FUNCTIONAL AND RHEOLOGICAL PROPERTIES OF PROTEIN ENRICHED GLUTEN FREE COMPOSITE FLOURS

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ABSTRACT

Protein enriched composite flours comprising rice flour and soybean and pea protein isolates were made. Experimental design resulted in composite protein enriched blends with different functional, rheological, mechanical and surface related textural properties. The enzyme transglutaminase (TG) was added for reinforcing the protein network. Protein isolates induced a significant (α<0.01) increase in the water absorption of the composite blends, having also a synergistic effect and a decrease of the storage (G′) and viscous (G″) moduli. Protein isolates also modified the mechanical and surface related textural properties. Soybean protein isolate showed the most significant effect on the functional properties, rheometer and surface related textural responses. Crosslinking activity of the transglutaminase led to a significant decrease of the foaming activity and stability. Scanning electron micrographs of the composite blends showed that the usage of soybean, pea protein isolates and TG would be a promising approach to produce protein enriched blends for making fermented gluten free products.

Key words: rice flour, soybean, pea, proteins, transglutaminase, functional properties, rheology, microstructure.

INTRODUCTION

Yeasted bakery products addressed to coeliac patients require the development of complex matrixes with sufficient viscoelastic properties for holding the carbon dioxide released during the fermentation and enable to keep the structure during the expansion along baking. A considerable effort has been focussed in the design of functional polymer blends that meet the technological requirements of gluten free fermented products. In fact, rice based breads have been successfully developed using several combinations of hydrocolloids like carboxymethylcellulose and hydroxypropylmethylcellulose (HPMC) (Ylimaki et al., 1991; Gujral et al, 2003). The use of HPMC also confers good quality properties to gluten-free bread based on 70% sorghum flour, and 30% potato starch (Schober et al., 2007). Crosslinking enzymes (transglutaminase and glucose oxidase) have been proposed as processing aids for improving gluten free bread quality (Gujral and Rosell, 2004a,c). Other different proposed combinations have been a complex formulation including corn starch, brown rice, soy and buckwheat flour. (Moore et al., 2004), or a mix containing different proportions of rice flour with corn and cassava starches obtaining gluten-free bread with uniform and an even distribution of cells over the crumb as well as a pleasant flavor and appearance (Sanchez et al., 2002; Lopez et al., 2004). In general, those polymer blends are based predominantly on carbohydrates, with a very low proportion of proteins. Scarce information is available about the use of enriched protein blends as gluten free matrixes.

Different protein sources can be added for improving the nutritional quality of gluten-free products, given that celiac disease in some cases leads to malnutrition. Dairy and soybean proteins are the most used proteins in gluten-free bread formulations (Gallagher et al., 2003; Ribotta et al., 2004; Moore et al., 2006; Marco and Rosell, 2008). Legumes are a good supplement for cereal-based foods since both legume and cereal proteins are complementary in essential amino acids. Cereals are deficient in the essential amino acid lysine, while legumes have a high content of this amino acid.

On the other hand, cereal proteins complement legume proteins in the essential amino acid methionine (Iqbal et al., 2006).

Soybean is highly used in Asian diet and nowadays its presence in Western diets is increasing due to the association of soybean protein consumption with lower risk of cardiovascular diseases (FDA, 1999). Besides, soybean is used in food technology for supplying desirable functional properties such as emulsification, fat absorption, moisture holding capacity, thickening, and foaming (Wolf, 1970). Although the most used legume protein is from soybean, pea proteins can also be successfully used in bakery products, obtaining a protein enriched product with better amino acid balance (Tömösközi et al., 2001). In addition, it has been reported that the addition of soybean or pea proteins to rice flour modified the mechanical properties of the rice-proteins blend dough, inducing a significant increase in the elastic modulus recorded by the oscillatory tests (Marco and Rosell, 2008).

The aim of this study was to design a protein enriched composite flour comprising rice flour and soybean and pea protein isolates. Transglutaminase, a transferase with crosslinking activity, was added for creating a protein covalent network. Functional, rheological and microstructure properties of the resulting protein enriched matrixes were determined.

MATERIALS AND METHODS

Commercial rice flour, from Harinera Belenguer SA (Valencia, Spain), had moisture, protein, lipid and ash contents of 12.2, 11.5, 1.0 and 1.0%, respectively. Protein isolates (pea and soybean) were from Trades SA (Barcelona, Spain). Moisture, protein, lipid and ash contents of the pea protein isolate were 6.2, 90.6, 1.1, 4.8%, respectively, and 6.0, 91.2, 0.4, 4.8%, respectively, in the soybean protein isolate. Composition of the different ingredients was determined following the AACCI Approved Methods (1995). 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.

