BREADMAKING PERFORMANCE OF PROTEIN ENRICHED GLUTEN FREE BREADS

Cristina Marco, Cristina M. Rosell

Cereal Group, Institute of Agrochemistry and Food Technology (IATA-CSIC), P.O. Box 73, 46100 Burjassot, Valencia, Spain. E-mail: crosell@iata.csic.es

Running title: Protein enriched gluten free bread

Correspondence should be sent to:

Cristina M. Rosell

Food Science Department

Institute of Agrochemistry and Food Technology (IATA-CSIC)

P.O. Box 73, 46100 Burjassot, Valencia, Spain.

E-mail: crosell@iata.csic.es

Tel 34 963900022

Fax 34 963636301

ABSTRACT

Soybean enriched rice based gluten free breads were designed incorporating a structuring agent (hydroxypropylmethylcellulose HPMC) and a processing aid (transglutaminase TG). At dough level the effect of increasing amounts of soybean protein isolate (SPI), HPMC and water was studied in the Mixolab. Mixing and thermal characteristics showed the significant effect induced by water, soybean protein isolate, HPMC and TG, allowing the selection of the appropriate amounts for the breadmaking performance of enriched gluten free breads. The single addition or in combination of 4% HPMC, 13% soybean and 1% TG produced significant changes in the physical properties of the rice based gluten free breads. The presence of SPI blended with rice flour produced a significant decrease in the specific volume of the bread, although this detrimental effect was partially counteracted by its combination with HPMC, decreasing also the crumb hardness. The micrographs of the crumb showed the beneficial effect of the HPMC, obtaining a more open aerated structure. Protein enriched gluten free breads can be obtained with a combination of SPI, HPMC and TG.

Key words: rice flour, soybean proteins, hydroxypropylmethylcellulose, transglutaminase, bread, gluten free.

INTRODUCTION

The ability of wheat proteins to develop a viscoelastic matrix is what converts wheat in the most appropriate cereal for breadmaking. The protein fractions involved in the development of gluten are prolamins and glutelins that comprise 40 and 46% of the total proteins, respectively [1]. This viscoelastic matrix is able to retain the gas produced during the fermentation process, yielding an aerated crumb bread structure. However, gluten must be eliminated of the diet of celiac sufferers, since after its ingestion they suffer very important intestinal damage. Rice flour is a gluten free cereal frequently used for producing fermented products [2-4]. Rice flour has soft taste, colourless, low levels of sodium, easily digestible carbohydrates and low hypoallergenic properties. Nevertheless, rice flour is unable to develop a network with similar properties to gluten, likely due to its different storage protein ratio than the one found in wheat. In rice the major storage proteins are the glutelins (65-85%) while prolamins are the minor fraction [5]. In order to overcome the problems associated to the lack of viscoelasticity different gums -hydroxypropylmethylcellulose (HPMC), locust bean gum, guar gum, carrageenan, xanthan gum, agar- and starches (corn, cassava and potato) are often incorporated in the recipes of fermented gluten free products [6-10]. Among the hydrocolloids used, the HPMC is one of the most appropriate to improve the volume and texture of the rice based gluten-free breads [2, 11-14]. In addition, proteins from different sources such as soybean, egg albumen and dairy proteins can also be added to gluten-free flours in order to increase the nutritional value [15-18]. Soybean is a good counterpart for cereals, since legume and cereal proteins are complemented in the essential amino acids lysine and methionine improving the protein biological value of the product, [19]. Moreover, some health benefits are attributed to soy products, like the decrease of the risk for coronary heart disease associated to the consumption of soy protein due to the reduction in total low-density lipoprotein cholesterol and also in triacylglycerols [20]. However, high levels of soybean flour may lead to a decrease in the specific volume and sensory properties of

the bread [21]. Also a negative effect of high levels of soybean in wheat bread quality has been reported [22-23]. The deleterious effect of the addition of soybean has been associated to the lack of interaction between soy and gluten proteins [24]. However, the increase in the molecular size of the soybean proteins, produced by physical modifications leads to stronger soybean added wheat dough that yields larger loaf volumes [25]. Transglutaminase (TG) (protein-glutamine γ -glutamyltransferase, EC 2.3.2.13) is an enzyme whose the most dominant reaction is the covalent crosslinking between proteins through the reaction between an ϵ -amino group on protein-bound lysine residues and a γ -carboxyamide group on protein-bound glutamine residues. Soybean has shown to be substrate for the TG [26-29], obtaining protein chains with higher molecular weight that might improve the rheological properties of rice bread.

