RHEOLOGICAL AND TEXTURAL PROPERTIES IN A BAKERY PRODUCT AS A FUNCTION OF THE PROPORTIONS OF THE EGG YOLK FRACTIONS: DISCUSSION AND MODELLING.

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Abstract

With the aim of widening our knowledge about the structural changes induced by the components of egg yolk granules and their relevance in baked goods, rheological and other physical parameters have been studied in a real food system and correlated with the proportion of plasma/granules added in each case.

For this purpose, the whole egg yolk content was progressively substituted by its high protein-content granular fraction in a muffin recipe until a 100% granular recipe was obtained. Five different formulas, corresponding to different plasma/granules ratios, were used and another formula with 100% granules and containing mono- and diglycerides of fatty acids (E471) was tested too.

Flow curves at 25 °C and mechanical spectra at 90 °C were obtained for each substitution in order to evaluate the effect of the granules on the structure of the batter. In addition, other physical parameters of the baked muffins, such as hardness, were determined. The effects of the granular fraction on the aeration of the batter and on the baked muffins were also assessed by means of image analysis.

The progressive addition of granular proteins resulted in a non-linear increase in the consistency coefficient, the strength of the interactions and the hardness of the baked muffins, particularly from a plasma/granules ratio lower than 0.75. The addition of emulsifiers reverted the effects observed. These results suggest that the desirable effects of the egg yolk on the textural properties and shape of the muffin are due mainly to the effect of the plasma fraction.

Keywords

Egg yolk; granules; muffins; rheology; texturometry; additives.

1. Introduction

Egg yolk is a key ingredient in many food products, such as, for example, in sweet bakery formulations. These bakery products have a high fat content, which requires the addition of ingredients that act as emulsifiers, this being one of the main characteristics of egg yolk (Mine, 2002). Another functional property of egg yolk is the ability of its proteins to coagulate and thus form gels that may affect the texture and other qualities of cakes (Paraskevopoulou & Kiosseoglou, 1997). Furthermore, the influence of egg yolk on other cake parameters such as colour, flavour and appearance is well known.

Egg yolk can be easily separated into two fractions: plasma and granules (Laca, Paredes, & Díaz, 2010). The plasma fraction contains around 75-81% of egg yolk solids (Le Denmat, Anton, & Beaumal, 2000). It is composed mostly of low density lipoproteins (LDL) which are spherical particles with a core rich in triglycerides and cholesterol esters, and externally covered by a monofilm of phospholipids and apoproteins (Nakamura, Hayakawa, & Sato, 1977). In this fraction, the content in lipids and cholesterol is high.

On the other hand, the granular fraction contains mainly high density lipoproteins (HDL). These HDLs provide 70% of the granular content (McCully, Mok, & Common, 1962) and show a globular-like protein folding. Furthermore, these HDLs form a supramolecular structure with themselves and with phosvitin through phosphocalcic bridges, which can only be dissolved in media with high ionic strengths (>0.5 M NaCl) (Anton & Gandemer, 1997; Burley & Cook, 1961; Causeret, Matringe, & Lorient, 1991). These granular aggregates have a variable composition, forming different granule subclasses with different sedimentation behaviour (Strixner & Kulozik, 2013). Additionally, this fraction shows a high protein content and low levels of cholesterol, and it has functional properties that could allow its use as a whole egg yolk substitute in foods such as mayonnaises, having a cholesterol content-lowering effect (Laca, Sáenz, Paredes, & Díaz, 2010).

It is also remarkable that these two fractions have a noticeably different microstructure and composition, resulting in different viscosity profiles at high temperatures (Ulrichs & Ternes, 2010) and consequently, their behaviour during the heating of egg-based products should be different too.

In previous research, the total substitution of the egg yolk by its granular fraction in a gluten-free muffin recipe was studied (Marcet, Paredes, & Díaz, 2014). However, how the different parameters vary with different plasma/granules ratios (P/G), and therefore to what extent these muffin parameters are affected by each sub-fraction remains unstudied. Hence, the aim of this work is to study, in a muffin recipe used as a model for an egg-based bakery product, the effect of different plasma/granules ratios on the structure of the batter and the baked product. Furthermore, the study of a muffin recipe made with only egg yolk granules and with food additives (E471) to reverse the effect of the egg yolk granules on the physical properties of the batter and of the muffins was also performed. Finally, the rheological and other physical parameters were modelled for a better understanding of the described phenomena.

2. Materials and methods

2.1. Obtaining egg yolk granules

Egg yolk and albumen were manually separated and the albumen residuals were eliminated from the yolk employing blotting paper (Laca, Sáenz, Paredes, & Díaz, 2010). Egg yolk material was mixed with water (1:1.5 v/v) and the pH of the diluted egg yolk was adjusted to 7 using NaOH (1 N). Then, it was kept overnight at 4 ^{o}C and centrifuged later at 10000 x g for 45 min to separate it into plasma and granules fractions.

2.2. Muffin preparation

The muffins were prepared and baked according to a traditional muffin recipe. The basic formulation includes 100 g of wheat flour containing 10.32% proteins and 1.2% lipids; 3 g of baking powder containing disodium diphosphate (E-450i), sodium bicarbonate (E-550ii), sodium carbonate (E-500i) and calcium sulphate (E-516); 65 g liquid pasteurized egg white; 35 g of fresh egg yolk; 100 g of refined sunflower oil and 100 g of sugar. All the ingredients were acquired from a local market.

Egg yolk and egg white were whipped for 3 min employing a 180 WATT hand blender (Morphy Richards HB01 Hand blender, UK) at maximum speed. Sugar and oil were added and mixed for 2.5 min with a 200 WATT mixer (Severin eletrogeräte, Germany), adjusting the speed control to level 3. Finally, flour and baking powder were added and mixed for 1.5 min using the same 200 WATT mixer. The batter was poured manually into the paper muffin cups, weighing out 38 g of batter each time. The muffins were always placed in the same place in a conventional oven, and baked for 24 min at 180 °C.

Six different formulas of muffins were then prepared. As shown in Table 1, in each muffin the whole egg yolk (35 g) was progressively substituted by the granular fraction, which produces different plasma/granules ratios (P/G). Furthermore, in preparation P/G 0(+E471), 2.5% (w/w flour) of the emulsifier E471 (mono- and diglycerides of fatty acids) were added. The amount of emulsifier was previously tested at 0.5%, 1.5% and 2.5% (w/w flour), obtaining the best performance at 2.5% (data not shown). This emulsifier is a food grade additive and it is used in the industry to improve the mixing of the ingredients and the sponginess of the bakery products.

Table 1.

2.3. Specific gravity (SG) and image analysis of the batter

The specific gravity of each sample was calculated in duplicate as follows. A standard container was filled with batter and its weight was recorded and divided by the weight of the same container filled with water. Regarding the image analysis of the batter, a method similar to that of Gómez, Ruiz and Oliete (2011) was carried out. An amount of fresh batter was placed on a microscope slide and a cover slip was used to create a thin layer of preparation. To maintain the same thickness in each case, two paperclips were used between the slips. The micrographs of the batters were obtained using an Olympus BX50 light microscope with 10x magnification. The number of bubbles was calculated in a surface of 4 mm^2 of raw batter using the ImageJ software. Furthermore, bubbles were distributed in three groups according to their size.

