Rheological and microstructural characterization of batters and sponge cakes fortified with pea proteins

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Abstract

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Keywords	liquid foams; stability; free-drainage; CLSM; volume air fraction

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Nantes, November 18th, 2019

Dear Editor, dear Susana

This letter goes with our resubmission (R3) of our paper FOODHYD_2019_1235_R2, entitled :

Rheological and microstructural characterization of batters and sponge cakes fortified with pea proteins

by Mélissa Assad-Bustillos, Camille Jonchère, Catherine Garnier, Anne-Laure Reguerre, and myself as corresponding author.

As you will see in the enclosed file, we have strived to perform the minor change (highlighted in blue in the text of the legend) requested by the reviewer in the graphical abstract. We appreciated his/her help, and also yours, in improving the manuscript and we hope that it is finally accepted for publication in Food Hydrocolloids.

Thanking you in advance, I remain,

Yours sincerely,

Guy DELLA VALLE

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Authors' answers to Comments from the reviewer about **FOODHYD_2019_1235_R2** " Rheological and microstructural characterization of batters and sponge cakes fortified with pea proteins" **by Assad-Bustillos et al**

-Reviewer 1

In graphic abstract, the authors added the scale bar values of the images in the legend '(upper row, scale bar 50 µm) and sponge-cakes (lower row, scale bar 200 µm)'; however, scale bar of the right lower row picture is 100 µm. From the data of Fig 7, scale '100 µm' seems to be correct, but the authors should show photos of the same row in same magnification. In addition to this correction, the authors should put the scale bar in the same position (the position is different in only the left lower row picture).

OK. We thank the reviewer for her/his accuracy.
We apologize not being able to deliver an image at same magnification (x2) on the same row, but we really think that it does not create any ambiguity for the reader since the message we want to convey is to "illustrate the jamming of the foam structure through the creation of PP network **" and not to focus on other microstructure details such as bubbles or other particles. Anyway , we have modified the legend by adding "**and 100µm for last micrograph at right**" for lower row of images.**

Finally we alo put the scale bar on the same position on left / lower row image.

Highlights

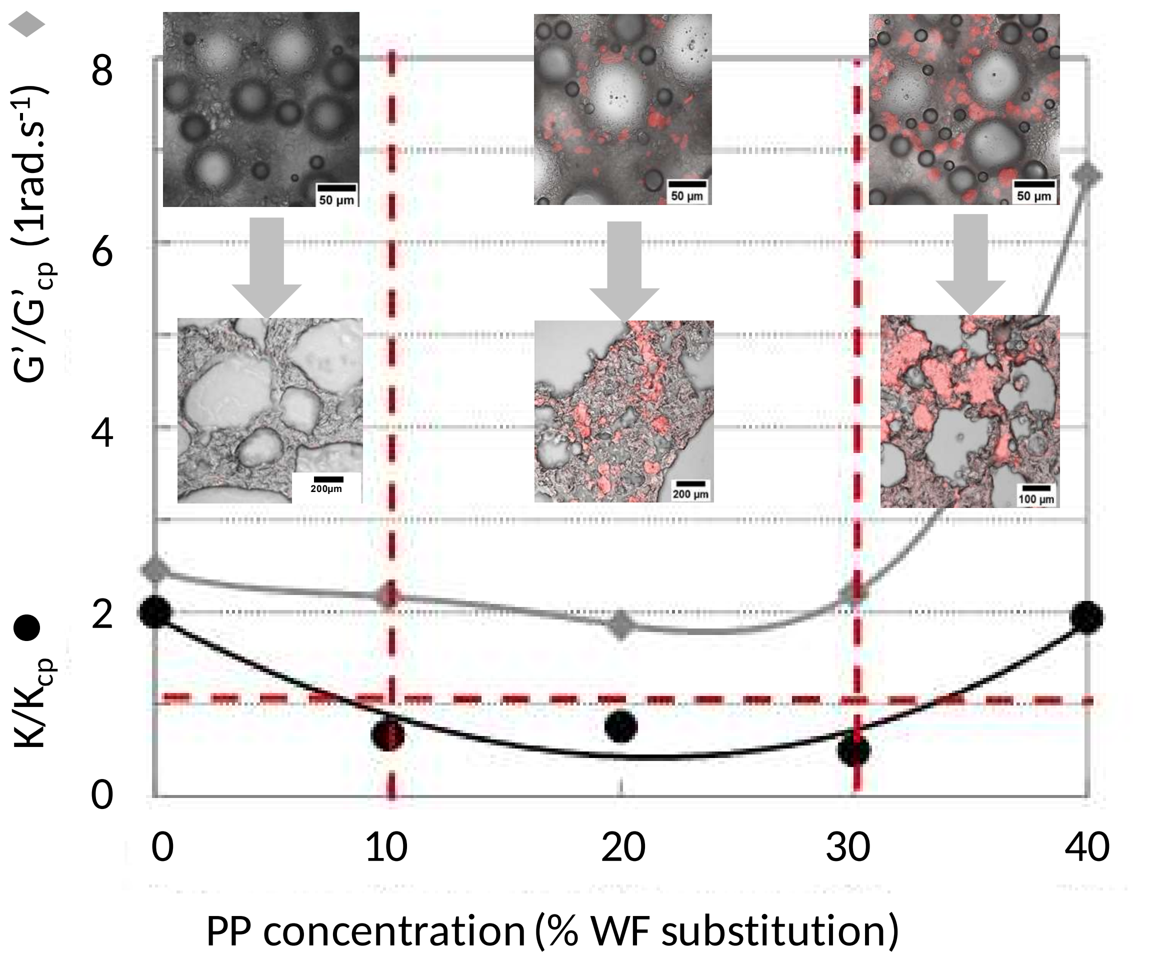
* sponge cake properties are directly impacted by batter air volume fraction (Φ_a)

* addition of pea protein isolates (PP) decreases Φ_a and increase batter viscosity

* addition of pea protein isolates (PP) leads to more stable batters

* CSLM imaging suggests that viscosity increase is first due to PP filler effect

 * at higher levels of substitution, PP can form a non-covalent network



Variations of the normalized rheological properties and micrographs of batters (upper row, scale bar 50 µm) and sponge-cakes (lower row, scale bar 200 µm, and 100µm for last micrograph at right) as a function of PP concentration (PP particles are stained in red) illustrate the jamming of the foam structure through the creation of PP network. Red dotted lines indicate some characteristic values.

Rheological and microstructural characterization of batters and sponge cakes fortified with pea proteins

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Abstract

The effect of pea protein fortification on the rheological properties of sponge-cake batters and their continuous phases, as well as their relationship with the final product properties were studied. Foams made out of whole egg and sugar were prepared in a planetary mixer; wheat flour (WF) was added to form a typical sponge-cake batter. Pea protein isolates (PP) were added in substitution to WF to form five batters with various PP concentrations expressed as the percentage of WF substitution: 0, 10, 20, 30 and 40%. The batter air volume fraction decreased when increasing PP concentration, which led to an increase in the cake density and apparent Young modulus. All batters and their respective continuous liquid phases showed shear-thinning behavior, which was modeled by a power law. They showed a predominant elastic behavior at intermediate frequencies, and a cross-over point at high frequencies. All of the rheological properties increased by a factor of about 10 when the PP concentration increased from 0 to 40%. Free-drainage experiments showed that batter stability increased with increasing PP concentration. PP had larger particles than WF, and showed a higher water binding capacity than WF, but no significant difference in solubility. Observations of the batter and cake microstructure revealed PP formed a network of interconnected “bridged” particles in the continuous phase. These results suggest that PP first acted as fillers that swelled and connected to each other in the continuous phase, and were the main driver for the increase of rheological properties.

Keywords: liquid foams, stability, free-drainage, CLSM, volume air fraction.

Nomenclature

CLSM	Confocal scanning laser microscopy
$d_{4,3}$	Mean volume diameter (m)
E*	Apparent Young modulus (Pa)
G’, G’’	Viscoelastic storage and loss modulus, respectively (Pa)
H_f , H_{liq}	height of foam (Initial) and liquid, respectively (m)
K	Consistency index ($Pa.s^n$)
n	Flow index
PP	Pea protein isolates
PSD	Particle size distribution
S	Solubility
SNK	Student-Newman-Keuls test
tan δ	Phase shift factor
VEL	Viscoelastic linear domain
WC	Water content
WF	Wheat flour
WHC	Water holding capacity
Ø	Diameter
Φ_a	Air Volume fraction
ρ, ρ^*	liquid and batter (or) crumb density, respectively ($kg.m^{-3}$)

1. Introduction

The fortification of cereal products with pulse proteins, such as pea protein, is a good way to improve their nutritional properties by equilibrating the essential amino acid profile (Young & Pellett, 1994), but it may have a negative effect on their texture properties (Monnet, Laleg, Michon & Micard, 2019).

