Assessing influence of protein source on characteristics of gluten-free breads optimizing their hydration level

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Abstract

Most gluten-free products have lower protein content than their counterparts with wheat flour. The addition of exogenous proteins could not only be a good option to compensate for this reduction but also a tool to create gluten-free products rich in protein. However, the different water binding capacities of proteins modify dough rheology, which also affects bread volume. Therefore, this study aimed to analyse the incorporation of a high percentage (30%) of several proteins (rice, pea, egg white and whey protein) in gluten-free breads whose hydration levels were adjusted for each protein to achieve the maximum volume. In this way, the breads with vegetal proteins required a higher amount of water than the breads with animal proteins. Moreover, all enriched breads exhibited lower maximum volume values than control, and the ones with whey protein presented the lowest volumes overall. From these results, the rheological behaviour and characteristics (colour, texture and weight loss) of optimized doughs and breads were measured. The doughs with whey protein presented the highest G' and G'' values due to their low hydration level, and the ones with egg white protein were very watery. Regarding colour, the addition of protein led to darker crusts, with the ones with whey protein being the darkest. With respect to the control, breads with animal proteins exhibited higher hardness, especially with whey protein, while the ones with vegetal proteins did not present significant differences.

Keywords: protein, gluten-free, bread, volume, hydration.

1. Introduction

Wheat protein has an essential role in bread making, since it allows for the formation of the gluten network when hydrated and subjected to mechanical work. This gluten network is responsible for imparting cohesiveness and extensibility in doughs, enabling the handle of dough and retention of gas during fermentation and baking (Delcour et al. 2012). Besides these characteristics, wheat protein accomplishes other roles during bread making. For one, it participates in Maillard reactions, which have a key role in bread colour and flavour (Smak 1972), and it also has well-known nutritional functions (Friedman 1996). During recent decades, interest in the development of gluten-free products has increased due to the existence of groups who must follow a gluten-free diet, such as patients with wheat allergy, celiac disease, non-celiac gluten sensitivity, among others (Reilly 2016). Nowadays, most gluten-free breads are elaborated with starches or starch-rice flour blends as main ingredients (Conte et al. 2016; Masure et al. 2016). As a result, commercial gluten-free breads show a lower protein content than their equivalent products elaborated with wheat flour (Conte et al. 2016; Miranda et al. 2014). This fact could contribute to several typical problems of gluten-free breads, such as their paler colour and poorer texture. To compensate for these lacks, the addition of different proteins in starch-based breads (Horstmann et al. 2017; Kittisuban et al. 2014; Krupa-Kozak et al. 2013; Witczak et al. 2017; Ziobro et al. 2013, 2016), rice flour-based breads (Marco and Rosell 2008a; Nozawa et al. 2016; Phongthai et al. 2016; Shin et al. 2010; Storck et al. 2013) and rice flour and starch mixture-based breads (Aprodu et al. 2016; Crockett et al. 2011; Gallagher et al. 2003; Smerdel et al. 2012) has been suggested. None of these studies included a protein level higher than 10% except Crockett et al. (2011) and Krupa-Kozak et al. (2013), who studied the addition of 15% egg white protein and 15-30% dairy proteins, respectively. The results obtained are often contradictory as, for the same protein, in some studies its addition did not modify bread specific volume, whereas in other studies it was found to decrease or increase specific volume. This could be due to the use of different formulations as well as the lack of adjustment of dough moisture for each elaboration. In fact, most studies used a formulation with the same amount of water for all elaborations.

Several studies have demonstrated that the amount of water used in the formulation of gluten-free breads significantly affects their specific volume and final characteristics (de la Hera et al. 2013; McCarthy et al. 2005; Ylimaki et al. 1988). Thus, Mancebo et al. (2017) observed that when greater amounts of water were included in the formulation, the specific volume of breads increased until achieving a maximum value for which the dough volume during fermentation or baking decreased. This effect was related to the influence of water on dough rheology, since a similar effect was observed when oil was added in doughs.

In the case of breads elaborated with wheat flour, the optimal hydration of doughs is determined by farinograph or other equipment. However, in the case of gluten-free products there is no validated equipment for this purpose. Most investigations used formulations with constant hydration, although in some cases the hydration level was optimized based on rheology (Martínez et al. 2014; Nunes et al. 2009; Ziobro et al. 2013, 2016) or modified according to previous tests which were not clearly explained. Only Ronda et al. (2015) optimized the volume for each sample in an investigation with β-glucan concentrates by adjusting the hydration level.