2.4. Rheological properties of batter mixes

Batters were kept for 60 min at 25 ^oC before the rheological test. Rheological properties of batters were determined with a Haake MARS II rotational rheometer using a Peltier unit to control the temperature. A plate/plate measuring system (PP60) was used with a gap of 1 mm where samples were left for 25 min to relax stress and stabilize the temperature. The excess of sample was removed, and a glass hood and silicone oil were employed to protect against dehydration during the experiment. Rheological measures were calculated in duplicate for two different batches.

2.4.1. Flow properties

Flow properties were measured at 25 ^{o}C. Apparent viscosity was obtained as a function of shear rate from 0.01 1/s to 100.0 1/s. The experimental time was adjusted to 300 sec. 100 points were collected with a logarithmic distribution and two flow curves of different batches of every formulation were obtained. Duplicates presented differences lower than 10%. Data obtained were adjusted to the Ostwald model (Martinez-Cervera, Salvador, Muguerza, Moulay, & Fiszman, 2011)

$$\eta = K\ \dot{\gamma}^{\,n-1} \qquad (1)$$

Where η is the apparent viscosity, $\dot{\gamma}$ is the shear rate, K (Pa.s^{n}) is the consistency coefficient, and n is the flow behaviour index.

2.4.2. Dynamic tests

Frequency dependence tests were conducted at 90 ^{o}C in the range of linear viscoelasticity. The frequency range tested was from 10.0 Hz to 0.01 Hz. The experimental data obtained were adjusted to the following power law equation according to Gabriele, de Cindio and D'Antona (2001).

$$G^{*} = \mathbb{A}\cdot\omega^{1/z} \qquad (2)$$

Where G* is the complex modulus in Pa. The complex modulus is a measure of the overall resistance of the batter to deformation, ω the frequency in Hz, Z (dimensionless) the coordination number and $\mathbb{A}$ the proportional coefficient (Pa).

2.5. Physical measurements of baked muffins

Physical measurements were made on 8 muffins from 2 different batches.

2.5.1. Height and middle section area of baked muffins

Baked muffin height was calculated from the highest point to the bottom employing a calliper, after 3 h of cooling at room temperature. To calculate the middle section area the upper half was removed and the muffin diameter was measured at the mould level. The area of the muffin surface at the cut level was calculated.

2.5.2. Air cell number determination

Muffins were cut at the mould level, the higher half was removed and an image was obtained of the crumb using a flatbed scanner (Model HP PSC 1610, Hewlett Packard, USA). Air cell number was calculated from a 4 cm side square surface by image analysis using the ImageJ software.

2.5.3. Texture profile analysis (TPA)

Texture analysis was performed with a TA.XT.plus Texture Analyser (Stable Microsystems, UK). Two centimetres of the lower half of each muffin was evaluated; the upper half was discarded. Texture parameters were set at 1.0 mm/s, and a double compression of 50% of the original height was carried out with a flat-ended cylindrical probe (P/75). The parameters obtained from the TPA graph were hardness, springiness, cohesiveness, chewiness and resilience.

Hardness is the force necessary to produce a given deformation. It was calculated with reference to the TPA graph as the maximum value in the first compression cycle. Springiness is the recuperation rate from a deformation after the applied force has been removed, and it was calculated as the distance on the time axis between the point where the probe begins to exert a force and the peak detected in the second compression, divided by the corresponding distance in the first bite. The cohesiveness parameter shows how much the material can be deformed before it ruptures. The cohesiveness value was obtained by dividing the total area of the second compression cycle by the total area of the first compression cycle. Chewiness is the energy required to disrupt a solid material until it is ready for swallowing. It was calculated as the product of the hardness and the springiness. Finally, resilience is the initial effort of a product to recover its original position. It was measured from the TPA graph by dividing the area during the withdrawal by the area of the first compression.

2.6. Statistical analysis

Analysis of variance (ANOVA) was applied. Least significant differences (LSD) were calculated by Fisher´s test to determine significant differences among the tested samples. Pearson correlation coefficients (r) for the relationships between all properties were also calculated. These analyses were performed using the statistical software Statgraphics® v.15.2.06. Fittings of the experimental data to models were carried out using Micromath Scientist® Software.

3. Results and discussion

3.1. Raw batter

3.1.1. Flow curves, specific gravity, batter image analysis

Flow curves centred in the power-law region (shear rate from 0.01 to 100 1/s) are shown in Figure 1. In this figure, it can be observed how the viscosity decreased within the shear rate range for each formulation, which indicates the pseudoplastic behaviour of all the batters. Furthermore, differences between the samples were detected when the flow curves were fitted to the Ostwald model. All the data were successfully fitted ($r^2 > 0.99$), and the consistency coefficient (K) and the flow behaviour index (n) obtained are shown in Figure 2. The flow behaviour index (n) is related to the degree of structuring of the sample; values close to 1 are a feature of newtonian fluids, while lower values are related to more structured samples. In the batters tested, no significant statistical differences were detected between the flow behaviour index values, showing that all the samples had similarly entangled structures and resistances to flow.

Figure 1.

Figure 2.

The consistency coefficient (K) is related to viscosity, since changes in K values correspond to changes in the apparent viscosity (Rao & Kenny, 1975). In the tested muffin formulations, it was observed that the higher the degree of egg yolk substitution by granules, the higher were the K values (Figure 2), which could be a positive indication about the improvement in the capacity of the batter to retain air during baking when the plasma/granules ratios are low (Tan, Chin, Yusof, Taip, & Abdullah, 2014). Furthermore, in other investigations into fat and flour replacements in cakes, the decrease in the apparent viscosity caused by the variations in the formulations has been associated with a more consistent crumb and smaller cake size (Baixauli, Sanz, Salvador, & Fiszman, 2008; Lakshminarayan, Rathinam, & KrishnaRau, 2006). This is probably because low viscosities correspond to unstable batters, which encourage the buoyancy of the bubbles and their release to the surrounding atmosphere.

Focussing now on the specific gravity data shown in Figure 3, this quality is related to the air retention capacity of the batters during mixing of the ingredients, with high values indicating poor air retention. This decrease in the air retention of the batters with a decrease in the P/G ratio was also confirmed by image analysis (Figure 1S). In the muffin batters, the incorporation of bubbles is promoted by the batter whisk during the mixing of ingredients. Thus, the size of the bubbles and their number depend on the mixing time and energy. In Figure 3, it can be observed that the reduction in whole egg yolk content and its substitution by granules resulted in a gradual decrease in the total number of bubbles, mainly due to the reduction in the number of the smallest ones (Table 1S). The smallest bubbles in the raw batter provide nucleating sites for the CO_2 produced by the baking powder, and after starch gelatinization and protein denaturation, they give rise to the porous crumb. On the other hand, the biggest bubbles are more susceptible to coalescence phenomena, and they tend to disappear during cooking. Therefore, the reduction in the population of the smallest bubbles should have some effects on the muffin texture and size.

All these changes in the rheology of the batters and in the air retention during the mixing of the ingredients can be explained, at least partially, by considering the limited capacity of the granular protein to occupy the oil-water and air-water interfaces in comparison with the proteins from the plasma fraction (Anton & Gandemer, 1997), taking into account that it is the egg yolk proteins which have an important role in foam formation and stabilization (Cauvain & Young, 2008).

