FORMATION OF HOMOPOLYMERS AND HETEROPOLYMERS BETWEEN WHEAT FLOUR AND SEVERAL PROTEIN SOURCES BY TRANSGLUTAMINASE CATALYZED CROSSLINKING

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ABSTRACT

The effect of different protein sources (soy flour, lupin flour, egg albumin, gelatin powder, protein-rich beer yeast flour) on wheat dough functionality was tested by determining gluten index, texture properties and Mixolab parameters. Transglutaminase was also added for improving the dough functionality by forming crosslinks. The presence of protein sources induced significant effect on the gluten index, with the exception of lupin flour. Gelatin and the presence of transglutaminase resulted in significant single effects on the texture properties of the wheat-protein dough. All the protein sources tested significantly modified the mixing characteristics of the dough and/or the thermal behaviour, measured by the Mixolab. Capillary electrophoresis studies of the water soluble, salt soluble and glutenin proteins indicated that interactions were mainly within proteins, thus homologous polymers. Scanning electron microscopy studies of the doughs made from blends of wheat and protein sources doughs supported the formation of heterologous structures in the wheat-lupin blends. The combination of TG and lupin would be a promising method to be used on the treatment of insect-damaged or weak flours, to increase the gluten strength.

Key words: protein, transglutaminase, functional properties, wheat dough.

INTRODUCTION

A common practice in food processing is the incorporation of protein ingredients in the product formulation for increasing the product quality, particularly flavour, texture and storage stability. Soybean flour is probably the most widely employed functional ingredient, used, for instance, in ground or emulsified muscle foods (Ramirez-Suarez et al 2003). Soy proteins are macromolecular food ingredients with the ability to form gels, required in many food applications. This gel forming property is considered to be responsible, not only for texture, but also for holding water and other components in the protein three-dimensional network (Furukawa et al 1979). Gelatin, the product of collagen denaturation and hydrolysis, is widely used as a gelling ingredient in food products. The gelatin is a reversibly crosslinked biopolymer network held together predominantly by hydrogen bonded junction zones (Babin and Dickinson 2001). Viscosity of gelatin solutions, gel strength, gelling and melting temperatures, govern its usage. Several authors also reported the usage of lupin flour as an additive to increase the nutritional quality of doughs. Doxastakis et al (2002) and Dervas et al (1999) reported that lupin flour (5% substitution levels from wheat flour) increased the stability and the tolerance index of the dough. Pollard et al (2002) reported that loaf height and structure were maintained when lupin flour substituted wheat flour at levels up to 5%. Finally, it has been reported that addition of lupin flour increases the protein content and total essential amino acids (especially lysine), as well as in vitro digestibility (Mubarak 2001).

However, very often proteins do not meet the requirements for food processing, and additional modifications are necessary. Modification of proteins using diverse enzymes is a promising method to improve the functional properties and nutritive values of currently available food proteins. The incorporation of new protein crosslinks offers a way by which the food industry can modify the functional properties of food without damaging, or even improving the nutritional quality (Gerrard 2002).

Transglutaminase (TG) catalyzes an acyl-transfer reaction in which the γ-carboxyamide groups of peptide-bound glutaminyl residues are the acyl donors. Primary amino groups in a variety of compounds may act as acyl acceptors with the subsequent formation of monosubstituted γ-amides of peptide-bound glutamic acid. ε-Amino groups of lysyl residues in proteins can also serve as substrates, generating intra- or intermolecular ε-(γ-glutamyl)lysyl crosslinks, which are isopeptide bonds (Zhu et al 1995, Jong and Koppelman 2002). TG catalyses the crosslinking of a wide amount of proteins, including those from milk, soy, casein, conoalbumin, lactalbumin, gelatin, myosin, pea legumin or oat globulin (Ikura et al 1980, Larre et al 1993, Babiker et al 1996, Yildirim, M. & Hettiarachchy 1997, Takahashi et al 1999, Siu et al 2002a, 2002b, Nieuwenhuizen et al 2003, Kolodziejska et al 2004, Fan et al 2005). The crosslinking of wheat proteins has been widely investigated in numerous studies, which reported both the biochemical and rheological effects of the enzyme catalyzed reaction on dough (Gerrard et al 1998, Larre et al 1998, Gerrard et al 2000, Larre et al 2000, Gerrard et al 2001, Basman et al 2002a, 2002b, Tseng and Lai 2002, Bauer et al 2003a, 2003b, Mujoo and Ng 2003, Rosell et al 2003, Collar and Bollain 2004, Autio et al 2005). Lately, it has been

suggested that TG in baked products may act upon the gliadin proteins in dough to generate the epitope associated with the celiac response, nevertheless this hypothesis has not been confirmed yet (Gerrard and Sutton 2005). TG has the ability to restore the functional and biochemical properties of damaged wheat or wheat that suffered hydrolysis by proteases (Babiker et al 1996, Bonet et al 2005, Caballero et al 2005). However, the majority of those studies describe the effect of transglutaminase generating crosslinks in homogeneous protein systems.

The crosslinking reaction could be applied to the glutamines and lysines of 2 different types of proteins (Jong and Koppelman 2002). Several authors described indirect evidences of the formation of heteropolymers by TG. Nonaka et al (1997) described the crosslinking between casein and gelatin based on the completely different pH solubility profile of the crosslinking mixture, than that obtained with each protein separately. Yildirim and Hettiarachchy (1997) reported the formation of heterologous and homologous biopolymers from whey protein isolate and soybean 11S globulin.

The aim of the present study was to study the effect of different protein sources on the functional properties of wheat dough and to examine the effectiveness of a microbial transglutaminase as a catalyst for the formation of heteropolymers of wheat and wheat-exogenous proteins. If any of the proteins assessed would show the formation of heteropolymers, it could be possible to improve the rheological properties and nutritive value of doughs.

