1 **EFFECT OF DIFFERENT PROTEIN ISOLATES AND TRANSGLUTAMINASE ON RICE**
2 **FLOUR PROPERTIES**

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11 **Running title:** Rice flour and protein isolate blends properties

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ABSTRACT

The effect of the addition of different protein isolates (pea, soybean, egg albumen and whey proteins) on the viscosimetric and rheological properties of the rice flour dough, and the development of a protein network through the use of microbial transglutaminase (TG) were evaluated. Protein isolates significantly ($p<0.05$) modified the gelatinization and gelling behaviour of the rice starch determined in the rapid viscoanalyser (RVA). Pea, soybean and whey proteins significantly ($p<0.05$) decreased the final viscosity in addition, whey protein also promoted a significant decrease (27.3%) in the peak viscosity. The elastic modulus value (G') recorded in the oscillatory tests was significantly ($p<0.01$) affected by both the protein isolates and the TG. The extent of the effect was dependent on the protein source; pea and soybean increased this parameter, whereas egg albumen and whey protein drastically decreased it. A modification in the emulsifying properties was also observed by the addition of protein isolates and the TG treatment. The decrease in the amount of free amino groups after TG treatment confirmed the protein crosslinking catalysed by TG. Therefore, the use of protein isolates and TG broads the applications of rice flour in the bakery industry, and brings about an increase in the protein content with the subsequent nutritional improvement of the resulting products.

Key words: rice flour, protein isolates, transglutaminase, rice-protein blends properties, rheology.

INTRODUCTION

Rice flour has soft taste, colourless, hypoallergenic properties, low levels of sodium and easy digestible carbohydrates. Because of these properties, rice flour is the most suitable cereal to make gluten free products (Gujral, Guardiola, Carbonell & Rosell, 2003a; Gujral, Haros & Rosell, 2003b; Gujral & Rosell, 2004a,b; Lopez, Pereira & Junqueira, 2004). However, gluten free cereals like rice do not meet the requirements necessary for processing fermented food products because their proteins cannot develop a viscoelastic network like gluten, enable to retain the CO₂ produced during fermentation process. Because of this, different strengtheners agents mainly included in the category of gums and starches have been proposed (Nishita, Roberts & Bean, 1976; Kang, Choi and Choi, 1997).

The addition of starches, gums and hydrocolloids to gluten free products provide the necessary network for obtaining fermented bakery products. The effect of several hydrocolloids -hydroxypropylmethylcellulose (HPMC), locust bean gum, guar gum, carrageenan, xanthan gum, and agar- on rice bread has been already tested (Kang et al., 1997). All of them gave successful formation of rice bread, but the addition of HPMC yielded rice breads with the optimum volume expansion, up to 4% of HPMC might be added for obtaining rice dough with similar rheological properties to wheat flour dough and good rice bread specific volume (Gujral et al., 2003a; Gujral & Rosell, 2004a,b; Sivaramakrishnan, Senge & Chattopadhyay, 2004; McCarthy, Gallagher, Gormley, Schober & Arendt, 2005). Starches from corn, cassava and potato are usually found in gluten free bread formulations (Sánchez, Osella & de la Torre, 2002; Lopez et al., 2004; Schober, Messerschmidt, Bean, Park & Arendt, 2005; McCarthy et al., 2005). Diverse flours are mixed with a high proportion of different starches affecting the crumb bread structure and also the staling process due to the different starch crystallites formed during storage (Osella, Sánchez, Carrara, de la Torre & Buera, 2005). Nevertheless, gluten free products resulting from mixing starches and gums with a proportion of gluten free cereals have a very low protein content and are lysine deficient. Proteins from different sources