In a simplified model made with water and oil, similar emulsifying properties were attributed to the plasma and the granular fraction, but the granular fraction had to be dissolved in 0.55 M NaCl previously (Anton, Beaumal, & Gandemer, 2000; Le Denmat, Anton, & Beaumal, 2000). In distilled water, the plasma fraction was found to be a better emulsifier, since the apoproteins of LDL have a labile structure that can be easily disorganized when they are in contact with the surface of the oil droplets (Anton, Martinet, Dalgalarrondo, Beaumal, David-Briand, & Rabesona, 2003; Martinet, Saulnier, Beaumal, Courthaudon, & Anton, 2003; Mine, 1997, 1998). Furthermore, these LDL apoproteins are very flexible and hydrophobic, and can effectively occupy the oil-water interface, enhancing their surface-activity (Kiosseoglou & Sherman, 1983). According to Anton et al. (Anton, Beaumal, & Gandemer, 2000) if the appropriate amount of NaCl is not added, the granules can be adsorbed to the oil-water interface, but in their native form. These native granules have decreased emulsifying properties in comparison with the previously dissolved granules, the latter producing finer and more homogeneous dispersions. In accordance with the results obtained, it is likely that the formulation of the muffins does not produce the optimal dissociation of the egg yolk granules, resulting in a poorer emulsifying performance of the granular fraction in comparison with the egg yolk plasma.

Furthermore, and together with the different emulsifying properties of the egg yolk fractions, the increase in the K values associated with the progressive addition of granules could be enhanced by protein interactions that increase the viscosity in the batter system, since structural associations are related to increments in this parameter (Damodaran, 1997; Meyers, 1989).

Figure 3.

3.1.2. Mechanical spectra at 90 °C

Stress sweeps at 90 °C were carried out previously to verify the lineal viscoelastic range of the batters (data not shown).

When the batter is heated in the oven above the starch gelatinization onset temperature, its rheological properties undergo great changes. In these conditions the batter constituents, mainly the flour starch and proteins, as well as the egg proteins, cause a rise in the viscosity, with a paste being formed. Therefore, the interactions between biopolymers at high temperatures are different to those observed for temperatures lower than the gelation temperature. In a previous study (Marcet, Paredes, & Díaz, 2014), it was shown that at 25 °C the substitution of all the whole egg yolk by the granular fraction in a muffin recipe has no effect on the viscoelastic properties of the raw batter. For this reason, and in order to evaluate the rheological effect of the granular lipoproteins on the structure of the baked muffins at different plasma/granules ratio, mechanical spectra were obtained at 90 °C (Figure 4).

Figure 4.

In Figure 4a) and 4b), within the frequency range from 0.1 to 10 Hz the batter behaviour corresponds to a soft-gel, with a slight frequency dependence of G´and G´´, and tan δ values are higher than 0.1. In addition, in the frequency range studied it was observed that values for G´ were higher than for G´´ for all the formulations. This behaviour is typical of cross-linked polymer networks. The substitution of egg yolk by granules causes an increase in the degree of compaction, which results in a stronger and more structured batter. In fact, the highest viscoelastic modulus belongs to preparation P/G 0, while the unsubstituted recipe (P/G 3.5) showed the lowest degree of structuring. So, the results revealed a positive effect of the granules, since a batter with a high viscosity and elasticity at high temperatures is commonly associated with a better baking process. In this sense, a lower degree of structure has been associated with batter collapse during cooking (Sahin, 2008; Shelke, Faubion, & Hoseney, 1990).

Loss tangent values show the relative contributions of the elastic and viscous moduli to the batters' characteristics (Figure 4c). In the tested formulations, the progressive substitution of egg yolk led to a reduction in tan δ values, and therefore, to an increase in the relative importance of the elastic modulus over the viscous one. This confirms the structuring effect of the granular protein, with the system behaviour approaching that found in solids (tan δ = 0). Therefore, the substitution changed the viscoelastic behaviour of the batters.

Data obtained from the mechanical spectra at 90 °C were adjusted to equation 2 and presented in Figure 5. The $\mathbb{A}$ parameter indicates the strength of the interactions in the network of the soft-gel, whereas the Z value is related to the number of rheological units.

Figure 5.

The progressive decrease in the P/G ratio caused by the substitution of egg yolk by granules, produced an increase in the strength of the interaction in the batters ($\mathbb{A}$) as well as an increase in the network extension (Z). At 90 °C the gelation of the egg yolk protein and the starch is expected. However, the gelation characteristics of the emulsions using whole egg yolk are different to those obtained using granules in their formulations: the use of the granular fraction produces a strengthening of the heated-emulsion gels compared with those made with whole egg yolk only. According to Anton et al. (Anton, Le Denmat, Beaumal, & Pilet, 2001) the LDL apoproteins of the plasma fraction orientate their hydrophobic residues to the oil-water interface. Thus, these are not available to interact with the polymers of the gel matrix, behaving as an inactive filler (Van Vliet, 1988) and limiting the strengthening of the gel. However, when the granular fraction was tested, the opposite effect was obtained. A possible explanation for this behaviour is that the phosvitin that remains in the gel matrix could interact through its hydrophobic and phosphor-seryl residues with the HDL which are present in the oil-water interface. These interactions would produce an increase in the strength of the interactions in the batters ($\mathbb{A}$) and in the network extension (Z), this egg yolk fraction behaving as an active filler. Furthermore, in the complex food matrix of the muffins, these interactions between proteins could be disturbed by the presence of other ingredients. As can be observed in Figure 5, a significant degree of substitution of the whole egg yolk by granules was required to obtain a noticeable increase in the strength of the interactions (preparations P/G 0.3 and P/G 0).

3.2. Baked muffins

3.2.1. Physical properties and image analysis

Physical measurements for baked muffins are presented in Figure 6. As regards the height of the muffins (Figure 6a), it increased as the level of egg yolk substitution rose. This means that the 100% granules recipe (P/G 0) gave the highest muffins, while the 75%-25% egg yolk-granules mix (P/G 1.6) and the 100% egg yolk formulation (P/G 3.5) gave the lowest ones. This increase in the height was statistically significant, although the difference between the highest and the lowest is around 6 mm, being barely perceptible to the eye. Regarding the surface area of the central section of the muffin, the addition of granules produced a decrease in this parameter, as can be observed in Figure 6b. This suggests crumb compaction.

To confirm this, crumb images were taken (Figure 2S). These images showed changes in the distribution of the air cells that can be visually appreciated, particularly from preparation P/G 0.75 to P/G 0. To characterize the air cell population (Table 1S) and the total number of bubbles (Figure 6c), software image analyses were carried out in the baked muffins for each formulation. Regarding the smallest and most numerous air cells, their numbers were progressively reduced when the granule content increased, which also caused a reduction in the total number of air cells. This decrease in the number of bubbles has undesirable effects on the textural parameters of the bakery products (Bennion, Bent, & Bamford, 1997).

These results are in accordance with the number of bubbles in raw batter shown in section 3.1.1. In conclusion, a higher proportion of egg yolk granules produced muffins with less middle section surface area, more compact crumb and probably with a harder texture.

Figure 6.

3.2.2. Texture profile analysis

The hardness values obtained from the texture profile analyses of baked muffins are shown in Figure 7. As can be observed, the addition of granules from egg yolk produced a large increase in muffin hardness, specifically in the 100% granules preparation (P/G 0), where the hardness was more than twice the value obtained for the 100% egg yolk preparation (P/G 3.5). In the preparation of gluten-free muffins, this textural parameter changes considerably, depending on the protein source used in the recipe (Matos, Sanz, & Rosell, 2014), so in this case, the hardening of the muffins is a tendency that can be associated with the increase in the amount of granular lipoprotein from recipe P/G 3.5 to recipe P/G 0. Furthermore, the hardness parameter is affected by the degree of aeration of the baked batter too (Handleman, Conn, & Lyons, 1961b) and the increase in the granular lipoproteins together with the decrease in the plasma lipoproteins affects the aeration of the muffins, as is shown in Figure 3 and 6c. Finally, the increase in the strength of the interactions ($\mathbb{A}$) related to the decrease in the P/G ratio (previously discussed in section 3.1.2) contributes to increasing the hardness of the muffins.

