Influence of protein source on the characteristics of gluten-free layer cakes

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Abstract

The aim of this study was to examine the effect of four commercial proteins (pea, rice, egg white and whey) on the characteristics of gluten-free layer cakes. Rice flour was partially substituted with 15, 30 and 45% protein. Hydration properties, batter density and viscosity, cake characteristics (weight loss, specific volume, texture and colour) and consumer acceptability were analysed. In general, the addition of protein increased the viscosity of the batters, with higher protein contents exhibiting greater effects and with pea protein presenting the highest effect overall. The addition of egg white protein led to the hardest cakes ($p<0.05$) and whey protein, which also increased the cake hardness ($p<0.05$), gave rise to cakes with the highest specific volume. Both animal proteins increased the cake cohesiveness and springiness ($p<0.05$). On the contrary, pea and rice protein hardly modified hardness, colour and specific volume of cakes overall, but reduced their cohesiveness ($p<0.05$). Regarding sensory evaluation, all protein-enriched cakes presented lower acceptability with respect to control cake ($p<0.05$), but this effect was more pronounced when rice and egg white protein were added due to their taste, odour and texture. Whey protein cakes were, among the enriched samples, the ones with the highest acceptability.

Keywords: Protein enrichment, batter, texture, volume, acceptability.

1. Introduction

Over recent years, the market demand for gluten-free products has increased significantly. One of the reasons for this increasing trend is the rise in diagnosed cases of celiac disease (Gallagher, Gormley, & Arendt, 2004). Although the prevalence of celiac disease is approximately 1% of the general population in Europe and the United States, the sole treatment is a unique gluten-free diet (Niewinski, 2008). However, there are also people who follow a gluten-free diet without suffering any celiac pathology, as they believe in its benefits for health orweight loss. (Staudacher & Gibson, 2015).

Currently, gluten-free products have lower protein content than their equivalent products with gluten (Miranda, Lasa, Bustamante, Churruca, & Simon, 2014; Wu et al., 2015). This lack should be compensated, as proteins are one of the basic nutrients necessary for various anabolic processes in the body (Hoffman & Falvo, 2004). In addition, some authors reported that a greater protein intake could have beneficial health effects for athletes (Phillips, 2014) and elderly people (Nowson & O'Connell, 2015). Due to this, the addition of different proteins to gluten-free bakery products could be a way to address the potential consequences of the current market demand.

Cakes are an interesting matrix in which proteins can be incorporated due to their high acceptability. Furthermore, their worldwide market is currently growing at a rate of about 1.5% per year (Wilderjans, Luyts, Brijs, & Delcour, 2013). Cake batter could be considered as a complex oil-in-water emulsion with a continuous aqueous phase containing dissolved or suspended dry ingredients (Ronda, Oliete, Gómez, Caballero, & Pando, 2011). The correct formation of the emulsion depends on both the aeration of the aqueous phase (Brooker, 1993) and the retention of this air incorporated. The efficacy of air retention in the batter is inversely

proportional to batter viscosity (Sahi & Alava, 2003) and it is known that flour characteristics affect it (Gómez, Ruiz-París, & Oliete, 2010). If the batter viscosity is too low, the air bubbles escape of the batter to the surface during baking, as the batter can not retain them. On the other hand, if the batter viscosity is too high, the air bubbles can not extend in the oven and the cake volume is low. Therefore, the study of the effect of substituting the flour by different proteins on batter viscosity and cake volume would be needed.Regarding cakes elaborated with wheat flour, studies can be found about substituting flour with whey protein (Jyotsna, Manohar, Indrani, & Rao, 2007), gluten protein (Wilderjans, Pareyt, Goesaert, Brijs, & Delcour, 2008) or soy protein (Majzoobi, Ghiasi, Habibi, Hedayati, & Farahnaky, 2014; Sung, Park, & Chang, 2006). As for gluten-free cakes, there are few authors who have studied the influence of protein on product characteristics (Matos, Sanz, & Rosell, 2014; Ronda et al., 2011; Shevkani, Kaur, Kumar, & Singh, 2015; Shevkani & Singh, 2014). However, none of these studies included protein levels exceeding 15%, except that of Ronda et al. (2011) who added 20% of protein, although starch was used instead of flour. On the other hand, only Matos et al. (2014) compared vegetal and animal proteins. To cover the existing gaps, the aim of this study was to study how three percentages (15, 30 and 45%) of different vegetal (pea and rice) and animal (egg white and whey) commercial proteins affected batter (density and viscosity), physical characteristics (specific volume, weight loss, texture and colour) and acceptability of gluten-free layer cakes.