(soybean, pea, egg albumen, whey) can be added in order to increase the nutritional value of gluten free products (FAO, 1981). With that purpose, dairy ingredients have been used in the production of gluten free products resulting in nutritional benefits and improved volume, appearance and sensory aspects of the loaves (Gallagher, Gormley & Arendt, 2003a; Gallagher, Kunkel, Gormley & Arendt, 2003b; Sánchez, Osella & de la Torre, 2004). Nevertheless, the addition of increasing amounts of skim milk powder induced a decrease in loaf height (Schober et al, 2005). Soy and egg proteins have also been added to gluten free products resulting also in better loaf volume (Gallagher et al, 2003a; Ribotta, Aussar, Morcillo, Pérez, Beltramo & León, 2004; Kobylanski, Perez & Pilosof, 2004). However, although initially the aim of addition of proteins was to increase the nutritional value of gluten free products, lately, it has been reported that the formation of a continuous protein phase is critical for obtaining an improvement in the quality of gluten-free bread (Moore, Schober, Dockery & Arendt, 2004). Therefore, the selection of the protein source with the appropriate functionality seems to play an important role in the breadmaking process of gluten free breads. In addition, if a network-like structure could be created with those proteins and the gluten free cereal proteins, that could hold the dough structure during proofing, resembling the gluten network obtained in wheat bread dough.

Protein functionality can be modified by forming intramolecular or intermolecular crosslinks (Gerrard, 2002; Jong & Koppelman, 2002). Transglutaminase (TG) catalyses the reaction between an ϵ -amino group on protein-bound lysine residues and a γ -carboxamide group on protein-bound glutamine residues, leading to the covalent crosslinking of the proteins. This is the most dominant reaction, but TG also catalyses two other reactions: in the presence of primary amines, TG crosslinks the amine to a γ -carboxamide group on protein-bound glutamine residues. In the absence of amine substrates, TG catalyses the hydrolysis of the γ -carboxamide group of glutamine, resulting in deamidation. This enzyme has been used for improving the baking quality of

the weak flours ([Autio, Kruus, Knaapila, Gerber, Flander & Buchert, 2005](#)), bringing about an improvement in the texture of the loaves ([Gerrard, Fayle, Wilson, Newberry, Ross & Kavale, 1998](#)). In addition, some improvement in the rice protein functionality was observed when they were crosslinked with TG ([Gujral & Rosell, 2004a](#)), which allowed decreasing the levels of HPMC needed to obtain acceptable rice bread.

The aim of the present study was to screen the effect of the addition of different protein isolates on the properties of the rice flour dough, and the contribution of the transglutaminase activity to the thermal viscosimetric profile and viscoelastic behaviour of the rice flour-protein blends.

MATERIALS AND METHODS

Commercial rice flour, from Harinera Belenguer SA (Valencia, Spain), had moisture, protein, fat and ash contents of 13.4, 7.5, 0.9 and 0.6%, respectively. Protein isolates (pea, soybean, egg albumen and whey) were from Trades SA (Barcelona, Spain). Microbial transglutaminase (100 units/g) was from Apliena, SA (Terrasa, Barcelona, Spain). Composition of the different protein isolates was determined following the ICC-Standard methods (2004) (Table 1). All reagents were of analytical grade.

Pasting properties

Pasting properties were determined using a Rapid Visco Analyser (RVA) (Newport Scientific model 4-SA, Warriewood, Australia) by following the AACC Approved Method No 61-02 ([AACC, 1995](#)). RVA analyses were performed on rice flour mixed with 5% (w/w, flour-protein blend basis) of the different protein isolates. When TG was present, 1% (w/w, flour-protein blend basis) of TG was mixed with the rest of the powdered ingredients. Viscosity was registered during a heating-cooling cycle: heating from 50 to 95 °C in 288 s at a rate of 9.4 °C/min, holding at 95 °C for 150 s and cooling to 50 °C in 228 s; the paddle speed was 960 rpm for 10 s and then decreased to 160 rpm for the rest of the cycle. The plots of the samples containing the different protein isolates are showed in Figure 1. The

parameters determined were: peak viscosity, final viscosity, breakdown and setback (difference between final viscosity and peak viscosity).

