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Effect of microalgae addition on mineral content, colour and mechanical properties of breadsticks

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Abstract

Microalgae has recently attracted considerable attention due to its potential as a high source of proteins, lipids, vitamins, minerals and phytochemicals. Hence, it can be a useful ingredient intended to increase the nutritional and technological value of food products. The aim of the present study was to evaluate the effect of the addition of microalgae biomass (*Chlorella vulgaris* and *Arthrospira platensis*) on mineral content, colour and mechanical properties of breadsticks, and the colour, texture and rheology of doughs. Microalgae were shown to affect texture and rheology in doughs showing greater hardness values, although, a decrease in hardness, toughness, crispiness and brittleness parameters was seen in breadsticks. The main effects observed with microalgae addition were changes in dough colour, with visual colour perception, in all cases, of distinguished green colour tones. Breadstick colour was evaluated over 15 days of storage and showed colour stability. Furthermore, mineral content of breadsticks increased, specifically, iron and selenium, both important compounds involved in human body functions. The incorporation of Chlorella or Spirulina in the formulation allows for the production of breadsticks classed as "high in iron and selenium food" and more stable in colour and texture.

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Keywords: mineral content, *Spirulina platensis*, *Chlorella vulgaris*, breadstick, colour, rheology, mechanical properties.

1. Introduction

Microalgae are unicellular microorganisms and are commonly found in fresh and salt water and vary in shape and size, with lengths or diameters ranging, approximately, between 3 – 10 µm.[1] Microalgae have gained more attention from researchers due to their rich source of biochemicals, namely protein, vitamins, minerals, pigments, plus many more.[2,3] The first commercialised microalgae were *Chlorella vulgaris* and *Spirulina platensis*, being two important microalgae used in food applications, and were promoted as health foods in Japan, Taiwan and Mexico.[4] *Chlorella vulgaris* is a green microalga belonging to the *Chlorophyta* group, having important nutritional value and consumed worldwide.[5] Chlorella contains high amounts of chlorophyll, proteins, polysaccharides, vitamins, minerals and essential amino acids.[6] Spirulina is filamentous in nature and is a microscopic blue-green microalgae type, which is found intensively in alkaline waters.[7] Spirulina has a high content of micro and macronutrients. Its dry weight chemical composition includes 60 – 70 % proteins, carbohydrates, vitamins and minerals (iron, calcium, chromium, copper, magnesium, manganese, phosphorus, potassium, sodium and zinc).[8] Therefore, microalgae, due to its phenomenal composition, is a novel healthy food ingredient with biologically active compounds[9] and when used in food production, those such foods are determined functional.[10] Functional foods can be defined as foods that are similar in appearance to conventional foods, are consumed as part of a regular diet, and can contribute to increase the health condition of a person to a higher degree of that expected from regular nutritional intake from a common food source.[11] Currently, bakery products are the most widely consumed foods in the world and have great potential for delivery of macroalgae and microalgae functional ingredients.[12,13] In recent years, the demand for snacks with improved nutritional and functional properties has increased,[14] due to the

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high practicality, convenience and acceptability of these foods, especially among children.[14,15] The present study aimed to evaluate the effect of the addition of two microalgae biomass (*Chlorella vulgaris* and *Arthrospira platensis*) on mineral content, colour, textural and rheological properties of breadsticks. It should be noted that breadsticks are traditionally baked snacks in the Valencian Community, Spain, although they are also widely consumed in other Mediterranean countries and the world. For this, doughs and breadsticks were evaluated to study and improve the quality and nutritional content of the bakery product upon the incorporation of microalgae. Considering that, the formulation was varied with the type of microalgae while retaining the same level of concentration.

2. Material and methods

2.1. Raw materials

Freeze dried *Arthrospira platensis* (Spirulina) and *Chlorella vulgaris* were supplied from AlgaEnergy S.A., Madrid, Spain. Wheat flour, salt, yeast and sunflower oil were purchased from a local supermarket (Alcampo, Valencia, Spain).

2.2. Dough formulation and breadstick preparation

Three dough samples of 60 g were prepared by mixing microalgae and wheat flour, the ratio was calculated by percentage; control dough (CD) contains 0 % microalgae and 100 % wheat flour; Spirulina dough (SD), 1.5 % *Arthrospira platensis* and 98.5 % wheat flour, and Chlorella dough (CHD), 1.5 % of *Chlorella vulgaris* and 98.5 % wheat flour. The other components of the dough were 14 g of sunflower oil, 7 g of fresh yeast, 0.4 g of salt and 18.6 g of water. The ingredients were mixed in a food processor (Kenwood chef classic, KM400/99 plus, Kenwood Corporation, Tokyo, Japan), kneading for 15 min at low speed. Dough samples were fermented for 1 hour at 33 ºC in a controlled temperature oven (Convotherm OES 6.06 mini CC, Convotherm Elektrogeräte GMBH, Eglfing, Germany). The breadsticks were shaped by hand into 10 cm length and 10 g

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weighted sticks and fermented once again for 30 min at 33 °C. After the second fermentation, samples were baked on rectangular baking sheets at 180 °C for 28 min in a steamer oven (Convotherm OES 6.06 mini CC, Convotherm Elektrogeräte GMBH, Eglfing, Germany). Breadsticks were cooled for 2 h and placed in heat-resistant polyethylene plastic pouches (Cryovac® HT3050) to be stored at room temperature (25 °C) for 15 days.