2. Materials and methods

2.1 Materials

Rice flour (12.83% moisture, 8% protein and 85.4 μm particle size) was supplied by Harinera la Castellana S.A (Medina de Campo, Valladolid, Spain), Nutralys BF pea protein (78.13% protein and 134.0 μm particle size) by Roquette (Leutrem, France), Remypro N80+G rice protein (79% protein and 95.2 μm particle size) by Beneo (Mannheim, Germany), egg white powder (81.66% protein and 30.7 μm particle size) by EPS S.P.A (Occhiobello, Italy) and Provon 295 IP whey protein (92% protein and 39.7 μm particle size) by Glanbia (Kilkenny, Ireland). Particle size was measured with a laser diffraction particle size analyser (Mastersizer 3000, Malvern Instruments, Ltd., Worcestershire, UK) and it was expressed as D(4;3), which reflects the size of those particles which constitute the bulk of the sample volume. Other ingredients were white sugar (AB Azucarera Iberica, Valladolid, Spain), UHT whole milk (President, Lactalis Food Service Iberia, S.L.U., Madrid, Spain), liquid pasteurized egg (Ovopack, Álvarez Camacho, S.L., Sevilla, Spain), refined sunflower oil (Langosta, F. Faiges, S.L., Daimiel, Ciudad Real, Spain) and baking powder “25 × 1” (Puratos, Gerona, Spain).

2.2 Methods

2.2.1 Cake elaboration

The layer cake was elaborated using the following formulation: 350 g rice flour, 315 g sugar, 210 g milk, 175 g liquid pasteurized egg, 105 g sunflower oil and 10.5 g baking powder. In the protein-enriched formulations, the rice flour was replaced by 15, 30 or 45% of each commercial protein (rice, pea, egg white and whey). The moisture of flour and flour-protein blends was adjusted to 13%. All ingredients were mixed using a KitchenAid Professional mixer (Kitchen Aid, St. Joseph, Michigan, USA) for 10 min: 1 min at speed 4 and 9 min at speed 6. The cake batter (185 g) was placed into oil-coated aluminium pans (159 × 119 × 35 mm) and baked at 190 °C for 25 min. After baking, the cakes were removed from the pan, left to cool for 1 h at room temperature and packaged in polyethylene bags to be stored at 24 ºC. All the cake elaborations were performed twice.

2.2.2 Batter measurements

Batter density (at 20 °C) was determined by an Elcometer 1800 pycnometer (Manchester, UK) with a known volume (100 cm^3). The density value was calculated as the relation of the weight (g) of batter placed in the filled pycnometer and the volume capacity of the container.

Batter viscosity was measured using a Rapid Viscoanalyser (RVA-4) (Newport Scientific model 4-SA, Warriewood, Australia). A batter sample (28 g) were placed in an RVA aluminum canister with a plastic paddle and submitted to a viscosity analysis (160 rpm at 30 °C).

For batter microstructure, a drop of the batter was placed on a microscope slide and covered with a coverslip. The slides were compressed under a constant weight (1 kg) to achieve a layer of batter of uniform thickness, removing the highest amount of air bubbles as possible. The batter samples were examined using a DM750 microscope (Leica Microsystems, Wetzlar, Germany). The microscope was equipped with a Leica EC3 video camera, and images were captured using LAS-EZ V1.7.0 for Windows software (Leica Microsystems, Heerbrugg, Switzerland).

