EFFECT OF TRANSGLUTAMINASE ON THE PROTEIN ELECTROPHORETIC PATTERN OF RICE, SOYBEAN AND THEIR BLENDS

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ABSTRACT

The interactions taking place in composite dough containing rice flour and soybean proteins (5%, w/w) in the presence of transglutaminase, an enzyme with crosslinking activity, were studied by using different electrophoretic analysis. The interaction between rice proteins and soybean proteins was intensified by the formation of new intermolecular covalent bonds catalysed by transglutaminase and the indirect formation of disulfide bonds among proteins. The main protein fractions involved in those interactions were both β-conglycinin and glycinin of soybean and the glutelins of the rice flour, although albumins and globulins were also crosslinked. The addition of soybean proteins to rice flour improves the amino acid balance and also they might play an important role on the rice dough properties since soybean proteins interact with rice proteins yielding protein aggregates of high molecular weight.

Key words: rice, proteins, soybean, transglutaminase, electrophoresis.

INTRODUCTION

Rice, due to its properties, is an appropriate cereal to elaborate gluten free cereal products (Gujral et al 2003a, 2003b). The protein content of rice is lower than any other cereals. Rice flour has a protein content around 7.0-8.5%, compared to the 10-15% found in wheat flour. In addition, from the nutritive point of view, rice, as the rest of the cereals, is deficient in lysine, an essential amino acid for the human diet. The combination of cereal proteins with legume proteins has been used for increasing their nutritive value (Ribotta et al 2005). Rice proteins are deficient in lysine but high in methionine, while legume proteins are high in lysine and deficient in methionine, thus they are complementary regarding the essential amino acids (Wolf 1970). The combination of rice and soybean would result in gluten free products with better amino acid balance.

The use of rice to obtain fermented products has been very limited because rice proteins are unable to develop gluten, mainly due to the nature of its proteins. The major storage proteins in wheat are prolamins or gliadins (40%) and glutenins (46%) (Orth and Bushuk 1972). These proteins are the main compounds of the gluten, which confers the viscoelastic properties necessary for the dough expansion during the fermentation. Conversely, the major storage proteins in rice are the glutelins (65-85%) while prolamins are the minor fraction (Huebner et al 1990).

Soybean is used in food technology for supplying desirable functional properties such as emulsification, fat absorption, moisture holding capacity, thickening, and foaming (Wolf 1970). However, Ribotta et al (2005) reported that soy compounds interfere in the gluten formation, decrease dough strength, and diminish dough gas retention capacity, in consequence decrease the bread quality. The negative effects of the soybean might be related with the type of interaction between soy and gluten proteins (Bonet et al 2006, Marco and Rosell, 2008), although it can be improved by a physical modification

of soy flour (Maforimbo et al 2006). In the case of gluten free bread, the addition of active soybean flour can improve the bread volume, either due to the role of its proteins or the enzyme activities present in the soybean flour (Ribotta et al 2004). The main storage proteins in soybean are globulins, which show two major fractions: 7S or β-conglycinin and 11S or glycinin. Despite the gene of soybean glycinin is derived from common ancestor gene of rice glutelins (Utsumi 1992), rice proteins lack of the functional properties of the soybean proteins. This behaviour is assigned to the way rice glutelins polymerise through disulfide bonds and hydrophobic interactions yielding very large macromolecular structures (Utsumi 1992). Rice and soybean proteins lack of good baking properties, but probably as they have complementary amino acids profile, some interactions between them could led to a better network.

Transglutaminase (TG) is an enzyme that catalyses the reaction between an ε-amino group on protein-bound lysine residues and a γ-carboxyamide group on protein-bound glutamine residues, leading to the covalent crosslinking of the proteins. This is the predominant reaction, but TG also catalyses two other reactions: in the presence of primary amines, TG crosslinks the amine to a γ-carboxyamide group on protein-bound glutamine residues. In the absence of amine substrates, TG catalyses the hydrolysis of the γ-carboxyamide group of glutamine, resulting in deamidation. The transglutaminase has been used for improving the baking quality of the weak and/or insect damaged wheat flours (Caballero et al 2005, Bonet et al 2005), bringing about an improvement in the texture of the loaves. In addition, some improvement in the rice protein functionality was observed when they were crosslinked with transglutaminase (Gujral and Rosell 2004, Marco and Rosell 2008). Therefore, the use of TG might crosslink rice and soybean proteins to develop a structure similar to the network of gluten. The aim of this study was to understand the interaction between rice and soybean proteins and the

crosslinking effect of transglutaminase on the rice-soybean blends by quantifying the proteins content and separating them by using the electrophoresis under different conditions.

