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Effect of microalgae incorporation on physico-chemical and textural properties in wheat bread formulation.

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Effect of microalgae incorporation on physico-chemical and textural properties in wheat bread formulation.

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ABSTRACT

The objective of this study was to evaluate the effect of the incorporation of different micoalgae on physico-chemical and textural properties of bread. Four species of microalgae *Isochrysis galbana* (Ig), *Tetraselmis suecica* (Ts), *Scenedesmus almeriensis* (Sa) and *Nannochloropsis gaditana* (Ng) were used in this study. Properties as water activity, pH, microbiological counts, viscosity and color were analyzed to determine the effect of microalgae addition on sourdough. The technological quality of breads was analyzed in terms of physico-chemical properties, color, texture profile and porosity. The main effect of microalgae addition was changes in bread color, crust and crumb that implies an increase of browning and an evolution to more green-yellow tonalities. The textural parameters of breads as hardness, chewiness and resilence are not modified by microalgae addition.

Key words: microalgae, bread, color, texture

1. Introduction

Bread is the most frequently consumed bakery product in many countries. In recent years, different healthy ingredients have been used in the production of bread to enhance its nutritional profile or to confer functional properties. Microalgae have received increasing attention because they represent one of the most promising sources of compounds with biological activity that could be used as functional ingredients (Buono et al., 2014; Da Silva Vaz et al., 2016; Pulz and Gross, 2004). Their balanced chemical composition (good quality proteins, balanced fatty acid profiles, vitamins, antioxidants and minerals) and their interesting attributes can be applied in the formulation of novel food products (Da Silva Vaz et al., 2016; Spolaore et al., 2006).

Microalgae are photosynthetic microorganisms with an important role as primary producers in most aquatic food webs. They are characterized by a high diversity and are potentially a great source of natural compounds for several biotechnological and therapeutic applications (Borowitzka, 2013; Guedes et al., 2011; Raja et al., 2008). In fact, they convert inorganic substances into organic matter richin lipids, proteins, carbohydrates and other molecules. Because of their high nutritional value, microalgae have been widely used in aquaculture as food for molluscs, zooplankton and early life-stages of crustaceans and small fishes (Hemaiswarja et al., 2011). On the other hand, microalgae are considered one of the prospective biological species for producing biofuel (Brennan and Owende, 2010). Among microalgae, the species *Isochrysis galbana* (*I. galbana*) is known to have good nutritional quality, and has received increasing interest in aquaculture mainly due to its polyunsaturated fatty acid content (Liu and Lin, 2001; Sánchez et al., 2000; Yoshioka et al., 2012). It also has beneficial effects on human health and provides a good source of fish oil substitution in the human diet (Batista et al., 2013; Yu et al., 2010). In addition, *I. galbana* showed promising

curative effects inducing weight loss, decreased glucose, triacylglycerol and cholesterol levels in diabetic rats (Nuño et al., 2013). Cultivation of *I.galbana* has also been suggested for biodiesel production (Sánchez et al., 2013). *Tetraselmis suecica* (*T. suecica*) is a marine based microalgae and is known to be able to tolerate salinity of 25-35%. The microalgae species has a relatively high lipid concentration compared to other microalgae species. Tolerance to high salinity, high specific growth rate and lipid content of *T. suecica* makes it a good candidate for biofuel production (Zainan et al., 2015). The microalgae *Scenedesmus almeriensis* (*S. almeriensis*) was isolated in Almería (Spain). Some remarkable characteristics of this species are its high lutein (0.53% dry mass (DM)) and carotenoid (0.69% DM) content, which make *S. almeriensis* an interesting alternative for the production of carotenoids (Sánchez et al., 2008), the high productivity (3.6 mg L^{-1} day^{-1}), and its tolerance to a wide range of environmental growth conditions. From a nutritional point of view, furthermore, this specie presents considerable protein content (49.4% crude protein on DM basis) and a fatty acid profile with substantial amounts of linolenic acid, 18:3n3 (Sánchez et al., 2008). The high protein content, together with remarkable productivity, turns *S. almeriensis* biomass into a potential protein ingredient. *Nannochloropsis gaditana* (*N. gaditana*) is found in a wide range of temperature and irradiance conditions in natural environments, with high pigment contents (carotenoids), thus suggesting that this microalgae is able to regulate its photosynthetic apparatus as a function of culture conditions (Camacho-Rodríguez et al., 2015; Lubián et al., 2000). Carotenoids have antioxidant properties and are therefore used in food and health preservation (Cerón et al., 2007). *N. gaditana* is also appreciated for its ability to accumulate proteins, lipids (Fábregas et al., 2002) and polyunsaturated fatty acids (PUFAs) (Camacho-Rodríguez et al., 2015). The microalgae used in this work (*Isochrysis galbana* (Ig), *Tetraselmis*

suecica (Ts), *Scenedesmus almeriensis* (Sa) and *Nannochloropsis gaditana* (Ng)) are not recognized as GRAS at the moment.

Then, the use of natural ingredients as microalgae exhibiting functional properties, providing specific health benefits, beyond traditional nutrients, is a very attractive way to design new food products, with an important market niche of the healthier foods, growing exponentially. One of the main issues regarding the application of functional ingredients in novel food products is their stability and resistance to severe processing conditions (e.g. high temperatures in bread production). So far, little research has been carried out regarding the effects of whole microalgae incorporation in flour based products. Gouveia et al. (2007, 2008) studied the use of *Chlorella vulgaris* as colouring source in traditional butter cookies and the use of *Isochrysis galbana* as source of PUFA in traditional biscuits. Rodríguez De Marco et al. (2014) use spirulina biomass to enhance the nutritional profile of bread wheat pasta. Ketabi et al. (2008) analysed the effect of microalgae exopolysaccharides in rheological properties of sourdoughs.

The aim of this work was to use four different microalgae biomass (*I. galbana*, *T. suecica*, *S. almeriensis* and *N. gaditana*) as new functional ingredients to evaluate how the addition of microalgae to bread dough affected the technological quality of this product.