The microscope was also equipped with a PE120 System equipped with PE95 controller, ECP water circulator (Linkam Scientific Instruments, Surrey, United Kingdom) and a GS3-U3-23S6C-C camera (FLIR Systems, Wilsonville, USA) to capture the videos. For that, the batter, prepared in the same way as that to capture images, was heated until reaching 30 ºC and was then maintained at this temperature for 3 min. Next, the sample was heated further until reaching 85 ºC with a heating rate of 14 ºC/min. Finally, the sample was warmed up until attaining 100 ºC (at 2.5 ºC/min heating rate) and was maintained at this temperature for 10 min. This temperature ramp was used to simulate the heating process that the batter undergoes during baking. All measurements were realized twice.

2.2.3 Cake characteristics

Cake volume was obtained from two pieces of each elaboration using a laser sensor with the BVM-L 370 volume analyser (TexVol Instruments, Viken, Sweden). Specific volume and weight loss were calculated according to de la Hera, Martinez, Oliete and Gómez (2013).

Crumb texture was determined using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) with the “Texture Profile Analysis (TPA)” test. A 25-mm-diameter cylindrical aluminium probe was used in a double compression test, penetrating to 50% of the initial height at a speed of 2 mm/s, with a 30-s delay between the first and second compression and the measurements were realized on the centre of cake slices. From each cake batch, two cakes were sliced into slices of 20 mm in thickness and the three central ones of each cake were analysed. Hardness (N), cohesiveness and springiness were calculated from the TPA graphic.

Colour was measured using a Minolta CM-508i spectrophotometer (Minolta Co., Ltd, Japan) with the D65 standard illuminant and the 2º standard observer. Crust colour was measured at five points of the surface of two cakes from each elaboration. Crumb colour was obtained from the measurements of two slices of two cakes from each preparation. Both results were expressed in the CIELAB colour space with the lightness coordinate L^*, a green-red-oriented coordinate a^* ($-a^*$: greenness, $+a^*$: redness) and a blue-yellow-oriented coordinate b^* ($-b^*$: blueness, $+b^*$: yellowness) (Witt, 2007).

The measurement of cake characteristics was carried out 24 h after baking.

2.2.4. Consumer test

Sensorial evaluation of cakes was completed by 100 volunteers, who were from 16 to 65 years of age and usual consumers of cakes. Cakes were divided into pieces of 2 cm wide and presented on white plastic plates coded with four-digit numbers and served in random order. One entire cake was presented on the principal table for its appearance to be evaluated. The cakes were evaluated based on consumer acceptance of their appearance, odour, texture, taste and overall acceptability. This evaluation was completed by using a hedonic scale of 9 points. This scale ranged from “I like very much” (9 score) and “I dislike very much” (1 score).

2.2.5 Statistical analysis

The results obtained were assessed by an analysis of variance (one-way ANOVA) with the Fisher’s least significant differences (LSD) test and with significance level of 95% ($p<0.05$). Statistical analysis was completed using Statgraphics Centurion XVI software (StatPoint Technologies Inc, Warrenton, EEUU).

3. Results and Discussion

3.1. Batter characteristics

Batter characteristics are shown in Table 1. In general, the incorporation of rice and egg white proteins reduced the density of the batters. Considering batter microstructure (Figure 1), the incorporation of rice protein increased the size of bubbles due to evident coalescence phenomena, above all with 45% incorporation of protein. In the case of batters with egg white protein, the addition of this protein gave rise to the highest amount of bubbles with the lowest size because of its very good foaming properties (Richert, 1979). In both cases, these different phenomena could explain the lower density of the batter. On the other hand, pea protein had no effect on batter density. The batters with pea protein presented a greater number of bubbles, although they were smaller than the control sample. This outcome was more pronounced with higher percentage of protein. The compensation between the number of bubbles and their size lead to there are not significant differences of density in relation to control ($p>0.05$). However, whey protein exhibited a different behaviour, as low amount of protein increased the density of the batters while high amount reduced it drastically.