The other textural parameters are shown in Table 1S. Regarding chewiness, this parameter rose with each increment in the proportion of granules in the recipe, as did the hardness parameter. In broad terms, the progressive substitution of egg yolk by granules caused only small changes in the other textural parameters evaluated. Thus, with high levels of substitution, the muffins were harder and more difficult to chew.

Figure 7.

3.3. Effect of the mono- and diglycerides of fatty acids (E471) on the muffin properties

As it is shown in Table 2, the addition of this emulsifier to the 100% muffin granules recipe (P/G 0) reversed the values of the parameters studied, returning them to those found in the 100% egg yolk preparation (P/G 3.5). The mono- and diglycerides reduced the oil-water interfacial tension more than the granular protein alone did, facilitating the mixing of the ingredients, and according to Table 2, lowering the consistency coefficient (K) of the batter. Furthermore, the number of bubbles in the batter (bi) was increased too. Emulsifiers are widely used in the bakery industry because they decrease the surface tension of the aqueous phase favouring air retention (Handleman, Conn, & Lyons, 1961a) and providing gas bubble stability until starch gelatinization occurs (Turabi, Sumnu, & Sahin, 2008; Zhou, Faubion, & Walker, 2011). These facts explain the high number of bubbles found after the baking of the muffins (bf) when the E471 was added. Regarding the strength of the interactions ($\mathbb{A}$), the values of this parameter decreased after the addition of additive, being them more similar to those found in the whole egg yolk recipe. This could be explained by the displacement of the granular protein from the oil-water interface to the gel matrix, with the interfacial area preferably being occupied by the mono- and diglycerides, and limiting the active filler behaviour of the granular proteins. All these phenomena produced a softer crumb, with hardness (H), middle section area (M) and height (h) similar to those obtained in the egg yolk recipe. However, in this case and according to Marcet, Paredes and Díaz (2014) the removal of the most fat-soluble carotenoids which are contained in the plasmatic fraction leads to muffins with a slightly more yellowish crust and a crumb that is less reddish than that seen with the whole egg yolk recipe. These differences in the visual characterization of the muffin could be an interesting attribute to consider in the design of bakery products.

Table 2.

3.4. Modelling the effects of the plasma/granules ratio on the physical parameters. Relationships among the parameters obtained.

As can be seen in Figure 3S, the progressive substitution of the egg yolk by the granular fraction causes an increase in the total protein content in the formula. This increment is principally due to the granular protein (mainly HDL), while the plasma protein content (mainly LDL) is reduced. In addition, the progressive substitution produces a decrease in the total lipid content. In fact, in formula P/G 3.5, the amount of lipid provided by the egg yolk is 71% of the total egg yolk dry matter (lipids of the granular and the plasma fraction), whereas in formula P/G 0 the lipid constitutes only 42% of the dry matter and is provided only by the granular fraction. These variations in the total protein and lipid contents are the main causes of the changes in the different parameters observed in previous sections for both raw batter and baked muffin.

In order to model the effect of the plasma and granules on the different parameters, models according to equation 3 (if the parameter increases with the amount of granules) or to equation 4 (if the parameter decreases) have been proposed. Table 3 shows the values of C_i obtained in the fitting and the regression coefficients.

$$\frac{Y_{i,100\%\,substitution} - Y_i}{Y_{i,100\%\,substitution} - Y_{i,egg\,yolk}} = \frac{X}{C_i + X} \qquad (3)$$

$$\frac{Y_{i,egg\,yolk} - Y_i}{Y_{i,egg\,yolk} - Y_{i,100\%\,substitution}} = \frac{X}{C_i + X} \qquad (4)$$

Y_i being the parameters obtained in sections 3.1 and 3.2; X the P/G ratio and C_i the fitting parameter calculated for each case.

In the case of the raw batter, the strength of the interactions ($\mathbb{A}$) and the consistency index (K) values were successfully fitted to equation 3 (r^2 = 0.99) according to the plasma/granules ratio in each formula. Experimental and modelled data are compared in Figures 2 and 5. As can be seen in Figure 2, when the proportion P/G decreases from 3.5 to 0.75, the K values only increase by 20%. However, for P/G ratios lower than

0.75, small increments in the amount of the granular fraction involved considerable changes in the consistency index. The $\mathbb{A}$ values vary in a similar way to the K values. This could be explained taking into account that the LDL apoproteins have a high proportion of amphipathic α-helix chains (Anton, Martinet, Dalgalarrondo, Beaumal, David-Briand, & Rabesona, 2003). Due to this, these proteins show a high degree of flexibility and the capacity to spread at the oil-water interface, displacing other proteins (Mine & Keeratiurai, 2000). At high P/G ratios, the presence of the globular-like granular protein at the oil-water interface is probably restricted, with no significant effect on the $\mathbb{A}$ and K parameters. Regarding the hardness values, it was reported in a previous study using gluten-free muffins that the total substitution of the whole egg yolk by granules also produced a noticeable increment in the muffin hardness (Marcet, Paredes, & Díaz, 2014). As can be seen in Figure 7, the evolution of this parameter with variations in the P/G ratio can be successfully fitted to equation 3 ($r^2 = 0.99$), following the same behaviour as the strength of the interactions ($\mathbb{A}$) and the consistency index (K). Furthermore, as can be seen in Table 4, the two rheological parameters $\mathbf{A}$ and K are positively correlated with the textural parameter of hardness (H), with a correlation coefficient of $r = 0.98$ ($P < 0.01$), indicating that these rheological parameters could be used individually to predict the hardness of the baked muffins.

The number of bubbles in the batter and in the baked muffin are shown in Figures 3 and 6c respectively. These numbers also vary with the relative amounts of plasma and granules in the recipe; the higher the P/G ratio, the higher the number of bubbles both in batter and in the baked muffins. Using equation 4 in these cases (bubbles in batter, $r^2 = 0.93$; bubbles in baked muffins, $r^2 = 0.94$), it can be concluded that the number of bubbles in the raw batter and baked muffins is very sensitive to changes in P/G ratio, particularly for values of this parameter lower than 1.6 and for the raw batter. So, for a P/G ratio change from 3.5 to 0.3, 53% of the bubbles in batter were lost, this reduction being 27% in the case of the baked batter. This means that the air retention due to the granules during the mixing of ingredients is low, but their capacity to maintain the air bubbles during cooking is higher than in the case of the plasma. In addition, and as can be deduced from Table 4, there is a correlation ($r = 0.95$, $P < 0.05$) in the number of bubbles before and after the baking, since, as has been mentioned above, the bubbles in the baked muffins originate from air bubbles entrapped in the raw batter.

Regarding the parameters which describe the variations in the shape of the muffins, that is, the middle section area (Figure 6a) and the height of the baked muffins (Figure 6b), the first was successfully fitted to equation 4 ($r^2 = 0.99$) and the second to equation 3 ($r^2 = 0.94$). As can be observed in these Figures, the experimental muffin height and middle section area vary in a similar way but in opposing directions, which indicates that the muffins were becoming higher and narrower when the degree of substitution was increased. According to Table 4, the middle section area (M) is highly correlated with the strength of the interactions ($\mathbb{A}$) and the consistency index (K) ($p < 0.01$, $r = -0.97$ in the two cases), suggesting their strong inter-relationships. In a similar way, the height of the muffins and the rheological parameters are highly correlated too.

