Influence of chitosan on thermal, microstructural and rheological properties of rice and wheat flours-based batters

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ABSTRACT

Wheat flour replacement by rice flour is a key strategy in gluten-free batter production. Rice flour needs hydrocolloids to offset the development of the network of the mix. In this context, the aim of this work was to analyze the influence of chitosan (0 to 1 g/100 g of batter) addition on the microstructural, rheological and thermal properties of wheat:rice flours batters (100:0; 70:30, 30:70 and 0:100 (g/g)). Results showed that increasing replacement of wheat flour by rice one decreased the consistency (*K*) and the yield stress (τ_0), and increased the flow behavior index (*n*) because of the absence or lower gluten content. However, the addition of only 0.25 g/100 g chitosan to rice flour formulation (0:100 (g/g)) increased its viscosity (from 371 to 1006 mPa·s), exhibiting a rheological behavior similar to wheat flour formulation (100:0 (g/g) (1050 mPa·s). Chitosan enhanced consistency and structural agglomeration, and the interaction among ingredients, especially in batters with high content of rice flour (30:70 and 0:100 (g/g)). Lastly, chitosan incorporation did not significantly modify thermal properties, excepting in rice-flour batters (0:100 (g/g), reducing T_m, ΔH_m, and thus, increasing the bound water content (from 17 to 32 g/ 100 g).

Keywords: Batter coating; chitosan; gluten-free; physical properties

1. INTRODUCTION

Batters are complex liquid systems composed mainly of flour and water, in which the food product is dipped before frying. Commonly, other ingredients such as starch, hydrocolloids, salt and seasoning are incorporated to improve their functionality and sensory properties. During frying, the uniform layer covering the product generates a crispy crust as a result of a rapid loss of water. This crust entails a barrier effect to further water loss and oil gain. Minimizing the oil uptake is one of the key proposals to obtain healthier fried-products. Despite wheat flour is the main solid ingredient in batter formulations, rice flour has been lately incorporated because its addition enhances some properties of frying batters. Proteins and starch from rice flour have the particularity to be gluten-free and to retain less oil, resulting in a final product with less calories as well as with lower acrylamide content compared with wheat flour-based batter (Shih & Daigle, 1999; Shih, Boué, Daigle, & Shih, 2004). However, rice flour batters form thin slurries which require additives to develop adequate viscosity and other desirable batter properties (Shih & Daigle, 1999). For this reason, some authors considered advantageous the use of hydrocolloids in batters (Albert & Mittal, 2002; García, Ferrero, Bértola, Martino, & Zaritzky, 2002; Sahin, Sumnu, & Altunakar, 2005; Garmakhany, Mirzaei, Nejad, & Maghsudlo, 2008). Hydrocolloids are substances characterized by the capability to link water increasing the viscosity of a solution. This property causes changes in the coating pick-up and yield; and after cooking, hydrocolloids affect freeze-thaw stability and improve mechanical resistance of the crust, and thus, the final texture (Varela & Fiszman, 2011). Additional benefits have been described related to the use of hydrocolloids. Zeng et al., (2010) reported a decrease of acrylamide generation in model systems, crackers and fried potatoes when pectin or alginic acid were used. Acrylamide is a potentially carcinogenic compound which is generated during frying or baking as a consequence of Maillard reactions (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Romani, Bacchiocca, Rocculi, & Dalla Rosa, 2009). Recently, Sansano, Castelló, Heredia, & Andrés (2016) reported a reduction of 61 ± 7 % in acrylamide formation when 5 g/kg of chitosan was added as an ingredient in fried batters. The mechanism of acrylamide reduction that authors proposed is based on the richness of amino groups of chitosan, which compete with asparagine amino groups to bind carbonyls (e.g. reducing sugars) (Sansano et al., 2016; Sansano, Castelló, Heredia, & Andrés, 2017).

However, there are no previous studies focused on the influence of chitosan addition to rice flour batters on their microstructural, rheological and thermal properties. Ansarifar, Shahidi, Mohebbi, Razavi, & Ansarifar, (2015) studied the flow behavior of wheat flour batter formulations for chicken nuggets, and reported higher viscosities of the batters related with chitosan content, due to its high water binding capacity. Moreover, changes in the rheological properties of a material reveal changes in its molecular structure. Consequently, the rheological properties of a batter influence its flow characteristics and are themselves influenced by structural changes generated by the process or formulation (Xue & Ngadi, 2007a).

Since the type of flour used provides different rheological characteristics, it is necessary to study how they affect these and other properties. Interactions between components of batter formulations and their influence during heating treatment determine physical and chemical changes that can be analyzed by studying thermal properties. Chitosan incorporation in batters and specifically its interactions with the other components have not been analyzed in terms of thermal properties.

In this context, the aim of this work was to analyze the influence of chitosan addition on the microstructural, rheological and thermal properties of raw rice and wheat flours-based batters.

2. MATERIALS AND METHODS

2.1. Batter formulations

Battering formulations consisted in different combinations of wheat and rice flours, 2.5 g/100 g of salt and 3.1 g/100 g of sodium bicarbonate (dry weight basis) in a water-to-dry mix proportion of 1.2:1 (g/g). The rice and wheat flours were combined in the following ratios (g/g) of wheat:rice flours: 100:0; 70:30, 30:70 and 0:100. The batter systems were formulated with 0, 0.25, 0.5 and 1 g/100g chitosan, adding a 2 g/ 100 g of chitosan solution, made as follows: 2 g of chitosan were dissolved in 198 g of 1 g/100g acid lactic solution and stirred at 40ºC during 24h. Lactic acid and water were added to complete their final content (0.545 g/100 g and 54.54 g/100 g wet basis, respectively). Batters were manually mixed during 60 s to guarantee uniformity and were kept at room temperature for 30 min before analyzing.