MATERIALS AND METHODS

Commercial rice flour from Harinera Belenguer SA (Valencia, Spain) was used in this study. The rice flour had moisture, protein, lipid and ash contents of 13.4, 6.5, 0.7 and 0.5%, respectively. The moisture, protein, lipid and ash contents were determined following the AACCI Approved Methods No 44-19, No 46-13, No 30-25 and No 08-01, respectively (AACCI 1995). Soybean protein isolate was from Trades SA (Barcelona, Spain). The protein isolate had moisture, protein, lipid, ash and carbohydrates (calculated by difference) contents of 6.9, 80.8, 0.2, 3.6 and 8.5%, respectively. Microbial transglutaminase of food grade (Activa™ TG) (100 units/g) was provided by Apliena, S.A. (Terrasa, Barcelona, Spain). All reagents were of analytical grade.

2.1 Rice dough preparation

The dough was made in a 50g bowl Farinograph (Brabender, Germany), mixing 50 g of rice flour with 90% of water (flour basis, corrected to 14% moisture basis) for 15 min. When soybean protein isolate was present, rice flour was replaced by 5% (w/w, flour-protein blend basis) protein isolate. The effect of TG was studied by adding 1% (w/w, flour-protein blend basis) TG. The resulting dough was used for the determination of the protein content and the rest of the dough was frozen and freeze-dried.

2.2 Protein quantification

The protein fractions from the doughs were extracted following a sequential extraction with different solvents. Albumin-globulin extraction was carried out by adding 100 mL of 5% (w/v) NaCl to 20 g of dough, the suspension was homogenized for five minutes and

then centrifuged at 5,500 x g for 10 min. After albumin-globulin extraction, the alcohol soluble fraction was extracted from the residue adding 100 mL of 50% (v/v) 1-propanol, following the same procedure as was described for the albumin-globulins. The insoluble proteins were extracted with 100 mL of 0.1N NaOH containing 0.5% (w/v) sodium dodecyl sulfate (SDS) and 0.6% (v/v) β-mercaptoethanol (ME) (Sugimoto et al 1986, Ju et al 2001). Each extraction was repeated twice in order to increase the protein extraction and the supernatants were collected. Protein contents in the doughs and protein fractions were determined following the micro-Kjeldahl method approved by the AACC (No 46-13) (AACCI 1995), using 5.95 as the protein conversion factor.

2.3 SDS-PAGE protein electrophoresis

Electrophoresis was carried out under non reducing and reducing conditions in order to determine differences due to the presence of disulphide bonds. For total protein extraction under non reducing conditions, a buffer containing 0.063 M tris(hydroxymethyl)aminomethane (Tris/HCl, pH 6.8), 2% (w/v) SDS, 10% (v/v) glycerol and 0.01% (w/v) bromophenol blue) was added to freeze-dried dough (1 mL buffer/50 mg of rice dough and 1 mL/30 mg of rice-soybean blend) and soybean protein isolate (1 mL /5 mg). Suspensions were vortexed for 2.5 hours, heated in a boiling-water bath for 5 min and cooled at room temperature. Then, they were centrifuged at 400 x g, for 5 min. The proteins remained in the supernatants. Sequential extraction of the different protein fractions was made using the solvents previously described. Under reducing conditions, the procedure was as in the non reducing conditions, but the buffer solution also contained 3% (v/v) β-mercaptoethanol.

Supernatants extracted under non reducing conditions were used for simple SDS-PAGE and multistacking SDS-PAGE (preparative and analytical). The supernatants extracted under reducing conditions were used only for analytical SDS-PAGE.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 12% separating gels with 4% stacking gels according to Laemmli (1970). In multistacking electrophoresis (Khan and Huckle 1992), two stacking gels of 4 and 8% (w/v) acrylamide concentration (0.108 and 0.216% w/v bisacrylamide concentration, respectively), and one resolving gel of 12% (w/v) acrylamide (0.48% (w/v) bisacrylamide), were prepared. Gels of 0.75-mm width were prepared for analytical purposes, and gels of 1.5-mm width were used for preparative analysis. A Mini Protean II Slab Cell (Bio-Rad Laboratories, Richmond, CA) vertical unit was used**.** The standard proteins were from Bio-Rad (Low range, Bio-Rad Laboratories, Hercules, USA) and consisted of phosphorilase b (97,000), bovine serum albumin (66,000), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500) and lysozyme (14,400). The gels were stained with 0.25% Coomassie Brilliant Blue R in methanol:water:acetic acid (4:5:1, v/v/v) and were de-stained in the same solvent excluding the dying reagent. The gels from preparative multistacking were not stained, instead, they were cut initially into three pieces (corresponding to the different acrylamide concentration) that were separately submerged into buffer solution containing ME, then they were vortexed for 48 hours at ambient temperature. The resulting mixtures were placed in a water bath at 100 °C for 10 min. Protein composition was analyzed by SDS-PAGE (stacking gel of 4% (w/v) acrylamide and resolving gel of 12% (w/v) acrylamide). Runs were performed in the same equipment as described above. Gels were quantified using an Image Master VDS (Pharmacia Biotech, USA) equipped with Image MasterVDS software (Pharmacia Biotech, USA) providing the integrated optical density (IOD) values.