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) at a paddle speed of 90 rpm for 15 min. When protein isolates were 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 to the solids. Rice dough was used for the dynamic oscillatory test and the rest of the dough was frozen and freeze-dried. Dry rice dough was milled with a refrigerated micro-hammer mill resulting in a powder with a particle size not exceeding 250 μm .

Oscillatory measurements

Dynamic rheological measurements of the dough were determined on a controlled stress rheometer (Rheostress 1, Termo Haake, Germany). The measuring system consisted of parallel plate geometry (60 mm diameter, 1 mm gap). The rice dough was placed between the plates within 1 hour after mixing and the test started after 5 minutes resting. The rim of the sample was coated with Vaseline oil in order to prevent evaporation during the measurements. Measurements were performed at 30 °C ([Gujral & Rosell, 2004a](#)). Stress sweeps at 1 Hz frequency were carried out to determine the linear viscoelastic zone. Frequency sweep tests were performed from 0.01 to 10 Hz to determine the storage modulus (G'), loss modulus (G'') and loss tangent ($\tan \delta$) as a function of frequency. Two replicates of each measurement were made.

Emulsifying properties

The emulsifying properties of the rice dough, in the presence and absence of TG and protein isolates, were determined by the method of [Pearce and Kinsella \(1978\)](#). To

prepare the emulsion, 2 mL refined sunflower oil and 6 mL freeze-dried samples suspension (0.5%) in 0.1M phosphate buffer (pH 7.0) were shaken together and homogenized in a T18 Ultra Turrax (Wilmington, NC, USA) at 22,000 rpm for 1 min at 20 °C (Gujral & Rosell, 2004c). 50 µL of the emulsion were taken each 10 min and added to 5 mL of sodium dodecylsulphate solution (0.1%, w/v). The absorbance of the diluted solutions was read at 500 nm. The emulsifying activity was expressed as the absorbance measured at 0 min, and the emulsion stability was expressed as ES (%) = $(\text{Abs}_{60\text{min}}/\text{Abs}_{0\text{min}}) \times 100$ (Ahn, Kim & Ng, 2005; Babiker, 2000). Four replicates of each measurement were made.

Quantification of free amino groups

The amount of free amino groups was quantified in the freeze dried samples in order to confirm the formation of covalent bonds catalysed by TG. This method is based on the reaction between primary amino groups and o-phthaldialdehyde (OPA) (Nielsen, Petersen & Dambmann, 2001; Gujral & Rosell, 2004a). Freeze-dried rice dough (0.1 g) were suspended in 1.0 mL KCl (0.1 M, pH 1.0), vortexed for 10 min and centrifuged for 5 min at 16,000 x g. To 50 µL of the clear supernatant, 250 µL of OPA reagent were added. It was allowed to react for two minutes and the absorbance was determined at 340 nm in a microplate reader. To avoid the ammonia interference, the absorbance at 340 nm was also determined after the precipitation of the proteins with cold trichloroacetic acid (TCA) 20% (w/v). 450 µL of the supernatant were mixed with 50 µL of TCA, kept at 4°C for 1h, and centrifuged 10 min at 16,000 x g. Then, to 50 µL of the clear supernatant, 250 µL of OPA reagent were added and, after two minutes, the absorbance was read (Bonet, Caballero, Gómez & Rosell, 2005). The results were calculated against a serine standard curve. Four replicates of each measurement were made.

