Influence of acidification on dough viscoelasticity of gluten-free rice starch-based dough matrices enriched with exogenous proteins

Felicidad Ronda[1*], Marina Villanueva[1], Concha Collar[2]

(1) Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain.

(2) Cereal Group, Food Science Department, Instituto de Agroquímica y Tecnología de Alimentos, IATA-CSIC, Avda. Catedrático Agustín Escardino 7, 46980 Paterna, Spain.

*Corresponding author: Felicidad Ronda. College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain. Tel: +34979108339; Fax: +34 979108200. E-mail: fronda@iaf.uva.es

Abstract

The impact of acid incorporation (acetic+lactic, 0.5%) into rice starch-based doughs enriched with different proteins (egg albumin, calcium caseinate, pea protein and soy protein isolates) at different doses (0, 5 and 10%) has been investigated on dough viscoelastic and pasting profiles. Oscillatory (stress and frequency sweeps) and creep-recovery tests were used to characterise the fundamental viscoelastic behaviour of the doughs, and thermomechanical assays were performed to assess dough viscometric performance. Supplementation of gluten-free doughs with proteins from vegetal sources led to more structured dough matrices (higher viscoelastic moduli and steady viscosities, and lower tan δ, instantaneous and retarded elastic compliances) effect being magnified with protein dose. Acid addition decreased these effects. Incorporation of proteins from animal source resulted in different viscoelastic behaviours according to the protein type, dosage and acidification, especially for casein. Acidification conferred lower dough deformation and notably higher steady viscosity and viscoelastic moduli for 5 %-casein-added dough. Protein-acid interaction favoured higher viscosity profiles, particularly for doughs with proteins of vegetable origin and lower dosage. Dough acidification decreased the pasting temperatures and the amylose retrogradation. Acidification of protein-enriched rice-starch doughs allowed manipulation of its viscometric and rheological properties which is of relevant importance in gluten-free bread development.

Keywords: Acetic acid; Gluten-Free Doughs; Lactic acid; Proteins; Rheology

Abbreviations:

a: Exponent from fitting power law to G’ data

b: Exponent from fitting power law to G’’ data

BD: Breakdown viscosity

c: Exponent from fitting power law to *tan* δ data

FV: Final Viscosity

$G_1^{'}$: Elastic modulus at a frequency of 1 Hz obtained from fitting power law to G’ data

$G_1^{''}$:Viscous modulus at a frequency of 1 Hz obtained from fitting power law to G’’ data

J_{0c}: Instantaneous compliance obtained from creep test

J_{0r}: Instantaneous compliance obtained from recovery phase

J_{1c}: Retarded compliance obtained from creep test

J_{1r}: Retarded compliance obtained from the recovery phase.

LVR: Linear Viscoelastic Region

λ_{1c}: Retardation time in the creep phase

λ_{1r}: Retardation time in the recovery phase

μ_0: Steady state viscosity

PV: Peak Viscosity

PT: Pasting Temperature

SB: Setback

$(tan\ \delta)_1$: Loss tangent at a frequency of 1 Hz obtained from fitting power low to *tan* δ data

TV: Through Viscosity

ω: Oscillation Frequency

1. Introduction

Gluten-free (GF) products are a growing sector in the food industry, and the related research constitutes a prioritised and challenging topic in cereal-based goods area. ~~The unequivocal need for~~ The development of new GF products is emerging not only because daily dietary requirements for essential nutrients of celiac disease patients are not fully covered at present by existing products (Mandala ~~and~~ & Kapsokefalou, 2011). The target group of GF products is currently expanding to ~~adhere~~ join, in addition to celiac patients (1-3% of the population), people looking for nonallergenic ingredients, leading to a new market that needs a variety of products. Also, GF products can function as prototypes/templates for the development of other products addressed to specific vulnerable groups of population with special nutritional needs (e.g., diabetics). GF product approaches include: (1) reformulations (e.g., high-fiber gluten-free versions of traditional antecedents), (2) new forms of existing products (e.g., frozen and part-baked), (3) repackaging of existing products, and (4) innovative products (e.g., use of novel cereals) (Kelly, Moore, Elke, & Arendt ~~et al.~~, 2008). Concerning the first approach, complex formulations that appear promising in terms of technological improvement and nutritional quality have been developed so far, with variable success/failure regarding sensory appreciation and technological constraints. The formulations mainly involve the incorporation of starches of different origin, other non-gluten proteins such as dairy proteins, gums, and their combinations (Mariotti, Lucisano, Pagani, & Ng ~~et al.~~, 2009). These ingredients can mimic the viscoelastic properties of gluten and may result in improved structure, mouthfeel, acceptability, and shelf life of these products (Gallagher, Gonnley, & Arendt ~~et al.,~~, 2004).

Rice flour is considered one of the most suitable cereal flour for preparing gluten-free products associated to its several significant properties such as natural, hypoallergenic, colorless, and bland taste. It has also very low level of protein, sodium, fat, fiber and high amount of easily digested carbohydrates. Since most of the rice contain relatively small amount of prolamin (2.5–3.5%) (Gujral ~~and~~ & Rosell, 2004), it is necessary to use some sort of gum, emulsifier, enzymes or dairy products together with rice flour for achieving desired viscoelastic mixture (Demirkesen, Mert, Sumnu, & Sahin ~~et al.,~~, 2010). Gum type additives, such as hydroxy~~l~~ propyl methyl cellulose (HPMC) (Sivaramakrishnan, Senge, & Chattopadhyay ~~et al.,~~, 2004) and the enzyme glucose oxidase (Gujral ~~and~~ & Rosell, 2004) resulted in successful formation of rice bread

Formatted: Strikethrough

showing the optimum volume expansion and a general improvement of bread quality, respectively (Nikolić, Dodić, Mitrović, & Lazić, ~~et al.,~~ 2011). Proteins from different sources can be added to increase both nutritional and functional values of GF products. Protein incorporation leads to the formation of a continuous protein phase (Moore, Tilman, Dockery, & Arendt ~~et al.~~, 2004), and are added to GF applications (Crockett, Ie, & Vodovotz ~~et al.~~, 2011) to increase elastic modulus by cross linking, to improve perceived quality by enhancing Maillard browning and flavour, to improve structure with gelation and to aid in foaming (Moore, Dal Bello, & Arendt ~~et al.~~, 2008). These result in bread with increased loaf volume, improved crumb regularity and improved sensory characteristics (Moore et al., 2008). The use of dairy powder in gluten-free baked product formulations has resulted in improved volume as well as better appearance and sensory aspects of the loaves (Gallagher et al., 2004). Soy protein isolate and dried egg white solids were investigated due to their foam-stabilizing activity and use in GF applications (Marco ~~and~~ & Rosell, 2008; Moore et al., 2004). According to Stathopoulos (2008), the most used ingredients in gluten-free baked product formulations are caseinates, skim milk powder, dry milk, whey protein concentrate and milk protein isolate. It follows that the selection of the proteins used in a gluten-free formulation is a critical issue (Mandala ~~and~~ & Kapsokefalou, 2011). Soybean protein isolates increases the nutritional value of rice cassava bread and increases elastic modulus, resulting in enhanced gas retention and loaf volume, and improves water binding in the bread loaves. Other authors stated that the addition of soybean protein isolate to an HPMC-treated rice cassava bread reduced dough stability by suppressing HPMC functionality, altering water distribution within the dough, weakening HPMC interactions with the starch matrix and reducing foam stability (Crockett et al., 2011). Green pea protein has been used in less extent than the soybean protein in GF breads evidencing also an increase in the elastic modulus (Marco ~~and~~ & Rosell, 2008). Acetic and lactic acids confer suitable properties to final breads in terms of odour and taste either when produced by the exogenous microflora or added to breadmaking matrices, increasing in addition protease and amylase activities that lead to a retarded staling during storage (Moore et al., 2008).

