD1 and GSD1, respectively. In the absence of denaturing and reducing agents as SDS or DTT, a high content of total protein was significantly ($p<0.05$) extracted in gluten prepared with emulsifiers, comparing to control gluten (G), especially with SSL (GS1). These results were in agreement with the more quantity of aggregates solubilised from SSL-gluten samples (Fig. 1). These results suggest that a high level of SSL induce changes in gluten protein structures, leading to a more depolymerised or labile gluten network.

Protein composition of the soluble aggregates was analyzed by bidimensional electrophoresis (Fig. 2). Denaturation and dissociation of aggregates were performed treating protein extracts with SDS and β-mercaptoethanol. Results show that soluble aggregates of molecular mass >100 kD, present in all samples, were formed by monomers of molecular mass between 35 and 67 kD (Fig. 2, arrow). Gluten (G) extracts presented high molecular weight aggregates compound by 67, 65, 61 and 47 kD monomers. Gluten with SSL (GS1) had also 40 and 36 kD monomeric polypeptides (Fig. 2, arrow). All gluten samples contained monomeric polypeptides of 67, 52, 35, 33, 25, 20, 17 and 12 kD. SSL induced the extraction of high proportion of monomers of 52, 35 and 25 kD, and less quantity of proteins of 20, 17 and 12 kD, comparing to G (Fig. 2). SSL dissociates or do not allow the formation of large insoluble aggregates, therefore these polymers are able to be solubilised in the extraction buffer. At the same time, the relative less quantity of peptides of 12 and 17 kD extracted (Fig. 2), suggest that SSL would induce association of these peptides, possibly throughout hydrophobic or ionic bonds. The interaction between high amounts of SSL (1%) and glutenins (HMW and LMW glutenins) via hydrophobic linkages would interfere with the union between these glutenin molecules, leading to the formation of a weakened gluten structure, with more content of soluble polymers, as it was also observed in Fig. 1.

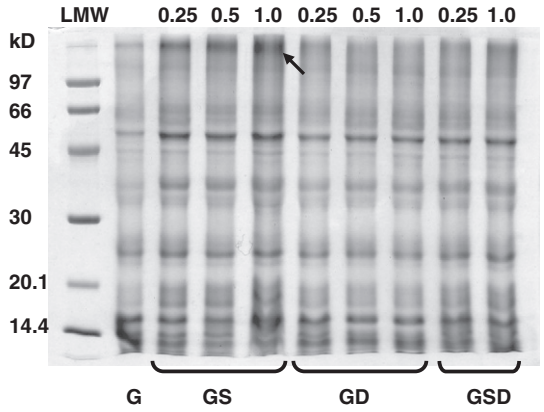

Fig. 1. 1D-SDS-PAGE of total soluble protein extracted from gluten. G: control gluten, GS: gluten-SSL, GD: gluten-DATEM, GSD: gluten-SSL + DATEM. Levels of emulsifiers: 0.0, 0.25, 0.5, 1.0% w/w. Low molecular weight markers (LMW): phosphorylase b (94 kD), albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20.1 kD) and α-lactalbumin (14.4 kD).

Bidimensional electrophoresis profile of gluten prepared with 1% of DATEM (GD1) was similar to that observed for G and GS1; although a great proportion of peptides of 67, 25, 20, 17 and 12 kD were observed (Fig. 2). High molecular mass aggregates (Fig. 2, arrow) were mainly formed by the 67 kD protein, and a high proportion of this monomer was also solubilised. Gluten prepared with the mixture of emulsifiers (GSD1) presented an intermediate behavior of that observed for GS1 and GD1 (data not shown).

Different soluble protein aggregates present in gluten matrix could be separated by a multistaking electrophoresis (MS-SDS-PAGE). Protein aggregates were separated by size and were retained in different stacking gels (4, 6, 8 10, 12, 14%) (Huang & Khan, 1997). After separation, polymers were treated inside the gels with SDS and β-mercaptoetanol in order to cleave non covalent and disulfide linkages. Fig. 3 shows MS-SDS-PAGE profiles of G, GS1 and GD1 samples. Each lane contains monomers that were forming polymers of different size. In order to facilitate interpretation, stacking lanes were divided in three groups: group I (4, 6, 8 and 10%), group II (12%) and group III (14%). Stacking gels of each group presented similar electrophoretic profiles.