2.3. Analysis

2.3.1. Water content

Water content (g water/100 g sample) was determined by vacuum oven drying at 105 °C until constant weight,[16] both for dough and for breadstick samples. Samples were analysed in triplicate.

2.3.2. Colour Measurement

The colour of dough and breadstick samples were measured instrumentally using a Konica Minolta CM-700d colourimeter (Konica Minolta CM-700d/600d series, Tokyo, Japan) with standard illuminant D65 and a visual angle of 10°. For dough colour, three breadstick shaped doughs were measured at three equidistant points for each formulation. For breadstick colour, three breadsticks of each formulation were measured on each side, bottom and top, at three equidistant points, at day 0 and day 15 of storage. The results were expressed in terms of L* (brightness: L* = 0 (black), L* = 100 (white)), a* (- a* = greenness, + a* = redness), and b* (- b* = blueness, + b* = yellowness), according to the CIELab system (CIE, 1986). Chroma, C^*_{ab} (saturation) and hue angle, $h°_{ab}$, were also calculated, defined by: $C^*_{ab} = [(a^{*2} + b^{*2})]^{1/2}$; $h°_{ab} = \arctan (b^* / a^*)$. The total colour difference (ΔE) between samples with and without microalgae, and of both doughs and breadsticks, was determined using L*a*b* values according to: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. For breadstick samples, ΔE

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measurements between positions (top and bottom) were calculated, as well as between 0 and 15 days of storage.

2.3.3. Rheological Measurements

The rheological behaviour of doughs was studied using a Kinexus pro+ rotational rheometer (Malvern Instruments, Worcestershire, UK) and rSpace software. The rheometer was equipped with a parallel plate geometry (25 mm diameter) set to a 2 mm gap. Prior to oscillatory testing, dough samples were loaded onto the geometry plate and rested for 300 s before measurement. Dynamic rheological characterisation of the samples was subsequently performed. Firstly, the linear viscoelastic region was determined, followed by oscillatory stress sweeps at 20 °C with a frequency range of 0.1 - 10 Hz for each sample using a constant strain of 0.5 %. Values of elastic modulus (G' [Pa]), viscous modulus (G'' [Pa]) and tan δ (G''/G') were obtained for different frequency values (ω [Hz]). Samples were analysed in triplicate.

2.3.4. Textural properties

The texture of doughs and breadsticks was measured using a TA-XT2 Texture Analyser (Stable Micro Systems Ltd, Godalming, UK) and software Texture Exponent (version 6.1.12.0). A dough piece (60 g) was placed in a standard size back extrusion container (50 mm diameter) immediately after the second dough fermentation. An extrusion disc (35 mm diameter) attached to a 50 kg load cell was positioned centrally over the container compressing the sample to a thickness of 5 mm at pre-test and test speeds of 1.0 mms^{-1} and post-test speed of 10 mms^{-1}. Six samples were measured from each blend at day 0, and the results were averaged. The parameters analysed were a maximum force (N), the gradient of force-time (N/s) and area under the curve force-time (N.s). A snap test was used to evaluate the textural properties of the breadsticks and observe any changes which occurred during storage. The texture analyser was equipped with a 50 kg load cell and a three-point bending rig (HDP/3PB).

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The two adjustable supports of the base plate were set 20 mm apart, and the sample was placed centrally over the supports just prior to testing. The upper blade moved downwards at a pre-test and test speeds of 1.0 mms^{-1} and 3.0 mms^{-1}, respectively. Hardness, toughness, crispiness and brittleness, an index of breadstick break strength was measured with six breadsticks per batch. The test was performed on days 0 and 15 of storage.

2.3.5. Ash and mineral content

Total ash content was determined following method 930.05 of AOAC procedures.[16] A sample of 500 mg was incinerated with high pressure in a microwave oven (Muffle P Selecta Mod.367PE) for 24 h at 550 ºC, and ash was gravimetrically quantified. The residue of incineration was extracted with HCl (hydrochloric acid) (50 % v/v) and HNO_3 (nitric acid) (50 % v/v) and made up to an appropriate volume with distilled water,[17] also minerals were measured using standard solutions for calibration purposes. The multi-mineral determination was performed by using inductively coupled plasma optical emission spectrometer, model 700 Series ICP-OES from Agilent Technologies (Santa Clara, United States), with axial viewing and a charge coupled device detector. The instrumental parameters used for the multi-element determination were as follows: RF generator of 40 MHz, a power of 1 kW, plasma gas flow rate of 15 $Lmin^{-1}$, auxiliary gas flow rate of 1.5 $Lmin^{-1}$ and nebuliser gas (One Neb 2) pressure of 200 kPa. The elements and the analytical spectral lines (nm) used were: P (214.914), K (766.491), Ca (317.933), Na (589.592), Mg (285.213), Fe (238.204), Zn (213.857), Cu (327.395), Mn (259.372), and Se (196.026). Mineral composition (macro and microelements) were expressed as mg/100 g. Samples were analysed in triplicate.