MATERIALS AN METHODS

Commercial wheat flour was provided by Harinera La Meta (Lérida, Spain). Soy flour, gelatin powder, egg albumin and lupin flour were provided by Bayogar (Madrid, Spain), while protein-rich beer yeast flour was provided by Bispan (Madrid, Spain). Transglutaminase (TG, protein-glutamine gamma-glutamyl transferase EC 2.3.2.13) (100 U/g) was a gift from Apliena SA (Barcelona, Spain). Chemical reagents were purchased from Sigma (St. Louis, MO) and were of the highest purity. Composition of the wheat flour and the different protein sources were determined following the ICC-Standard methods (Table I).

Dough preparation

Doughs were prepared on a 50g bowl Brabender farinograph, previously determining the water absorption and the optimum development time to give a consistency of 500 Brabender Units (BU). Protein sources were tested at 5 levels (0, 1, 5, 10, 20% w/w wheat flour-protein blend basis) and TG was tested at two levels (0, 1% w/w wheat flour-protein blend basis).

Rheological properties

Dough machinability was assessed both by texture profile analysis (TPA) and dough stickiness determination in a TA-XT2i texturometer as described Collar and Bollaín (2005) using the Chen & Hoseney cell. The cohesiveness was measured in the absence of dough adhesiveness by using a plastic film on the dough surface to avoid the distortion induced by the negative peak of adhesiveness (Collar and Bollain 2005).

Mixolab measurements

Mixing and pasting behaviour of the wheat flour dough was studied using the Mixolab (Chopin, Tripette et Renaud, Paris, France) which measures in real time the torque (expressed in Nm) produced by passage of dough between the two kneading arms, thus allowing the study of its physico-chemical behaviour. Rosell et al (2005) reported a detailed description of the equipment and the parameters registered. The instrument allows analysing the quality of the protein network, and the starch behaviour during heating and cooling. For the assays, 50 grams of wheat flour or wheat flour-protein blends (using 10% w/w flour-protein blend basis of the protein sources) were placed into the Mixolab bowl and mixed. After tempering the solids, the water required for optimum consistency was added. Special attention was paid to the determination of the water absorption, in order to ensure the complete hydration of all the components. The settings used in the test were 8 min at 30ºC, temperature increase at 4ºC/min until 90ºC, 8 min holding at 90ºC, temperature decrease at 4ºC/min until 55ºC, and 6 min holding at 55ºC; and the mixing speed during the entire assay was 73 rpm. Two repetitions were made of each blend and control. Parameters obtained from the recorded curve were: water absorption (%) or percentage of water required for the dough to produce a torque of 1.1 Nm, dough development time (min) or time to reach the maximum torque at 30°C, stability (min) or elapsed time at which the torque produced is kept at 1.1 Nm, mechanical weakening (Nm) or the torque difference between the maximum torque at 30°C and the torque at the end of the holding time at 30°C, minimum torque (Nm) or the minimum value of torque produced by dough passage subjected to mechanical and thermal constraints, thermal weakening (Nm) or

the difference between the torque at the end of the holding time at 30°C and the minimum torque, peak torque (Nm) or the maximum torque produced during the heating stage, cooking stability (Nm) calculated as a ratio of the torque after the holding time at 90°C and the maximum torque during heating period, and setback (Nm) the difference between the torque produced after cooling at 50°C and the one after the heating period. In addition, the slopes of ascending and descending torques and the angle between ascending and descending curves were calculated. Then, those angles were used to determine α, β, γ and δ, which correspond to the arc tangent of the four curve angles, respectively. Two repetitions were made for each blend.

Gluten Index determination

Gluten index was determined according to the Approved Method (AACC International 2000). Four repetitions were made of each blend.

High performance capillary electrophoresis analysis

Water soluble (WS) and salt soluble (SS) proteins were prepared following the method described by Bean and Tilley (2003). Blends of flour and exogenous protein (200mg in total) with or without TG were mixed with 100µl distilled water for 5 min, and incubated at 37ºC for 60 min. After the incubation, 900µl of water were added and WS and SS proteins were extracted following the reported procedure (*42*). The final pellet was used for extracting the glutenins following the method described by Bean and Lookhart (1998).

Electrophoretic separations of the proteins were made using a Beckman MDQ instrument. Uncoated fused silica capillaries (Composite Metal Services Ltd,

Worcester, UK) of 50μm i.d. x 27 cm (20 cm L/D) were used for all separations. High-performance capillary electrophoresis (HPCE) of glutenins was performed using 50mM iminodiacetic acid (IDA) in acetonitrile, hydroxypropylmethyl-cellulose (HPMC) and water (20:0.05:79.95, v/v) at 45ºC and 30kV (Bean and Lookhart 2000). The electrophoretic separation of WS and SS flour proteins were performed by HPCE as described Bean and Tilley (2003). Three repetitions were made for each determination.

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to examine the dough structure. After a resting time of 10 min, small dough samples (500mg) containing 20% (w/w, wheat flour- basis) of each protein source with and without 1% (w/w) TG, were fixed with glutaraldehyde 5%(v/v) in phosphate buffer 0.2M pH 7.0 (5ml) during 24h at 4ºC. Glutaraldehyde was decanted and samples were dehydrated using solutions with increasing ethanol concentrations. Finally, acetone (5ml) was added, and dehydration was finished by using a critical point dryer. Dehydrated dough samples were manually fractionated, mounted on stubs and coated with gold in a JEE-400 vacuum dryer (JEOL, Japan) during 2h. Samples were observed with a JSM-5200 (JEOL, Japan) scanning electron microscope with an accelerating voltage of 10kV.

Statistical analysis

Multiple analysis of variance for the identification of all single effects was performed by using Statgraphics Plus V 7.1 Statistical Graphics Corporation, UK).

Fisher's least significant differences (LSD) test was used to describe means with 95% confidence.

RESULTS AND DISUSSION

Wheat gluten quality

The determination of gluten index was used to assess the effect of different protein sources and TG crosslinking activity on the gluten quality. Figure 1 shows the results of gluten index of non-TG-treated and TG-treated flour containing 20% (w/w, wheat flour-protein blend basis) of wheat-exogenous proteins. Each protein tested affected in different extent the quality of gluten. While soy and egg albumin significantly ($p < 0.05$) increased the quality of gluten, the presence of gelatin and protein-rich beer yeast flour decreased this parameter. No effect was induced with the addition of lupin flour.Those differences could not be explained only considering the different chemical composition of the protein sources, therefore also the nature of their proteins might be responsible of the results. In the case of gelatin and protein-rich beer yeast, those protein sources might interfere in the formation of the gluten network yielding a drastic decrease of the gluten index.