2. Materials and methods

2.1. Materials

Isochrysis galbana (Ig), *Tetraselmis suecica* (Ts), *Scenedesmus almeriensis* (Sa) and *Nannochloropsis gaditana* (Ng) used in this study (Fig. 1) were provided by Universidad de Almeria (Department of Chemical Engineering, University of Almería, Almería, Spain). The microalgae were obtained from the Marine Culture Collection of

the Institute of Marine Sciences of Andalucía (CSIC, Cádiz, Spain) and produced in an industrial size outdoor tubular photobioreactor, in continuous mode on Almería (Spain). The biomass was daily harvested by centrifugation, then being freeze-drying and stored at 18 ºC. The content of protein, carbohydrates and lipids (P/C/L) in percentage for freeze-dried microalgae used was 53 / 13.5 / 13 for Ig; 37.6 / 31.6 / 6.7 for Ts; 48.3 / 38.3 / 10 for Sa and 52 / 12 / 28 for Ng. Freeze-drying biomass was used as raw material (Acién et al., 2012). The level considered of its use in sourdoughs was 1.5% to obtain a final level of 0.6 g of microalgae in 150 g of bread. *Chlorella vulgaris* and *Arthrospira platensis* (Spirulina) were taken as reference to define this level of use because they are recognized as GRAS as an ingredient in food in a level of use of 1.35 g (dairy intake) for *Chlorella* and 0.5 to 3 g/serving for Spirulina (USFDA, 2011; USFDA, 2012).

Wheat flour (Haricecu type H) was provided by Harinera Segorbina S.L. (Castellón, Spain). The alveographic parameters were W 110 ± 18 and P/L 0.2 ± 0.1. Salted water (Mediterranea Agua de Mar) was provided by Marevendis Agua de Mar S.L. (Alicante, Spain). It is a commercial solution of purified and sterilized seawater with a 5% salt content (86% NaCl and 14% other minerals).

2.2. Sourdough and bread preparation

A local master baker (Horno San Bartolomé, Valencia, Spain) provided sourdough starter. This starter was made with water and apple 50% and flour 50% and spontaneously fermented. Five sourdoughs were prepared, one without microalgae (control) and four with 7.5 g of microalgae (Ig, Ts, Sa or Ng) added in 500 g of sourdough starter (1.5% w/w). The mix was stored 24 h at 8 ºC and 80% relative humidity. Apart from the first fermentation, the operation named “back-slopping” (or “refreshment”), consisting in the inoculation of flour and water with an aliquot of

previously fermented dough, is repeated before each fermentation step (De Vuyst et al., 2009). After 24 h, sourdoughs were tempered 2 h at 25 ºC and feed with water and flour (15 g of both) to raise its activity. This mix was used to study the characteristics of sourdough with microalgae. For bread elaboration, 40 g of each sourdough were mixed with 100 g of wheat flour and 50 g of salted water with a spiral mixer (Kenwood Classic Chef KM331, Kenwood Limited, New Lane, UK) during 10 min. This dough (190 g) was shaped into loaves (63 g) and left to ferment at 9 ºC during 20 h (slow fermentation) at 75% relative humidity. After, doughs were maintained at room temperature (25 ºC) during 2 h and 75% relative humidity. Finally, the leavened products were baked in rectangular molds (11 cm long, 6 cm wide, 3 cm height) at 210 ºC during 25 min in an oven steamer (Convotherm OES 6.06 mini CC, Convotherm Elektrogeräte GMBH, Eglfing, Germany) and cooled. Three breads for each formulation were obtained with this process. The process was replicated as necessary to obtain enough samples to analyze the different parameters considered in this study.

2.3. Physico-chemical analysis

The moisture content was determined by vacuum oven drying at 70 ºC until constant weight (AOAC, 1997). For determination of pH, 10 g of bread sample was homogenized in 90 mL distilled water in the ratio 1:9 (w/v) using laboratory warring blender. The measurement of pH in sourdough was made directly on a sample of 100 mL. The pH was measured using a Consort C830 pH-meter (Consort n.v. Parklaan, Turnhout, Belgium) by inserting the electrode into the homogenates (AOAC, 1995). Water activity was determined using an AquaLab Dewpoint Water Activity Meter 4TE (Decagon Devices, Inc., Pullman, USA) (AOAC, 1998). Viscosity was measured with a rotational viscometer Fungilab Alpha L Smart Series (Fungilab S.A., Barcelona, Spain) and results were expressed in cP. Measurements were taken at 20 ºC using a spindle L4

at constant rate of 12 rpm. Total titratable acidity (TTA) was determined using an automatic titrator Metrohm 902 (Gomensoro S.A., Madrid, Spain) after homogenization of 10 g of bread with 190 mL of distilled water, and expressed as the amount (mL) of 0.1 M NaOH per gram of sample needed to reach the value of pH of 8.5. Samples were analyzed by triplicate.

2.4. Microbiological analysis

Microbiological counts of fermented sourdoughs were determined. Ten grams of sample were homogenized with 90 mL of sterile peptone water (1% [w/v] of peptone and 0.9% [w/v] of NaCl) solution. Total aerobic mesophilic bacteria (TAMB) counts were determined using plate count agar (PCA, Scharlau, Barcelona, Spain), after incubating for 48 h at 30 ºC (ISO, 2013). Lactic acid bacteria (LAB) were determined on De Man Rogosa Sharpe (MRS) agar (Scharlau, Barcelona, Spain) after incubation at 30 ºC for 48 h (ISO, 1998). Cell densities of yeasts and moulds were estimated on glucosed Sabouraud medium (Scharlau, Barcelona, Spain) after incubation at 25 °C for 5 days (ISO, 1987).

2.5. Color

Color was measured using a Minolta CM3600d colorimeter (Minolta Corp., Ramsey, NY, USA). The instrument was calibrated against a ceramic reference, illuminant C, before use. Results were given in the CIELab system for illuminant D65 and a 10° angle of vision (CIE, 1986). Registered parameters were L* (brightness: L* = 0 [black], L* = 100 [white]), a* (–a* = greenness, +a* = redness) and b* (–b* = blueness, +b* = yellowness). Also for sourdough and breads, the total color difference (ΔE) between samples with microalgae and control was calculated. For bread samples, the browning index (BI) in crust and crumb was calculated.

$$BI = [100(x - 0.31)]/0.172$$

Where:

$x = (a^* + 1.75L^*)/(5.654L + a^* - 3.012b^*)$

The browning index (BI) represents the purity of brown color and is reported as an important parameter in processes where enzymatic or non-enzymatic browning takes place (Palou et al., 1999)

For sourdough color four dough were measured in three different points for each formulation. For crust and crumb color four breads of each formulation were measured in three different points.

2.6. Textural properties

Texture Profile Analysis (TPA) was performed in bread slices (25 mm thin) without crust using a TA-XTPlus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) and software Texture Exponent Lite 32 (version 4.0.8.0). The samples were placed on the base plate of the analyzer with a cylindrical aluminum probe (SMS P/75, 7.5 cm in diameter) using a 50 kg load cell. The crosshead speed was 1.7 mm/s, with a rest period of 15 s between cycles, and the deformation was 40% of the original length (Jekle and Becker, 2012). Six textural parameters were determined from each curve: hardness, adhesiveness, springiness, cohesiveness, chewiness and resilence (Bourne, 1978). Six different breads for each formulation were used.