The goal of this work was to improve the rheological properties of soybean enriched gluten-free doughs for making rice based bread, by the addition of a structuring agent (HPMC) and a processing aid (TG), and to optimise the breadmaking process.

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% (dry basis), respectively. The moisture, protein, lipid and ash contents were determined following the AACC Approved Methods No 44-19, No 46-13, No 30-25 and No 08-01, respectively (AACC 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. Hydroxypropylmethylcellulose (HPMC) (Methocel K4M from Dow Chemical, USA) has 22.7% methyl groups and 11.2% hydroxypropyl groups. The HPMC viscosity of 2% solution in water was 4664 mPa s at 20 °C. Microbial transglutaminase from *Streptomyces* spp. from Ajinomoto

Co. Inc. (Tokyo, Japan) (100 units/g) was kindly supplied by Apliena, S.A. (Terrasa, Barcelona, Spain). All reagents were of food grade.

Mixolab measurements

Mixing and pasting behaviour of the rice flour dough and blends were studied using the Mixolab (Chopin, Tripette et Renaud, Paris, France), which measures in real time the torque (expressed in Nm) produced by passage of dough between the two kneading arms, thus allowing the study of its physico-chemical behaviour (Figure 1). [Rosell, Collar and Haros \[30\]](#) reported a detailed description of the equipment and the parameters registered. For the assays, 50 grams of rice flour were placed into the Mixolab bowl and mixed. The effect of variable amounts of water, soybean protein isolate, HPMC, and TG was tested using the Mixolab. The settings used in the test were 8 min for initial mixing, temperature increase at 2.3°C/min until 90°C, 7 min holding at 90°C, temperature decrease at 4°C/min until 50°C, and 5 min holding at 50°C; and the mixing speed during the entire assay was 80 rpm. Parameters obtained from the recorded curve give information about the protein stability subjected to mechanical and thermal constrain and both the gelatinization and gelling of starch. Parameters obtained from the recorded curve were (Figure 1): initial consistency (C1), stability (min) or elapsed time at which the torque produced is kept constant, minimum torque (Nm) or the minimum value of torque produced by dough passage subjected to mechanical and thermal constraints (C2), peak torque (Nm) or the maximum torque produced during the heating stage (C3), the minimum torque during the heating period (Nm) (C4) and the torque obtained after cooling at 50°C (C5) (more information about recorded parameters in [Bonet, Blaszcak and Rosell \[28\]](#); [Rosell, Collar and Haros \[30\]](#); [Collar, Bollain and Rosell \[31\]](#)).

Breadmaking process.

The formulations used are showed in table 1. Five hundred grams of rice flour and 550 mL of boiling water were mixed during 5 min in a 1-kg arm mixer. The dough was let to rest till the temperature decreased to 30 °C. Then, the rest of the flour (500 g) and the other ingredients, and water (550 mL) were added and mixed during 5 min. Dough pieces of 400 g were put in well-greased pans and proofed for 60 min at 30 °C and 85% RH. The dough pieces were baked for 1 hour at 175 °C. Then, the loaves were removed from the pans and cooled at room temperature for one hour. Loaves packaged in polypropylene bags were stored at 24 °C for 24 hours and then used for bread quality assessment.

Bread quality assessment

In order to determine the bread quality, the volume (rapeseed displacement), weight, height/width ratio of the slices, crust and crumb colour, and moisture content were quantified. Crust and crumb colour were determined using a colorimeter (Chroma Meter CR-400/410, Konica Minolta, Japan). Moisture content was determined following the ICC Method. Besides, a texture profile analysis (TPA) of the bread crumbs was performed by a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK). A bread slice of 1 cm-thickness was compressed up to 50% of its original height at a crosshead speed of 1 mm/s with a cylindrical stainless steel probe (diameter 25 mm). Measurements were performed after 24 h of baking. Values were the mean of four replicates.

Statistical analysis

Multiple sample comparison was used for the statistical analysis of the results, which 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.

SEM analysis

The structure of the crumb bread was analysed by scanning electron microscopy (SEM). Samples were freeze-dried and then mounted on metal stubs 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.

