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Impact of *Chlorella vulgaris* on the rheology of wheat flour dough and bread texture

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ABSTRACT

Modern foods lead to healthier, cheaper and more convenient products to increasingly demanding consumers. Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications and can be used to enhance the nutritional and technological value of food products. The enrichment of bread with microalgae biomass is a great challenge resulting from its impact on the development of the gluten structure. The addition of *Chlorella vulgaris* in a wheat flour dough was studied, to evaluate its influence on the dough rheology and bread texture. Microalgae contents from 1.0 to 5.0 g per 100 g of wheat flour were tested and it was observed that up to 3.0 g of microalgae biomass addition, a positive impact on dough rheology and viscoelastic characteristics, with strengthening of the gluten network, was observed. For higher microalgae content, a negative effect on dough rheology, bread texture and flavor was noticed and an acceleration on bread aging was relevant. No impact on the kinetics of yeast fermentation, neither on the time required for fermentation, was induced by the biomass addition.

1. Introduction

The search for new healthy food ingredients is a strategy to overcome the food shortages expected for the next decades. Microalgae are at the bottom of the food chain and can be considered an interesting source of nutrients and bioactive compounds. The production of food products enriched with microalgae has increasingly gaining attention, as people become more aware of the relation between diet and health (Roohinejad et al., 2017).

Spirulina maxima, *Chlorella vulgaris*, *Haematococcus pluvialis*, *Diacronema vlkianum*, and *Isochrysis galbana* are some of the most interesting algae with potential bioactive properties, revealing a well-balanced chemical composition as well as a source of highly valuable molecules, such as polyunsaturated fatty acids, pigments (e.g. carotenoids, chlorophylls), sterols, vitamins, hydrocolloids and other biologically active compounds (Batista, Gouveia, Bandarra, Franco, & Raymundo, 2013). All of them present specific functional and technological properties and recent studies also revealed antimicrobial potential against pathogenic and spoilage microorganisms in food, namely in bread (Pina-Pérez, Rivas, Martínez, & Rodrigo, 2017). *Spirulina maxima* and *Chlorella vulgaris* assume special relevance to be used as food ingredients since they are allowed for that propose by the European Food Safety Authority.

Several traditional products, such as pasta (Fradique et al., 2010), biscuits (Gouveia, Batista, Miranda, Empis, & Raymundo, 2007; Gouveia et al., 2008b), puddings/gelled desserts (Batista, Gouveia, Nunes, Franco, & Raymundo, 2008) and mayonnaises/salad dressings (Gouveia, Batista, Raymundo, Sousa, & Empis, 2006; Raymundo, Gouveia, Batista, Empis, & Sousa, 2005) have been developed with the incorporation of microalgae in their formulation, and expectations about this practice are promising for the food industry (Pina-Pérez et al., 2017). However, few studies have been done on the incorporation of microalgae in bread, despite its high interest as a staple food with great importance in human nutrition. It is a source of carbohydrates, proteins, dietary fibre, vitamins, micronutrients and antioxidants, being appreciated for its taste, versatility, convenience, texture and appearance (Rubel, Pérez, Manrique, & Genovese, 2015).

In fact, the incorporation of new ingredients into a dough matrix is always a significant technological challenge. The unique bread-making properties of wheat flour can be mainly attributed to the ability of its gluten proteins to form a viscoelastic network when mixed with water. Several studies suggested that the weakening of this network, due to the presence of foreign proteins on wheat flour dough, was the result of a dilution of the gluten structure, that resulted in lower loaf volume and subsequently a negative effect on other quality attributes, such as crumb grain and tenderness (Belitz, Kieffer, Seilmeier, & Wieser, 1986;

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Dervas, Doxastakis, Hadjisavva-Zinoviadi, & Triantafillakos, 1999; Mohammed, Ahmed, & Senge, 2012). A similar effect was also reported for the addition of soluble dietary fibre (inulin, pectin and maltodextrins) to wheat flour dough for bread-making (Arufe et al., 2017; Noort, Mattila, Katina, & Willem van der Kamp, 2017; Sirbu & Arghire, 2017), where the negative impact of fibre addition on dough rheology and bread texture was evidenced and a critical dosage should be establish to ensure the acceptable dough rheological behaviour and consequent quality of the enriched bread.

The negative effect resulted from the incorporation of non-traditional ingredients to the dough matrix, related to the disturbance of the dough structure. Dough has a foam-like structure, consisting of a dispersion of discrete gas cells in a continuous starch–protein matrix that are incorporated by the occlusion of air during mixing, and later expand as carbon dioxide (CO_2) is produced by yeast fermentation (Gan, Ellis, & Schofield, 1995). Bread dough shows viscoelastic behaviour, which is mainly attributed to gluten, the major protein source in wheat flour (Mirsaeedghazi, Emam-Djomeh, & Mousavi, 2008) and has a major impact on bread quality. Disturbing the gluten network by adding other ingredients such as protein or fibres will affect physical and rheological properties of the wheat flour dough and its final products. A similar effect is expected for the microalgae addition, considering their composition, namely the high contents of protein (Batista et al., 2013).

Physical dough testing devices have been extensively applied to evaluate the bread making potential and performance characteristics of formulations enriched with different types of ingredients (Arufe et al., 2017; Rebellato et al., 2017; Sirbu & Arghire, 2017). Information about rheological properties of dough will be useful for predicting the potential application of the microalgae and also the quality of the produced bread. The quality of wheat flour bread is determined by the rheological behaviour of dough (Serpil, Kevser, Bengihan, & Hamit, 2008) and is strongly related to bread characteristics and consumer acceptance (Sirbu & Arghire, 2017).