Regarding the addition of TG, control samples showed a significant increase ($p < 0.05$) of the gluten index values after the treatment. These results agree with those obtained by Rosell et al (2003) and Bonet et al (2005) when wheat flour was treated with TG. The same effect was observed in the blends of wheat flour and lupin flour, which showed an improvement on gluten quality after the TG treatment, either due to homologous crosslinking within wheat or lupin proteins or the heterologous crosslinking between wheat and lupin proteins. Conversely,

wheat-gelatin blends showed a significant ($p < 0.05$) decrease in the gluten index, which could be related to the deamidation activity of the transglutaminase, making difficult, or even hindering the formation of the gluten network. The differences observed among the protein sources could be attributed to their content in lysine residues and also to the three dimensional structure of the proteins, because some of the lysine residues can be no accessible to the enzyme activity.

Rheological measurements

Figure 2 shows the value of hardness obtained for the texture profile analysis (TPA) of non-TG-treated doughs (A) and TG-treated doughs (B). Doughs containing gelatin powder showed a steady increase of hardness when increasing the percentage of protein, likely due to the viscosity and gelling properties of this protein. The addition of TG did not induce a significant change in the hardness of the wheat-gelatin dough. Doughs were prepared using the optimum water absorption for each blend, thus this result could not be ascribed to different hydration of the compounds; instead some physical interactions between the proteins could be responsible of this behaviour.

Although lupin and soy flours did not significantly modify the hardness of the resulting dough, an increase of dough hardness was detected in the wheat-lupin dough and wheat-soy dough when TG was added. Mugurama et al (2003) reported the improvement of chicken sausage texture by adding soybean and milk proteins modified by TG, due to the formation of network structures that increased the hardness of the sausage gels. In addition, Furukawa et al (1979) and Fan et al (2005) described the crosslinking of soy proteins and its effect on

texture of gels. Therefore, the increase of the hardness values obtained in this study could result either from the crosslinking of the soy or the wheat proteins separately, or from the formation of covalent bonds between heterologous proteins. The absence of effect observed on the gluten index of wheat-soy dough treated with TG drives to consider that covalent bonds would be formed within each protein forming homologous polymers. Regarding to the rest of protein sources, no clear trend was detected on the values of hardness, with the exception of egg albumin, which induced a decrease in the dough hardness, and that effect was not counteracted in the presence of TG.

Table II shows the effect of the different protein sources on the texture parameters of the wheat dough. Gelatin-wheat flour dough showed significantly ($p < 0.001$) lower cohesiveness than the wheat dough, although the trend changed when 20% of the wheat flour was replaced by gelatin. Protein-rich beer yeast flour source significantly ($p < 0.05$) decreased the cohesiveness values by 23% when 20% (w/w) of the wheat flour was replaced. The addition of TG to wheat dough induced a significant ($p < 0.001$) increase on the cohesiveness, which agree with results reported by Collar and Bollaín (2004). Thus, the TG treatment that could involve the formation of high molecular weight homopolymers significantly modified cohesiveness.

Stickiness is also an important factor that affects handling convenience in dough processing. The presence of gelatin significantly ($p < 0.001$) decreased the stickiness of the wheat-gelatin dough by 67% when gelatin replaced 20% (w/w) of the wheat flour. The addition of TG resulted in a significant ($p < 0.001$) decrease of the dough stickiness, which agrees with results reported by Tseng

and Lai (2002) who noted a decrease of 12-22% in the values of stickiness of different types of wheat flour dough after the treatment with TG.

Mixolab results

This instrument measures the behaviour of both the wheat proteins and starch when subjected to a dual mechanical shear stress and temperature constraint (Rosell et al 2005). Therefore, effect of the protein sources and their possible crosslinking by TG on the dough mechanical changes due to mixing and heating could be registered. Figure 3 shows a typical Mixolab curve, in which different stages can be distinguished. Firstly, the initial mixing (8 min) where the hydration of the compounds occurs together with the stretching and alignment of the proteins, bringing about the formation of a three dimensional viscoelastic structure. The interactions between polymeric proteins resulted from disulfide linked polymer proteins and hydrogen-bonding aggregates play the main role in this structure (Aussenac et al 2001). The period of barely constant torque determines dough stability. In the second stage (from 8 to 23 min), the combined effect of the mechanical shear stress and the temperature constraint induced a decrease in the torque due to the beginning of the protein destabilization and unfolding (Rosell et al 2005). As the temperature increases, the contribution of the proteins to the torque is masked by the starch changes (3rd stage). During this stage, the swelling and gelatinization of the starch granules occurs until the physical breakdown of the granules accompanied of a reduction in the torque (4^{th} stage). A further increase in the torque, when the temperature decreases (stage 5^{th}), is associated to the recrystallization of the starch and it has been related to the retrogradation of the starch molecules.

From the chart, it can be calculated four slopes (α, β, γ, δ). The slope α related to the protein weakening during a period of steady temperature rise, β related to the starch gelatinisation, γ related to starch breakdown, and finally, δ related to starch recrystallization during paste cooling.

Data from the Mixolab parameters were submitted to the analysis of variance to determine the single effects of the different protein sources and the transglutaminase (Table III). The single presence of gelatin or lupin significantly increased the water absorption of the wheat dough by 4% and 15%, respectively; whereas the single addition of egg protein source significantly decreased this parameter by 13%. Likely, the nature of the proteins is responsible of this behaviour, since proteins are the component mainly involved in the water adsorption. The addition of these protein sources (gelatin, egg and lupin) induced a significant ($p < 0.001$) increase in the development time or time necessary for hydrating all the compounds. The blends of wheat and lupin or protein-rich beer yeast flour induced a significant reduction of the dough stability. When dough is simultaneously subjected to mechanical shear stress and the temperature constraint, a reduction in the dough torque was produced and with the exception of egg proteins, the presence of different protein sources resulted in a significant increase of the time required to reach the minimum torque. In opposition, the addition of transglutaminase significantly reduced the time to reach de minimum torque, likely the formation of new covalent bonds favours the protein aggregation and unfolding (Schofield et al 1983). The presence of gelatin significantly increased by 7% the temperature at which the minimum torque was reached. Wheat protein aggregation due to heating becomes

evident at 50 °C (Hayta and Schofield 2004), and the largest protein weakening can be modified by the presence of additives (Rosell et al 2005).