Flours were bought in the local market, and their composition were: 77.4 g/100 g of carbohydrates, 0.5 g/100 g of fat and 7.1 g/100 g of proteins for the rice flour and 75 g/100 g of carbohydrates, 1.2 g/100 g of fat and 9 g/100 g of proteins, for the wheat flour. Particle size was analyzed with the Mastersizer 2000 (Malvern Instruments, Herrenberg, Germany) coupled with the Scirocco 2000 module for dry measurement. Rice and wheat flours had particle sizes *d*(0.5) lower than 178 and 88 µm and *d*(0.9) lower than 310 and

191 µm, respectively. Chitosan (Poly (D-glucosamine)*Deacetylated chitin), high molecular weight, was purchased from Sigma-Aldrich (St. Louis, MO, USA), and lactic acid was from Panreac (Barcelona, Spain). Chitosan molecular weight and deacetylation degree were analyzed in a previous work (Sansano, et al., (2017)) and resulted in 1460 KDa, and 64.8 %, respectively.

2.2. Rheological measurements

Rheological properties were studied using a strain/stress control rheometer MRC 102 (Physica/Anton Paar, GmbH, Graz; Austria) equipped with a plate-plate (50 mm of diameter). The gap between plates was fixed to 1 mm. The free surface of samples edges was covered with a thin film of silicone oil.

Apparent viscosity (Pa·s) was measured in triplicate, at 20 ºC as a function of increasing shear rate (γ) from 0 to 150 s^{-1} after 5 min of stabilization time. The obtained flow curves were evaluated and fitted according to the Herschel-Buckley model, as equation *(I)*:

$$\tau = \tau_0 + K \cdot \gamma^n \qquad (I)$$

where τ is the shear stress (Pa), τ_0 the yield stress (Pa), *K* the consistency index ($Pa \cdot s^n$) and *n* the flow behavior index.

2.3. Microstructural analysis

The microstructure of samples was observed by using a light microscope (Nikon, Shinjuku, Japan) at 10x of magnification, taking ten micrographs for each sample. The magnification was chosen after preliminary trials to obtain a wider field of view to see the whole structure and the interaction between particles. One drop of dispersion (previously diluted with hexane) was placed on a glass side and covered with a cover slip carefully placed over the sample, parallel to the plane of the slide and centered to ensure sample thickness was uniform. Micrographs were captured using a digital camera (Model 2.1 Rev 1; Polaroid Corporation, NY, USA). The acquired images were subsequently elaborated using the software Image Pro-plus 6.0 (Media Cybernetics Inc, Rockville, USA). Particles size were determined according with Glicerina, Balestra, Dalla Rosa, & Romani, (2013), by evaluating the Feret diameter, defined as the distance between two tangent lines to the two opposite sides of the particles (Allen, 1997). An Euclidean Distance Map (EDM) was further generated in order to evaluate the distance between particles. The map indicates, for each pixel in the image (black points) the shortest distance between them (Danielsson, 1980; Bayod, 2008; Glicerina, Balestra, Dalla Rosa, & Romani, 2016). The distance between black points (particles) was expressed as grey values. On the other hand, the white points represented the empty space. For this reason, applying an EDM to the original image is possible to obtain information about the minimum distance between particles and about the amount and distribution of void spaces (Krislock & Wolkowicz, 2012).

2.4. Thermal properties

Thermal properties of batter formulations were analyzed with an Auto Q20 Differential Scanning Calorimeter (T.A. Instrument, Hüllhorst, Germany). Glass transition temperature, temperature and enthalpy of gelatinization and ice-melting were analyzed. 26 ± 1 mg of sample were placed in hermetic aluminum pans and an empty pan was used as the reference.

The ramps were calibrated 10 ºC/min with indium, and then, the thermal profile was performed as follows: from 15 ºC to 120 ºC at 10 ºC/min (to obtain gelatinization temperature and enthalpy), and cooling until -50 ºC. It included an isotherm step during 3min and then a heating to thawing, until 40 ºC at 10 ºC/min to obtain, the glass transition temperature followed by melting temperature and melting enthalpy. Non-freezable water content (*UFW*, g water/g solids) was analyzed following the equation *II* (Laaksonen & Roos, 2000):

$$UFW = \frac{w_{tot} - \frac{\Delta H_{mtot}}{\Delta H_{mw}}}{C_{tot}} \qquad (II)$$

where w_{tot} is total amount of water (g), ΔH_{mtot} is total heat of melting of ice (J), ΔH_{mw} is latent heat of melting ice (334 J/g) and C_{tot} is total amount of solids (g).

2.5. Statistical analysis

The influence of rice-wheat flours ratio and chitosan content on microstructural, thermal and rheological properties of batter formulations was analyzed using Statgraphics Centurion XVI (StatPoint Technologies, Inc., Warrenton, USA). Variance was evaluated by a one-way analysis for microstructural and rheological properties, and a multifactorial analysis was carried out for thermal properties, with a significance level of 95 %.