Statistical analysis

Multiple analysis of variance for the identification of all single effects and interactions 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 DISCUSSION

Effect of different protein isolates on the rice dough viscosity profile during a heating and cooling cycle

Viscosity of rice dough was measured during a heating-cooling cycle to evaluate the effect of the protein isolates from several sources (Figure 1) and the TG treatment on the pasting properties of the rice flour. Data from the RVA parameters were submitted to the analysis of variance to determine the single effects of the different protein isolates and the transglutaminase (Table 2). The presence of whey protein significantly decreased the peak viscosity of the rice-protein blends by 27% when compared with rice sample. A decrease in the viscosity was also observed when prolamin proteins were added to rice flour (Baxter, Blanchard & Zhao, 2004), likely due to the dilution effect on the starch concentration, since a negative correlation had been established between the protein content and the peak viscosity in rice flour (Lim, Lee, Shin & Lim, 1999; Tan & Corke, 2002). However, the dilution effect could not be the unique responsible for explaining the decrease in the peak viscosity promoted by whey proteins, because the extent of that was higher than the one observed with the other protein isolates, thus some other properties like the nature of the whey proteins might be responsible of the observed effect. Numerous studies have been reported pertaining to the thermal induced gelation process of the proteins after the aggregation of the polymer chains that induces huge modifications in the rheological behaviour of the proteins, being the gelation process temperature sensitive for each protein (Paulsson, Dejmek & van Vliet, 1990; Ziegler & Foegeding, 1990; Puyol, Cotter & Mulvihill, 1999; Ngarize, Adams & Howell, 2004).

The breakdown, related to the ability of the starches to withstand heating at high temperature and shear stress, showed a significant ($p<0.05$) modification in the presence of different protein isolates, whereas the addition of TG did not induce a significant change in this parameter. The presence of the egg albumen and whey protein isolates significantly decreased the breakdown by 34 and 36%, respectively, compared to the control in absence of these proteins. Final viscosity was also significantly ($p<0.05$) modified due to the presence of proteins. Pea, soybean and whey proteins promoted a significant decrease of the final viscosity by 20, 14 and 12%, respectively, when compared to the values of rice flour. However, in this case, the decrease in the viscosity induced by whey protein was lower than the one observed in the peak viscosity. It may be due to the gelation process. Whey proteins do not aggregate easily at room temperature, but they have a high ability to form gel during heating, getting the higher viscosity during cooling. Egg albumen proteins have also this ability, showing higher viscosity than whey proteins (Ngarize et al., 2004). The increase of viscosity during cooling or setback, usually related to the crystallization of the amylose chains when starches are studied, could be affected by the reorganization of the denatured proteins from the protein isolates. In fact, the protein isolates factor resulted in a significant ($p<0.01$) changes of the setback. Pea and whey proteins significantly modified the setback; but while pea decreased this parameter by 61%, the presence of whey proteins increased it by 74% compared with the values obtained in the absence of protein isolates.

The transglutaminase factor did not result in any significant difference in the RVA parameters of the rice flour-protein isolate blends. Collar and Bollaín (2004) reported a progressive decrease in the peak viscosity and final viscosity in wheat dough with the increase of the TG level up to 0.5%. The different nature of the cereal proteins is greatly responsible for that different behaviour.

Effect of protein isolates and transglutaminase on the viscoelastic properties of rice flour

The viscoelastic properties of the rice dough containing different protein isolates were studied by dynamic oscillatory test. The mechanical spectra of all the samples showed storage or elastic modulus (G') values higher than loss or viscous modulus (G'') at all the frequency range tested, which suggest a viscoelastic solid behaviour of the doughs (Figure 2). G' was independent of the frequency in control sample (without protein isolate) and in samples with pea or soybean proteins. Egg albumen and whey protein showed a slight increase in G' with frequency. G'' showed lower values at intermediate frequency in control sample and in the presence of whey protein, whereas G'' of the egg albumen sample showed a slight increase with frequency; in the case of pea and soybean, G'' was independent of frequency. TG hardly modified the shape of the mechanical spectra (data not shown).