Concerning the effect of the different protein sources on the physicochemical changes of starchy compounds, with the exception of egg protein, the wheat-protein enriched blends had a significant reduced peak torque. Wheat- egg dough showed a significant increase in the peak torque. Starch gelatinization is modified with the presence of different additives like hydrocolloids (Rojas et al 1999, Funami et al 2005a, 2005b), less information is available pertaining to the effect of the presence of different proteins. Results show that the increase in the amount of proteins modifies the gelatinization of the starch in dough, where the amount of water is limited. No significant effect was observed on the cooking stability of the dough. The setback or the torque difference during the cooling period was significantly affected by the presence of soy flour or egg proteins. In the case of soy flour, likely the lipid content of the flour affected the amylose retrogradation, whereas the emulsifying properties of the egg proteins might be responsible of this effect.

Studies performed with wheat dough containing different hydrocolloid combinations indicated that their overall effect on the mechanical shearing and thermal treatment of the wheat dough can be studied using the arc tangent of the different slope angles (Rosell et al 2005). The parameter α described the effect of the combination of mechanical shearing and slight thermal treatment on the wheat dough. Whereas the parameters β, γ and δ indicated the behaviour of wheat dough during heating, holding at 90 °C and cooling, respectively, and thus, mainly associated to starch changes. The presence of soy flour only significantly decreased the changes during cooling. Gelatin in the wheat dough

blends induced significant changes in the gelatinization process and during the holding period at 90 °C. Wheat dough enriched with egg proteins resulted in significant changes during the holding period at 90 °C and cooling stage, whereas the presence of protein-rich beer yeast flour significantly modified changes occurred during the holding period at 90 °C.

Dough microstructure determined by SEM

SEM has the potential of examining the structure of the starch/protein in dough matrix. Microscopic analysis are in relation to the results obtained from rheological and biochemical measurements, and could help to discern between homologous and heterologous protein polymers crosslinked by TG. The SEM observations indicate that addition of different proteins to the dough modified it microstructure. Dough treatment with TG evoked significant changes especially in microstructure of protein.

Figure 4 shows the dough micrographs obtained for non-TG-treated (A,C,E,G,I) and TG-treated (B,D,F,H,J) dough samples containing 20% (w/w, wheat flour - protein blend basis) of protein sources. Microstructure of non-TG-treated dough with soy flour addition (Figure 4A) is formed by starch granules, namely, large A-starch granules of lenticular shape and smaller, more spherical B-ones distributed in protein matrix that presents discontinuous as well as heterogeneous character (Rojas et al 2000, Blaszczak et al 2004). The protein matrix demonstrated two different kinds of structures; apart from flat-like porous structures, some protein strands could also be distinguished. Treatment of dough with TG resulted in significant changes in protein microstructure (Figure 4B). These changes were mainly related to formation of more compact and

homogeneous protein network. Basman et al (2002a) reported a better compatibility of soy proteins at the TG active site compared to wheat proteins, which would result in the hindrance of TG-catalyzed crosslinking reaction among wheat proteins. Han and Damodaran (1996) suggested that heterologous crosslinking between two proteins by TG probably depends on the thermodynamic compatibility of the substrate proteins at the enzyme's active site. A lack of differences in microstructure between gluten and soy proteins could result from a fact that TG affected soy proteins during treatment. Increased aggregation of soy gels when treated with TG was reported by Fan et al (2005), who analysed their structure using SEM. Concerning gelatin and egg albumin, in the absence of TG, it was observed heterogeneous and discontinuous protein matrix consisting of gluten and gelatin proteins (Figure 4C) or egg albumin (Figure 4E). TG-treated dough with gelatin addition (Figure 4D) showed fine, filamentous-like structures bound with other coarser ones. More compact and homogenous structure of protein was observed in the case of TG-treated dough with egg albumin.

Structures of gluten, starch granules mixed with yeast cells can be observed in the microscopy pictures of dough with protein-rich beer yeast flour (Figure 4G). After the TG treatment (H), only coarser structures resulted from crosslinking of gluten strands were observed. Autio et al (2005) observed an enhanced protein network when analysed TG-treated wheat dough by scanning electron microcopy. Another effect reported by these authors was that the protein network was unevenly distributed because the protein strands were not extended as much as they were in the control dough. The typical structure of crosslinked gluten was observed on TG-treated doughs when adding protein-

rich beer yeast flour, gelatin and egg albumin. This would indicate the absence of heterologous crosslinks between these proteins and wheat proteins.

Dough with lupin flour addition (Figure 4I) showed a significantly different microstructure compared to the one obtained in the presence of TG (Figure 4J). The structure obtained after the TG crosslinking was not as dense as the one observed with the wheat-soy blends. However, a continuous structure was observed without no longer differentiation between wheat and lupin independent protein structures, which might be attributed to the formation of heteropolymers between these two types of proteins.

HPCE analysis

Figure 5 shows the results obtained by capillary electrophoresis quantification of glutenins, water soluble (WS) and salt soluble (SS) proteins, from blends of wheat flour and 20% (w/w, wheat flour -protein blend basis) exogenous protein sources.

The presence of different protein sources on wheat dough significantly reduced the extraction of the alcohol soluble protein fractions , suggesting the formation of protein aggregates with low solubility in the conditions of glutenin extraction. Except on the control dough, results for glutenin extractability did not show any significant ($p < 0.05$) difference between TG-treated and non-TG-treated samples. The decrease in the extractability of the glutenins from TG-treated control flour was mainly due to the formation of large aggregates between the high molecular weight glutenin subunits (HMW-GS) favoured by the formation of new covalent bonds, and in less extent the formation of some aggregates between the low molecular weight glutenin subunits (LMW-GS) (Larre et al

2000, Gerrard et al 2001, Bauer et al 2003a, Mujoo and Ng 2003, Rosell et al 2003, Bonet et al 2005).