The combined effect of acid addition and protein supplementation in GF matrices has not been described so far despite inter ande intra-molecular interactions established between exogenous proteins and starch molecules that are the main responsible for dough structurization, certainly depend on dough pH. In addition, despite several rheological techniques, including oscillation, stress relaxation, creep and creep-recovery measurements have been used extensively for assessing fundamental mechanical properties of gluten, the use of dynamic rheometry in studies of GF-dough rheological behavior has only been applied over the last decade (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis ~~et al.~~, 2007; Ronda, Pérez-Quirce, Angioloni, & Collar ~~et al.~~, 2013). Fundamental and empirical rheological properties of doughs inform about interactions among ingredients and the creation of structure at macromolecular and macroscopic levels, respectively. In addition, quality attributes of breads such as volume and texture can be correlated with dough rheological properties (Sahin, 2008; Pérez-Quirce, Collar, & Ronda, 2014).

This paper is intended to know the impact of acid incorporation (acetic:lactic, 0.1:0.4 ~~%, w:w~~g/100 g starch+protein basis) into GF rice starch-based dough matrices enriched with different proteins (egg albumin, calcium caseinate, pea protein and soy protein isolates) at different doses on dough viscoelastic, and pasting profiles, prior to assess comparatively the structure promoting ability in GF matrices of exogenous proteins in absence/presence of acid.

2. Material and methods

2.1. Materials

Rice starch (9.9 % moisture, 0.2 % ash and 0.5 % protein) from Ferrer Alimentación S.A. (Barcelona, Spain), and salt, sugar (Azucarera Ebro, Spain) and sunflower oil (branded Coosur Premium) purchased from the local market, were used to make gluten-free doughs. Hydroxypropylmethylcellulose (HPMC, Methocel K4M Food Grade) was provided by Dow Chemical (Midland, EEUU). Proteins used in gluten-free formulations were: soybean isolate Supro 500-E IP from Proveedora hispano-holandesa S.A. (Barcelona, Spain), calcium caseinate from Armor proteines (Saint-Brice-en-Coglès, France), egg albumin in dry powder from Eurovo (Valladolid, Spain) and pea protein isolate branded Pisane C9, from Cosucra (Warcoing, Belgium). Acetic acid and lactic acid (analytical grade; Panreac, Barcelona) were used as a source of hydrogen ions.

2.2. Methods

2.2.1. Dough preparation

A straight dough process was performed using the following formula on a 100 g rice starch (or rice starch+protein) basis: 6 g/100 g% oil, 5 % g sucrose, 1.5 % g salt, 2 % g HPMC and 80 % g water**.** All proteins were added at 0 %, , 5 % and 10 % g/100 gw/w (starch+protein basis) levels. Doughs were supplemented with (0.1 % g/100 g + 0.4) % g/100 g (w/w starch+protein basis) of acetic and lactic acid, respectively, when acid-treatment was applied. The experimental design is shown in Table 1. GF dough-making was achieved by blending first solid ingredients and oil in a kitchen-aid professional mixer (KPM5). Then water was added and hand mixed. Finally the dough was mixed with dough hook at a speed 4 for 8 min. Acid blend, when added, was diluted in a small part of water (7 % of total) and adjusted to the dough before the mixer was powered on.

2.3 Dough measurements

Oscillatory and creep recovery tests

Oscillatory and creep-recovery tests were carried out with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) with parallel plate geometry (60 mm diameter) of serrated surface and with 3 mm gap. The excess of batter was removed and vaseline oil was applied to cover the exposed sample surfaces. Before the measurement, the batter was rested for 10 min to allow relaxation. Frequency sweeps were carried out from 20 to 0.1 Hz in the linear viscoelastic region (LVR) previously established for each batter by means of stress sweeps from 0.1 to 1000 Pa at 1 Hz. The frequency sweeps of all batters were carried out at stress values between 2 Pa and 10 Pa. Temperature was 25 ºC. Frequency sweep data were fitted to the power law model as in previous works (Ronda et al., 2013):

$$G'(\omega) = G'_1 \cdot \omega^a ;\; G''(\omega) = G''_1 \cdot \omega^b ;\; \tan\ \delta(\omega) = \frac{G''(\omega)}{G'(\omega)} = \left(\frac{G''}{G'}\right)_1 \cdot \omega^{(b-a)} = (\tan\ \delta)_1 \cdot \omega^c$$

The coefficients G_1', G_1'', and $(\tan \delta)_1$, represent the elastic and viscous moduli and the loss tangent at a frequency of 1 Hz. The a, b and c exponents quantify the dependence degree of dynamic moduli and the loss tangent with the oscillation frequency, ω. Creep tests were performed by imposing a sudden step shear stress in the LVR for 150 s. In the recovery phase the stress was suddenly removed and the sample was allowed for 300 s to recover the elastic (instantaneous and retarded) part of the deformation. Each test was performed in triplicate. The data from creep tests were modelled to the 4-parameter Burgers model (Lazaridou et al., 2007) given by:

$$J_c(t) = J_{0c} + J_{1c}\left(1 - \exp\left(\frac{-t}{\lambda_{1c}}\right)\right) + \frac{t}{\mu_0}$$

In the equation, J_c (t) is the creep compliance (strain divided by stress), J_{0c} is the instantaneous compliance, J_{1c} is the retarded elastic compliance or viscoelastic compliances, λ_{1c} is the retardation time and μ_0 gives information about the steady state viscosity. Similar equations were used for the recovery compliance $J_r(t)$. As there is no viscous flow in the recovery phase, equations consist only of parameters describing the elastic response after removal of the shear stress. The data from creep tests were modelled to the 3-parameter Burgers model given by:

$$J_r(t) = J_{\max} - J_{0r} - J_{1r}\left(1 - \exp\left(\frac{-t}{\lambda_{1r}}\right)\right)$$

J_{max} is the maximum creep compliance obtained at the end of the creep step.

Thermoviscous test: Viscometric profile

Viscometric profiles (gelatinization, pasting, and setback properties) of formulated starch rice doughs were obtained with a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) using ICC Standard 162. Freeze-dried hydrated samples (3.5 g, 14 % moisture basis) were transferred into canisters and ≈25 ± 0.1 mL of distilled water were added and processed following standard method. The pasting temperature (PT), peak time (when peak viscosity occurred) (VT), peak viscosity (PV), holding strength or trough viscosity (TV), breakdown (BD), final viscosity (FV) and setback (final viscosity minus peak viscosity) (SB) were calculated from the pasting curve (Collar, 2003) using Thermocline v. 2.2 software. For each viscometric measurement, 3 samples were used.

2.4. Statistical analysis

Statgraphics Centurion v.6 (Bitstream, Cambridge, MN, USA) was used for multivariate non-linear regression and Pearson correlation matrix. STATISTICA package (Tulsa, OK, EEUU) v.6, allowed performance of MANOVA analysis, and LSD (Least Significant Difference) test was used to evaluate significant differences ($p<0.05$) between samples.

3. Results and discussion

Table 2 and 3 show the single and 2nd order interactive effects of protein and acid addition on pH and rheological and pasting properties of GF doughs. Protein presence increased the dough pH between 7 % and 12 % with respect to the control dough, depending on the dose. The lower increase was obtained with albumin. The acidification of protein-enriched doughs resulted in pH values 15 % – 34 % higher than the acid-added control dough.

3.1. Fundamental rheology

3.1.1. Dynamic oscillatory rheology

Protein–enriched rice starch-based doughs were submitted to both stress and frequency sweeps in the linear visco-elastic region (LVR), which oscillatory rheological behaviour for selected samples is illustrated in Figures 1.a. and 1.b., respectively. Stress sweep tests allowed to know the maximum stress (τ_{max}) that GF matrices can tolerate in the LVR -from 6 to 108 Pa- providing structure preservation. Lower τ_{max} values corresponded to control samples without protein addition and to albumin-enriched samples regardless either acid or protein level addition, whereas higher τ_{max} were obtained for no acid/10 g/100 g~~%~~ pea protein or 10 g/100 g~~%~~ soya protein enrichment and for acid/10 g/100 g~~%~~ casein incorporation. Except for the egg albumin, the presence of protein encompassed a significant ($p<0.01$) increase in τ_{max} values as a result of dough structurization. Increased dosage from 5 to 10 g/100 g~~%~~ promoted τ_{max} for doughs enriched with vegetal proteins (+63 % soya, +89 % pea), whereas only acidified matrices containing casein underwent a relevant structure promotion with protein dose (+160 %). For vegetal proteins, dough acidification led to a weakening effect regardless the dose, and consequently to a decrease in $\tau_{max,}$ more prominent in pea enriched samples (-54 %) than in soya samples (-37 %). Samples supplemented with casein proteins observed a strengthening effect in acid medium when added at 10 g/100 g~~%~~ (+37 %), but underwent weakening impact with acid addition when added at 5 g/100 g~~%~~ to the doughs (-47 %).