The farinograph is one of the rheological tests that was invented in the 1930s, as the first of the physical dough testing instruments (Janssen, Van Vliet, & Vereijken, 1996) and it is the most popular of the empirical rheological instruments. It is widely used in baking industry to determine flour quality and relates to the desired end product and its manufacturing process. Another reliable empirical method, suitable for wheat flour quality testing, is the alveograph, where a biaxial extension is applied during dough bubble inflation, from the process of dough stretching. The alveograph has been used to evaluate bread making quality of wheat flours (Duyvejonck, Lagrain, Dornez, Delcour, & Courtin, 2012) through the extensibility (L) and tenacity (P) or resistance to extensibility parameters. From the side of the fundamental rheology testing dynamic oscillatory measurements with small amplitude are commonly useful to obtain information on the viscoelastic properties of the food materials. Nevertheless, few work reported on the use of these methodologies to evaluate the dough rheology (Lee & Campanella, 2013; Verheyen, Jekle, & Becker, 2014).

The aim of the present work was to evaluate the impact of the *Chlorella vulgaris* addition on wheat flour dough and the respective impact on bread texture and aging kinetics. Different contents of microalgae were added to wheat flour and the impact on dough rheology was measured by the farinograph and alveograph as well as using small amplitude oscillatory measurements. The breads with the same levels of microalgae incorporation were prepared and its initial texture, texture decay over time, and appearance was evaluated to establish storage time and the maximum content of *Chlorella vulgaris* with potential application in market terms.

2. Experimental

2.1. Raw materials

Bread was prepared using commercial wheat flour - WF (Espiga T65) with 9.1 g/100 g protein and 1.6 g/100 g ash. Commercial white crystalline saccharose (Sidul), sea salt, fresh yeast (Levital), SSL-E481-sodium stearoyl-2 lactylate (Puratos). *Chlorella vulgaris* (*Cv*) powder (Iswari by Allma - Portugal) was used, with the following nutritional composition: 60.7 g/100 g protein, 12.4 g/100 g fiber, 2.3 g/100 g lipids, 13.8 g/100 g carbohydrates, and 0.2 g/100 g salt. Presence of vitamin B12, 220 μg/100 g (Cobalamina), iron, 120 mg/100 g and chlorophyll, 2183 mg/100 g was also referred in the information available on package label.

2.2. Bread doughs preparation

A control dough, without *Chlorella vulgaris* addition, was prepared using the following composition for 100 g of dough: 59.3 g wheat flour, 1.0 g salt, 0.3 g SSL (E481), 2.35 g of fresh yeast, 0.6 g of sugar and 36.45 g of water, a quantity that was determined according to the farinograph tests. The breads enriched with *Cv*, were prepared considering incorporations of 1.0, 2.0, 3.0, 4.0 and 5.0 g *Cv*/100 g WF of the wheat flour. As before, water content was adjusted according to the farinograph measurements.

Six formulations were prepared by mixing the solid ingredients with water, following the current procedure for bread production: Bread dough preparation was performed in a thermo-processor (Bimby-Vorwerk, Cloyes-sur-le-Loir, France). First, the fresh yeast was activated in warm water on the processor cup, during 30 s at position 3. The rest of the solid ingredients were added and homogenized during 60 s at position 6, and the kneading was carried out during 120 s. Bread dough was placed in a rectangular bread container (5.0 cm × 20.0 cm x 8.5 cm), previously oiled and floured, following up the fermentation during 60 min at 37 °C (optimal yeast activity time/temperature, previously optimized), in an electric oven (Ariana). Breads were then baked in the oven at 160 °C during 30 min. After cooling, the breads were stored in a plastic bag, at room temperature, in a cupboard protected from light.

2.3. Dough rheological characteristics

Farinograph characteristics were determined according to AACC International Method 54–21.02 (AACC International, 2011a). The following parameters were determined in a farinograph equipped with a 300 g mixing bowl (Brabender, Duisburg, Germany): water absorption, i.e. percentage of water required to yield dough consistency of 500 BU (Brabender Units), dough development time (DDT) or time to reach maximum consistency, stability (time at a consistency of 500 BU) and degree of softening (difference, in BU, between 500 and the medium line of the curve 12 min after development time). For each sample three measurements were performed.

Alveograph tests were performed using AlveoLab (Chopin Technologies, Villeneuve-La-Garenne, France) according to the AACC International Method 54–30.02 (AACC International, 2011b). The following alveograph parameters were automatically recorded by the respective computer software program: P - tenacity or resistance to extension (the pressure inside the dough bubble in mmH_2O) as the height of the peak in the alveogram and L - dough extensibility (in mm), as the width of the curve. The curve configuration ratio (P/L), that express the equilibrium between the tenacity and the extensibility. For each sample five measurements were performed.

Viscoelastic behaviour of dough during fermentation was accessed using a controlled stress rheometer (Haake Mars III – Thermo Scientific, Karlsruhe, Germany), with an UTC - Peltier system to control temperature, using a serrated parallel-plate sensor system (PP20) and 2 mm gap between plates, to overcome the slip effect. The gap was previously studied and fixed at 2 mm to reduce the interference of normal stresses during the fermentation process, in agreement with the conditions previously optimized by Verheyen et al. (2014).

Dough fermentation *in situ*, on the rheometer plates, was carried out to evaluate the impact of *Cv* addition on fermentation time, by applying a time sweep according to the following conditions: initial temperature of the rheometer at 5 °C, during 120 s, to avoid the start of the fermentation before the desired time, increasing the temperature up to 37 °C (optimal temperature of the yeast), and follow up of the fermentation, by the change on storage modulus (G'), at 1 Hz of frequency and constant shear stress, within the linear viscoelastic region, previously determined by a stress sweep test. This test was performed during 5400 s to obtain an equilibrium of the viscoelastic functions.