Due to its high versatility and airy texture, sponge-cake is a very popular food among the consumers. Its simple composition and processing makes it easily available. Its porous structure allows it to absorb liquids such as syrups and jams; for those reasons it is apt for a large variety of applications and constitutes the base of many bakery specialties worldwide (Díaz-Ramírez et al., 2013). The structure of sponge-cake is originated during the mixing of cake batter, before it becomes a soft solid foam after thermal setting during baking. Briefly, the batter process consists in forming a liquid foam by introducing air into a continuous liquid phase made of egg and sugar, to which wheat flour and other minor solid components are folded in to form the batter. In the industry, this process is known as two-stage mixing (Wilderjans, Luyts, Brijs, & Delcour, 2013). Like other egg based cakes, the final airy structure of sponge-cake depends heavily on the air trapped within the continuous phase (Conforti, 2014; Wilderjans et al., 2013). For that reason, the rheological properties, and particularly the viscosity of the batter are key factors that determine its capacity to retain air bubbles during processing (Sahi & Alava, 2003). Indeed, the air volume fraction, or porosity (Φ_a) is a critical factor that is strongly linked to the rheological behavior of the batter (Allais, Edoura-Gaena, Gros, & Trystram, 2006), and allows its classification into two categories: bubbly liquids (Φ_a<0.64) and aqueous (or wet) foams (0.64<Φ_a<0.95) (Cantat et al., 2013). In sponge-cake and other egg based foams, Φ_a values commonly situate them in the aqueous foam domain; for instance Φ_a =0.68 for a typical foam made of whole egg; and Φ_a =0.74 if made only of egg white (Spencer, Scanlon, & Page, 2008).

Aqueous foams are made by highly packed air bubbles that form an interconnected structure, composed of films, Plateau borders and nodes (Cantat et al., 2013). This unique structure is responsible for their viscoelastic behavior at low strains (Cohen-Addad, Hoballah, & Höhler, 1998) and their shear-thinning behavior at large strains, sometimes presenting a yield stress if the bubble packing is “jammed” (Gopal & Durian, 1999). These rheological properties are intimately related to Φ_a

and to the bubble size distribution (Kraynik, Reinelt, & van Swol, 2004). Moreover, since foams are not thermodynamically stable systems, they are ubiquitous and their rheology is time-dependent (Cipelletti & Ramos, 2002; Marze, Guillermic, & Saint-Jalmes, 2009); therefore, the destabilization phenomena that occur during the time scale of batter processing are to be taken into account, as they can have an influence on the structure and final properties of the cake crumb (Foegeding, Luck, & Davis, 2006). The main mechanisms that lead to foam destabilization are drainage- driven by gravity, coarsening - due to pressure differences between bubbles of different sizes and coalescence, which is the bursting of liquid films separating neighboring bubbles (Cantat et al., 2013; Murray, 2007). In aqueous foams, the extent to which a particular aging mechanism dominates foam destabilization also depends considerably on Φ_a (Saint-Jalmes, 2006). For instance, Spencer et al., (2008) showed that destabilization of sponge-cake batters was dominated by drainage when the systems were classified as bubbly liquids, whereas coalescence was dominant in foams. In any case, the study of the stability of cake batters gives important information about their internal structure and their macroscopic behavior during processing; moreover, it can be assessed in a relatively simply manner, for instance *via* free-drainage experiences (Saint-Jalmes & Langevin, 2002).

Even though all of the above mentioned phenomena have been relatively well described in physical chemistry and soft matter sciences, the cake making industry could benefit from basic insights taken from said disciplines in order to improve its processing for applications such as protein and fiber fortification, sugar or fat reduction (Mezzenga, Schurtenberger, Burbidge, & Michel, 2005). In the particular case of protein fortification, the consequences of adding pulse flours or isolates have been recently reviewed for their impact on sensory properties (Foschia, Horstmann, Arendt, & Zannini, 2017) and on their structuring during baking (Monnet et al., 2019). However, there is still a lack of understanding of the mechanisms leading to those changes during the whole process chain.

In this context, the objectives of this study were: i) to characterize the changes induced by the addition of pea protein isolates, at different levels of wheat flour substitution, on the rheological, stability and air incorporation properties of sponge-cake batters and their continuous liquid phase; ii) to relate them to the structural and mechanical properties of the corresponding baked cakes; iii) to provide insight on the possible mechanisms at the origin of these changes; iv) to discuss the possible corrective actions that could be implemented to avoid them.

2. Materials and methods

2.1 Batter and cake formulation

Five batters were prepared according to the formulae detailed in Table 1. Pasteurized liquid eggs (HORECA, France) and white sugar (Daddy, France) were bought one week before the experiment at a local store; eggs were conserved at 4°C. Wheat flour (T55, Decollogne, Aiseray, 21, France), and pea protein isolates (PP, NUTRALYS BF, Roquette, Lestrem, 62, France) were bought 2 months before the experiment and stored in closed containers at 4°C. Due to the extraction process, PP are not known as functional, i.e. they do not exhibit large gelling or emulsifying properties. Ingredients were weighed separately in sufficient quantity to prepare 1 kg of batter. To prepare the foams, first, eggs are mixed with sugar in a planetary mixer with rotating whisk (N50, HOBART, USA) at intermediate speed (281 rpm arm and 124 rpm whisk) during 10 minutes, plus 5 minutes at high speed (580 rpm arm + 255 rpm whisk). Then wheat flour (WF) and pea protein isolates (PP) are added progressively to the egg-sugar foam to form the batter while mixing manually and gently to avoid bubble coalescence and collapse.

For each formula, batter properties were characterized right after being prepared. Additionally, a small amount (≈15 mL) of batter was poured in a plastic test tube that was placed inside a vacuum desiccator system (without any silica particles) to remove the air bubbles during 2 hours. The properties of the airless batters, considered as their continuous phase, were characterized as for regular batters. The properties of the egg-sugar foam previous to the addition of flour and isolates were also characterized.

Since the characterization trials of batter are destructive, cakes were prepared from new batter batches by following the same formulae shown in Table 1. Additionally, 1% of a commercial chemical leavening agent was added ($Na_2CO_3/Na_2H_2P_2O_7$ powder mix, DGF, France) to each formula, modifying minimally their composition. For each formula, 4 cakes were prepared. Circular aluminum molds with a diameter (Ø) of 25 cm were sprayed with oil to avoid sticking, then 250 g of batter was weighed on each mold; the latter were placed on a tray and baked in a pre-heated electric deck oven at 200°C during 20 min, as recommended by our partner Cerelab®, sponge cake manufacturer. The cakes were un-molded and cooled at room temperature for 3 hours, then wrapped in plastic foil and left to rest for 24 h before the characterization of their properties.

2.2 Batter density and air volume fraction

A 20 mL plastic container was filled with freshly prepared batter and weighed. The measurement was performed thrice. From this data, the batter density (ρ*), in g·cm^{-3}, was calculated by:

$$\rho^* = \frac{m_{container+batter} - m_{container}}{m_{container+water} - m_{container}} \times \rho_w \qquad (1)$$

Where:

$m_{container}$, is the mass of the empty container, in g;

$m_{container+batter}$, is the mass of the container filled with the batter, in g;

$m_{container+water}$, is the mass of the container filled with water, in g, and water density $\rho_w \approx 1 g.cm^{-3}$).

The batter air volume fraction, or posrosity (Φ_a) was calculated from the relation expressed in (2):

$$\Phi_a = 1 - \frac{\rho^*}{\rho} \qquad (2)$$

Where:

ρ^* is the batter density, in g·cm^{-3} , calculated as detailed above;

ρ is the density of the continuous phase, in g·cm^{-3}, calculated from the material density of each individual component in relation to their proportion in the continuous phase:

$$\rho = \frac{1}{\frac{\chi i}{\rho_i} + \frac{\chi j}{\rho_j} + \frac{\chi k}{\rho_k} \ldots} \qquad (3)$$

Where

$\chi_{i,j,k\ldots}$ are the mass fractions of the individual components *(i, j, k…)* of the continuous phase, and;

$\rho_{i,j,k\ldots}$ is the material density of each individual component *(i, j, k…),* in g·cm^{-3}.

2.3 Batter rheological properties

The rheological properties of the batters and their continuous phases were measured with a controlled strain rheometer (ARES, TA Instruments, USA) equipped with a parallel plate geometry (discs Ø= 40 mm) and a 1 mm gap at 25 °C. Approximately 2 g of the sample were carefully placed with a spatula in the bottom plate, and the gap was narrowed at the minimal loading speed to avoid damaging its structure. Paraffin oil was used to cover the geometry in order prevent sample drying during the test. The measurements were performed thrice.

2.3.1 Viscoelastic properties

The mechanical spectra of the samples were determined by frequency sweeps (0.01 to 100 rad·s^{-1}) within the viscoelastic linear domain (VEL) at a strain of 0.6%; this strain value was previously determined on a different sample by performing a strain sweep from 0.1 to 1% at a frequency of 1 rad·s^{-1}. From the mechanical spectra, the values of the storage modulus G' (Pa) and the phase shift factor (tan δ) at 1 rad·s^{-1} were extracted and used to characterize the structure properties of the sample at "rest".

2.3.2 Flow properties

Following the determination of viscoelastic properties, the shear viscosity η (Pa·s) of the samples was measured in the range of 0.01 - 600 s^{-1} and was fitted with the Ostwald-de Waele power law model:

$$\eta = K * \dot{\gamma}^{n-1} \quad (4)$$

The consistency index K (Pa·s^n) and the flow index (n) of each sample was used to characterize flow properties.