Frequency sweep tests of unacidified and acidified 5 g/100 g~~%~~ casein added dough matrices are illustrated in Figure 1.b. Visco-elastic behaviour of dough samples corresponded with no exception to solid-like samples with storage modulus values (G'_1) higher (from 2568 to 70665 Pa) than loss modulus values (G''_1) (from 477 to 10465 Pa), slight frequency dependence, and values for tan δ (G''/G') under 1, in good accordance with earlier results found for rice doughs enriched with protein isolates

(Gujral and & Rosell, 2004). In this work, protein addition affected dough viscoelasticity, the extent of the changes being dependent on the type and the dose of protein and on the absence/presence of acid (Table 2), and on the interactive effects of protein x acid (Table 3). Interactions between starch and proteins depend upon the molecular structure of protein, the starch: state of the granules and the amylose/amylopectin ratio, the composition of protein and starch, as well as the phase transition temperatures of starch gelatinization and protein denaturation. There is also an electrostatic association between the two polymers. Anionic polysaccharide and protein are incompatible at pH values above the protein's isoelectric point (point of minimum solubility, pH ~ 5.1) and completely compatible below it due to the net opposite charges they carry (Rao, 2007). Factors affecting protein-polysaccharide compatibility and the characteristics of their complexes include the molecular characteristics of the two molecules (e.g., molecular weight, net charge, and chain flexibility), the pH, ionic strength, temperature, the protein/polysaccharide ratio, rate of acidification, and rate of shear during acidification (Rao, 2007). Vegetal proteins significantly increased ($p<0.01$) both the elastic and viscous components in doughs (Table 2), increments being larger in soya protein samples (+143 % G', +94 % G'') than in pea protein matrices (+109 % G', +78 % G'') by increasing the dose from 5 to 10 g/100 g%, starch-protein basis. Acid addition modulated dough viscoelasticity in soya protein matrices at higher dose, so that a weakening effect denoted by a significant drop in G' (-61 %) and G'' (-40 %) with a concomitant increase in tan δ (+52 %) was observed (Table 3). Animal proteins significantly modified mechanical spectra of protein-enriched matrices depending on the type of protein, when compared to both unacidified and acidified control doughs. Casein addition observed a dependence on the frequency for both dynamic moduli (Figure 1.b), a higher consistency than the control and albumin enriched samples, but a lower predominance of G'_1 over G''_1, (higher tan δ values) compatible with a more viscous nature (Table 2). The acidification of casein supplemented samples increased G' (+52 %) when added at 5 g/100 g~~%~~ and decreased G'' depending on the dose of addition (-34 % at 5 g/100 g~~%~~, -25 % at 10 g/100 g~~%~~) (Table 3). Doughs enriched with albumin exhibited a different behaviour with lower mechanical spectra profiles than unsupplemented protein-samples, regardless the dose of addition and the absence/presence of acid (Table 2 and Table 3). Slight dependence of the moduli on angular frequency (a and b values ranged 0.11-0.28) and values of phase shift tangent (tan δ) varying in the range $0.1 < \tan\delta < 0.4$ are both characteristic features for the systems which so called weak gels (elastic behaviour). This is in agreement with earlier observations regarding viscoelastic properties of GF dough (Witczak, Korus, Ziobro, & Juszczak ~~et al.~~, 2010). Significant variation in dough viscoelastic moduli was also observed by Nunes, Ryan, and Arendt ~~et al.~~ (2009) who supplemented GF bakery products with milk and whey proteins. In the case of albumin a significant decrease of G' and G'' was accompanied with a slight, but statistically significant increase of phase shift tangent when added at 5 g/100 g~~%~~. All other protein preparations caused significant increase of moduli G' and G'' (Table 2). Although the addition of pea protein resulted in a significant growth of G' and G'', it caused only a slight shift of phase shift tangent in the range of low frequencies, in accordance with previous reports (Ziobro, Witczak, Juszczak, & Korusa ~~et al.~~, 2013). In oscillatory studies, Crockett et al. (2011) observed an increase of storage modulus accompanied by the drop in phase shift tangent of the dough supplemented with soy protein isolate, which was potentially due to protein aggregation within the medium. The application of casein significantly modified rheological image of dough structure, shifting its properties toward values typical for strong gels, probably caused by its special arrangement, in which regularly occurring amino acid sequence favoured the formation of tight polypeptide-strands stabilized by covalent and hydrogen bonds, as described for collagen (Gómez-Guillén, Giménez, López-Caballero, & Montero ~~et al.~~, 2011). Current results in agreement with previous studies (Crockett et al., 2011; Ziobro et al., 2013) are compatible with the creation of a robust crosslinked structure by added proteins, especially supported in the case of soya protein by glicinin and a high water retention ability (Crockett et al., 2011). In studies using acid in rice flour based doughs, chemical acidification encompassed a dough softening effect highly dependent on both the final dough pH and the type of acid (Blanco, Ronda, Pérez, & Pando ~~et al.~~, 2011). Some authors have reported an increase in wheat flour dough stiffness (viscosity or complex shear modulus) with decreasing pH in the range 6-5.6 to 4 (Jekle ~~and~~ & Becker, 2012) probably as result of the change in the conformation of the proteins. The decreased pH would lead to the change in the overall net charge from neutral (near the isoelectronic point) to positive. A neutral charge causes less repulsion forces and less space for water molecules between the proteins. This repulsion forces increase with increasing charge and more water molecules can be attached to the protein strands whereby less mobile water is available in the dough system (Jekle ~~and~~ & Becker, 2012).

3.1.2. Creep-recovery tests

Creep-recovery tests were also conducted on formulated GF doughs. Stress applied in the LVR ranged from 2 Pa to 10 Pa, and were maintained for 150 s, sufficient for the sample to reach the steady-state flow. Creep-recovery curves of GF doughs exhibited a typical viscoelastic behaviour combining both viscous fluid and elastic components (Figure 1.c), similar to the corresponding curves obtained previously for rice flour

(Sivaramakrishnan et al., 2004) and other gluten-free doughs (Lazaridou et al., 2007; Ronda et al., 2013).

Creep parameters for all GF dough formulations are summarized in Table 2. Major impact on creep-recovery parameters was associated to vegetal proteins and albumin incorporation. Increased vegetal protein incorporation led to significantly lower instantaneous (J_0) and retarded (J_1) elastic compliance in both creep and recovery phases associated to a lower dough deformation submitted to a constant stress, and a higher recovery when stress is removed, respectively. Maximum depletion in compliance values was observed for soya protein enriched matrices at 10 g/100 g~~%~~ of addition: -70 % (J_{0c}), -54 % (J_{1c}), -70 % (J_{0r}), -72 % (J_{1r}). For animal protein supplemented doughs, albumin incorporation notably promoted J values compared to control doughs, increases being magnified with protein dosage; whereas casein inclusion in dough formulation only affected J_{0c} when added at 10 g/100 g~~%~~, encompassing a 40 % decrease in values (Table 2).

Addition of protein from both animal and vegetal source encompassed higher retardation times in the creep phase (λ_{1c}) and lower retardation times in the recovery phase (λ_{1r}), indicating a slower and quicker retarded elastic response, respectively (Table 2). pH decrease as a result of acidification significantly affected major creep-recovery parameters (Table 3). In unacidified doughs, J_{1c} values were higher in presence of animal proteins but similar or even 50-60 % lower in presence of vegetal proteins, in accordance with a higher deformation at a constant stress with time for animal proteins encompassing a lower dough consistency. Dough acidification led to a decrease in J_{1c} when albumin or casein was incorporated while for vegetal protein addition, the opposite effect was observed. Protein addition to unacidified matrices significantly increased values of λ_{1c} except for doughs supplemented with 5 g/100 g~~%~~ pea protein.