2.7. Crumb porosity

Breads baked with different microalgae were cut into slices vertically. The slices were placed over the glass of a scanner (HP Officejet 4636, USA) having a resolution of 300 dpi. The scanned image was analyzed using the software Image J (http://rsb.info.nih.gov/ij/; Abramoff et al., 2004; Braadbaart and Van Bergen, 2005) that uses the contrast between the two phases (pores and solid part) in the image. The scanned color image is first converted to gray scale. Using bars of known lengths, pixel

values are converted into distance units. The largest possible rectangular cross-section of the bread slices was cropped. After adjusting the threshold, pore area as fraction of total area was determined using the software (Datta et al., 2007). Six samples were analyzed for each formulation.

2.8. Statistical analysis

The effect of the different treatments, on several variables was obtained by analysis of variance, one-way ANOVA using the statistical package Statgraphics Centurion XVI ver. 16.2.04 (StatPoint Technologies Inc., Virginia, USA). In those cases where the effect was significant ($p<0.05$) the mean values were compared using Multiple Range Tests and Fisher's least significant difference (LSD) procedure at 95% confidence level ($p<0.05$).

3. Results and discussion

3.1. Sourdough properties

The initial value of pH for sourdough before fermentation was 4.35 ±0.02. After 24h at 8 ºC, control sourdoughs showed values of pH of 3.72 ±0.02 and a_w values of 0.9854±.0006) (Table 1). Compared to sourdough with microalgae, very slight decreases ($p<0.05$) of the a_w values occurred in samples with Ig, Sa and Ng, and only in sourdough with Ig a slight increase ($p<0.05$) of pH was detected (0.1 pH units). The pH values in the sourdoughs were similar those reported in other studies (Gül et al., 2005; Şimşek et al., 2006).

Fig. 2 shows apparent viscosity of sourdoughs. In general, the addition of microalgae increases viscosity of sourdough. The great increase was obtained with the addition of Ig. Adesanya et al. (2012) studying rheological properties of microalgae for the production of biofuels showed that even at low concentrations microalgae interaction

plays a role in terms of viscosity enhancement. This is an effect to take into account because an excessive increase of viscosity in the sourdough can affect the characteristics of foam structure in the dough.

The results of the microbiological counts of the sourdoughs are reported in Fig. 3. The total bacterial counts of the samples showed significant differences ($p<0.05$) and ranged from 1.5 x 10^7 CFU/g (control) to 6.3 x 10^7 CFU/g (Sa). These results indicated a lighter increase of TAMB in the samples with microalgae. However, levels of TAMB were similar that observed in other studies (Gül et al., 2005; M'hir et al., 2007; Şimşek et al., 2006). Moulds and yeast counts showed significant differences ($p<0.05$) between the different samples analyzed. The reached values oscillated between 5.0 x 10^6 CFU/g for sourdoughs with Ng to 7.9 x 10^6 CFU/g for control and samples with Ig. Similar counts of these microorganisms have been show in others studies (Gül et al., 2005; Şimşek et al., 2006). The values of LAB counts ranged between 3.9 x 10^8 CFU/g to 1.0 x 10^9 CFU/g and the sourdoughs with Ts and Sa showed higher values of LAB counts. As a rule, LAB is the predominant microorganisms and in many cases, yeasts are present in significant numbers (Vogel et al., 1999; Vogel et al., 1996). LAB and yeasts are often associated in sourdough. The LAB/yeasts ratio generally varied between 10:1 and 100:1 (Gobbetti et al., 1994; Ottogalli et al., 1996; Pepe et al., 2004). In this study, the ratio was between 100/0.63 in the samples with Ts and Sa and 100/2 with control. In the case of samples with Ng this relation was 100/1. It is generally considered that the optimal ratio should be about 100:1 for optimal leavening and acidification activities (Rehman et al., 2006).

In good bakery practice, the levels of LAB and yeasts should be of 10^8 CFU/g to 10^9 CFU/g and 10^6 CFU/g to 10^7 CFU/g for acidification and leavening action of dough, respectively (De Vuyst and Neysens, 2005; Rehman et al., 2006).

The incubation of sourdough at low temperature (8 ºC) can significantly limit the growth of many LAB species (De Vuyst and Neysens, 2005; Rizello et al., 2016). Furthermore, as already observed (Corsetti and Settanni, 2007), the ratio between yeast and LAB populations in sourdough is greatly affected by temperature. Changes in color are the most significant effect of microalgae addition in sourdoughs. The analysis of L* (Table 1) shows that lightness decreased significantly ($p<0.05$) with microalgae addition, which means that the addition of microalgae biomass resulted in darker sourdoughs. However, differences ($p<0.05$) are found between microalgae been Ng the ones that provide more light sourdoughs and Ts and Sa the ones that provide more dark samples. The evaluation of a* parameter shows that microalgae, except Ts, significantly ($p<0.05$) increase the sourdoughs green color (negative a* values) and the yellow tonality (b*) (Table 1). The ΔE values show perceivable color differences ($\Delta E>3$) with control for all sourdoughs with microalgae. In spite of that, the addition of the microalgae to the sourdough implies darker sourdough with an increase of yellow-green tonality.

3.2. Physico-chemical properties of bread

After baking at 210 ºC during 25 min, control doughs presented aw values of 0.9492±0.0051, moisture of 33±1% and pH of 10.2±0.2, corresponding to values of TTA of 5.4±0.4 mL 0.1 M NaOH/10g of dough (Table 2). Compared to breads with microalgae there are few differences between samples. The final aw values and TTA were similar for all samples only small differences were obtained for moisture and pH values. That means that the addition of microalgae in the levels studied in this work not implies a significant change on physicochemical properties of bread.

3.3. Bread color

The variation in the crumb and crust color parameters of the breads due to the addition of microalgae are summarized in Table 3. In the same way than for sourdough, ΔE values show perceivable color differences in crust and crumb with control for all breads with microalgae. A ΔE>3 implies perceivable color difference for a consumer (Witzel et al., 1973). The crust color parameters showed a decrease in whiteness (L*) and a statistically significant increase in browning index (BI) with the addition of microalgae. Crust color depends on the physico-chemical characteristics of the raw dough (i.e. water content, pH, reducing sugars and amino acid content), ingredients of the dough and on the operating conditions applied during baking (i.e. temperature, air speed, relative humidity, heat transfer conditions) (Purlis and Salvadori, 2007; Zanoni et al., 1995). Crust browning is due to caramelization and Maillard reactions, belonging to the non-enzymatic or non-oxidative browning category (Fennema, 1996). In this case, degradation of microalgae pigments as chlorophyll and carotenoids due to heat also contribute to color changes in samples with microalgae. The samples with lower values of L* were breads with Sa, followed by Ig and Ts and finally with Ng. This differences can be explained by the different pigment concentration in microalgae (Bai et al., 2011), when higher pigment concentration more changes occurs. Parameter a* (redness) and b*(yellowness) showed significant differences ($p<0.05$). In general addition of microalgae implies a decrease of a* (redness) and an increase of b*(yellowness), except for breads with Ts in which a* increase, this effect was the same observed in the sourdough. This can be due to the high quantity of carotenoids that are present in these microalgae (Garrido and Rodríguez, 2009). The addition of microalgae produces changes in crust color that implies increase of browning index and an evolution to more green-yellow tonalities. Related to changes in crumb color, addition of microalgae caused a statistically significant ($p<0.05$) decrease in whiteness (L*), redness (a*) and