In this investigation, the effect of high percentages of several proteins on characteristics of gluten-free breads was studied. Moreover, a new focus of gluten-free bread research has been proposed, involving the optimization of specific volume to modify the hydration level of doughs. For this work, an initial formulation was selected in which maize starch was substituted by 30% of several proteins (rice, pea, egg white and whey). For each type of protein, the hydration level was changed until identifying the optimized value for which the specific volume was maximized. The rheological and physical characteristics (texture, weight loss and colour) of the optimized enriched breads were measured.

2. Materials and methods

2.1 Materials

The maize starch (Miwon; 7.91% moisture, 0.745 water binding capacity; Daesang Co., South Korea) was purchased at a local market. The proteins used were: rice protein (Remypro N80+6; >79% protein, 5.17% moisture, 0.01% solubility, 2.47 water binding capacity; Beneo, Germany), pea protein (Nutralys F85M; 78.13% protein, 6.16% moisture, 0.38% solubility, 5.40 water binding capacity, Roquette, France), egg white powder (81.66% protein, 6.18% moisture, 1.11% solubility, 0.00 water binding capacity; EPS S.P.A., Italy) and whey protein (Provon 295 IP; >92% protein; 6.02% moisture, 0.54% solubility, 0.00 water binding capacity; Glanbia, Ireland). Water binding capacity of the maize starch and the proteins was measured using AACC method 56.30 (AACC International 2012) and protein solubility was evaluated by the Quick Start™ Bradford Protein Assay (Bio Rad, Hercules, California, United States).

Other ingredients were white sugar (AB Azucarera Iberica, Valladolid, Spain), refined sunflower oil (Langosta, F. Faiges, S.L., Daimiel, Ciudad Real, Spain), salt (Disal, Unión Salinera de España S.A, Madrid, Spain), instant dry baker's yeast (Dosu Maya Mayacilik A.Ş, Istanbul, Turkey) and hydroxypropyl methylcellulose (HPMC) K4M (Rettenmaier & Sohne, Rosenberg, Germany).

2.2 Methods

2.2.1 Bread formulation

The following ingredients were used in bread making (g/100 g of starch): maize starch (100 g), oil (6 g), sugar (5 g), yeast (3 g), HPMC (2 g) and salt (1.8 g). The moisture of starch and starch-protein blends was adjusted to 11%, by adding extra water, to compensate for moisture differences of proteins and starch and to achieve all of mixtures had the same starting moisture conditions. This water addition was independent of the water amount used for each hydration level. Moreover, in enriched samples, maize starch was substituted by 30% protein.

All ingredients, except the yeast, were mixed for 1 minute at speed 1 using a KitchenAid Professional mixer (Kitchen Aid, St. Joseph, Michigan, USA) with a dough hook (K45DH). The instant yeast was rehydrated and mixed with the rest of ingredients for 8 minutes at speed 2. The bread batter (150 g) was placed into oil-coated aluminium pans (159 × 109 × 39 mm) and fermented at 30°C and 90 % RH for 60 min. Next, doughs were baked at 190ºC for 40 min. After baking, the loaves were removed from the pan, left to cool for 1 h at room temperature and then packaged in polyethylene bags. The loaves were stored at 24ºC for 3 days for being analysed the next day and the day 3 after baking. All the bread elaborations were performed twice.

2.2.2. Optimization of the hydration level of breads

The optimal hydration level (OHL) (g of water/100 g of protein-starch) for each sample was established after elaborating breads with different hydration levels and measuring their specific volume. Those hydration levels with which the breads achieved the maximum specific volume values were considered as the OHL. . In all cases, the hydration level was increased by 10% until observing a decrease of specific volume and achieving a maximum volume. After that, modifications of the hydration level by 5% around the maximum volume also were also made. From the results obtained, the variation of specific volume was modelled depending on the hydration level of doughs for each formulation. In this way, the optimized doughs (OD) and breads (OB) were deemed those that contained the amount of water needed to obtain the highest specific volume values. The bread volume was measured using a laser-based scanner (Volscan Profiler 300; Stable Microsystems, Surrey, UK) and determined on two loaves from each elaboration. Specific volume was calculated as relation between the bread volume and its weight (Horstmann et al. 2017; Mancebo et al. 2017; Martínez et al. 2014) , both parameters measured the day after baking.