Acidification induced longer λ_{1c} with respect to control doughs only in doughs formulated with casein, pea protein or soya protein added at 10 g/100 g~~%~~ (Table 3). Viscosity at steady state (μ_0) marked increased with soya protein addition although decreased with the remaining proteins. It decreased notably with dough acidification in soya protein presence (-67 % for 5 g/100 g~~%~~ and -72 % for 10 g/100 g~~%~~) and slightly, but significantly, in presence of 10 g/100 g~~%~~ pea protein and 5 g/100 g~~%~~ albumin. Rice starch control dough also showed a decreased viscosity at lower pH. Doughs with 10 g/100 g~~%~~ albumin, 5 ~~%~~ or 10 ~~%~~ casein or 5 g/100 g~~%~~ pea protein observed the opposite trend. In acidified doughs, vegetal protein incorporation led to increased values for μ_0 while animal proteins, except casein at 5 g/100 g~~%~~ dose, encompassed a significant decrease (Table 3). As it was established for cake batters (Sahi and Alava, 2003) there is probably an optimum consistency for gluten-free doughs, more similar to batters than to wheat doughs, to achieve breads of high volume. A proper consistency, with high enough G' and G'' moduli and viscosity, μ_0 , helps to hold the carbon dioxide produced during fermentation. Too strength doughs, with too low J_0 and J_1 compliances, can restrict dough expansion and lead to less developed breads (Pérez-Quirce et al., 2014).

3.2. Visco-metric profile

Impact of protein addition and acidification (Table 2) and interactive effects of protein x acid (Table 3) on the RVA primary parameters evidenced significant changes on the pasting and gelling behaviour of protein-enriched rice starch-based matrices. Major single effects on cooking and cooling parameters were provided by casein and vegetal proteins, especially by pea protein (Table 2). Pasting occurs when the starch granules absorb sufficient water and swell after gelatinization. The initial increase in viscosity with temperature during heating could be attributed to the increase in the leachates from the starch granules and the formation of a homogeneous mass resulting from the remaining fragile starch granules (Atwell, Hood, Lineback, Marston, & Zobel et al., 1988). A sharp decrease in peak viscosity was observed with the addition of casein and vegetal proteins with a concomitant general increase in pasting temperature, with changes being magnified with increased dose of protein (Table 2). The importance of protein in the initialization of pasting (Meadows, –2002) as well as in peak and final viscosity (Fitzgerald, Martin, Ward, Park, & Shead et al., 2003) has been strongly evidenced in rice. In addition, protein-starch linkages established in presence of proteins stabilise starch structure, and hence delayed the gelatinization process (Crockett et al., 2011). Lower values for pasting viscosities are an indication of a reduction in starch available for gelatinization. This reduction is likely due to a general reduction in the starch content of the pastes because of replacement with proteins that can additionally retain water from the starch granules. The reduction of available water in the system would reduce initial starch granule swelling and, hence, add to the explanation of lower peak viscosities of the pastes. In addition to the retention of the integrity of the starch granules, it is suggested that a reduction in pasting characteristics may be associated with a reduced enthalpy of starch gelatinization as observed in dietary enriched biscuits (Brennan and & Samyue, 2004). Acidification decreased pasting temperature in protein-free and protein-enriched doughs with the exception of both soya and pea proteins added at 10 g/100 g%. Effects of acid incorporation on peak viscosity revealed a decrease in protein-free doughs and an increase in protein enriched doughs with the exception of soya protein, where no significant effects were observed. The viscosity of the paste that had been gelatinized in acetic/lactic acid solution was decreased by shearing thinning effect caused by stirring in the RVA test. Takahashi (1974) mentioned that the part where the molecular associative strength was weak in starch granule collapsed and dispersed when gelatinized starch paste was sheared by mechanical power. In the presence of acetic/lactic acid, the structure of the starch became more fragile by stirring, resulting in the decrease of viscosity and the increase of breakdown. It was considered that the residual proteins prevented the increase in viscosity and the collapse of starch granules during heating. Proteins mainly exist among the starch granules as protein bodies. Proteins around starch granules might indirectly disturb the gelatinization of starch (Ohishi, Kasai, Shimada, & Hatae ~~et al.~~, 2007).

Upon subsequent cooling, a gel is formed that consists of an amylose matrix in which amylopectin enriched granules are embedded (Miles, Morris, Orford, & Ring ~~et al.~~, 1985). Effects of protein supplementation and acidification on the parameters characterizing the gelling process were particularly significant for the final viscosity on cooling (Table 2, Table 3). This parameter sharply decreased in presence of increasing amounts of either vegetal or animal protein except for albumin. Dough acidification promoted the decrease in final viscosity values for unsupplemented and supplemented protein matrices particularly for soya protein, except for casein-enriched samples that underwent an increase (Table 3). In earlier reports, final viscosity of the rice paste with acetic acid was lower than that with distilled water. It was suggested that cooked rice with acetic acid might exhibit less tendency to retrogradation when rice was soaked in acetic acid solution; proteins were eluted from rice grains and degraded by aspartic proteinase and carboxypeptidase (Ohishi et al., 2007). The different nature of added proteins may be responsible for the different behaviour. General results are in accordance with those reported by others for protein isolates (Ribotta ~~and~~ & Rosell, 2010) and acetic acid incorporation (Ohishi et al., 2007).

3.3. Correlations between fundamental and empirical rheological parameters

Multivariate data handling of rheological variables supplied useful information on the significantly correlated viscoelastic and viscometric characteristics of GF dough samples. Using Pearson correlation analysis, a range of correlation coefficients (r) (from 0.46 to 0.95) was obtained for the relationships between fundamental and empirical properties of protein-free and protein-supplemented rice starch-based matrices with/without acid addition (Table 4). A significant interdependence ($0.51<r<0.98$) within both rheometer and mimetic measurements was found. This is especially true for parameters retrieved from the same fundamental (oscillatory measurements and creep-recovery features) and mimetic (pasting and gelling) tests. Storage and loss moduli, indicators of dough strengthened structure and solid-like behavior, strongly correlated ($p<0.001$, $r=0.81$). The loss tangent tan δ indicating solid-like or liquid like nature, is highly connected to the "a" exponent ($p<0.001$, $r=0.98$), indicating a correspondence between less structured doughs with high viscous nature expliciting elastic component G' more dependent on the frequency. As expected, a strong correlation was found between creep compliance parameters and the recovery phase counterparts ($p<0.001$), since the creep-recovery tests were carried out in the LVR (data not shown). In addition it was observed that factors increasing viscosity at the steady state (μ_0) decreased compliance values J_0 ($r=-0.56$) and J_1 ($r=-0.66$), in good accordance with previous observations (Lazaridou et al., 2007; Ronda et al., 2013), and increased G'_1. The larger the maximum stress τ_{max} providing structure integrity, the greater are the dynamic moduli, the poorer are the instantaneous and retarded compliance, and the lower is the visco-metric profile of the corresponding doughs.

4. Conclusions

A gluten-free formulation based on rice starch can be obtained with a suitable combination of different proteins (egg albumin, calcium caseinate, pea protein and soy protein isolates) and acid. Supplementation of GF doughs with proteins from vegetal sources led to more structured dough matrices (higher viscoelastic moduli and steady viscosities, and lower tan δ, instantaneous and retarded elastic compliances) effect being magnified with protein dose. Acid addition produced weakening of the structure dough matrices. Acidification of soya-added doughs decreased G’ and G’’ (20–60 % depending on the dose) and the steady viscosity (60-70 %) and increased the loss tangent (up to 50 %) and the elastic compliances, J_{0c} (30 – 120 %) and J_{1c} (30 % - 230 %). The effect of acidification on pea protein-enriched doughs was similar although the changes in viscoelastic moduli and loss tangent did not result significant. Incorporation of proteins from animal source resulted in different viscoelastic behaviours according to the protein type, dosage and acidification, especially for casein. Acidification conferred lower dough deformation and notably higher steady viscosity, G’ and G’’ for dough with 5 g/100 g~~%~~ casein. Protein-acid interaction favoured higher viscosity profiles, particularly for doughs with proteins of vegetable origin and lower dosage. Dough acidification decreased the pasting temperatures and the amylose retrogradation. It can be concluded that acidification of protein-enriched rice-starch doughs allows manipulation of dough rheological properties which is of relevant importance in GF bread development.

Acknowledgement

Authors gratefully acknowledge the financial support of the Spanish Institutions Ministerio de Economía y Competitividad (Projects AGL2012-35088 and AGL2011-22669) and Comunidad de Castilla y León (Project VA252A12-2).