In this context, it has been emphasized that the functional properties of the egg yolk are largely due to its protein composition. So, in the plasma fraction the apo-LDLs play a major role in forming emulsions, more than the phospholipids and cholesterol (Mizutani & Nakamura, 1984, 1987). With regard to the granular fraction, as has been discussed in the previous sections, its emulsifying properties depend mainly of the aggregation state of the granular protein on the oil-water interface area. For these reasons and in order to obtain a deeper knowledge of the effect of proteins and lipids, the parameters measured in the raw batter and in the baked muffins were also successfully fitted to the granular protein/lipids and plasma protein/lipids ratios according to equation 5.

$$Y_i = J_i\left(\frac{Gp(g)}{Gl(g)+Pl(g)}\right) + B_i\left(\frac{Pp(g)}{Gl(g)+Pl(g)}\right) \qquad (5)$$

Where Gp is the granular protein content, Gl is granular lipid content, Pp is the plasma protein content and Pl is the plasma lipid content.

The values of the fitting parameters J_i and B_i obtained in each case are shown in Table 3. These J_i and B_i values reveal the weighted effect on parameter Y_i of the proteins from the plasma and the granules, respectively. That is, a high J_i value indicates that a gram of proteins from granules have a more intense effect on the parameter Y_i than a gram of proteins from plasma, and vice versa. The progressive substitutions tested in this work involve at each step the progressive reduction in plasma protein and the addition of larger amounts of granular protein. Keeping this in mind, it is possible to find equations where coefficient J_i is lower than coefficient B_i and the parameter Y_i increases with the degree of substitution, because the amount of granular protein added offsets the low value of its coefficient. This can be observed in the cases of the number of bubbles in the raw batter ($b_{i,}$ $r^2 = 0.98$), the middle section area (M, $r^2 = 0.99$), the height of the muffins (h, $r^2 = 0.99$) and the bubbles in the baked muffins ($b_{f,}$

r^2 = 0.99). Furthermore, there are other parameters with similar J_i and B_i coefficients, such as the strength of interactions ($\mathbb{A}$, r^2 = 0.99), and the hardness (H, r^2 = 0.99). In these cases, the effect of the granular protein is more evident, since the amount of granular protein added is not offset by its coefficient, and its relevance must be greater than that of the plasma protein. Finally, in the case of the consistency index (K, r^2 = 0.99), coefficient J_i is greater than coefficient B_i, so the granular protein was found to have a higher effect on this parameter than the plasma protein.

Table 3.

Table 4.

4. Conclusions

The progressive substitution of the plasma fraction by the granular fraction produces a non-linear variation in the rheological and textural parameter values of muffins. In these bakery products, a substitution of 50% of the whole egg yolk by egg yolk granules is possible without significant changes in their physical properties and with an average increase of 4 mm in the muffin height. Therefore, the granular proteins may occupy the oil-water interfacial area, modifying the physical properties of the muffins, only when the amount of plasma protein is low enough. This suggests that the plasma fraction is the egg yolk fraction which gives the textural and shape properties to these egg-based bakery products.

Furthermore, it is possible to determine the degree of substitution and to know with precision the variations produced in each case using the equations presented in this work. These findings allow us to predict the changes in the rheology and in consequence the textural and other physical parameters of the egg-based bakery products when varying the plasma/granules ratio included in their recipe.

Finally, and since the granular fraction has an significant nutritional value in comparison with the plasma fraction, a muffin recipe with only granules instead of whole egg yolk is possible, but the inclusion of an emulsifier (E471 in this case) is necessary to obtain a product that is physically similar to that obtained using whole-egg yolk.

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Table 1.

	P/G					
	3.5	**1.6**	**0.75**	**0.3**	**0**	**0(+E471)**
Egg Yolk % (w/w)[a]	100	75	50	25	X	X
Granules % (w/w)[a]	X	25	50	75	100	100
Emulsifier E471 % (w/w)[b]	X	X	X	X	X	2.5

[a] Percentage referring to the egg yolk content in the original recipe.

[b] Percentage of emulsifier with respect to flour.

Table 2.

	K (pa.s^n)	**$\mathbb{A}$ (Pa)**	**b_i**	**b_f**	**h (mm)**	**H (g)**	**M (mm^2)**
P/G 3.5	111000 (700)a	884 (49)a	297 (23)a	736 (17)a	37 (0.8)a	1018 (77)a	3981 (123)a
P/G 0	448000 (17600)b	2008 (125)b	137 (14)d	532 (42)c	43 (1)b	2394 (143)b	3094 (74)b
P/G 0 (+E471)	108000 (1815)a	1137 (87)c	282 (35)a	707 (45)ab	35 (1)c	1073 (33)a	3811 (45)c

Values are expressed as means ± SD. Different letters in the same column indicate significant differences ($P < 0.05$). K: consistency coefficient; A: strength of the interactions; bi and bf: bubbles in the raw batter and in the baked muffins; h, H and M: height, hardness and middle section area of the baked muffins respectively.

Table 3.

Y_i	**C_i**	**r^2**		**J_i**	**B_i**	**r^2**
K(pa.s^n)	0.204	0.99		312630	114547	0.99
A (Pa)	0.27	0.99		1454	2501	0.99
b_i	0.812	0.93		78	1222	0.98
b_f	1.04	0.94		360	2895	0.99
h(mm)	0.55	0.94		31.8	143	0.99
M(mm^2)	0.43	0.99		2210	16391	0.99
H(g)	0.233	0.99		1693	2890	0.99

K: consistency coefficient; A: strength of the interactions; bi and bf: bubbles in the raw batter and in the baked muffins; h, M and H: height, middle section area and hardness of the baked muffins respectively.

Table 4.

	$\mathbb{A}$	bf	bi	H	height	K	M
$\mathbb{A}$		-0.87 ns	-0.86 ns	0.98 **	0.89 *	0.98 **	-0.97 **
bf			0.95*	-0.85 ns	-0.88 *	-0.83 ns	0.94 *
bi				-0.86 ns	-0.97 **	-0.84 ns	0.94 *
H					0.89 *	0.98 **	-0.97 **
height						0.89 *	-0.94 *
K							-0.97**

ns: not significant. * $P < 0.05$; ** $P < 0.01$. $\mathbb{A}$: strength of the interactions; bf and bi: bubbles in the baked muffins and in the raw batter; h, H and M: height, hardness and middle section area of the baked muffins respectively; K: consistency coefficient.

