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IMPACT OF ALGAE ADDITION ON BREAD PROPERTIES AND CONSUMERS BEHAVIOR-PRELIMINARY RESEARCH

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Article history:	ABSTRACT
10 March 2019	The paper presents some preliminary results related to the effects of
Accepted:	Spirulina (S. platensis) powder addition in ratios of 1%, 3% and 5%,
20 March 2019	respectively on some properties of white bread. Experimental data indicate
	that the fortification of bread results in significantly higher values of
Keywords:	polyphenols and proteins contents, antioxidant activity and minerals level,
Spirulina powder	the higher Spirulina ratio the higher values were obtained. On the other
Spirulina bread	side, the consumers studies indicated that spirulina-bread is not accepted,
Functional bread	the highest ratio of addedd Spirulina the lowest scores for sensorial
Consumer acceptance	attributes being obtained. The main reasons indicated by the consumers for
	the spirulina-breads rejection were the grey-green color of breads often
	associated with mold presence, the caverns in the bread crumb and the
	"herbal" taste. Further investigations are required to find spirulina-bread
	formulations able to meet both the high functionality and consumers
	sesnsorial expectations.

1. Introduction

Bread is the staple food in many countries important source of complex as an carbohydrates and proteins. Its main disadvantage is related to the low nutritional content because the vitamins and minerals are in the bran and germ which are discarded during milling. Bread fortification (EU regulation 1925/2006) corrects the nutrients deficiencies, balances the total nutrient profile and restores nutrients lost during baking. Although fortified flours with iron exist on the market of 68 countries (around 2 billions beneficiaries) the resulted products are usually rejected by the consumers due to poor organoleptic and sensory quality and side effects (digestive distress) caused by the minerals addition in inorganic state (sulphate, oxide). Multiples studies demonstrated that organic bond minerals are absorbed more efficiently than inorganic compounds (Rider et al., 2003). Spirulina (*Spirulina platensis*), a blue-green microalgae has arisen as a promising sustainable, balanced and complete source of organic bioactive compounds which help nurture and sustain physical and mental health (Ama Moor et al., 2016).

The paper presents some preliminary research related to the impact of *Spirulina* (*Spirulina platensis*) powder addition on some nutritional values of bread as well on the consumer acceptance of spirulina-bread.

2. Materials and methods

Spirulina (Spirulina platensis) powder was purchased from Hofigal Company whils the other ingredients (wheat flour, salt, yeast) from the local market.

2.1. Bread preparation

Four bread formulations in which the amount of *Spirulina* powder was 1%, 3% and

5% were prepared in respect of the same amounts of flour, white yeasts and water (100g, 5 g, 50 mL). The ingredients were mixed for 30 minutes and the dough was allowed to rest for about 30 minutes at room temperature. After moulding in round shape, dough pieces were proofed at 30°C for 45 minutes, remixed 2 minutes, moulded in rectangular shape put into baking tins at 30°C for another 25 minutes. The tins were kept for 30 minutes at 220°C in a MATINA Oven prior pre-heated at 150°C. After baking, the bread loaves were allowed to cool at ambient temperature for 3 h and then slices and send to analysis.

2.2. Polyphenols content

Bread extract was prepared accordint to the method proposed by Michalska et al. (2007). According to it, 1g of bread samples was mixed with 10 mL methanol for 2h at 37°C and the centrifuged at 12,000 x g rpm for 15 minutes. The supernatant was used for both polyphenol and DPPH analyzes.

For polyphenol content 0.25 mL of bread extract was mixed with 0.25 mL of diluted Folin-Ciocalteu's reagent (water 1:1 v/v), 0.5 mL of sodium carbonate (Na₂CO₃) solution (0.2 g/ml) and 4 mL of water and allowed to rest for 25 minutes at room temperature. After centrifugation for 10 minutes at 2,000 x g the absorbance of supernatant was red at 725 (UV-VIS spectrometer Lambda 35) against gallic acid standards Michalska et al. (2007). The results were expressed as mg gallic acid/g of bread.