High molecular mass aggregates of group I, were formed by subunits of molecular mass between 35 and 105 kD (Fig. 3). Low proportion of high molecular mass polymers (4 and 6%), higher amounts of polymers of intermediate size (8%) and mainly those of low size (10%) were extracted from G. This behavior suggests that large polymers (macro polymers) are insoluble and they are forming gluten matrix.

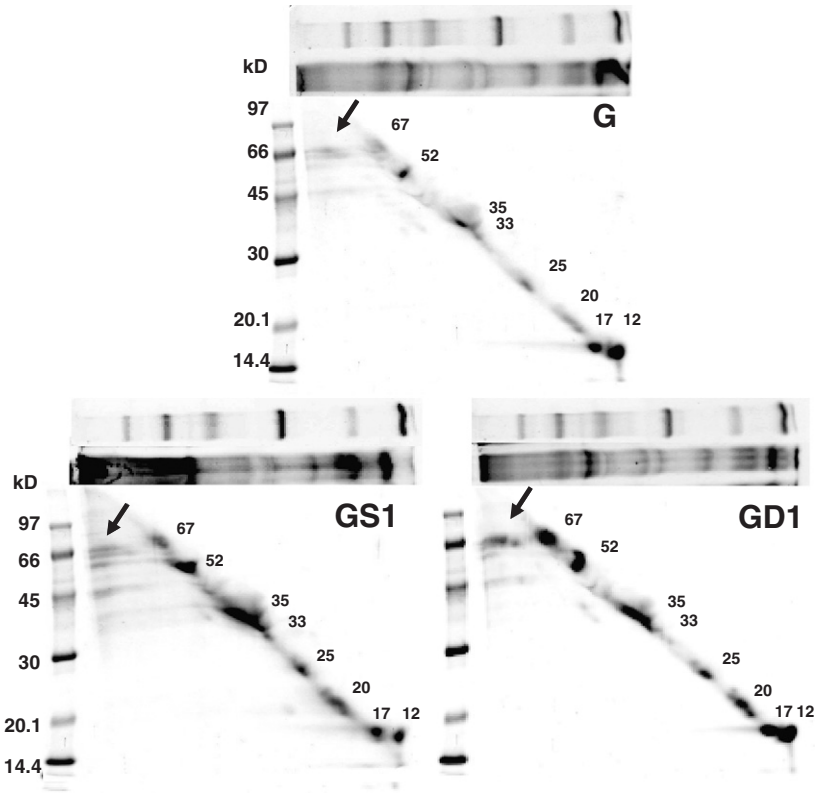

Fig. 2. 2D-SDS-PAGE of total soluble protein extracted from gluten after treatment with SDS and β-mercaptoethanol. G: control gluten, GS1: gluten-SSL 1% w/w, GD1: gluten-DATEM 1% w/w, GSD1: gluten-SSL + DATEM 1% w/w. LMW: Low molecular weight markers.