2.2.3. Rheological characteristics of OD

The rheological behaviour of OD was studied using a Thermo Scientific Haake RheoStress controlled strain rheometer (Thermo Fisher Scientific, Schwerte, Germany) with a constant temperature (25ºC) controlled by a Phoenix II P1-C25P water bath (Thermo Fisher Scientific, Schwerte, Germany). Parallel-plate geometry (60 mm diameter titanium serrated plate-PP60 Ti) with 3 mm gap was used. The dough was rested for 300 s before measuring. Strain and frequency sweeps were carried out at 25ºC. The strain sweep test, with a strain range of 0.1 to 100 Pa and a constant frequency (1 Hz), was performed to identify the linear viscoelastic region. A strain value included in the linear viscoelastic region was used in a frequency sweep test with a frequency range of 10 to 0.1 Hz. Values of elastic modulus (G′[Pa]) and viscous modulus (G″[Pa]) were obtained based on frequency values (ω [Hz]). The samples were analysed in duplicate.

2.2.4. Physical characteristics of OB

Crumb texture was determined using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) with the software “Texture Expert”. A 25-mm diameter cylindrical aluminium probe was used in a “Texture Profile Analysis” (TPA) double compression test to penetrate to 50% of the depth at a speed of 2 mm/s and with a 30-sec delay between the first and second compressions. From each bread batch, two loaves were sliced into pieces of 20 mm thickness and the two central ones were used in the measurement. Hardness (N), springiness, cohesiveness, chewiness and resilience were calculated from the TPA graphic. ΔHardness (%) was calculated as the percentage related to the hardness of breads measured one day and three days after baking.

Weight loss of breads was calculated as the difference, expressed as a percentage, between the weight of batter placed into the pan (150 g) and the weight of the bread after, divided by the weight of batter.

Crust colour of breads was measured using a Minolta CM-508i spectrophotometer (Minolta Co., Ltd, Japan) with the D65 standard illuminant and the 2° standard observer. Bread colour measurements were made at the centre of the upper surface of four loaves from each batch preparation. The results were expressed in the CIE L*a*b* colour space.

The measurement of weight loss and crust colour was made one day after baking.

2.2.5 Statistical analyses

To study the results obtained, an analysis of variance (one-way ANOVA) was used. Fisher's least significant differences (LSD) test was selected to differentiate the medians with signification level of 95% ($p<0.05$). The statistical analyses were completed using Statgraphics Centurion XVI software (StatPoint Technologies Inc, Warrenton, EEUU).

3. Results and Discussion

3.1. Bread optimization

The different specific volume of breads and their modelling graphics based on the hydration level of the doughs are shown in Figure 1. The highest specific volume was obtained by the control sample. Among protein-enriched breads, those with vegetal proteins and egg white protein showed similar specific volume, although the ones with rice protein presented higher specific volume than the ones with egg white and pea protein (Table 1). On the other hand, the breads with whey protein exhibited the lowest specific volume.

In general, previous studies about the addition of protein in gluten-free breads reported contradictory results. Regarding the incorporation of egg white protein, some authors shown an increment of bread volume (Crockett et al. 2011; Ziobro et al. 2013, 2016) and others did not observe significant differences (Phongthai et al. 2016; Storck et al. 2013). As for whey protein, Kittisuban et al. (2014) observed a decrease in volume, Shin et al. (2010) observed an increase in volume and Krupa-Kozak et al. (2013) found a clear improvement in volume with low percentages which was reduced as the amount of protein was increased. The results of studies with vegetal proteins also are contradictory, but in general a reduction of specific volume was observed (Crockett et al. 2011; Marco and Rosell 2008a; Witczak et al. 2017; Ziobro et al. 2013, 2016), which agrees with the results observed in our study. However, as shown on Figure 1, these results could vary depending on the hydration level, since in most of studies this parameter was not modified. For all proteins, the discrepancies of results among studies could be due to the different formulations used, especially the use of hydrocolloids and the different amount of protein added (other studies included lower percentage of protein than our study), just like the fact that the hydration level was not adjusted for each protein. Previous studies have already observed that, in the elaboration of gluten-free breads, a low dough consistency improved the final bread volume (Mancebo et al. 2017). This suggests that the use of different formulations could produce changes in the dough rheology, and these lead to differences in volume values.