Frequency sweep, to evaluate the effect of the *Cv* added on dough structure and the evolution of the dough after fermentation time, the storage (G′) and loss (G″) moduli were obtained ranging the frequency from 0.001 Hz to 10.0 Hz, at a constant shear stress within the linear viscoelastic region of each sample. All determinations were repeated at least two times to ensure that reproducible results were obtained.

2.4. Bread texture

Texture characterisation of bread was performed using a texturometer TA-XTplus (Stable MicroSystems, Surrey, UK), applying a texture profile analysis (TPA) in penetration mode, which consists of compressing a piece of food two times in a reciprocating motion that imitates the action of the jaw (Bourne, 2002, pp. 182–186). Each bread sample was cut into slices 20 mm high of a 120 × 100 mm rectangular shape and rested for 15 min before testing. An acrylic cylindrical probe with 10 mm diameter pierced 5 mm of the sample at 1 mm/s of crosshead speed, with a load cell of 5 kg. Comparison of bread texture with different *Chlorella vulgaris* content was performed in terms of firmness, which was considered the most representative parameter from the texture profile. Firmness (N) of the bread crumb was considered as the maximum resistance to the penetration of the probe and was calculated as the height of the first force peak. The evaluation of firmness during the storage time was also performed and the aging kinetic of the bread was described as a function of biomass incorporation. To discuss the variation of bread texture, the bread moisture was also determined, according to the standard method AACC 44–15.02 (AACC International, 1999).

2.5. Statistical analysis

The experimental results were statistically analyzed by determining the average value, standard deviation, and the significance level was set at 95%, for each parameter evaluated.

Statistical analysis was made by applying variance analysis, the one factor (ANOVA), and multiple comparisons (Tukey test).

3. Results and discussion

3.1. Effect of Chlorella vulgaris content on farinograph parameters

The effect of *Cv* addition on the farinographic characteristics of the wheat flour dough can be seen in Fig. 1. Water absorption (Fig. 1A) steadily increased up to 3.0 g *Cv*/100 g WF addition due to the presence of extra-protein from the microalgae. After this concentration, further addition of *Cv* results in a slight, but significant ($p < 0.05$) reduction of water absorption. These results suggest that the *Cv* cells, together with the gluten proteins, up to a certain level, i.e. 3.0 g *Cv*/100 g WF, required more water to achieve the standard dough consistency (500 BU). For additions above this value, aggregation between *Cv* cells should reduce the water absorption capacity. Similar results were observed by Dervas et al. (1999), Mohammed et al. (2012) and Turfani, Narducci, Durazzo, Galli, and Carcea (2017), with addition of legume proteins to wheat flour. An increase in water absorption resulting from the incorporation of vegetable protein or other protein sources, concentrates or isolates, to wheat flour, has been attributed to the water absorbing capacity of these proteins, and their ability to compete for water with other constituents in the dough system, resulting in doughs which exhibit increased farinograph water absorption (Dervas et al., 1999; Doxastakis, Zafiriadis, Irakli, & Tananaki, 2002). The quantity of added water is considered to be very important for the distribution of the dough materials, their hydration and the gluten protein network development, as well as to increase the production yield. The time required for the dough development (DDT), or time necessary to reach 500 BU of dough consistency, was almost constant until 3.0 g *Cv*/100 g WF and slightly increased ($p < 0.05$) for higher concentrations of *Cv* (Fig. 1B). This was followed by the dough stability index (Fig. 1B) which, over 3.0 g *Cv*/100 g WF addition showed a sudden significant ($p < 0.05$) decay to about half of the initial value (from 8.7 to 4.6). This means that from 3.0 g *Cv*/100 g WF over of microalgae incorporation, the structure of the wheat dough is somehow disrupted, which resulted in lower dough strength and loaf volume with a negative effect on the quality attributes of bread (Fig. 2), such as less alveoli on crumb grain, and on the crust shape on the top of the loaf, as previously reported for the addition of other protein sources to bread (Knorr & Betschart, 1978; Mis, Grundas, Dziki, & Laskowski, 2012). Similar results have been previously reported by other authors, for the addition of vegetable proteins (Mohammed et al., 2012; Lazo-Velez, Chuck-Hernandez, & Serna-Saldívar, 2015) or hydrocolloids (Doxastakis et al., 2002) on bread formulations. Dough softening degree (Fig. 1C) shows the same pattern, i.e., a sudden increase ($p < 0.05$) up to twice the initial value at over 3.0 g *Cv*/100 g WF addition. Similar changes of this parameter were reported for blends with cowpea flour and chickpea flour by Fernandez and Berry (1989), and Sharma, Bajwa, and Nagi (1999).

Wheat flour doughs with different *Cv* concentrations from 0 to 5.0 g/100 g of wheat flour were formulated, using the water absorption values from the farinographic analyses, to be baked and the resulting breads can be seen on Fig. 2. The impact of the *Chlorella vulgaris* addition on the bread volume, as well as on the alveoli structure of the crumb and crust color, are noticeable. The *Cv* bread internal structure revealed an increase on the size of the gas cells, compared to the control bread, indicating that the incorporation of microalgae disturbs the dough internal structures. Again, for additions of over 3.0 g *Cv*/100 g WF, the most relevant effect is observed on the crumb structure, with a coalescence of alveoli in a less homogeneous structure with big holes. This important feature, induced by the microalgae addition, can be attributed to the decrease of dough stability, due to the dilution effect of the gluten matrix and phase separation of the excess of *Cv* particles with possible disruption of the gluten network. Dervas et al. (1999) and Doxastakis et al. (2002), also reported a loss on bread crumb structure with increasing levels of lupin or soy flour and attributed this decrease to the dilution of the wheat gluten by the legume proteins. According to Mohammed et al. (2012) this decrease in bread crumb quality was justified by the combined effects of gluten dilution and mechanical disruption of the gluten network structure by the chickpea proteins.