2.4 Batter stability: free-drainage

The destabilization kinetics of the batters was measured by following the apparition of liquid at the bottom of a transparent non-graduated test tube (Ø= 2.5 cm, h=21 cm) during a free drainage experience. The tube containing the freshly prepared sample was placed between a white halogen light source (KL 2500 LCD, Schott, Germany), and a monochrome CMOS camera (EXO SVS-250MGE, D-Vistek, Germany) equipped with a 35 mm f/1.4 lens (Myutron, Japan). The camera was set to acquire images of the entire tube at t=0 and then, automatically, every 10 min during 12h. The images were processed with the ImageJ freeware (https://imagej.nih.gov). A threshold was applied to distinguish the liquid in the image from the remaining dry foam. This allowed the quantification of the height of liquid (H_{liq}) in every image, which was normalized by the initial height of the foam (H_f). The results were expressed as the evolution of the ratio of Hl_{iq} / H_f over time and were used to characterize the stability properties of the samples. The measurement was performed twice.

2.5 Measurement of pH

The pH of the continuous phases was measured using a pH meter (905 Titrando, Metrohm, Switzerland). The measurement was performed thrice.

2.6 Cake density, water content and mechanical properties

Following cooling and rest during 24 h, the cakes were weighed and their volume was measured by the rapeseed displacement method (AACC, 2009a). Their density ρ* (g·cm^{-3}), was calculated as their mass to volume ratio. The water content of the cakes (WC), expressed as a wet basis percentage, was determined by placing 2 g of crumb taken from the center of the cake inside an oven during 2 h at 135 °C as recommended by (AACC, 2009b). The apparent Young modulus E*(kPa), of the cake crumbs were characterized from the slope of the linear part of the stress –strain curve obtained by performing a uniaxial compression test with an universal testing machine (Adamel Lhomarghy, France) on cylindrical crumb samples (Ø=40 mm, h=30 mm) taken from the center of the cake, and using the same test conditions described by Assad-Bustillos et al. (2019). All measurements were performed thrice.

2.7 Pea protein isolates and wheat flour characterization

2.7.1 Particle size distribution

The particle size distribution (PSD) of pea protein isolates (PP) and wheat flour (WF) in dry dispersion was determined with a light scattering instrument (Mastersizer 2000, Malvern Instruments®, UK). The PSD in wet dispersion of PP and WF in distilled water (5% w/v) was also determined within a time scale relevant to product processing (t=15 min). The refractive index was set at 1.45 as estimated for proteins, and water was set at 1.33. The mean volume diameter ($d_{4,3}$) was used to characterize the particle size of the samples. Measurements were performed thrice.

2.7.2 Solubility and water holding capacity

The solubility (S) and water holding capacity (WHC) of the samples were measured for 10% (w/v) dispersions of PP and WF, based on the methods described by Peters, Vergeldt, Boom, & van der Goot (2017). Briefly, this method consisted in preparing the dispersions in Eppendorf tubes that were mixed with a vortex during 15 minutes (3 intervals of 5 minutes) to be representative of processing conditions. Subsequently, the tubes were centrifuged at 3000 rpm (845 g) for 20 min. The supernatant was separated and placed in an oven at 130°C to determine its dry mass; the pellet was weighed and afterwards placed in the oven to determine its dry mass as well. The measurements were performed five times. This data allowed the calculation of S, expressed as the percentage of solids retained in the supernatant on a dry basis, and WHC, expressed as the ratio of absorbed water to dry matter of the samples):

$$WHC = \frac{m_{wet\ pellet} - m_{dry\ pellet}}{m_{dry\ pellet}} \quad (5)$$

$$S = \frac{m_{dry\ supernatant}}{m_{dry\ pellet}} \times 100 \quad (6)$$

Where:

$m_{wet\ pellet}$ is the mass of the pellet (g) ;

$m_{dry\ pellet}$ is the mass of the pellet after desiccation (g) ;

$m_{dry\ supernatant}$ is the mass of the supernatant after desiccation (g).

2.8 Pea protein isolate localization in batters and cakes

Observations of the batter and crumb microstructure were made by confocal scanning laser microscopy (CSLM), using a confocal laser scanning microscope (A1, Nikon, Japan); the excitation wavelength was set to 488 nm and the emission was recorded between 520 and 600 nm in order to identify the auto-fluorescence of PP samples, as reported by Nunes, Raymundo, & Sousa (2006); no fluorophore or dying agent was added to the samples. To prepare the samples, a small amount of freshly prepared batter was carefully placed on a microscope slide within a spacer frame of 250 µm in thickness (Thermo Fisher Scientific, USA) that was sealed with a plastic cover right after to preserve the structure of the foam without crushing it and to prevent the air from escaping. The observations were made immediately after sealing. For cakes, a cubic crumb sample taken at the center of the cake (5 cm^3) was used to obtain slices of 120 µm in thickness with a cryo-microtome (HM 500 OM, Microm, France). The slices were placed on microscope slides and were left to rest for 3 days before the observations. To make sure the observed fluorescent particles correspond to PP, isolates were prepared in dry and wet 5% (w/v) dispersion, placed on a microscope slide, and covered with a classic cover slip. All samples were observed using a x20 lens (Plan APO with numerical aperture of 0.5) and a 5x digital zoom, when needed. For each sample, 3 to 5 images were taken for illustrative purposes.

2.9 Statistical treatment

One-way analyses of variance (ANOVA) were performed in order to investigate the differences in properties between the different cakes, batters and continuous phases of different composition. Student t-tests were performed to compare the solubility and the water holding capacity between two samples (wheat flour *vs* pea protein isolates). For all statistical tests, a significance level of $\alpha=0.05$ was used. When significant effects were found, Tukey and Student-Newman-Keuls test were used for post-hoc treatment. Since similar outcomes were obtained, only SNK results were reported to avoir redundancy. All statistical analyses were performed with XLSTAT software (v.2016 18.06, Addinsoft, USA).

3. Results and discussion

3.1 Air volume fraction and impact on cake properties

The addition of PP induced some changes in the cake crumb appearance, with more individual visible gas cells at 40% substitution (Fig. 1a). The air volume fraction (Φ_a) of the egg-sugar foam is significantly ($p<0.01$) reduced after wheat flour (WF) is added, from 0.75 to 0.71 (Fig. 1 b). This is caused by the rupture of the air / water interfaces leading to bubble coalescence during the mixing in the presence of starch, as previously reported by Bousquières, Michon, & Bonazzi, (2017), who observed an augmentation of sponge-cake batter density when the mixing time after starch addition was increased. Interestingly, when pea protein isolates (PP) are added in substitution of WF, Φ_a is furtherly reduced, reaching a value of 0.61 for the highest concentration of PP. Such a value of Φ_a is low enough (Φ_a<0.64) for the system to be considered a bubbly liquid, rather than a foam (Cantat et al., 2013). These results are in line with those reported by Gómez, Doyagüe, & de la Hera, (2012), where the addition of pea flour in sponge-cakes caused an increase in batter density, reflecting less air incorporation. This decrease in aeration could be responsible for the increase in the cake density reported by the same authors. Indeed, such relationship had already been reported by Bousquières et al. (2017) in regular sponge-cake batters, which suggests the dependence of the cake density on the air volume fraction is not exclusive of protein fortified systems. In our case, substituting WF by PP led to a significant (p<0.001) increase in the cake density (ρ^*), from 0.21 ± 0.01 to 0.35 ± 0.02 g·cm^{-3}. Cake density values were directly correlated batter density (Fig 1 c, r^2=0.92), then to the batter air volume fraction Φ_a, which confirmed the importance of the initial aeration in determining the final cake properties. Moreover, the apparent Young modulus (E^*) of the cakes also increased, from 5 ± 1 to 17 ± 2 kPa (Fig. 1 c). Also, the values of E^* are highly correlated to the crumb density ρ^* with an exponent close to 2 (Fig. 1 c, r^2=0.9), in accordance with Gibson & Ashby's scaling law for solid foams (1988), suggesting there is little influence of the intrinsic cell wall in the final cake properties. In other words, the texture changes caused by the substitution of WF by PP in the batter composition may be imparted to the increase of density, rather than to a change of mechanical properties of the intrinsic material, the sugar-starch-proteins matrix, itself. In baked products, high values of E^* are undesirable since they are associated with the perceived firmness of the crumb (Lassoued, Delarue, Launay, & Michon, 2008), and the latter may impact negatively the consumer's acceptability (Angioloni & Collar, 2009; Monnet et al., 2019). Finally, the water content (WC) of the cake crumb (not shown) increased slightly with PP addition, but not significantly (p>0.05), from 28 ± 2% in the reference cake (S0), to 30 ± 2 % for the highest level of fortification (S40).

