Establishing the function of proteins on the rheological and quality properties of rice based gluten free muffins

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Abstract

The incorporation of proteins has been long established in the bakery industry to obtain enriched products, but they also take active part on the making process of sweet baked goods. This study was focused on assessing the role of proteins on the rheology and quality of wheat free muffins by using rice flour. Six rice based formulations were used: one without added protein (No-Protein) and five with different protein sources: soy protein isolate (SPI), pea protein isolate (PPI), egg white protein (EWP), casein(C), and for comparing purposes vital wheat gluten (VWG) was included. Proteins effects were established by evaluating the rheological behaviour of batters measuring the storage modulus (G') and the loss modulus (G''), and the technological characteristics of the muffins obtained (specific volume, colour, and texture). The addition of SPI, PPI and C significantly ($P<0.05$) increased G', but this was not modified in batters containing EWP. Casein and EWP increased the specific volume of the muffins. SPI did not have effect on hardness, springiness, cohesiveness, chewiness, and resilience of the muffin, while PPI containing muffins were softer and springier. The overall results indicated that both the rheological properties of the batters and the technological characteristics of the muffin are dominated by the presence of the type of protein used in the formulations. Therefore the source of protein included in the formulation is fundamental to ensure the proper texture and other technological properties of these products.

Highlights

- Rice based batter and muffins are evaluated by rheological and quality parameters.
- Protein source discriminates rice flour-based muffins

- Rheological characteristics of the batters are dominated by the type of protein.

Keywords: Muffins; Gluten-free; Rice flour; Protein sources; Batter, Rheology; Quality.

1. Introduction

Muffin is a popular breakfast or afternoon snack food, which is sold in many bakeries. Muffins are sweet, high-calorie baked products highly appreciated by consumers due to their good taste and soft texture. Muffins batter is a complex fat-in-water emulsion composed of an egg-sugar-water-fat mixture as the continuous phase and bubbles as the discontinuous phase in which flour particles are dispersed. Muffins are characterized by a typical porous structure and high volume, which confer a spongy texture. To obtain such a final structure, a stable batter lodging many tiny air bubbles is required (Martínez-Cervera, Sanz, Salvador, & Fiszman, 2012). Therefore, a large number of small cells provide high volume if the continuous phase of the batter is capable of retaining them during the baking process (Gómez, Ronda, Caballero, Blanco, & Rosell, 2007).

Traditionally, a muffin recipe is mainly composed of wheat flour, sugar, vegetal oil, egg and milk (Sanz, Salvador, Baixauli, & Fiszman, 2009). For this reason, persons with celiac disease (CD) are unable to consume this type of baked product since they are made with wheat flour. Gluten-free products were initially designed for people who have celiac disease. Today, there is an increasing number of people interested in wheat-free foods motivated by health concerns but also by the desire to avoid wheat in the diet (Nachay, 2010). However, the manufacture of baked goods products without gluten results in major technological problems for bakers. In fact, many gluten-free products available on the market are often of poor technological quality, exhibiting low volume, poor colour and crumbling crumb, besides great variation in the nutrient composition, with low protein and high fat contents (Matos & Rosell, 2011), particularly when compared to their wheat counterparts (Mariotti, Lucisano, Pagani, & Ng, 2009). Like bread, gluten-free muffins, cakes and other gluten-free baked goods have been commercially manufactured trying to resemble those made from wheat flour. However, these types of gluten-free baked products often present quality defects and low nutritional value.

Consumers adhered to gluten free products are increasingly demanding gluten free foods equivalent to the traditional gluten ones. As consequence, in recent years, there has been extensive research for the development of gluten-free sweet bakery products aimed to improve the structure, mouth feel, acceptability, shelf-life and nutritional quality of the finished products (Turabi, Sumnu, & Sahin, 2008a,b; Gularte, de la Hera, Gómez, & Rosell, 2012a,b; Park, Ha, & Shin, 2012). Gluten-free muffins, cake or cupcakes recipes contain rice flour as principal ingredient (Turabi et al., 2008a,b; Gularte et al., 2012a,b; de la Hera, Martinez, Oliete, & Gómez, 2012; Park et al., 2012), or different starches sources, such as rice, corn, potato and wheat (Ronda, Oliete, Gómez, Caballero, & Pando, 2011). Additionally, other ingredients such as sugar, egg white powder or egg white liquid, milk, baking powder, salt, vegetal oil, hydrocolloids and emulsifiers, can be incorporated on their formulations to improve the final quality product (Turabi et al., 2008a,b; Ronda et al., 2011; de la Hera et al., 2012; Park et al., 2012). The incorporation of dairy proteins has been long established in the bakery industry, but legumes such as soybean, can be also a good supplement for cereal based foods since they increased the protein content and complement the nutritional value of cereal proteins (Mariotti et al., 2009; Ronda et al., 2011; Gularte et al., 2012b). However, nutrition is not the only aim when adding proteins; they play a functional role, especially in muffins. In fact, Geera, Reiling, Hutchison, Rybak, Santha and Ratnayake (2011), when looking for egg replacers in wheat muffins, stated that egg is a critical ingredient in the muffins formulation to obtain expected product quality characteristics. Partial replacement of egg with commercial egg replacer changed product characteristics altering moisture retention, bulk volume, colour, texture and flavour, although those differences were not readily detected by sensory panellist. A review of the literature indicate that there is only a few published studies focused on the fundamental role of proteins in the technological properties of muffins (Ronda et al., 2011; Geera et al., 2011; Gularte et al., 2012b). .

The present study was focused on determining the role of proteins in the rheology and quality of muffins by by using rice flour, with the view to gain a better understanding on how to improve technological quality of gluten-free muffins.

2. Materials and Methods

2.1. Materials

Commercial rice flour was supplied by Harinera Derivats del Blat de Moro, S.L. (Parets del Vallés, Spain) had moisture and protein of 12.19 g/100g and 7.22 g/100g, respectively. Five commercial protein sources (all in dry powder form) were employed. Soybean protein isolate (Vicoprot) was from Trade, S.A (Barcelona, Spain). The soybean protein isolate had moisture and protein of 9.25 and 80.49 g/100g, respectively. Pea protein isolate (Pisane C9) from Cosucra Group Warcoing (Warcoing, Belgium) had moisture and protein of 4.45 g/100g and 77.85g/100g, respectively. Vital Wheat Gluten from Roquette (Keokuk, IL) had moisture and protein of 9.23g/100g and 72.4 4 g/100g, respectively. Casein from Cargill (Spain) had moisture and protein of 5.43g/100g and 84.54 g/100g, respectively. Egg white protein (EWP) from EPSA Aditivos Alimentarios (Valencia, Spain) had moisture and protein of 6.83 g/100g and 79.38 g/100g, respectively. Composition of the different ingredients was determined following the AACCI Approved Methods (2000). Xanthan gum (Satiaxane CX-91) food grade was supplied by Cargill (Spain). Sodium bicarbonate and citric acid were purchased from Martínez SA (Valencia, Spain). Refined sunflower oil was acquired from Coosur (Jaen, Spain). Sugar and salt were purchased from the local market. All reagents were of analytical grade.