Data from the viscoelastic test were submitted to the analysis of variance to determine the main effects of the protein isolates and the transglutaminase and their interaction (Table 3). The presence of protein isolates significantly ($p < 0.001$) changed the viscoelastic properties of the rice dough, whereas the presence of TG only induced a significant ($p < 0.001$) change of the elastic modulus. A significant ($p < 0.001$) synergistic effect of the protein and TG was also observed on the elastic modulus. The extent of the effect of the protein isolates was greatly dependent on the nature of the proteins. Pea and soybean significantly increased G' by 39 and 69%, respectively. Whereas the opposite trend was observed with the presence of egg albumen or whey protein, they significantly decreased the value of G' by 94% in the case of egg albumen and 98% in the case of whey protein. Regarding to viscous modulus, the same tendency than the one observed in the elastic modulus was obtained. Pea and soybean proteins induced a significant large increase of G'' , whereas the opposite trend was observed with the presence of egg albumen or whey protein. The loss tangent was also significantly modified by the presence of egg albumen and whey proteins. This result agrees with the findings of [Dogan, Sahin and Sumnu](#)

(2005a), who observed higher viscosity in corn-wheat batters when adding soybean protein than in the presence of whey protein or egg albumen. The increase in the viscosity in the presence of soybean proteins was attributed to its higher water binding capacity (Dogan, Sahin & Sumnu, 2005b).

The addition of TG only induced a significant increase on the elastic modulus of the rice-proteins blends. The other parameters did not show any significant difference. Contradictory results have been reported pertaining to the effect of TG on the viscoelastic properties of different matrix. Larré, Deshayes, Lefebvre and Popineau (1998) and Larré, Denery-Papini, Popineau, Deshayes, Desserre and Lefebvre (2000) and Gujral and Rosell (2004a) reported an increase in G' values when cereal proteins were treated with TG. An increase in the resistance of the wheat-soybean blends dough was observed when they were treated with TG (Basman, Köksel & Ng, 2003). Köksel, Sivri, Ng and Steffe (2001) reported an increase in the complex modulus (G^*) determined by dynamic rheological measurements when wheat doughs containing 10% of bug-damage flour were treated with 1.5% TG, but the addition of soy protein isolate did not increase the extent of crosslinking catalysed by TG. Wilcox and Swaisgood (2002) did not observe any crosslinking when whey protein treated with TG were analysed by electrophoresis, but in the presence of reducing agent whey gels adopted more solid-like characteristics. Likely, increasing the accessibility of the amino acid residues in the presence of the reducing agent favours the formation of covalent links within the protein chains. Truong, Clare, Catignani and Swaisgood (2004) did not observe difference in G' and G'' values after treating whey proteins with 0.12 unit/g of immobilized TG, but a decrease of G' was observed when the TG concentration was increased, probably viscoelastic changes can be only observed after an extensive crosslinking of the protein.

Emulsifying properties

The effect of the addition of protein isolates and TG treatment on the emulsifying properties of the proteins was also determined. The presence of egg albumen or whey proteins increased the emulsifying activity of the rice flour, while pea and soybean proteins hardly modified this parameter (Table 4). The stability of the emulsion significantly ($p < 0.05$) decreased when egg albumen and whey proteins were present. Whey proteins are widely used because their emulsifying properties (Hsieh, Regenstein & Rao, 1993), so the presence of these proteins greatly contributes to the emulsifying activity of the rice proteins. Contradictory results have been reported about the emulsion properties of the proteins tested. Jackman and Yada (1989) observed better emulsifying activity and stability of the emulsion with whey protein compared with pea protein. Pearce et al. (1978) also reported higher emulsion activity of the whey protein compared with soybean protein, but egg albumen showed lower emulsion activity than soybean. However, Webb, Naeem and Schmidt (2002) observed better emulsifying properties in soybean than in dairy proteins. Pea proteins have relatively good emulsifying properties with low emulsion stability compared to the behaviour of the soybean proteins (Tömösközi, Lásztity, Haraszi & Baticz, 2001). These differences may be due to the process followed when producing the protein isolates, since this process can affect the solubility and the degree of hydrophobicity, modifying the functional properties of the proteins (Petrucelli & Aqon, 1994). In the present study, likely the initial properties of the protein isolates are masked by the dilution effect of the rice flour.