3.2 Rheological properties of the batter and their continuous phases.

3.2.1 Viscoelastic properties

The mechanical spectra of the batters show a high frequency dependence with a predominance of the storage over the loss modulus (G'>G''), which denotes an elastic dominant behavior; two cross-over points can be observed at low and high frequencies (Fig. 2 a). This behavior reflects mainly the non-covalent bonding of the molecules present in the sample (Steffe, 1996). In the particular case of foams, the second cross-over point, corresponding to a relaxation time, is likely a consequence of the foam aging mechanisms (mainly coalescence), that cause changes in the size of the bubbles (Cohen-Addad et al., 1998). According to Gopal & Durian (2003), the relaxation time of a foam corresponds to its “unjamming” point, where elasticity completely vanishes and the bubbles are no more closely packed. The G’ and G’’ values of the wheat flour (WF) batter across the whole spectra are about 4 times larger when than those of the egg-sugar foam, and they are increased when the pea protein isolates (PP) are added, by a factor of about 15 at the highest PP level (S40) (Table 2). Moreover, the tan δ value (=G’’/G’) at 1 rad·s^{-1} decreases significantly from 0.6 in the reference formula (S0), to 0.3 in the highest PP formula (S40), reflecting the enhancement of the elastic character of the batters with the addition of PP.

A similar behavior is observed for the liquid continuous phases, but with G’’ closer to G’ (Fig. 2 b). An additional minor difference is that the egg-sugar continuous phase displays spectra (not shown) reflecting a dominant viscous behavior (G’’>G’) (Table 2). Overall the G’ and G’’ values are lower for the continuous phases than for batters, but they still rise by a factor of 5 when the maximum PP concentration is reached (S40). Also, no significant differences were observed in the tan δ values at 1 rad·s^{-1} between the different formulae, which varied between 0.8 and 0.9, meaning the continuous phases show a more viscous character than the batters, which is coherent with the shift of the second cross-over point in the spectra towards lower frequencies, close to 1 rad·s^{-1}.

3.2.2 Flow properties

Regarding the shear flow properties, both batters and continuous phases showed a non-Newtonian shear-thinning behavior (Fig. 3), as previously encountered by several authors in cake batters of similar composition (Bousquières et al., 2017; Chesterton, de Abreu, Moggridge, Sadd, & Wilson, 2013; Edoura-Gaena, Allais, Trystram, & Gros, 2007; Meza et al., 2011; Sanz, Salvador, Vélez, Muñoz, & Fiszman, 2005). Although the flow curves are not fully regular, likely as a consequence of inner structural changes, like breakdown of foam structure, they could be fitted by a power law.

The shear viscosity (□) of both batters and continuous phases, increases significantly with the addition of WF and PP, as reflected by their consistency index (K) values (Table 2). The consistency K of WF batter (S0) is increased by a factor of 6 compared to (egg + sugar) batter and it then increases by a factor of 8 for the highest concentration of PP (S40). Oppositely, the flow index (n) decreases after the addition of WF (S0), meaning the shear-thinning character is accentuated, but remains constant for the increasing PP formulae. For the continuous phases (Fig. 3 b), the initial increase of K after the WF addition (S0) is much larger, by a factor of 60. Indeed, the K and n values of the egg-sugar continuous phase are very close to those reported for liquid whole egg (K=0.16 and n=0.84 at 20°C), which is known for its low shear viscosity and for a high flow index that makes it nearly Newtonian (Gosset, Rizvi, & Baker, 1983). In fact, adding sugar and flour to a liquid egg solution increases its viscosity in order to increase its ability to retain air bubbles (Wilderjans et al., 2013). Otherwise, the value of K of the continuous phases was multiplied by about 8 when the highest concentration of PP was reached (S40); this order of magnitude is similar to the one observed for batters. Surprisingly, the K values of the continuous phases were higher than those of batters, excepting for the reference formula (S0) and the highest PP concentration (S40). These results are in contradiction to what has been previously reported by authors who have compared foams to their corresponding “slurries”, i.e. their continuous liquid phase, where the K values always were higher for foams (Chesterton et al., 2013; Meza et al., 2011). However, these studies were carried out on batters with traditional ingredients, which did not include proteins from any other source than wheat. In our case, the presence of pea proteins could be responsible for the observed irregularities in flow properties, as reflected by the slight slope changes at larger shear rates ($\approx 10^2\ s^{-1}$), which could be attributed to the rearrangement of protein aggregates in the case of the continuous liquid phase. An in-depth discussion on this effect is provided in section 3.6.

3.3 Batter stability

As seen from the kinetics of liquid apparition (Fig.4 a), the stability of the egg-sugar foam increases significantly when WF is added to the egg-sugar foam. This is reflected by both, the increase of the time of liquid apparition during the free drainage experience, and by the more progressive and less abrupt destabilization curve of S0, as compared to egg-sugar. Also, the stability of the batters containing PP is significantly augmented when increasing PP concentration. Similar to what occurs in S0, destabilization of samples S10 and S20 occurs progressively. In contrast, despite their larger values of liquid apparition time, the destabilization curve becomes abrupt again for the two highest PP concentrations (S30 and S40). To explain this behavior, we hypothesize that the higher viscosity of their continuous phases promotes the local accumulation of drained liquid within the Plateau borders; this type of local drainage is not macroscopically visible and does not immediately induce liquid apparition at the bottom of the tube: the liquid will continuously accumulate until its volume is high enough to cause a disruption in the internal equilibrium forces, resulting in the abrupt liberation of the accumulated liquid. Illustrations of both progressive and abrupt liquid apparition during free drainage are shown in Figure 4 (b, c).

A possible explanation for the increase of stability could rely on the migration of pea proteins and their adsorption at the water/air interface, like previously encountered by Turbin-Orger et al. (2015) in the case of wheat flour dough liquor. However, adsorption at the interface requires proteins to be soluble in the continuous phase (Raikos, Neacsu, Russell, & Duthie, 2014). In fact, vicilin and convicilin, the proteins found in pulses, are highly insoluble in the native state around their isoelectric point ($4<\text{pH}<6$), thus showing optimum solubility at acidic and alkaline pH values (Boye, Zare, & Pletch, 2010; Gueguen, 1983). In our case, PP are rather under the form of aggregates due to the extraction process, and the pH of the continuous phases was found to be 7.5 ± 0.3 and did not differ significantly ($p>0.05$) between samples of different composition. Since this value remains close to the proteins isoelectric point, solubility may not be optimal, and thus the hypothesis of protein adsorption at the water/air interface seems less likely.

On the other hand, the low solubility of PP could be responsible for the viscosity increase of the continuous phase of the batters, since PP could act as filler like solid particles in a suspension. Therefore, we hypothesize the viscosity increase of the continuous phase is the main mechanism that drives the stabilization of batters. However, in order to validate this hypothesis, the characterization of the solubility, particle size and water holding capacity of both WF and PP is needed.

3.4 Pea protein isolates characterization

From Table 3 it can be seen that the solubility (S) of wheat flour (WF) and pea protein isolates PP does not differ significantly ($p>0.05$), being very low in both cases. In WF, this is not surprising, since its main components are starch and gluten, both of which are insoluble in water at pH=7 (Buleon, Colonna, Planchot, & Ball, 1998; Shewry, Tatham, Forde, Kreis, & Miflin, 1986). The low solubility of PP, indicating that they are in the form of aggregates ,was also low as expected from the pH value of the continuous phase and as discussed previously in section 3.3. Conversely, in terms of water holding capacity (WHC) the difference between PP and WF is significant ($p<0.01$): PP absorbs about 2 times more water than WF. In terms of particle size distribution (PSD) in dry dispersion, WF showed a bimodal distribution (see Appendix), with a coarse fraction (1st mode ≈105 µm) and a fine one (2nd mode ≈24 µm), where the second fraction seems to correspond with starch granules, which diameter is found within the range of 25-29 µm (Buleon et al., 1998). Conversely, for PP, the distribution was monomodal (see Appendix). The bimodal distribution of WF illustrates its wide particle heterogeneity as compared to PP, which is not well captured by its mean volume diameter ($d_{4,3}$). In any case, the differences of particle diameter between WF and PP were highly significant ($p<0.001$), independently on the dispersion medium used. However, the wet dispersion method seems more appropriate and relevant to our processing. Then, it is possible to conclude that the particle diameter of PP determined by wet dispersion, is significantly larger than WF.

The auto-fluorescence of commercial pea protein isolates at an excitation wavelength of 488 nm has been reported previously by Nunes et al. (2006). However, it is unclear which components in the isolates are responsible for the fluorescence. According to Monici (2005), many endogenous fluorophores are present in plant cells such as proteins containing aromatic amino-acids, NADPH, flavins, lipo-pigments, chlorophylls, flavonoids and other cell wall components, such as lignin, cutin, etc. In Figure 5, we show confocal laser scanning microscopy (CLSM) images that captured the fluorescence (λ=488 nm) of PP in dry and wet dispersions. Clearly, PP show fluorescence emission that is bright enough to allow their localization within the batters and the cakes without the need to perform any staining that could damage or perturb the structure of the sample.

3.5 Localization of pea protein isolates in batters and cakes

Bubbles in the egg-sugar foam have a round shape and seem to be loosely packed (Fig.6 a). After WF is added, large spherical bubbles (≈50µm) can still be seen, but there are less small bubbles, and, consequently the area of water / air interface is decreased. These observations are in agreement with the decrease of air volume fraction (Φ_a) from 0.75 to 0.71, reported in 3.1. After adding WF, small starch granules appear finely dispersed in the continuous phase (Fig. 6 b). When PP is added, they are preferably localized near the air/water interface of the bubbles, and, to a lesser extent, in the continuous phase (Fig. 6 c). When the PP concentration is increased, the organization of the continuous liquid phase changes and PP appear to form a more or less continuous network of particles, in the liquid phase (Fig.6 d). PP particles may be linked by non-covalent interactions as previously used and described in the case of colloidal systems that stabilize emulsions (Horozov & Binks, 2006; Stancik, Kouhkan, & Fuller, 2004). Clearly, the formation of this type of network may be linked to the observed changes in the rheological properties of the batters and in their continuous phases, which were particularly significant at the highest level of fortification (S40).