It is also important to note that the incorporation of *Cv* transmitted a marine taste, more intense for higher concentrations, that can be considered a depreciative attribute as it was found in a previous work about the incorporation of *Cv* on pasta (Fradique et al., 2010).

3.2. Influence of Chlorella vulgaris on the alveograph characteristics

The effect of *Cv* addition on the alveograph parameters of wheat flour dough is shown in Fig. 3. Dough resistance to deformation - tenacity (P) is a predictor of the ability of the dough to retain gas (Rosel, Rojas, & Barber, 2001). This parameter shows a tendency to decrease with the microalgae addition, being similar to the control for 1 g *Cv*/100 g of WF, slightly reduced for the 2 and 3 g *Cv*/100 g WF and subsequently reduced for 4 and 5 g *Cv*/100 g WF additions, as can be seen from Fig. 3. An opposite effect was observed on the extensibility (L), an indicator of the handling characteristics of the dough, that was greatly increased with *Cv* addition, to almost double of the control

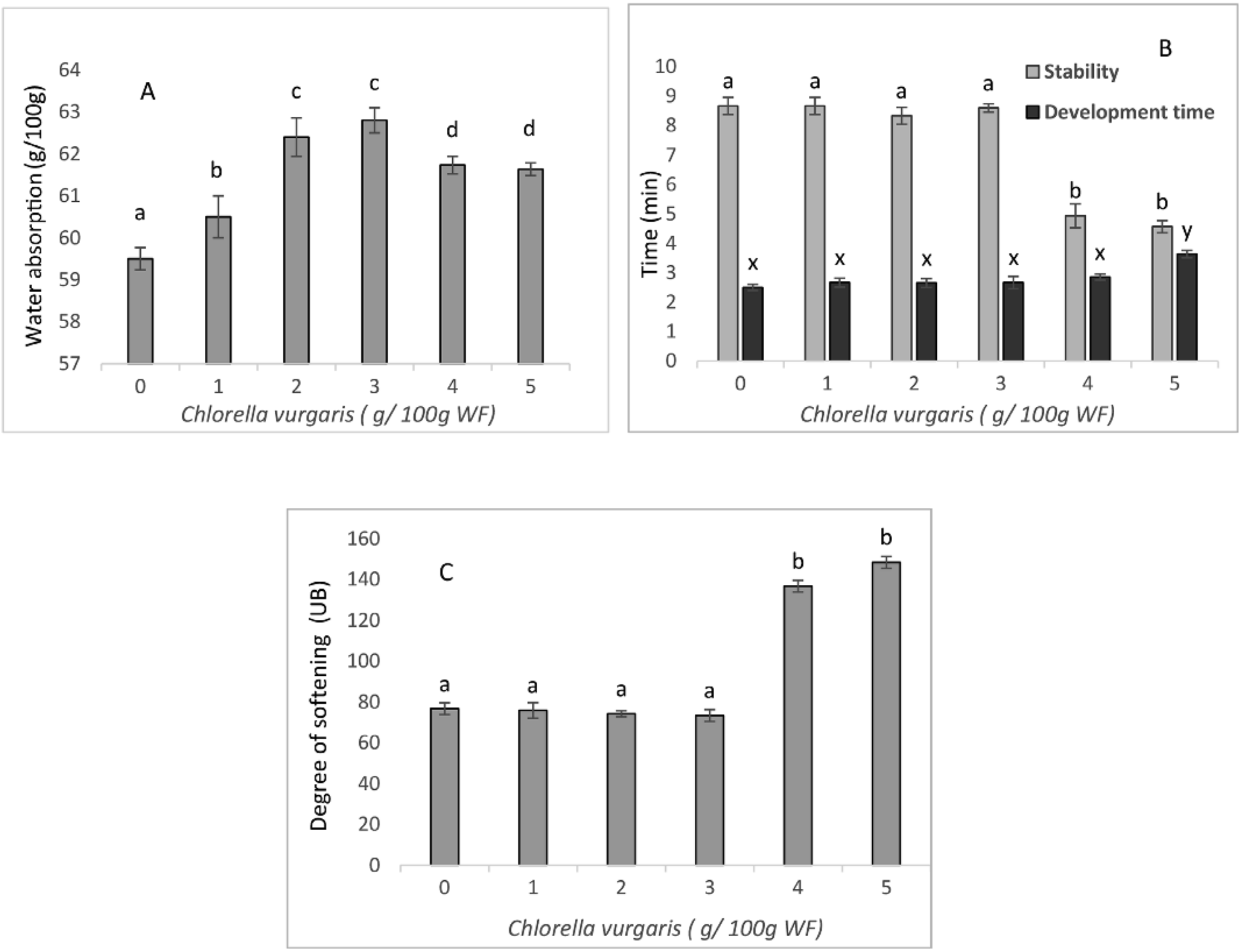

Fig. 1. Effect of *Chlorella vulgaris* addition on the farinograph characteristics of the wheat dough: A - Water absorption; B - Time required for the dough development (DDT) and Dough stability; C - Dough softening degree.