Batters containing both rice flour and different vegetal protein sources (VPS): vital wheat gluten (VWG), soy protein isolate (SPI) and pea protein isolate (PPI); and batters containing rice flour and different animal protein sources (APS): egg white protein (EWP), and casein(C) were prepared.

2.2 Methods

2.2.1 Batter preparation

Rice flour-based batters were prepared without adding any external protein source (No-Protein) or with one of the following five different protein sources: vital wheat gluten (VWG), soy protein isolate (SPI), pea protein isolate (PPI), egg white protein (EWP), and casein (C). The formulation of batters included 100g rice flour; 100g water; 17.3 g protein added (75% protein); 75g sugar; 46g refined sunflower oil; 4g sodium bicarbonate; 3g citric acid; 1.5g salt; 0.5g xanthan gum. The amount of added protein (13%) was calculated based on the percentage of protein provided by both milk and egg in a muffins formulation (Sanz et al. 2009). It was considered a contribution of 75% of protein for the selected protein sources. In this way, the amount of protein that should be added to each formulation was obtained [(13 x 100)/75 = 17.3 g]. In addition, this amount of added protein kept the same solid content in all formulations. The samples were identified as No-Protein (without exogenous protein added), VWG, SPI, PPI, EWP, and C, according to the type of protein added.

The rice flour-based batters were prepared by the modified method of Sanz et al. (2009). The batters were prepared in a mixer (Kenwood Major Classic Model KM800, UK), in which the rice flour, protein (depending on the formulation), sodium bicarbonate, sugar, citric acid, salt and xanthan gum, were incorporated in the first place, and sunflower oil was gradually dripped in; finally the water was added. The batter was beaten for 10 min at speed 4 (380 rpm) until smooth. The batter was used for both the rheological test and to prepare the gluten-free muffin. Each formulation was prepared twice (two replicates), on different days.

2.2.2. Batter properties

The specific gravity (SG) of batter was measured as the ratio of the weight of a standard container filled with batter (W2) to that of the same container filled with water (W1). Two different batches were employed and each formulation was measured in triplicate.

The rheological behaviour of the batter was evaluated. Properties of the rice flour-based batter were studied using an AR G2 controlled-stress rheometer (TA Instruments, Crawley, UK). The batters were all kept at 25°C for 60 min after batter preparation before the rheological test. Temperature was controlled by a Peltier system. The samples were allowed to rest in the measurement cell for 5 min as stabilization time. Parallel plate geometry (60 mm diameter) with 1 mm gap between the plates was employed.

An oscillatory stress sweep was made at a constant frequency of 1 Hz over an oscillatory stress range of 1.0×10^{-3} to 20 Pa for each batter sample. Frequency sweep test was performed from 0.01 to 10 Hz at a constant oscillatory stress within the linear viscoelastic range at 25°C. The oscillatory stress applied was selected to guarantee the existence of a linear viscoelastic range of each batter sample. The applied oscillatory stress varied among formulations and was between 0.12 and 0.32 Pa. To study the effect of heating in the batter structure, temperature sweeps were performed from 25°C to 95°C at a heating rate of 1.0°C/min and a constant strain. The strain applied was selected to guarantee the existence of linear viscoelasticity along the complete temperature range according to previous stress sweeps. The applied strain varied from $1.0\text{x}10^{-4}$ to $3.8\text{x}10^{-4}$, depending on the specific batter sample. Vaseline oil (Panreac, Spain) was applied to the exposed surfaces of all the samples, in order to prevent their drying during the measurements. The storage modulus (G'), loss modulus (G''), phase angle, and loss tangent (tanδ), were measured. Three replicates of each test were run with samples prepared on different days. Results are means of three replications from different batches of each formulation.

2.2.3. Rice flour-based muffins preparation

Rice flour-based muffins were prepared according to methods described by Sanz et al. (2009). Muffins without added protein (No-Protein) and with different protein sources (VWG, SPI, PPI, EWP, and C) were prepared from the gluten-free muffin batters. The batter was poured into a dosing machine (Edhard Corp., Hackettstown, USA). Quantity of batter dispensed was of 65.0±0.2 g in each 60 mm diameter and 36 mm muffin paper cups. Twelve cups were arranged in three rows of four in a baking tray and baked for 20 min at 180 ºC in a conventional electric oven (Fagor Elegance 2H-114B, Guipúzcua, Spain) that had been preheated to this temperature for 10 min. The oven, the tray and the tray position in the oven were identical in each case.

The muffins were left to cool down at room temperature for 1h on rack. Then, they were packed in polypropylene bags (O_2 permeability at 23°C = 1650 cm^3/m^2.day; water vapour permeability at 38°C and 90% humidity = 9 g/m^2 day; thickness=65μm) (HUECOGRABADO FINA, S.A., Valencia, Spain) and stored at 20ºC for 1day, until determinations were conducted. The muffins from each formulation were prepared twice, on different days, with 12 muffins in each batch.

2.2.4. Rice flour-based muffins properties

Samples were directly milled prior to analytical determinations. The moisture and protein contents were determined according to ICC corresponding standard methods (ICC, 1994). The muffins were weighed before baking (W3) and after baking and 1-h cooling (W4). The weight loss upon baking was calculated (W3-W4). Height was measured with a digital calliper from the highest point of the muffin to the bottom of the paper cup after cooling for 1-h cooling at room temperature. Volume was determined by rapeseed displacement. Specific volume of individual muffins was calculated by dividing volume by weight. Images of the muffins were captured using a flatbed scanner equipped with the software HP PrecisoScan Pro version 3.1 (HP Scanjet 4400C, Hewlett–Packard, USA). Values were the mean of at least three replicates for each formulation.