Rice dough preparation

Rice flour was replaced by combinations of proteins and transglutaminase following a central composite design for sampling (Table 1). Design factors (quantitative independent factors) tested at five levels (-1.68, -1, 0, +1, +1.68), included soybean protein (from 1 to 25g/100g composite flour), pea protein (from 1 to 25g/100g composite flour) and transglutaminase (from 0.1 to 1.5g/100g composite flour). The model resulted in 17 different combinations of composite flours prepared in a Brabender farinograph (Duisburg, Germany) bowl (50g flour capacity) by mixing for 15 min all the ingredients. All the composite doughs were prepared at constant consistency. Dough was freshly prepared for the dynamic and static rheological properties. Whereas for determining the functional properties, composite flour doughs were freeze dried till further analysis.

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 (rough plate 35 mm diameter, 1 mm gap). Rice dough, placed between the plates, rested for five minutes before starting the test. The rim of the sample was coated with Vaseline oil in order to prevent evaporation during the measurements that were performed at 30 ºC (Gujral et al., 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 δ) as a function of frequency. Two replicates of each measurement were made.

Mechanical and surface related texture properties

Dough machinability was assessed by assessing the texture profile analysis (TPA) and dough stickiness in a TA-XT2i texturometer as described Armero and Collar (1997) using the Chen & Hoseney cell. The primary textural properties were 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 (Armero and Collar, 1997; Collar and Bollaín 2004). The adhesiveness was measured without the plastic film. Parameters registered included: hardness, cohesiveness, resilience, springiness, gumminess, adhesiveness and stickiness (for detailed information about those parameters see Armero and Collar, 1997). Three and ten repetitions for the TPA parameters and stickiness were made, respectively.

Functional properties

Composite flour doughs previously freeze-dried and ground with a refrigerated micro-hammer mill (powder with a particle size not exceeding 250 μm) were used for determining the functional properties.

The emulsifying properties of the composite flour doughs, 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 one minute at 20 ºC (Gujral & Rosell, 2004c). Aliquots of fifty microliters of the emulsion were taken at zero and 30 minutes 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 (%) = $(Abs_{30min}/Abs_{0min})x100$ (Ahn et al., 2005; Babiker, 2000). Four replicates of each measurement were made.

The foaming properties of the different doughs were determined following the method of Miller and Groniger (1976) with slight modifications. Fifty milligrams of freeze-dried sample were added to 5 ml 0.02 M phosphate buffer pH 7.0 and homogenized for one minute at 18,000 rpm in a T18 Ultra Turrax (Wilmington, NC, USA). Then, the blend was transferred to a measuring cylinder to determine the volume at zero and 60 minutes after homogenizing. Foaming capacity (FC) and foam stability (FS) were determined. The foaming capacity was expressed as the increased volume of mixture after the homogenization. Foam stability was calculated as FS(%) = (FC_{60min}/ $FC_{initial}$) x 100. At least two replicates of each measurement were made.

The protein solubility of the freeze-dried samples was determined at pH 4 and pH 6. Samples (0.2%, w/v) were suspended in 0.05 M acetate buffer (pH 4) or 0.05 M phosphate buffer (pH 6), and after vortexing for 10 min the turbidity was measured at 500 nm, following the method of Babiker (2000). Four replicates of each measurement were made.

Scanning electron microscopy

The structure of the composite flours was analysed by scanning electron microscopy (SEM). Fragments of the freeze-dried samples were mounted on aluminium specimen stubs using doubled tape and sputter-coated with 100-200Å thick layer of gold and palladium by Ion Sputter (Bio-Rad SC-500). Samples analysis was performed at an accelerating voltage of 10kV with a SEM Hitachi 4100 from the SCSIE department of the University of Valencia.

Statistical analysis

The analysis of variance for each functional and rheology characteristic (response) was conducted using Statgraphics V.7.1 program (Bitstream, Cambridge, MN), in order to determine significant differences among the factors combination. Analytical data were fitted to multiple regression equations using the desing factors as independent variables. For each response with significant differences response surface plots were generated from the regression equations by using the Statgraphics program. Response surface plots were obtained by holding the independent variable with least significant effect on the particular response at constant value and changing the other two variables.

RESULTS AND DISCUSSION

Analytical data obtained from the central composite design samples on functional and rheological responses were fitted to second-order polynomial models using added principles (design factors) as independent factors in order to estimate response surfaces of dependent dough variables. Experimental data were submitted to the analysis of variance to determine the main effects of the protein isolates and the transglutaminase and their interaction.