Statistical analysis

Multiple sample comparison was used for the statistical analysis of the results (Statgraphics Plus 5.1, Statistical Graphics Corporation, UK). Fisher's least significant differences (LSD) test was used to describe means with 95% confidence.

RESULTS AND DISCUSSION

Quantification of the protein fractions

In order to determine the protein fractions affected by the crosslinking activity of the TG, the protein content in each protein fraction was determined by the Kjeldahl method. As it was expected, the glutelins were the major protein fraction in rice, representing about 77.8% of the total proteins (Table 1). The content in salt soluble proteins (albumin-globulin) was 15.5% and the alcohol soluble protein (prolamin) content 4.3%. Those values fall within the results reported previously (Gorinstein et al 1999, Ju et al 2001). When 5% (w/w) soybean protein isolate was blended with rice flour, the proportion of salt soluble proteins increased up to 18.0%, thus soybean proteins were mainly extracted with the albumin-globulin fraction. The amount of alcohol soluble proteins decreased as a consequence of the rice protein replacement by soybean, whereas SDS soluble fraction showed barely the same value, indicating that soybean proteins were partially extracted with the SDS solvent overcoming the dilution effect. The major part of the soybean proteins has been classified as globulins (Wolf 1970, Gorinstein et al 1999), the increase observed in the SDS soluble fraction might be ascribed to the production process of the protein isolate, since it may cause physical and chemical changes and, therefore, they may affect the solubility of the proteins (Arrese et al 1991).

In the presence of TG, a decrease in the extraction of the albumin/globulin and glutelins and an increase in the residue content were observed. The more affected fractions were the salt soluble proteins in both samples (rice and rice-soybean blends) and the SDS soluble proteins in the rice-soybean blend. Similar behaviour was

obtained when pea proteins were enzyme crosslinked with rice flour proteins (Marco et al 2007). Likely, the crosslinking action of the TG induced changes in the extractability of the proteins. The extent of the TG effect was higher in the rice-soybean protein blend, where the residue increased from 1.4% in absence of TG to 27.7% after TG treatment. The rice-soybean protein blend was better substrate for the TG than the rice alone, which could be related with the lysine content that is necessary for the crosslinking reaction catalyzed by TG. Rice has lysine values of about 4.0g/16.8g N (Cagampang et al 1976) compared to the soybean that has lysine values of about 6.0g/16.8g N (El-Moniem et al 2000), and in rice the lysine amino acids are mainly concentrated in the albumin fraction (Villareal and Juliano 1978).

SDS-PAGE analysis

SDS-electrophoresis was performed in different conditions for evaluating the nature of the interactions between proteins from rice and soybean due to the transglutaminase activity. The SDS-PAGE analysis was performed under non-reduced and reduced conditions. When electrophoresis was performed in nonreduced conditions (without ME), two major bands at 53,700 and 22,400 (molecular weight obtained from the densitometric analysis) were found in the rice sample and also some polypeptides of high molecular weight that were unable to enter the stacking and separating gels (Figure 1a, lane 2). In the presence of TG, a decrease in the intensity of the protein bands was observed, with the exception of a band about 35,300, which showed a great increase in the intensity (from 554 to 1193 IOD units) after the TG treatment (Figure 1a, lane 3). Probably, the formation of a new polymer due to the crosslinking reaction produced this increase in the band at 35,300.

Soybean protein isolate, showed the characteristics bands of this legume: α', α and β subunits at 85,000, 76,200 and 51,400 corresponding to the β-conglycinin, and A and B

subunits at 37,400 and 22,500 corresponding to the glycinin (Figure 1a, lane 6) (Ribotta et al 2005, Tang et al 2006). The lane of the rice flour-soybean protein blend (Figure 1a, lane 4) did not show the band corresponding to the A glycinin subunit of soybean, probably due to it is prone to interact with other proteins. In the presence of TG this sample also showed a decrease in the intensity of the bands (Figure 1a, lane 5). The α′ (MW 86,800) and α (MW 79,000) subunits corresponding to the β-conglycinin did not appeared after TG treatment and again the band of about 33,700 showed higher intensity than that without TG treatment, likely due to the aggregation of small polypeptides. Soybean has been reported as a good substrate for TG, giving better results than wheat and barley (Basman et al 2002). The crosslinking by TG involves both β-conglycinin and glycinin of soybean, mainly affecting the acidic subunits of the β-conglycinin, because the basic subunits are not readily accessible for the transglutaminase (Tang et al 2006). In addition, the treatment with TG induced a decrease in the protein located on the top of the stacking and separating gels. Protein extractability can be reduced due to both the protein crosslinking catalysed by TG or the indirect formation of disulfide bonds, because the crosslinking reaction may bring near the sulphur containing amino acids, making easier the formation of these bonds (Gujral and Rosell 2004, Marco et al 2007).