A Konica Minolta CM-3500 spectrocolorimeter was used to measure the crumb colour parameters (L^*, a^*, b^*) of the muffins. The results were expressed in accordance with the CIELAB system (D65 illuminant and 10° viewing angle). The measurements were made with a 30 mm diameter diaphragm inset with optical glass. The parameters measured were L^* (L^*= 0 [black], L^*=100[white] indicates lightness, a^* indicates hue on a green ($-a^*$) to red ($+a^*$) axis, and b^* indicates hue on a blue ($-b^*$) to yellow ($+b^*$) axis. Additionally, hue or hue angle (h) and Chroma (C^*) values were obtained. Hue angle is the angle for a point calculated from a^* and b^* coordinates in the colour space. Chroma is the quantitative component of the colour, which reflected the purity of colour in the CIELAB space (Kane, Lyon, Swanson, & Savage, 2003). The muffins were cut in half on a plane parallel to its base and the colour of crumb was measured at several points on the cut surface. Data from three slices per sample were averaged.

The instrumental texture measurements of the muffin samples were made with a TA.XT.plus Texture Analyzer (Stable Microsystems, Godalming, UK) provided with Texture expert software. The muffins were cut horizontally at the height of the cup, the upper half was discarded and the 1.5 cm high lower halves were removed from the paper cup. A double compression test (texture profile analysis) was performed with a 75 mm diameter flat-ended cylindrical probe (P/75) and compression to 50% of the initial height at a speed of 1 mm/s with 5s waiting time between the two cycles. The parameters obtained from the curves were hardness, springiness, cohesiveness, chewiness, and resilience. Values were the mean of at least three replicates for each formulation, which were prepared twice (two batch), on different days.

2.2.5. Statistical analysis

For each parameter evaluated, a one way analysis of variance (ANOVA) was applied using Statgraphics Plus V 7.1 (Statistical Graphics Corporation, UK). Bonferroni's multiple comparison procedure was used to assess significant differences ($P<0.05$) among samples that might allow discrimination among them.

3. Results and Discussions

To determine the role of proteins in gluten free batters and muffins making, several proteins from different sources were selected and wheat gluten was used for comparison purposes. Overall the experimental results showed some common peculiarities within vegetal proteins and the same within animal source proteins, because of that the discussion of the results has been carried out grouping the proteins regarding their vegetal or animal origin.

3.1. Effect of protein source on specific gravity, and dynamic viscoelastic properties of rice flour-based batters.

According to the ANOVA results, it was observed that SG was significantly affected ($P<0.05$) by the protein type (Table 1). The highest SG value was obtained in the batter prepared with casein protein (C). On the contrary, batter in presence of egg white protein (EWP) had the lowest SG, which showed that more air was incorporated and retained during mixing (Turabi et al., 2008; Ronda et al., 2011; Martínez-Cervera et al., 2011). VWG, PPI and SPI, proteins from vegetable origin, showed similar effects on SG. Conversely, EWP and C, from animal origin, did not have the same effect on SG. Differences observed could be attributed to the functional properties of the proteins, like emulsifying activity or foam stability. Egg albumen or whey proteins increased the emulsifying activity of rice flour, while pea and soybean proteins hardly modified this parameter, whereas the stability of the emulsion significantly decreased when egg albumen and whey proteins were present (Marco & Rosell, 2008).

The viscoelastic properties of the rice-based muffins batter containing different protein sources were studied by dynamic oscillatory test. The mechanical spectra of all the batters (Figure 1 and 2) revealed the typical behaviour of soft gels with values of the storage modulus (G') higher than the values of loss modulus (G'') and slight dependence of both moduli with frequency (Figure 1). Marco and Rosell (2008) reported that the mechanical spectra of rice flour dough samples (without and with protein isolate) showed G' values higher than G'' at the frequency range tested (0.1-10 Hz), suggesting a viscoelastic solid behaviour of the dough.

The addition of the proteins affected the batter viscoelastic behaviour and the extent of the effect was protein source dependent. The presence of all vegetable proteins modified the elastic and viscous component of the rice-based muffins batter, inducing a hardening effect (increase in G' and G'') on the batters. Batters containing PPI and SPI showed the highest increase in G' and G'' values, whereas VWG batter only showed values of G' and G'' slightly higher to those obtained with the No-Protein batter. Therefore, leguminous proteins induced a major hardening effect on the batter structure.

The animal proteins also modified the dynamic mechanical spectra of the rice based muffin batter, with a clear different trend between egg white powder and casein (Figure 2). The addition of casein induced a very noticeable change in the batter viscoelastic behaviour. In C batter both moduli showed higher frequency dependence than in the No-Protein and EWP batters. Also the predominance of G' over G'' was lower in the C batter indicating a more viscous and less elastic behaviour of this batter in comparison to No-Protein and EWP. However, values of both moduli in the C batter were higher than the No-Protein.

Viscoelastic data at a frequency of 1 Hz were submitted to analysis of variance to determine the main effects of the protein isolates on viscoelastic properties of rice based muffin batters (Table 1). The presence of the different protein types significantly ($P<0.05$) changed the viscoelastic properties of the batter. As already mentioned, values of G' were always higher than values of G''. The presence of SPI, PPI and C, significantly ($P<0.05$) increased the G' modulus, and the other proteins tested did not modify it. The extent of the effect of the added protein was greatly dependent on the nature of the added protein. Batters containing vegetable proteins had higher G' value, although in the case of cereal protein it was not significant, indicating similarities between the gluten protein and the rice proteins. The presence of leguminous proteins induced a large increase of the G' modulus, being higher with PPI. Those results agree with those of Ronda et al. (2011) and Marco and Rosell (2008).

Regarding animal proteins, C induced a significant increase of G', whereas this was not significantly modified by EWP. The same trend was observed for the G''. Complex modulus (G^*) significantly increased due to the addition of proteins, and it showed the same trend observed in G', indicating low contribution of the viscous component (G'') to the viscoelastic properties of the batter systems.

The loss tangent (tanδ) was also significantly ($P<0.05$) modified by the presence of the protein isolates. Considering that all batter showed $G' > G''$, the loss tangent was lower than 1. Both animal proteins significantly decreased the batter viscoelasticity (values of tanδ closer to 1), being the effect much more evident for casein. Contrarily, the vegetable proteins, SPI and PPI induced a significant reduction in the loss tangent with no significant differences between them.

Therefore, EWP and specially C led to structures with less solid like character than the rice batter alone, whereas leguminous protein isolates led to more structured and solid like (lower tanδ) batters. In cake batters made of wheat flour, also values of tanδ lower than 1 has been reported (Baixauli, Sanz, Salvador, & Fiszman, 2007). The presence of protein in layer cake batter decreased significantly the loss tangent, with a major diminution when using the SPI than the wheat protein (Ronda et al., 2011). In all batters evaluated, phase angle was lower than 45°, which indicates that the material behaves more like a solid (Rosell & Foegeding, 2007). SPI and PPI batters showed the lowest values of the phase angle, without significant differences between them. Nevertheless, the presence of the other protein significantly ($P<0.05$) increased the phase angle, with a major increase in the batter containing C (31.67), reflecting, as already mentioned that in the presence of casein the rice based batter increases its viscous component.

