**RHEOLOGICAL PROPERTIES OF RICE-SOYBEAN PROTEIN COMPOSITE
FLOURS ASSESSED BY MIXOLAB® AND ULTRASOUND**

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ABSTRACT

Rheological behaviour of gluten free composite flours formulated with rice flour and increasing amounts of soybean protein isolate (0-2%, w/w, flour blend basis) in the presence and absence of transglutaminase (1%, w/w), was assessed using two complementary approaches, the Mixolab® device and ultrasonic measurements. Dough was subjected to dual mechanical and thermal constraint, namely mechanical changes due to mixing and heating, in the Mixolab® and in parallel measurements of ultrasonic attenuation and velocity were performed at two different temperatures (25°C and 65°C). Main differences were observed during the mixing process, where soybean proteins (SP) and transglutaminase (TG) induced an important increase of the dough consistency. Results from ultrasonic measurements were in accordance with those obtained with the Mixolab® device, being able both approaches to assess the empirical rheological behaviour of gluten free matrixes and even to detect the effects induced by protein ingredients and processing aid.

Practical Applications

Two complementary approaches, the Mixolab® device and ultrasonic measurements are proposed for assessing rheological behaviour of gluten free doughs. Mixolab® provides information of gluten free dough empirical rheology following the dough physico-chemical changes associated to dual mechanical shear stress and temperature constraint. Ultrasound may be also used to determine consistency changes in gluten free dough, resulting in an interesting potential for future development of a quality control system intended to the industrial production process of dough. Consequently, Mixolab® and ultrasounds could be regarded as promising tools in order to investigate rice flour dough properties and the changes induced by other ingredients and processing aids.

Key words: rice flour, Mixolab®, ultrasound, soybean protein, TG, rheology.

INTRODUCTION

Breadmaking is a dynamic process where physico-chemical changes are taking place induced by mechanical work, heating and the biochemical activity of the baker's yeast. The mechanical work and the heat treatment applied during the breadmaking process affect the physicochemical properties of the dough components, determining the quality of the end product. The rheological properties are the more relevant in this process, due to the important role of a viscoelastic matrix (Collar and Armero, 1996; Uthayakumaran et al., 2000). Rheological changes produced in the dough during this process, have been the focus of numerous studies, although independent devices had to be used in order to fulfill the dough performance along the different breadmaking stages. The deformations in wheat dough produced during the mixing and during the fermentation process have been well characterized (Bollaín and Collar, 2004; Rosell and Collar, 2008). Large amount of studies have been focused on the different mechanical dough parameters determined by different devices (Bollaín and Collar, 2004; Mikhaylenko et al., 2000; Rosell et al., 2001; Uthayakumaran et al., 2000). In fact, rheological analysis has been successfully applied as predictor of the functionality of the gluten and starch in breadmaking wheat dough performance (Armero and Collar, 1997; Collar and Bollaín, 2005; Bollaín et al., 2006). However, in contrast to wheat doughs, scarce studies have been reported on gluten-free matrixes. Gluten free dough lacks of natural viscoelastic network, named gluten, which makes necessary to completely design polymeric matrixes to meet breadmaking requirements. The most recent alternative when working with gluten-free matrixes is the use of enzymes (Gujral and Rosell, 2004a, 2004b; Moore et al., 2006) and different protein sources (Gallagher et al., 2003; Marco and Rosell, 2008a, 2008b) for building a polymeric network in order to use these blends in baking processes. Fundamental rheology has been used to determine the viscoelastic behaviour of gluten free doughs, defining a solid elastic-like behavior of the rice flour dough with higher elastic modulus (G') than the viscous modulus (G'') (Gujral and Rosell, 2004a, Marco and Rosell, 2008a). However, it has been stated for wheat flour dough that mixing involves large deformations, that are beyond the linear viscoelastic limit; only large deformation

measurements can provide information about the extent of the contribution of long-range (protein-protein) and short range (starch-starch, starch-protein) interactions to the rheological behavior of dough (Collar and Bollaín, 2005).