2.4. Statistical analysis

Analysis of variance (ANOVA), with a confidence level of 95 % ($p < 0.05$), using Statgraphics Centurion XVII Software, version 17.2.04, was applied to evaluate the

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differences among breadstick samples. Furthermore, a correlation analysis among the rheological and textural properties of doughs studied, and the textural properties of breadsticks produced in the study, with a 95 % significance level, was carried out (Sta Statgraphics Centurion XVII). A factor analysis was applied to the values of rheological and textural parameters of doughs studied, using SPSS program version 16.0.

3. Results and Discussion

3.1. Dough properties

Doughs obtained according to section 2.2 were evaluated with regards to water content, rheological and textural properties, and colour. The water content of three doughs was similar (≈ 28 %) without significant ($p > 0.05$) differences among them.

Dough rheological behaviour is considered the predominant characteristic for the effective production of bakery products, therefore rheological properties of dough significantly influence the final quality of baked products.[18] Dough rheological parameters are shown in Figure 1 and Table 1. In all cases, storage modulus (G') was greater than loss modulus (G'') throughout the frequency range (0.1 to 10 Hz), which shows a predominant solid viscoelastic behaviour of all the breadstick doughs studied.[19] Additionally, the loss factor ($\tan \delta = G''/G'$) represents the relationship between loss modulus and storage modulus, where a high value of $\tan \delta$ corresponds to materials with predominant viscous behaviour. The mean values of G', G'' and $\tan \delta$ at 1 Hz are presented in Table 1.

The cyanobacterium Spirulina is well recognised as a potential food supplement for humans because of its high levels of protein (60 – 70 % of dry weight).[20] Chlorella, also containing elevated levels of protein is a potential food supplement,[9] along with Spirulina. Protein addition increases G' and was observed when wheat flour was substituted for flours with a higher protein content in cookies formulations.[21] Other authors[22] suggest that content up to 3.0 g of Chlorella per 100 g wheat flour showed higher G' values, indicating a possible strengthening effect of the dough structure,

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believed to be due to a reinforcement of the viscoelastic protein matrix from the addition of microalgae, with high protein content. Microalgae addition increased G' values (Figure 1) when compared with the control sample without significant differences ($p > 0.05$), and when values were compared at 1 Hz (Table 1). Relating to tan δ, the values observed in Table 1 show that samples with microalgae are significantly lower ($p < 0.05$), indicating a greater elastic behaviour.

The textural properties of doughs were determined in terms of maximum force (N), the gradient of force-time (N/s) and area under the curve force-time (N.s) (Table 1). The addition of microalgae biomass contributed to a significant increase ($p < 0.05$) on the textural characteristics of the doughs studied. Therefore, doughs with microalgae showed a greater hardness in comparison with control doughs, with SD being the hardest sample.

A factor analysis (Figure 2) was used to explore the relationships between rheological and textural parameters of doughs studied, as well as the links between them. The first two factors showed eigenvalues higher than 1 and the consideration of both factors accounted for 95.40 % of the total variability. The first factor (F1), explaining 67.05 % of the variability, was associated with maximum force ($r = 0.97$), area ($r = 0.97$), tan δ ($r =0.95$) and gradient ($r = 0.94$) values. The second factor (F2) accounted for 28.35 % of the variability and was mainly associated with G' ($r = 0.98$) and G'' ($r = 0.82$) values. F1 separated CD and dough from microalgae on the left-hand side, because of their lower values of F_{max}, area, gradient and their higher values of tan δ. This analysis clearly separates the doughs depending on the use of microalgae in their formulation, as ingredient affecting higher resistance and hardness as well as elasticity. F2 shows higher viscoelastic behaviour in SD, in comparison with the other samples. The results from current analysis confirm that the addition of microalgae in dough reinforces their elastic behaviour.

The results obtained for the dough colour parameters, lightness (L*), green/red (a*), blue/yellow (b*), hue (h*) and chroma (C*) are presented in Table 2. The

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evaluation of parameter L* shows that lightness decreased significantly ($p < 0.05$) with microalgae addition. Also, for both microalgae doughs (Chlorella and Spirulina) at the same concentration (1.5 %) significant ($p < 0.05$) differences were found between them, with the addition of Spirulina resulting in a darker dough than Chlorella. Parameter a* (red) and b* (yellow) showed significant differences ($p < 0.05$) and microalgae addition significantly ($p < 0.05$) increased the doughs green colour (negative a* values) compared with control dough. ΔE values show perceptible colour differences when comparing the control dough to both doughs with microalgae (ΔE1), and when comparing Spirulina and Chlorella doughs (ΔE2) together. A value of ΔE > 3 implies perceptible colour difference for a consumer,[23] however; the visual colour perception in all cases is very pleasant, as can be observed in Figure 3.

3.2. Breadstick characterisation

Breadsticks produced were evaluated according to water and ash content, textural and optical properties, and mineral content. All breadsticks presented water content values ranging from 0.45 to 0.65 % without significant differences ($p > 0.05$), which is typical for this type of dried foods. No significant changes ($p > 0.05$) were observed in the breadsticks' ash content upon microalgae addition (0.89 – 1.12 %).