dough extensibility. Similar results were observed by Mis et al. (2012), with additions of the carob fiber and oat wholemeal to wheat flour. Wheat proteins include albumins, globulins, gliadins and glutenins but only the last two participate in the formation of a continuous viscoelastic network, i.e., the gluten network (Shewry, Halford, Belton, & Tatham, 2002). Glutenin polymer chains provide strength and elasticity to bread dough development, whereas globular proteins, such as gliadins, contribute to the viscosity of dough. Gliadins interact with the glutenin polymers via non-covalent hydrophobic interactions and hydrogen bonding being the structure of both proteins crucial to the breadmaking process (Sivam, Sun-Waterhouse, Perera, & Waterhouse, 2012). The incorporation of *Cv* in wheat flour seems to favor the action of gliadin, contributing to more extensible dough (L), as it can see in Fig. 3A. In contrast, it appears to have an adverse effect on the glutenin action, verifying a significant decrease in the dough tenacity (P) at over 3 g *Cv*/100 g WF. Therefore, it can be considered that the *Cv* proteins disturb the gluten matrix of the bread dough.

As a result of the microalgae action on both dough resistance and dough extensibility, the P/L ratio, which gives information about the elastic resistance and extensibility balance of a wheat flour dough, was reduced in all doughs (Fig. 3). Similar results were observed by Lazo-Velez et al. (2015), with soybean proteins in yeast-leavened breads. An ideal P/L ratio for baking varies between 0.5 and 1.20 (Arozanena, Iguaz, Noriega, Bobo, & Virseda, 2012). The control dough has a 1.04 P/L ratio, which is classified as balanced and suitable wheat flour for bread production. According to the results obtained (Fig. 3B), the blends within this range of P/L are the ones with 1.0, 2.0 and 3.0 g *Cv*/100 g WF. Blends with over 3.0 g *Cv*/100 g WF showed a reduction on P/L ratio and are not suitable for bread making. Nevertheless, these mixtures can be used for other higher valued applications, such as cookies and biscuits.

Comparing these findings with the works performed by Dervas et al. (1999), Rosel et al. (2001) and Mohammed et al. (2012), related to the addition of chickpea and lupin, a similar effect was observed, and the reduction of P/L was attributed to the reduction of gluten strength to a dilution effect of the gluten proteins.

3.3. *Fermentation behaviour of doughs containing Chlorella vulgaris*

The changes of dough viscoelastic properties during fermentation time were monitored by a controlled stress rheometer, following the storage modulus variation (G′). From Fig. 4 (A), a considerable decrease of G’ up to 3600 s, was observed for all the doughs tested. After this time, the viscoelastic parameter remained almost constant in all cases. The first stage, about 1 h, corresponds to the high fermentation activity, with CO_2 production, which impacts on the dough structure, with deformation induced by the growing of the gas cells. On the next stage, the fermentation process reached a steady state. From these results, it can also be said that the *Cv* addition does not markedly influence the fermentation kinetics, neither the time required for fermentation.