Effect of protein isolates and transglutaminase on the farinograph water absorption of the composite blends

Regression coeffients of the added principles obtained from the stepwise regression fitting model and analysis of variance are included in Table 2. The value of the determination coefficient (R^2) indicated that the model as fitted explained 99.84% of the variability in the water absorption. The water absorption determined by the farinograph was significantly ($\alpha<0.01$) affected by the amount of soybean and pea protein isolates. The higher the amount of protein isolate added, the higher the water absorption was (Figure 1). Besides, the addition of both proteins simultaneously produced a significant synergistic effect. The soybean protein showed greater effect than the pea protein (Figure 1). The addition of soybean protein at the maximum level tested (+1.68179) and pea protein at the minimum level tested (-1.68179) induced an increase in the water absorption of 84.9 %. While in the opposite side, when pea protein was added at highest level and soybean at the lowest, the increase in this parameter was 38.9 %. The addition of both protein isolates at the maximum level produced an increase of 149.7 %. The increase in the water absorption produced by the addition of protein isolate, might be related with their water holding capacity, which is about 2.7-2.8 g/g for pea protein isolate and 4.0-5.0 g/g for the soy protein isolates (Vose, 1980). The same effect has been observed when 20% soy flour was added to wheat flour (Ahn et., 2005).

The addition of the transglutaminase resulted in a positive quadratic effect on the water absorption. The crosslinking activity of transglutaminase induces the formation of protein polymers with greater water holding capacity (Wang et al., 2007)

Effect of protein isolates and transglutaminase on dynamic 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, which has already been described for rice based doughs (Gujral and Rosell, 2004a).

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 value of the determination coefficients (R^2) indicated that the models as fitted explained 77, 76 and 98% of the variability in the G´, G´´ and tan δ, respectively. The addition of different levels of soybean and pea protein isolates significantly affected the viscoelastic properties of the composite flour dough determined by the rheometer. The increase in the amount of soybean produced a significant linear decrease in the storage (G´) and the loss (G´´) moduli. The same effect was induced by the pea protein but derived from a significant quadratic effect. Besides, the presence of both protein isolates, soybean and pea, produced a significant increase in the tan δ, due to their positive linear and negative quadratic effect on that response (Figure 2). The interaction between soybean and pea protein produced a significant antagonist effect in the tan δ. The addition of soybean or pea protein isolate at maximum level when the other protein isolate was at the minimum level led to an increase in the tan δ of 77.9 and 77.7%, respectively (Figure 2). However, the addition of both proteins at the highest levels studied led to an increase in the tan δ of 72.2%.

Marco and Rosell (2008) observed a significant increase in G´ and G´´ when added 5% of pea or soybean protein to rice flour whereas the opposite trend was observed with the presence of egg albumen or whey protein. Differences can be attributed to the diverse water absorption applied in both studies, since constant water absorption (90%) was used in Marco and Rosell (2008), whereas the present studied was carried out at constant dough consistency, adjusting the amount of water needed for obtaining dough with the same consistency. Results previously presented regarding the effect of pea and soybean proteins on the water absorption confirmed this assumption, since those proteins had a significant effect on the water absorption.

Transglutaminase (TG) only had a significant (α<0.05) negative and quadratic effect on the viscous modulus. Different findings have been reported pertaining the effect of the TG on cereal proteins. Some authors reported an increase in G´ values when cereal proteins were treated with TG (Larré et al., 1998; Larré et al., 2000; Gujral et al., 2004a). Marco and Rosell (2008) also reported a significant increase in G´ when TG was added to rice flour enriched with 5% of different protein sources. In addition, some others described an increase in G´ and G´´ when wheat gluten solutions were treated with TG (Wang et., 2007), but Truong et al. (2004) did not observe any difference in G´ and G´´ values after treating whey proteins with 0.12 unit/g of immobilized TG. However, the same authors observed a decrease of G´ when the TG concentration was increased; probably changes on the viscoelastic properties can be only observed after an extensive crosslinking of the protein. In the present study, a tendency to increase the storage and loss moduli and to decrease the tan δ values was observed when the level of TG increased, although the changes produced were not significant. Maybe, the great effect of the protein isolates is masking some of the changes produced by the TG.

Effect of protein isolates and transglutaminase on the mechanical and surface related textural properties of rice composite flours

Rheological assessment is a good indicator of polymer molecular structure and thus of end-use performance (Marin and Monfort, 1996). In the case of wheat dough, rheological analysis has been successfully applied as indicator of the molecular structure of gluten and starch, and as predictors of their functionality in breadmaking performance (Armero and Collar, 1997; Collar and Bollaín, 2005; Bollaín et al., 2006). Despite gluten free matrixes are structurally different than gluten dough, rheological assessment of the gluten free matrixes might give an indication of its further functionality. The experimental data obtained from the texturometer were statistically analyzed to determine the significance of the independent factors on the surface related textural responses of the protein enriched composite flours (Table 4). Mechanical and surface related texture parameters were dependent on the factors studied, soybean and pea protein isolate and TG, being particularly significant for hardness (R^2=0.7970), springiness (R^2=0.9350), gumminess (R^2=0.7920) and stickiness (R^2=0.9610).