In addition, the assessments of the functional properties of the gluten free matrixes have provided information about emulsifying and foaming activities and stabilities (Marco and Rosell, 2008a, 2008b). However, it is always difficult to characterize the performance of gluten free dough in real time and using the matrixes with the consistency of gluten free bread dough.

Other alternative techniques, scarcely applied to food technology, due to their novelty like the Mixolab® device or their uncommon use in cereals like the ultrasound measurements, could be very promising approaches for assessing the rheological behavior of gluten free doughs. The Mixolab® works with real dough systems giving information about protein and starch changes along mixing, heating and cooling, recording the mixing and pasting properties of the flours (i.e. flour behaviour under mechanical and thermal constraints). The use of this device has been successfully applied to wheat dough characterization (Bonet et al., 2006; Kahraman et al., 2008), and to assess the effect of different additives (proteins and hydrocolloids) and processing aids (α -amylase, xylanase and transglutaminase) (Bonet et al., 2006; Collar et al., 2007; Rosell et al., 2007).

Ultrasound techniques may offer some advantages over conventional methods of dough testing due principally to its relatively lower cost and its non-destructive, hygienic and almost real time performance (Alava et al., 2007). In addition, there is some scientific literature that deals with the potential of ultrasound testing in the determination of some wheat flour dough properties (Elmehdi et al. 2003; Garcia-Alvarez et al. 2006; Lee et al., 2004; Letang et al., 2001; Kidmose et al., 2001; Scanlon and Zghal, 2001; Scanlon et al., 2002). Some of these works compare extensional and/or low strain rheology with ultrasound analysis, showing good agreement between these methods. In addition, good agreement was also found between the results obtained by shear oscillatory tests and ultrasound measurements (Ross et al. 2006). Despite the

reported potential of ultrasound to characterize wheat dough, in our knowledge there is no information available about ultrasound characterization of some other kinds of dough, such as gluten free dough. Since the ultrasound properties of wheat flour dough are strongly influenced by its gluten network, besides its gas content (Scanlon et al., 2002), it is feasible to consider that the acoustic properties of rice flour dough could also be related with the properties of their protein matrix. Therefore, ultrasound could be sensitive to the role of SP and TG in the properties of rice flour dough given that the addition of these ingredients may affect the properties of its protein network.

The objective of this study was to assess the rheological properties of gluten free rice flour based dough using two complementary approaches, the Mixolab® device and ultrasound measurements, and their potential to distinguish the changes induced by protein ingredients like soybean protein isolate and processing aids like the transglutaminase.

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 AACCI Approved Methods No 44-19, No 46-13, No 30-25 and No 08-01, respectively (AACCI 1995). Soybean protein isolate was from Trades SA (Barcelona, Spain). The protein isolate had moisture, protein, lipid and ash contents of 6.0, 91.2, 0.4 and 4.8% (dry basis), respectively. The microbial transglutaminase of food grade (Activa™ TG) (100 units/g) was provided by Apliena, S.A. (Terrasa, Barcelona, Spain).

Determination of rheological behaviour using Mixolab®

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 rheological behaviour (Figure 1). For the assays, 50 grams of rice flour were placed into the Mixolab® bowl and mixed with the amount of water needed for obtaining 65% (v/w, flour basis) water absorption. The effect of soybean protein isolate, and TG was tested using the Mixolab®. The settings used in the test are detailed in Table 1. Parameters obtained from the recorded curve (Figure. 1): give information about the protein stability subjected to mechanical and thermal constraint and both the gelatinization and gelling of starch. Those parameters included initial maximum consistency (Nm) (C1), stability (min) or time until the loss of consistency is lower than 11% of the maximum consistency reached during the mixing, amplitude (Nm) or the bandwidth at C1 related to dough elasticity (Gras et al., 2000; Rosell and Collar, 2008), the mechanical weakening defined as the loss of consistency due to shearing (previous to the heating stage), minimum torque (Nm) or the minimum value of torque (Nm) produced by dough passage subjected to mechanical and thermal constraints (C2), thermal weakening or the loss of consistency mainly due to heating (torque at the end of 30 °C stage – 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 (Nm) obtained after cooling at 50°C (C5). The different slopes of the curve during the assay are related to different properties of the flour: speed of the weakening of the protein network due to heating (alfa); gelatinization rate (beta); cooking stability rate (gamma), and amylose retrogradation (delta). More information about recorded parameters in Bonet et al. (2006), Rosell et al. (2007) and Collar et al. (2007). Results are the average of duplicate measurements.