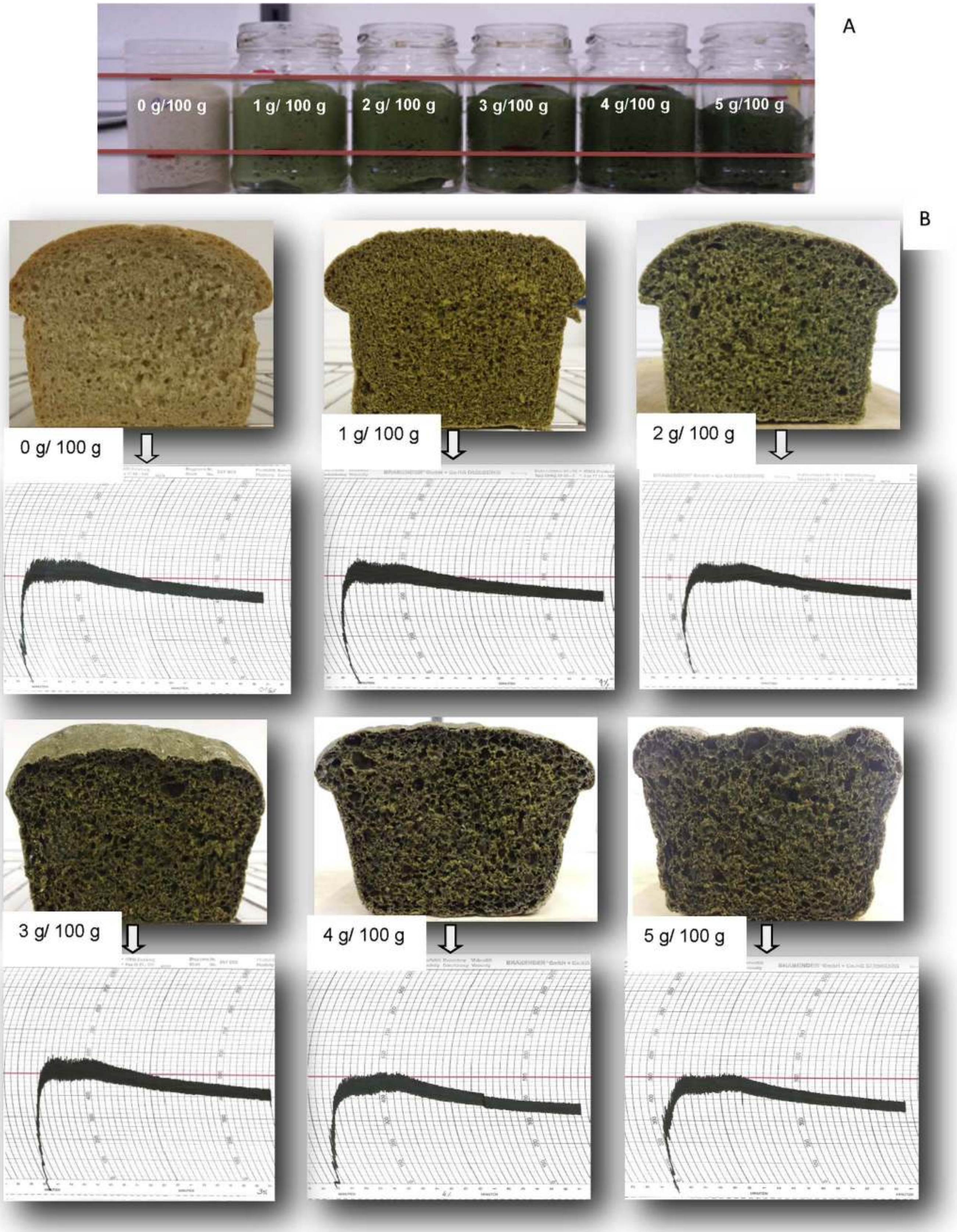

Fig. 2. Variation of the bread dough volume with different concentrations of *Chlorella vulgaris*, after 1 h of fermentation at 37 °C (A) and farinograms obtained by farinograph analysis for each flour blend in study: Control Bread (without *Cv*) and bread produced with 1, 2, 3, 4 and 5 g *Cv*/100 g WF (B).

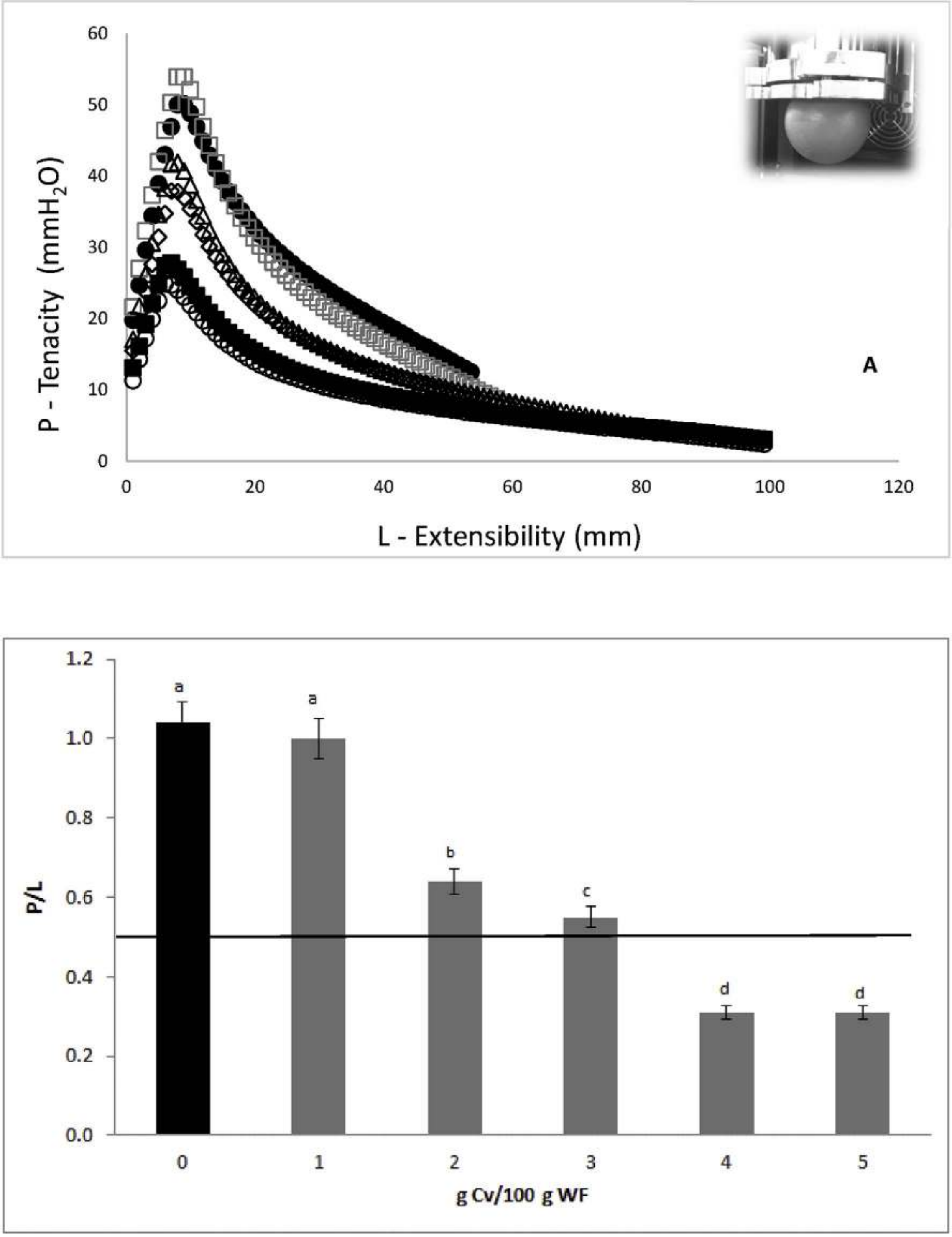

Fig. 3. Effects of *Chlorella vulgaris* addition on wheat flour on the alveograph parameters: A) P - dough tenacity; L - dough extensibility and B) P/L ratio; 0 g/100 g WF; 1 g/100 g WF; 2 g/100 g WF; 3 g/100 g WF; 4 g/100 g WF; 5 g/100 g WF; The black horizontal bars indicate the range of P/L for breadmaking. Different letters mean statistically different values ($p < 0.05$).