Table 3 shows the mean values of hardness, brittleness, area, toughness and crispiness of breadsticks studied at 0 and after 15 storage days. In general, the addition of microalgae provokes a decrease in all textural parameters studied in breadsticks. In the same way as Shahbazizadeh *et al.,*[15] who reported that the firmness of cookies generally decreased by enhancing microalgal biomass content, as a consequence of the formation of weaker gels when Spirulina biomass was added to the batter.[24] However, after 15 storage days, breadsticks with microalgae were harder and presented higher areas than the control breadsticks. While the control's hardness and area were decreasing, these parameters in breadsticks with microalgae were

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increasing (SB) or maintaining (CHB). Other authors observed this trend in cookies enriched with 2 % of Spirulina, after eight weeks of storage.[25]

To explain the relationship of the rheological and textural properties of doughs studied with the textural properties of breadsticks produced, correlation statistical analyses were performed (Table 4). Textural properties of doughs (F_{max}, area and gradient) showed negative Pearson's correlation coefficients with textural properties of breadsticks. Doughs with microalgae put up more resistance; nevertheless, the related breadsticks were less hard. In general, there is a negative relationship between area and maximum force of doughs and breadstick hardness. On the other hand, the highest significant ($p < 0.05$) correlation was observed between tan δ and area of dough and crispiness in breadsticks. Dough with high tan δ (CD) showed high values of area and crispiness in the breadsticks samples (CB).

Colour is an important characteristic of baked products because together with texture, it contributes to consumer preference.[26] Mean values of colour parameters of breadstick samples were shown in Table 5. The degradation of microalgae pigments such as chlorophyll and carotenoids due to heat contributes to colour changes in samples with microalgae.[27] This fact can be observed comparing Table 2 (dough colour) and Table 5 (breadstick colour). The typical green colour of microalgae dough (Table 2) was lost after baking and the addition of microalgae decreased luminosity significantly ($p < 0.05$), when compared to the control, that is, it caused darkening of the breadstick. The values for a* and b* also decreased significantly ($p < 0.05$) in samples with microalgae. This behaviour was observed by other authors[28] in cookies because of Spirulina biomass addition. Comparing the colour measurement position (top and bottom) in table 5, there were no significant ($p > 0.05$) differences in total colour differences between the type of microalga added (ΔE_2). Chroma values for the three samples were significantly ($p < 0.05$) different according to the colour measurement position, both top and bottom positions for chroma values of samples were ordered as CB > CHB > SB.

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L*, a* and b* variation of storage time and the total colour differences observed for stored breadstick samples are showed in Table 6. There are no significant ($p > 0.05$) differences of L*, a* and b* colour coordinates over storage. These trends were also observed by Gouveia *et al.*,[24] in cookies enrichment with Spirulina. Samples total colour difference due to storage on the top position were below the perceptible sensory limit ($\Delta E > 3$) according to Bodart *et al.*[23] Nevertheless, on the bottom position, the control's total colour differences, due to storage, were significantly ($p < 0.05$) higher than the rest of the samples. These differences were higher than 3 units, so were perceptible by the human eye.[23] In short, breadstick enrichment with Chlorella or Spirulina were more stable in colour terms.

Figure 4 shows the mineral content of the studied breadsticks. All samples presented similar macronutrient composition (Figure 4a), however potassium content (K) in breadsticks with Chlorella was significantly ($p < 0.05$) higher than the rest of the samples. Potassium, along with sodium and calcium, are present as salts in body fluids, where these have the physiological function of maintaining osmotic pressure.[29] Also, the addition of microalgae results in a significant increase ($p < 0.05$) in iron (Fe) and selenium (Se), as can be observed in Figure 4b, however, without significant differences ($p>0.05$) between Spirulina and Chlorella. Other authors[7] also observed a significant increase of iron content in bread when they added Spirulina in its formulations. This fact is an important benefit to the enrichment of breadsticks with microalgae since one of the most important minerals in human nutrition is iron according to Latham (2002). Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid synthesis, and electron transport.[30]

World Health Organization (WHO) estimates that two billion people are anaemic worldwide and attribute approximately 50 % of all anaemia to iron deficiency.[31] On the other hand, the essential trace mineral, selenium, is of fundamental importance to human health. As a constituent of selenoproteins, selenium has structural and enzymic

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roles, best-known as an antioxidant and catalyst to produce active thyroid hormone. Selenium is needed for a properly functioning immune system and appears to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS. It is required for sperm motility and may reduce the risk of miscarriage. Deficiency has been linked to adverse mood states. Furthermore, selenium is presented both as an antioxidant and anti-inflammatory agent.[32]

The iron dietary needs are approximately ten times the physiological requirements of the body, if a healthy man or post-menopausal woman requires 1 mg of iron per day, due to iron losses, the dietary needs are around 10 mg per day.[29] The Council directive on the 24 September 1990, on nutrition labelling for foodstuffs,[33] in the annex, specify that the recommended daily allowance of iron is 14 mg. According to this directive and the regulation No. 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made in foods.[34] breadstick enrichment with Chlorella or Spirulina are a food “high in iron”. With respect to selenium, the intake of this micromineral required to achieve plateau concentrations of plasma is 55 µg per day for both men and women.[32] In the same way as iron, breadsticks enriched with Chlorella or Spirulina can be considered as a “high in selenium” food. Subsequently, the content of other studied microminerals was not affected by the microalgae incorporation and do not need classification.