About the OHL for each protein, the results shown in Figure 1 indicate that the breads with vegetal proteins required higher hydration level to obtain maximum values of specific volume, with respect to control samples and the ones with animal proteins. Among vegetal proteins, pea protein required greater amount of water (150%) than rice protein (115%). This results agree with (Ziobro et al. 2013) who confirmed that, to achieve the same farinograph consistency, the doughs with pea protein needed an higher hydration level than the control sample. On the other hand, control samples required a higher hydration level (90%) than egg white protein breads (85%) and these, in turn, had an OHL greater than the ones with whey protein (40%). Gluten-free doughs are more like batters than doughs with gluten, so viscosity is a very important parameter to keep in mind. The lower the batter viscosity is, the higher the bread volume (Mancebo et al. 2015), but there is always a viscosity limit value from which the volume bread drops during baking (Ziobro et al. 2016). This drop is caused by a reduction of dough viscosity during first steps of baking because a fraction of the starch leaches out of the granules (McClements 2005). Therefore, the initial viscosity should not be too low, since the dough would be not able to retain the bubbles within the dough during baking, and a volume drop is produced (Miś et al. 2018). This explanation, together with the positive correlation water binding capacity-viscosity observed by Martínez and Gómez (2017), could explain the different behaviour between the animal and vegetal proteins. Vegetal proteins agreed with the previous explanation since pea protein had higher WBC than rice protein and the doughs with the first protein required higher hydration level. Nevertheless, in the case of animal proteins, the amount of water accepted by the egg white protein was higher than whey protein despite their WBC were similar. Thus, the egg white protein doughs was very watery but it was able to produce a bread with high volume. This fact could be explained by the coagulation process that egg white protein experiences during baking (Kiosseoglou and Paraskevopoulou 2006), which contribute to cell wall structure development and stabilization of the expanding bubbles, counteracting the viscosity decrease.

Finally, it is interesting to highlight that the breads with different proteins showed differences with regards to their stability when the hydration level was modified, meaning the variations on hydration level affect them differently. In general, hydration level had the lowest influence on the breads elaborated with egg white protein, since the reduction of specific volume in relation to hydration level was less pronounced (Figure 1). On the contrary, the control bread presented the lowest stability and, although its volume was the highest respect to the rest of samples, the variations on the hydration level significantly affected it, decreasing the specific volume when the hydration level was not close to OHL. As it is observed in Figure 1, if a hydration level lower than 70% had been used in our study, the specific volume of the egg white protein bread would not have been modified so much, but it would be higher than control bread. Thus, an excess or lack of water seems to more negatively affect the control bread than the bread with egg white protein. Therefore, a correct hydration level for the breads with egg white protein would be excessive for the control breads.

On the other hand, results shown in Figure 1 indicate that the breads with vegetal proteins required higher hydration level to obtain maximum values of specific volume, with respect to control samples and the ones with animal proteins. In addition, among vegetal proteins, pea protein was the protein that required greater amount of water. This results agree with (Ziobro et al. 2013) who confirmed that, to achieve the same farinograph consistency, the doughs with pea protein needed an higher hydration level than the control sample.

The differences between animal and vegetal proteins could be due to the insoluble character of the first ones and their low water binding capacity (Amagliani et al. 2017; Horstmann et al. 2017), which also agrees with our measurements. Martínez and Gómez (2017) observed that the higher the water binding capacity values, the greater the consistency of gluten-free doughs. Moreover, several studies found a negative correlation between dough consistency and specific volume of gluten-free breads (Mancebo et al. 2015, 2017; Nishita et al. 1976). Regarding breads with animal proteins, these needed a lower hydration level than control sample to obtain maximum values of volume and the breads with whey protein were absorbed the lowest amount of water. The same way as previously, these differences could be explained by the soluble character of egg white and whey protein, their low water binding capacity and their influence on dough rheology.

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3.2. Dough rheology

The rheological behaviour of OD is shown in Figure 2, in which the G’ and G’’ values at determined frequency (1Hz) were included. First, it is important to point out that the doughs with egg white protein had a consistency too low for the methodology suggested and, due to this fact, are not included. Ziobro et al. (2013) and Crockett et al. (2011) also observed a significant decrease of storage (G’) and loss (G’’) moduli when albumin was added in gluten-free doughs. However, the measurement could be done since the level of substitution (less than 15%) and hydration level was lower than in our study. For all samples, the storage modulus (G’) was higher than loss modulus (G’’) throughout the frequency range, indicating that they presented a solid-like behaviour. Moreover, all formulations showed an increase in G’ and G’’ when the frequency was increased, so that the doughs could be considered shear-dependent.