Ultrasonic measurements

Samples for the ultrasound analysis were extracted from the Mixolab when reaching the temperature of 25°C and 65°C. Then, each sample was divided into three similar size sub-samples and tested with no resting time.

The set-up used for ultrasonic characterization is depicted in Figure 2. It consists of a signal generator (HP33120A) connected to an ultrasonic shear wave emitter transducer (Panametrics)

and a digital oscilloscope (LeCroy LT344) linked to the ultrasound receiver transducer (Panametrics). The nominal resonant frequency of the shear transducers is 100 kHz in order to find the lowest level of attenuation in the experiments. The dough sample was placed between both transducers. The relative high attenuation that rice dough samples present leads to reduce the maximum distance between transducers to less than 2 mm in order to ensure sufficient signal strength. Furthermore, a sine burst excitation was used to avoid transducer overheating and the absence of standing waves was checked by observing the exponential increase of the signal amplitude and the linear decrease of the phase when transducers approach each other. So as to compensate the changes in ultrasound velocity and attenuation introduced at every interface between materials with different acoustic impedance, differential measurements have been carried out. Using that measurement procedure, detailed in (Alava et al., 2007), the same number and type of interfaces are kept in measurements at two fixed distances, d_1 and d_2 , and the effects of these interfaces can be thus easily compensated. The equations used to obtain the shear ultrasound velocity and attenuation are, respectively:

$$v = \frac{d_2 - d_1}{t_2 - t_1} \quad \text{Eq. 1}$$

$$\alpha = \frac{20 \cdot \log\left(\frac{A_2}{A_1}\right)}{d_2 - d_1} \quad \text{Eq. 2}$$

where A_1 and A_2 are the amplitude of the received signal at the distances d_1 and d_2 respectively. The time point t_1 and t_2 are the position of the first zero-crossing points of the received signals at the corresponding distances. If the small strain hypothesis (Letang et al., 2001) is verified ($\alpha v / \omega < 1$), the ultrasound rheological parameters' G' and G'' can be obtained through the following equations (Kono, 1960; Letang et al., 2001; Lee et al., 2004), respectively:

$$G' = \frac{\rho v^2 \left(1 - \frac{\alpha^2 v^2}{\omega^2}\right)}{\left(1 + \frac{\alpha^2 v^2}{\omega^2}\right)^2} \quad G'' = \frac{2\rho v^2 \left(\frac{\alpha v}{\omega}\right)}{\left(1 + \frac{\alpha^2 v^2}{\omega^2}\right)} \quad \text{Eq. 3 and Eq. 4}$$

Statistical analysis

In order to study the relationships between ultrasound and Mixolab® parameters, linear correlations were considered for all variables. These statistical analyses were performed using the Statgraphics (V5.5) program.

RESULTS AND DISCUSSION

Thermomechanical behaviour of gluten free dough

There is general consensus that mixing characteristics are strongly related to wheat dough rheological properties, and they can be recorded as torque versus time curves obtained from small scale mixers (Zheng et al. 2000; Dobraszczyk and Morgenstern, 2003). The Mixolab® plot provides useful information about dough behavior submitted to thermo mechanical constraints, namely dual mechanical shear stress and temperature constraint, recording the torque produced by the passage of the dough between the two kneading arms (Figure 1). The rheological behaviour of rice flour containing different amounts of soybean protein isolate (SP), in the presence and in the absence of transglutaminase (TG), was studied using the Mixolab®. Main effects induced by soybean protein isolate (Figure 3) and transglutaminase (Figure 4) addition were observed along the mixing process, and differences among samples were minimized along heating and cooling. In the initial stage of mixing, an increase of consistency was observed in the presence of soybean and TG, likely the water amount was a limiting factor. It has been previously reported the important role of the water during the mixing process and that was even more predominant when working with gluten free matrixes (Marco and Rosell, 2008b; Rosell and Marco, 2007). The increase in the consistency due to the TG effect also agrees with the higher hydration required for the doughs treated with this enzyme (Gerrard et al., 1998; Gujral and Rosell, 2004a).