4. Conclusions

Breadsticks are a carbohydrate-based snack, which are consumed widely in Mediterranean countries. Breadstick enrichment with Chlorella and Spirulina are foods classed “high in iron and selenium”. In addition, this study found microalgae dough presents an attractive and innovative appearance with tonalities close to green. However, the effects of baking provoke changes in colour toward brown tonalities, but the incorporation of Chlorella or Spirulina in the formulation allows for the production of breadsticks, more stable in colour and texture.

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Figure Caption

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Figure 1. Frequency sweeps of doughs. Storage modulus (G') with a continuous line and loss modulus (G'') with a dotted line of studied doughs. CD: control dough; SD: dough with Spirulina (1.5 %); CHD: Dough with Chlorella (1.5 %)

Figure 2. Factor analysis plot for studied dough: rheological and textural parameters.

Figure 3. Visual colour differences among the three studied doughs: a) CD: Control dough, b) SD: Spirulina dough and c) CHD: Chlorella dough

Figure 4. Mean values and standard deviation of macrominerals (a) and microminerals (b) content of each formulation (CB, SB and CHB). Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for each mineral.

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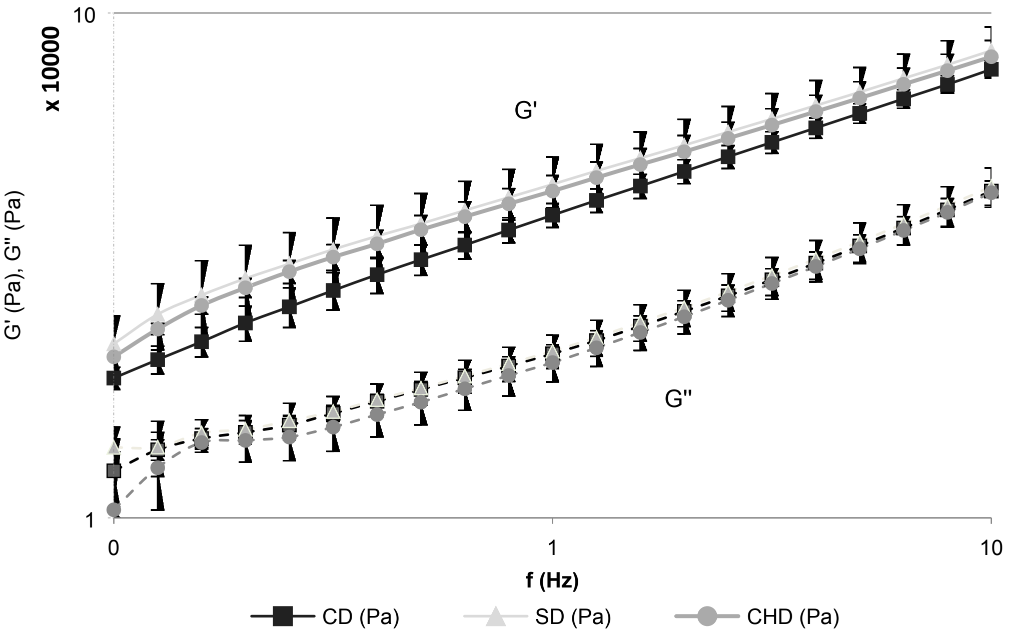

Figure 1.

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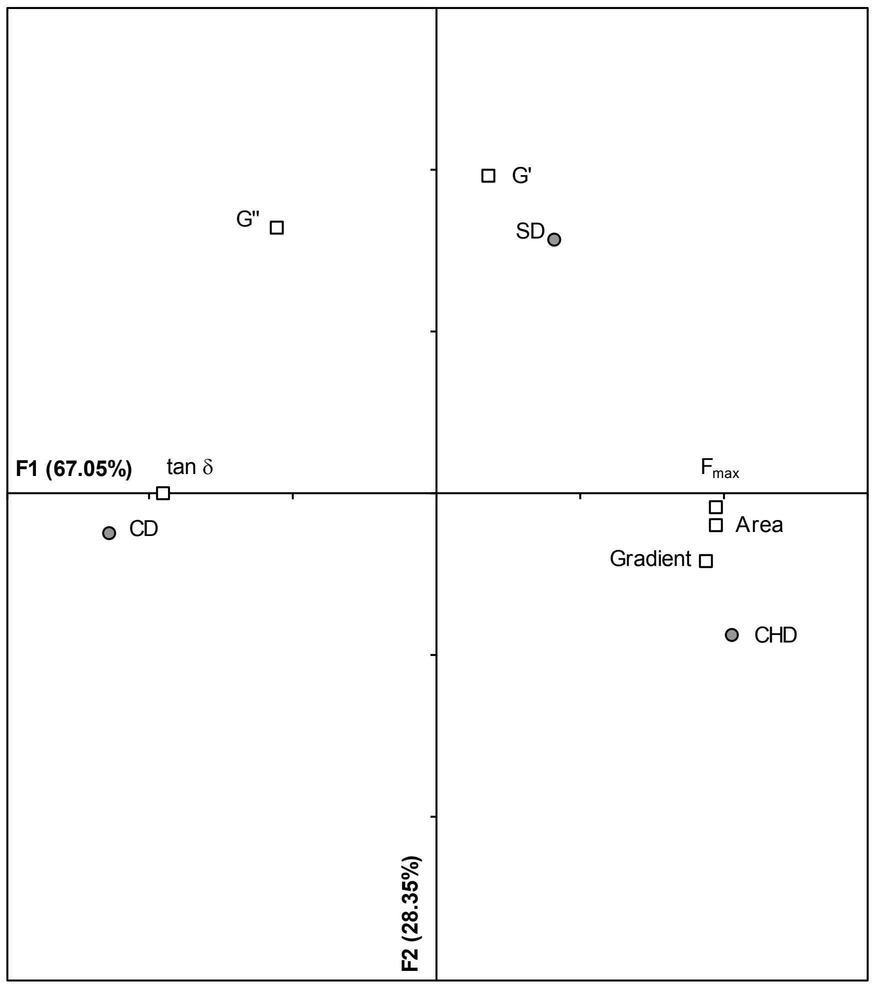