Under reducing conditions, a higher number of bands corresponding to rice and soybean proteins was observed (Figure 1b). In the rice sample, the intensity of the band of about 53,000, which was the major band in non-reducing conditions, showed a great decrease when electrophoresis was performed in the presence of ME, indicating the existence of disulfide bonds in that fraction (Figure 1b, lane 2). The three major protein bands of the rice appeared at 15,100, 22,300 and 32,700 that are very close to those reported by Steenson and Sathe (1995) in the *Basmati* rice (14,500, 20,400 and

33,100) and by Villareal and Juliano (1978) in *Indica* rice (16,000, 25,000 and 38,000). Regarding to rice-soybean proteins blends (Figure 1b, lane 4), it was possible to observe the band corresponding to the A glycinin subunit, which was not observed under non-reducing conditions, confirming the interaction of this subunit by disulfide bonds yielding polymers with higher molecular weight. In rice and rice-soybean blend samples, the TG activity promoted an increase in the protein unable to enter the stacking and separating gels and also a decrease in the intensity of the bands, with the exception of the band around 32,700 in the rice sample and the bands around 34,700 and 23,400 in rice-soybean blend, where the relative intensity showed an increase in the presence of TG (Figure 1b, lanes 3 and 5), again probably due to the aggregation of small polypeptides. The more affected bands by TG were those of MW about 76,800 and 65,700 (α and α´ conglycinin subunits**)**, 51,400 (β conglycinin subunit), and 37,900 (A glycinin subunit) in rice-soybean blends. Those bands were not present after TG treatment, what means the involvement of these proteins in the crosslinking reaction. Several authors have studied the crosslinking between glycinin and conglycinin (Ikura et al 1980) and also with other globular proteins (Yildirim et al 1996), describing that both soybean proteins were able to form polymers through covalent bonds catalysed by transglutaminase and that the crosslinking of glycinin proceeded faster than that of conglycinin (Ikura et al 1980). In addition, the basic subunits of glycinin remain almost intact after TG treatment, while the acidic subunits are the most affected proteins (Ikura et al 1980, Tang et al 2006). This difference between the reactivity of the acidic and basic subunits from glycinin could be related with the native structure of glycinin, since the basic subunits of glycinin are buried in the interior of hexamers of glycinin, what could difficult the access of this subunit to the active site of the TG (Nielsen 1985, Tang et al 2006). In addition, compared to the acidic polypeptides, the basic polypeptides

278 contain relatively low levels of glutamine and lysine, the necessary amino acids for the TG crosslinking reaction (Nielsen 1985).

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The extent of the TG effect in each protein fraction, obtained from a sequential 282 extraction, was determined. TG promoted an increase in the band intensity on the top of stacking and separating gel in the albumin-globulin fraction of the rice sample 284 (Figure 2a, lane 3). Conversely, the band of about 90,800 disappeared after TG treatment and a decrease in the intensity was observed in the bands with lower MW. 286 The trend of the albumins-globulins in the rice-soybean protein blend treated with TG was the same (Figure 2b, lane 3). The majority of the bands in this fraction were from 288 rice, although a disappearance of a band about 37,000 corresponding to soybean protein was noticed and the disappearance of the soybean bands between 66,200- 290 97,400 kDa. It indicates that the TG is crosslinking proteins yielding an increase of the molecular weight of the polymers retained on the top of the stacking and separating 292 gel. Prolamins fraction only showed a slight decrease in intensity due to the TG activity. The glutelins extraction was made in two steps: firstly in the absence of ME (non- 294 reducing conditions) and secondly in the presence of ME for increasing the protein extraction. Therefore, besides of the crosslinking catalysed by TG, the activity of the 296 enzyme also induced an increase in the disulfide bonds between proteins (Gujral and Rosell 2004, Marco et al 2007) that made necessary the use of a reducing agent to 298 favour its extraction. Under reducing and non-reducing conditions of extraction, it was obtained the same electrophoresis pattern. Rice sample showed two major bands with 300 molecular weight of about 34,000 and 22,700 (Figure 2a, lanes 6 and 8) that corresponded to the acidic and basic polypeptides of rice glutelins, respectively 302 (Villareal and Juliano 1978, Steenson and Sathe 1995, Jahan et al 2005). Soybean protein isolate showed the β-conglycinin and glycinin subunits (Figure 2b, lanes 7 and 304 10), although it was expected that these proteins were extracted in the salt soluble