When analyzing the individual effect of soybean protein isolate, it was observed that its addition resulted in an increase in the development time (time to reach C1) (Table 2), that agrees with the results reported by Bonet et al. (2006) and Marco and Rosell (2008b), when protein sources were added to wheat or rice flour, respectively. The development time is related to the time necessary to hydrate all the compounds, being the proteins the most involved compounds in the water absorption. Soybean protein isolate was tested at 0.5, 1 and 2%, showing an enhancement in the maximum torque (C1) from 1.09 Nm to 1.47 Nm in the presence of 2% SP (w/w, f.b.). This effect might be related to the high water holding capacity of this protein (4.0-5.0 g/g) (Vose, 1980), limiting the water available for the rest of the components. Marco and Rosell (2008b) also reported an increase in the water absorption determined by the farinograph as the amount of soybean protein added to rice doughs increased, working at constant consistency. The presence of TG (1%), an enzyme with strengthening effect, promoted an increase in the rice dough consistency along mixing (Table 2). The consistency of the rice doughs containing soybean proteins and TG was higher than their counterparts without TG (Figure 3, 4). This effect was more noticeable during the mixing before the heating stage. The greater water holding capacity of the protein polymers formed by the enzyme crosslinking activity seems to be responsible of the increase in the consistency in the presence of TG (Wang et al., 2007). Regarding to the amplitude, related to the dough elasticity, no trend was observed when SP or TG were added, although in wheat doughs it has been observed a direct relationship between amplitude or bandwidth and consistency (Rosell and Collar, 2008). The stability decreased in the presence of SP or TG. No statistically significant trends were observed regarding the amount of the SP and TG added singly (Table 2). Collar et al. (2007) observed an increase of the stability when transglutaminase was added to wheat flour dough. The mechanical weakening increased when soybean protein was added to rice flour dough (Table 2). Ribotta et al (2005) reported that the addition of SP to wheat dough interferes in the gluten matrix formation, decreasing the dough strength, what could be happening in these gluten free matrixes that showed an increase in the mechanical weakening in the presence of SP. The

addition of TG, single or in combination with SP, also produced an increase in the protein weakening (Table 2). The thermal weakening (%) decreased when SP or TG were added individually or in combination (Table 2). In wheat dough performance, the mechanical and thermal weakening have been associated to the role of proteins, being mechanical weakening attributed to overmixing (Rosell et al., 2007), which led to less elastic and more sticky wheat doughs (Sliwinsky et al., 2004). Namely, the thermal weakening is ascribed to the aggregation and further denaturation of the proteins due to heating (Rosell and Foegeding, 2007). The nature of the exogenous protein and the interactions produced between proteins, also intensified by the TG treatment, would be responsible of the effects observed in the presence of SP or TG. The minimum torque was obtained around 54°C, thus, the decrease of the thermal weakening in the presence of SP might be due to the higher temperature required for SP aggregation (Petrucelli and Añón, 1994). The time required for reaching the minimum torque during the heating (C2) decreased by the single addition of SP or TG (Table 2), although no tendency was observed with the increasing amount of SP. Bonet et al. (2006) also reported a decrease of this parameter due to the use of TG in wheat doughs, and the addition of high amount (10%, wheat flour basis) of SP isolates induced an increase in this parameter.

The protein breakdown rate (alfa) increased as the SP amount increased (Table 2), and this effect was intensified with the TG treatment, inducing an increase in absolute values from 0.0591 to 0.0889 when SP was added at the maximum level studied in the presence of TG.