The increment of the protein added led to greater amount and size of bubbles, which was related to the drop of density whenever the percentage of whey protein went up. The addition of 45% whey protein increased the number of bubbles compared to control and their size was the highest, which led to the fact that this sample had the lowest density.

Table 1. Characteristics of batters and layer cakes elaborated with three percentages of pea (P), rice (R), egg white (E) and whey (W) proteins.

Sample	*Density (g/ml)*	*Viscosity (10-3 Pa·s)*	*Specific volume (ml/g)*	*Weight loss (g/100g)*
CONTROL	0.99±0.01ef	2349.0±80.61ab	2.21±0.06bcd	10.52±0.19ef
R15	0.94±0.01cd	2428.0±70.71ab	2.30±0.04def	11.79±1.00gh
R30	0.91±0.00bc	3097.5±6.36cd	2.25±0.02cde	11.13±1.17fg
R45	0.89±0.00b	3393.5±143.54de	1.99±0.01a	10.24±0.11def
P15	0.99±0.00ef	3193.5±304.76cd	2.31±0.04def	10.63±0.73efg
P30	1.01±0.01f	5447.5±635.69g	2.08±0.06ab	9.81±0.42cde
P45	0.99±0.03ef	8375.0±601.04h	2.04±0.15a	10.19±0.34cdef
E15	0.95±0.01de	2483.5±125.16ab	2.13±0.06abc	9.3±0.76bcd
E30	0.94±0.02cd	2752.5±28.99abc	2.39±0.07ef	7.71±0.19a
E45	0.91±0.02bc	4277.5±118.09f	2.33±0.01def	7.42±0.13a
W15	1.08±0.00g	2180.5±50.20a	2.44±0.06fg	9.03±0.08bc
W30	1.0±0.03f	2802.0±67.88bcd	2.57±0.05g	8.24±0.11ab
W45	0.58±0.05a	3981.5±232.64ef	3.45±0.13h	12.89±0.11h
St. Error	0.01	194.35	0.05	0.39

Data are expressed as means ± SD of duplicate assays. The numbers that appear in the sample name column indicate the percentage of protein added. The values with the same letter in the same column do not present significant differences (p<0.05).

Comparing animal and vegetal proteins, the main difference between them was the presence of coalescence phenomena, which only occurred with vegetal types. This different behaviour could be explained because whey and egg white proteins have good foaming properties, favouring bubble formation and stabilization (Richert, 1979).

Regarding viscosity, the addition of protein increased batter viscosity of all samples, but this effect was greatest with pea protein. In fact, the increment of viscosity with animal proteins only was significant ($p<0.05$) with the highest percentage of substitution. Wilderjans et al. (2008) and Ronda et al. (2011) also observed an increase in batter viscosity when increasing protein (gluten and soy, respectively) content in cake formulation. Our results also agree with those of Matos et al. (2014) who observed an increase in G' and G'' of batters with pulse proteins, while gluten and egg white protein did not modify them. Shevkani and Singh (2014) and Shevkani et al. (2015) also observed and increment of G' and G'' with the incorporation of vegetal proteins. Thus, as Majzoobi et al. (2014) reported, the increase in the water-binding capacity (WBC) of mixtures reduces the amount of free water available to assist the movement of particles in batters and, accordingly, leads to higher viscosity. This could explain the higher viscosity of the batters with vegetal proteins since this increased the WBC of the blends (Mancebo, Rodriguez, & Gómez, 2016; Shin, Gang, & Song, 2010). However, animal proteins usually reduce the WBC (Sarabhai & Prabhasankar, 2015), so the previous explanation is not applicable to these proteins. Thus, the effect of animal proteins on batter viscosity could be explained by their foaming capacity, as this was further supported by the presence of a greater amount of bubbles in batters with higher percentages of protein. In fact, it is know that the higher amount of incorporated air in batters, the greater their consistency (Torres, Hallmark, & Wilson, 2015).