fraction, since globulins are the major storage proteins in soybean. Presumibly, the change in the solubility of these proteins was due to the process of the soybean protein production, which can modify the characteristics of the resulting protein isolate (Arrese et al 1991). In rice-soybean protein blend (figure 2b, lanes 8 and 11), the basic polypeptides of the rice glutelins appeared at the same molecular weight that the basic subunit of the soybean, since the genes of both proteins come from common ancestor gene (Utsumi 1992). In addition, a small amount of high molecular weight components (around 58,000), likely comprised of residual albumins and globulins, was observed together with a major band at approximately 14,000. The low molecular weight fraction probably was a prolamin polypeptide, typically reported as the principal contaminant of the glutelin preparations from rice (Krishnan and Okita 1986). In the TG-treated samples of rice-soybean protein blend, the bands with molecular weight about 70,300 and 40,700, both from soybean disappeared after the TG treatment, and the intensity of the bands was higher under reducing conditions probably because more proteins remained insoluble in TG sample after the extraction in non reducing conditions (Figure 2b, lanes 9 and 12). The effect of the TG in the SDS soluble fraction was more noticeable in the rice-soybean protein blend than in rice sample, where the effect of the enzyme was hardly noticed in the low molecular weight proteins. In the SDS soluble fractions, the intensity on the top of the stacking and separating gel was higher in the absence of TG when the extraction was made without ME (Figure 2b, lane 8), in opposition, when the reducing agent was used to extract the rest of the SDS soluble proteins, more aggregates were observed on the top of the gel in the sample with TG (Figure 2b, lane 12), what indicates the formation of disulfide bonds in great extent in samples with TG. The protein aggregates that remained on the top of the stacking and separating gel in the TG treated samples confirms the intermolecular crosslinking between the proteins extracted with SDS. The crosslinking reaction among proteins catalysed by TG may be intermolecular or intramolecular, only resulting in higher

molecular weight of the proteins when intermolecular linkages are formed (Basman et al 2002). Numerous studies related to the effect of TG reported a decrease in the intensity or disappearance of some bands of the proteins and an increase in the protein aggregates retained on the top of the stacking and separating gel, supporting the intermolecular crosslinking (Yildirim et al 1996, Babiker 2000, Basman et al 2002, Mujoo and Ng 2003, Fan et al 2005, Marco et al 2007).

The increase in the molecular weight of the proteins and, therefore, the increase in the interactions between proteins (disulfide bonds or other covalent interactions) modifies or improves the viscoelastic and functional properties of rice-soybean doughs (Marco and Rosell 2008). A relationship has been also established between the increase in the proportion of high molecular weight proteins of soybean proteins and their potential for being used in wheat flour breadmaking (Maforimbo et al 2006).

4.3 Multistacking SDS-PAGE

The proportion of proteins (relative IOD "integrated optical density") retained in the multistacking SDS-PAGE as a function of their molecular weight is showed in Table 2. In the absence of TG, the blend of soybean with the rice flour induced an increase in the proportion of proteins with high molecular weight (retained in the 4 and 8% gels) was observed, probably due to the association of the soybean proteins with rice proteins yielding protein aggregates. In the presence of TG, a decrease in the proportion of aggregates with higher MW was observed in the presence of TG, as a consequence of a decrease in the solubility of the aggregates crosslinked by the enzyme. Only the monomers with lower MW could be extracted and separated in the 12% gel.

The preparative multistacking allows determining the proteins retained in the different gel concentrations (4, 8 and 12%). In rice and rice-soybean protein blends, the same

protein bands were observed in the three concentrations of the gels, with the exception of two bands (*) that only appeared in the lane corresponding to the 12% gel (Figure 3 a and b). These proteins were from rice and they did not contribute to the formation of high molecular weight aggregates in the rice-soybean protein blend. However, in the presence of TG, the intensity of these bands decreased (Figure 3a and 3b, lane 6), likely due to their crosslinking forming insoluble aggregates; and that result was supported by the increase in the intensity on the top of the stacking and separating gels in the lane corresponding to 4% gel (Figure 3a and 3b, lane 4). Therefore, the intensity of all bands decreased in the presence of TG, and some of the bands disappeared due to the crosslinking, being the proteins of high molecular weight the most affected.

CONCLUSIONS

The addition of soybean proteins to rice flour provides a protein enriched dough with better amino acid balance and also those proteins might play an important role on the rice dough properties since soybean proteins interact with rice proteins yielding protein aggregates of high molecular weight. The interaction between rice proteins and soybean proteins can be intensified by the formation of new intermolecular covalent bonds catalysed by transglutaminase and also the indirect formation of disulfide bonds. The main protein fractions involved in those interactions are both β-conglycinin and glycinin of soybean and the glutelins of the rice flour, although albumins and globulins were also crosslinked.

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FIGURE CAPTIONS

480

Figure 1. Electrophoregrams obtained from the analysis of the SDS-polyacrylamide
482 gels of the proteins from rice and rice-soybean protein blends in the absence and the presence of 1% (w/w) transglutaminase. The values in the MW standard are expressed
484 in kDa. (a) unreduced conditions; (b) reduced conditions. IOD: Integrated optical density.

486

488 **Figure 2a.** SDS-PAGE analysis of the protein fractions of rice in the absence (lanes 2, 4, 6, 8) and the presence of TG (lanes 3, 5, 7, 9). MW standard (lane 1), albumins-
490 globulins (lanes 2, 3), prolamins (lanes 4, 5), glutelins step 1 (lanes 6, 7) and glutelins step 2 (lanes 8, 9). **2 b**. SDS-PAGE analysis of the protein fractions of soybean protein
492 isolate (without TG) (lanes 1, 4, 7, 10) and of rice-soybean blend in the absence (lanes 2, 5, 8, 11) and the presence of TG (lanes 3, 6, 9, 12). Salt soluble proteins (lanes 1, 2,
494 3), alcohol soluble proteins (lanes 4, 5, 6), SDS soluble proteins step 1 (lanes 7, 8, 9), SDS soluble proteins step 2 (lanes 10, 11, 12). MW standard (lane 13).