The frequency sweep performed after *in-situ* rheometer fermentation was performed to evaluate the impact of *Cv* microalgae addition on dough structure after fermentation. The mechanical spectra presented in Fig. 4 (B and C), suggest that the microalgae have different effects on dough properties, according to the *Cv* content:

i) until 3.0 g *Cv*/100 g WF, higher G' values, are an indication of a possible strengthening effect of the dough structure, probably due to a reinforcement of the protein matrix, resulted from the addition of the microalgae with a high protein content;
ii) from 3.0 g *Cv*/100 g WF over of microalgae, the strengthening effect induced by microalgae is replaced by a destructuring effect probably due to a phase separation of the added biomass with disruption of the gluten matrix.

All the results obtained, i.e., from the farinograph, alveograph and

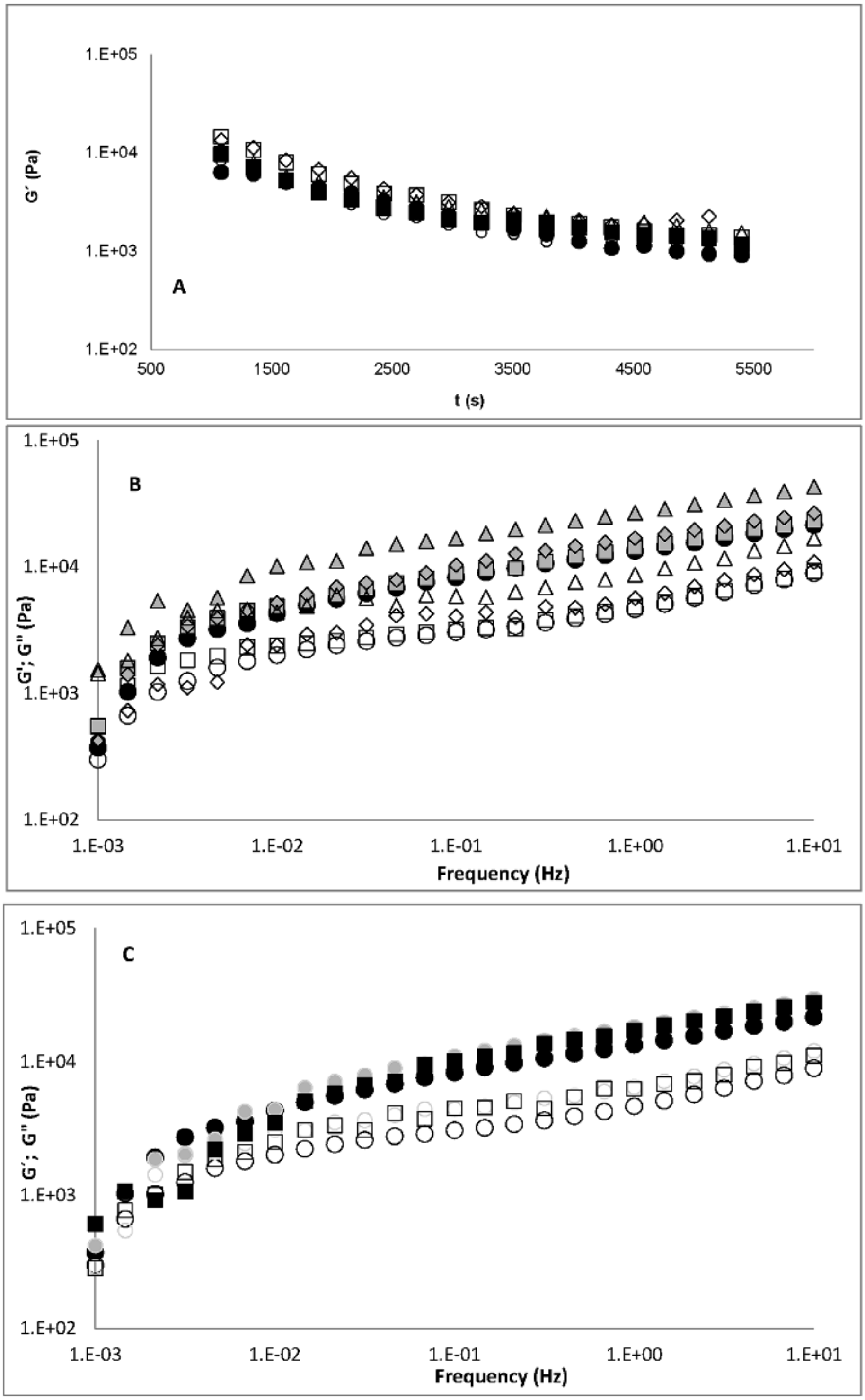

Fig. 4. A) Changes in storage modulus (G') on bread dough, during fermentations: 0 g/100 g WF; 1 g/100 g WF; 2 g/100 g WF; - 3 g/100 g WF; 4 g/100 g WF; 5 g/100 g WF; and B) mechanical spectra at 5 °C - variation of storage (G'-full symbols) and loss (G" – open symbols) moduli measured by after fermentation stage, comparing the control (without *Cv*) with1, 2 and 3 g *Cv*/100 g WF addition:; 0 g/100 g WF,; 1 g/100 g WF,; 2 g/100 g WF,; 3 g/100 g WF; and C) comparing the control with 4 and 5 g *Cv*/100 g WF addition:; 4 g/100 g WF,; 5 g/100 g WF.

rheometer are in agreement and a protein network model, dependent on microalgae concentration, can be proposed: up to 3.0 g *Cv*/100 g WF an increase of the viscoelastic parameters is observed and this can be attributed to the reinforcement of the viscoelastic protein network with the *Cv* particles evenly distributed within the gluten matrix, according to the swollen network model earlier proposed by Morris (1998).