The images of cake microstructure (Fig. 7) are coherent with the observations on batters. They help to interpret the influence of batter structure on final cake properties. During baking, starch gelatinizes, granules swell, and, as water evaporates, air cells expand. As a result, the continuous solid phase dries and concentrates, and the swollen starch granules become the building bricks of a “brick and mortar” type structure, which is held together by strands of coagulated egg protein (Bousquières et al., 2017; Wilderjans et al., 2013). This type of structure is visible in the reference cake (S0) (Fig. 7 a).

However, when PP are added, this structure seems less continuous, or at least two co-continuous phases of swollen starch granules and PP particles may coexist (Fig. 7 b, c, d). Like starch in WF, PP swell and this generates a competition for water in the continuous phase. Therefore, starch granules may only achieve partial gelatinization, as previously encountered by Hesso et al. (2015), which could partially explain the disrupted appearance of the crumb microstructure (Fig. 7 b). Additionally, when the PP concentration increases, and as previously observed in the batter, the PP particle network is still present, and may have become covalently linked after baking, as observed for heated pea proteins (Mession, Sok, Assifaoui, & Saurel, 2013). This PP network (Fig. 7 c) might limit the air expansion process, since the air cells surrounded by the PP network appear smaller than those who are not. Many of these hypotheses should be confirmed by complementary experiments. Overall, this qualitative approach allowed us to formulate a coherent hypothesis about the organization of PP in batter cake systems, to be discussed in the following section (3.6).

3.6 Overall discussion

By integrating all the obtained results, it is possible to plot the diagram presented in Figure 8. This representation shows the evolution of the rheological properties of batters (B) normalized by those of their continuous phases (CP), e.g. the ratios K/K_{CP} and $G'/G'_{CP.}$ From this diagram, it can be seen that both viscoelastic and flow properties evolve in a coherent manner, although K/K_{CP} is lower than 1 for intermediate levels of PP concentration. This relation allows defining three regions that describe rheological behavior as a function of PP concentration. In the first region (PP<10% WF substitution), K/K_{CP} >1, which indicates that, at low levels of fortification, PP has not a major impact on batter structure and will behave like WF. To interpret the second (K/K_{CP} <1, 10 ≤ PP ≤30%) and third regions (K/K_{CP} >1, PP >30%), the existence of a non-covalent network formed by PP during batter formation may be inferred, as suggested in the preceding section (3.5). Indeed, since PP have a larger particle diameter and higher water holding capacity than WF, they are likely responsible for the viscosity increase in the continuous liquid phase. When the concentration is high enough (third region), PP may form a non-covalent network, even in the presence of bubbles (Fig. 6 d), so both K and K_{CP} increase, which explains K/K_{CP} >1. Conversely, in the second region, the non-covalent network is not formed, because of steric hindrance caused by the presence of bubbles. For this reason, K does not increase as much as in the third region. On the other hand, in the continuous liquid phase, PP may act like solid particles in a liquid, which explains the more pronounced increase of K_{CP}. This would explain the higher viscosity of the continuous phase and therefore K/K_{CP} <1. Recently, Assad-Bustillos (2019) has shown by X-ray tomography experiments that, compared with a standard product, a sponge cake prepared with the addition of 5% PP (substitution of 20% wheat flour) had slightly larger density (0.23 instead of 0.21 g.cm^{-3}), close mean cell wall thickness values (≈ 75μm) but lower mean cell width, about 250 µm instead of 300 µm. Considering the strong correlation between cake and batter densities (Fig.1c), this result confirms that the increase of viscosity, due to PP addition, decreases the amount of air incorporated in the batter, leading to smaller gas cells in the batter and then after baking. Finally, to increase the Φ_a of batters to which PP have been incorporated, it could be suggested to modify the mixing time or to use more powerful mixers in order to facilitate air incorporation as shown by Chesterton et al. (2013), or to lower the pH of the batter by adding citric acid in order to increase PP solubility (Zhang et al., 2012).

CONCLUSIONS AND PERSPECTIVES

We have confirmed that sponge cake properties are directly impacted by their density, or by the air volume fraction of the batter (Φ_a). The addition of pea protein isolates (PP) in substitution of wheat flour decreased Φ_a and increased the viscosity of the continuous phase of the batter, as it became more difficult to incorporate air into the continuous phase. However, once PP incorporated, the high viscosity of the batters conferred them a large stability, far beyond the time scales that are relevant for processing. Indeed, depending on the concentration of PP, the rheological properties of the batters suggest two different behaviors. It is first hypothesized that the viscosity increase mechanism by PP is due to their "filler" effect, caused by a combination of their larger particle diameter and higher water holding capacity as compared to wheat flour. Then, at higher levels of substitution, PP showed the capacity to form a non-covalent network that could be also responsible for the observed rheological behavior, and final sponge cake structure. Overall, our results suggest that the fortification, by pea proteins, of sponge-cakes and other egg foam foods can be carried with limited negative consequences on the cake properties, and some processing strategies can be envisioned to prevent or correct those changes.

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List of figures

Figure 1: Images of sponge cake cross section with increasing PP content from left to right (a) (a) and variations of (b) the air volume fraction (Φ_a) of batter as a function of PP concentration (red square (■ stands for the Φ_a of the egg-sugar (e+s) foam) and (c) of batter density (ρ*,●) and apparent Young modulus (E*, ■) as a function of cake density. Dotted lines reflect fitting via power law. All measurements were performed thrice.

Figure 2: Typical mechanical spectra obtained at strain = 0.06% (♦, G'; □, G'') of batters (a) and their respective continuous liquid phase (b) at 0.6% strain, for different composition and PP concentration: egg-sugar foam (red); S0 (light gray); S20 (medium gray); S40 (black), from bottom to top. All measurements were performed thrice. For the sake of visibility, not all samples are shown.

Figure 3: Typical flow curves representing the variations of shear viscosity of batters (a) and their respective continuous phase (b) for different PP concentration: egg-sugar foam (red dotted line ●●); S0 (●);S20 (●);S40 (●), from bottom to top. Straight lines represent fitting by power law (eq.1) using K and n values reported in Table 2. All measurements were performed thrice. For the sake of visibility, curves are not shown for all samples.

Figure 4. (A) Destabilization kinetics of the batter samples represented by the evolution of the normalized liquid fraction over time for egg-sugar foam (e+s, ●●), and for different PP concentration (S0, ■), (S10, ■), (S20, ■), (S30, ■), (S40, ■), from left to right. Measurements were performed twice Example of progressive S0 (b) *vs.* abrupt S40 (c) liquid apparition.

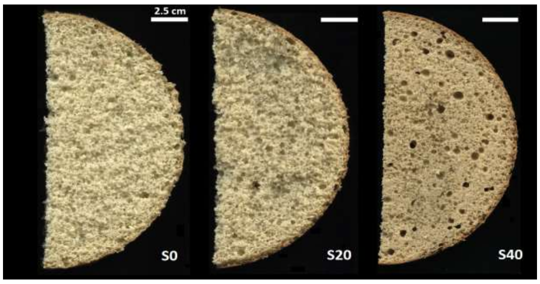
Figure 5. Typical CLSM images (λex = 488 nm) showing the autofluorescence of PP (colored in red) in dry (a) and 5% w/v wet (b) dispersions. Three to five images were taken for each sample.

Figure 6. Typical CLSM images (λex=488 nm) showing the following batter samples: (a) egg-sugar foam; (b) reference batter (S0); (c) S20; and (d) S40. Particles colored in red represent PP isolates. Three to five images were taken for each sample.

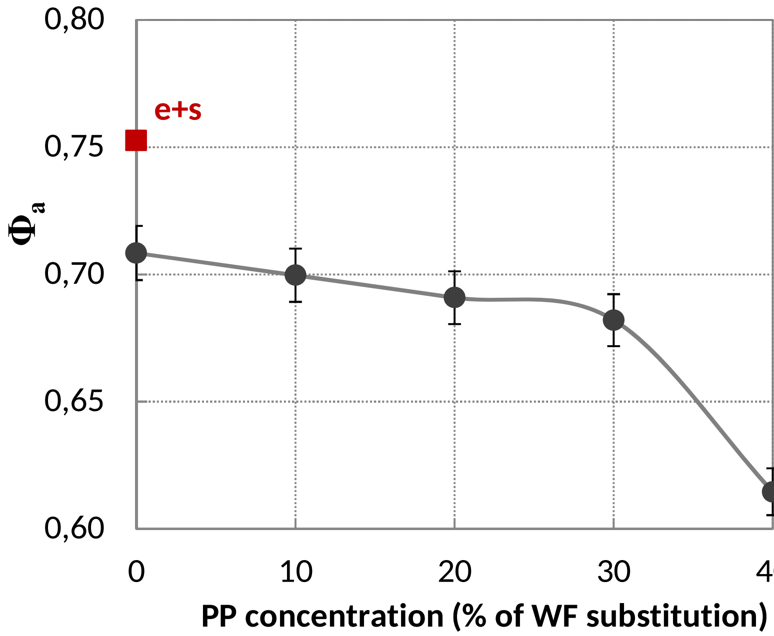
Figure 7. Typical CLSM images (λex = 488 nm) showing the following sponge-cake samples: (a) S0; (b) S20; (c, d) S40 at two different scales. Three to five images were taken for each sample.