3. RESULTS AND DISCUSSION

3.1. Effect of wheat-rice flours combinations and chitosan on flow behavior of batters.

Rheological parameters corresponding to all batter formulations were obtained by fitting the curves to Herschel-Bulkley model (table 1). Samples made of rice flour (RF) with respectively 0.5 and 1 g/100 g of chitosan could not be analyzed because of their excessive consistency and hardness. The incorporation of chitosan above 0.25 g/100 g together with the particle size of rice flour negatively limited the flow behavior of batters. Formulations without chitosan exhibited significant differences in rheological parameters depending on the type of flour. The presence of rice flour in batters (RF, 70WF/30RF and 30WF/70RF) decreased consistency (K) and the yield stress (τ_0) and increased the flow behavior index (n). This fact is related to a decrease of gluten, which contributed to water retention, as a consequence of wheat flour replacement by rice flour (Mukprasirt, Herald, & Flores, 2000; Dogan, Sahin, & Sumnu, 2005; Xue & Ngadi, 2006).

It is noteworthy that the formulation of rice flour (RF) without chitosan showed a visible syneresis because of its inability to retain water, due to its lack of gluten.

The incorporation of chitosan increased τ_0 values, *K* and apparent viscosity and decreased *n*. This effect has been previously reported for other hydrocolloids, whose presence favors an increase of viscosity and consistency. Concretely, the addition of 2 g/kg of xantham gum and 0.1 g/kg of methylcellulose significantly increased the consistency index of rice batter formulations, due to a higher amount of free water available to encourage the hydration of the hydrocolloid than wheat flour-based formulations (Xue & Ngadi, 2007a). Other ingredients such as phosphorylated starch or gelatinized rice flour have been used to increase poor thickening properties of rice flour and reduce oil uptake (Shih & Daigle, 1999). Baixauli, Sanz, Salvador, & Fiszman, (2003) reported that 0.15 g/kg dried egg addition also increased consistency and reduced flow index of wheat flour-based batters at different temperatures, while dextrin was not effective.

However, formulation made with rice flour (RF) and 0.25 g/100 g chitosan showed similar rheological behavior to wheat flour–based formulations, in particular apparent viscosity values at 20 s^{-1} were 1050 and 1006 for samples without and with chitosan respectively. The addition of 0.25 g/100 g chitosan greatly affected the flow behavior of the different formulations tested. As shown in Figure 1, wheat flour (WF) and rice flour (RF) had very different flour behavior. The addition of 0.25 g/100 g of chitosan increased shear stress in both formulations; while rice flour (with 0.25 /100 g of chitosan) showed values close to wheat flour batter (WF) without chitosan.

Regarding the rheological properties, it has been found that an absence of gluten was offset by the addition of 0.25 g/100 g chitosan in rice flour batters. A similar viscosity would mean a similar pick-up and stickiness of a batter formulation. These results can be used as a new strategy to produce gluten-free batters based on rice flour.

3.2. Microstructural analysis of batters made with different wheat-rice flours combinations and chitosan.

In order to better explain rheological results, microstructural analysis was also performed. The formulations related to the extremes of the experimental plan were analyzed to obtain the most representative information on the interactions between different percentage of rice and wheat flour with (1 g/100 g) or without chitosan. Moreover, as previously mentioned, because of the high consistency and hardness, it was not possible to perform rheological analysis on sample made with rice flour (RF) and 1 g/100 g of chitosan, that was however characterized from a structural point of view. In Figure 2 (A, B, C, D) the micrographs of the different batter samples are shown.

In formulation without chitosan, a reduction in the structure aggregation was observed as wheat flour was replaced by rice one. The decrease in wheat amount parallel to the increase in rice flour involves a reduction in the contact point between particles and an increase of void spaces between particles and aggregates. This effect is attributed, as reported in the rheological section, to a decrease of gluten presence that reduce the batter's water holding capacity, and thus the network formation (Lai, 2002). As known by literature in fact gluten proteins absorb water twice its own weight and tend to hold it through complex chemical bonds, that give arise to a more aggregate structure (Sozer, 2009). Even though rice flour has low capacity of absorbing water, it is one of the most suitable cereal flour used in gluten-free products, because it is natural, hypoallergenic, colourless and with bland taste (Ronda, Villanueva, & Collar, 2014).