As heating proceeded further, differences between dough samples were minimized due to the predominant role of the starch, major compound in all the gluten free matrixes tested. It has been reported in wheat flour dough that minimum torque is detected in the range 52-58°C, within that temperature range and beyond that protein changes have minor influence, becoming the changes pertaining to starch granules the main responsible for further torque variations (Rosell et al., 2007). Starch granules swell due to the absorption of the water available in the medium and, amylose chains leach out into the aqueous intergranular phase

promoting the increase in the viscosity and thus the increase in the torque. That enhancement continues until the mechanical shear stress and the temperature constraint promote the physical breakdown of the granules, which is associated with a reduction in viscosity (Rosell et al., 2007). In the present study, all the formulated samples (with the exception of 0.5% SP) had higher consistency than the control dough after starch gelatinization (C3) (Table 3). Bonet et al. (2006) reported different trends for protein (10% w/w) enriched wheat doughs depending on the protein source; soybean, gelatine and lupin proteins produced a decrease in C3, whereas egg protein produced an increase in this parameter. The higher consistency in the presence of SP might be due to gel formation of the SP as the dough temperature raise. The thermal induced gelation process of the proteins after the aggregation of the polymer chains induces huge modifications in the rheological behaviour of the proteins, being the gelation process temperature sensitive for each protein (Ngarize et al., 2004). The gelation temperature of the soy protein isolate suspensions ranged around 71-93 °C, depending on the pH; also the protein concentration affects the gelation temperature (Renkema et al., 2001). The TG treatment also produced an increase in the peak torque (C3) (Table 3, Figure 4), which might be due to the formation of protein polymers with higher molecular weight by the crosslinking, reaching the highest consistency when TG was combined with SP at 2%.

The rate of starch gelatinization (beta) increased in the presence of SP (Table 3). Also the use of TG produced an increase in that rate, which was even higher in the presence of 0.5 or 2% SP.

The effect of the SP or TG in the minimum torque during the heating period (C4) was less evident than during the initial heating, although in this case also the sample with TG and SP showed lower torque values than their counterparts without TG (Table 3) (Figure 3, 4). During this stage, the physical breakdown of the starch granules due to the mechanical shear stress and the temperature constraint is the main responsible of the decrease in viscosity (Rosell et al., 2007). The cooking stability range (C3-C4) increased in the presence of 1 or 2% SP (Table 3). The addition of TG also produced an increase in the cooking stability range, obtaining more

stable doughs when TG was combined with 1 or 2% SP. The addition of TG also produced an increase in the cooking stability rate (γ), but no trend was observed with the amount of SP.

During the cooling step, the individual addition of SP led to an increase of the final consistency (C5), increasing the consistency as the amount of SP increased (Table 3). However, the presence of TG added singly or in combination with SP resulted in a reduced consistency after cooling (C5). The recrystallization of the amylose chains is the main responsible of the increase in the consistency during cooling. This increase is known as setback (C5-C4). An increase in the cooling setback was observed in the presence of SP (Table 3). The reorganization of the denatured proteins from the protein isolates could affect the starch gelation, obtaining different trends depending on the protein source (Marco and Rosell, 2008a). In previous studies using the Rapid Visco-Analyzer (RVA), no significant effect was observed on the setback when soybean protein isolate was added to rice flour (Marco and Rosell, 2008a). The different behaviour might be attributed to the different hydration conditions used when the assays are performed using the RVA (suspension) or the Mixolab (dough). The addition of TG produced a decrease in the setback possibly related to the increase in the molecular weight of the rice and soybean protein polymers resulting from the crosslinking activity of the TG (Marco et al., 2008), making more difficult the reorganization of the amylose chains. The TG also decreased the cooling setback rate (δ) (Table 3), likely the bigger size of the protein chains reduce the movement of the starch and protein chains.

Gluten free dough consistency by ultrasounds

Ultrasound measurements in dough samples at two temperatures and with different SP and TG content were also carried out. As can be seen in Figure 5, ultrasound velocity generally increases and attenuation decreases with SP content, especially for SP values above 2%, which were tested for confirming the trend. It is known that doughs with lower values of attenuation