3.2. Effect of protein source on the viscoelastic properties of batters during heating

In order to understand the effect of protein type in the changes occurred during the thermal treatment of the rice-based batters, the viscoelastic properties were studied during the application of a temperature sweep. The storage modulus (G') values during heating from 25 °C to 95°C are shown in Figures 3 and 4.

The presence of vegetable proteins produced changes in the slope of the heating curves that have been associated with starch gelatinization and protein coagulation processes in different muffin batter formulas (Martínez-Cervera et al., 2011; 2012). As expected, No-Protein batter exhibited an early onset of starch gelatinization (61-78 °C), estimated as the first increase in the elastic component when the temperature rises. A similar behaviour was displayed by the batter containing gluten protein, but in this case the onset of gelatinization was reached in the range 70 and 83°C. It is well known that the gelatinization of rice starch occurs at around 70-71°C; while the protein denaturation occurs at temperature above 60 °C, depending of each protein type. Rosell and Foegeding (2007) reported that when heating gluten a decrease of G' is produced, reaching a minimum at 57°C, and further increase of the temperature induced the formation of a more elastic gluten network, as indicated the increase of G'. These authors explained that gluten proteins show a progressive loss of strength due to protein unfolding, resulting in a decrease of the elastic modulus and undergoes a thermal transition around 60°C.

In this study, the conformational changes experimented by both the rice starch and the added proteins were largely responsible for the predominant elastic behaviour of the batters. The addition of wheat proteins did not drastically affected the rheological properties of the batter at temperatures lower than 70°C; however at higher temperatures that batter showed less elastic behaviour, reflecting the development of hindered rice starch three-dimensional internal structure. Additionally, the underlining phenomena that determine the observed reduction in rigidity would be the dissociation and denaturation of the proteins (Sorgentini, Wagner, Arrese, & Añón, 1991). The starch dilution effect also would explain the storage modulus decrease of the batter containing gluten protein.

SPI batter showed a progressive increase of G' as the temperature rises, indicating the formation of a more rigid network (Figure 3). In general, G' increased with SPI, which can be associated with the development of an internal SPI structure. The heating of SPI dissociated the compact glycinin (11S) and β-conglycinin (7S) oligomers into monomers and therefore, the hydrophobic group are exposed (Tseng, Xiong, & Boatright, 2008), leading to an aggregation process and later the formation of a gel. Particularly, in this curve was not detected any point of inflection, probably the commercial SPI used could be greatly denatured, which allows greater capacity for interaction within active groups that may be present in the system.

In regard to PPI batter, the thermal profile revealed different stages (Figure 3), in which G' upward or downward were detected along the temperature increase. The different stages observed could be indicating the effect of the distinct protein fraction present in the pea protein isolate, since they have different structures, molecular properties and different functional properties. Pea proteins, similarly to soybean proteins, are mainly storage proteins comprised of albumins and two globulins (11S and 7S). The globulins (>80% of total proteins) consist of legumin, vicilin and convicilin (Choi & Han, 2001; Andrade, Azevedo, Musampa, & Maia, 2010). Batter containing PPI showed a marked inflection peak around 88°C, which could be associated with the pea protein coagulation, which ranged from 88.9 to 94.5°C (Choi & Han, 2001). The results indicate that the behaviour of batter containing mainly SPI and PPI is notably dominated by the presence of the protein network. Though both SPI and PPI are leguminous proteins, these proteins yielded different response on heating, likely due to the distinct thermal stability of the protein fractions (Sorgentini et al., 1991; Sirtori, Isak,, Resta, Boschin, & Arnoldi, 2012).

The animal proteins also influenced the development of storage modulus of the rice-based muffin batters (Figure 4). At 25°C only the batter containing casein, showed *G′* values higher than those obtained in the No-Protein batter. EWP containing batter showed similar trend than No-Protein batter at temperature lower than 65°C and a rapid increase was observed from 84°C until the end of the experiment, indicating the formation of a more rigid network. This increase might result from the progressive formation of higher molecular weight products (Kokini et al., 1994). The thermal profile revealed that, again the process of protein denaturation governs the evolution of the storage modulus. Egg white contains as many as 40 different proteins, among them; the major proteins imparting functionality are ovalbumin (54%), conalbumin (12%), ovomucoid (11%) and lysozyme (3.5%). It has been reported that, the denaturation temperature of ovalbumin is close to 84°C, while conalbumin (ovotransferrin) denaturation occurs about 60°C and the denaturation temperature of lysozyme is around 70-75°C (Arzeni, Pérez, & Pilosof, 2012). Therefore, the changes observed in *G′* behaviour clearly can be associated with the coagulation phenomena of the different egg white proteins. Regarding to batter containing casein, it showed a completely different behaviour than the EWP batter. As heating progresses, the storage modulus value rose until approximately 70°C, where a maximum was detected, then decreased rapidly indicating that the structure was highly prone to weakening, and no increase associated to starch gelatinization was detected. Casein containing batter had very hard consistency, indicating the great water absorption of this protein. In consequence, limited amount of water was available for starch gelatinization. The presence of denatured casein could be inducing a drastic effect on the structure of the batter, yielding a weak gel. However, G' has a plateau value from 85°C until the end of the experiment, indicating that the gel structure behaves stable in this temperature range.

To further evaluate the effect of temperature in the viscoelastic properties the evolution of tan δ was evaluated. Figure 5 and 6 showed the values of tan δ versus temperature. The effect of temperature in viscoelasticity was dependent on the protein type. The tan δ values of the no protein batter were practically not affected by the temperature increase. In the presence of the vegetable proteins, a higher, although still small, influence of temperature in tan δ in comparison to the no protein batter was observed. In VWG, values of tan δ softly decreased with temperature, reflecting an increase in the predominance of the elastic component. In SPI the values of tan δ remained almost constant until approximately 80ºC where a decrease in tan δ (higher viscoelasticity) was observed. In the animal proteins the effect of temperature in the viscoelastic properties was more evident. Incorporation of both EWP and C induced a clear increase in the solid like properties of the batter (higher viscoelasticity) with the increase in temperature reflecting a clear change in the type of structure associated to the effect of temperature in the protein structure.

3.3. Effect of protein source on quality characteristics of rice flour-based muffins.

3.3.1. Protein and moisture contents of the gluten-free muffins

As it was expected, the addition of the different protein sources increased the protein content of the muffins. Muffins containing SPI, EWP and C showed the highest protein content (11.55 g/100g, dm); VWP and PPI containing muffins had 10.43 and 10.96 g/100g dm, respectively. Significant differences were also observed in the moisture content of the muffins (results not showed).