The hardness was significantly affected by the level of addition of the three factors studied. The increase in the addition of soybean produced a decrease in the hardness, whereas the addition of pea protein or TG produced an increase in this parameter (Figure 3). The effect of TG on the hardness may be explained by the increase in the molecular weight of the proteins resulted from the crosslinking action of this enzyme, obtaining larger polymers (Marco et al., 2007, 2008). Wang et al. (2007) also observed higher values of hardness, adhesiveness, cohesiveness and chewiness in gluten gels containing TG than in those obtained without TG.

The cohesiveness was significantly (α<0.05) affected only by soybean protein content. The increase of cohesiveness promoted by soybean proteins agrees with the negative correlation between hardness and cohesiveness reported by Armero and Collar (1997) when studied wheat dough, because of harder dough experiments greater permanent damage to internal structure than less hard dough when both are exposed to the same strain. In wheat doughs, dough cohesiveness has been reported as a good predictive parameter of fresh bread quality, since more cohesive wheat doughs give softer breads with higher specific volume (Armero and Collar, 1997).

Both soybean and pea protein isolates produced doughs with higher springiness when the level of the protein increased (Figure 3). Conversely, the interaction between soybean and pea protein produced a significant (α<0.01) antagonist effect on the springiness.

Pea protein resulted in a positive linear effect on gumminess and the adhesiveness was only significantly reduced by the transglutaminase (Figure 3). The stickiness determined by the Chen & Hoseney cell was significantly (α<0.01) modified by the three factors. All of them decreased the stickiness of the doughs when the level of addition was increased (Figure 3). The stickiness showed a quadratic positive dependence on the addition of pea protein isolate and TG. The same effect of TG on dough stickiness has been already reported in wheat doughs (Tsen and Lai, 2002; Collar and Bollaín, 2004).

Functional properties of the composite blends

The value of the determination coefficients (R^2) indicated that the models as fitted explained 81 and 74% of the variability in the emulsifying activity and the emulsion stability, respectively (Table 2). The emulsifying activity of the composite blends was statistically affected by the level of addition of soybean protein isolate. An increase in the amount of soybean protein resulted in a decrease of this parameter. The emulsion stability was also significantly affected by the level of soybean protein isolate. The addition of this protein led to a negative linear and a positive quadratic effect on this response, as a consequence, the emulsion stability reached a minimum at 0.47 level of soybean protein (Figure 4). Therefore, that minimum should be avoided when looking for an increase in the emulsion stability. Soybean proteins shows higher emulsifying activity and emulsion stability than the pea proteins (Tömösközy et al., 2001). The addition of 20% soy flour to wheat produced a significant positive effect on the emulsifying activity of the samples (Ahn et al., 2005). Marco and Rosell (2008) reported that the addition of 5% of pea or soybean protein isolate to rice flour hardly modified the emulsifying activity of rice flour dough. These differences may be attributed to the different hydration of the composite blends, since water acts as a plasticizer defining the functional properties of the dough (Rosell and Marco, 2007). The effect of the TG in the emulsifying activity was almost statistically significant (α=0.0530) (Table 2, Figure 4). Siu et al. (2002) and Ahn et al. (2005) observed a decrease in the emulsifying activity when cereal or soy proteins were treated with TG, which has been attributed to the loss of solubility of crosslinked proteins. The increase in the molecular weight of polypeptide chains may lead to some loss of flexibility and reduces the protein ability to unfold at the oil-water interface (Siu et al., 2002; Marco et al., 2007, 2008). The presence of TG also induced an increase in the emulsifying stability, although that effect was no statistically significant (Table 2).

The regression models as fitted explained 83 and 96% of the variability in the foaming activity and the foam stability, respectively (Table 2). The foaming capacity and the foam stability were significantly affected by the addition level of the three factors studied in this experimental design. Both protein isolates, soybean and pea, showed a linear positive effect in these responses but the enzyme produced the opposite effect, an increase in the level of addition of TG resulted in a significant decrease in these properties (Figure 4). Besides, the foam stability showed a negative quadratic dependence on the soybean protein and TG level (Figure 4). The interaction of the protein isolates with the TG was also significant in the foam stability. The interaction between soybean and TG had a synergistic effect, whereas the interaction between pea and TG showed an antagonist effect. Similar results regarding the effect of soybean on foaming activity and foam stabilty have been reported by Tömösközi et al. (2001). The decrease in the foaming activity and foam stability produced by the TG might be due to the increase in the molecular weight and the loss of the flexibility protein chains produced by the crosslinking activity (Marco et al., 2007, 2008).