RESULTS AND DISCUSSION

Effect of the different ingredients and processing aids on the rice dough consistency

Mixing characteristics are strongly related to dough rheological properties, and they can be recorded as torque versus time curves obtained from small scale mixers [32]. It is well known that the presence of biochemical constituents like the added ingredients, additives, and technological aids in dough formulation modify wheat dough characteristics influencing baking performance [33-34]. In order to make a preliminary assessment of the individual effect of water content, HPMC, soybean protein isolate and transglutaminase on the breadmaking performance of rice flour, blends were subjected to a dual mechanical shear stress and temperature constraint using the Mixolab device. Information concerning mechanical and thermal protein weakening, starch gelatinization and starch gelling can be extracted from the recorded curves (Figure 1) [30-31]. The effect on the dough rheological behaviour of the addition of different amounts (65, 70, 75, 85 and 95%) of heated water (65°C) to rice flour was determined by the Mixolab (Table 2). The purpose was to find out the maximum amount of water that could be absorbed by the flour constituents, since the plasticizer effect of the water is crucial in the breadmaking of gluten-free rice and low-protein starch breads [35-36]. The sample with 95% of water showed the lowest development time, which is the time to reach the maximum torque during the mixing stage. As it was expected, the addition of increasing amounts of water produced a decrease in the

maximum torque (C1) reached by the dough during the first mixing step. The highest decrease in the maximum torque or maximum consistency was observed when the added water increased from 65% to 70%, where the torque decreased from 1.45 to 0.7 Nm. The addition of 95% water produced a decrease up to 0.18 Nm. The increase in the amount of water added produced a decrease in all the recorded parameters, with the exception of the amplitude (dough elasticity), which showed a decrease with the increase in the water absorption but an inflexion point was detected at 85% water absorption. Amplitude defined as the bandwidth of the mixogram could be indicative of the role of water in the lubrication during mixing as it happens in the case of the mixograph and has been related to extensional properties of the dough during mixing [37]. A decrease in the minimum torque (C2), peak torque (C3), the minimum torque reached during cooling (C4) and the torque after cooling at 50°C (C5) was also observed when increasing amounts of water were added, but this decrease was lower than the decrease observed during the mixing period previous to heating. The setback or the increase in the torque during the cooling (up to 50°C) decreased when the amount of water increased. Further studies were carried out using 95% of water absorption because dough gave good amplitude and enough peak torque during heating, consequence of the starch gelatinization.

When the effect of the addition of increasing amounts of HPMC was tested (Figure 2), a new consistency peak was detected before the gelatinization peak of the rice starch. The HPMC peak appeared at around 65°C when greater amount than 2% was added. The HPMC is a hydrocolloid with a two steps gelation process. The first step involves the formation of a pregel that starts around 30°C and the subsequent gelation produces a stable gel at around 75 °C. The transition from pregel to gel of methylcellulose derivatives occurs at around 50°C [38], but it seems that only at higher temperature is observed a change in the consistency. The maximum consistency of the dough increased with the addition of the hydrocolloid. The consistency of the doughs was

higher in the presence of HPMC during the entire assay, but the difference was most noticeable during the mixing stage. The effect of the HPMC on the rice starch gelatinization decreased as long as the amount of hydrocolloid increase, observing simultaneously a progressive increase of the HPMC gelation peak. Finally, 4% HPMC was selected as the most convenient hydrocolloid amount because it gave enough consistency increase during mixing with a minor gelation peak of HPMC.

Soybean protein isolate was tested at two levels of rice flour replacement 13% and 25% (flour-soybean proteins blend basis) (Table 3). The addition of the protein produced an increase in the development time, or the time necessary for hydrating all the compounds. The maximum consistency increased from 0.18 Nm in the absence of soybean to 0.75 or 1.34 Nm in the presence of 13% or 25% of soybean proteins, respectively. During the heating-cooling period, the effect of the soybean proteins was highly dependent on the amount of protein isolate present on the formulation, inducing opposite effects the presence of 13% or 25% of soybean proteins. The addition of soybean proteins increased setback, although this increase was higher with 13% than with 25% soybean. [Bonet, Blaszcak and Rosell \[28\]](#) also described an increase in the development time induced by the addition of protein sources (gelatin, egg and lupin) to wheat flour, that attributed to the nature of the proteins, since proteins are the main components involved in water absorption. Regarding to the peak torque, [Bonet et al. \[28\]](#) observed different effects depending on the protein source and the setback was significantly affected by the presence of soybean flour. The peak torque during heating was detected at around 65°C when soybean proteins were included in the formulation, which differed from the 90°C found when only rice flour was tested. This behaviour was likely due to the soybean protein aggregation during heating treatment. Glycinin and conglycinin are the major proteins of soybean and both have the ability to form ordered gel structures. The behaviour of the commercial soybean protein isolates may be quite different from native soy proteins, due to processing conditions causing denaturation and various states of aggregation [\[39\]](#).