The effect of *Chlorella vulgaris* addition over 3.0 g/100 g WF on dough structure characteristics can be attributed to the depletion flocculation, or phase separation phenomena (Morris, 1998), as it was also previously reported by Nunes, Raymundo, and Sousa (2006) for other complex gelling systems, where the particles segregate each other and phase separate with an antagonistic effect in the dough network structure.

3.4. Bread texture and aging kinetic

The texture of the bread produced with different contents of *Chlorella vulgaris* was evaluated through a texture profile analysis (TPA) and it was verified that firmness was the parameter that efficiently discriminate the different formulations, since the other TPA parameters were not able to discriminate the different formulations, showing no significant differences for all the samples. The evaluation of firmness

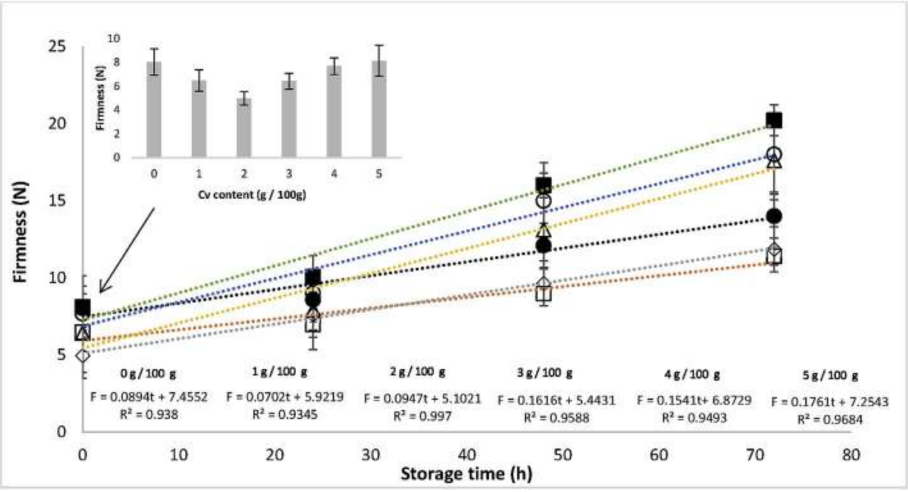

Fig. 5. Variation of firmness (N) over time, for wheat flour breads prepared with different contents of Cv addition and respective linear equations: 0 g/100 g WF; 1 g/100 g WF; 2 g/100 g WF; 3 g/100 g WF; 4 g/100 g WF; 5 g/100 g WF.

during the storage time (72 h) was also performed, to observe the effect of microalgae addition on the kinetic of bread aging (Fig. 5). A similar procedure was followed by Noort et al. (2017), to study the impact of different processing conditions on bread quality.

From Fig. 5 it can be observed that, for all the microalgae content, a positive linear relation is observed between bread firmness at storage time, according to a linear equation ($R > 0.9$):

$$\text{Firmness} = A * \text{time} + B \tag{1}$$

where A can be considered the aging velocity and B the initial firmness.

It can be noticed that for the time zero, initial firmness (B) is not substantially affected by microalga concentration ($p > 0.05$). Nevertheless, the results follow the reverse tendency of the dough water absorption (Fig. 1 A), i.e., less firm with more absorbed water. In terms of bread moisture, a significant ($p < 0.05$) increase was observed: 37 up to 46 g water/100 g bread, when *Chlorella* content increased from 2 up to 4 g/100 g flour. However, a noticeable increase of the bread aging velocity (A) with *Cv* content was observed – from 0.0894 (N/h) to 0.1761 (N/h), representing an increase of 97%. Nevertheless, for 1.0 and 2.0 g *Cv*/100 g wheat flour, only a slight variation of aging velocity was observed. This behaviour has an important impact for the commercial purpose and it should be explained by changes in the moisture distribution in the system, as it was early reported by Noort et al. (2017). As it was observed in Fig. 1, a markedly increase of water absorption with biomass addition was observed for 4.0 and 5.0 g *Cv*/100 g WF, this competition for water absorption by the *Cv* components should cause an acceleration in the aging of bread, expressed in terms of firmness increase within short times.

4. Conclusions

Given the present results, based on empirical and fundamental experiments, it was found that the addition of *Chlorella vulgaris* had an impact on the rheological features of the bread dough, depending on the concentration level: up to 3.0 g *Cv*/100 g WF, a positive impact on viscoelastic characteristics, with strengthening of the gluten network was observed. This behaviour was also supported by the increase of farinograph water absorption, presumably due to greater water requirement to hydrate *Cv* proteins. At this concentration range, the P/L ratios are within the range of values appropriate for baking. No impact on the kinetics of yeast fermentation, neither on the time required for fermentation was induced by the biomass addition. For this range of *Cv* content, the bread firmness is close to the reference wheat bread, and a similar aging kinetic was observed. From the commercial purpose it is also important to refer that overall, up to 3.0 g *Cv*/100 g WF addition, resulted in breads with an interesting appearance, but with higher *Cv* contents a markedly depreciation of the global aspect was noticed.

The use of *Chlorella vulgaris* as a food ingredient is a promising way to enrich bread in bioactive compounds, resulting from its well-known composition. A technological limitation for biomass incorporations higher than 3.0 g *Cv*/100 g WF was noticed. The addition of microalgae at concentrations above 3.0 g *Cv*/100 g WF originated doughs with lower strength, lower tenacity (elasticity) and more extensible and consequently with limiting P/L ratios for bread application. However, in such cases the blends can still be exploited for the production of other bakery products such as biscuits, which requires lower P/L values.

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