The solubility of the composite blends enriched with proteins was studied at pH 4 and pH 6. The R^2 values indicated that the models as fitted explained 80 and 81% of the variability in the solubility at pH 4 and pH 6, respectively (Table 2). Soybean protein isolate was the unique independent factor that significantly affected the solubility at these pHs. In both cases, increasing soybean protein content produced a decrease in the solubility. The solubility obtained by the addition of soybean at the maximum level tested when the pea protein and TG were at the minimum value was 0.72 at pH 4 and 1.21 at pH 6. This agrees with the solubility profile of soybean given by Tömösközi et al. (2001) that showed higher solubility at pH 6 than at pH 4.

Microstructure analysis

The objective of the microstructure analysis was to elucidate the relationships between dough handling properties and food structure as suggested by Autio and Laurikainen (1997). Rice flour dough observed by scanning electron microscopy (SEM) showed a disrupted-like structure where starch granules were hold together by the proteins (Figure 5A). When transglutaminase (1%, w/w) was added, an uniform distribution of the starch granules through a more compact rice flour dough structure was observed (Figure 5B). Autio et al (2005) and Bonet et al (2006) observed an enhanced protein network when analysed TG-treated wheat dough by scanning electron microscopy. Soybean proteins displayed a gel-like structure (Figure 5C), whereas pea proteins presented aggregates of a distorted spherical structures (Figure 5D). When all the independent factors (protein isolates and transglutaminase) were mixed together at (+1), (+1), (+1) coded levels, a composite protein enriched blend with a significantly affected microstructure was obtained (Figure 5E). Rice flour constituents and pea proteins seemed to be integrated in a compact structure surrounded by the soybean proteins, making difficult the differentiation between rice and proteins isolate as independent structures. The effect of TG was not readily evident, although some protein strands were observed but it was not possible to decipher if they were from soybean proteins or consequence of the enzyme crosslinking. Increased aggregation of soy gels when treated with TG was reported by Fan et al. (2005), who analysed their structure using SEM. 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 (Bonet et al., 2006).

CONCLUSIONS

Composite protein enriched flours can be designed using rice flour, soybean and pea protein isolates. The addition of transglutaminase to the composite blends reinforced the network structure, although its effects were greatly masked by the high amount of protein isolates. Experimental design resulted in composite protein enriched blends with different functional, rheological, mechanical and surface related textural properties. Soybean protein isolate showed the most significant effect on the functional properties, rheometer and surface related textural responses. Scanning electron micrographs of the composite blends showed that the usage of soybean, pea protein isolates and TG would be a promising approach to produce protein enriched blends for making fermented gluten free products.

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FIGURE CAPTIONS

Figure 1. Response surface plots of water absorption . Effect of the addition level of the studied factors. SP: soybean protein, PP: pea protein, TG: transglutaminase.

Figure 2. Response surface plot of tan δ determined by dynamic rheology. Effect of the addition level of the studied factors. SP: soybean protein, PP: pea protein, TG: transglutaminase.

Figure 3. Response surface plots of the surface related textural properties. Effect of the addition level of the studied factors. SP: soybean protein, PP: pea protein, TG: transglutaminase.

Figure 4. Response surface plots of functional properties. Effect of the addition level of the studied factors. SP: soybean protein, PP: pea protein, TG: transglutaminase.

Figure 5. Scanning electron micrographs (x500) of different composite rice flour-protein blends. A: hydrated rice flour, B: hydrated rice flour in the presence of 1% (w/w) TG, C: hydrated soybean protein isolate, D: pea protein isolate, E: composite blend containing SP, PP and TG at +1, +1, +1 coded levels, respectively.

Table 1. Central composite design for sampling. Design factors are soybean protein isolate (SP), pea protein isolate (PP) and transglutaminase (TG). –1.68179, -1, 0, +1 and +1.68179 indicate coded levels of design factors. Axial distance: 1.68179. Values are expressed as g/100g of composite flours.

Factors	Symbols	Coded-variable levels				
		-1,68179	-1	0	+1	+1,68179
Soybean protein (g/100)	SP	1.0	5.9	13.0	20.1	25.0
Pea protein (g/100)	PP	1.0	5.9	13.0	20.1	25.0
Transglutaminase (g/100g)	TG	0.1	0.4	0.8	1.2	1.5

Table 2. Regresión equation[a] coefficients and analysis of variance for dough functional properties.