Figure 8. Variations of the batter rheological properties normalized by their values for the continuous liquid phase (elastic modulus, ♦; consistency ●) as a function of pea protein isolate concentration. Red dotted lines merely indicate some characteristic values. Curves linking experimental points result from polynomial linear fitting and only illustrate the trend of variations.

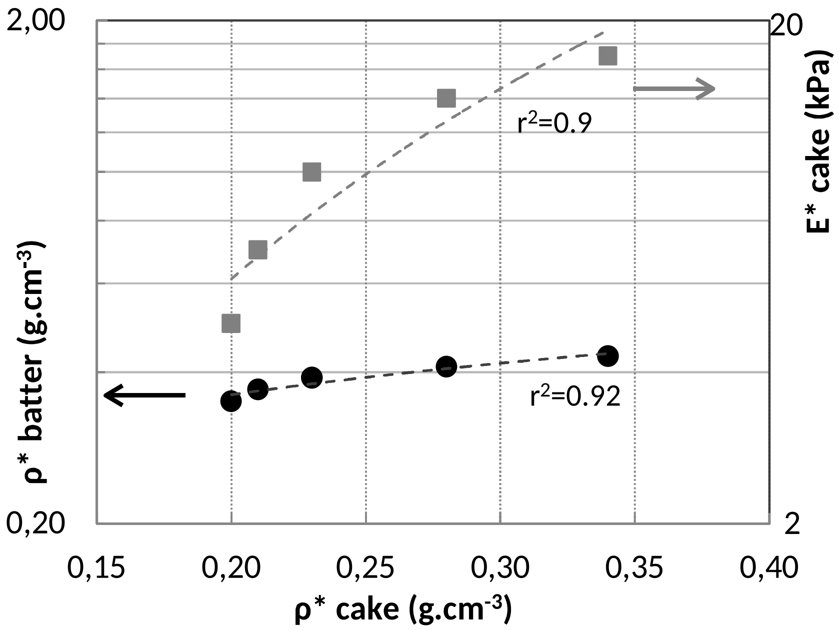
(a)


2.5 cm
S0
S20
S40

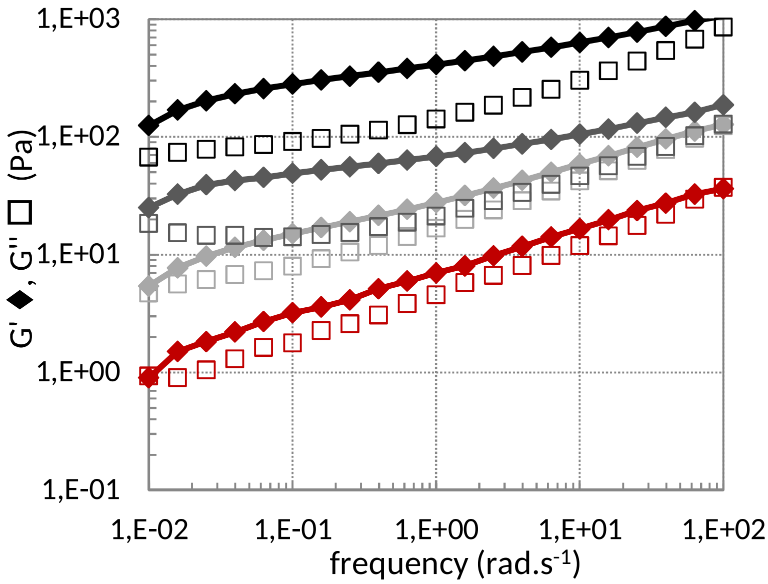
(b)


0,80
0,75
0,70
0,65
0,60
e+s
Φa
0
10
20
30
40
PP concentration (% of WF substitution)

(c)

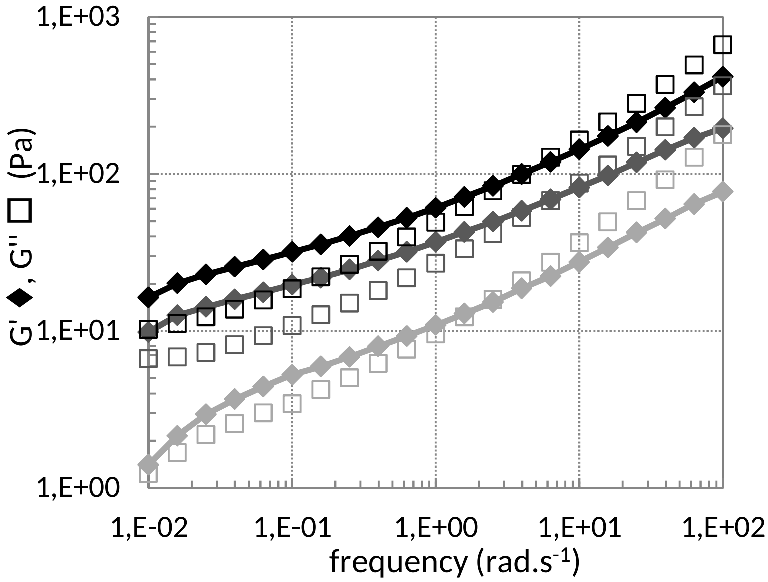

2,00
0,20
20
2
r2=0.9
r2=0.92
ρ* batter (g.cm-3)
E* cake (kPa)
0,15
0,20
0,25
0,30
0,35
0,40
ρ* cake (g.cm-3)

(a)



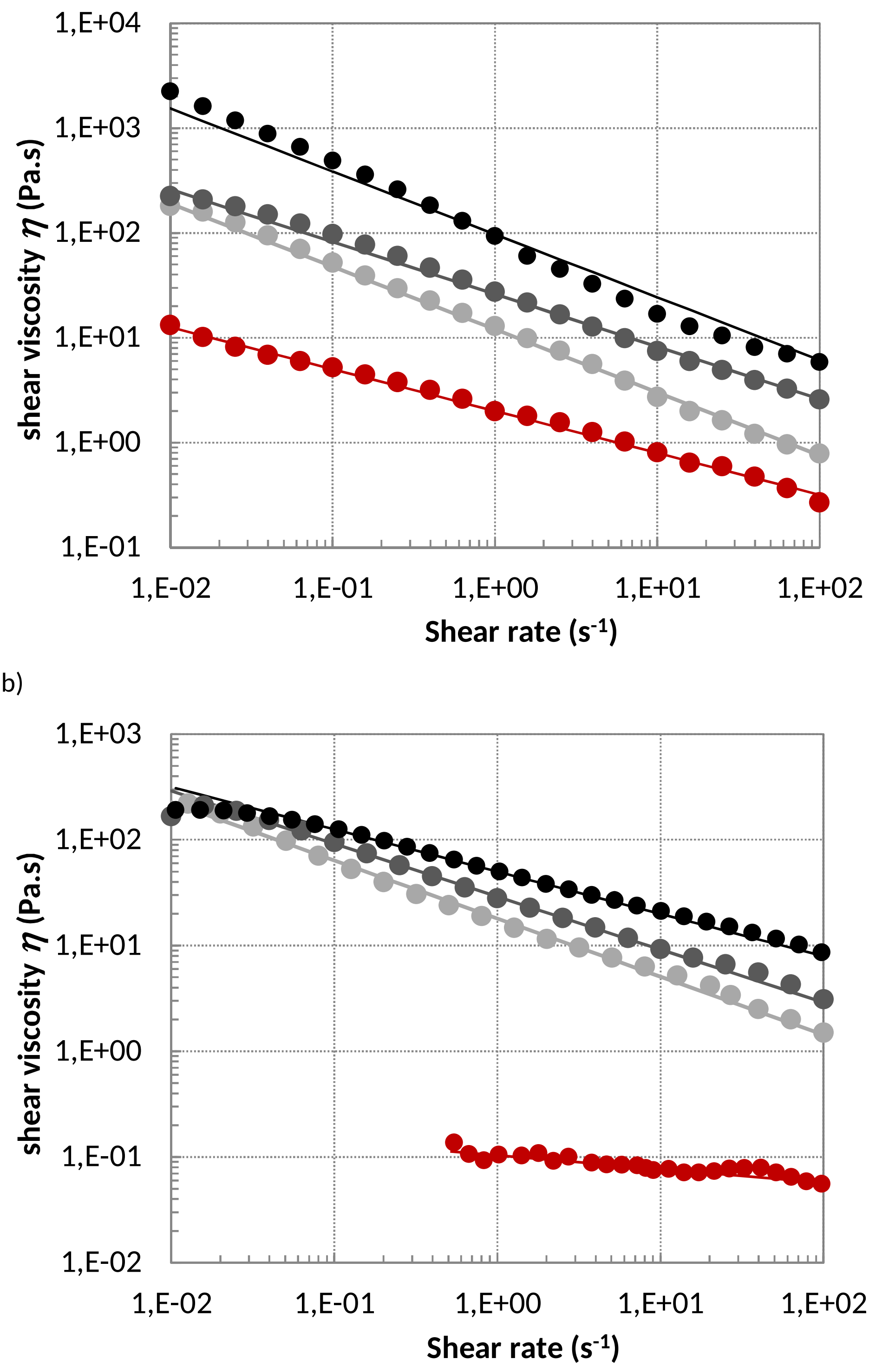
1,E+03
1,E+02
1,E+01
1,E+00
1,E-01
G' ◆, G'' □ (Pa)
1,E-02
1,E-01
1,E+00
1,E+01
1,E+02
frequency (rad.s-1)