References

Atwell, W. A., Hood, L. F., Lineback, D. R., Marston, E. V., & Zobel, H. F. (1988). The terminology and methodology associated with basic starch phenomena. *Cereal Foods World*, *33*, 306-311.

Blanco, C. A., Ronda, F., Pérez, B., & Pando, V. (2011). Improving gluten-free bread quality by enrichment with acidic food additives. *Food Chemistry, 127*, 1204-1209.

Brennan, C. S., & Samyue, E. (2004). Evaluation of starch degradation and textural characteristics of dietary fibre enriched biscuits. *International Journal of Food Properties, 7*, 647-657.

Collar, C. (2003). Significance of viscosity profile of pasted and gelled formulated wheat doughs on bread staling. *European Food Research and Technology, 216(6)*, 505-513.

Crockett, R., Ie, P., & Vodovotz, Y. (2011). Effects of soy protein and egg white solids on the physicochemical properties of gluten-free bread. *Food Chemistry, 129*, 84-91.

Demirkesen, I., Mert, B., Sumnu, G., & Sahin, S. (2010). Rheological properties of gluten-free bread formulations. *Journal of Food Engineering, 96*, 295-303.

Fitzgerald, M. A., Martin, M., Ward, R. M., Park, W. D., & Shead, H. J. (2003). Viscosity of rice flour: A rheological and biological study. *Journal of Agricultural and Food Chemistry, 51(8)*, 2295-2299.

Gallagher, E., Gonnley, T. R., & Arendt, E. K. (2004). Recent advances in the formulation of gluten-free cereal-based products. *Trends in Food Science & Technology, 15*, 143-152.

Gómez-Guillén, M., Giménez, B., López-Caballero, M., & Montero, M. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: a review. *Food Hydrocolloids, 25(8)*, 1813-1827.

Gujral, H. S., & Rosell, C. M. (2004). Improvement of the breadmaking quality of rice flour by glucose oxidase. *Food Research International, 37*, 75-81.

Jekle, M., & Becker, T. (2012). Effects of Acidification, Sodium Chloride, and Moisture Levels on Wheat Dough: I. Modeling of Rheological and Microstructural Properties. *Food Biophysics, 7*, 190-199.

Kelly, A. L., Moore, M. M., Elke, K., & Arendt, E. K. (2008). New product development: the case of gluten-free food products. In E. K. Arendt, & F. Dal Bello (Eds.), *Gluten-free: cereal products and beverages* (pp. 413-431). New York: Academic Press.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering, 79*, 1033-1047.

Mandala, I., & Kapsokefalou, M. (2011). Gluten-Free Bread: Sensory, Physicochemical, and Nutritional Aspects. In V. R. Preedy, R. R. Watson, & V. B. Patel (Eds.), *Flour and breads and their fortification in health and disease prevention* (pp. 161-169).

Marco, C., & Rosell, C. M. (2008). Functional and rheological properties of protein enriched gluten free composite flours. *Journal of Food Engineering, 88*, 94-103.

Mariotti, M., Lucisano, M., Pagani, M. A., & Ng, P. K. W. (2009). The role of corn starch, amaranth flour, pea isolate, and *Psyllium* flour on the rheological properties and the ultraestructure of gluten-free doughs. *Food Research International*, *42*, 963-975.

Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research*, *135(2)*, 271-281.

Moore, M., Tilman, T. S., Dockery, P., & Arendt, E. K. (2004). Textural comparisons of gluten-free and wheat-based doughs, batters, and breads. *Cereal Chemistry, 81(5)*, 567-575.

Moore, M., Dal Bello, F., & Arendt, E. K. (2008). Sourdough fermented by *Lactobacillus plantarum* FST 1.7 improves the quality and shelf life of gluten-free bread. *European Food Research and Technology*, *226*, 1309-1316.

Nikolić, N., Dodić, J., Mitrović, M., & Lazić, M. (2011). Rheological properties and the energetic value of wheat flour substituted by different shares of white and brown rice flour. *Chemical Industry and Chemical Engineering Quarterly, 17(3)*, 349-357.

Nunes, M. H. B., Ryan, M. A. L., & Arendt, E. K. (2009). Effect of low lactose dairy powder addition on the properties of gluten-free batters and bread quality. *European Food Research and Technology*, *229*, 31–41.

Ohishi, K., Kasai, M., Shimada, A., & Hatae, K. (2007). Effects of acetic acid on the rice gelatinization and pasting properties of rice starch during cooking. *Food Research International*, *40*, 224-231.

Pérez-Quirce, S., Collar, C., & Ronda, F., (2014). Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads *International Journal of Food Science and Technology*, *49*, 1375–1382.

Rao, M. A. (2007). Rheology of Food Gum and Starch Dispersions. In M. A. Rao (Ed.), *Rheology of Fluid and Semisolid Foods Principles and Applications* (pp. 153-214). New York: Springer Science+Business Media.

Ribotta, P. D., & Rosell, C. M. (2010). Effects of enzymatic modification of soybean protein on the pasting and rheological profile of starch–protein systems. *Starch*, *62*, 373-383.

Formatted: Font: Italic

Ronda, F., Pérez-Quirce, S., Angioloni, A., & Collar, C. (2013). Impact of viscous dietary fibres on the viscoelastic behaviour of gluten-free formulated rice doughs: A fundamental and empirical rheological approach. *Food Hydrocolloids, 32*, 252-262.

Sahi, S.S., Alava, J.M., (2003). Functionality of emulsifiers in sponge cake production. *Journal of the Science and Agriculture, 83*, 1419–1429.

Sahin, S. (2007). Cake batter rheology. In: Summu, S.G., Sahin, S. (Eds.), *Food Engineering Aspects of Baking Sweet Goods* (pp. 99–118). London. CRC Press.

Sivaramakrishnan, H., Senge, B., & Chattopadhyay, P. K. (2004). Rheological properties of rice dough for making rice bread. *Journal of Food Engineering*, *62*, 37-45.

Stathopoulos, C. E. (2008). Dairy-based ingredients. In E. K. Arendt, & F. Dal Bello (Eds.), *Gluten-free cereal products and beverages* (pp. 464). Elsevier, Academic Press.

Takahashi, R. (1974). Physical properties of starch granules and their uses. *Journal of the Japanese Society of Starch Science, 21*, 51-60.

Ziobro, R., Witczak, T., Juszczak, L., & Korusa, J. (2013). Supplementation of gluten-free bread with non-gluten proteins. Effect on dough rheological properties and bread characteristic. *Food Hydrocolloids, 32*, 213-220.

Witczak, M., Korus, J., Ziobro, R., & Juszczak, L. (2010). The effects of maltodextrins on gluten-free dough and quality of bread. *Journal of Food Engineering*, *96*, 258-265.

Table

Table 1.- Experimental design

Fórmula	Protein				Acetic/Lactic acid
	CA	EA	SPI	PPI	
1	0	10	0	0	0.1/0.4
2	0	5	0	0	0
3	0	0	10	0	0
4	0	0	5	0	0
5	0	10	0	0	0
6	0	5	0	0	0.1/0.4
7	0	0	0	0	0
8	0	0	5	0	0.1/0.4
9	0	0	0	5	0
10	0	0	0	10	0.1/0.4
11	0	0	0	0	0.1/0.4
12	10	0	0	0	0.1/0.4
13	5	0	0	0	0
14	5	0	0	0	0.1/0.4
15	0	0	0	5	0.1/0.4
16	0	0	10	0	0.1/0.4
17	0	0	0	10	0
18	10	0	0	0	0

CA: Calcium Caseinate; EA: Egg Albumin;
SPI: Soya Protein Isolate; PPI: Pea Protein Isolate
Amounts are in % w/w, starch +protein basis

Table 2. Single effects of design factors at different levels on the dynamic oscillatory, creep-recovery, visco-metric parameters and pH of protein-enriched rice starch-based gluten-free doughs.