In order to better highlight the state of aggregation of the batter matrices, Euclidean distance maps (EDM) were obtained (Figure 2: A1, B1, C1, D1). By using an EDM it was possible to highlight the distribution of particles (black areas) and void spaces (white areas), and to evaluate the minimum distance between particles and therefore, their state of aggregation related to their interactions (Glicerina et al., 2016). In Table 2, the particle Feret diameters and the minimum distance between particles of the batter formulations with and without chitosan are reported. It is possible to notice that wheat (WF) and 70WF/30RF samples have greater particles size compared to 30WF/70RF and rice (RF) did with higher amount of rice flours. However, despite as expected from literature, (Prasad et al., 2003; Afoakwa, Paterson, Fowler, & Vieira, 2009), the minimum distance between particles increased as he particle size decreased. These results confirm rheological ones. In fact, samples characterized by a less aggregate structure and more distance between particles (30WF/70RF and RF) had a lower consistence index (K) and yield values (τ_0) compared to WF and 70WF/30RF samples. This means that the amount of energy needed to allow the sample to start flowing was lower in the two former samples. In Figure 3 (E, F, G, H) are shown the micrographs of the different batter formulations with of 1 g/100 g of chitosan and the same pictures elaborated by using EDM (E1, F1, G1, H1). Adding 1 g/100 g of chitosan in the batter formulations it was highlighted an increase in the structure aggregation from sample E to H. As shown in batter mixtures made up without chitosan, a reduction in particle size was noticed as the rice flour amount increased (table 2). However, the presence of chitosan induced a reduction in particle size proportional to a decrease in the distance between them. An increase in the contact point between particles was observed from sample E (WF) to H (RF), and the presence of high agglomeration areas was highlighted as rice amount increased (Fig 3). As known by literature, hydrocolloids, such as chitosan, are hydrophilic compounds, that can dramatically increase the viscosity of products in which are presents, due to their interactions with the water molecules through hydrogen – bonding (Kapoor, Khandal, Seshadri, Aggarwal, & Kumar Khandal, 2013). At sufficiently high concentrations, the hydrocolloids become entangled with each other, forming loose networks that change the flow properties of the solution (Cassiday, 2012). For these reasons, the structure of samples with 1 g/100 g chitosan were more aggregate than in batter mixtures without this compound. Moreover, hydrocolloids such as pectin, guar gum, arabic gum, galactomannans, methylcellulose, etc, are frequently used in gluten free baked product in order to form structural equivalent of gluten network in wheat dough (Sanchez, Osella, & Torre, 2002; Ahlborn, Pike, Hendrix, Hess, & Huber, 2005; McCarthy, Gallagher, Gormley, Schober, & Arendt, 2005). Many characteristics of gluten-free bread depend on the amount and type of non-starch hydrocolloids used as gluten replacers (Eidam, Kulicke, Kuhn, & Stute, 1995; Funami et al., 2005). For this reason, one of the goals for researchers is to evaluate the optimum proportion of hydrocolloids in gluten-free bread production. As demonstrated here from the rheological and microstructural obtained results, the addition of 1 g/100 g of chitosan to the different batter formulations give arise to a product with high yield stress values and aggregation state, that become limiting factors, especially in the case of rice flour (RF). The presence or the addition of protein in rice flour coupled to the hydrocolloids (in right proportions) give arise to more compact structures compared to wheat flour matrices with high moisture content (Nammakuna, Barringer, & Ratanatriwong, 2015). However, the difference in the microstructural characteristics and aggregation state, observed between batters made with the same chitosan amount can be attributed to the different amount of gluten in samples (higher in WF and 70WF/30RF). Gluten, in fact, competing with hydrocolloids for water absorption, could retain a part of water that cannot be bound by chitosan. In the mixtures RF and 30WF/70RF, instead, the low amount or the absence of gluten, make available water for chitosan, creating intra or inter –hydrogen bonding, give arise to very aggregate structures (Figure 3 E, F, E1,F1) (Xue & Ngadi, 2007b).

3.3. Effect of wheat-rice flours combinations and chitosan on thermal properties of batters

In Table 3 the different parameters related to the starch gelatinization of the studied wheat-rice-chitosan flours mixtures are reported: the peak temperature (T_p) ranging from 70.25 to 83.4 ºC, the onset temperature (T_o) from 62.5 to 74.5 ºC and the corresponding enthalpy (AH_G) varied from 3.8 to 6.2 J/g. Control samples (without chitosan) with rice as main flour (30WF/70RF and RF) exhibited the highest gelatinization temperatures (T_p and T_o) and enthalpy values. The reduction of gluten presence in the formulations based in rice flour, increases the amount of available water able to interact with starch (Wang, Choi, & Kerr, 2004). Xue & Ngadi, (2007b) reported similar results in batter systems formulated with different blends of wheat and rice flours and also, in corn and wheat flour mixtures. Chitosan incorporation to blends did not significantly modify either gelatinization temperatures (T_p and T_o) or enthalpy (ΔH_G). Chitosan presence, however, contributed to homogenate the onset temperature in formulations with wheat flour as main ingredient (WF and 70WF/30RF). Chitosan might have contributed to a better transfer and control of water in the gluten net, managing the starch hydration process. Furthermore, the incorporation of 1 g/100 g of chitosan to rice flour (RF) notably reduced the T_o. In RF without chitosan, a visible syneresis took place due to the lack of interaction between water and rice flour; while RF made with 1 g/100 g of chitosan was more stable and phase-separation did not occur, evidencing how chitosan addition facilitates starch hydration. However, other hydrocolloids such as hydroxypropyl methylcellulose (HPMC), pectin, alginate, guar and xanthan gum, added in similar concentrations (1 g/100g) to wheat flour batters increased T_o and decreased gelatinization enthalpy (ΔH_G). Apparently, the strong interaction between hydrocolloids and starch induces a stable structure that requires higher temperatures to start starch gelatinization (Rojas, Rosell, & Benedito de Barber, 1999).

Glass transition temperature (T_g') was analyzed during thawing step, appearing close to water melting endothermic transition (Table 4). Obtained results showed that wheat-flour replacement of by rice-flour, with the consequent gluten reduction, increased the T_g' of the batters due to an increase of available water compared to wheat-flour batters (WF). Furthermore, rice-starch granule size is smaller than the wheat-starch granule size, contributing negatively to water retention (Xue & Ngadi, 2007b). However, water retention in batters seemed to increase when chitosan was added to the formulations, being this effect more noticeable in rice flour batters (RF) with an increase of T_g' from -12.42 to -10.66 ºC.