2.3. Antioxidant activity (DPPH method)

The method proposed by López-de-Dicastillo et al. (2010) adapted to our study was used to measure the antioxidant activity of bread varieties. 5 mL of bread extract was put in contact with 5 mL DPPH for 30 minutes in the darkness at room temperature. The absorbance of sample was read at 515 nm using as compared to blank made of 5 mL ethanol and 5 mL DPPH. The absorbance was calculated as:

Antioxidant activity (%) = $(1 - Abs_s/Abs_b)x100$ (1)

where: Abs_s, Abs_b are the absorbance of sample and blank, respectively;

2.4. Mineral content (Ca, Mg, Fe, Zn)

Bread digestion occurred according to the method described by Berghof (MWS–2/ Food, Pharma, Cosmetics which provides the contact of 300 mg of bread with 6 mL of HNO₃ and 1 mL HCl followed by heating in the microwave oven (MWS-2) for 5, 10 and 15 minutes at 145°C, 170°C and 200°C. The content of Ca, Mg, Zn and Fe was read by using atomic absorbtion spectrometer Perkin Elmer AAnalyst 800.

2.5. Proteins content

Kjeldahl method was used to determine the protein in bread samples (AOAC 945.18-B method). Around 2 g of ground bread, sieved at 2 mm and dried at 105°C to constant weight was digested (7 g K₂SO₄, 5 mg powder of Se, 12 mL H₂SO₄, 5 mL H₂O₂ 35%) for 20 minutes at 420°C. After cooling and dilution with 50 ml of deionised water the solution was distilled and the distilled was collected in 25 mL boric acid solution of 4%. After titration with HCl 0.2 N the protein content was calculated according to the Eq (2):

Protein content (mg N-NH₃)= $V \cdot 2.803$ (2)

where V is the volume of HCl used for titration, mL; 2.803 is the amount of N-NH₃ corresponding to 1 mL of HCl 0.2 N

2.6. Consumer acceptance study

Fifty assessors of which 25 were females and 25 male were included in the sensorial analysis of the spirulina-bread varieties. Prior to analysis they were instructed in order to develop a consensual vocabulary for the interested issues: color, taste, texture, smell and overall acceptability. They were asked to rank each descriptor according to their intensity in a range of 1 (weak) to 5 (strong).

2.7. Statistically processing of experimental data

All analyzes were performed in triplicate and results are expressed as mean \pm standard deviation. The statistical significance of the differences obtained between bread varieties was evaluated using the student *t*-test. Probability value p < 0.05 was considered statistically significant.

3.Results and discussions

3.1.Pholyphenols content and antioxidant activity

As in Figure 1 is displayed, the highest level of polyphenols can be noticed in the bread enriched with *Spirulina* powder. By increasing the amount of incorporated *Spirulina* with 1%, 3% and 5% the polyphenols content rises 1.19-fold, 1.41-fold and 2.-73-fold as compared with un-supplemented bread.

Higher antioxidant activity was also obtained after the addition of Spirulina in the bread (Figure 1). It increased to 11.06% in bread with 1% Spirulina, 13.21% in bread with 3% Spirulina and 15.46% in bread with 5% Spirulina from 10.24% in plain bread. Several bioactive compounds founded at high level in Spirulina (phycocyanin, total carotenoids and β -carotenoids, phenolic compounds) can be responsible considered for the high polyphenols content and antioxidants activity of fortified bread (Saharan and Joode, 2017; Marco et al., 2014). Good correlation was observed between polyphenols content and antioxidant activity of spirulina-bread (R= 0.9369). Our values for polyphenols content and antioxidant activity are close to those obtained by Saharan and Joode (2017) of which bread formula included Spirulina

powder at 98:2, 96:4, 94:6 and 92:8 (yeast 3g, sugar 10g, salt 1.75g, water \pm 60 ml).