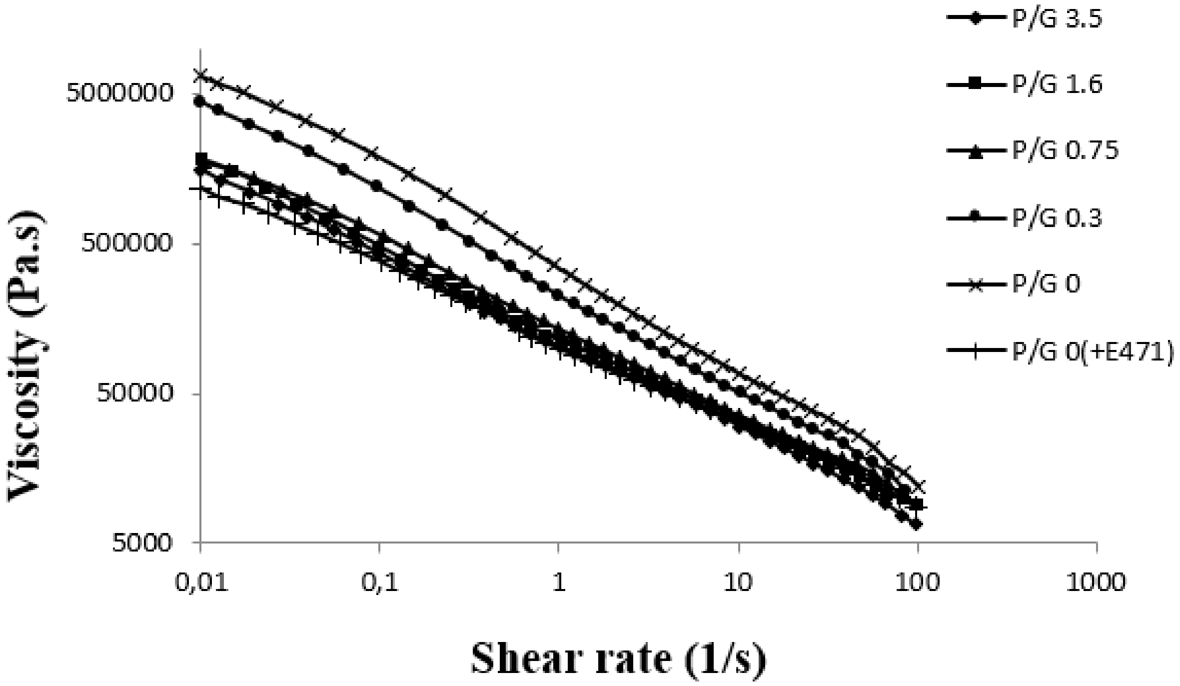

Viscosity (Pa.s)
5000000
500000
50000
5000
0,01
0,1
1
10
100
1000
Shear rate (1/s)
P/G 3.5
P/G 1.6
P/G 0.75
P/G 0.3
P/G 0
P/G 0(+E471)

Figure 1.

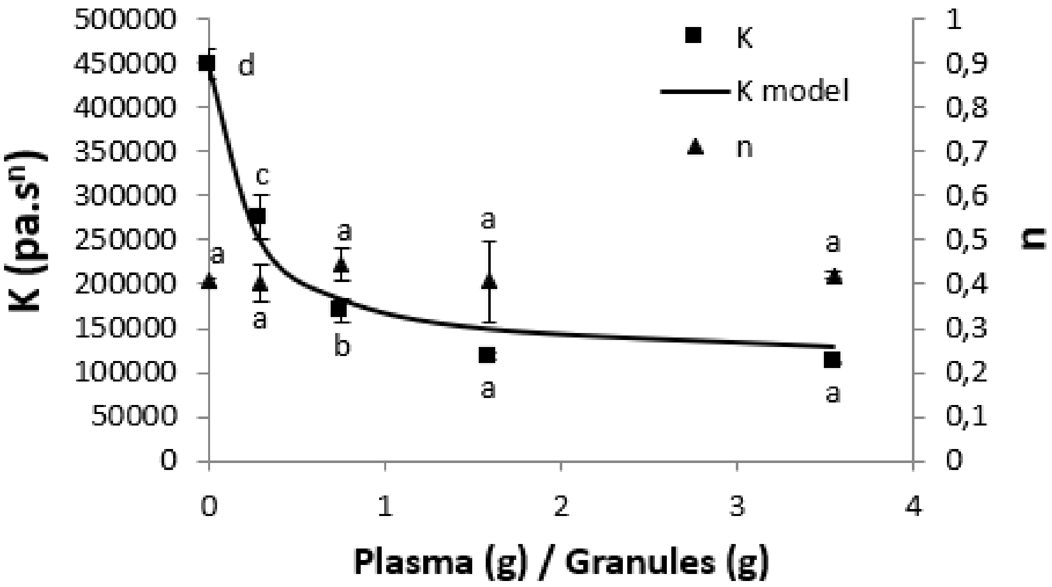
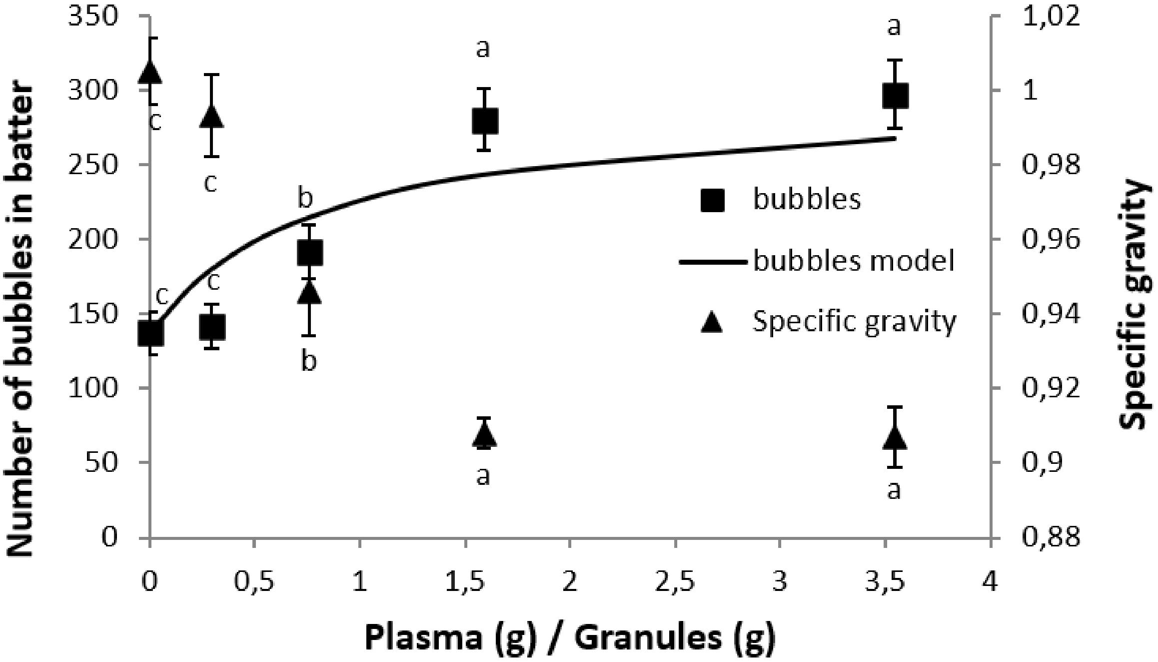

500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
K (pa.s^n)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
n
0
1
2
3
4
Plasma (g) / Granules (g)
K
K model
n
d
c
b
a
a
a
a
a
a
a
a

Figure 2.



Number of bubbles in batter
350
300
250
200
150
100
50
0
Specific gravity
1,02
1
0,98
0,96
0,94
0,92
0,9
0,88
Plasma (g) / Granules (g)
0
0,5
1
1,5
2
2,5
3
3,5
4
■ bubbles
bubbles model
▲ Specific gravity
a
a
b
c
c
c
c
b
a
a

Figure 3.