Coefficient	Functional properties						
	water absorption (%)	emulsifying activity (AU)	emulsion stability (%)	foaming capacity (ml)	foam stability (%)	solubility pH 4 (AU)	solubility pH 6 (AU)
b_0	240.9	0.309	61.23	0.43	62.60	0.650	0.740
b_1	41.2**	-0.054**	-17.36*	0.09*	19.22**	-0.237**	-0.219**
b_2	21.8**	-0.004	-2.58	0.07*	5.55*	-0.092	-0.059
b_3	1.3	-0.030	2.66	-0.08*	-11.21**	0.083	0.110
b_{11}	0.4	0.026	16.43*	-0.04	-11.35**	0.070	0.084
b_{22}	-1.2	0.025	-6.01	0.01	-3.17	0.028	0.033
b_{33}	2.3*	-0.002	-2.48	-0.03	-12.23**	-0.048	-0.018
b_{12}	3.3**	-0.002	2.84	0.02	-3.97	-0.018	-0.063
b_{13}	1.5	-0.007	-5.71	0.03	8.09*	-0.025	-0.071
b_{23}	1.3	0.005	-4.90	-0.01	-7.27*	0.068	0.092
R-SQ	0.9984	0.8085	0.7404	0.8256	0.9600	0.8014	0.8069

[a] $y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$
where x_1 = SP, x_2 = PP, x_3 = TG.
AU: Absorbance units.
* Significant at $\alpha<0.05$, ** significant at $\alpha<0.01$.

Table 3. Regresión equation[a] coefficients and analysis of variance for dough rheometer parameters.

Coefficient	Rheometer parameters		
	G´	G´´	Tan δ
	Pa	Pa	
b_0	68,868.5	11,566.0	0.169
b_1	-14,776.5*	-1,816.0*	0.010**
b_2	-1,219.6	203.1	0.010**
b_3	3,065.4	515.1	-0.001
b_{11}	-2,641.1	-946.6	-0.006**
b_{22}	-12,667.9*	-2,170.1*	-0.003*
b_{33}	-11,183.0	-1,850.8*	-0.001
b_{12}	-2,999.3	-870.1	-0.007**
b_{13}	2,386.3	392.0	0.002
b_{23}	5,755.0	935.6	0.002
R-SQ	0.7650	0.7580	0.9800

[a] $y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$
where x_1 = SP, x_2 = PP, x_3 = TG.
*Significant at $\alpha<0.05$, ** significant at $\alpha<0.01$.

Table 4. Regresión equation[a] coefficients and analysis of variance for surface related textural responses of the composite flours.

Coefficient	Surface related textural responses						
	hardness	cohesiveness	resilience	springiness	gumminess	adhesiveness	stickiness
	N				N	N x m	force, N
b_0	47.2	0.209	0.062	0.854	9.8	0.518	0.121
b_1	-5.6 *	0.030 *	0.004	0.115 **	0.3	-0.088	-0.021 **
b_2	5.6 *	0.022	0.001	0.104 **	2.5 **	-0.006	-0.038 **
b_3	5.3 *	0.002	-0.006	0.024	1.5	-0.192 *	-0.016 **
b_{11}	4.0	-0.002	-0.002	-0.013	0.6	-0.094	-0.002
b_{22}	0.1	0.007	0.006	-0.036	0.6	0.078	0.010 *
b_{33}	0,3	0.017	0.007	0.011	0.9	-0.060	0.011 *
b_{12}	-1.0	0.022	-0.004	-0.105 **	-1.3	0.072	0.004
b_{13}	0.1	-0.011	-0.008	0.033	-0.4	-0.073	0.004
b_{23}	1.7	0.017	0.006	0.025	1.4	-0.060	-0.009
R-SQ	0.7970	0.7380	0.4000	0.9350	0.7920	0.7250	0.9610

[a] $y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$
where x_1 = SP, x_2 = PP, x_3 = TG.
* Significant at $\alpha<0.05$, ** significant at $\alpha<0.01$.

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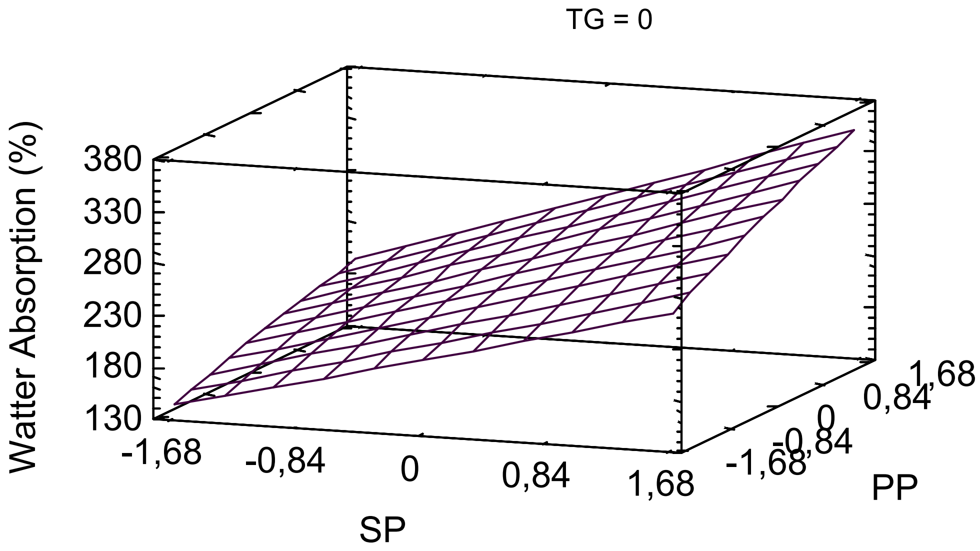