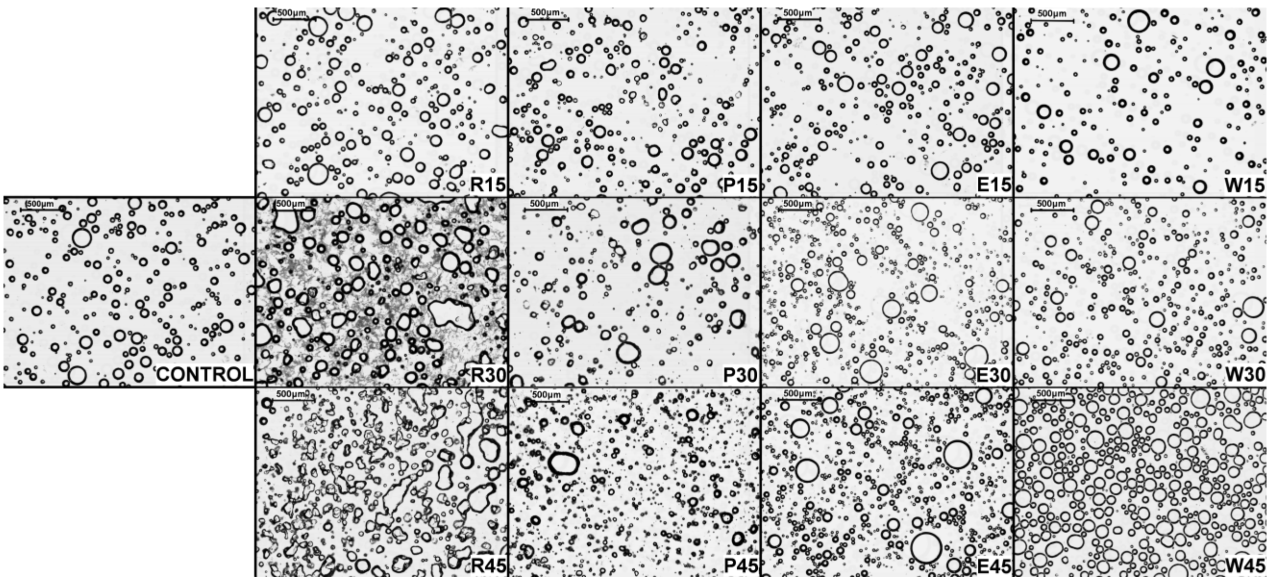

Fig. 1. Batter microstructure 100 times magnification of layer cakes elaborated without (control) and with three percentages of pea (P), rice (R), egg white (E), and whey (W) proteins.