an increase in yellowness of crumb compared to the control sample. These changes are related, as in the case of the crust, with the content of pigments in microalgae. Sa is characterized by its high lutein content, up to 0.53% DM (Sánchez et al., 2008). In this case the addition of Sa implies an increase of b*(yellowness) in crust and crumb. Ig is rich in chlorophyll a (Valenzuela-Espinoza et al., 2002) that implies lower values of a* (greenness) and an increase of b*(yellowness). Ng is a source of β-carotene and vaucheriaxanthin (Camacho-Rodríguez et al., 2015).

3.4. Structural properties of bread

Table 4 shows the crumb textural properties of bread prepared from different microalgae obtained from TPA test. The textural parameters of breads suggested that the structure was not largely affected by the incorporation of microalgae. With respect to crumb properties, hardness, chewiness and resilence are not modified by microalgae addition ($p<0.05$). Adhesiveness increase and springiness decrease in samples with Ig and cohesiveness decrease in samples with Ng.

Crumb cohesiveness reflects internal cohesion of the material. Bread with high cohesiveness is desirable because it forms a bolus, rather than disintegrates during mastication whereas low cohesiveness indicates increased susceptibility of the bread to fracture or crumble. Crumb elasticity is described by springiness and resilience (Bourne, 2002). A reduction in resilience or springiness characterizes loss of elasticity. Resilience is defined as the ratio of the area under the curve of the second half of the first cycle (upward stroke) to the first half (downward stroke) (Moore et al., 2004). On the other hand, springiness refers to the distance that the food recovers its height during the time that elapses between the end of the first bite (or compression) and the start of the second bite (Bourne, 2002). Since springiness is time-dependent, its value is dependent on the

time interval between the compression cycles. It is therefore obvious that breads can recover their springiness completely (i.e. 100%) if the time interval between the compression cycles is sufficiently long. However, this point of view is irrelevant under practical conditions because an adult makes about 1 chew s^{-1} (Bourne, 2002), which is considerably lower than the 5 s used in our Texture Profile Analysis study. Changes in crumb chewiness reflected the changes in firmness, cohesiveness and springiness because chewiness is a product of the three texture parameters (Bourne, 2002). Crumb chewiness reflects the energy required to masticate food to a state ready for swallowing. Chewy foods tend to remain in the mouth without rapidly breaking up or dissolving (Bourne, 2002). Adhesiveness is not a desired crumb attribute because its sensory equivalent is an undesirable moist and sticky crumb.

Crumb porosity of different breads with microalgae and control is shown in Fig. 4. The obtained values of porosity were 16±5% for Is, 19±3% for Ts, 18±4% for Sa, 14±1% for Ng and 12±4% for control samples. There is no significant change in porosity ($p<0.05$) comparing with the control porosity measured as percentage of surface space occupied by holes by surface unit. Porosity is caused by the production of the CO_2 gas by yeast and some heterofermentative lactic acid bacteria. The CO_2 content increases during fermentation as the pH drops (Farnworth, 2003). In the samples with microalgae the gas was released as the same way than in control samples.

4. Conclusions

Microalgae represents a new ingredient in bread formulation as source of important natural compounds for human nutrition. Under conditions used in this study, their incorporation in bread formulation did not modify significantly physico-chemical

properties or microbiological counts of the sourdough. In general addition of microalgae implies changes in color of bread related with a decrease of a* (redness) and an increase of b*(yellowness). These changes are related with the content of pigments in microalgae. The textural parameters of breads as hardness, chewiness and resilence are not modified by microalgae addition.

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References

A.O.A.C. Association of Official Analytical Chemists. (1995). Official Methods of Analysis. (16th ed.). Washingtong, DC.

A.O.A.C. Association of Official Analytical Chemists. (1997). Official Methods of Analysis. Washington, DC.

A.O.A.C. Association of Official Analytical Chemists. (1998). Official Methods of Analysis. Washington, DC.

Abramoff MD, Magelhaes PJ and Ram SJ. (2004). Image processing with imageJ. *Biophotonics International* 11(7): 36–42.

Acién FG, Fernández JM, Magán JJ and Molina E. (2012). Production cost of a real microalgae production plant and strategies to reduce it. *Biotechnology Advances* 30: 1344-1353.

Adesanya VO, Vadillo DC and Mackley MR. (2012). The rheological characterization of algae suspensions for the production of bioguels. *Journal of Rheology* 56: 925-939.

Bai M-D, Cheng C-H, Wan H-M and Lin Y-H. (2011). Microalgal pigments potential as byproducts in lipid production. *Journal of the Taiwan Institute of Chemical Engineers* 42: 783-786.

Batista AP, Gouveia L, Bandarra-Narcisa M, Franco JM and Raymundo A. (2013).Comparison of microalgal biomass profiles as novel functional ingredient for food products. *Algal Research* 2: 164-173.

Borowitzka MA. (2013). High-value products from microalgae, their development and commercialization. *Journal of Applied Phycology* 25: 743-756.

Bourne M. (2002). Food texture and viscosity. Concept and measurement. New York, USA: Academic Press, Elsevier Science.

Bourne MC. (1978). Texture Profile Analysis. *Food Technology* 32: 62- 66.

Buono S, Langellotti AL, Martello A, Rinna F and Fogliano V. (2014). Functional ingredients from microalgae. *Food & Function* 8: 1669-1685.

Braadbaart F and Van Bergen PF. (2005). Digital imaging analysis of size and shape of wheat and pea upon heating under anoxic conditions as a function of the temperature. *Vegetation History and Archaeobotany* 14: 67-75.

Brennan L and Owende P. (2010). Biofuels from microalgae. A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews* 14: 557-577.

Camacho-Rodríguez J, Cerón-García MC, Fernández-Sevilla JM and Molina-Grima E. (2015). The influence of culture conditions on biomass and high value product generation by *Nannochloropsis gaditana* in aquaculture. *Algal Research* 11: 63-73.