After TG treatment, control sample showed an increase in the emulsifying activity. An increase in this parameter was also showed when the samples with soybean or pea proteins were treated with TG. However, the emulsion stability decreased in these samples. Conversely, the samples with egg albumen or whey protein showed a decrease in the emulsifying activity but did not modify the emulsion stability after TG treatment. Different behaviours on the emulsifying properties have been reported when proteins are

319 treated with TG. [Siu, Ma, Mock and Mine \(2002\)](#) reported a decrease in the emulsifying
320 activity index and the emulsion stability index when oat globulin was treated with TG. In
321 addition, [Ahn et al. \(2005\)](#) observed a slight decrease in the emulsifying activity but an
322 increase in the emulsion stability after the treatment of cereals flours with TG. The
323 decrease in the emulsifying activity and the emulsion stability caused by TG may be due
324 to the loss of solubility of the crosslinked proteins. The increase in the molecular weight of
325 polypeptide chains may lead to some loss of flexibility and reduces the protein ability to
326 unfold at the oil-water interface ([Siu et al., 2002](#)).

328 **Quantification of free amino groups**

329 In order to evaluate the extent of the effect of TG, the free amino groups of the proteins
330 from both rice flour dough and rice-protein blends were quantified.

331 Transglutaminase (TG) catalyses the reaction between an ϵ -amino group on protein-
332 bound lysine residues and a γ -carboxyamide group on protein-bound glutamine residues
333 leading to covalent crosslinking of the proteins. Because of the involvement of the amino
334 groups in the crosslinking reaction, a decrease in the amount of these groups would show
335 that TG is catalysing this reaction. A decrease in the amount of the free amino groups was
336 reported by [Gujral and Rosell \(2004a\)](#) and [Bonet et al. \(2005\)](#) when cereal proteins were
337 treated with TG.

338 As was expected the presence of protein isolates resulted in an increase in the amount of
339 the free amino groups, as a consequence of the increase in the protein content (Figure 3).
340 After TG treatment, no statistically significant difference was observed in the control
341 (without protein isolates) neither in the presence of pea proteins. However, in the
342 presence of soybean or whey proteins, a decrease in the amount of free amino groups
343 was observed after TG treatment. The reduction in the amount of amino groups confirmed
344 the protein crosslinking catalysed by TG. This crosslinking may be between homologous
345 or heterologous protein chains. Surprisingly, the sample containing egg albumen protein
346 showed an increase in free amino groups after TG treatment. The increase in the amount

of the amino groups might indicate an increase in the solubility of the proteins resulted from the deamidation reaction (Babiker, 2000).

Overall results indicate that the presence of different protein isolates modifies in different extent the properties of the rice flour. The use of transglutaminase changes the viscoelastic behaviour of the rice flour-protein blends getting different trends depending on the protein origin. Therefore, the use of protein isolates and TG broadens the applications of rice flour and leads to an increase in the protein content with the subsequent nutritional improvement.

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FIGURE CAPTIONS

Figure 1. Variation of elastic (A) and viscous (B) moduli with the frequency for blended rice dough with or without protein isolates. Legends: ● : control; ■ : pea; ▲ : soybean; ◆ : egg albumen; x : whey proteins.

Figure 2. Effect of the addition of 5 % (w/w, flour-protein blend basis) protein isolate and 1 % (w/w, flour-protein blend basis) transglutaminase on the amount of free amino groups of rice dough. Solid bars: in the presence of TG. White bars: in the absence of TG. Different letters denote statistically significant differences ($p < 0.05$).

Table 1. Proximate composition of the protein isolates tested.