Data related to melting transition are reported in table 5. Melting temperature (T_m) was, in general, non-dependent on the type of flour or chitosan percentage in the batter. The influence of chitosan presence on melting enthalpy (ΔH_m), melting temperature (T_m), and thus non-freezable water content, was only noticeable in rice-flour batters (RF). The incorporation of chitosan at 0.5 and 1 g/100 g gradually decreased ΔH_m, T_m, and increased the bound water content. These results pointed out the relevance of the interactions between chitosan and water molecules when rice flour is present in high quantity or is the only flour in batter formulations 30WF/70RF and rice flour (RF), what increased non-freezable water content.

4. CONCLUSIONS

Gluten-free formulations based on rice flours present poor adhesive properties, being a limitation for their use as batter coating. The results of this study show that chitosan addition would compensate the lack of adhesiveness and improve batter functionality and performance. Concretely, the addition of 0.25 g/100 g of chitosan to rice-flour formulation enhanced its viscosity and consistency. From a microstructural point of view, a reduction in structure aggregation occur when wheat flour is replaced by rice-flour in battering formulation. Newly, chitosan addition is presented as an effective strategy to improve ingredient-interactions in the matrix system.

Chitosan incorporation to blends does not significantly modify either gelatinization temperatures (T_p and T_o) or enthalpy (ΔH_G). However, batters formulated with rice flour present higher T_g' than those made of wheat flour (WF), thus, chitosan addition to rice flour-batters would improve frozen stability.

The influence of chitosan presence in batters on melting enthalpy (ΔH_m), melting temperature (T_m), and thus non-freezable water content, is only noticeable in rice-flour batters (RF), reducing ΔH_m, T_m, and increasing the bound water content.

Finally, the contribution of rice flours and chitosan to the quality of related products, such as battered nuggets, as well as the functionality of the modified batter in the final fried product should be evaluated.

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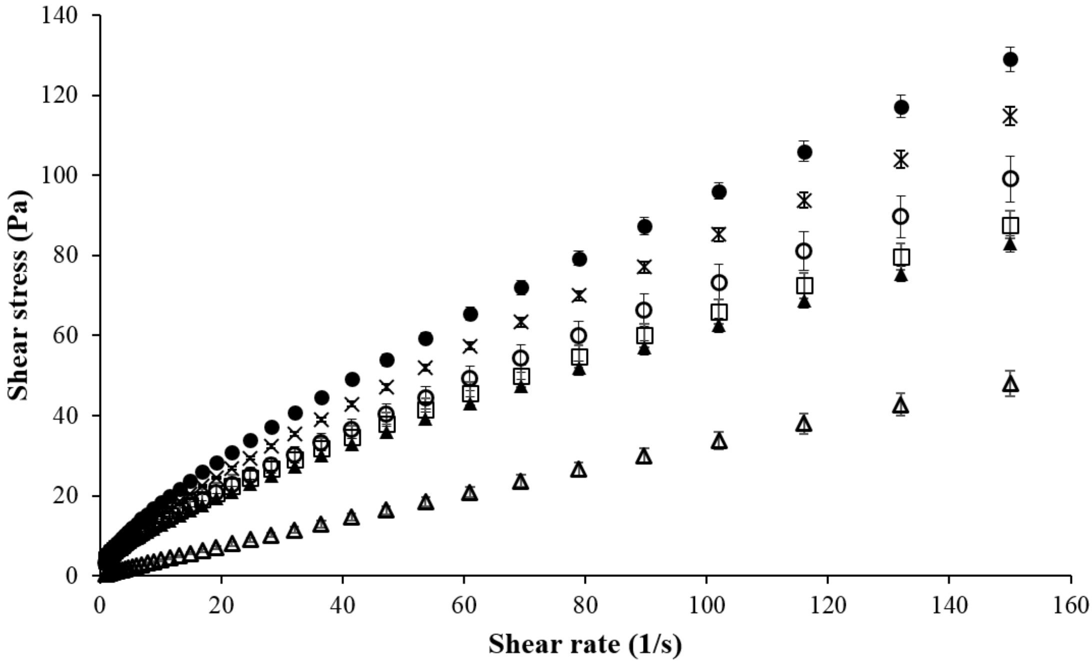

Figure 1. Flow behavior properties of the following batter samples: (○) wheat flour (WF) and (Δ) rice flour (RF) without chitosan; and formulations: (●) WF; (×) 70WF/30RF; (□) 30WF/70RF; and (▲) RF with 0.25 g/100 g of chitosan.