3.3.2. Height, weight loss, and specific volume

Rice flour-based muffins obtained from different recipes presented important differences in relation to height, weight loss, and specific volume (Table 2). Muffin height was significantly (P<0.001) affected by the protein type. The largest effect on height was found with EWP, which caused a significant increase in this parameter. The incorporation of proteins did not significantly affect the weight loss parameter, with exception of the decrease induced by casein, which supports the view that casein containing muffin was more capable of binding water during cake making. The No-Protein sample and the muffins containing vegetal protein source (VWG, SPI and PPI) did not differ significantly (*P*<0.01) in specific volume. Conversely, muffins with the highest specific volume were those prepared with animal protein sources, and the greatest effect was observed with EWP, likely due to that more air was incorporated and retained during mixing and baking. Geera et al. (2011) reported that muffins made with dry whole egg formulation had the highest height and volume and the lowest density. Park et al. (2012) found that the specific volume of the rice cupcakes ranged from 2.97 to 3.25 mL/g; while Turabi et al. (2008a) found specific volume ranged from 1.08 to 1.66 mL/g in rice cake formulated with different gums and an emulsifier blend. In another study, Gularte et al. (2012b) found that the incorporation of legume flour (chickpea, pea, lentil and bean) did not significantly affect the weight loss of the cake; but with the exception of chickpea cake, all legumes flour increased the specific volume. Ronda et al. (2011) evaluated layer rice cake made with SPI and wheat protein reporting that SPI did not modify volume but wheat proteins improved volume.

3.3.3. Colour parameters

Results from the crumb colour parameters are presented in Table 3. The L^*, a^* and b^* values for crumb colour showed significant (*P*<0.05) differences among the different protein enriched muffins. Lightness of muffin crumb was significantly (*P*<0.05) decreased by VWG, SPI, PPI and C proteins; while the EWP addition increased L^* value. The lowest L^* was obtained for PPI containing muffin, which was due to the darker colour of the protein isolate (data no showed). Consequently, the L^* values can be associated to the original colour of both rice flour and protein isolates. Colour in baked goods could come from different sources: intrinsic colour imparted by individual ingredients (Gularte et al., 2012b), developed colour resulting from the interaction of ingredients (Acosta, Cavender, & Kerr, 2011), like Maillard or caramelization reactions, besides processing changes associated to chemical or enzymatic reactions. Regarding a^* values, all samples showed positive a^* values, indicating hue on red axis, and all were higher than those of the No-Protein, with the exception of EWP sample that showed negative a^*. The b^* scale showed positive values (yellow hue) for all samples evaluated. However, EWP muffin did not exhibited significant (*P*<0.05) differences when compared to No-Protein sample. PPI, followed by SPI showed higher b^* value than the other samples, it could be derived from the original yellowish pigment of the pea and soy protein powder added as ingredient in each formulation. Results agree with previous studies (Gómez, Moraleja, Oliete, Ruiz, & Caballero, 2010 ; Gularte et al., 2012b). In relation to hue angle, h and chroma, C* colour attributes, great variation was observed (Table 4). All the muffins presented positive hue angle values (81.64 - 92.29°) reflecting their yellow-orange hue. Additionally, the PPI and SPI muffins increased chroma compared with all other samples, which revealed their higher purity of colour related to major intensity of the yellow component.

3.3.4. Global appearance of the muffins

Muffin images clearly revealed differences among crumb muffins samples, mainly related to shape, crumb porosity, crumb colour and degree of collapse on surface of muffins by effect of type of protein added (Figure 7). Great variation in the appearance of the crumb structure between the samples was observed. No-Protein and VWG containing muffin showed denser matrix, indicating more compact crumb than other muffins samples. Contrarily, muffins containing EWP and C protein showed higher number of air bubbles than No-Protein, showing more spongy and light structure. Addition of casein produced muffins with stable network structure with homogeneous air cell but showed higher degree of collapse on surface, in addition these muffins showed a soft and humid appearance. SPI and PPI muffins did not show collapse during baking, but presented compact crumb.

3.3.5. Instrumental texture

The effect of protein on the texture parameters of rice flour-based muffins is shown in Table 4. According to ANOVA results, muffins differed significantly ($P<0.05$) in crumb hardness, springiness, cohesiveness, chewiness and resilience. The incorporation of protein sources increased significantly ($P<0.05$) springiness and cohesiveness of muffins samples, except with addition of SPI, which showed the same values as the No-Protein sample. The hardness significantly ($P<0.05$) increased only in presence of casein. It was also observed that hardness and chewiness showed similar trend for all samples, with exception of muffins containing EWP, which had the highest chewiness value.

In general, the addition of vegetal protein sources did not induce a clear tendency on crumb hardness. However, PPI containing muffins showed the lowest hardness, and the highest springiness value among the samples made from vegetable proteins. A significant (P<0.05) increase in the springiness and cohesiveness was observed in VWG and PPI containing muffins, while only the sample containing VWG showed a significant (P<0.05) increase in the chewiness, indicating more difficulty in chewing the sample. All muffins containing vegetal proteins showed low resilience value; however no significant differences were observed in this parameter when compared with No-Protein. Dense masses with lower number of gas cell led to lower resilience values, implying that it will take more time for the structure of the muffins to recover after compression (Martínez-Cervera et al., 2011). It has been reported that the incorporation of legumes flour (chickpea, pea, lentil and bean) significantly (P<0.05) increased the hardness and chewiness in rice based cakes, except with the addition of lentil (Gularte et al., 2012b).

Regarding the animal proteins, a significant (P<0.01) increase in the hardness was observed in C containing muffins. Additionally, a significant (P<0.05) increase in the springiness, cohesiveness, and resilience was observed in the presence of EWP and C muffins, indicating more elasticity. The increase in springiness, cohesiveness and resilience values could be also reflecting higher specific volume values, and more aerated structure, which was found for these samples. It is known that, springiness is associated to fresh, aerated and elastic product, and in the case of muffins high springiness values are linked to high quality (Sanz et al., 2009).

In general, muffins made from animal proteins were springier, more cohesive and chewy than those made from vegetal protein source. Results clearly revealed great variability on texture quality of the rice-based muffins made from different protein sources.