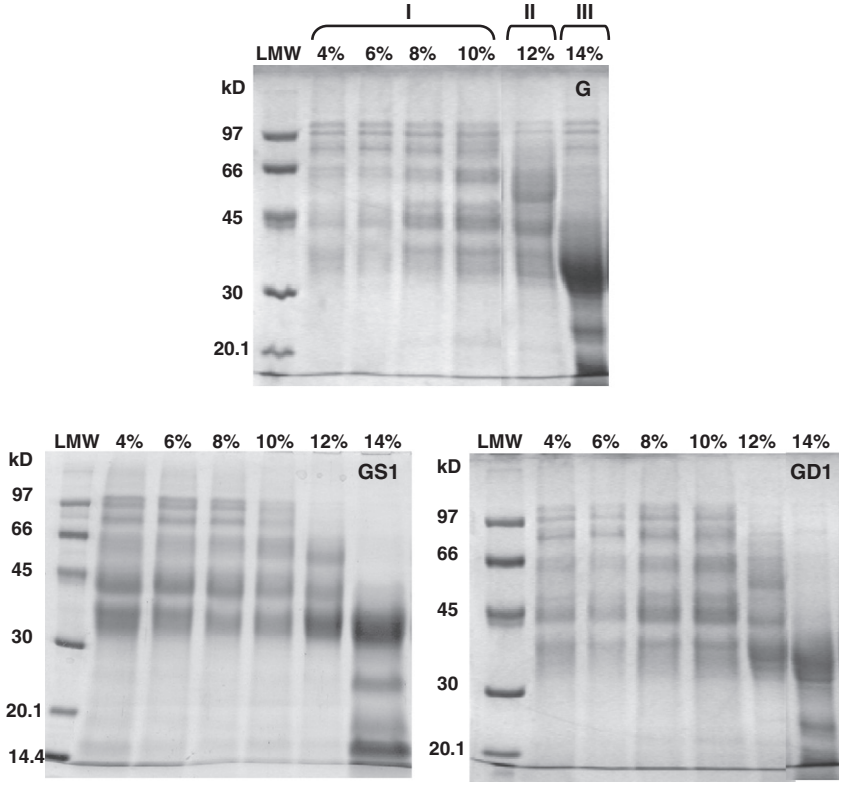

Fig. 3. SDS-PAGE of proteins extracted from gluten samples, separated according to MS-SDS-PAGE and treated with SDS and β-mercaptoethanol. G: control gluten, GS1: gluten-SSL 1% w/w, GD1: gluten-DATEM 1% w/w, GSD1: gluten-SSL + DATEM 1% w/w. I, II, III: stacking lines groups. LMW: Low molecular weight markers.

SSL caused the formation of a gluten network capable to release a great amount of high molecular mass polymers retained in 6% gel and mainly in 4% stacking gel. These polymers were mainly constituted by peptides of 35 and 40 kD and in a less degree with those of 60, 80, 97 and 103 kD. An appreciable amount of polymers of medium size (8 and 10%) was also observed. Proteins retained in 10% stacking gel were mainly conformed by the 35 kD protein, while in G sample these aggregates were composed by proteins higher than 40 kD.

Electrophoretic profile of 1% w/w DATEM-gluten sample (GD1) was similar to that observed for control gluten. These results show that DATEM did not caused structural changes in gluten network capable of releasing high molecular mass aggregates as in the case of SSL.

This behavior suggests a distinct type of interaction of each emulsifier with gluten proteins, with a predominance of bonds of different nature. A more hydrophobic molecule as DATEM would interact via hydrophobic bonds without disrupting gluten network; while the more hydrophilic SSL molecule would link gluten proteins through ionic interactions of its polar residue, allowing the release of soluble polymers from gluten matrix.

Group II profile was also different for all samples. For G sample proteins retained in the 12% stacking gel were composed by the 35 kD, but mainly by those of 42 and 52 kD polypeptides. This last protein band was absent in large polymers (<12%). This protein band of 52 kD would correspond to gliadins (ω-gliadins) or to monomers of LMW-glutenins (D-type) (Gianibelli, Larroque, Macritchie, & Wrigley, 2001; Shewry & Lookhart, 2003). The GS1 gluten sample presented a 12% profile with dominance of the 35 kD protein, suggesting that small polymers formed in the presence of SSL contained a different peptide composition than those of control gluten sample (G). Monomer profile of group II (12% stacking) for DATEM-gluten (GD1) resulted almost similar to GS1 sample, indicating that emulsifiers, in a certain degree, link proteins in the same way; probably through the fraction of the molecule that present similar structure.

Profiles of 14% stacking (group III) resulted similar for all gluten samples, suggesting that in this gel only free monomers of low molecular mass (<45 kD) were retained (Fig. 3). These monomers would not be engaged or were slightly bonded to oligomers, polymers and/or aggregates. They would belong to LMW-GT (30–40 kD) and/or α, β-gliadins (33–40 kD).