The addition of transglutaminase only resulted in a increase of the development time and the dough stability (Table 3), the rest of the parameters recorded were similar to the ones observed in the rice flour.

The combined effect of HPMC, soybean protein isolate and TG was also studied (Table 3). HPMC was added at 4% level, soybean was added at 13% and TG at 1%. The addition of all the components gave doughs that showed a remarkable increase in the development time, and also in the values of stability and maximum torque during mixing. During the heating and cooling stage the increasing effect was not as notable as during mixing. This increase allowed increasing the addition of water till 110% (w/w, rice-protein blends basis). In those conditions, doughs with higher torque during the mixing stage compared to the rice dough were obtained. This combination was the one used for the breadmaking processes, although also the individual effect of the ingredients and processing aid studied were evaluated.

Effect of the different ingredients and processing aid on the quality of the gluten free bread

The effect of the individual presence of soybean protein isolate, HPMC and TG was studied on the quality of gluten-free bread. The values obtained for crust and crumb colour, specific volume, height/width ratio of the slices and moisture content are showed in Table 4. The presence of soybean protein blended with rice flour produced a significant ($P<0.05$) decrease in the specific volume of the bread, in opposition, the addition of HPMC produced an increase in the specific volume. The presence of TG did not modify the specific volume of the fresh rice-based bread. The detrimental effect of the soybean proteins on the specific volume was partially counteracted by its combination with HPMC. Therefore, the addition of HPMC allowed obtaining protein

enriched gluten free breads with acceptable specific volume, similar to the one observed in the rice based bread.

The loaves obtained with the addition of HPMC showed significantly higher height/width ratio of the slices. And that effect was reduced when soybean proteins were added in the formulation of the gluten free bread. When all the components were added together, the values obtained for this parameter were similar to those obtained for the rice alone. The single addition of TG or soybean proteins did not produce any significant effect in this parameter. When soybean proteins and TG were added together a significant ($P<0.05$) decrease was observed. [Ribotta et al. \[16\]](#) reported that rice-cassava based gluten-free breads with inactive soybean flour showed very dense crumb structure and low volume, whereas bread volume improved when active soybean flour was added. Also a negative effect in the bread volume was reported when soy products were added to wheat flour, where the soybean produced a loss of gas retention properties due to dough weakening and soy-interrupted gluten [\[40\]](#).

The improving effect of hydrocolloids on the bread volume has been reported by several authors [\[10, 12, 14, 41-42\]](#). The addition of hydrocolloids (carboxymethylcellulose, agarose, xanthan, β -glucan, pectin) at 1% supplementation level produced an increase in the volume of gluten-free bread except for xanthan and pectin, although when the hydrocolloid dosage increased from 1% to 2% the opposite effect was observed, except for pectin [\[10\]](#). [McCarthy et al. \[14\]](#) also reported a slight decrease in the gluten-free bread volume when increasing the addition level of HPMC. However, [Gujral and Rosell \[12\]](#) obtained higher volume when the addition level of HPMC to rice flour was increased until 4%, obtaining bread loaves with 2.5 cm³/g specific volume. The improving effect of the HPMC on the bread volume might be due to their ability to retain water and also the formation of a gel network during heating in the breadmaking process. This network will increase the viscosity and also will give strength to the expanding cells of the dough; as a result, gas retention during baking improves, obtaining better bread volume [\[43\]](#).

The addition of HPMC produced a significant ($P<0.05$) decrease in the moisture content of the bread, due to the ability of the hydrocolloids to retain water molecules. Opposite results were reported by [Bárcenas and Rosell \[41\]](#) when HPMC was added in the formulation of partially baked bread, because an extra amount of water was added to keep constant the dough consistency and compensate the water retention capacity of the HPMC. In the present study, the reduction in the moisture content induced by the HPMC was partially masked when part of the rice flour was replaced by soybean proteins; where rice starch molecules were replaced by protein molecules.