and higher values of velocity usually have higher consistency (Alava et al., 2007), so these ultrasound measurements may point out the hardening of the dough samples as the SP content increases. Moreover, the dough samples at 65°C offered generally lower values of velocity and higher values of attenuation (dashed lines) than the samples measured at an ambient temperature of 25°C (solid lines), which can indicate the softening of the sample with the increase of temperature to that level. These results seem in accordance with the Mixolab® measurements shown in Figure 3. Considering only the results at ambient temperature (25°C), in most measurements velocity was higher and attenuation was lower when TG was added to the sample. These effects, clearly observed in samples over 1% of SP, showed the increase in consistency that the samples can experiment when TG is added, reported in both Figure 3 and Figure 4. Nevertheless, the role of both SP and TG was not as strong in the ultrasound measurements carried out with the samples at 65°C. In spite of the fact that also velocity tended to increase and attenuation generally decreased with SP content, these tendencies were less severe in samples at 65°C than the reported ones at 25°C. As it can be observed in Figure 5, the variation in attenuation and velocity values, close to 42% and 23% respectively at ambient temperature, were of scarcely 13% and 4% in their corresponding curves at 65°C. Moreover, the addition of TG seems also to have a weak influence in the ultrasound measurements at 65°C, regarding to the relative closeness of the curves of both attenuation and velocity that can be observed in Figure 5, especially for SP contents over 2%.

It should be noted that some ultrasound parameters, such as the ultrasound velocity, are also affected by temperature itself, being this influence not easy to differentiate from the caused by changes in the structure of the sample. However, the general agreement between the reported tendency of the dough consistency and the ultrasound velocity with SP and TG at the two temperatures considered seems to point out that velocity measurements are significantly more sensitive to changes in the structure of the sample than to temperature itself.

In order to perform a further study of the ultrasound sensitivity to consistency in dough, the individual values of velocity and attenuation for all dough samples, tested at ambient temperature and at 65°C, were plotted against each other in Figure 6. As can be seen, the ultrasound results can be sorted in two clearly differentiated groups. The dough samples at 65°C were placed at the upper left corner while doughs at 25°C tended to be located at the opposite corner. According to this chart, dough samples at 65°C, with high attenuation and low velocity values, should have lower consistency than most of the doughs at 25°C, which agrees with the results obtained by using the Mixolab® shown in Figure 3. Moreover, the same trend was also observed with the softest and hardest doughs within a group of samples at the same temperature. Thus, a combination of both ultrasonic attenuation and velocity parameters could be used as an indicator of dough consistency. In this sense, a multiple regression analysis with ultrasound and Mixolab® parameters (C1 and torque at 65°C) was performed to study this link. Considering only the ultrasound attenuation and velocity and the mechanical torque, the measurements can be clearly divided into two groups depending on the temperature of the sample, 25°C or 65°C. While the level of correlation between ultrasound and rheological parameters is not statistically significant for the samples at 65°C, likely due to the mentioned masking effect of the starch on the torque measurements, fairly high linear correlation between attenuation and velocity with dough torque, $r=-0.73$ ($P\leq 0.05$) and $r=0.72$ ($P\leq 0.05$) respectively, was found with the samples at 25°C. Moreover, the combination of these two ultrasound parameters allows attaining a level of correlation with torque of $r^2=0.927$ ($P\leq 0.01$), with a relationship described by the next equation:

$$Torque = 1.205 - 2.145 \cdot 10^{-3} \cdot Attenuation + 7.239 \cdot 10^{-3} \cdot Velocity \quad \text{Eq.5}$$

with the torque in Nm, the attenuation in dB/cm and the velocity in m/s.

Some other considerations can also be made with regard these ultrasound measurements. Firstly, the set of samples tested at 25°C offered a wider range of values of both velocity and

attenuation than the measured at 65°C, observed also in Figure 5, which could point out some reduction of the effect of SP and TG in dough consistency in samples tested at 65°C. Secondly, dough samples with only 6% of SP could reach similar levels of consistency than doughs with 2% of SP but 1% of TG at ambient temperature. Therefore in some occasions the effect of TG could be replaced by an increment of SP, as has been previously observed in wheat doughs (Bonet et al., 2006). The obtained range of values of both ultrasound velocity and attenuation, shown in Figure 6, were similar to the ones obtained in some samples of wheat flour dough with relatively low consistency (high water content) (Alava et al., 2007).