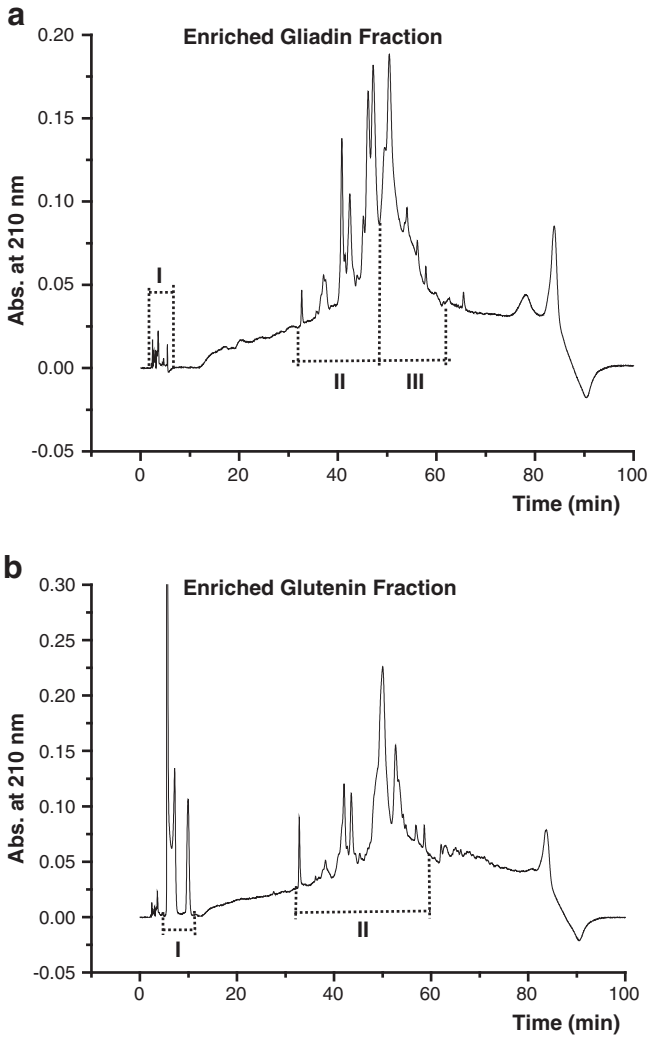

Fig. 4. RP-HPLC profiles of enriched gliadins fraction and enriched glutenins fraction, extracted from gluten samples. I, II, III: different groups of gliadins/glutenins.

3.2. Gluten proteins: Gliadins and glutenins

Enriched fractions containing gliadins and glutenins were extracted from gluten samples and separated by RP-HPLC. Chromatograms are shown in Fig. 4. Three (I, II, III) and two (I, II) groups of different range of retention times were identified in each chromatogram for gliadins and glutenins, respectively. The effect of emulsifier level on gluten structure and consequently in the percentage of area of each group of gliadins and glutenins fractions, was analyzed. Gliadins were separated by RP-HPLC and identified according to their molecular mobility (Lookhart & Albers, 1988; Lookhart, Albers, & Bietz, 1986; Seilmeier & Wieser, 2003; Wieser, 1998). Three groups of gliadins were identified: group I (2–6 min), presumably associated to ω-gliadins; group II (30–45 min) related to α and β-gliadins and group III (45–62 min), corresponding to γ-gliadins (Fig. 4a). In general, percentage of extracted gliadins presented the following trend: α, β > γ > ω. No significant differences in percentage of protein extracts of different type of gliadins between GS1 and control gluten were observed. This different behavior, comparing to the major quantity of soluble aggregates observed in Fig. 1 for GS1, could be attributed to the different type of medium extract for total proteins or gliadin/glutenin fractions. The extraction of proteins of group I was favored in gluten prepared with 1% SSL (GS1) respect to sample with 0.5% SSL (GS0.5) (Fig. 5a), while less amount of the fraction containing α and β-gliadins (group II) was extracted from GS0.5 and GD0.5 (Fig. 5b). Levels of 0.5% w/w of emulsifiers (GS0.5 and GD0.5) allowed a higher quantity extraction of γ-gliadin proteins, respect to G (Fig. 5c). The interaction of emulsifiers (0.5% w/w) with gluten proteins favored the release of higher amounts of low molecular mass-hydrophobic proteins, as γ-gliadins, especially in the case of SSL.

In the case of glutenins, profiles (Fig. 4b) were associated to HMW and LMW glutenins according to Kruger, Marchylo, and Hatcher (1988). Although differences were not significant respect to control sample, a tendency to an increase in protein percentage of HMW-glutenins was detected when DATEM was present in 0.5% in gluten (Fig. 6a). On the other hand, SSL tended to decrease the percentage of HMW glutenins in the extract. Highest and lowest quantity of LMW glutenins (group II, 30–62 min) was extracted from GS0.5 and GD0.5, respectively (Fig. 6b). Results suggest that the more polar emulsifier (SSL) interact with HMW-glutenins mainly via ionic bonds, releasing from gluten matrix the more hydrophobic proteins (LMW-glutenins). The high protein solubility, the type of soluble aggregates formed, and the differential gliadin/glutenin extraction profile, suggest the formation of a more labile gluten network due to SSL action.