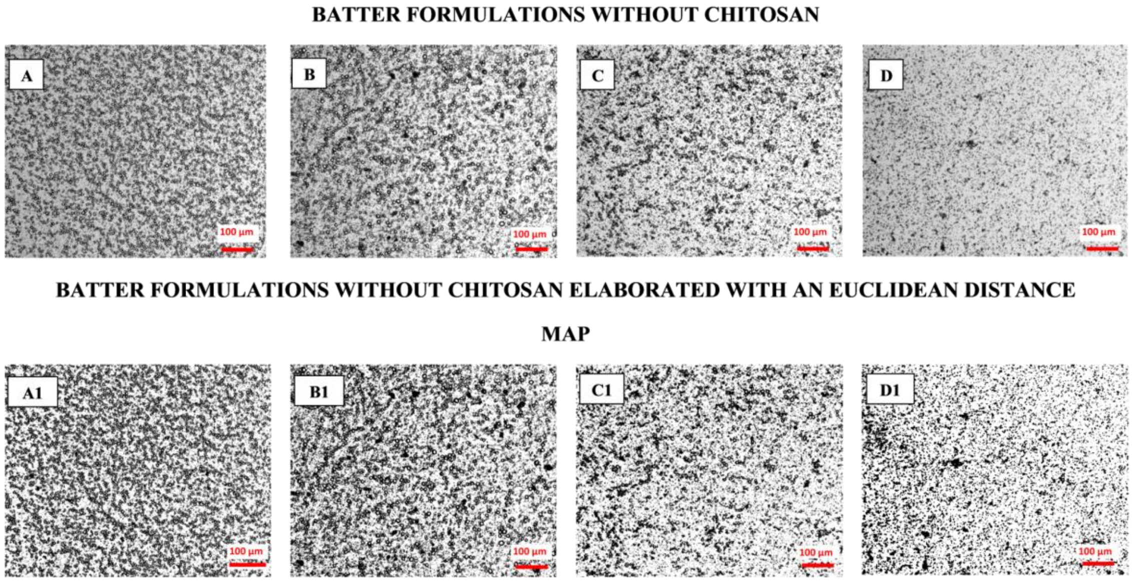

Figure 2. Micrographs of different batter formulations without chitosan, acquired at 10x of magnification. Samples A, B, C, D correspond to formulations respectively made of: wheat flour (WF); 70WF/30RF; 30WF/70RF and rice flour (RF). Samples A1, B1, C1, D1 represent respectively the same formulations elaborated with an Euclidean Distance Map.

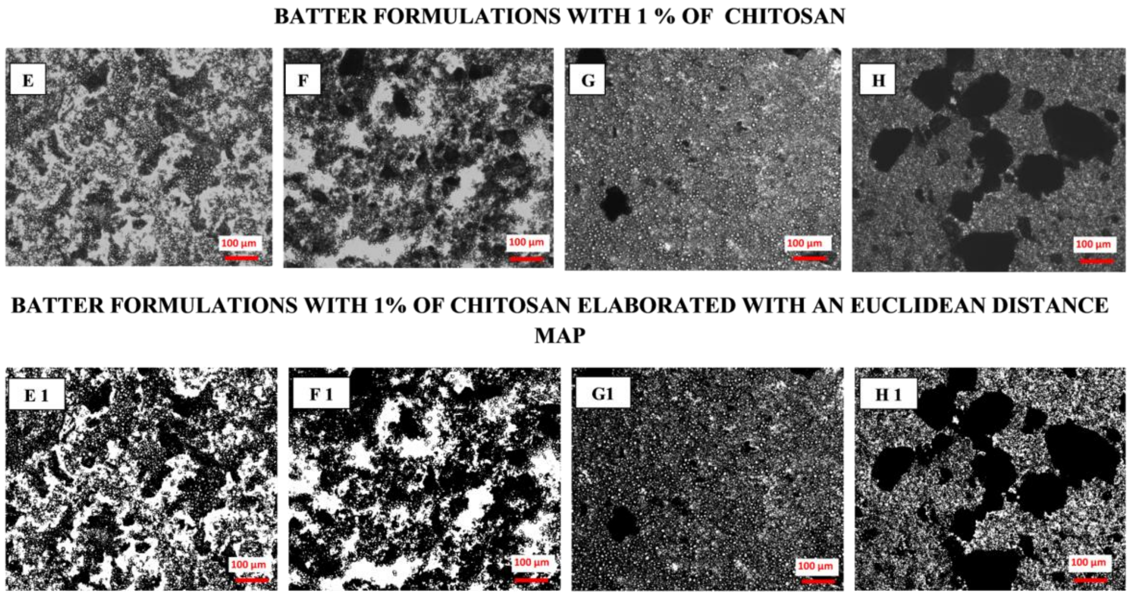

Figure 3. Micrographs of different batter formulations with 1 g/100 g of chitosan, acquired at 10x of magnification. Samples E, F, G and H correspond to formulations made of wheat flour (WF); 70WF/30RF; 30WF/70RF and rice flour (RF), respectively. Samples E1, F1, G1, H1 represented respectively the same formulations elaborated with an Euclidean distance map.

Table 1. Rheological parameters: yield stress (τ_0), the consistency index (K) and flow behavior index (n) obtained from Herschel–Bulkley model depending on batter samples formulated with different type of flour (rice and wheat flours) and chitosan amount. Mean values (n=3) and standard deviation. Different letters indicate differences between homogenous groups, in capital letters (A, B, C, D) as a function of wheat –rice flour formulation, and small letters as function of chitosan content (a, b, c, d).