Figure 2.

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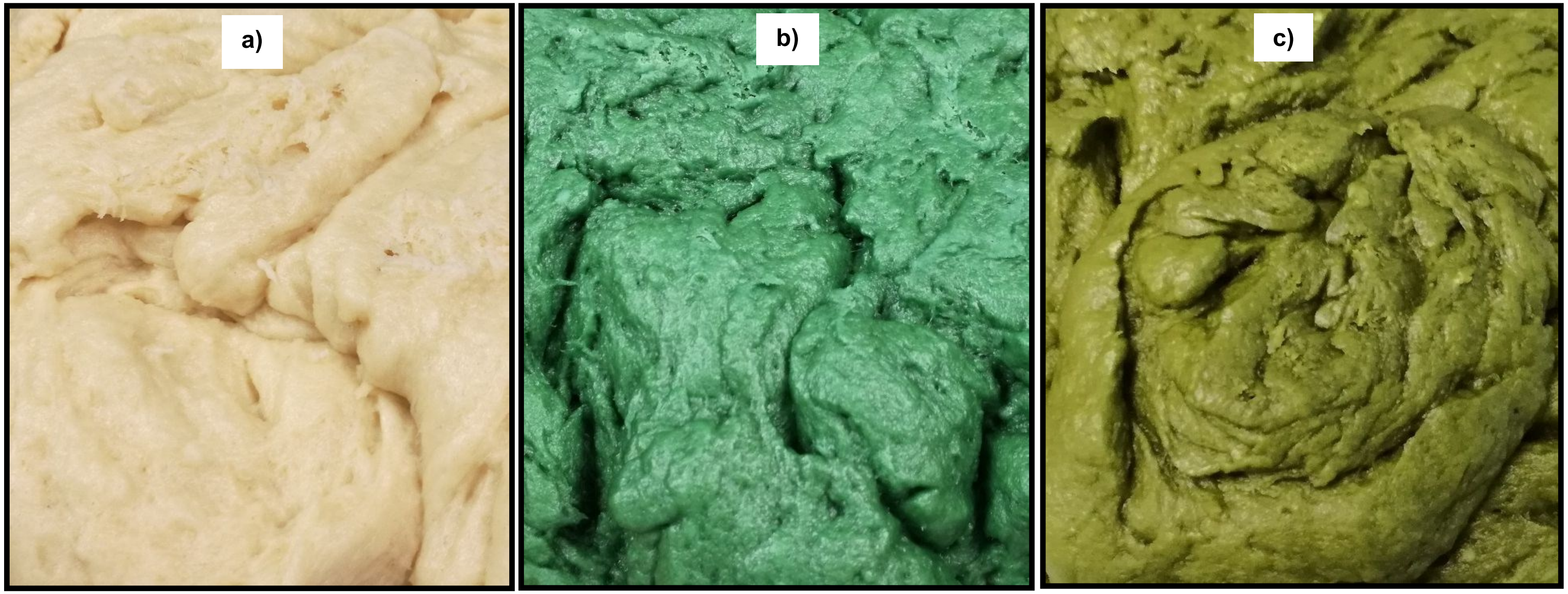

Figure 3.