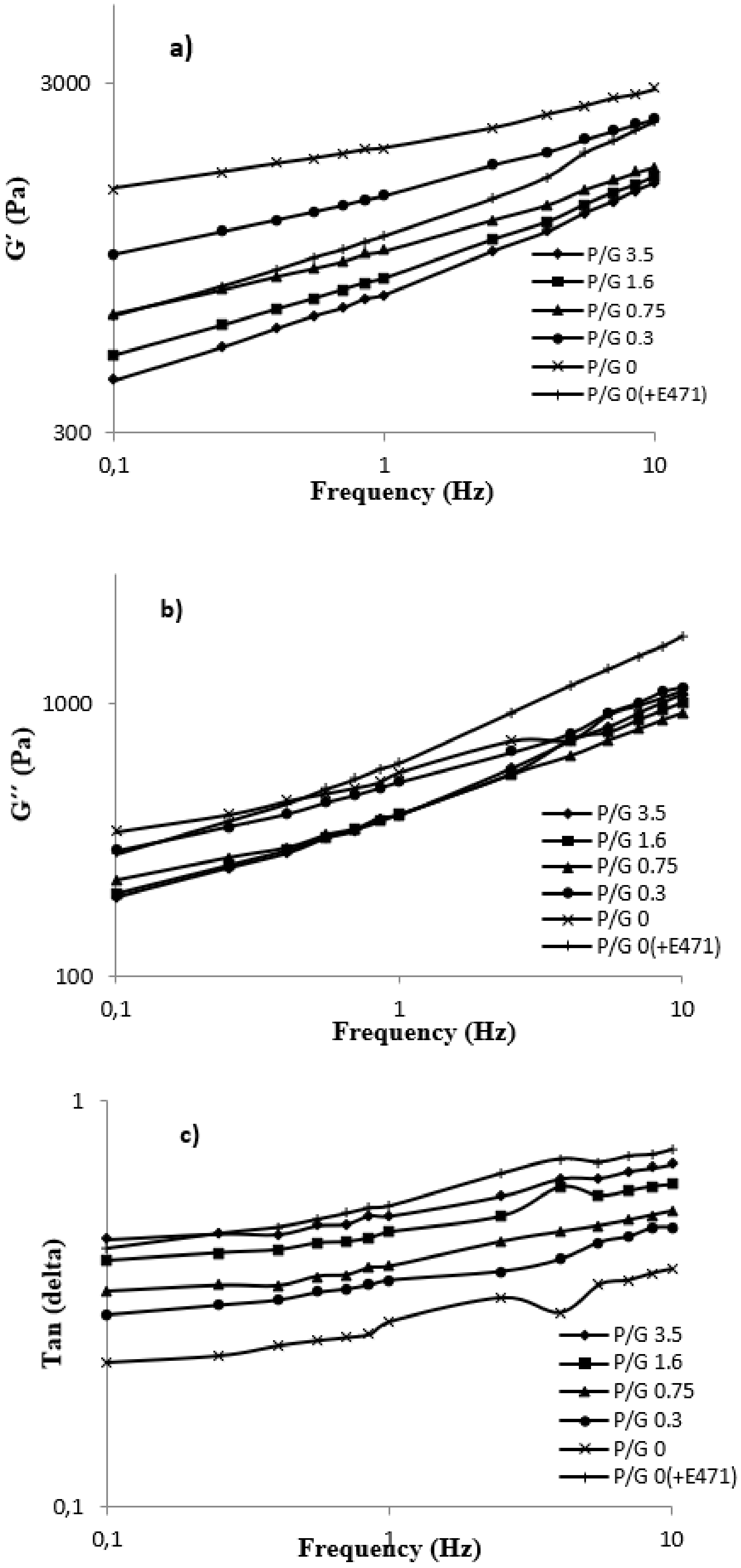

a)
G′ (Pa)
3000
300
0,1
1
10
Frequency (Hz)
P/G 3.5
P/G 1.6
P/G 0.75
P/G 0.3
P/G 0
P/G 0(+E471)
b)
G′′ (Pa)
1000
100
0,1
1
10
Frequency (Hz)
P/G 3.5
P/G 1.6
P/G 0.75
P/G 0.3
P/G 0
P/G 0(+E471)
c)
Tan (delta)
1
0,1
0,1
1
10
Frequency (Hz)
P/G 3.5
P/G 1.6
P/G 0.75
P/G 0.3
P/G 0
P/G 0(+E471)

Figure 4.

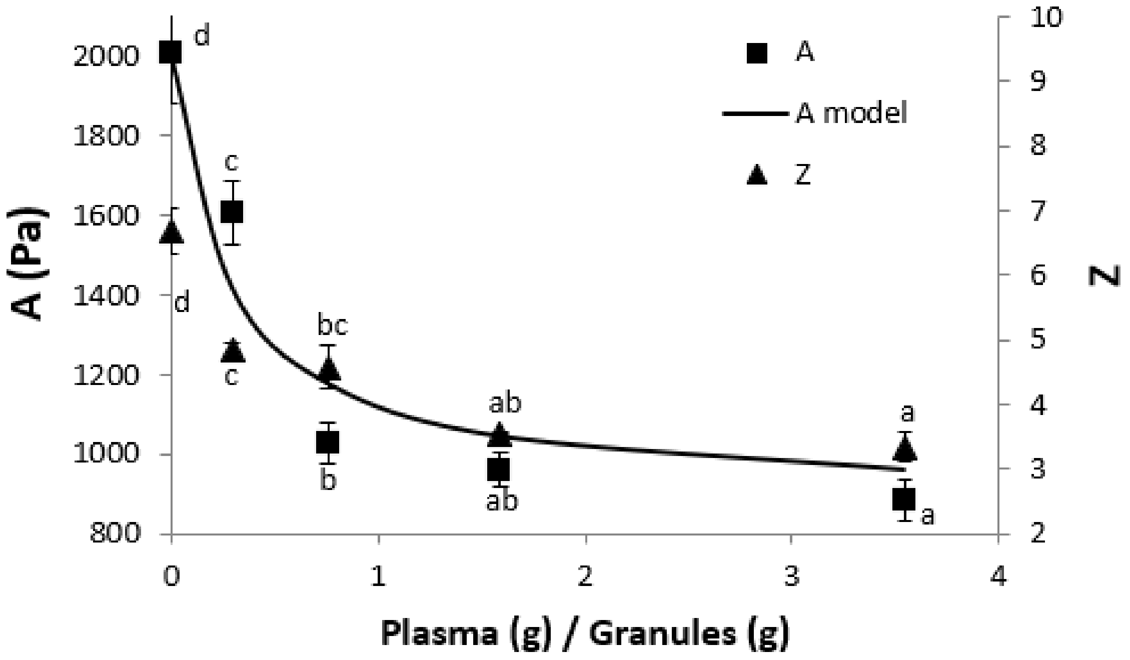

A (Pa)
2000
1800
1600
1400
1200
1000
800
Z
10
9
8
7
6
5
4
3
2
0
1
2
3
4
Plasma (g) / Granules (g)
A
A model
Z
d
d
c
c
bc
b
ab
ab
a
a

Figure 5.