The bread colour also was affected by the addition of the studied components. The lower values of *L* (lightness) of the crust were obtained in the presence of TG. The breads containing TG had darker crust, which might be attributed to the maltose present in the commercial preparation of the TG that produces an increase in the non-enzymatic browning during the baking. The combination of HPMC and soybean produced the breads with the highest *L* value. The *a* scale varies from negative values (green hue) to positive values (red hue); and the *b* scale varies from negative values (blue hue) to positive values (yellow hue). The *a* values of the crust were all positive. The individual addition of HPMC did not promote any effect on the *a* parameter of the crust. But the single addition of soybean protein or TG or in combination with the other compounds produced an increase in this parameter, being the effect of TG higher than the one induced by the soybean. Presumably, the increase in the value of *a* obtained by soybean and TG were due to the increase of lysine groups and maltose, respectively, that are involved in the non-enzymatic browning reaction or Maillard reaction. The colour of the commercial soybean protein preparation also conferred some dark colour to the bread. The *b* values for the crust were positive (yellow hue) for all the samples. The presence of TG in the gluten free formulation yielded the lowest *b* values of the crust. The presence of soybean protein produced an increase of this parameter, and the crust bread with soybean and HPMC showed the highest *b* value.

Regarding to the crumb, *L* increased when any of the components used in this study was incorporated to rice bread formulation. The *a* values were positive in the presence of soybean protein and negative in its absence. The single addition of HPMC or TG produced a decrease in this parameter. The *b* value increased in the presence of soybean (single or in combination). The addition of TG or HPMC produced a slight increase in this parameter.

The values obtained for the textural parameters of the rice bread are showed in the Table 5. All the components incorporated significantly ($P<0.05$) affected the hardness. The addition of HPMC produced a significant decrease of the crumb hardness. Conversely, the addition of soybean proteins induced a significant increase of the crumb hardness, only compensated when HPMC was included in the formulation. The hardness also increased in all the samples that contained TG, although in lesser extent than the effect induced by the soybean proteins. The hardness increase resulted with the addition of TG has been reported previously in gluten-free rice based breads and it might be attributed to the protein crosslinking catalyzed by the TG, increasing the strength of the crumb [11]. The softening effect of the HPMC has been directly related to its effect on the specific volume of the bread [11-12, 41], because there is a positive relationship between the bread specific volume and the crumb hardness. The increase in the hardness due to the addition of soybean protein isolate agrees with results of Ahlborn et al. [35], who reported that the force needed to obtain compression values were higher for added-protein bread than for standard wheat bread.

Regarding the adhesiveness (how the bread stuck to the palate, tongue, and teeth), the highest absolute value was obtained when soybean and HPMC were added jointly but it decreased significantly in the presence of TG. A significant decrease in the cohesiveness (related to how the bread held together as it was masticated) was

observed when soybean proteins and TG were added together, likely due to the hindering effect of the soybean proteins on the enzyme activity, which agrees with previous results of [Ahlborn et al. \[35\]](#) in protein enriched wheat bread. Opposite results have been reported by [Collar et al. \[44\]](#), when TG was added to wheat breads. TG produced suitable effects on low extraction rate flours (increased cohesiveness, volume, aroma intensity, typical taste and crumb cell ratio and decreased cell number), whereas the effects were adverse on high extraction rate flours (decreased volume, typical taste and crumb cell ratio and increased crumb hardness, chewiness and cell number). Regarding the resilience, it significantly decreased in the presence of soybean (single or in combination), while a significant increase in this parameter was observed when HPMC or HPMC+TG were added. The TG did not show any significant effect in the resilience.

Microstructure of gluten free bread crumbs

Scanning electron microscopy was used in order to see the effect of the addition of soybean protein isolate, HPMC and TG on the microstructure of the rice crumb bread. Rice crumb bread showed a large number of very small gas cells in a interrupted protein matrix, presenting a dense structure (Fig 3A). In the presence of soybean was even more disaggregated, and the shape of the gas cells were very irregular (Fig 3B). When TG was added, the structure of the crumb looked less disaggregated, showing less gas cells (Fig 3D). This appearance might be attributed to the protein crosslinking that leads to a more continuous protein matrix. A more compact and homogeneous protein network due to the protein crosslinking was reported by [Bonet, Blaszcak and Rosell \[28\]](#) in wheat doughs with soy flour when they were treated with TG. The addition of HPMC resulted in a more continuous matrix with improved structure, since less number and bigger size of cell gas were observed (Fig. 3C, E, G, H). The beneficial effects of the hydrocolloids on the dough or crumb structure have been previously reported in frozen doughs or partially baked bread [\[41, 45\]](#). The HPMC

seems to hold the constituents of the crumb covering them by a veil-like film [41], although also the physical interaction between the HPMC and the proteins or the starch granules could be possible [46-47, 38]. In addition, the use of hydrocolloids, such as xanthan gum, in gluten free bread produces a web-like structure similar to the structure of the standard wheat bread [35]. However, the hydrocolloids cannot produce this web-like structure when they are used in starch based gluten free breads, indicating the importance of the proteins to form a continuous phase since the hydrocolloids alone do not seem to be enough to stabilize gas cells [35]. In the present study, the crumb of the bread containing HPMC and TG (Fig. 3G) and also soybean protein (Fig. 3H) showed the best structure, with more aerated structure.