Water-soluble protein fraction significantly increased with the presence of gelatin, albumin or protein-rich beer yeast flour. The addition of TG resulted in a significant ($p < 0.05$) decrease of the WS fraction from dough containing gelatin, egg albumin, lupin flour and protein-rich beer yeast flour, likely due to the crosslinking action of the TG that resulted in the formation of insoluble polymers. The extractability of the SS protein fraction was significantly increase in the presence of gelatin and lupin flour, whereas this fraction decreased when the wheat blends contained soy and protein-rich beer yeast flour. The presence of TG in the wheat dough resulted in an increase of the SS protein fraction, likely the formation of glutenin aggregates catalysed by TG might affect the extractability of the diverse protein fractions. The opposite effect was observed when gelatin was present in the wheat dough. SDS-PAGE studies of wheat-soy blends treated with TG showed a decrease in the relative intensity of protein bands from 7S and 11S of soy, and the gliadins and LMW-GS from wheat, confirming the crosslinking within heterologous proteins (Basman et al 2002a). However, a large incubation period was necessary for the formation of those polymers, since TG showed higher compatibility for the soy proteins (Basman et al 2002a). In the present study, the extractability of the different protein fractions from wheat-soy blends was not modified due to the addition of TG, likely the polymers formed did not change the protein solubility. Concerning gelatin, the presence of TG resulted in a decrease of the WS and SS protein fractions, indicating the formation insoluble aggregates. TG only brought about a reduction of the WS protein fraction from wheat-egg albumin, wheat-lupin and

wheat-protein-rich beer yeast flour blends. Wheat WS proteins, generally regarded as non-dough forming proteins, would be involved in the formation of covalent bonds catalysed by TG.

CONCLUSIONS

From all the protein sources assessed (soy flour, egg albumin, gelatin, protein-rich beer yeast flour and lupin flour) only doughs made with lupin flour seem to form heteropolymers in the presence of TG. Increasing of gluten quality and texture, decreasing of extractability of WS proteins, SEM micrographs, and results obtained from the Mixolab instrument, supported that TG catalyzed heterologous crosslinking on wheat-lupin doughs. Gelatin powder and soy flour blends showed an homologous crosslinking, which would hinder the TG activity on wheat proteins. Likely, the gelatin, a reversible crosslinked polymer prompted to interact within its structure is responsible of that behaviour. Egg albumin and protein-rich beer yeast flour blends showed homologous crosslinking but to a lower extent, which did not affect the rheological properties of doughs. Nevertheless, the addition of soy flour or egg albumin, in the presence of absence of TG also provides certain mprovement of the wheat flour rheological properties. Further studies to determine the specific interaction between these proteins will be undertaken.

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1

2

FIGURE CAPTIONS

Figure 1. Effect of 1% (w/w) TG on the gluten index of wheat flour and protein blends. 20% of the protein sources (w/w, flour-protein blend basis) were added to each dough. Different letters indicate significant ($p < 0.05$) differences between bars.

Figure 2. Effect of TG (1%, w/w) on the hardness of wheat and wheat-protein dough measured by texture profile analysis (TPA). A: non-TG-treated, B: TG-treated. Soy flour (◀), gelatin powder (○), egg albumin (▽), lupin flour (▼), protein-rich beer yeast flour (■).

Figure 3. Typical Mixolab curve showing the α, β, γ and δ slopes related to the protein weakening, starch gelatinisation, starch breakdown and starch retrogradation, respectively.

Figure 4. SEM micrographs (magnification x2000) of wheat–protein dough samples containing 20% (w/w, wheat-protein blend basis) of different protein sources in the absence of TG treatment (A: soy, C: gelatin, E: egg albumin, G: protein-rich beer yeast flour, I: lupin) and their counterparts in the presence of TG treatment (B: soy, D: gelatin, F: egg albumin, H: protein-rich beer yeast flour, J: lupin).

Figure 5. Extractability of glutenins, water soluble (WS) and salt soluble (SS) proteins, measured by HPCE on doughs with 20%(w/w) of wheat-exogenous protein sources and with or without TG 1% (w/w). Different letters indicate significant ($p < 0.05$) differences between bars.

1 **Table I**. Composition (%) of the wheat flour and the protein sources tested in
2 this study.
3

	Wheat flour	Soy	Gelatin	Egg albumin	Lupin	Yeast
Moisture	14,2	6,5	11,0	6,5	7,3	4,2
Protein	9,9	37,8	95,5	81,4	38,3	47,4
Lipid	1,2	22,2	0,0	0,2	8,1	3,7
Ash	0,7	4,2	1,7	3,3	2,7	6,9
Carbohydrates[a]	74,0	29,3	-	8,6	43,6	37,8

4 [a] Calculated by difference.

Table II. Effect of different protein sources on the texture properties of the resulting wheat-protein dough determined with the texturometer.

TPA parameters	Overall mean	Level	soy	gelatin	egg albumin	lupin	yeast	TG
Cohesiveness	0.443	0		0.399***			0.516**	0.382***
		1		0.408			0.523	0.504
		2		0.362			0.440	
		3		0.436			0.395	
		4		0.608			0.341	
Stickiness (g)	15.2	0		33.4***				21.6***
		1		21.8				8.8
		2		10.1				
		3		5.6				
		4		5.0				
Hardness (g)	2787	0	2685**	1055***				2547***
		1	2431	1445				3027
		2	2543	2233				
		3	2838	4078				
		4	2435	5127				