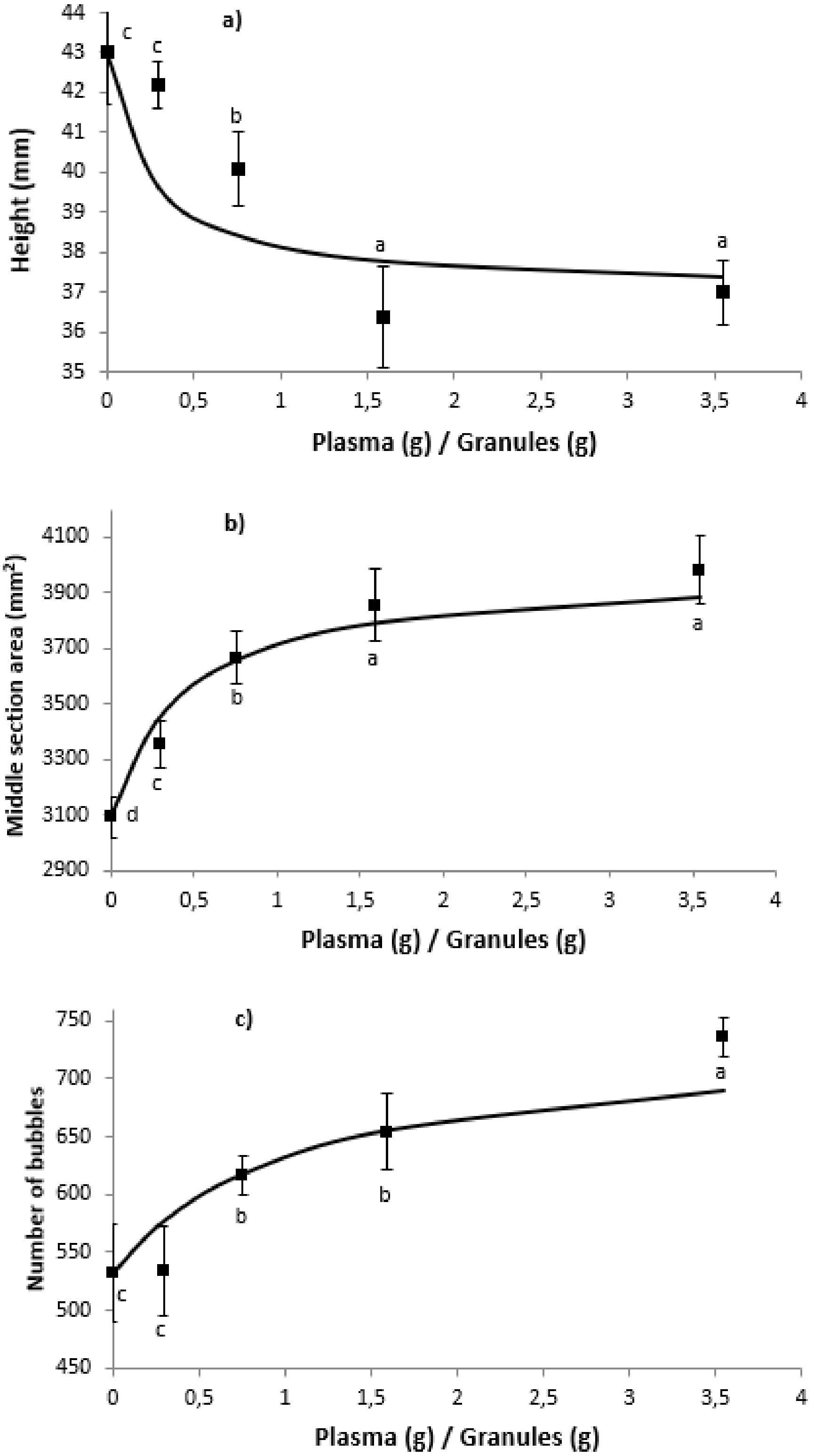

a)
Height (mm)
44
43
42
41
40
39
38
37
36
35
c
c
b
a
a
0
0,5
1
1,5
2
2,5
3
3,5
4
Plasma (g) / Granules (g)
b)
Middle section area (mm^2)
4100
3900
3700
3500
3300
3100
2900
d
c
b
a
a
0
0,5
1
1,5
2
2,5
3
3,5
4
Plasma (g) / Granules (g)
c)
Number of bubbles
750
700
650
600
550
500
450
c
c
b
b
a
0
0,5
1
1,5
2
2,5
3
3,5
4
Plasma (g) / Granules (g)

Figure 6.

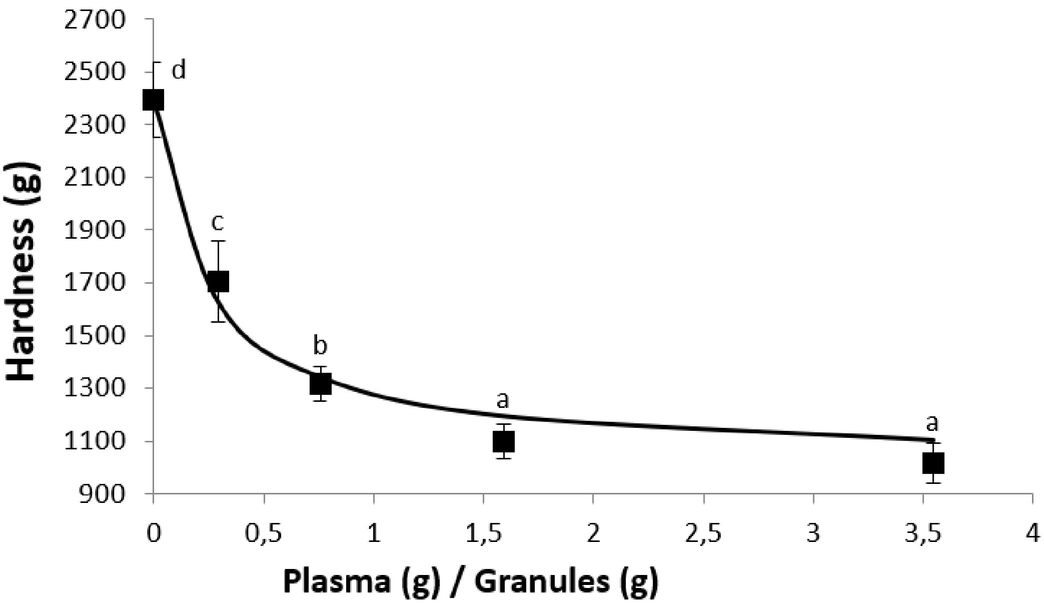

Hardness (g)
2700
2500
2300
2100
1900
1700
1500
1300
1100
900
d
c
b
a
a
0
0,5
1
1,5
2
2,5
3
3,5
4
Plasma (g) / Granules (g)

Figure 7.

Figure 1. Plots of batter viscosity with different Plasma/granules ratios vs the shear rate at 25 ^{o}C.

Figure 2. Consistency coefficient (K) and flow behaviour index (n) obtained from Figure 1. Model curve for consistency coefficient was obtained from equation 3. Different letters in the same experimental parameter indicate significant differences (P < 0.05).

Figure 3. Total number of bubbles and specific gravity in each batter. Total number of bubbles was obtained from images presented in Figure 1S. Model curve for bubbles was obtained using equation 4. Different letters in the same experimental parameter indicate significant differences (P < 0.05).

Figure 4. Mechanical spectra of the tested batters at 90 °C. Storage modulus (4a), loss modulus (4b) and loss tangent values (4c) are plotted.

Figure 5. Soft-gel model applied to data obtained from mechanical spectra at 90 °C. $\mathbb{A}$ and Z values were obtained from Figure 4. Model curve for $\mathbb{A}$ was obtained from equation 3. Different letters in the same experimental parameter indicate significant differences (P < 0.05).

Figure 6. Physical properties of baked muffins. Height (a), middle-section area (b) and number of bubbles in the muffin crumb (c). Solid lines were obtained from eq. 3 (6a) and eq. 4 (6b and 6c). Different letters in the same experimental parameter indicate significant differences (P < 0.05).

Figure 7. Muffin hardness parameter. Hardness was obtained from the TPA analyses of the baked muffins. Model curve for hardness was obtained from eq. 3. Different letters in the same experimental parameter indicate significant differences (P < 0.05).