Cerón MC, García-Malea MC, Rivas J, Acién FG, Fernández JM, Del Río E, Guerrero MG and Molina E. (2007). Antioxidant activity of *Haematococcus pluvialis* cells grown in continuous culture as a function of their carotenoid and fatty acid content. *Applied Microbiology Biotechnology* 74: 1112-1119.

CIE (Commission Internationale de l'Eclairage) (1986) Colorimetry: technical report. 2nd edn, CIE Pub. No.15. Pp. 35–36. Vienna, Austria.

Corsetti A and Settanni L. (2007). Lactobacilli in sourdough fermentation. *Food Research International* 40: 539-558.

Da Silva Vaz B, Moreira JB, Morais MG and Costa JAV. (2016). Microalgae as a new source of bioactive compounds in food supplements. *Current Opinion in Food Science* 7: 73-77.

Datta AK, Sahin S, Sumnu G and Keskin O. (2007). Porous media characterization of breads baked using novel heating modes. *Journal of Food Engineering* 79: 106-116.

De Vuyst D and Neysens P. (2005). The sourdough microflora: biodiversity and metabolic interactions. *Trends in Food Science and Technology* 16: 43-56.

De Vuyst L, Vrancken G, Ravyts F, Rimaux T, Weckx S. (2009). Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. *Food Microbiology* 26: 666-675.

Fábregas J, Maseda A, Domínguez A, Ferreira M and Otero A. (2002). Changes in the cell composition of the marine microalga, *Nannochloropsis gaditana*, during a light:dark cycle. *Biotechnology Letters* 24: 1699-1703.

Farnworth ER. (2003). *Handbook of fermented functional foods*. Boca Raton, FL: CRC.

Fennema OR. (1996). *Food chemistry* (3rd ed.). New York: Marcel Dekker.

Garrido JL and Rodríguez F. (2009). Occurrence of loroxanthin, loroxanthin decenoate, and loroxanthin dodecenoate in *Tetraselmis* species (Prasinophyceae, Chlorophyta). *Journal of Phycology* 45: 366-374.

Gobbetti M, Corsetti J, Rossi F, La Rosa F and De Vicenzi S. (1994). Identification and clustering of lactic acid bacteria and yeasts from wheat sourdoughs of central Italy. *Italian Journal of Food Science* 1: 85-94.

Gouveia L, Batista AP, Sousa I, Miranda A, Empis J and Raymundo A. (2007). *Chlorella vulgaris* biomass used as colouring source in traditional butter cookies. *Innovative Food Science and Emerging Technologies* 8: 433-436.

Gouveia L, Coutinho C, Mendonça E, Batista AP, Sousa I, Bandarra NM and Raymundo A. (2008). Sweet biscuits with *Isochrysis galbana* microalga biomass as a functional ingredient. *Journal of the Science of Food and Agriculture* 88: 891-896.

Guedes AC, Amaro HM and Malcata FX. (2013). Microalgae as sources of high added-value compounds. A brief review of recent work. *Biotechnology Progress* 27(3): 597-613.

Gül H, Özçelik S, Sağdiç O and Certel M. (2005). Sourdough bread production with lactobacilli and *S. cerevisiae* isolated from sourdoughs. *Process Biochemistry* 40: 691-697.

Hemaiswarja S, Raja R, Kumar RR, Ganesan V and Anbazhagan C. (2011). Microalgae: A sustainable feed source for aquaculture. *World Journal of Microbiology and Biotechnology* 27: 1737-1746.

International Standards Organization – ISO (1998). *Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 ºC, ISO 15214*. Geneva, Switzerland: The International Organization for Standardization.

International Standards Organization- ISO (2013). *Microbiology of the food chain, Horizontal method for the enumeration of microorganisms, Part 1: Colony count at 30 °C by the pour plate technique, ISO 4833-1*. Geneva, Switzerland: The International Organization for Standardization.

International Standards Organization- ISO (1987). *Microbiology: General guidance for enumeration of yeasts and moulds: Colony count technique at 25 ºC, ISO 7954*. Geneva, Switzerland: The International Organization for Standardization.

Jekle M and Becker T. (2012). Effects of acidification, sodium chloride, and moisture levels on wheat dough: II. Modeling of bread texture and staling kinetics. *Food Biophysics* 7: 200-208.

Ketabi A, Soleimannian-Zad S, Kadivar M and Sheikh-Zeinoddin, S. (2008). Production of microbial exopolysaccharides in the sourdough and its effects on the rheological properties of dough. *Food Research International* 41: 948-951.

Liu CP and Lin LP. (2001). Ultrastructural study and lipid formation of *Isochrysis sp.* CCMP1324. *Botanical Bulletin of Academia Sinica* 42: 207-214.

Lubián LM, Montero O, Moreno-Garrido I, Huertas IE, Sobrino C, González-del Valle M and Parés G. (2000). *Nannochloropsis* (Eustigmatophyceae) as source of commercially valuable pigments. *Journal of Applied Phycology* 12: 249-255.

M'hir S, Mejri M and Hamdi M. (2007). Microflora distribution and species ratio of Tunisian fermented doughs for bakery industry. *African Journal of Biotechnology* 6(18): 2122-2129.

Moore MM, Schober T, Dockery P and Arendt EK. (2004). Textural comparisons of gluten-free and wheat-based doughs,batters and breads. *Cereal Chemistry* 81: 567-575.

Nuño K, Villarruel-López A, Puebla-Pérez AM, Romero-Velardec E, Puebla-Morad AG and Ascencio F. (2013). Effects of the marine microalgae *Isochrysis galbana* and *Nannochloropsis oculata* in diabetic rats. *Journal of Functional Foods* 5: 106-115.

Ottogalli G, Galli A and Foschino R. (1996). Italian bakery products obtained with sourdough: characterization of the typical microflora. *Advances in Food Science* 18: 131-144.

Palou E, López-Malo A, Barbosa-Cánovas GV, Welti-Chanes J and Swanson BG. (1999). Polyphenoloxidase activity and color of blanched and high hydrostatic pressure treated banana puree. *Journal of Food Science* 64: 42-45.

Pepe O, Blaiotta G, Anastasio M, Moschetti G, Ercolini D and Villani F. (2004). Technological and molecular diversity of *Lactobacillus plantarum* strains isolated from naturally fermented sourdoughs. *Systematic and Applied Microbiology* 27: 443-453.

Pulz O and Gross W. (2004). Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology* 65: 635-648.

Purlis E and Salvadori VO. (2007). Bread browning kinetics during baking. *Journal of Food Engineering* 80: 1107-1115.

Raja R, Hemaiswarya S, Kumar NA, Sridhar S and Rengasamy R. (2008). A perspective on the biotechnological potential of microalgae. *Critical Reviews in Microbiology* 34: 77-88.

Rehman SR, Paterson A and Piggott JR. (2006). Flavour in sour dough breads: a review. *Trends in Food Science and Technology* 17: 557-566.