CONCLUSIONS

The rheological properties of the soybean enriched rice doughs can be modified by the use of structuring agents, such as HPMC, and processing aids, such as TG. The protein crosslinking produced by the TG reaction is reflected in a increase in the hardness of the bread crumb and a more continuous structure of the crumb observed in the micrographs. The use of HPMC allowed improving the volume of the bread, compensating the detrimental effect of the soybean, observing a more aerated structure closer to the wheat bread structure.

ACKNOWLEDGEMENTS

This work was financially supported by Comisión Interministerial de Ciencia y Tecnología Project (MCYT, AGL2005-05192-C04-01) and Consejo Superior de Investigaciones Científicas (CSIC). C. Marco gratefully acknowledges the Ministerio de Educación y Ciencia for her grant.

REFERENCES

1. Orth RA, Bushuk W (1972) Cereal Chem 49:268-275

2. Gujral HS, Guardiola I, Carbonell JV, Rosell CM (2003) J Agric Food Chem 51:3814-3818
3. Gujral HS, Haros M, Rosell CM (2003) Cereal Chem 80(6):750-754
4. Lopez ACB, Pereira AJG, Junqueira RG (2004) Brazilian Arch Biol Technol, 47:63-70
5. Huebner FR, Bietz JA, Webb BD, Juliano BO (1990) Cereal Chem 67:129-135
6. Nishita KD, Roberts RL, Bean MM (1976) Cereal Chem 53:626-635
7. Kang MY, Choi YH, Choi HC (1997) Korean J Food Sci Technol 29:700-704
8. Kobylański JR, Pérez OE, Pilosof AMR (2004) Thermochim Acta 411:81–89
9. Schober TJ, Bean SR, Boyle DL (2007) J Agric Food Chem 55:5137-5146
10. Lazaridou A, Duta D, Papageorgiou M, Belc N, Biliaderis CG (2007) J Food Eng 79(3):1033-1047
11. Gujral HS, Rosell CM (2004) J Cereal Sci 39:225-230
12. Gujral HS, Rosell CM (2004) Food Res Intl 37:75-81
13. Sivaramakrishnan HP, Senge B, Chattopadhyay PK (2004) J Food Eng 62(1):37-45
14. McCarthy DF, Gallagher E, Gormley TR, Schober TJ, Arendt EK (2005) Cereal Chem 82:609-615
15. Gallagher E, Kunkel A, Gormley TR, Arendt EK (2003) Eur Food Res Technol 218:44-48
16. Ribotta PD, Ausar SF, Morcillo MH, Perez GT, Beltramo DM, Leon AE (2004) J Sci Food Agric 84:1969-1974
17. Moore MM, Heinbockel M, Dockery P, Ulmer HM, Arendt EK (2006) Cereal Chem 83(1):28-36
18. Marco C, Rosell CM (2008) J Food Eng 84:132-139
19. Iqbal A, Khalil IA, Ateeq N, Khan MS (2006) Food Chem 97:331-335
20. Anderson JW, Johnstone BM, Cook-Newell ME (1995) The New England J Med 333:276-282
21. Sánchez HD, Osella CA, de la Torre MA (2004) Food Sci Technol Int 10(1):5-9