Parameter	Unit	Overall Mean	Level	Egg albumin	Calcium caseinate	Isolated pea protein	Isolated soya protein	Acid
Dynamic Oscillatory Rheometry								
G'_1	Pa	21858	0	14382 b	14382 a	14382 a	14382 a	ns
			1	3945 a	19330 a	17987 a	23138 a	
			2	3042 a	28690 b	37600 b	56313 b	
G''_1	Pa	4152	0	2738 b	2738 a	2738 a	2738 a	ns
			1	888 a	5443 b	3519 b	4104 a	
			2	603 a	9146 c	6254 c	7952 b	
tan δ		0.2070	0	0.19 a	0.19 a	0.19 b	0.19 b	ns
			1	0.23 b	0.31 b	0.20 b	0.18 ab	
			2	0.20 a	0.32 b	0.17 a	0.15 a	
Creep recovery test								
J_{oc}	Pa^{-1}	1.19×10^{-4}	0	0.99×10^{-4} a	0.99×10^{-4} b	0.99×10^{-4} c	0.99×10^{-4} c	ns
			1	2.70×10^{-4} b	0.81×10^{-4} b	0.64×10^{-4} b	0.55×10^{-4} b	
			2	3.84×10^{-4} c	0.53×10^{-4} a	0.34×10^{-4} a	0.30×10^{-4} a	
J_{1c}	Pa^{-1}	1.14×10^{-4}	0	0.54×10^{-4} a	ns	0.54×10^{-4} c	0.54×10^{-4} b	ns
			1	2.64×10^{-4} b		0.41×10^{-4} b	0.33×10^{-4} a	
			2	3.44×10^{-4} c		0.24×10^{-4} a	0.25×10^{-4} a	
λ_{1c}	s	20	0	17 a	17 a	17 a	17 a	22.37 b
			1	21 b	18 a	18 b	29 b	18.57 a
			2	17 a	21 b	20 c	24 ab	
μ_0	Pa·s	3.69×10^{6}	0	4.33×10^{6} b	4.33×10^{6} b	4.33×10^{6} a	ns	ns
			1	0.63×10^{6} a	3.02×10^{6} ab	3.34×10^{6} a		
			2	0.40×10^{6} a	0.86×10^{6} a	5.50×10^{6} b		
J_{or}	Pa^{-1}	1.45×10^{-4}	0	1.14×10^{-4} a	ns	1.14×10^{-4} c	1.14×10^{-4} c	ns
			1	3.23×10^{-4} b		0.71×10^{-4} b	0.59×10^{-4} b	
			2	4.67×10^{-4} c		0.39×10^{-4} a	0.36×10^{-4} a	
J_{1r}	Pa^{-1}	1.16×10^{-4}	0	0.90×10^{-4} a	ns	0.90×10^{-4} c	0.90×10^{-4} b	ns
			1	2.26×10^{-4} b		0.42×10^{-4} b	0.35×10^{-4} a	
			2	3.17×10^{-4} c		0.27×10^{-4} a	0.25×10^{-4} a	
λ_{1r}	s	77	0	121 b	121 b	121 b	121 b	ns
			1	65 a	97 ab	60 a	65 a	
			2	63 a	69 a	72 a	83 a	
Viscometric profile								
PV	mPa.s	2340	0	ns	2860 c	2860 c	2860 b	ns
			1		2222 b	2426 b	2518 a	
			2		1665 a	2035 a	2294 a	
TV	mPa.s	1804	0	2429 b	2429 c	2429 c	2429 c	ns
			1	2225 ab	1821 b	1679 b	1955 b	
			2	1986 a	1377 a	1216 a	1545 a	
FV	mPa.s	2683	0	ns	3228 c	3228 c	3228 b	ns
			1		2655 b	2557 b	2822 ab	
			2		2164 a	2090 a	2349 a	
PT	ºC	81.54	0	ns	79.31 a	79.31 a	79.31 a	82.87 b
			1		83.94 b	77.78 a	82.03 b	80.21 a
			2		87.30 c	79.63 a	83.69 b	
pH of the medium								
pH		5.20	0	4.54 a	4.54 a	4.54 a	4.54 a	5.73 b
			1	5.08 b	5.22 b	5.23 b	5.16 b	4.73 a
			2	5.27 c	5.47 c	5.53 c	5.43 ab	

Levels: 0, absence; 1, 5% protein addition (starch + protein basis) or acetic/lactic acid addition (0.1/0.4, w/w, starch + protein basis); 2, 10% protein addition (starch + protein basis). ns: non significant effects p>0.05. Within each parameter, different letters in the corresponding column mean statistically differences between means at p<0.05.

Abbreviations used for the measured parameters are presented in the materials and methods section.

Table 3. Selected second order interactive effects (protein x acid) on the dynamic oscillatory, creep-recovery, visco-metric parameters and pH of protein-enriched rice starch-based gluten-free doughs

Parameter	Unit	Overall Mean	Level protein	Level acid	Albumin x acid		Caseinate x acid		Pea protein x acid		Soya protein x acid	
Dynamic Oscillatory Rheometry												
G'_1	Pa	21858	0	0	ns		15763	a	ns		15763	a
			0	1			11620	a			11620	a
			1	0			15360	a			27920	b
			1	1			23300	b			20748	ab
			2	0			30480	c			70665	c
			2	1			26900	bc			27610	b
G''_1	Pa	4152	0	0	ns		2852	a	ns		2852	ab
			0	1			2511	a			2511	a
			1	0			6568	c			4903	c
			1	1			4317	b			3705	b
			2	0			10465	e			9184	d
			2	1			7826	d			5487	c
tan δ		0.2070	0	0	ns		0.18	a	ns		0.18	b
			0	1			0.22	b			0.22	c
			1	0			0.43	e			0.18	b
			1	1			0.19	a			0.18	b
			2	0			0.34	d			0.13	a
			2	1			0.29	c			0.20	bc
Creep recovery test												
J_{oc}	Pa^{-1}	1.19 x 10^{-4}	0	0	0.88 x 10^{-4}	a	0.88 x 10^{-4}	c	0.88 x 10^{-4}	e	0.88 x 10^{-4}	e
			0	1	1.10 x 10^{-4}	b	1.10 x 10^{-4}	e	1.10 x 10^{-4}	f	1.10 x 10^{-4}	f
			1	0	2.45 x 10^{-4}	c	1.05 x 10^{-4}	d	0.57 x 10^{-4}	c	0.47 x 10^{-4}	c
			1	1	2.96 x 10^{-4}	d	0.57 x 10^{-4}	b	0.71 x 10^{-4}	d	0.62 x 10^{-4}	d
			2	0	4.52 x 10^{-4}	f	0.54 x 10^{-4}	ab	0.31 x 10^{-4}	a	0.19 x 10^{-4}	a
			2	1	3.16 x 10^{-4}	e	0.51 x 10^{-4}	a	0.36 x 10^{-4}	b	0.42 x 10^{-4}	b
J_{1c}	Pa^{-1}	1.14 x 10^{-4}	0	0	0.44 x 10^{-4}	a	0.44 x 10^{-4}	b	0.44 x 10^{-4}	d	0.44 x 10^{-4}	d
			0	1	0.65 x 10^{-4}	b	0.65 x 10^{-4}	c	0.65 x 10^{-4}	e	0.65 x 10^{-4}	e
			1	0	2.69 x 10^{-4}	d	2.38 x 10^{-4}	f	0.40 x 10^{-4}	c	0.29 x 10^{-4}	b
			1	1	2.60 x 10^{-4}	cd	0.37 x 10^{-4}	a	0.43 x 10^{-4}	d	0.38 x 10^{-4}	c
			2	0	4.33 x 10^{-4}	e	1.27 x 10^{-4}	e	0.23 x 10^{-4}	a	0.12 x 10^{-4}	a
			2	1	2.55 x 10^{-4}	c	0.85 x 10^{-4}	d	0.26 x 10^{-4}	b	0.39 x 10^{-4}	c
λ_{1c}	**s**	20	0	0	16	a	ns		ns		16	a
			0	1	17	ab					17	ab
			1	0	22	c					40	e
			1	1	19	b					18	b
			2	0	19	b					25	d
			2	1	16	a					22	c
μ_0	**Pa·s**	3.69 x 10^{6}	0	0	5.79 x 10^{6}	f	5.79 x 10^{6}	f	5.79 x 10^{6}	f	5.79 x 10^{6}	d
			0	1	2.87 x 10^{6}	e	2.87 x 10^{6}	d	2.87 x 10^{6}	a	2.87 x 10^{6}	a
			1	0	0.67 x 10^{6}	d	0.56 x 10^{6}	a	3.08 x 10^{6}	b	9.09 x 10^{6}	e
			1	1	0.60 x 10^{6}	c	5.47 x 10^{6}	e	3.61 x 10^{6}	c	3.68 x 10^{6}	b
			2	0	0.33 x 10^{6}	a	0.62 x 10^{6}	b	5.52 x 10^{6}	e	13.6 x 10^{6}	f
			2	1	0.47 x 10^{6}	b	1.10 x 10^{6}	c	5.48 x 10^{6}	d	3.85 x 10^{6}	c
J_{or}	Pa^{-1}	1.45 x 10^{-4}	0	0	0.97 x 10^{-4}	a	0.97 x 10^{-4}	d	0.97 x 10^{-4}	e	0.97 x 10^{-4}	e
			0	1	1.30 x 10^{-4}	b	1.30 x 10^{-4}	e	1.30 x 10^{-4}	f	1.30 x 10^{-4}	f
			1	0	2.96 x 10^{-4}	c	1.74 x 10^{-4}	f	0.67 x 10^{-4}	c	0.49 x 10^{-4}	b
			1	1	3.50 x 10^{-4}	d	0.67 x 10^{-4}	a	0.75 x 10^{-4}	d	0.69 x 10^{-4}	d
			2	0	5.39 x 10^{-4}	f	0.84 x 10^{-4}	c	0.37 x 10^{-4}	a	0.20 x 10^{-4}	a
			2	1	3.94 x 10^{-4}	e	0.74 x 10^{-4}	b	0.41 x 10^{-4}	b	0.52 x 10^{-4}	c
J_{1r}	Pa^{-1}	1.16 x 10^{-4}	0	0	1.02 x 10^{-4}	b	1.02 x 10^{-4}	d	1.02 x 10^{-4}	d	1.02 x 10^{-4}	e
			0	1	0.78 x 10^{-4}	a	0.78 x 10^{-4}	b	0.78 x 10^{-4}	c	0.78 x 10^{-4}	d
			1	0	1.83 x 10^{-4}	c	2.66 x 10^{-4}	f	0.41 x 10^{-4}	b	0.31 x 10^{-4}	b
			1	1	2.68 x 10^{-4}	d	0.66 x 10^{-4}	a	0.43 x 10^{-4}	b	0.39 x 10^{-4}	c
			2	0	3.51 x 10^{-4}	f	1.37 x 10^{-4}	d	0.28 x 10^{-4}	a	0.13 x 10^{-4}	a
			2	1	2.83 x 10^{-4}	e	0.92 x 10^{-4}	c	0.25 x 10^{-4}	a	0.37 x 10^{-4}	c
λ_{1r}	**s**	77	0	0	153	f	153	f	153	f	153	e
			0	1	888	e	88	d	88	e	88	c
			1	0	50	a	69	b	66	c	63	a
			1	1	80	d	124	e	53	a	67	b
			2	0	52	b	62	a	83	d	100	d
			2	1	75	c	75	c	60	b	67	b