Rizello CG, Lorusso A, Montemurro M and Gobbetti M. (2016). Use of sourdough made with quinoa (*Chenopodium quinoa*) flour and autochthonous selected lactic acid bacteria for enhancing the nutritional, textural and sensory features of white bread. *Food Microbiology* 56: 1-13.

Rodríguez De Marco E, Steffolani ME, Martínez CS and León AE. (2014). Effects of spirulina biomass on the technological and nutritional quality of bread wheat pasta. *LWT-Food Science and Technology* 58(1): 102-108.

Sánchez A, Maceiras R, Cancela A and Pérez A. (2013). Culture aspects of *Isochrysis galbana* for biodiesel production. *Applied Energy* 10: 192-197.

Sánchez JF, Fernández JM, Acién FG, Rueda A, Pérez-Parra J and Molina E. (2008). Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis*. *Process Biochemistry* 43: 398-405.

Sánchez S, Martínez ME and Espinola F. (2000). Biomass production and biochemical variability of the marine microalga *Isochrysis galbana* in relation to culture medium. *Biochemical Engineering Journal* 6: 13-18.

Şimşek Ö, Çon AH and Tulumoğlu Ş. (2006). Isolating lactic starter cultures with antimicrobial activity for sourdough processes. *Food Control* 17: 263-270.

Spolaore P, Joannis-Cassan C, Duran E and Isambert A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering* 101(2): 87-96.

USFDA. (2011). Agency response letter GRAS notice no. GRN 000391. [online] http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm301318.htm (last accessed on June 8, 2016).

USFDA. (2012). Agency response letter GRAS notice no. GRN 000396. [online] http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm320210.htm (last accessed on June 8, 2016).

Valenzuela-Espinoza E, Millán-Núñez R and Núñez-Cebrero F. (2002). Protein, carbohydrate, lipid and chlorophyll a content in *Isochrysis* aff. *galbana* (clone T-Iso) cultured with a low cost alternative to the f/2 medium. *Aquacultural Engineering* 25: 207-216.

Vogel RF, Knorr R, Müller MRA, Steudel U, Gänzle MG and Ehrmann MA. (1999). Non-dairy lactic fermentations: the cereal world. *Antonie van Leeuwenhoek* 76: 403-411.

Vogel RF, Müller M, Stolz P and Ehrmann M. (1996). Ecology in sourdoughs produced by traditional and modern technologies. *Advances in Food Science* 18: 152-159.

Witzel RF, Burnham RW and Onley JW (1973). Threshold and suprathreshold perceptual color differences. *Journal of Optical Society of America* 63: 615-625.

Yoshioka M, Yago T, Yoshie-Stark Y, Arakawa H and Morinaga T. (2012). Effectof high frequency of intermittent light on the growth and fatty acid profile of *Isochrysis galbana*. *Aquaculture* 338-341: 111-117.

Yu CC, Chen HW, Chen MJ, Chang YC, Chien SC, Kuo YH, Yang FL, Wu SH, Chen J, Yu HH and Chao LK. (2010).Chemical composition and bioactivities of the marine alga *Isochrysis galbana* from Taiwan. *Natural Product Communications* 5: 1941-1944.

Zainan NH, Srivatsa SC and Bhattacharya S. (2015). Catalytic pyrolysis of microalgae *Tetraselmis suecica* and characterization study using *in situ* Synchrotron-based Infrared Microscopy. *Fuel* 161: 345-354.

Zanoni B, Peri C and Bruno D. (1995). Modelling of browning kinetics of bread crust during baking. *LWT-Food Science and Technology* 28(3): 604-609.

Table 1. Sourdough CIE L*a*b* coordinates, total difference of color (ΔE), a_w and pH values for samples with microalgae; *Isochrysis galbana* (Ig), *Tetraselmis suecica* (Ts), *Scenedesmus almeriensis* (Sa) and *Nannochloropsis gaditana* (Ng) and control. Lightness (L*), green-red chromaticity (a*), blue-yellow chromaticity (b*) (Medium values of twelve replicates for color parameters and three replicates for a_w and pH).

Sample	L*	a*	b*	ΔE	a_w	pH
Ig	59 ± 1^{b}	-2.36 ± 0.08^{a}	19.0 ± 0.3^{c}	18.9 ± 0.7^{b}	0.9803 ± 0.0009^{a}	3.80 ± 0.01^{b}
Ts	56 ± 1^{a}	0.85 ± 0.14^{d}	16.4 ± 0.7^{b}	20.4 ± 0.9^{c}	0.9837 ± 0.0011^{bc}	3.74 ± 0.02^{a}
Sa	56 ± 1^{a}	-2.41 ± 0.07^{a}	21.4 ± 0.8^{d}	23.0 ± 0.8^{d}	0.9823 ± 0.0019^{ab}	3.74 ± 0.06^{a}
Ng	64 ± 1^{c}	-0.94 ± 0.08^{b}	16.9 ± 0.6^{b}	13.6 ± 1.0^{a}	0.9804 ± 0.0010^{a}	3.72 ± 0.01^{a}
Control	76.3 ± 0.9^{d}	-0.61 ± 0.04^{c}	10.3 ± 0.1^{a}	-	0.9854 ± 0.0006^{c}	3.72 ± 0.02^{a}

Note: superscript characters (letters) indicate the effect of microalgae addition. Values in the same column for each formulation with the same letter are not statistically different according to the Tukey test ($p < 0.05$).

Table 2. Bread a_w, moisture, pH and total titratable acidity (TTA) values for samples with microalgae; *Isochrysis galbana* (Ig), *Tetraselmis suecica* (Ts), *Scenedesmus almeriensis* (Sa) and *Nannochloropsis gaditana* (Ng) and control (Medium values of three replicates).

Sample	a_w	Moisture (%)	pH	TTA (mL/10g)
Ig	0.9518 ± 0.0007^{a}	31 ± 2^{a}	10.1 ± 0.1^{a}	5.3 ± 0.1^{a}
Ts	0.9513 ± 0.0038^{a}	33.5 ± 0.8^{ab}	10.3 ± 0.1^{ab}	5.1 ± 0.2^{a}
Sa	0.9532 ± 0.0028^{a}	34 ± 2^{ab}	10.17 ± 0.07^{a}	5.0 ± 0.3^{a}
Ng	0.9528 ± 0.0030^{a}	35 ± 1^{b}	10.5 ± 0.1^{b}	5.1 ± 0.6^{a}
Control	0.9492 ± 0.0051^{a}	33 ± 1^{ab}	10.2 ± 0.2^{a}	5.4 ± 0.4^{a}

Note: superscript characters (letters) indicate the effect of microalgae addition. Values in the same column for each formulation with the same letter are not statistically different according to the Tukey test ($p < 0.05$).