Figure 2. Response surface plot of tan δ determined by dynamic rheology. Effect of the addition level of the studied factors. SP: soybean protein, PP: pea protein, TG: transglutaminase.

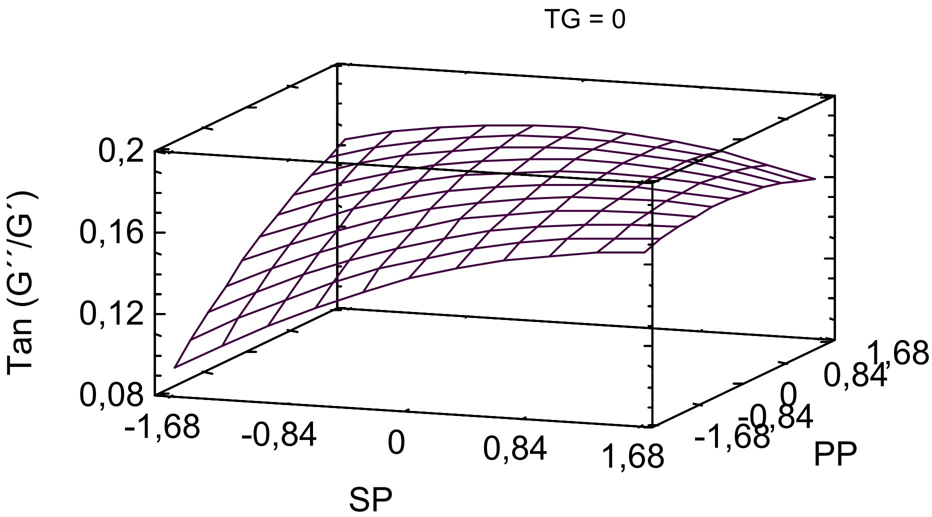


Figure 3. Response surface plots of the surface related textural responses. Effect of the addition level of the studied factors. SP: soybean protein, PP: pea protein, TG: transglutaminase.