Gluten structure was greatly modified by SSL. A major proportion of soluble high molecular mass aggregates was extracted with this emulsifier. The composition of the extracted monomeric proteins was also different for SSL and DATEM. For GS0.5 and

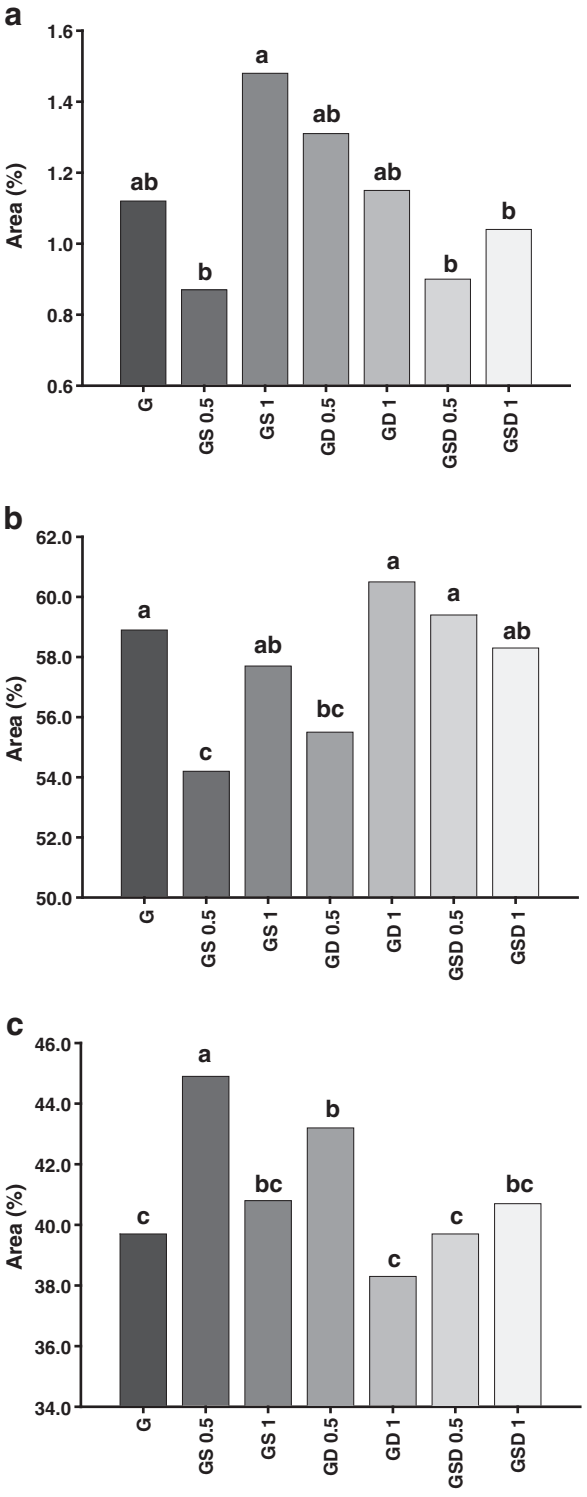

Fig. 5. RP-HPLC peak area of enriched gliadin fraction. a) ω-gliadins, b) α, β-gliadins, c) γ-gliadins. G: control gluten, GS: gluten-SSL, GD: gluten-DATEM, GSD: gluten-SSL + DATEM. Number after codified letters: level (% w/w) of emulsifier.