3.2. Minerals content

Significant improvement in the content of Ca, Mg, Fe and Zn of fortified bread as compared to plain bread was noticed (Figure 1). Thus, Ca increased 1.21-fold in 1% spirulina-bread, 1.88-fold in 3% spirulinabread and 2.06-fold in 5% spirulina-bread whilst Mg displayed higher growth increments of 1.39-fold in spirulina-bread, 2.42-fold in 3% spirulina-bread and 2.60-fold in 5% spirulinabread. The highest amount of Fe content was obtained in 5% spirulina-bread of 5.01 mg/100 as compared to 3.87 mg/100 g in 3% spirulinabread, 2.01 mg/100 g in 1% spirulina-bread and 0.96 mg/100 g in un-fortified bread. The increase in Zn content was 1.61-fold, 1.99-fold and 2.31-fold, respectively much higher than the results of Saharan and Joode (2017) who reported that the Zinc content was differed non-significantly. It is obviously that the addition of Spirulina powder resulted in the increase of minerals content in bread.

3.3. Proteins content

The fortification of bread with *Spirulina* powder also resulted in the increase of the proteins level up to 1.88-fold in the 5% spirulina-bread explained by the high level of proteins in the *Spirulina* powder (Simo et al., 2005). Ak et al. (2016) reported close values of proteins content in white bread enriched with 10% *Spirulina*.

3.4. Consumer acceptance study

The results of sensory evaluation study of spirulina-bread varieties are displayed in the Figure 2. According to it, the addition of *Spirulina* decreases all the sensory descriptors, the highest decrease being noticed in the case of bread enriched with 5% *Spirulina* and the lowest for the bread with 1% *Spirulina*.

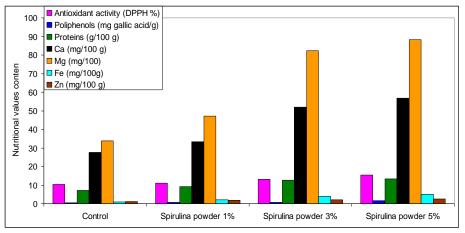


Figure 1. The characteristics of spirulina-bread

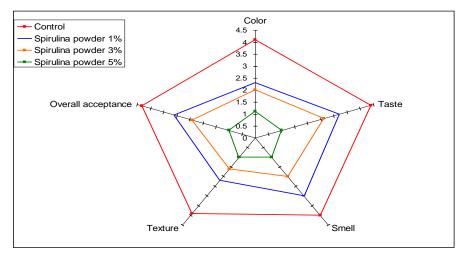


Figure 2. The sensorial chart related to spirulina-bread



Figure 3. Appearance of spirulina-breads

Thus, in the 1% spirulina-bread the color descriptor decrease 1.78-fold, taste 1.37-fold, smell 1.32-fold, texture 1.77-fold and overall acceptability 1.38-fold. In the 5% spirulina-bread, the decreases were 3.72-fold for color and 4-fold for the other descriptors. The main reasons indicated by the consumers for the spirulina-breads rejection were the grey-green color of breads often associated with the mold

presence, the caverns in the bread crumb and the "herbal" taste (Figure 3).

4. Conclusions

Preliminary research indicates that by addition of Spirulina the nutritional value of bread in terms of proteins, polyphenols and minerals levels is significantly enhanced. The antioxidant activity of bread also increases. These confer functional property to spirulinabread and recommend it not only for the vulnerable consumers (osteoporosis, calcium and magnesium defficiencies, anemia) but also to the healthy consumers interested in the prohealth-food.

On the other side, the consumers studies indicated that spirulina-bread is not accepted, the highest ratio of addedd *Spirulina* the lowest scores for sensorial attributes being obtained. Further investigations are required to find spirulina-bread formulations able to meet both the high functionality and consumers sesnsorial expectations.

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