Moreover, the ultrasound rheological parameters G' and G'' were calculated with the obtained values of shear ultrasound velocity and attenuation in dough, at ambient temperature and at 65°C (Kono, 1960). It should be noted that the frequency used in the ultrasonic experiments (100kHz) is some order of magnitude higher than those used in the traditional rheological tests, so the ultrasonic G' and G'' values will differ from those encountered through traditional rheological tests and therefore cannot be compared directly. These rheological parameters for doughs with different SP and TG can be observed in Figure 7. As can be observed, for both temperature conditions the rheological parameters usually increases with SP, which is in accordance with the general increase in consistency with SP observed in most doughs. Furthermore, in samples at ambient temperature it was clearly observed that the rheological parameters reached higher values if TG was added to the sample, which agrees with the higher consistency that samples reached with the addition of this enzyme. This trend was less marked in samples tested at 65°C. Moreover, ultrasound rheological parameters showed generally a more reduced range of variation with SP (and also with TG) at 65°C than to at ambient temperature. This seems to agree the already observed lower effect of SP and TG on ultrasound measurements performed in doughs at 65°C.

Conclusions

The rheological behaviour of gluten free rice flour based dough can be determined using the Mixolab® and ultrasounds measurements. Mixolab® provides information of gluten free dough empirical rheology following the dough physico-chemical changes associated to dual mechanical shear stress and temperature constraint. The addition of soybean protein isolate or/and a crosslinking enzyme (transglutaminase) resulted in substantial changes at the early stages of the mixing, and those were minimized at temperatures higher than 50°C, where starch acquired a major role due to the gelatinization process. Ultrasound measurements exhibited a general good agreement with the results obtained by using Mixolab®. Experimental results have demonstrated that ultrasound was sensitive to the effect on the rice flour dough properties caused by the addition of both SP and TG at different temperatures, although changes at high temperatures were less marked. Consequently, Mixolab® and ultrasounds could be regarded as promising tools in order to investigate rice flour dough properties and the changes induced by other ingredients and processing aids.

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FIGURE CAPTIONS

Figure 1. Typical Mixolab® curve showing the main parameters related to dough hydration and mixing (C1), the protein weakening (C2), starch gelatinisation (C3), starch breakdown (C4) and starch retrogradation (C5).

Figure 2. Ultrasonic setup.

Figure 3. Effect of soybean protein isolate (SP) on the rice flour dough consistency during a mixing-heating-cooling cycle determined by the Mixolab® device. Numbers in the legend indicated the level of addition expressed in percentage (flour basis).

Figure 4. Effect of soybean protein isolate (SP) and transglutaminase (TG) on the rice flour dough consistency during a mixing-heating-cooling cycle determined by the Mixolab® device. Numbers in the legend indicated the level of SP addition expressed in percentage (flour basis); transglutaminase was always added at 1% (w/w, flour basis).

Figure 5. Average values of ultrasound velocity (left) and attenuation (right) with SP (in %) for dough samples with 0% and 1% of TG (T0 and T1 respectively), at 25°C (solid lines) and 65°C (dashed lines).

Figure 6. Ultrasound measurements for doughs with 0% to 6% of SP and 0% to 1% of TG, at ambient temperature (solid line circle) and 65°C (dashed line circle).

Figure 7. Ultrasound rheological parameters G^* , G' and G'' with SP, for 0% (left) and 1% (right) of TG, at ambient temperature (top) and at 65°C (bottom).

555 TABLE 1.
 556 INSTRUMENTAL SETTINGS DEFINED IN THE MIXOLAB® FOR RUNNING THE
 557 SAMPLES.

558

559

Settings	Values
Mixing speed	80 rpm
Tank temperature	30 °C
Temperature 1st plateau	30 °C
Duration 1st plateau	8 min
Heating rate	4 °C/min
Temperature 2nd plateau	90 °C
Duration 2nd plateau	7 min
Cooling rate	4 °C/min
Temperature 3rd plateau	50 °C
Duration 3rd plateau	5 min
Total analysis time	45 min

560

TABLE 2.

EFFECT OF SOYBEAN PROTEIN (SP) AND TRANSGLUTAMINASE (TG) ON THE RICE DOUGH CHARACTERISTICS DURING MIXING DETERMINED BY USING THE MIXOLAB®. VALUES ON THE SAMPLES INDICATED THE AMOUNT OF ADDITION EXPRESSED IN % (W/W) FLOUR BASIS. A DETAILED DESCRIPTION OF THE PARAMETERS IS INCLUDED IN MATERIALS AND METHODS SECTION.