4. Conclusions

Results obtained allow concluding that both the rheological properties of the batters and the technological characteristics of the muffins obtained are notably dominated by the type of protein used in the formulations. All vegetal protein sources had similar effect on specific gravity of the batters, while EWP decreased the specific gravity. The presence of SPI, PPI and C significantly (P<0.05) increased the storage modulus. In general, G' showed large increase with the temperature when SPI, PPI and EWP were added. These differences can be attributed to the nature and the denaturation pattern of the protein fractions comprised within each protein isolate. Regarding the muffins quality, EWG increases the height and specific volume, and muffins colour was dominated by the colour of the added proteins. Concerning texture, PPI containing muffins were the softest and springier than the No-Protein and casein gave the hardest muffin. In general, muffins with best visual appearance were those containing egg white protein or casein.

The development of sweet-baked gluten-free product is greatly dependent on the protein source. The use of other proteins as egg and milk replacements, like soybean protein isolate or pea protein isolate, affects texture of baked goods. Therefore, the optimization of this type of formulations is fundamental to ensure the proper texture and good taste of this type of products. Additionally, future studies will be undertaken to determine the sensory quality and consumer acceptance of these gluten-free muffins.

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FIGURE CAPTIONS

Figure 1. Dynamic mechanical spectra of different rice based batters. Without protein (♦) and with various vegetal protein sources (▲VWG; ● SPI; and ■ PPI) measured 25°C. Closed symbols referred to storage modulus (*G′*) and open symbols designated loss modulus (*G″*).

Figure 2. Dynamic mechanical spectra of different rice based batters. Without protein (♦) and with various animal protein sources (● EWP and ▲ C) measured at 25°C. Closed symbols referred to storage modulus (*G′*) and open symbols designated loss modulus (*G″*).

Figure 3. Storage modulus (*G′*) as a fuction of increasing temperature in different rice flour batters. Without protein (◊) and with various vegetal proteins (Δ VWG ;○ SPI; and □ PPI).

Figure 4. Storage modulus (*G′*) as a fuction of increasing temperature in different rice flour batters. Without protein (◊) and with various animal proteins (Δ EWP and □ C).

Figure 5. Loss tangent (tan δ) as a fuction of increasing temperature in different rice flour batters. Without protein (◊) and with various vegetal proteins (Δ VWG ;○ SPI; and □ PPI).

Figure 6. Loss tangent (tan δ) as a fuction of increasing temperature in different rice flour batters. Without protein (◊) and with various animal proteins (Δ EWP and □ C).

Figure 1.

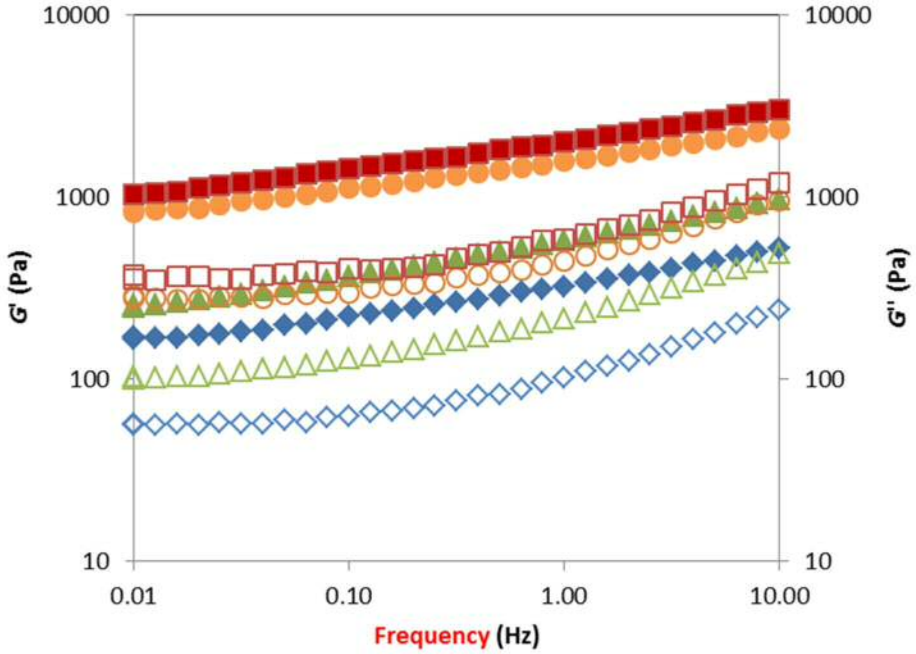


Figure 2.

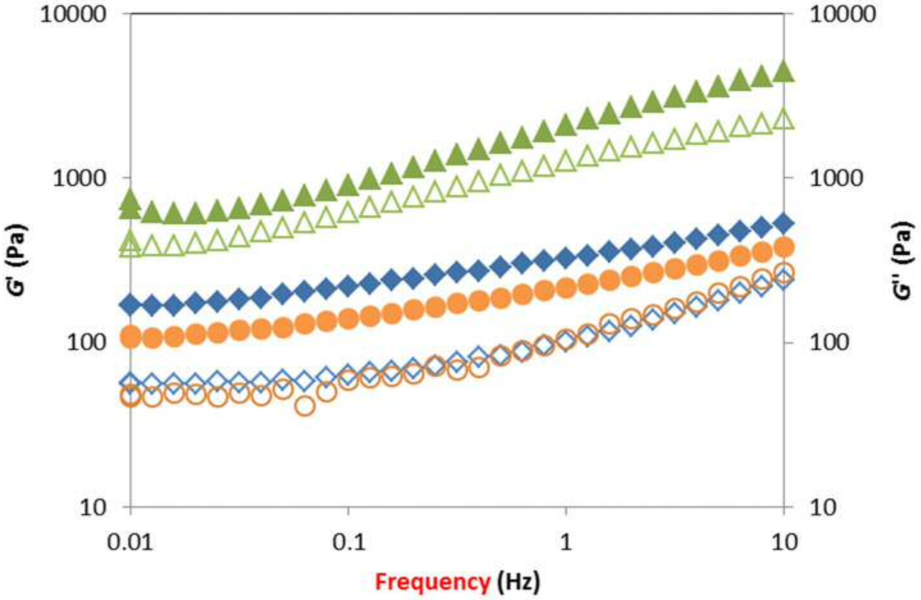
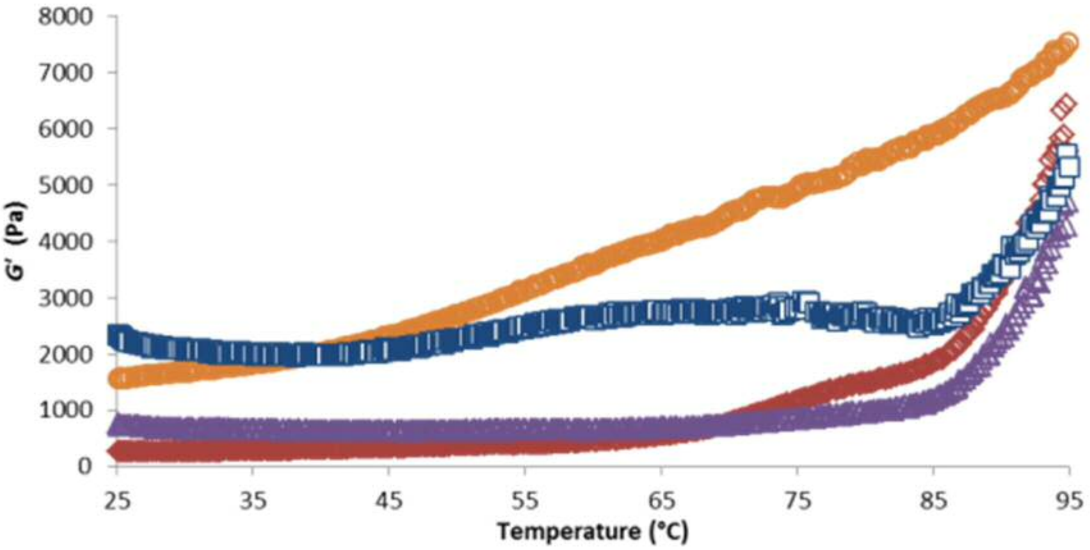