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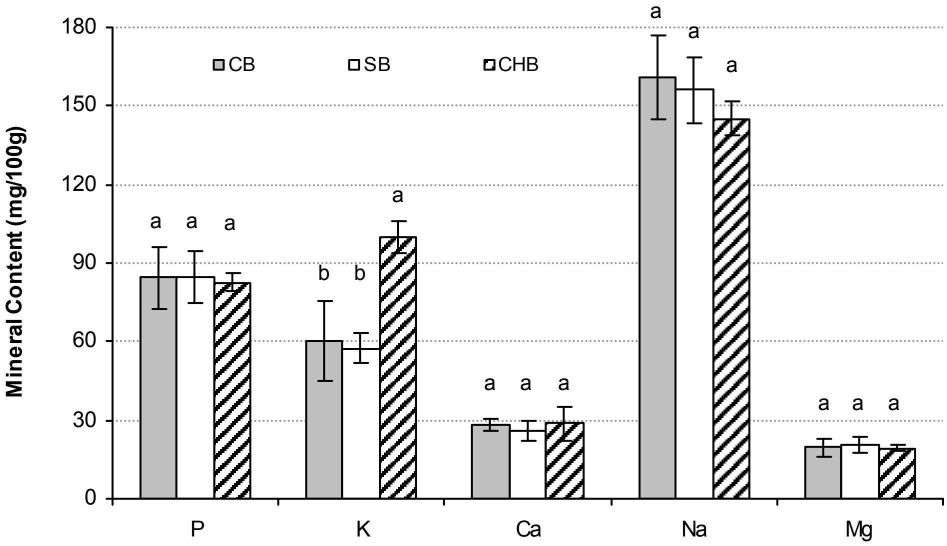
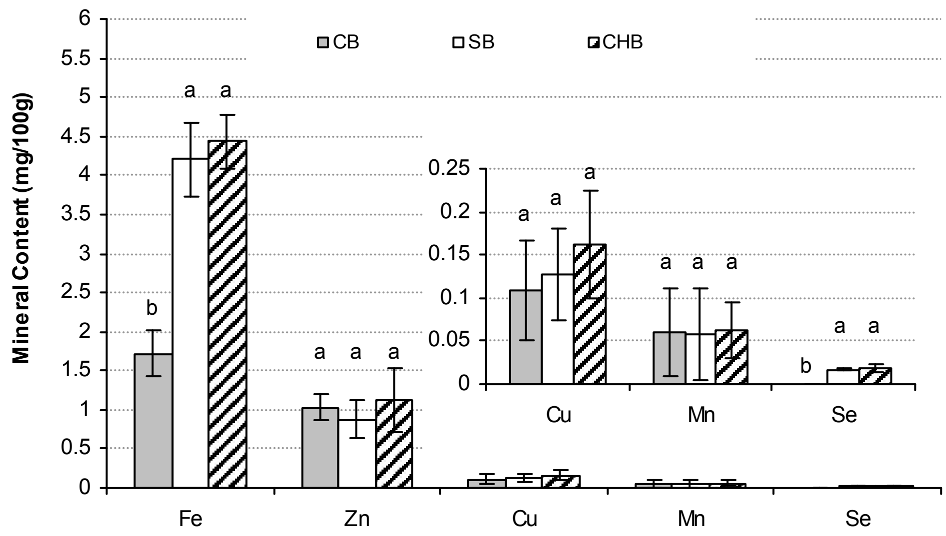


a)



b)

Figure 4.

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Table 1. Mean values (and standard deviations) of storage modulus (G'), loss modulus (G'') values and tan δ at 1Hz obtained from rheological measurements and maximum force (Fmax), area and gradient as textural properties of studied doughs.

Sample	G' (Pa)	G'' (Pa)	tan δ	Gradient (N/s)	Fmax (N)	Area (N.s)
CD	39777 (2180)[a]	21143 (238)[a]	0.53 (0.02)[a]	4.64 (0.18)[b]	25.7 (0.6)[c]	72 (3)[c]
SD	45780 (6067)[a]	21390 (1626)[a]	0.47 (0.03)[b]	5.44 (0.15)[a]	39.9 (0.6)[a]	81 (2)[a]
CHD	44395 (3260)[a]	20305 (1718)[a]	0.457 (0.005)[b]	5.2 (0.2)[a]	28.4 (0.8)[b]	77 (3)[b]

The same letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$).

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Table 2. Mean values (and standard deviation) of colour parameters of dough samples.

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Sample	L*	a*	b*	h*	C*	ΔE_1	ΔE_2
CD	58.5 (1.2)[a]	1.8 (0.4)[a]	20.0 (1.4)[a]	84.9 (0.9)[c]	20.1 (1.4)[a]	-	-
SD	35.1 (1.3)[c]	-4.1 (0.8)[c]	7.383 (0.997)[c]	119.1 (1.7)[a]	8.5 (1.2)[c]	27.3 (1.3)[a]	-
CHD	36.6 (1.4)[b]	-0.4 (0.3)[b]	10.9 (1.7)[b]	92.139 (1.096)[b]	10.9 (1.8)[b]	23.9 (1.9)[b]	5.874 (1.112)

The same letter in superscript within columns indicates homogeneous groups established by the ANOVA ($p < 0.05$).
Colour differences of doughs as compared to control (ΔE_1) and compared amongst Spirulina and Chlorella doughs (ΔE_2).

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Table 3. Mean values (and standard deviations) of Hardness (N), Brittleness (mm), Area (N.s), Toughness (N.s) and Crispiness (N) as textural properties of studied breadsticks.

Time (days)	Sample	Hardness (N)	Brittleness (mm)	Area (N.s)	Toughness (N.s)	Crispiness (N)
0	CB	40.6 (4.6)^{aA}	1.3 (0.2)^{aA}	9.7 (2.2)^{aA}	1.1 (0.5)^{aA}	2.67 (1.03)^{aA}
	SB	32.7 (2.8)^{bB}	0.89 (0.06)^{bB}	5.0 (0.6)^{bB}	0.36 (0.04)^{bB}	1.33 (0.82)^{bA}
	CHB	35.11 (3.11)^{bA}	1.1 (0.2)^{abA}	6.4 (1.9)^{bA}	0.7 (0.5)^{abA}	2.0 (1.3)^{abA}
15	CB	29.3 (1.9)^{cB}	0.93 (0.05)^{aB}	4.8 (0.6)^{bB}	1.1 (0.5)^{abA}	2.7 (1.6)^{aA}
	SB	41.8 (4.7)^{aA}	1.06 (0.10)^{aA}	7.6 (1.6)^{aA}	0.52 (0.08)^{bA}	2.33 (1.03)^{aA}
	CHB	34.0 (2.9)^{bA}	1.0 (0.3)^{aA}	5.9 (2.5)^{abA}	2.1 (1.7)^{aA}	2.3 (1.4)^{aA}

For each storage time (0 or 15 days), the same small letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$) comparing breadstick formulation.
For each breadstick (CB, SB or CHB), the same capital letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$) comparing storage time (0 or 15 days).