The OHL of whey protein was reached with the lowest hydration values (40%) and, thus, the G’ and G’’ values of the doughs with this protein were significantly higher than the rest of samples. For storage modulus (G’), the increase was 2.5 times greater than for loss modulus (G’’), what means that the addition of whey protein strengthened the elastic structure of the dough. Moreover, as it could be observed in Figure 2, the whey protein doughs showed less frequency dependence than the rest of samples. Some authors (Marco and Rosell 2008b) also observed that the addition of whey protein decreased the rheological parameters of doughs, but in this study the hydration level of samples was not modified. In our study, while one animal protein (whey protein) led to batters with the highest rheological parameters, the batters with egg white protein could not be measured due to being too watery. This difference between animal proteins could be explained by the different hydration level required of each protein, which is higher for egg white protein than whey protein. Among the rest of the doughs, significant differences were not observed between the control dough and the one with pea protein, even though the doughs with pea protein have higher hydration level. This fact could be attributed to the higher water binding capacity of pea protein and the well-known relation of water binding capacity-consistency (Martínez and Gómez 2017). Among vegetal proteins, doughs with rice protein presented lower G' and G'' values than pea protein samples. This protein presented a lower water binding capacity than pea protein, what could explain the need for lower hydration level to achieve the same consistency. However, it is notable that doughs with rice protein reach maximum volume with lower consistency than doughs with pea protein.

3.3. Bread Characteristics

In Tables 1 and 2, the physical characteristics of OB are shown. As indicated in Figure 1, the protein-enriched breads presented lower specific volume than control. There were no significant differences between breads with vegetal proteins and egg white protein, but the ones with whey protein displayed specific volume values clearly lower than the other samples (hese results have already been discussed in Section 3.1). However, although there were not differences between some samples, the bread structure is quite different (Figure 3). Unfortunately, it was not possible to obtain a slice of whey bread due to its high hardness, so that the image was not included in Figure 3. The structure of rice breads was formed by big holes, probably as result of the coalescence process occurred during baking. In the case of pea protein breads, the bread slice shows a structure less dense than control in which the holes of medium size. Finally, the addition of egg white protein gave rise to a bread structure with a regular number of holes, which have smaller size than control. The differences between proteins could be explained by their foaming properties, since a good foaming capacity favour the bubble formation and stabilization (Richert 1979). Regarding weight loss, the breads with animal proteins lost less water than the rest of samples, and lowest loss in weight was observed for whey protein. These results are logical since at a low hydration level, the amount of water available is lower. Moreover, the breads with whey protein presented lower volume and, therefore, a lower exchange surface which reduces the loss of water. This effect has also been observed in previous studies (Mancebo et al. 2017). Breads with vegetal proteins and the control sample also followed this trend since the higher the bread volume, the greater the weight loss. Thus, the control bread, with higher volume, exhibited higher weight loss, followed by breads with rice protein and pea protein, respectively. It is important to note that, despite having a higher OHL, the breads with vegetal proteins presented lower weight loss than the control. This fact could be explained not only by the lower volume of breads with vegetal proteins but also by the higher water binding capacity of these proteins, especially the pea protein.

The values of texture parameters during storage are shown in Table 2. Regarding texture, neither hardness at day 1 nor hardness after storage showed significant differences between control and vegetal protein-enriched samples. However, the breads with animal proteins, especially the ones with whey protein, were much harder and tended to harden over time. The hardness of breads generally is related to their specific volume (Gallagher et al. 2003; Mancebo et al. 2017; Martínez and Gómez 2017), and a correlation between initial hardness and its evolution during storage has been found in previous studies (Gómez et al. 2008). This could explain the higher hardness values of the breads with animal proteins, primarily those with whey protein, just like the greater increases in hardness of these breads. The breads with vegetal proteins did not present significant differences with respect to control because both of them had low initial hardness values. In the case of the breads with egg white protein, despite having similar specific volume as breads with vegetal proteins, they presented higher hardness than what was observed by other authors (Crockett et al. 2011; Phongthai et al. 2016; Ziobro et al. 2016). This fact could be due to the coagulation process of egg white protein during baking (Kiosseoglou and Paraskevopoulou 2006). During thermal coagulation, the endothermic enthalpy required by albumen for molecular unfolding is lower than for other proteins , so that egg white protein is readily unfolded by heating (Nozawa et al. 2016). In this way, following heating and molecular denaturation, disulphide bridges form between the albumen molecules and led to the formation of very strong and elastic gel network structure (Kiosseoglou and Paraskevopoulou 2006). Previous studies showed contradictory results, but in general, there are not similarities with our results. They usually agree on the fact that dairy proteins are able to reduce bread hardness (Gallagher et al. 2003; Krupa-Kozak et al. 2013) and vegetal proteins increase it (Marco and Rosell 2008a; Phongthai et al. 2016; Ziobro et al. 2016), although most of these studies used a constant hydration level. The increase of hydration level on breads is known to reduce the hardness, due to the fact that the volume is increased, so that the correction of hydration level could revert the general trend observed (Aprodu et al. 2016). This fact would explain our results, since the breads with vegetal proteins presented the highest OHL and the ones with whey protein presented the lowest. Finally, as it has already observed by several authors (Ronda et al. 2015; Ziobro et al. 2013, 2016), the crumb hardness of all breads increased after storing due to the loss moisture, starch retrogradation and physicochemical interactions between bread constituents (Korus et al. 2015).