496

Figure 3. Preparative multistacking SDS-PAGE of rice (a) and rice-soybean blend (b).
498 Acrylamide/bisacrylamide concentrations of 4, 8 and 12% without TG (lanes 1, 2 and 3, respectively) and with TG (lanes 4, 5 and 6) and MW standard (lane 0).

500 **Table 1**. Effect of transglutaminase (1%, w/w, solid basis) on the protein fraction content of rice flour dough and composite doughs containing rice flour and soybean
502 protein (5%, w/w).

	Salt soluble proteins (%)	Alcohol soluble proteins (%)	SDS soluble proteins (%)	Final residue (%)
Rice	15.5 b	4.3 b	77.8 b	2.4 a
Rice+TG	10.0 a	4.9 b	77.4 b	7.8 b
Rice-soybean blend	18.0 c	3.4 a	77.2 b	1.4 a
Rice-soybean blend+TG	10.4 a	2.9 a	59.1 a	27.7 c

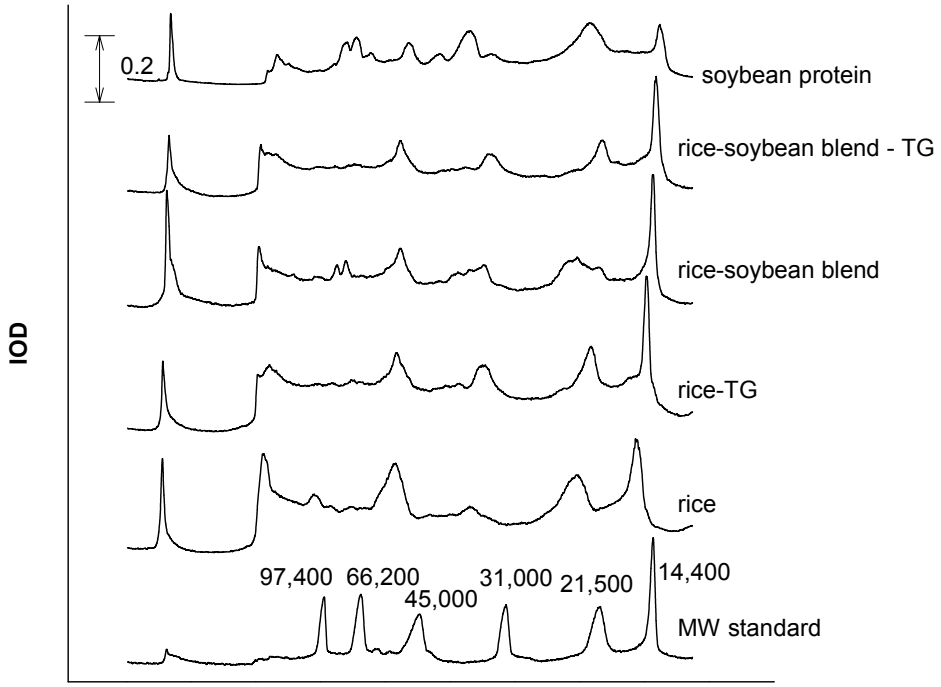
504 Means within columns followed by the same letter were not significantly different
506 ($P<0.05$).

Table 2. Relative IOD (integrated optical density) of the protein retained in the different concentrations of the multistacking gel. Rice-soybean blends were prepared by replacing 5% (w/w) of rice flour with soybean protein. In the transglutaminase treated samples, 1% (w/w) of transglutaminase was added.

	Relative IOD		
	4%	**8%**	**12%**
Rice	8.1 b	8.0 b	84.0 c
Rice+TG	2.8 a	2.3 a	94.9 d
Rice-soybean blend	17.3 d	13.1 d	69.6 a
Rice-soybean blend +TG	14.0 c	10.1 c	75.8 b

Means within columns followed by the same letter were not significantly different ($P<0.05$).