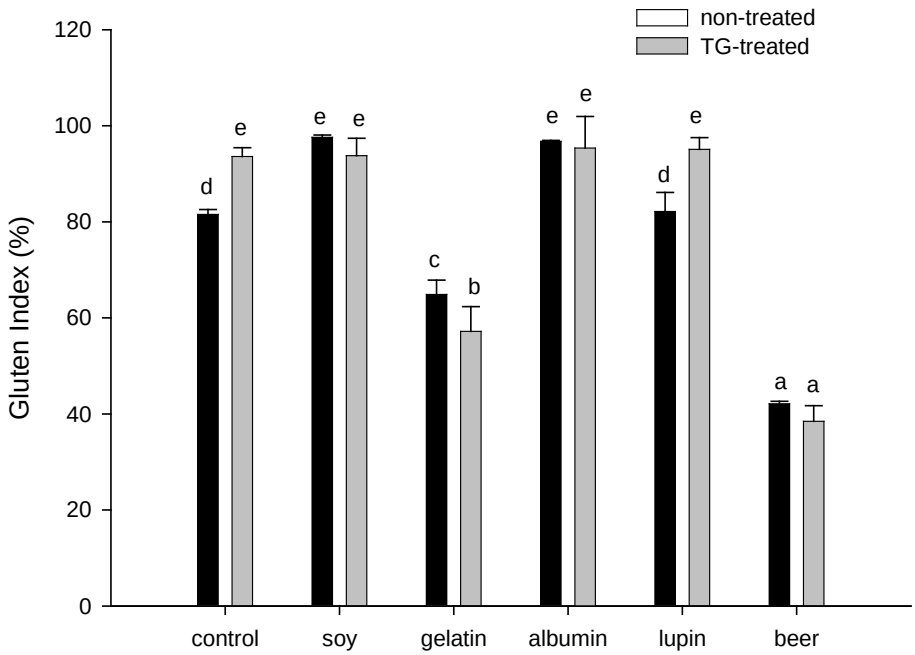
* p<0.05; ** p<0.01; *** p<0.001.

Table III. Effect of different protein sources on the thermo-mechanical properties of the resulting wheat-protein dough determined with the Mixolab.

Mixolab parameters	Overall mean	Level	soy	gelatin	egg	lupin	yeast	TG
Water absorption (%)	55.8	0		54.8**	59.5***	51.8***		
		1		56.8	52.0	59.8		
Development time (min)	5.2	0			3.8***	3.8***	4.3***	
		1			6.7	6.7	6.2	
Stability (min)	2.0	0				3.7*	4.1*	
		1				0.2	-0.1	
Time to minimum (min)	19.9	0	19.6**	19.0***		19.6**	19.4**	20.0*
		1	20.1	20.7		20.2	20.4	19.7
Minimum torque (Nm)	0.18	0		0.29**			0.22*	
		1		0.08			0.15	
Temperature at minimum (°C)	60.5	0		58.5*				
		1		62.4				
Protein weakening (Nm)	0.57	0					0.68*	
		1					0.47	
Temp at peak torque (°C)	80.3	0	82.2**		79.0*			
		1	78.4		81.6			
Peak torque (Nm)	1.30	0	1.45*	1.76***	0.84***	1.45*	1.44*	
		1	1.15	0.83	1.75	1.15	1.15	
Torque at 85 °C (Nm)	1.34	0	1.53*	1.73***	0.82***	1.52*		
		1	1.15	0.94	1.86	1.16		
Cooking stability (Nm)	0.93	0						
		1						
Setback (Nm)	0.55	0	0.68*		0.23***			
		1	0.43		0.87			
α (º)	-71.4	0						
		1						
β (º)	45.6	0		52.6**				
		1		38.6				
γ (º)	17.3	0		10.3*	1.8***		10.6*	
		1		24.3	32.7		23.9	
δ (º)	16.4	0	20.3*		7.6***			
		1	12.4		25.1			

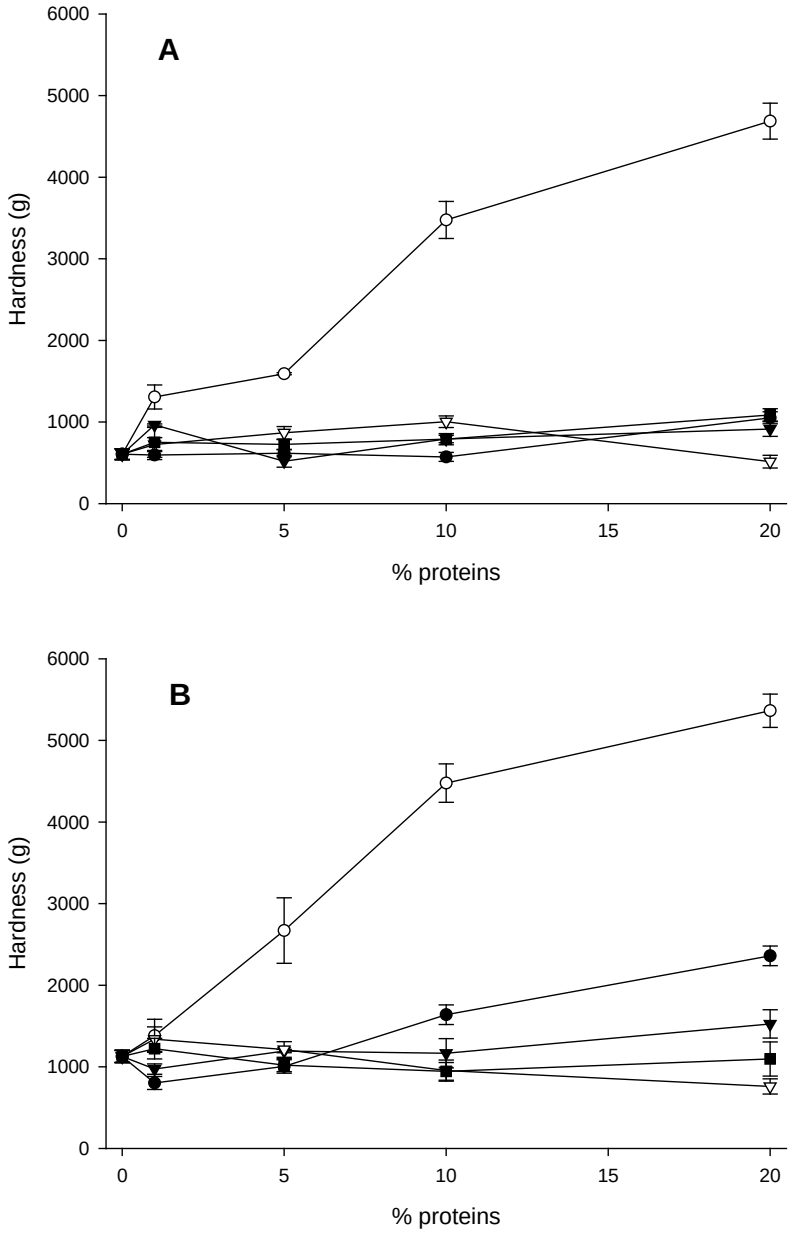
p<0.05; ** p<0.01; *** p<0.001.