Table 3. Browning index (BI), total difference of color (ΔE) and CIE L*a*b* coordinates for crust and crumb bread samples with microalgae; *Isochrysis galbana* (Ig), *Tetraselmis suecica* (Ts), *Scenedesmus almeriensis* (Sa) and *Nannochloropsis gaditana* (Ng) and control. Lightness (L*), green-red chromaticity (a*), blue-yellow chromaticity (b*) (Medium values of twelve replicates).

Crust	L*	a*	b*	ΔE	BI
Ig	64 ± 2^{c}	0.93 ± 0.39^{a}	27 ± 1^{c}	9 ± 2^{b}	53 ± 4^{c}
Ts	62 ± 1^{b}	3.08 ± 0.40^{d}	24 ± 1^{b}	11 ± 1^{c}	53 ± 5^{c}
Sa	59 ± 1^{a}	0.87 ± 0.23^{a}	28 ± 1^{d}	15 ± 1^{d}	64 ± 4^{d}
Ng	66 ± 1^{d}	1.53 ± 0.32^{b}	23 ± 1^{a}	7 ± 1^{a}	44 ± 3^{b}
Control	73.2 ± 0.7^{e}	2.72 ± 0.40^{c}	23 ± 1^{a}	-	39 ± 3^{a}
Crumb	**L***	**a***	**b***	**ΔE**	**BI**
Ig	70 ± 1^{b}	0.06 ± 0.18^{a}	20.4 ± 0.8^{d}	8 ± 1^{b}	33 ± 2^{c}
Ts	69 ± 1^{b}	1.80 ± 0.16^{d}	19.6 ± 0.7^{c}	8 ± 1^{b}	33 ± 2^{c}
Sa	67 ± 2^{a}	0.21 ± 0.16^{b}	23 ± 1^{e}	12 ± 2^{c}	40 ± 5^{d}
Ng	73 ± 1^{c}	0.62 ± 0.17^{c}	18.4 ± 0.7^{b}	4 ± 1^{a}	29 ± 2^{b}
Control	77 ± 1^{d}	1.96 ± 0.18^{e}	16.8 ± 0.6^{a}	-	26 ± 1^{a}

Note: superscript characters (letters) indicate the effect of microalgae addition. Values in the same column for each formulation with the same letter are not statistically different according to the Tukey test ($p < 0.05$).

Table 4. Crumb texture profile parameters of bread with microalgae; *Isochrysis galbana* (Ig), *Tetraselmis suecica* (Ts), *Scenedesmus almeriensis* (Sa) and *Nannochloropsis gaditana* (Ng) and control (Medium values of six replicates).

	Hardness (N)	Adhesiveness (N s)	Cohesiveness	Springiness	Chewiness (N)	Resilence
Ig	311 ± 51^{a}	-0.52 ± 0.32^{a}	0.65 ± 0.01^{ab}	0.93 ± 0.00^{a}	187 ± 30^{a}	0.32 ± 0.01^{a}
Ts	313 ± 6^{a}	-0.29 ± 0.26^{ab}	0.66 ± 0.01^{ab}	0.94 ± 0.00^{ab}	194 ± 6^{a}	0.34 ± 0.01^{a}
Sa	320 ± 22^{a}	-0.13 ± 0.16^{b}	0.66 ± 0.01^{ab}	0.94 ± 0.00^{b}	199 ± 17^{a}	0.34 ± 0.01^{a}
Ng	313 ± 43^{a}	-0.09 ± 0.09^{b}	0.64 ± 0.02^{a}	0.94 ± 0.00^{ab}	187 ± 29^{a}	0.33 ± 0.01^{a}
Control	299 ± 21^{a}	-0.09 ± 0.08^{b}	0.72 ± 0.10^{b}	0.94 ± 0.02^{ab}	202 ± 20^{a}	0.37 ± 0.06^{a}

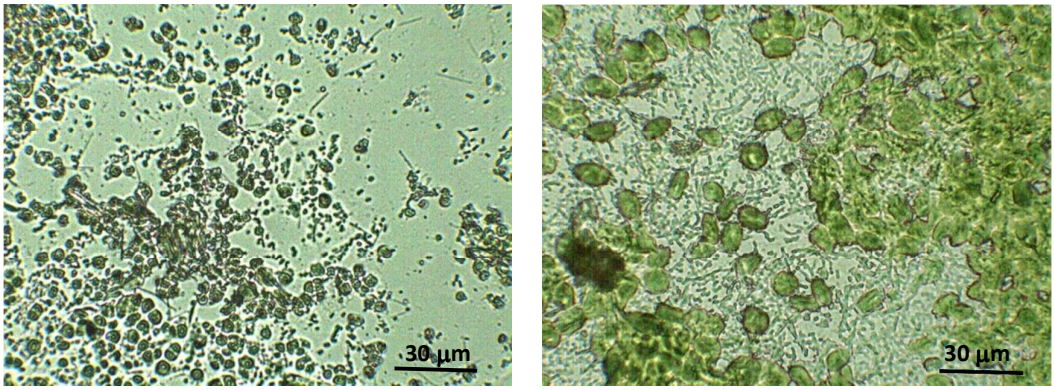
Note: superscript characters (letters) indicate the effect of microalgae addition. Values in the same column for each formulation with the same letter are not statistically different according to the Tukey test ($p < 0.05$).

Fig. 1. Micrographs of microalgae used. (a) *Isochrysis galbana* (Ig), (b) *Tetraselmis suecica* (Ts), (c) *Scenedesmus almeriensis* (Sa) and (d) *Nannochloropsis gaditana* (Ng).

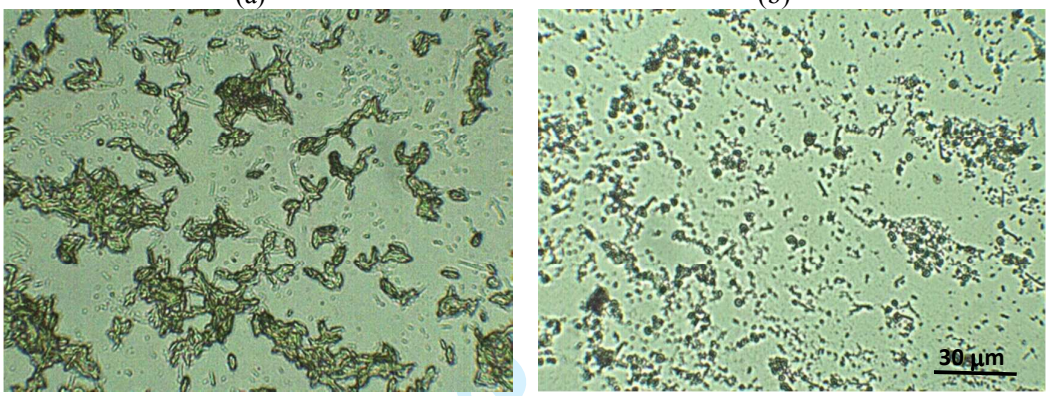
Fig. 2. Sourdough viscosity of samples with different microalgae. *Isochrysis galbana* (Ig), *Tetraselmis suecica* (Ts), *Scenedesmus almeriensis* (Sa) and *Nannochloropsis gaditana* (Ng).