3.2. Cake characteristics

The addition of vegetal proteins did not modify the specific volume of cakes in general, except for the highest percentage (45%), which reduced it (Table 1). Matos et al. (2014) also did not observe significant differences with 13% of pea protein ($p>0.05$), although Shevkani and Singh (2014) observed an increase in muffin volume with the incorporation of vegetal proteins. These differences could be attributed to the lower percentage of protein (10%) used by Shevkani and Singh (2014) and the fact that corn starch was used instead of rice flour, so it was a product with a lower protein content. The reduction of specific volume observed with the addition of vegetal proteins could be explained by the higher batter viscosity, and, accordingly, this effect was more significant with the pea protein. The greater batter viscosity would hinder the expansion of the bubbles during the first stage of baking, compensating for the low batter density with rice protein, and thus the incorporation of air. Nevertheless, it is important to consider that when substituting a part of the flour with protein, the starch content in the phase surrounding the bubbles decreases, and therefore, the consistency of this phase after starch gelatinization would also be reduced. This causes a greater mobility of bubbles in the last stage of baking (coalescence phenomena), generating bubbles with higher size and making it possible for them to escape out of the product. This effect was observed in the complementary videos (Video 1) and the Figure 1. In the case of animal proteins, a contrary effect was observed, asthe higher the protein substitution percentage, the greater the specific volume of cakes. The increasing effect was more significant with 45% whey protein, which is the cake with the highest specific volume, and this fact would be related to the lowest batter density as well as the distribution of bubbles However, the addition of 15% egg white protein did not produce significant differences with respect to control sample (p>0.05). Even so, Matos et al. (2014) already observed an increase in muffin volume with 13% egg white protein. This phenomenon of increased volume could be due to the stabilizing action of the bubbles by proteins. During baking, the coagulation of egg white protein (Kiosseoglou & Paraskevopoulou, 2006) and the aggregation process of whey proteins (Koo, Chung, Ogren, Mutilangi, & McClements, 2018) occurred. As egg white and whey are soluble proteins, they could surround the bubbles, stabilizing them and reducing the coalescence phenomena. In fact, in the Figure 1 and Video 1 it is observed that despite the high mobility of bubbles in the final stages of baking, once the starch is gelatinized, the bubbles do not join together and do not escape outwards.

Table 2. Textural characteristics of layer cakes elaborated with three percentages of pea (P), rice (R), egg white (E) and whey (W) proteins.

Sample	*Hardness (N)*	*Springiness*	*Cohesiveness*
CONTROL	6.38±0.21abc	0.89±0.02d	0.54±0.01f
R15	4.79±0.28a	0.85±0.01bc	0.45±0.01d
R30	5.49±0.23a	0.83±0.01b	0.4±0.04bc
R45	9.07±0.14bcd	0.74±0.02a	0.32±0.01a
P15	5.53±0.01ab	0.86±0.00cd	0.49±0.00e
P30	9.81±1.60cd	0.87±0.02cd	0.43±0.04cd
P45	12.30±2.44de	0.85±0.01bc	0.38±0.00b
E15	24.72±1.75g	0.94±0.00e	0.74±0.01h
E30	33.69±3.63h	0.95±0.01e	0.76±0.01h
E45	54.43±0.45i	0.94±0.01e	0.75±0.01h
W15	11.27±0.30de	0.94±0.00e	0.67±0.01g
W30	20.63±2.80f	0.93±0.01e	0.67±0.00g
W45	13.79±1.53e	0.93±0.01e	0.64±0.04g
St. Error	1.17	0.01	0.01

Data are expressed as means ± SD of duplicate assays. The numbers that appear in the sample name column indicate the percentage of protein added. The values with the same letter in the same column do not present significant differences (p<0.05).

In different studies, a positive correlation between specific volume and weight loss was observed (de la Hera et al., 2013), because of a higher surface in contact with air (Zhou & Therdthai, 2008). In our study, this trend was observed in cakes with vegetal proteins but not with animal proteins. In these cases, the higher surface of contact could be compensated for by differences in the internal structure of cakes, with finer cell grain, complicating the exit of water from the interior of the cakes.

Regarding texture (Table 2), the cakes with vegetal proteins followed a trend observed in other studies (de la Hera et al., 2013), in which the lower the specific volume, the higher the cake hardness. However, only the cakes with 45% pea protein showed significant differences with respect to control ($p<0.05$). The effect of animal proteins was very different because, in spite of their higher specific volume, increased hardness were observed in all cases, with the cakes incorporating egg white protein exhibiting the highest hardness. The effect of whey protein could be explained by Díaz-Ramírez et al. (2016). Theses authors attributed the increment of hardness with the phenomena of competition between sugar and whey protein. Due to the high solubility of whey protein, the available water for sugar solubility was lower, and crystallization phenomenon was produced when sugar was exposed to heat. Thus, this crystallinity could decrease the quality of the cake by modifying the texture. In the case of cakes with egg white protein, as well as crystallization phenomenon, a process of coagulation was produced where the proteins denatured around 85 ºC, forming a protein network (Kiosseoglou & Paraskevopoulou, 2006; Wilderjans et al., 2013). This phenomenon together with the starch gelatinization would increase the viscosity of the batter during baking and impart solid character to the cake (Guy & Pithawala, 1981).