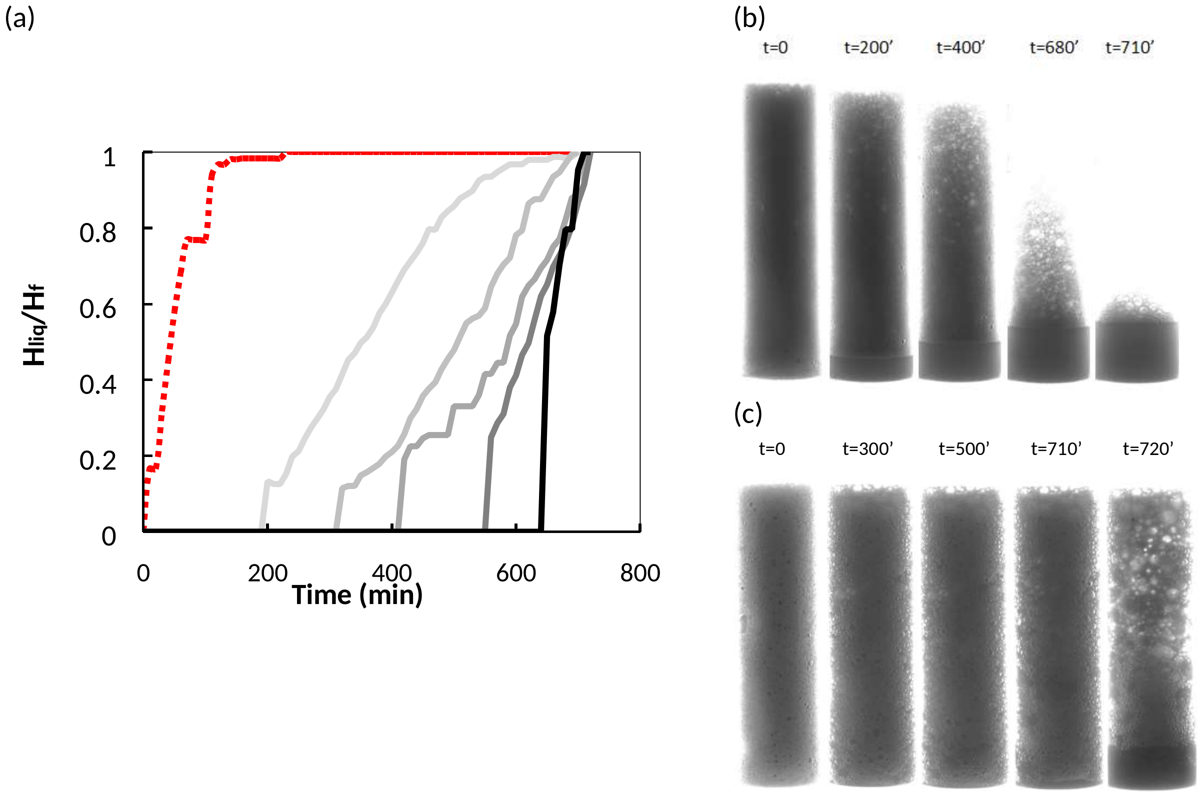
(b)



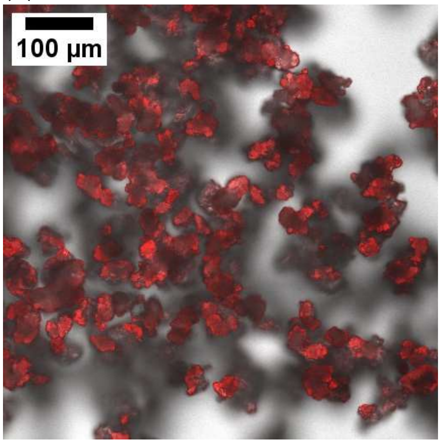
1,E+03
1,E+02
1,E+01
1,E+00
G' ◆, G'' □ (Pa)
1,E-02
1,E-01
1,E+00
1,E+01
1,E+02
frequency (rad.s-1)

(a)

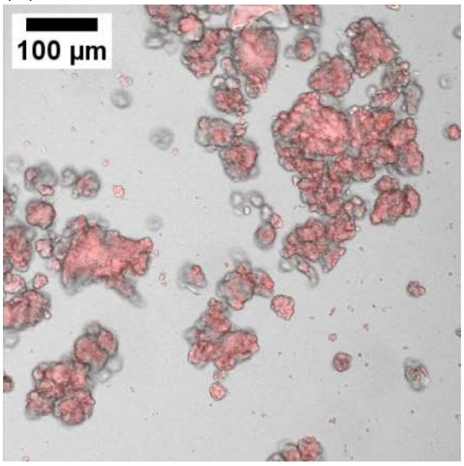

1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
1,E-01
shear viscosity η (Pa.s)
1,E-02
1,E-01
1,E+00
1,E+01
1,E+02
Shear rate (s-1)
(b)
1,E+03
1,E+02
1,E+01
1,E+00
1,E-01
1,E-02
shear viscosity η (Pa.s)
1,E-02
1,E-01
1,E+00
1,E+01
1,E+02
Shear rate (s-1)

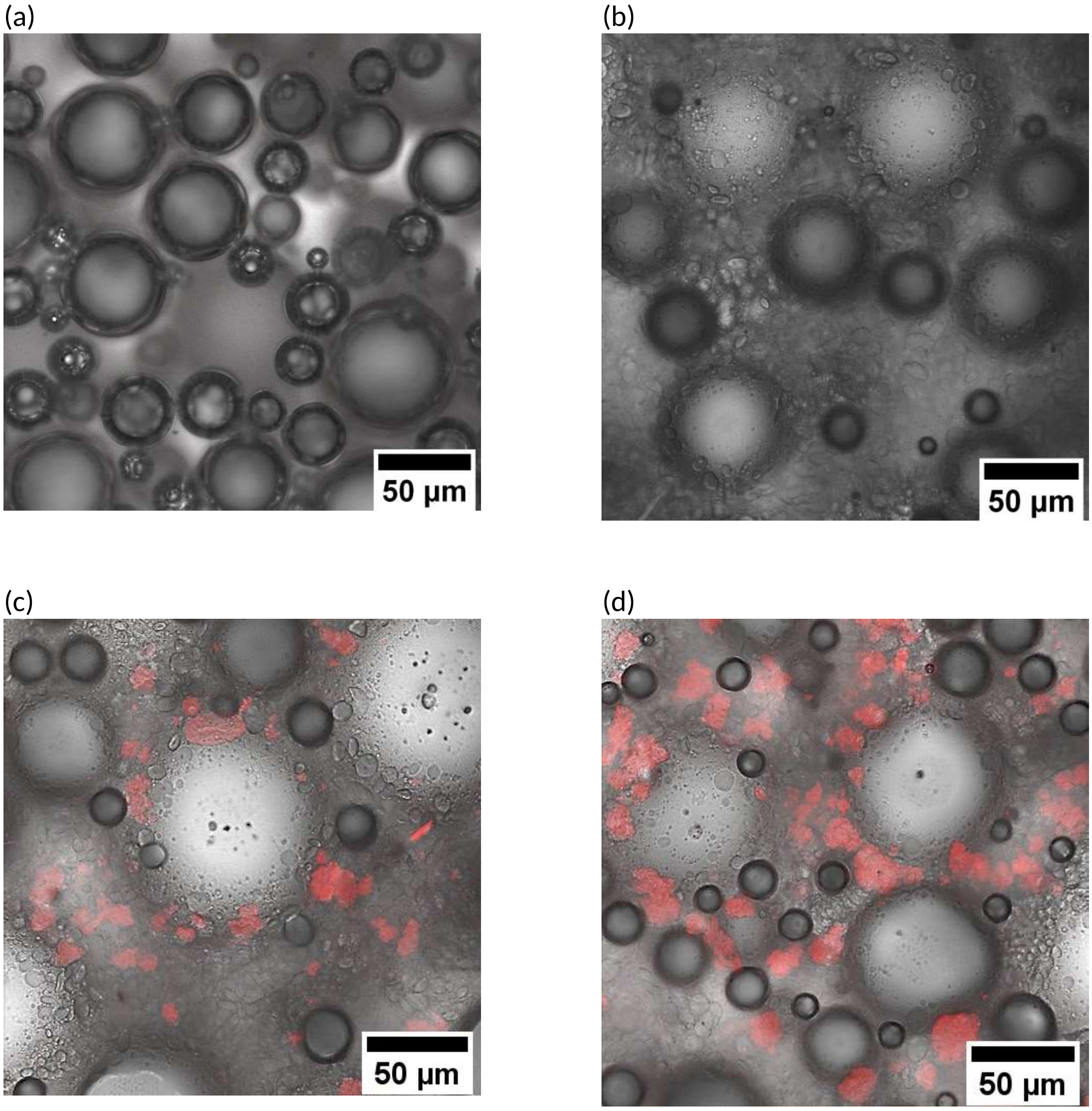

(a)
1
0.8
0.6
0.4
0.2
0
Hliq/Hf
0
200
400
600
800
Time (min)
(b)
t=0
t=200'
t=400'
t=680'
t=710'
(c)
t=0
t=300'
t=500'
t=710'
t=720'