Figure 3.



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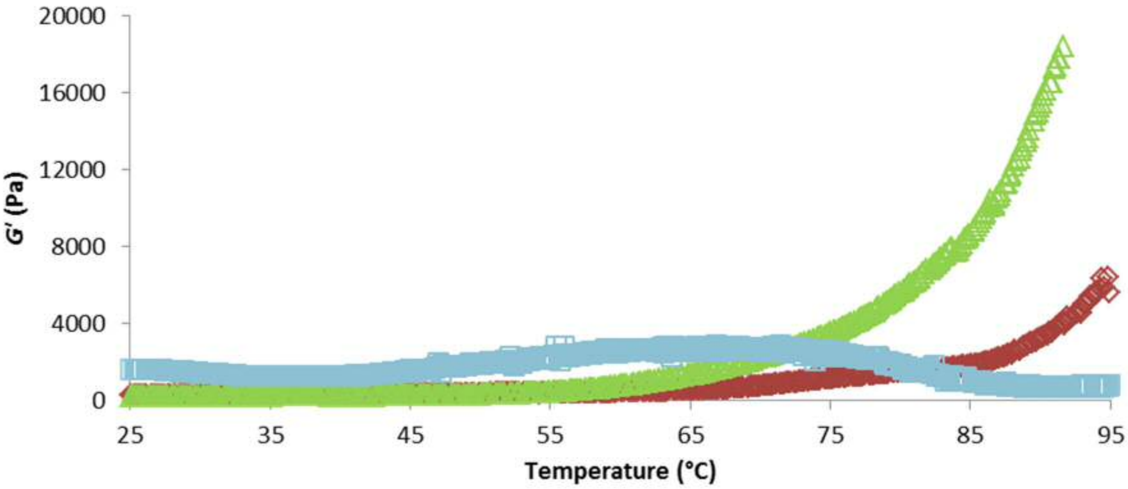

Figure 5

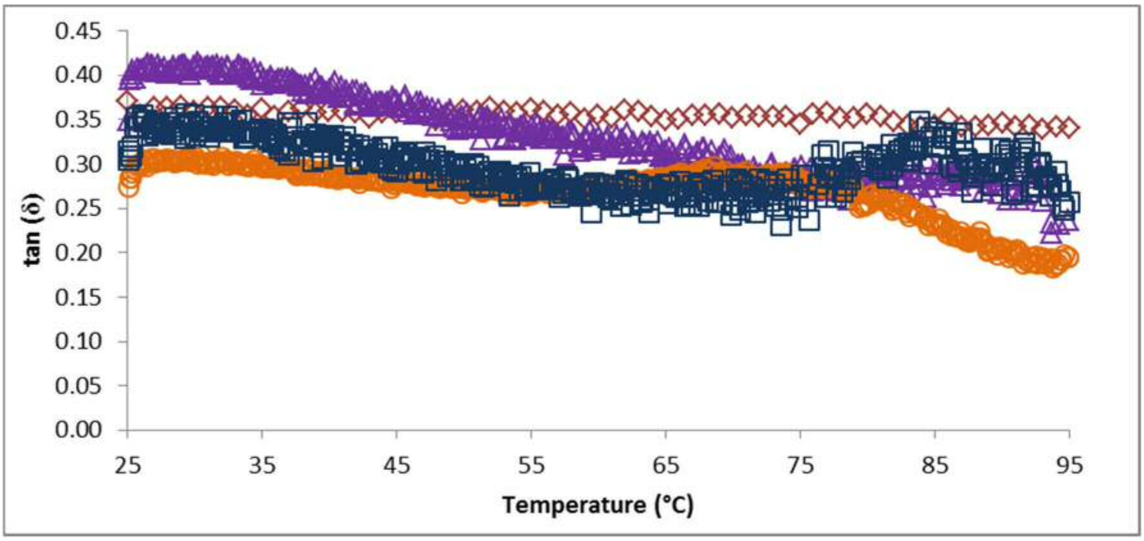

Figure 6.