Concerning the remaining texture values, the breads with egg white protein showed high values of springiness, cohesiveness and resilience, so that are less brittle than the control breads. This fact could be explained by the thermal coagulation that the albumen experiences during baking and results in a strong and elastic structure (Kiosseoglou and Paraskevopoulou 2006). Breads with whey protein exhibited low springiness and cohesiveness values, which could be due to the low OHL and, thus, the high dryness of the crumb. Texture parameters hardly changed throughout the days so that the crumb moisture have great influence in the crumb hardness. Among vegetal proteins, the samples with rice protein stood out by their high chewiness and resilience values with respect to the control sample and the ones with pea protein, by their greater springiness. Unlike the rest of samples, all textural parameters of vegetal protein breads, except hardness, was reduced during storage period, affecting negatively the bread quality.

The incorporation of all types of protein reduced the luminance values (L*) and increase the a* values, causing a darker crust that was more reddish, and this effect was higher with the addition of whey protein. Among vegetal proteins, the breads with pea protein presented darker colours than those with rice protein. Regarding yellow colour (b*), there were no significant differences between the control and the breads with vegetal proteins. However, the breads with egg white protein presented higher b* values than control, while the addition of whey protein reduced the b* values with respect to control. The reduction of luminance values with the incorporation of proteins agreed with other studies, both in breads with (Smak 1972) and without gluten with dairy proteins (Gallagher et al. 2003; Krupa-Kozak et al. 2013) and vegetal proteins (Phongthai et al. 2016). The explanation for this reduction is based on the participation of amino acids from proteins in Maillard reactions, which are responsible for the final crust colour (Bertram 1953; Purlis and Salvadori 2009). However, in previous studies with egg white protein (Phongthai et al. 2016) and soy protein (Marco and Rosell 2008a), there were no differences with respect to the control sample, and this could be due to the lower percentages of protein used in comparison with our study. The breads with whey protein were the darkest and this could be explained by the high lysine content of this protein (Peña-Ramos et al. 2004). The high lysine content would also explain the differences between the breads with pea and rice protein since, just as the whey protein, the pulse proteins have a higher lysine content than cereals (Pérez et al. 2013). Lysine has an important role in Maillard reactions since it is the primary reactive amino group that reacts with the reducing sugars (Kasran et al. 2013). Thus, the higher the amount of lysine, the greater the amount of Maillard reactions. The different a* and b* values of enriched breads could be attributed to the different amino acid composition of each protein, and therefore, due to the different compounds generated by Maillard reactions (Chen et al. 2015).

4. Conclusions

The method of analysing gluten-free breads by the optimisation of their specific volume provides additional information in comparison with the traditional methods of constant hydration or rheology. This kind of method permits knowledge of the optimal hydration level at which the bread volume is maximum, and thus, to study the rheological behaviour of the optimized doughs, the tolerance for the changes of hydration and its effect on bread characteristics. Moreover, in the case of protein enriched gluten-free breads, this method also could help explain the contradictory results obtained in previous studies. Regarding our results, the breads with vegetal proteins required a higher hydration level to obtain maximum volume than the ones with animal proteins. In addition, the maximum volume of breads with animal proteins, especially the one with whey protein, were lower than breads with vegetal proteins, and the egg white protein achieved bread with high volume and dough with very watery consistency.

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Table 1. Specific volume, weigth loss and colour parameters of optimized breads enriched with protein and control.