Figure 1


non-treated
TG-treated
Gluten Index (%)
120
100
80
60
40
20
0
d
e
e
e
c
b
e
e
d
e
a
a
control
soy
gelatin
albumin
lupin
beer

1
2

1 Figure 2



2
3
4

Figure 3

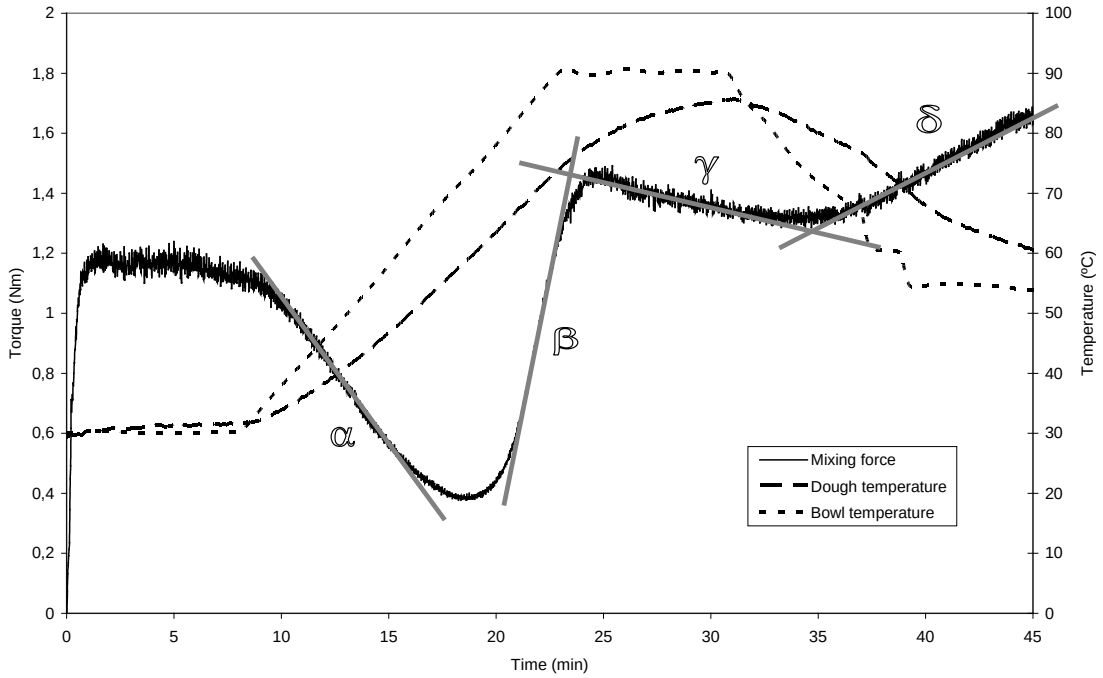
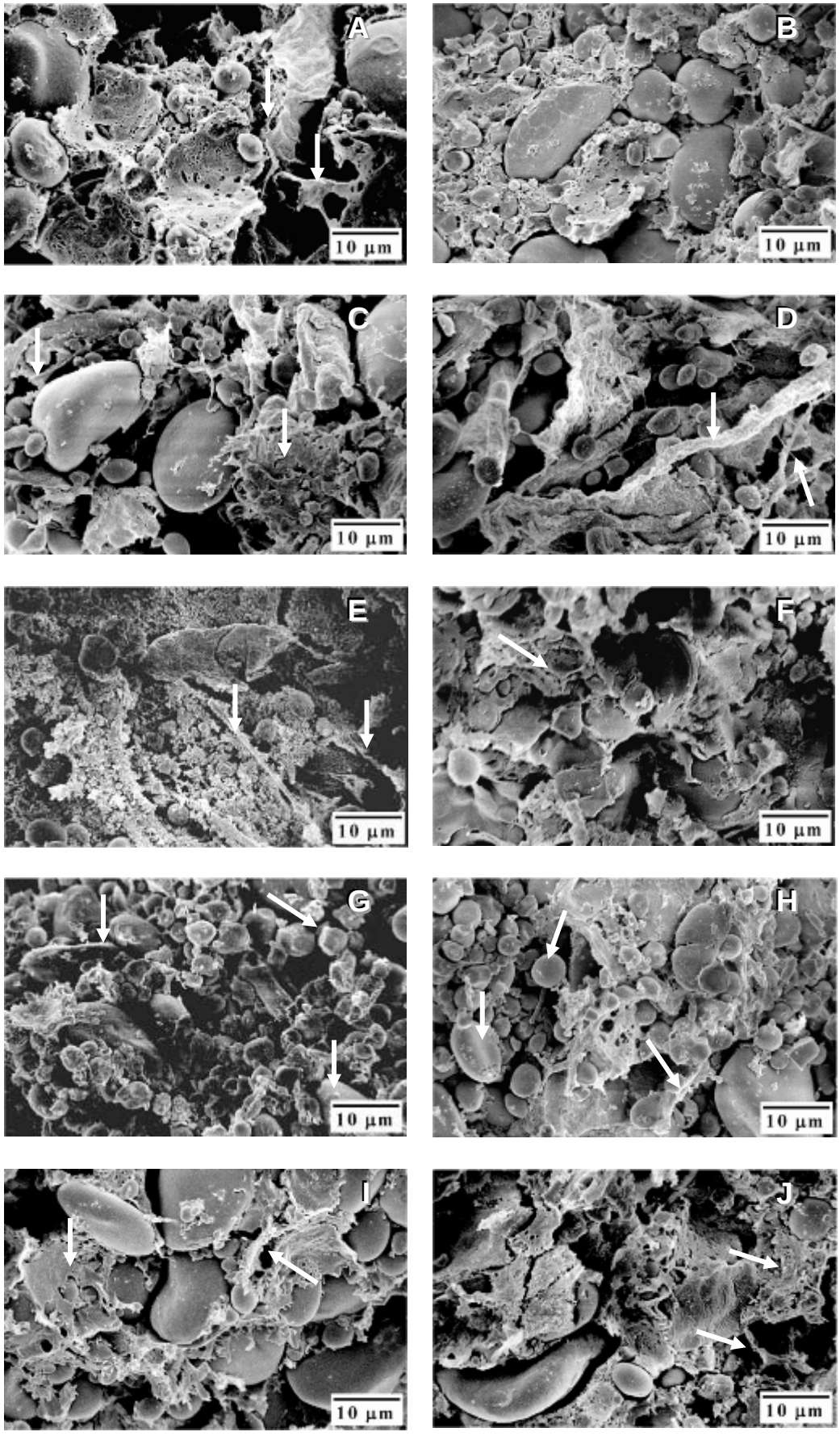
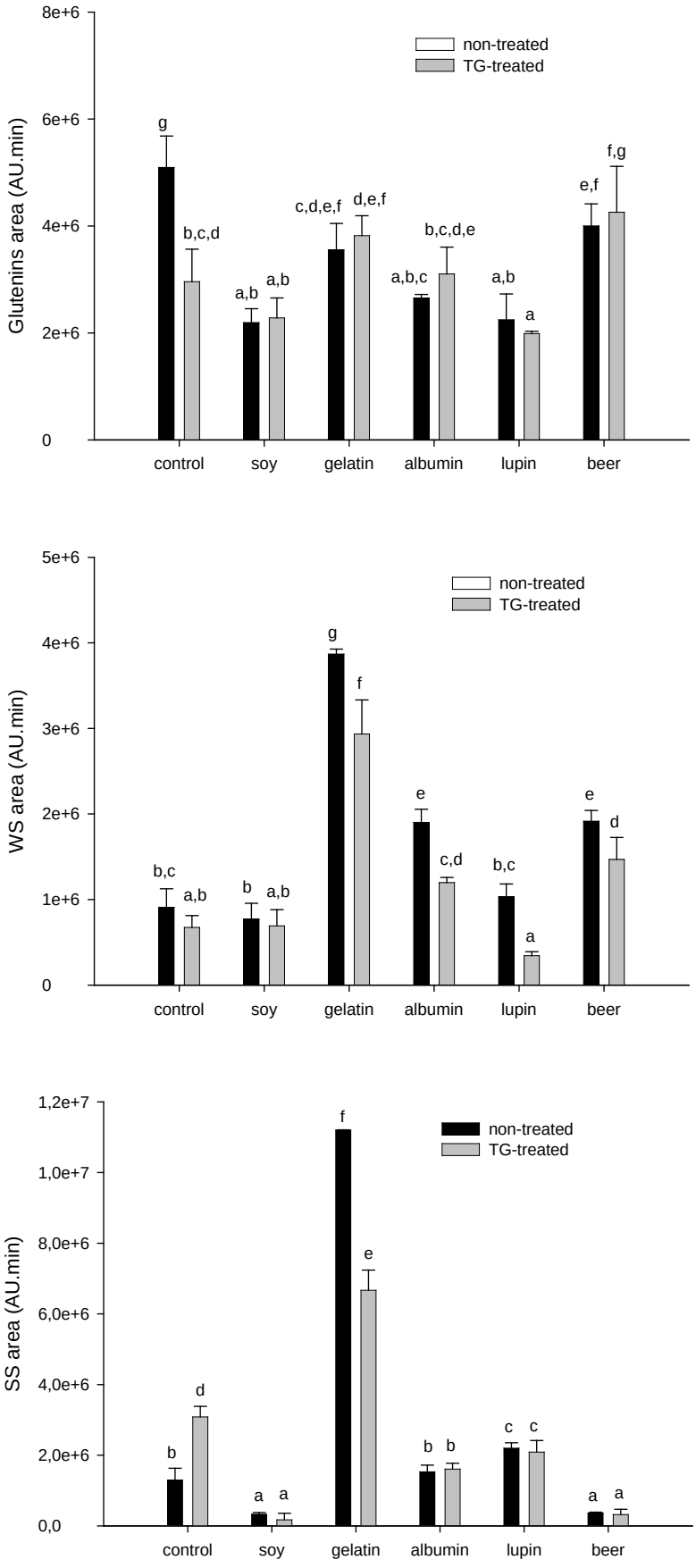

Torque (Nm)
Temperature (°C)
Time (min)
α
β
γ
δ
Mixing force
Dough temperature
Bowl temperature

Figure 4


A
10 µm
B
10 µm
C
10 µm
D
10 µm
E
10 µm
F
10 µm
G
10 µm
H
10 µm
I
10 µm
J
10 µm

1 Figure 5


Glutenins area (AU.min)
8e+6
6e+6
4e+6
2e+6
0
non-treated
TG-treated
g
b,c,d
a,b
a,b
c,d,e,f
d,e,f
a,b,c
b,c,d,e
a,b
a
e,f
f,g
control
soy
gelatin
albumin
lupin
beer
WS area (AU.min)
5e+6
4e+6
3e+6
2e+6
1e+6
0
non-treated
TG-treated
b,c
a,b
b
a,b
g
f
e
c,d
b,c
a
e
d
control
soy
gelatin
albumin
lupin
beer
SS area (AU.min)
1,2e+7
1,0e+7
8,0e+6
6,0e+6
4,0e+6
2,0e+6
0,0
non-treated
TG-treated
b
d
a
a
f
e
b
b
c
c
a
a
control
soy
gelatin
albumin
lupin
beer

2