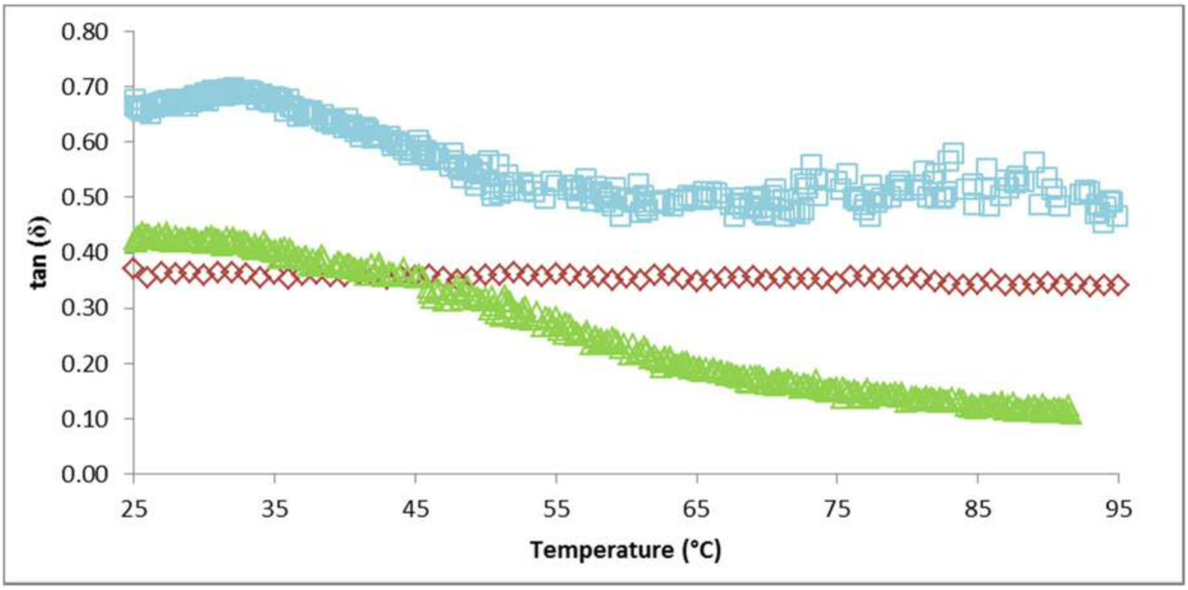

Table 1: Specific gravity (SG) and Viscoelastic parameters at 25ºC and 1 Hz (6.28 rad/s) of muffin batters prepared with different protein sources

Sample	SG (g/mL)	G' (Pa)	G'' (Pa)	G^* (Pa)	Phase angle(°)
No-Protein	1.03 ± 0.01 b	290 ± 50 a	100 ± 20 a	310 ± 60 a	19.0 ± 0.6 b
VWG	1.05 ± 0.01 c	580 ± 20 a	220 ± 10 ab	620 ± 20 a	20.5 ± 0.2 b
SPI	1.04 ± 0.01 bc	1580 ± 100 b	450 ± 10 bc	1640 ± 100 b	15.9 ± 0.8 a
PPI	1.04 ± 0.01 bc	2020 ± 105 bc	590 ± 25 c	2100 ± 110 bc	16.2 ± 0.2 a
EWP	0.97 ± 0.00 a	230 ± 30 a	100 ± 20 a	250 ± 35 a	22.9 ± 0.7 c
C	1.08 ± 0.00 d	2090 ± 350 c	1290 ± 190 d	2450 ± 390 c	31.7 ± 0.7 d
P- value	0.0001	0.0001	0.0001	0.0001	0.0001

Means ± standard deviation values followed by different letters within a column denote significant differences ($P<0.05$) (n=4).

No-Protein: Without protein; VWG: vital wheat gluten; SPI: soy protein isolate; PPI: pea protein isolate; EWP: egg white protein; C: casein

Table 2. Physical characteristics of protein enriched muffin prepared with different protein sources

Sample	Height (mm)	Weight loss (g)	Specific volume (mL/g)
No-Protein	37.1 ± 1.1 bc	7.5 ± 0.3 ab	1.56 ± 0.04 a
VWG	38.2 ± 1.0 c	7.6 ± 0.2 b	1.54 ± 0.05 a
SPI	35.2 ± 1.3 a	7.6 ± 0.3 ab	1.54 ± 0.04 a
PPI	36.4 ± 0.9 ab	7.5 ± 0.2 ab	1.54 ± 0.05 a
EWP	43.2 ± 2.2 d	7.4 ± 0.3 ab	2.19 ± 0.05 c
C	36.7 ± 1.3 abc	7.2 ± 0.4 a	1.74 ± 0.05 b
P- value	0.0001	0.0220	0.0001

Means ± standard deviation values followed by different letters within a column denote significant differences ($P<0.05$) (n=6)

No-Protein: Without protein; VWG: vital wheat gluten; SPI: soy protein isolate; PPI: pea protein isolate; EWP: egg white protein; C: casein

Table 3. Crumb colour parameters of protein enriched muffins.

Sample	L^*	a^*	b^*	C^*	$h(°)$
No-Protein	78.13 ± 0.59 d	0.38 ± 0.10 b	15.9 ± 0.4 a	15.9 ± 0.4 a	88.6 ± 0.3 e
VWG	73.82 ± 0.29 b	1.82 ± 0.08 d	20.3 ± 0.3 c	20.4 ± 0.3 c	84.9 ± 0.2 c
SPI	73.27 ± 0.40 a	2.57 ± 0.12 e	21.4 ± 0.4 d	21.5 ± 0.4 d	83.1 ± 0.3 b
PPI	72.83 ± 0.60 a	3.87 ± 0.30 f	26.3 ± 0.5 e	26.6 ± 0.6 e	81.6 ± 0.5 a
EWP	86.40 ± 0.30 e	-0.60 ± 0.04 a	15.7 ± 0.3 a	15.7 ± 0.3 a	92.2 ± 0.2 f
C	77.18 ± 0.48 c	0.66 ± 0.13 c	17.2 ± 0.5 b	17.2 ± 0.5 b	87.8 ± 0.4 d
P- value	0.0001	0.0001	0.0001	0.0001	0.0001

Means ± standard deviation values followed by different letters within a column denote significantly different levels ($P<0.05$) (n=9)

No-Protein: Without protein; VWG: vital wheat gluten; SPI: soy protein isolate; PPI: pea protein isolate; EWP: egg white protein; C: casein

Table 4. Texture parameters of protein enriched muffins prepared with different protein sources

	TPA parameters				
Samples	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience
No-Protein	104 ± 11 a	0.564 ± 0.042 a	0.411 ± 0.009 a	24 ± 2 a	0.186 ± 0.006 ab
VWG	104 ± 8 a	0.644 ± 0.046 bc	0.457 ± 0.010 c	31 ± 4 a	0.193 ± 0.006 ab
SPI	114 ± 14 ab	0.573 ± 0.037 a	0.412 ± 0.008 ab	27 ± 5 a	0.179 ± 0.005 a
PPI	97 ± 15 a	0.610 ± 0.029 ab	0.450 ± 0.025 abc	27 ± 4 a	0.191 ± 0.010 ab
EWP	114 ± 11 ab	0.818 ± 0.047 d	0.672 ± 0.063 d	63 ± 10 c	0.283 ± 0.040 c
C	123 ± 5 b	0.686 ± 0.028 c	0.494 ± 0.005 c	42 ± 3 b	0.209 ± 0.003 b
P- value	0.0013	0.0001	0.0001	0.0001	0.0001

Means ± standard deviation values followed by different letters within a column denote significant differences ($P<0.05$) (n=4)

No-Protein: Without protein; VWG: vital wheat gluten; SPI: soy protein isolate; PPI: pea protein isolate; EWP: egg white protein; C: casein