Table 3. (Continuation)

Parameter	Unit	Overall Mean	Level protein	Level acid	Albumin x acid		Caseinate x acid		Pea protein x acid		Soya protein x acid	
Viscometric profile												
PV	mPa.s	2340	0	0	3091	e	3091	f	3091	d	ns	
			0	1	2628	c	2629	e	2629	c		
			1	0	2384	b	1990	c	2239	b		
			1	1	2852	d	2454	d	2612	c		
			2	0	2086	a	1536	a	1869	a		
			2	1	2770	cd	1794	b	2200	b		
TV	mPa.s	1804	0	0	2771	d	2771	e	2771	f	2771	d
			0	1	2087	b	2087	d	2087	e	2087	c
			1	0	2158	bc	1615	c	1455	c	2008	bc
			1	1	2291	c	2028	d	1903	d	1902	b
			2	0	1876	a	1275	a	1151	a	1581	a
			2	1	2095	b	1479	b	1281	b	1509	a
FV	mPa.s	2682	0	0	3783	d	3783	e	3783	e	3783	d
			0	1	2672	a	2672	d	2672	d	2672	b
			1	0	3401	c	2558	c	2521	c	2986	c
			1	1	3128	b	2752	d	2593	cd	2658	b
			2	0	3077	b	2068	a	2227	b	2518	b
			2	1	2955	b	2259	b	1952	a	2181	a
PT	ºC	81.54	0	0	80.23	b	80.23	b	80.23	c	80.23	ab
			0	1	78.38	a	78.38	a	78.38	b	78.38	a
			1	0	81.05	b	86.03	d	81.30	c	83.52	c
			1	1	78.10	a	81.85	c	74.27	a	80.53	b
			2	0	83.57	c	88.10	e	79.00	b	83.05	c
			2	1	77.63	a	86.50	d	80.27	c	84.33	c
pH of the medium												
pH		5.20	0	0	5.21	d	5.21	d	5.21	c	5.21	d
			0	1	3.88	a	3.88	a	3.88	a	3.88	a
			1	0	5.56	e	5.71	e	5.73	d	5.68	e
			1	1	4.46	b	4.73	b	4.72	b	4.64	b
			2	0	5.73	f	5.84	ef	5.85	de	5.82	ef
			2	1	4.80	c	5.10	cd	5.20	c	5.03	c

Levels: 0, absence; 1, 5% protein addition (starch + protein basis) or acetic/lactic acid addition (0.1/0.4, w/w, starch + protein basis); 2, 10% protein addition (starch + protein basis). ns: non significant effects p>0.05. Within each parameter, different letters in the corresponding column mean statistically differences between means at p<0.05.
Abbreviations used for the measured parameters are presented in the materials and methods section.

Table 4: Correlations between dough functional properties

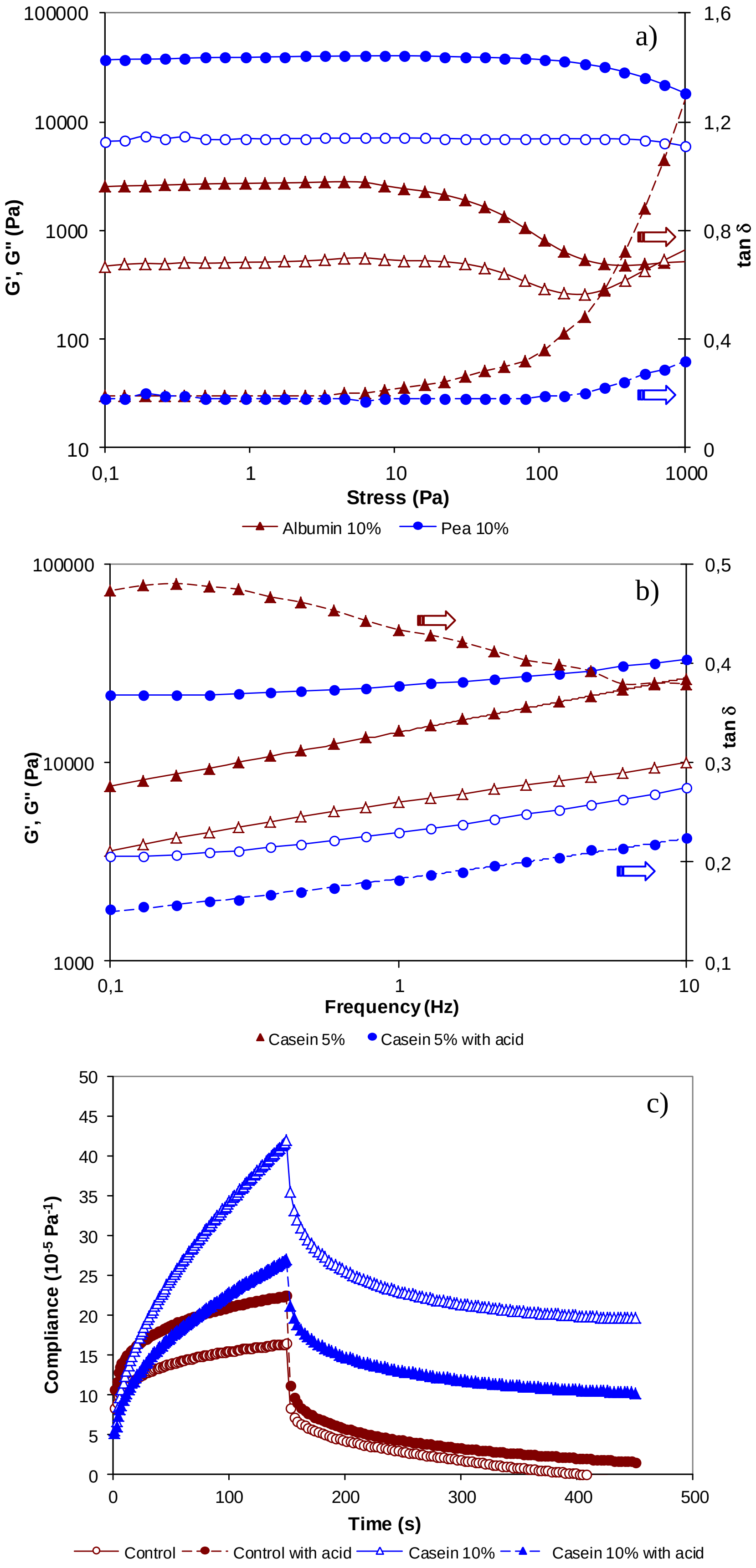
	a	G''$_1$	b	tan δ	c	τ_{max}	J_{0c}	J_{1c}	μ_o	PV	TV	BD	FV	SB	TP
G'$_1$	-	0.81***	-	-	-	0.75***	-0.70**	-0.66**	0.80***	-	-0.56*	0.52*	-0.55*	-	-
a		-	-	0.98***	-0,84***	-	-	-	-	-	-	-	-	-	-
G''$_1$			-	-	-	0.90***	-0.73***	-0.55*	-	-0.65**	-0.71***	-	-0.71***	-	0.59*
b				-	0,68**	-	0.66**	0.66**	-	-	-	-	-	0.65**	-
tan δ					-0,81***	-	-	-	-	-	-	-	-	-	-
c						-	-	-	-	-	-	-	-	0.47*	-
τ_{max}							-0.67**	-0.51*	-	-0.67**	-0.74***	-	-0.68**	-	0.57*
J_{0c}								0.95***	-0.56*	-	-	-	0.54*	-	-
J_{1c}									-0.66**	-	-	-0.52*	-	-	-
μ_o										-	-	0.56*	-	-	-
PV											0.85***	-	0.73***	-	-0.66**
TV												-	0.92***	-	-
BD													-	-	-0.46*
FV														0.50*	-
SB															-