The addition of vegetal proteins also reduced the springiness and cohesiveness, with greater effect seen with the highest amount of protein and in the case of rice protein before pea protein. In fact, the cakes with 15 and 30% pea protein did not show significant differences in springiness with respect to control ($p>0.05$). On the other hand, the animal proteins presented a contrary effect, as they increased the springiness and cohesiveness of cakes. This is in agreement with Matos et al. (2014) who compared cakes with 13% of pea and egg white protein.

The results obtained for crust colour are presented in Table 3. The addition of protein reduced the lightness values, giving rise to darker cakes than control sample. However, only the samples with animal proteins and the highest percentage of pea protein presented significant differences with respect to control ($p<0.05$). These samples also presented higher a^* values (reddish tones), while no clear trend of b^* values was observed. The lower values of L^* align with other studies related to the addition of proteins in cakes (Majzoobi et al., 2014; Subagio & Morita, 2008). In general, the colour of crust colour is due to Maillard reactions, which occurs between reducing sugars and amino groups during the baking process (Pérez, Matta, Osella, de la Torre, & Sánchez, 2013). Thus, the greater amount of protein increased amino groups' availability, increasing the colour development. Among amino acids capable for reacting with reducing sugar, lysine has an important role because its ε-amino groups are a source of free amino groups (Pérez et al., 2013; Tamnak, Mirhosseini, Tan, Ghazali, & Muhammad, 2016). In this way, the differences of lightness between proteins could be explained by the different lysine content of each one.

Table 3. Crust colour parameters of layer cakes elaborated with three percentages of pea (P), rice (R), egg white (E) and whey (W) proteins.

Sample	*Crust L**	*Crust a**	*Crust b**
CONTROL	67.50±1.56e	7.69±1.58ab	30.94±3.87b
R15	66.07±0.77e	8.20±1.82ab	31.03±4.29b
R30	68.46±0.97e	6.17±2.52a	29.62±1.42b
R45	63.71±2.48de	10.92±3.01b	28.62±0.99b
P15	63.72±2.50de	11.0±2.59bc	28.87±2.86b
P30	63.48±1.68cde	11.46±2.16bcd	25.56±1.93ab
P45	57.38±3.60abcd	15.02±1.78de	25.19±4.27ab
E15	52.69±6.94a	16.5±0.99e	26.67±9.09ab
E30	55.43±1.87ab	17.79±0.77e	27.05±0.15b
E45	55.80±5.17abc	17.90±0.19e	22.74±1.02ab
W15	53.98±5.94a	16.6±1.56e	28.04±3.80b
W30	52.14±4.57a	16.61±1.68e	18.51±5.75a
W45	62.45±1.75bcde	14.95±1.02cde	27.98±1.45b
St. Error	2.56	1.30	2.78

Data are expressed as means ± SD of duplicate assays. The numbers that appear in the sample name column indicate the percentage of protein added. The values with the same letter in the same column do not present significant differences (p<0.05).