Figure 1a



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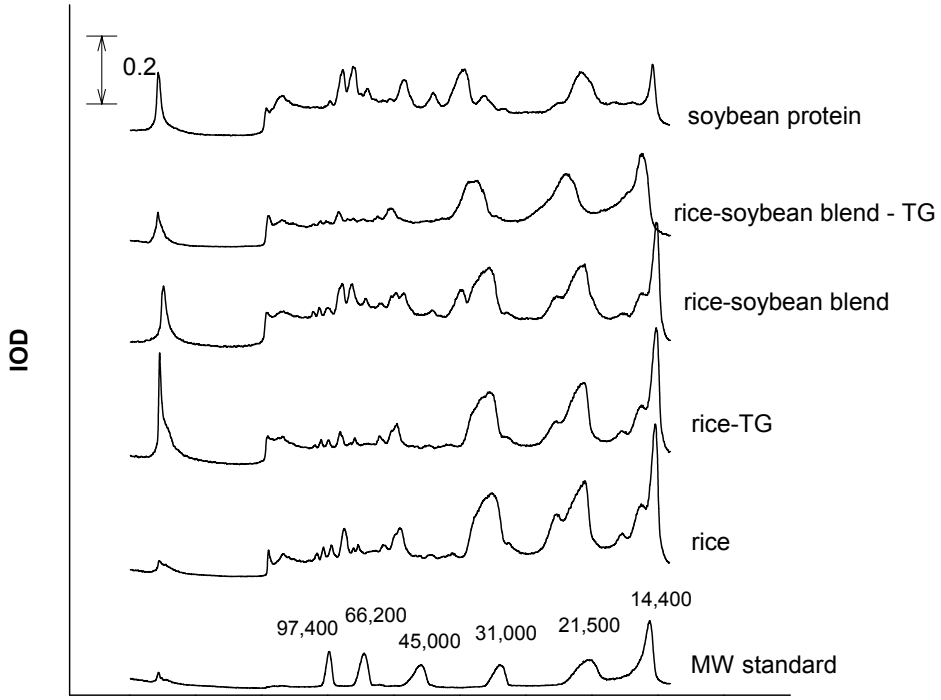
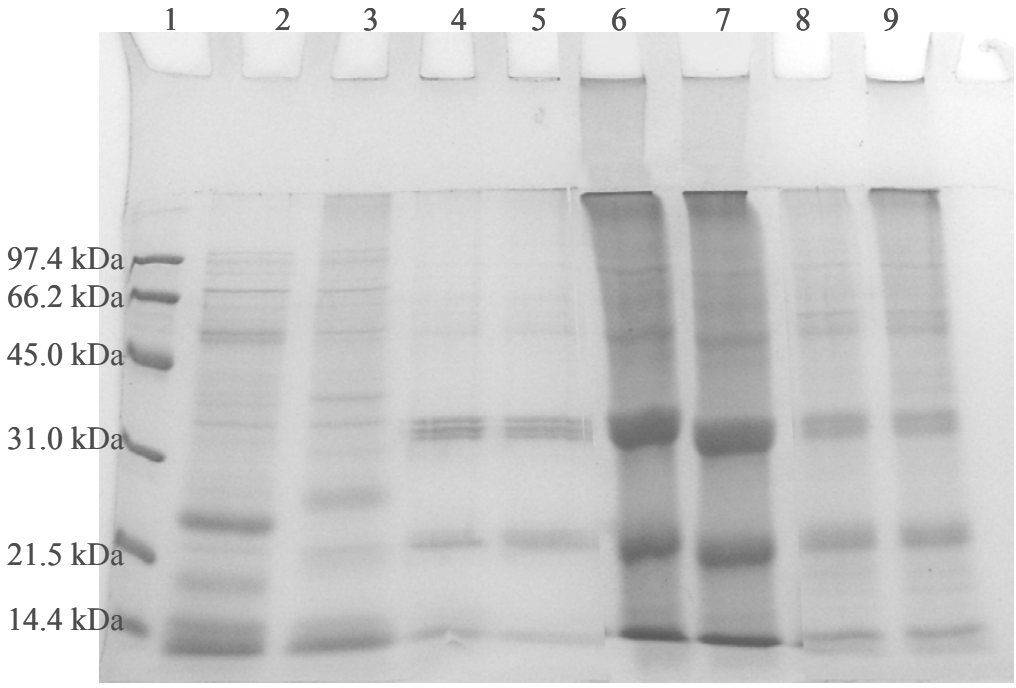
520 **Figure 1b**



522

Figure 2a.



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528 **Figure 2b**.