Protein: is referred to the dose of protein (0. 5. 10 %) independently of the type of protein; Acid: varied between 0 (without acid addition) and 1 (with addition); *p<0.05; ** p<0.01; *** p<0.001; ns: not significant
Abbreviations used for the correlated parameters are presented in the materials and methods section.

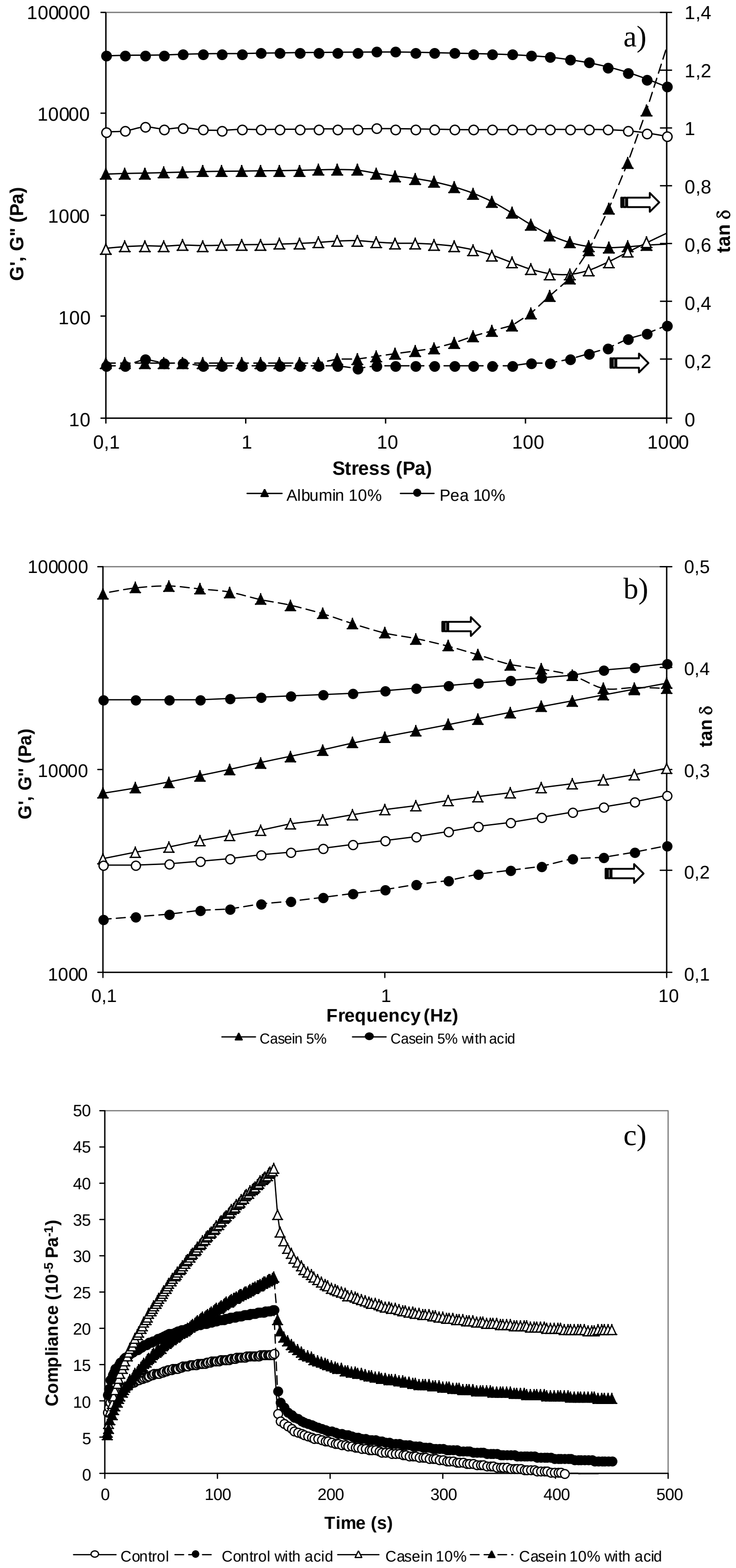
FIGURE CAPTIONS

Figure 1: (a) Stress sweeps of doughs with 10 g/100 g albumin (triangle) and pea protein (circle), (b) Frequency sweeps of doughs with 5 g/100 g casein without acid (triangle) and with acid (circle). Elastic modulus, G', is represented by solid points and the viscous modulus, G'', by void points. The loss tangent is represented by discontinuous lines in the secondary (right) scale.
(c) Creep-recovery tests of control doughs (circle) and doughs with 10 g/100 g casein (triangle), both with (solid points) or without (void points) acid.

Figure


a)
100000
10000
1000
100
10
1,6
1,2
0,8
0,4
0
G', G'' (Pa)
tan δ
0,1
1
10
100
1000
Stress (Pa)
Albumin 10%
Pea 10%
b)
100000
10000
1000
0,5
0,4
0,3
0,2
0,1
G', G'' (Pa)
tan δ
0,1
1
10
Frequency (Hz)
Casein 5%
Casein 5% with acid
c)
50
45
40
35
30
25
20
15
10
5
0
Compliance (10-5 Pa-1)
0
100
200
300
400
500
Time (s)
Control
Control with acid
Casein 10%
Casein 10% with acid

Figure



a)
G', G'' (Pa)
tan δ
Stress (Pa)
Albumin 10%
Pea 10%
100000
10000
1000
100
10
0,1
1
10
100
1000
1,4
1,2
1
0,8
0,6
0,4
0,2
0
b)
G', G'' (Pa)
tan δ
Frequency (Hz)
Casein 5%
Casein 5% with acid
100000
10000
1000
0,1
1
10
0,5
0,4
0,3
0,2
0,1
c)
Compliance (10^-5 Pa^-1)
Time (s)
Control
Control with acid
Casein 10%
Casein 10% with acid
50
45
40
35
30
25
20
15
10
5
0
0
100
200
300
400
500