Fig. 3. Microbiological counts of the sourdoughs. *Isochrysis galbana* (Ig), *Tetraselmis suecica* (Ts), *Scenedesmus almeriensis* (Sa) and *Nannochloropsis gaditana* (Ng). Superscript characters (letters) indicate the effect of microalgae addition in each parameter, the same letter are not statistically different according to the Tukey test ($p < 0.05$).

Fig. 4. Crumb porosity of different breads with microalgae. (a) *Isochrysis galbana* (Ig), (b) *Tetraselmis suecica* (Ts), (c) *Scenedesmus almeriensis* (Sa), (d) *Nannochloropsis gaditana* (Ng) and (e) control.



(a) (b)



(c) (d)

Fig.1

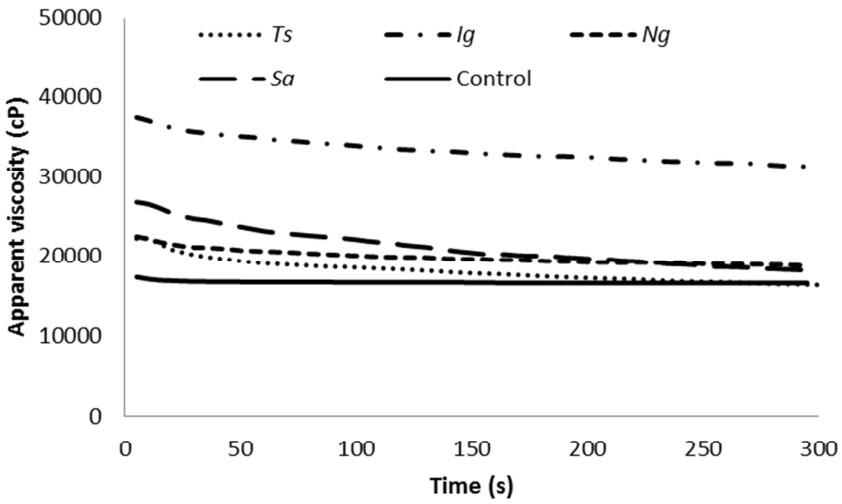


Fig. 2

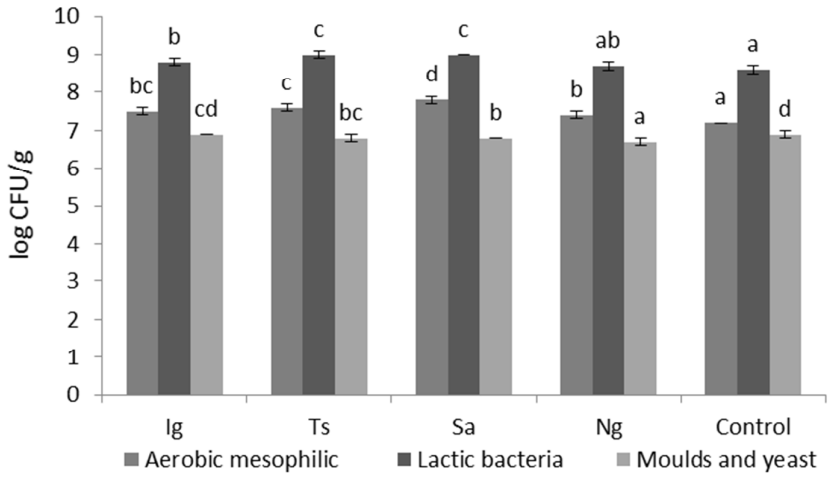
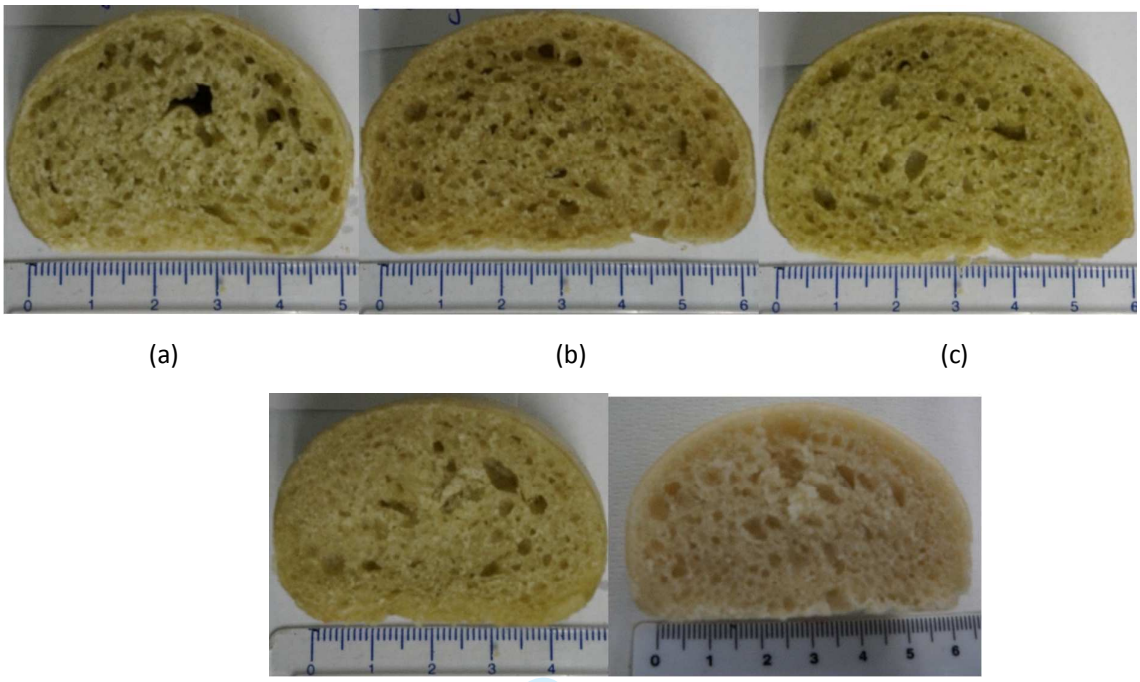


Fig. 3



(a) (b) (c)

(d) (e)

Fig. 4