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Table 4. Pearson correlation coefficients between rheological and textural properties of doughs and textural properties of breadsticks.

		Breadsticks				
		Hardness	**Brittleness**	**Area**	**Toughness**	**Crispiness**
Doughs	**F max**	-0.7355*	-0.609*	-0.6900*	-0.7042*	-0.4055
	Area	-0.7639*	-0.6346*	-0.7082*	-0.6816*	-0.5177*
	Gradient	-0.5714*	-0.4427*	-0.5804*	-0.6276*	-0.5227*
	G'	-0.1495	-0.3117	-0.2418	0.0445	-0.3526
	G"	0.3796	0.2328	0.3910	0.4422	0.2764
	Tan δ	0.7063*	0.7014*	0.8264*	0.5062	0.7906*

* Correlation is significant at the 0.05 level.

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Table 5. Mean values (and standard deviation) of colour parameters of breadstick samples.
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Sample	TOP			BOTTOM		
	CB	SB	CHB	CB	SB	CHB
L*	61 (2)^{aB}	42.6 (1.3)^{bB}	39.8 (1.7)^{cB}	63.8 (1.4)^{aA}	44.2 (1.2)^{bA}	44.2 (0.9)^{bA}
a*	5.3 (0.6)^{aA}	1.0 (0.2)^{cB}	1.8 (0.2)^{bA}	5.32 (1.06)^{aA}	1.6 (0.3)^{bA}	1.9 (0.2)^{bA}
b*	26.7 (0.7)^{aB}	13.72 (1.02)^{cA}	14.4 (1.5)^{bB}	27.7 (1.4)^{aA}	14.48 (1.13)^{cA}	17.47 (1.06)^{bA}
h*	78.7 (1.2)^{cA}	85.7 (0.9)^{aA}	82.9 (0.8)^{bB}	79.2 (1.6)^{bA}	83.55 (1.06)^{aB}	83.7 (0.5)^{aA}
C*	27.2 (0.7)^{aB}	13.759 (1.015)^{cB}	14.7 (1.5)^{bB}	28.3 (1.5)^{aA}	14.58 (1.15)^{cA}	17.57 (1.07)^{bA}
ΔE_1	-	22.8 (2.3)^{aA}	24.5 (2.4)^{aA}	-	24.0 (1.3)^{aA}	22.5 (1.3)^{bB}
ΔE_2	-	-	4.2 (1.5)^{A}	-	-	3.5 (1.4)^{A}

For each measurement position (top or bottom), the same small letter in superscript within rows indicates homogeneous groups established by ANOVA ($p < 0.05$) comparing breadstick formulation.

For each breadstick (CB, SB or CHB), the same capital letter in superscript within rows indicates homogeneous groups established by ANOVA ($p < 0.05$) comparing top and bottom.

Colour differences of breadsticks as compared to control (ΔE_1) and compared amongst Spirulina and Chlorella breadsticks (ΔE_2) for each measurement position (top or bottom).

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Table 6. Mean values (and standard deviation) of L*, a* and b* variation for storage and total colour differences for storage of breadstick samples.

Sample	TOP			BOTTOM		
	CB	SB	CHB	CB	SB	CHB
ΔL_{15-0}	-0.4 (2.6)^{aA}	0.8 (1.8)^{aA}	0.9 (1.9)^{aA}	-1.4 (2.9)^{aA}	0.1 (1.8)^{aA}	-0.01 (1.13)^{aA}
Δa_{15-0}	0.1 (0.8)^{aA}	0.1 (0.3)^{aA}	-0.1 (0.2)^{aA}	0.1 (1.9)^{aA}	-0.1 (0.4)^{aA}	-0.1 (0.3)^{aA}
Δb_{15-0}	0.313 (0.998)^{aA}	1.2 (1.5)^{aA}	0.9 (1.6)^{aA}	-0.3 (2.8)^{aA}	0.4 (1.4)^{aA}	-0.2 (1.2)^{aA}
ΔE_{15-0}	2.3 (1.7)^{aB}	2.4 (1.2)^{aA}	2.2 (1.7)^{aA}	4.2 (1.9)^{aA}	1.8 (1.5)^{bA}	1.4 (0.7)^{bA}

For each measurement position (top or bottom), the same small letter in superscript within rows indicates homogeneous groups established by ANOVA ($p < 0.05$) comparing breadstick formulation.
For each breadstick (CB, SB or CHB), the same capital letter in superscript within rows indicates homogeneous groups established by ANOVA ($p < 0.05$) comparing top and bottom.

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The addition of microalgae in breadsticks produces an increase in iron and selenium content

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