	Control	RP	PP	EWP	WP
Maximum specific volume (ml/g)	10.10±0.09e	6.79±0.10d	6.25±0.05b	6.48±0.06c	4.45±0.11a
Weight loss (%)	39.84±0.05e	30.90±1.09d	28.87±0.19c	25.49±0.30b	16.77±0.05a
L*	80.01±1.15d	58.23±6.51c	48.40±1.33b	56.60±2.82bc	38.20±0.47a
a*	1.20±0.47a	5.87±1.24b	10.06±0.20cd	8.49±0.31c	11.39±0.35d
b*	17.19±0.54b	17.85±3.10bc	16.56±0.42b	21.20±0.99c	11.54±0.35a

RP: rice protein; PP: pea protein; EWP: egg white protein. WP: whey protein. The values with the same letter in the same row do not present significant differences (p<0.05).

Table 2. Textural parameters of optimized breads enriched with protein and control.

		Control	RP	PP	EWP	WP
Day 1	Hardness (N)	1.18±0.05a	0.91±0.08a	0.77±0.03a	7.75±0.25b	14.08±0.58c
	Springiness	1.32±0.14b	1.33±0.13b	1.96±0.00c	1.94±0.03c	0.74±0.05a
	Cohesiveness	0.55±0.06b	0.34±0.04a	0.56±0.02b	0.72±0.00c	0.33±0.07a
	Chewiness (N)	0.62±0.04a	1.09±0.08a	0.87±0.31a	10.94±0.21c	3.39±0.39b
	Resilience	0.25±0.04a	0.99±0.11c	0.27±0.03ab	0.40±0.00b	0.15±0.05a
Day 3	Hardness (N)	1.57±0.04a	1.36±0.14a	1.28±0.11a	9.45±0.01b	28.91±2.60c
	Springiness	1.26±0.05c	0.92±0.02b	1.02±0.09b	1.19±0.10c	0.635±0.01a
	Cohesiveness	0.55±0.06c	0.33±0.03b	0.41±0.04b	0.65±0.01d	0.22±0.01a
	Chewiness (N)	1.14±0.03a	0.60±0.06a	0.52±0.09a	7.38±0.52c	3.72±0.21b
	Resilience	0.36±0.00c	0.145±0.01b	0.14±0.04b	0.345±0.01c	0.09±0.00a
	ΔHardness (%)	32.45±2.44ab	51.06±11.60bc	65.85±7.88c	22.00±3.83a	105.14±10.01d

RP: rice protein; PP: pea protein; EWP: egg white protein. WP: whey protein. The values with the same letter in the same row do not present significant differences (p<0.05).

Fig. 1 Modelling of specific volume of the protein enriched breads as a function of the hydration level of the doughs (●: control; Δ: rice protein; □: pea protein; ×: egg white protein; ◊: whey protein)

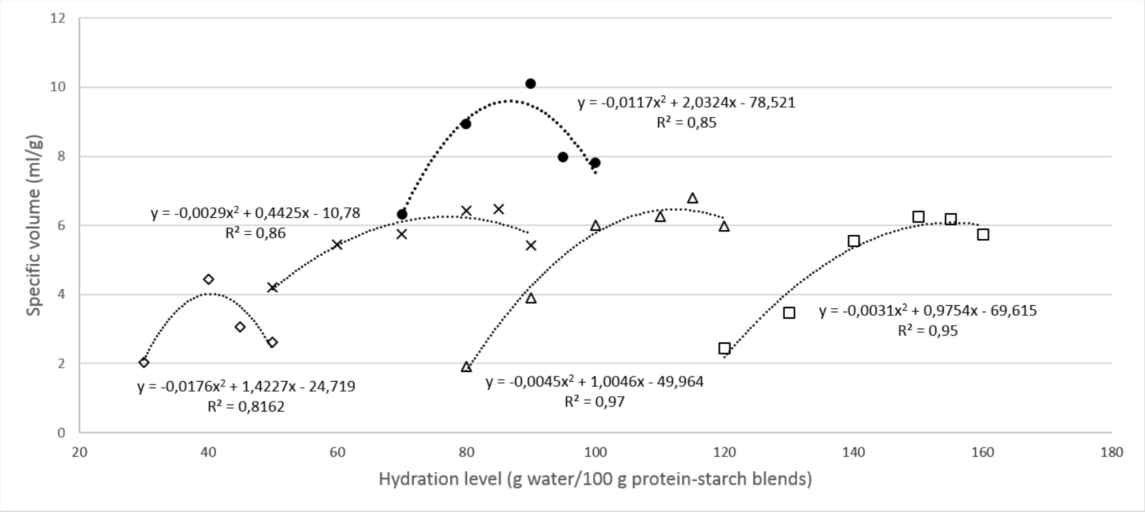

Fig. 2 Dynamic oscillatory properties of the optimized doughs enriched with protein and control (●: control; Δ: rice protein; □: pea protein; ◊: whey protein). G’ and G’’ values at frequency 1Hz are included.