(a)

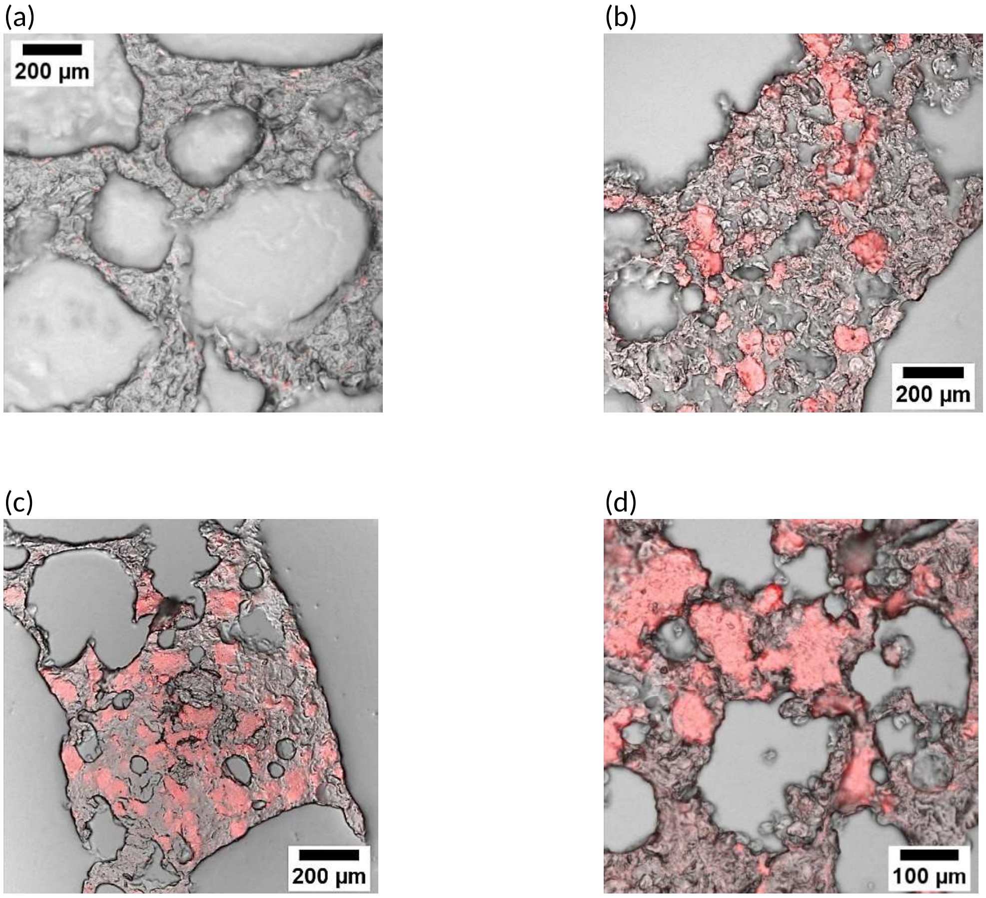


(b)

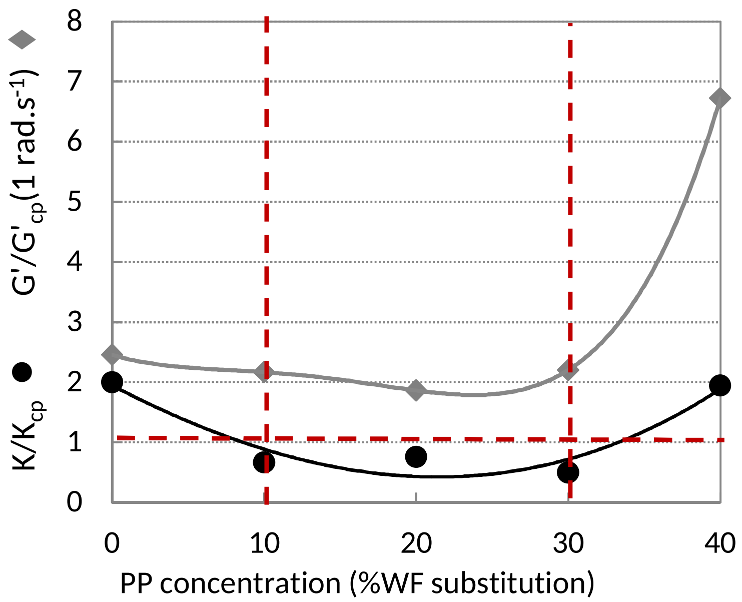




Assad-Bustillos et al Fig.6 Rheology Batter Pea Proteins



Assad-Bustillos et al Fig.7 Rheology Batter Pea Proteins



Assad-Bustillos et al Fig.8 Rheology Batter Pea Proteins

Table 1.Detailed formulae of the studied sponge-cake samples.

Batter composition (% w/w)	**Sample identification**				
	S0	**S10**	**S20**	**S30**	**S40**
Wheat flour (WF)	25	22.5	20	17.5	15
Sugar (sucrose)	25	25	25	25	25
Egg	50	50	50	50	50
Pea protein isolate (PP)	0	2.5	5	7.5	10
% of WF substituted by PP	**0**	**10**	**20**	**30**	**40**

Table 2. Summary of the rheological properties of the studied sponge-cake batters (B) and their continuous phases (CP). K and n values were extracted from Ostwald De Waele model (eq.1).

Sample	**G' at 1 rad·s^{-1} (Pa)**		**tan δ at 1 rad·s^{-1}**		**K (Pa·s^n)**		**n**	
	B	**CP**	**B**	**CP**	**B**	**CP**	**B**	**CP**
egg-sugar	7 ± 1^f	0.005 ± 0.002^a	0.7 ± 0.1^a	4.7± 0.1^a	2 ± 1^d	0.1 ± 0.05^f	0.6 ± 0.1^a	0.8 ± 0.1^a
S0	27 ± 4^e	11 ± 2^e	0.6 ± 0.1^a	0.8 ± 0.1^b	12 ± 2^c	18 ± 1^e	0.4 ± 0.1^b	0.5 ± 0.1^b
S10	39 ± 5^d	18 ± 3^d	0.4 ± 0.2^{ab}	0.9 ± 0.1^b	12 ± 2^c	14 ± 3^d	0.3 ± 0.1^b	0.5 ± 0.1^b
S20	67 ± 6^c	36 ± 5^c	0.3 ± 0.1^b	0.7 ± 0.1^b	22 ± 3^b	29 ± 4^c	0.5 ± 0.2^{ab}	0.5 ± 0.1^b
S30	141 ± 8^b	64 ± 4^b	0.4 ± 0.1^{ab}	0.7 ± 0.1^b	19 ± 3^b	38 ± 5^b	0.3 ± 0.1^b	0.5 ± 0.1^b
S40	410 ± 11^a	61 ± 6^b	0.3 ± 0.2^b	0.8 ± 0.1^b	97 ± 9^a	50 ± 5^a	0.4 ± 0.2^b	0.6 ± 0.2^b

Means within the same column labelled with the same letter are not significantly different (p<0.01) (Student Newman-Keuls test). Measurements were performed thrice.

Table 3. Comparison between wheat flour (WF) and pea protein isolate (PP) properties.

Sample	**WHC (g water/ g dry matter)**	**Solubility (%) pH=7**	**$d_{4,3}$ (µm)**	
			dry	wet 5% (w/v)
PP	2.6 ± 0.1^a	5.3 ± 0.5^a	79 ± 1^a	77 ± 3^a
WF	1.1 ± 0.1^b	6.1 ± 0.3^a	64 ± 1^b	45 ± 6^b

Measurements of WHC and solubility were performed five times, whereas particle size distributions were measured thrice.

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Mélissa Assad Bustillos : Conceptualization, Experimentation, Data treatment, Interpretation, Writing Original Draft

Camille Jonchère : Experimentation in Rheology, Validation, Reviewing Draft

Catherine Garnier : Validation, Interpretation, Reviewing Draft

Anne-Laure Réguerre : Experimentation in Imaging, Validation, Reviewing Draft

Guy Della Valle : Conceptualization, Data treatment, Interpretation, Validation, Writing, Reviewing, Editing, Supervision

APPENDIX

Particle size distributions of pea protein isolates (■) and wheat flour (●) on dry (A) and wet 5%w/v (B) dispersions.