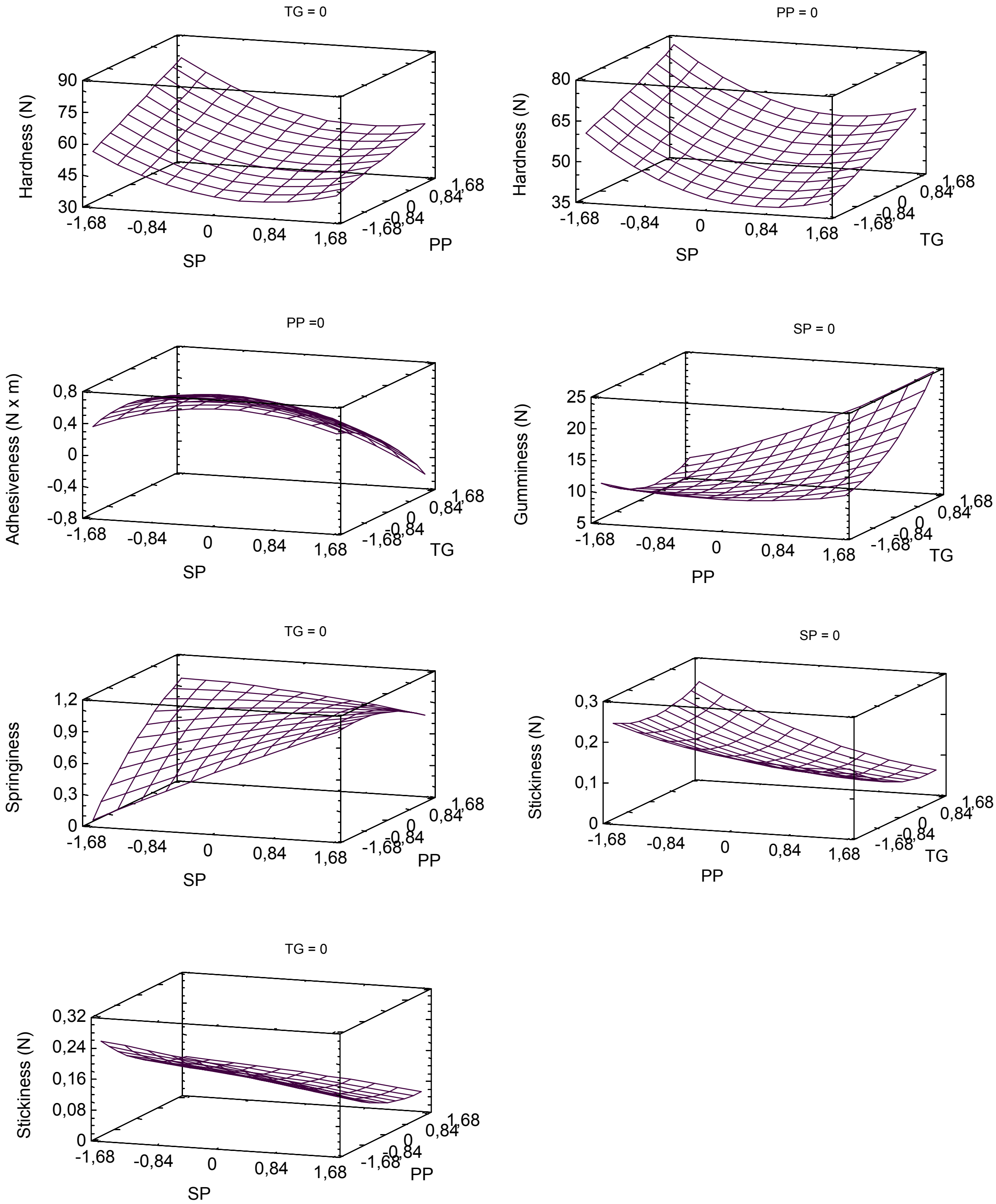
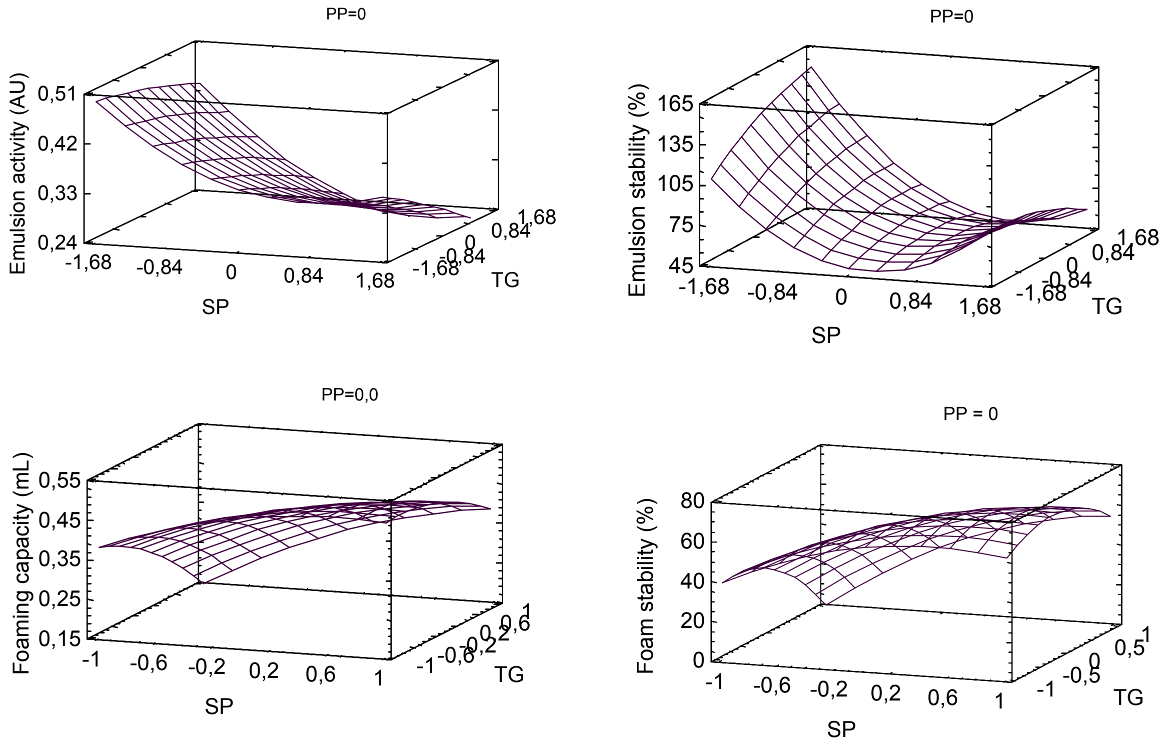


Figure 4. Response surface plots of functional properties. Effect of the addition level of the studied factors. SP: soybean protein, PP: pea protein, TG: transglutaminase.



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