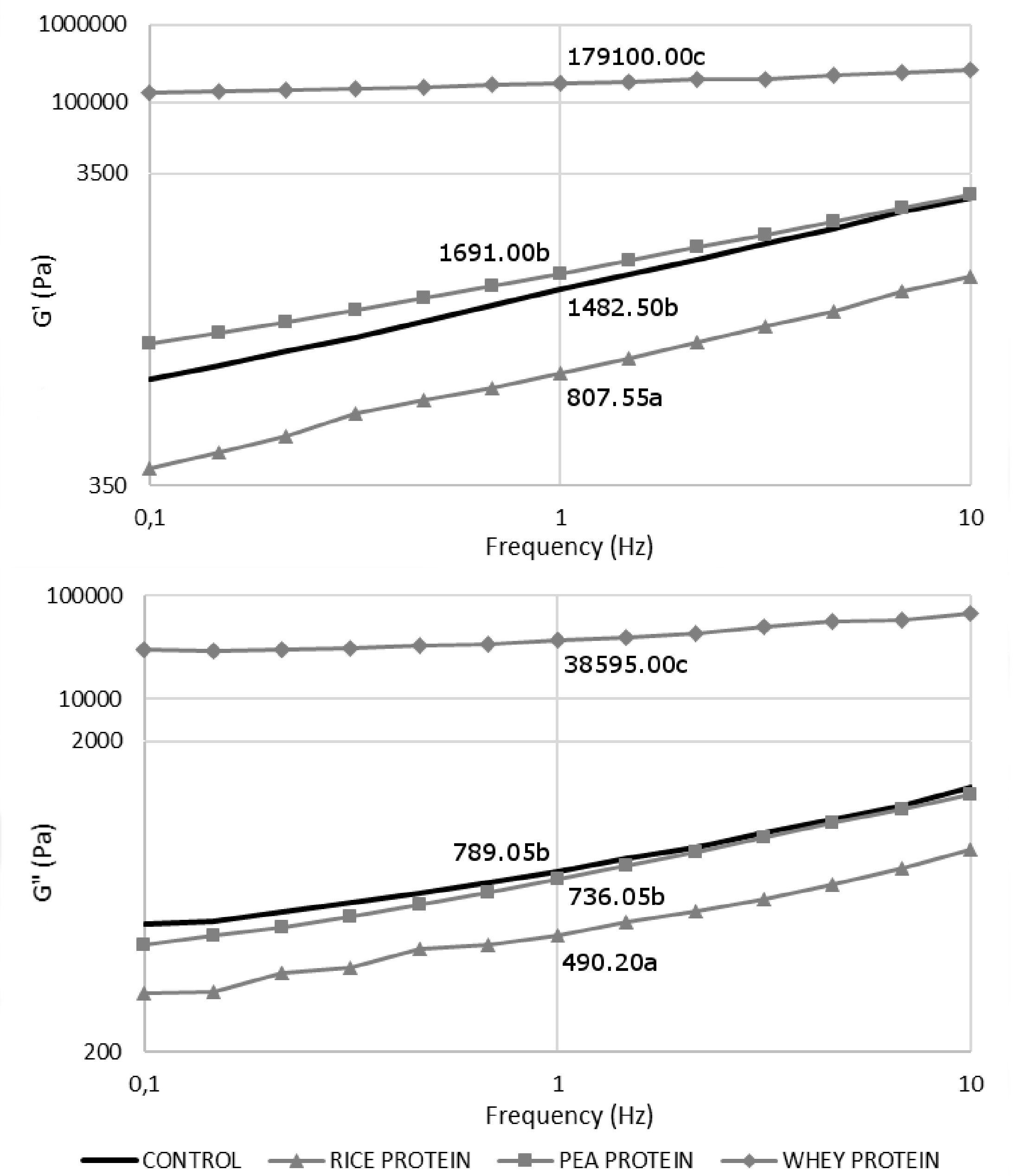

Fig. 3 Slices of control and protein enriched breads.