Formulation	Chitosan content (g/100 g)	τ_0 (Pa)	K (Pa s^n)	n	R^2	Apparent viscosity 20s^{-1} (mPa· s)
Wheat flour (WF)	0	1.6 ± 0.2 ^{Da}	1.8 ± 0.1 ^{Da}	0.796 ± 0.001 ^{Ac}	0.9994	1050 ± 5 ^{Da}
70WF/30RF	0	1.154 ± 0.009 ^{Ca}	1.380 ± 0.003 ^{Ca}	0.783± 0.004 ^{Ab}	0.9996	776 ± 9 ^{Ca}
30WF/70RF	0	0.78 ± 0.02 ^{Ba}	1.0539 ± 0.0004 ^{Ba}	0.790 ± 0.005 ^{Ac}	0.9995	596 ± 9 ^{Ba}
Rice flour (RF)	0	0.030 ± 0.007 ^{Aa}	0.45 ± 0.05 ^{Aa}	0.93 ± 0.02 ^{Bb}	0.9998	371 ± 20 ^{Aa}
Wheat flour (WF)	0.25	2.4 ± 0.1 ^{Aa}	2.47 ± 0.05 ^{Bb}	0.792 ± 0.002 ^{Ac}	0.9988	1476 ± 40 ^{Cb}
70WF/30RF	0.25	1.93 ± 0.03 ^{Aa}	2.205 ± 0.004 ^{ABa}	0.78 ± 0.01 ^{Ab}	0.9993	1296 ± 46 ^{Ba}
30WF/70RF	0.25	2.5 ± 0.9 ^{Aa}	2.0 ± 0.4 ^{Aa}	0.75 ± 0.04 ^{Abc}	0.9997	1081 ± 131 ^{Ab}
Rice flour (RF)	0.25	2.0 ± 0.4 ^{Ab}	1.9 ± 0.2 ^{Ab}	0.75 ± 0.02 ^{Aa}	0.9994	1006 ± 61 ^{Ab}
Wheat flour (WF)	0.5	6 ± 1^{Ab}	5.0 ± 0.2 ^{Ac}	0.75 ± 0.01 ^{Ab}	0.9995	2704 ± 55 ^{Ac}
70WF/30RF	0.5	8 ± 4 ^{Ab}	5 ± 1 ^{Ab}	0.73 ± 0.04 ^{Aa}	0.9985	2727 ± 303 ^{Ab}
30WF/70RF	0.5	9 ± 2 ^{Ab}	5.1 ± 0.5 ^{Ab}	0.70 ± 0.02 ^{Aab}	0.9987	2649 ± 186 ^{Ac}
Wheat flour (WF)	1	15 ± 1 ^{Ac}	9.57 ± 0.04 ^{Ad}	0.725 ± 0.008 ^{Ba}	0.9988	5133 ± 47 ^{Ad}
70WF/30RF	1	13 ± 2 ^{Ac}	8.9 ± 0.7 ^{Ac}	0.73 ± 0.06 ^{Ba}	0.9990	4740 ± 215 ^{Ab}
30WF/70RF	1	35 ± 5 ^{Bc}	13 ± 2 ^{Bc}	0.69 ± 0.03 ^{Aa}	0.9850	7513 ± 90 ^{Bd}

Table 2. Particles size (Feret diameter) and minimum distance between particles of different batters with 1 g/100 g and without chitosan, corresponding to formulations of wheat flour (WF), rice flour (RF) and their combinations, 70WF/30RF and 30WF/70RF. Mean values (n=3) and standard deviation in the same column followed by different letters differ significantly at a $p < 0.05$ level.

Formulation	Chitosan content (g/100 g)	Feret diameter (µm)	Minimum distance between particles (µm)
Wheat flour (WF)	0	39 ± 3^{d}	12 ± 1^{d}
70WF/30RF		31 ± 2^{c}	17 ± 1^{c}
30WF/70RF		20 ± 2^{b}	28 ± 2^{b}
Rice flour (RF)		9.1 ± 0.6^{a}	37 ± 3^{a}
Wheat flour (WF)	1	36 ± 2^{c}	10.1 ± 0.1^{c}
70WF/30RF		32 ± 2^{c}	11 ± 2^{c}
30WF/70RF		17 ± 2^{b}	5 ± 3^{b}
Rice flour (RF)		8.0 ± 0.5^{a}	1.98 ± 0.07^{a}

Table 3. Mean values (n=3) and standard deviation of gelatinization peak temperature (T_p), onset temperature (T_o), and enthalpy (ΔH_G) corresponding to formulations of wheat flour (WF), rice flour (RF) and their combinations, 70WF/30RF and 30WF/70RF; with 0 (control), 0.25, 0.5 and 1 g/100 g of chitosan. Homogenous groups are represented by the same letter (Multifactor ANOVA).

Formulation	Chitosan content (g/100 g)	Gelatinization temperature T_p (ºC)	T_o (ºC)	Gelatinization enthalpy ΔH_G (J/g)
Wheat flour (WF)	0	70.3 ± 0.2 ^{b}	62.6 ± 0.2 ^{c}	4.1 ± 0.2 ^{b}
70WF/30RF		71.0 ± 0.4 ^{b}	62.7 ± 0.2 ^{c}	4.9 ± 0.1 ^{b}
30WF/70RF		78 ± 2 ^{a}	67 ± 1 ^{b}	6.1 ± 0.8 ^{a}
Rice flour (RF)		81.4 ± 0.2 ^{a}	74.5 ± 0.5 ^{a}	5.4 ± 0.7 ^{a}
Wheat flour (WF)	0.25	70.33 ± 0.06 ^{b}	62.5 ± 0.2 ^{c}	4.2 ± 0.5 ^{b}
70WF/30RF		70.9 ± 0.3 ^{b}	62.75 ± 0.05 ^{c}	4.77 ± 0.09 ^{b}
30WF/70RF		76 ± 1 ^{a}	63.6 ± 0.5 ^{c}	5.14 ± 0.01 ^{a}
Rice flour (RF)		82.9 ± 0.5 ^{a}	74.1 ± 0.5 ^{a}	4.4 ± 0.5 ^{a}
Wheat flour (WF)	0.5	70.6 ± 0.5 ^{b}	62.5 ± 0.4 ^{c}	4.2 ± 0.1 ^{b}
70WF/30RF		70.8 ± 0.3 b	62.7 ± 0.3 ^{c}	4.6 ± 0.8 ^{b}
30WF/70RF		78 ± 1 ^{a}	63.4 ± 0.2 ^{c}	5.7 ± 0.3 ^{a}
Rice flour (RF)		83.4 ± 0.5 ^{a}	73.7 ± 0.7 ^{a}	6.1 ± 0.7 ^{a}
Wheat flour (WF)	1	71.1 ± 0.6 ^{b}	62.5 ± 0.3 ^{c}	4.1 ± 0.1 ^{b}
70WF/30RF		70.8 ± 0.2 ^{b}	62.6 ± 0.1 ^{c}	3.8 ± 0.6 ^{b}
30WF/70RF		79 ± 1 ^{a}	64.0 ± 0.8 ^{c}	6 ± 1 ^{a}
Rice flour (RF)		78 ± 2 ^{a}	65.7 ± 0.3 ^{bc}	6.2 ± 0.2 ^{a}