Proteins	Percentage (%)				
	Moisture content	Protein	Lipid	Ash	Carbohydrates ^a
pea	6.7	79.2	0.9	4.2	9.1
soybean	6.9	80.8	0.2	3.6	8.6
egg albumen	7.6	88.8	0.2	3.5	0.0
whey	8.9	84.2	0.2	2.2	4.5

^a Calculated by difference.

Table 2. Significant single effects of the protein isolates (5 %, w/w, flour-protein blend basis) and the transglutaminase (1 %, w/w, flour-protein blend basis) on the RVA parameters.

RVA parameters	Overall	protein						TG	
	mean	0	1	2	3	4		0	1
Peak viscosity (cP)	2471	2833 ^b	2468 ^{ab}	2601 ^b	2391 ^{ab}	2061 ^a	*	2426	2515
Breakdown (cP)	959	1075 ^b	1170 ^b	1147 ^b	714 ^a	689 ^a	*	947	971
Final viscosity (cP)	2988	3338 ^c	2667 ^a	2878 ^{ab}	3119 ^{bc}	2940 ^{ab}	*	2933	3043
Setback (cP)	517	505 ^b	199 ^a	277 ^{ab}	728 ^{cd}	879 ^d	**	507	528

* p<0.05; ** p<0.01.

Values followed by different letters in the same row are significantly different (P<0.05).

Protein levels: 0 (without protein isolate), 1 (pea), 2 (soybean), 3 (egg albumen), 4 (whey protein).

TG levels: 0 (without TG), 1 (1% TG).

Table 3. Significant single effects and binary interaction of the protein isolates and the transglutaminase on the viscoelastic parameters.

	G'		G''		Tan δ	
	F-ratio	P-Value	F-ratio	P-Value	F-ratio	P-Value
Protein isolate	159.26	0.0000	115.90	0.0000	25.02	0.0000
TG	28.70	0.0001	4.24	0.0601	1.04	0.3254
Interaction protein-TG	11.69	0.0003	2.05	0.1460	4.60	0.0156

	Level	Viscoelastic parameters		
		G' (Pa)	G'' (Pa)	Tan δ
Overall mean		5372	663	0,176
Protein	0	6460 ^b	719 ^b	0,112 ^a
	1	8992 ^c	1211 ^c	0,145 ^a
	2	10902 ^d	1273 ^c	0,118 ^a
	3	382 ^a	77 ^a	0,205 ^b
	4	126 ^a	33 ^a	0,298 ^c
TG	0	4412 ^a	611 ^a	0,168 ^a
	1	6333 ^b	714 ^a	0,183 ^a

Values followed by different letters in the same column within each factor are significantly different ($p < 0.05$).

Protein levels: 0 (without protein isolate), 1 (pea), 2 (soybean), 3 (egg albumen), 4 (whey protein).

TG levels: 0 (without TG), 1 (1% TG).

Table 4. Effect of different protein isolates or/and the presence of transglutaminase on the emulsifying properties of the rice flour (none) and the rice flour-protein blends. EA: emulsifying activity, Stability, of the emulsion after 60 min.

Protein	TG (%)	EA	Stability
none	0	0.216 ^a	98.1 ^e
	1	0.336 ^b	35.5 ^{ab}
pea	0	0.189 ^a	94.5 ^e
	1	0.320 ^b	55.2 ^{cd}
soybean	0	0.204 ^a	83.8 ^e
	1	0.347 ^b	63.8 ^d
egg albumen	0	0.404 ^c	30.8 ^{ab}
	1	0.329 ^b	41.6 ^{bc}
whey	0	0.905 ^e	18.8 ^a
	1	0.824 ^d	26.2 ^{ab}

Values are the mean of four replicates.

Values followed by different letters in the same column are significantly different ($p < 0.05$).

None: rice flour, pea: rice-pea blend, soybean: rice-soybean blend, egg albumen: rice-egg albumen blend, whey: rice-whey blend.