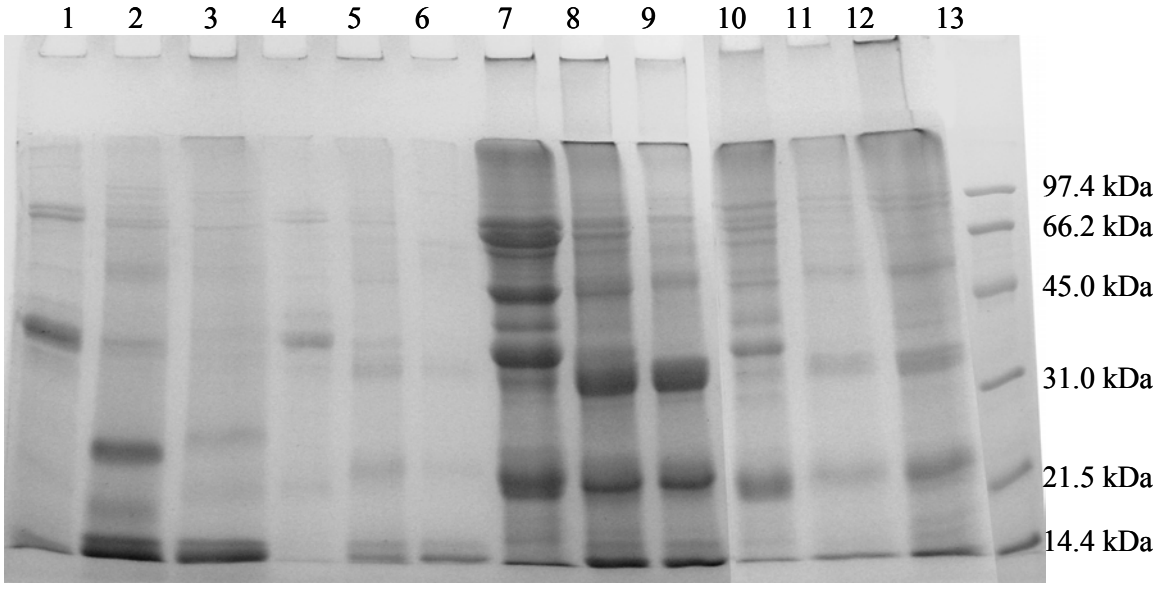

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Figure 3

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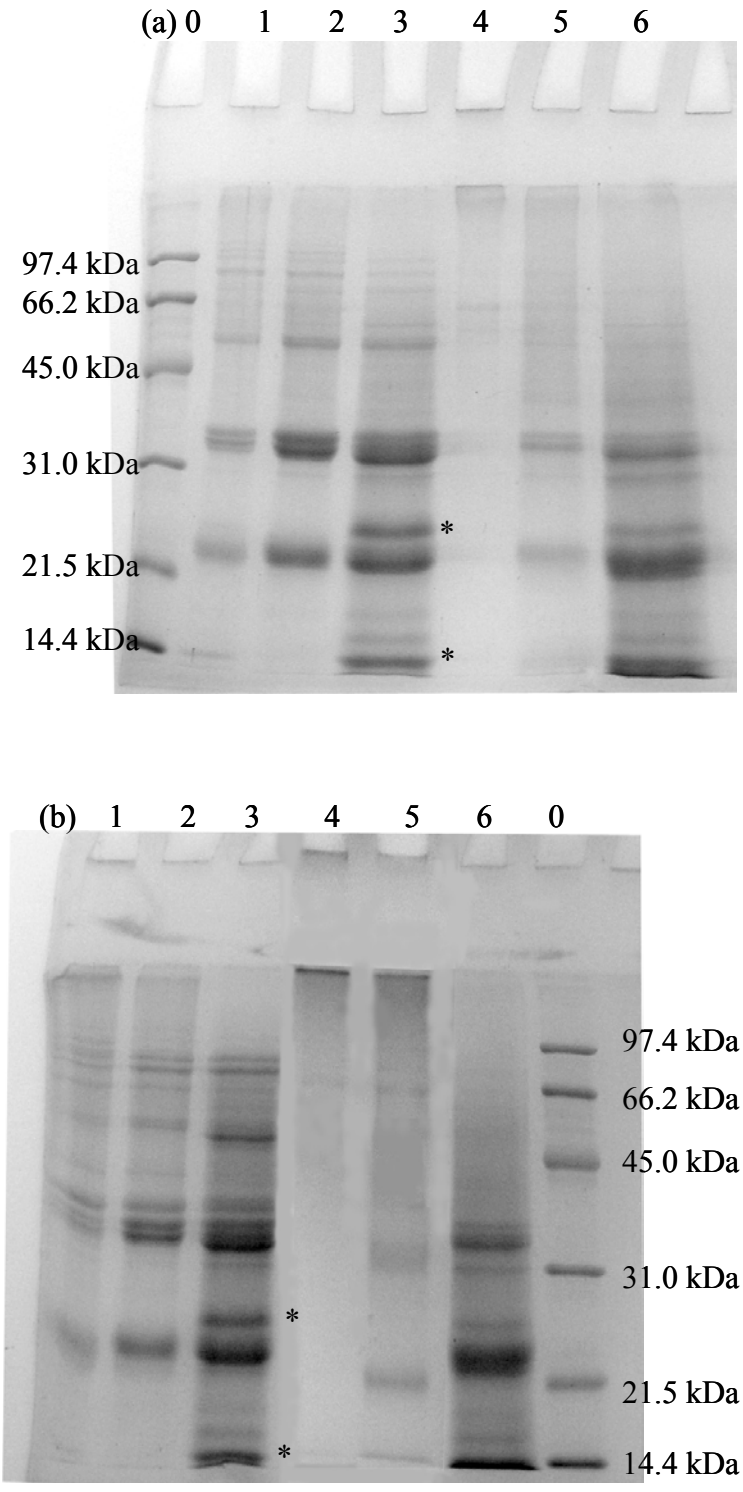

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