Table 4. Glass transition temperature (T_g') (mean n=3, and standard deviation) corresponding to formulations of wheat flour (WF), rice flour (RF) and their combinations, 70WF/30RF and 30WF/70RF; with 0 (control), 0.25, 0.5 and 1 g/100 g of chitosan. Homogenous groups are represented by the same letter (Multifactor ANOVA).

Formulation	Chitosan content (g/100 g)	Glass transition temperature, Tg' (ºC)
Wheat flour (WF)	0	-12.24 ± 0.02 [cd]
70WF/30RF	0	-12.4 ± 0.1 [d]
30WF/70RF	0	-11.70 ± 0.09 [bc]
Rice flour (RF)	0	-12.4 ± 0.1 d
Wheat flour (WF)	0.25	-12.4 ± 0.1 [d]
70WF/30RF	0.25	-12.1 ± 0.2 [cd]
30WF/70RF	0.25	-11.93 ± 0.06 [c]
Rice flour (RF)	0.25	-11.5 ± 0.2 [b]
Wheat flour (WF)	0.5	-12.2 ± 0.1 [cd]
70WF/30RF	0.5	-11.98 ± 0.08 [cd]
30WF/70RF	0.5	-11.76 ± 0.07 [bc]
Rice flour (RF)	0.5	-11.0 ± 0.1 [a]
Wheat flour (WF)	1	-11.85 ± 0.06 [c]
70WF/30RF	1	-11.99 ± 0.05 [cd]
30WF/70RF	1	-11.7 ± 0.1 [bc]
Rice flour (RF)	1	-10.7 ± 0.1 [a]

Table 5. Mean values (n=3) and standard deviation of melting temperature (T_m) and enthalpy (ΔH_m), and non-freezable water content corresponding to formulations of wheat flour (WF), rice flour (RF) and their combinations, 70WF/30RF and 30WF/70RF; with 0 (control), 0.25, 0.5 and 1 g/100 g of chitosan. Homogenous groups are represented by the same letter (Multifactor ANOVA).

Formulation	Chitosan content (g/100 g)	Melting enthalpy ΔH_m (J/g)	Melting temperature T_m (ºC)	Non freezable water (g water/100 g solid)
Wheat flour (WF)	0	143 ± 3 ^{b}	1.4 ± 0.3 ^{a}	24 ± 2 ^{b}
70WF/30RF		142 ± 3 ^{b}	1.5 ± 0.5 ^{a}	25 ± 2 ^{b}
30WF/70RF		141 ± 1 ^{b}	0.9 ± 0.3 ^{b}	25.9 ± 0.7 ^{b}
Rice flour (RF)		154 ± 11 a	1.4 ± 0.4 ^{a}	17 ± 4 ^{c}
Wheat flour (WF)	0.25	144 ± 7 ^{b}	1.2 ± 0.2 ^{b}	24 ± 4 ^{b}
70WF/30RF		144 ± 1 ^{b}	1.1 ± 0.3 ^{b}	23.5 ± 0.7 ^{b}
30WF/70RF		141.8 ± 0.5 ^{b}	1.0 ± 0.1 ^{b}	25.1 ± 0.3 ^{b}
Rice flour (RF)		143.0 ± 0.1 ^{b}	1.1 ± 0.1 ^{b}	24.3 ± 0.1 ^{b}
Wheat flour (WF)	0.5	142.2 ± 0.6 ^{b}	1.03± 0.04 ^{b}	24.8 ± 0.4 ^{b}
70WF/30RF		140.9 ± 0.6 ^{b}	0.9 ± 0.3 ^{b}	25.7 ± 0.4 ^{b}
30WF/70RF		141 ± 3 ^{b}	1.0 ± 0.1 ^{b}	26 ± 2 ^{b}
Rice flour (RF)		125 ± 2 ^{c}	0.6 ± 0.1 ^{c}	33 ± 1 ^{a}
Wheat flour (WF)	1	140 ± 4 ^{b}	1.5 ± 0.3 ^{a}	26 ± 2 ^{b}
70WF/30RF		145 ± 3 ^{b}	1.1 ± 0.1 ^{b}	23 ± 2 ^{b}
30WF/70RF		128.9 ± 0.2 ^{c}	1.2 ± 0.5 ^{b}	33.5 ± 0.1 ^{a}
Rice flour (RF)		131 ± 3 ^{c}	1.3 ± 0.4 ^{b}	32 ± 2 ^{a}