22. D'Appolonia BL (1997) *Cereal Chem* 54:53-63
23. Doxastakis G, Zafiriadis I, Irakli M, Marlani H, Tananaki C (2002) *Food Chem* 77:219-227
24. Ryan KJ, Homco-Ryan CL, Jenson J, Robbins KL, Prestat C, Brewer MS (2002) *Cereal Chem* 79:434-438
25. Maforimbo E, Skurray G, Uthayakumaran S, Wrigley CW (2006) *J Cereal Sci* 43:223-229
26. Basman A, Koksel H, Ng PKW (2002) *J Food Sci* 67(7):2654-2658
27. Tang CH, Wu H, Chen Z, Yang XQ (2006) *Food Res Int* 39(1):87-97
28. Bonet A, Blaszcak W, Rosell CM (2006) *Cereal Chem* 83(6):655-662
29. Marco C, Pérez G, León A, Rosell CM (2008) *Cereal Chem* 85:59-64..
30. Rosell CM, Collar C, Haros M (2007) *Food Hydrocolloids* 21:452-462
31. Collar C, Bollaín C, Rosell CM (2007) *Food Sci Tech Int* 13(2):99-107
32. Dobraszczyk BJ, Morgenstern MP (2003) *J Cereal Sci* 38:229-245
33. Collar C, Bollaín C (2005) *Eur Food Res Technol* 220:372-379
34. Collar C, Santos E, Rosell CM (2006) *Cereal Chem* 83:370-376
35. Ahlborn GJ, Pike OA, Hendrix SB, Hess WM, Huber CS (2005) *Cereal Chem* 82(3):328-335
36. Rosell CM, Marco C (2007) *Proc 57th Australian Cereal Chem Conf* 155-158
37. Gras PW, Carpenter HC, Anderssen RS (2000) *J Cereal Sci* 31:1-13
38. Rosell CM, Foegeding A (2007) *Food Hydrocolloids* 21:1092-1100
39. Hermansson AM (1986) *J Am Oil Chem Soc* 63:658-666
40. Ribotta PD, Arnulphi SA, León AE and Añón MC (2005) *J Sci Food Agric* 85(11):1889-1896
41. Bárcenas ME, Rosell CM (2005) *Food Hydrocolloids* 19(6):1037-1043
42. Haque A, Morris ER (1994) *Food Res Intl* 27(4):379-393
43. Bell DA (1990) *Cereal Foods World* 35:1001-1006
44. Collar C, Bollaín C, Angioloni A (2005) *J Food Eng* 70:479-488

45. Sharadanant R, Khan K (2006) Cereal Chem 83(4):411-417
46. Armero E and Collar C (1998) J Cereal Sci 28:165-174
47. Collar C, Armero E, Martínez J (1998) Z Lebensm Unters Forsch A 207:110-121

FIGURE CAPTIONS

Figure 1. Typical curve obtained in the Mixolab showing C1, C2, C3, C4 and C5 related to maximum dough torque, protein weakening, starch gelatinisation, starch breakdown and starch retrogradation, respectively.

Figure 2. Effect of the increasing amount of HPMC on the rice flour consistency determined by the Mixolab device.

Figure 3. Scanning electron micrographs (x100) of the crumb of different rice based breads. A: rice flour; B: rice flour + soybean protein isolate (SPI); C: rice flour + HPMC; D: rice flour + TG; E: rice flour + SPI + HPMC; F: rice flour + SPI + TG; G: rice flour + HPMC + TG; H: rice flour + SPI + HPMC + TG. In the formulation, SPI, HPMC and TG were used at 13, 4 and 1% (w/w), respectively.

Table 1. Formulation of the gluten free breads.

	Rice flour	SPI	HPMC	TG
Rice	100			
Rice+SPI	87	13		
Rice+HPMC	100		4	
Rice+TG	100			1
Rice+SPI+HPMC	87	13	4	
Rice+SPI+TG	87	13		1
Rice+HPMC+TG	100		4	1
Rice+SPI+HPMC+TG	87	13	4	1

Values expressed in % (w/w) (rice-protein blend basis).

SPI: soybean protein isolate, HPMC: hydroxypropylmethylcellulose, TG: transglutaminase.

1 **Table 2.** Effect of water absorption on the rice dough characteristics during mixing and heating determined by using the Mixolab.

Water absorption (%)*	Development time (min)	Maximum torque (C1) (Nm)	Amplitude (Nm)	Stability (min)	Minimum torque (C2) (Nm)	Peak torque (C3) (Nm)	C4 (Nm)	C5 (Nm)	Setback (C5-C4) (Nm)	Cooking stability (C4/C3)
65	0.77	1.45	0.16	1.78	0.70	1.58	1.46	2.16	0.70	0.92
70	0.78	0.70	0.07	1.70	0.43	1.37	1.24	1.76	0.52	0.91
75	0.87	0.52	0.06	0.70	0.32	1.26	1.15	1.56	0.41	0.91
85	0.87	0.38	0.04	0.55	0.06	1.01	0.90	1.20	0.30	0.89
95	0.50	0.18	0.06	0.33	0.02	0.82	0.75	0.96	0.21	0.91

2

3 * Amount of water expressed on rice flour basis.

Table 3. Effect of different ingredients and processing aid on the rice dough characteristics during mixing and heating determined by using the Mixolab. Assays were performed using 95% of water absorption (WA), unless otherwise stated.