3.3. Consumer test

After physical measurements, cakes with 30% of protein were chosen to be evaluated by consumers (Table 4). Cakes with animal proteins did not present significant differences compared to control on appearance score ($p>0.05$), whereas the addition of vegetal proteins reduced it ($p<0.05$). As it could be observed in Figure 2, the addition of vegetal protein led to an irregular surface of the cakes, reducing the appearance score. Moreover, as the results of colour test showed, the crust of cakes with animal proteins was more brown and this could improve the appearance score, resulting in similar values with respect to control sample. As for the odour and taste, the panelists considered that the incorporation of proteins made these parameters worse, although the samples with whey protein obtained scores similar to control. Maillard reactions affect the flavour of products (Zeng, Bai, Zhu, & Dong, 2017), thus this effect together with the sole taste of protein could have caused the lower taste scores of enriched cakes. Regarding texture, all enriched samples obtained lower scores than control and those with rice and egg white protein received the worst evaluation scores. This could be explained in relation to the results from the texture test, in which samples with egg white protein obtained the highest hardness and those with rice protein were the least cohesive. Control cakes were valued with the highest overall acceptability. Among protein-enriched cakes, those with whey protein presented the best evaluation, so it seems that the smell and taste have great importance in the global assessment of foods, something already observed in other studies (Pérez et al., 2013).

Table 4. Consumer test results of layer cakes elaborated with 30% of pea (P), rice (R), egg white (E) and whey (W) proteins.

Sample	*Appearance*	*Odour*	*Texture*	*Taste*	*Overall acceptability*
CONTROL	6.83±1.19b	6.8±1.31d	6.74±1.68d	6.65±1.50e	7.02±1.42d
R30	5.72±1.67a	4.55±2.03a	4.86±2.19b	3.49±1.93a	4.09±1.85a
P30	5.78±1.64a	5.34±1.83b	6.02±1.84c	4.73±1.95c	5.33±1.71b
E30	6.94±1.17b	4.78±1.61a	3.71±1.75a	4.02±1.89b	4.45±1.64a
W30	6.79±1.47b	6.01±1.51c	5.62±1.78c	6.07±1.82d	6.07±1.59c
St. Error	0.15	0.17	0.19	0.19	0.17

Data are expressed as means ± SD of duplicate assays. The numbers that appear in the sample name column indicate the percentage of protein added. The values with the same letter in the same column do not present significant differences (p<0.05).

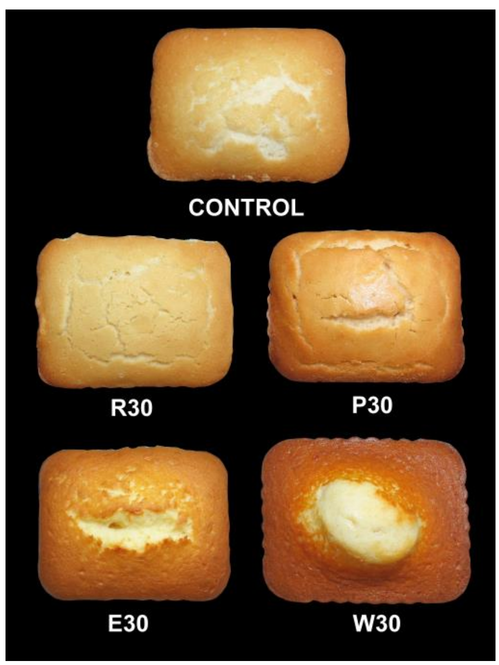

Fig. 2. Images of cakes without (control) and with 30% of pea (P), rice (R), egg white (E) and whey (W) proteins.

4. Conclusion

In general, it can be concluded that layer cakes can be a good vehicle for protein enrichment and that up to 45% of their flour can be substituted by protein. However, protein type has an important influence on the final quality of the product. Specifically, animal proteins improve cake volume and generate harder crumb, while vegetal proteins give cakes similar to the control but with crumbs less cohesive and elastic. Therefore, it would be interesting to investigate the effect of mixtures of vegetal and animal protein in the future. Regarding acceptability of enriched layer cakes, the ones with whey proteins received the highest rating. In this research, it was proven for the first time, by microscopy analysis, that, although vegetal proteins yield batters with air bubbles that are less stables to heating, animal ones (whey and egg) help to stabilize these bubbles.

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