Samples	C 1		Amplitude Torque	Stability Time	C 2		Mechanical weakening	Thermal weakening	alfa
	Time (min)	Torque (Nm)			Time (min)	Torque (Nm)			
control	0.5 a	1.09 a	0.11 b	4.1 b	18.2 a	0.34 b	0.27 (24.8)	0.48 (177.8)	-0.0591 a
0.5 % SP	1.1 b	1.32 c	0.12 b	2.6 a	17.8 a	0.36 b	0.40 (30.3)	0.56 (140.0)	-0.0652 a
1% SP	0.8 a	1.26 b	0.07 a	3.5 a,b	17.5 a	0.38 b,c	0.34 (27.0)	0.54 (158.8)	-0.0658 a
2% SP	0.9 a,b	1.47 d	0.11 b	3.0 a	17.5 a	0.41 c	0.44 (29.9)	0.62 (140.9)	-0.0782 b
1% TG	0.7 a	1.30 b, c	0.07 a	3.7 a,b	17.8 a	0.28 a	0.38 (29.2)	0.64 (168.4)	-0.0747 b
0.5 % SP + 1% TG	0.9 a,b	1.39 d	0.08 a	2.5 a	18.1 a	0.28 a	0.49 (35.3)	0.62 (126.5)	-0.0718 b
1% SP + 1% TG	0.6 a	1.39 d	0.15 c	3.2 a	18.2 a	0.29 a	0.44 (31.7)	0.66 (150.0)	-0.0831 b,c
2% SP + 1% TG	0.8 a	1.62 e	0.10 a, b	3.7 a,b	18.0 a	0.36 b	0.49 (30.3)	0.77 (157.1)	-0.0889 c

Data in parenthesis expressed in %.

Means sharing the same letter within a column were not significantly different ($P < 0.05$ ($n=3$))

TABLE 3.

EFFECT OF SOYBEAN PROTEIN (SP) AND TRANSGLUTAMINASE (TG) ON THE RICE DOUGH CHARACTERISTICS DURING HEATING DETERMINED BY USING THE MIXOLAB®. VALUES ON THE SAMPLES INDICATED THE AMOUNT OF ADDITION EXPRESSED IN % (W/W) FLOUR BASIS. A DETAILED DESCRIPTION OF THE PARAMETERS IS INCLUDED IN MATERIALS AND METHODS SECTION.

Samples	65 °C Torque (Nm)	C3 Torque (Nm)	C4 Torque (Nm)	C 5 Torque (Nm)	Cooking Stability range (C3-C4)	Setback (C5-C4)	beta (Nm/min)	gamma (Nm/min)	delta (Nm/min)
control	0.59 c	1.52 a	1.25 b	1.79 c	0.27	0.54	0.3105 a	-0.0365 a	0.0595 a,b
0.5 % SP	0.56 b,c	1.52 a	1.25 b	1.83 d	0.27	0.58	0.3810 b	-0.0358 a	0.0630 b
1% SP	0.58 c	1.56 b	1.26 b	1.88 e	0.30	0.62	0.3739 b	-0.0436 a	0.0635 b
2% SP	0.60 c	1.57 b	1.28 c	1.91 e	0.29	0.63	0.3554 a,b	-0.0394 a	0.0635 b
1% TG	0.43 a	1.58 b	1.25 b	1.77 c	0.33	0.52	0.4129 b	-0.0468 a	0.0544 a
0.5 % SP + 1% TG	0.49 b	1.57 b	1.22 a	1.73 b	0.35	0.51	0.4646 c	-0.0445 a	0.0550 a
1% SP + 1% TG	0.48 b	1.58 b	1.21 a	1.68 a	0.37	0.47	0.4196 b	-0.0490 a	0.0510 a
2% SP + 1% TG	0.49 b	1.62 c	1.25 b	1.77 c	0.37	0.52	0.4846 c	-0.0494 a	0.0546 a

Means sharing the same letter within a column were not significantly different ($P < 0.05$ ($n=3$))

|

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.