Samples	Development time (min)	Maximum			Minimum		Peak		Setback (C5-C4) (Nm)	cooking stability (C4/C3)
		torque	Amplitude	Stability	torque	torque	C4	C5		
		(C1)			(C2)	(C3)				
		(Nm)	(Nm)	(min)	(Nm)	(Nm)	(Nm)	(Nm)		
rice	0.50	0.18	0.06	0.33	0.02	0.82	0.75	0.96	0.21	0.91
rice + SPI- 13%	0.92	0.75	0.04	0.65	0.40	0.64	0.58	1.14	0.56	0.91
rice + SPI- 25%	1.30	1.34	0.09	2.52	0.99	1.23	1.00	1.31	0.31	0.81
rice + TG-1%	1.40	0.22	0.06	0.42	0.03	0.91	0.68	0.92	0.24	0.75
rice + SPI-13% + HPMC-4% + TG-1%	0.93	1.17	0.06	2.58	0.54	1.06	0.90	1.19	0.29	0.85
rice + SPI-13% + HPMC-4% + TG-1% (110% WA)	0.63	0.67	0.05	2.05	0.29	0.85	0.69	0.86	0.17	0.81

SPI: soybean protein isolate, HPMC: hydroxypropylmethylcellulose, TG: transglutaminase.

Values expressed in % (w/w) (rice-protein blend basis).

Table 4. Effect of individual and combined addition of different ingredients (HPMC and soybean protein isolate) and processing aid (transglutaminase) on the quality of rice flour based breads.

	Specific volume cm ³ /g	Height/width ratio	Moisture content (%)	Crust color parameters			Crumb color parameters		
				L	a	b	L	a	b
Rice	2.00 b	0.08 b	45.59 e	45.86 c	5.60 a	19.20 b	54.47 a	-0.87 b	7.07 a
Rice+SPI	1.59 a	0.08 bc	46.07 f	44.25 c	9.22 c	21.06 c	61.25 bcd	0.61 cd	14.54 d
Rice+HPMC	2.71 c	0.12 e	41.66 a	47.41 c	5.51 a	18.93 b	65.24 d	-0.97 b	7.80 b
Rice+TG	1.97 b	0.08 b	46.13 f	30.96 ab	12.73 e	14.56 a	62.76 bcd	-1.22 a	8.82 c
Rice +SPI+HPMC	1.95 b	0.10 c	46.08 f	54.33 d	7.77 b	23.10 d	64.48 cd	0.55 cd	14.56 d
Rice+SPI+TG	1.57 a	0.07 a	45.43 d	28.33 a	13.70 f	14.17 a	59.70 b	0.77 d	14.52 d
Rice+HPMC+TG	2.69 c	0.11 d	43.70 b	33.92 b	11.26 d	14.53 a	60.86 bc	-1.09 ab	7.65 ab
Rice+SPI+HPMC+TG	1.96 b	0.08 bc	45.06 c	31.39 ab	13.21 ef	14.27 a	62.76 bcd	0.49 c	15.09 d

SPI (soybean protein isolate), HPMC (hydroxypropylmethylcellulose) and TG (transglutaminase) added at 13, 4, and 1% (w/w) (rice-protein blend basis), respectively.

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

18 **Table 5.** Texture profile analysis of the bread crumbs obtained from different rice based gluten-free formulations.

19

	Hardness (N)	Adhesiveness (N*m)	Springiness	Cohesiveness	Chewiness (N*m)	Resilience
Rice	7.25 b	-0.0019 a	0.773 a	0.577 bc	0.0032 b	0.222 c
Rice+SPI	22.32 e	-0.0047 b	0.862 bcd	0.595 c	0.0097 d	0.172 a
Rice+HPMC	1.95 a	-0.0014 a	0.905 cd	0.691 d	0.0012 a	0.259 d
Rice+TG	17.69 d	-0.0017 a	0.779 ab	0.511 ab	0.0070 c	0.214 bc
Rice+SPI+HPMC	13.12 c	-0.0222 d	0.844 abc	0.610 c	0.0067 c	0.189 ab
Rice+SPI+TG	26.76 f	-0.0077 c	0.819 abc	0.505 a	0.0111 e	0.168 a
Rice+HPMC+TG	3.34 a	-0.0028 ab	0.936 d	0.692 d	0.0022 ab	0.266 d
Rice+SPI+HPMC+TG	14.28 c	-0.0055 bc	0.840 abc	0.522 ab	0.0062 c	0.179 a

20

21 SPI (soybean protein isolate), HPMC (hydroxypropylmethylcellulose) and TG (transglutaminase) added at 13, 4, and 1% (w/w) (rice-protein
22 blend basis), respectively.

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

Figure 1.

Figure 2.

Figure 3.