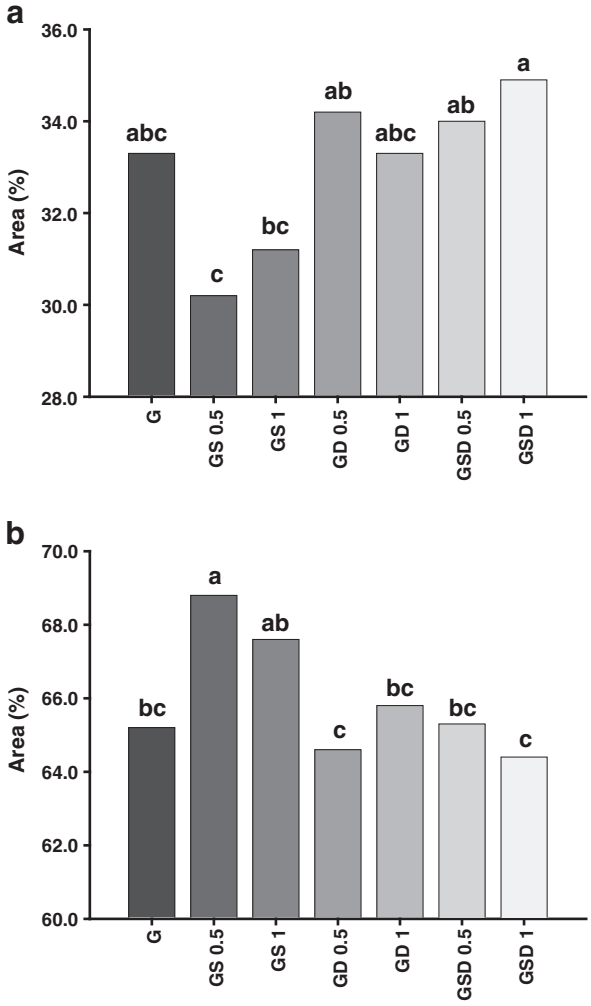

Fig. 6. RP-HPLC peak area of enriched glutenin fraction. **a)** HMW-glutenins, **b)** LMW-glutenins. G: control gluten, GS: gluten-SSL, GD: gluten-DATEM, GSD: gluten-SSL + DATEM. Number after codified letters: level (% w/w) of emulsifier.

GD0.5 samples, a higher and a lower quantities of α,β and γ-gliadins were extracted with 50%-propanol, respectively. In the case of GS0.5, DDT induced a greater extraction of LMW glutenins.

The observed differences suggest that the union of SSL (by hydrophobic and ionic interactions) and DATEM (mostly by hydrophobic interactions) to gluten proteins could be explained by a different mechanism of action of each emulsifier. Gluten proteins have a low charge density due to an approximately equal ratio between cationic and anionic amino acids. Some of the amino acids are hydrophobic and generate hydrophobic regions on gluten matrix surface. At low concentrations (0.25 and 0.5%) the lipophilic tail of the SSL binds to the hydrophobic regions, incorporating the negatively charge of its polar head to gluten matrix. This phenomenon would promote the aggregation of the protein in dough, due to the interaction between the polar head of the emulsifier and the positively charged amino acids of the gluten proteins. The addition of higher amounts of SSL (1%) caused two phenomenons: the unfolding of the gluten protein due to the repulsion generated by an excessive negative charge incorporated to the matrix, and an aggregation of proteins mediated by the hydrophobic interactions as a result of an excess of emulsifier.

Low concentrations of DATEM (0.25 and 0.5%), as in the case of SSL, favored the formation of hydrophobic bonds, while the interactions between the hydrophilic head of DATEM and charged amino acids of gluten proteins would be less effective due to this portion of the molecule acts as a weak acid partially ionized. Hydrophobic interactions led to the formation of a structured gluten network, of low degree of aggregation due to the low proportion of ionic interactions established with this emulsifier. Higher quantities of DATEM (1%) caused a major interaction with gluten proteins due to the greater amount of hydrophobic bounds generated; but no unfolding of gluten proteins as a consequence the low charge repulsion, as it was detected for SSL, was produced.

4. Conclusions

Emulsifiers as SSL and DATEM, widely used in improving bread quality, caused different changes in gluten structure. At the highest emulsifier concentration (1% w/w), SSL produced major degree of disorder than at 0.25% w/w, that was evidenced in a more opened and labile structure that allowed the solubilisation of a great proportion of polymeric aggregates. Those aggregates of high molecular mass (retained in 4 and 6% stacking gels) were formed by the 65 and 67 kD subunits, as well as by those of 45 and 35 kD, the last ones being absent in gluten and DATEM-gluten aggregates. SSL (0.5% w/w) also allowed the extraction of major percentage of γ-gliadins and LMW-glutenins than gluten and DATEM-gluten samples. These results support the evidence of the formation in the presence of 0.5% w/w of SSL of a gluten network stabilized mainly by HMW glutenins with low content of soluble aggregates, suggesting a low degree of structure disruption, while at high levels (1% w/w) a high tendency of depolymerisation was evidenced.

Differences in gluten structure produced by SSL or DATEM could be attributed to the distinct chemical structure of these emulsifier molecules, which condition their hydrophilic/lipophilic balance and therefore the kind of union that they establish with gluten proteins. These differences directly influence structural, rheological and thermal properties of dough, and consequently would behave in a different manner in improving bread quality.

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