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Effects of spirulina biomass on the technological and nutritional quality of bread wheat pasta

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ABSTRACT

The objective of this study was to evaluate the effect of the incorporation of spirulina on technological and nutritional quality of dried pasta. Wheat flour was substituted by spirulina biomass at three levels: 5, 10 and 20 g/100 g, and a sample without spirulina biomass was made as control. The technological quality was analyzed in terms of cooking properties and texture profile, while pasta surface was observed by confocal microscopy. In addition, protein content, *in vitro* protein digestibility, phenolic compound content and *in vitro* antioxidant activity were the major bio-functional characteristics measured. An *in vitro* starch digestion was performed in order to estimate the glycemic index. Only pasta with 20 g of spirulina / 100 g of flour did slightly modify technological quality parameters; microstructure studies revealed the impact of spirulina addition, resulting in a more heterogeneous surface. The glycemic index was not affected by the addition of spirulina. The incorporation of spirulina resulted in an increase of protein content; however, protein digestibility was reduced as microalgae content increased. Pasta with spirulina exhibited high phenolic compounds content and antioxidant activity compared to control pasta, which could be used to enhance the nutritional profile of the product.

1. Introduction

Pasta products are largely consumed worldwide, not only for their nutritional advantages as rich sources of complex carbohydrate, low sodium and fat contents, but also because of their sensory attributes (Chillo, Laverse, Falcone, & Del Nobile, 2008). Pasta is traditionally manufactured with durum wheat flour; however, since in central and north Argentine areas durum wheat is not grown, bread wheat is used in spaghetti and pasta in local industries (Martínez, Ribotta, Añón, & León, 2013). The extrusion-lamination forming process of pasta leads to the creation of a structured gluten network, the quality of the network will depend on the type of wheat and the properties of flour. Macroscopically, this compact structure is responsible for the slow starch degradation and the low glycemic index of pasta (Fardet et al., 1999).

In recent years, different healthy ingredients have been used in the production of pasta to enhance its nutritional profile or to confer functional properties. However, the amount of raw material that can be used as a substitute for wheat flour or can be added to wheat flour represents a compromise between nutritional improvement and satisfactory sensorial properties of pasta (Chillo et al., 2008). Cooked pasta of good quality is firm and resilient, with high elasticity and low stickiness, maintaining surface integrity after being cooked and little, if any, cooking losses (Bruneel, Pareyt, Brijs, & Delcour, 2010; Marchylo, Dexter, & Malcolmson, 2004).

Microalgae have received increasing attention due to the fact that they represent one of the most promising sources of compounds with biological activity that could be used as functional ingredients (Pulz & 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 (Spolaore, Joannis-Cassan, Duran, & Isambert, 2006).

Recently, studies have been carried out to improve the nutritional properties of pasta products with macro and microalgae. Prabhasankar et al. (2009) developed a seaweed (*Undaria pinnatifida*) dried extruded pasta with better bio-functional properties due to the higher content of fucoxanthin and fucosterol in these macroalgae. Fradique et al. (2010) incorporated *Chlorella vulgaris* and *Spirulina maxima* in fresh pasta, resulting in products with enhanced chemical compositions, without affecting the cooking quality.

Spirulina, filamentous blue-green microalgae or cyanobacteria, is well known as a source of protein (60–70 g/100 g) of high biological value, since it is a rich source of vitamins, mainly vitamin B_{12} and

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pro-vitamin A, minerals, especially iron, and γ-linolenic acid, essential fatty acid precursor for prostaglandins (Belay, Ota, Miyakawa, & Shimamatsu, 1993; Habib, Parvin, Huntington, & Hasan, 2008). Furthermore, spirulina contains such molecules as phycocyanin, β-carotene and xanthophyll pigments, γ-tocopherol and phenolic compounds, which are responsible for the antioxidant activities of these microalgae, as shown by several authors for *in vitro* and *in vivo* experiments (Miranda, Cintra, Barros, & Mancini-Filho, 1998; Patel, Mishra, & Glosh, 2006). Moreover, most research has focused on the health effects of spirulina as a dietary supplement for humans and animals. Many studies have shown the effects of these microalgae that may result in significant therapeutic applications: an anti-cancer effect (Hirahashi et al., 2002; Mao, Van de Water, & Gershwin, 2005), a hypolipidemic effect (Narmadha, Sivakami, Ravikumar, & Mukeshkumar, 2012; Ramamoorthy & Premakumari, 1996), and a protective effect against diabetes and obesity (Anitha & Chandralekh, 2010). These advantages make spirulina a good raw material for the healthy food.

So far, little research has been carried out regarding the effects of spirulina on cooked pasta, in terms of protein digestibility, *in vitro* starch hydrolysis or phenolic compound and the antioxidant activities after the drying and cooking processes.

The aim of this study was to evaluate how the addition of spirulina to dried pasta affected the technological and nutritional quality of this product, focusing on the changes produced at the level of protein digestibility, phenolic compounds content, antioxidant activity and glycemic index.

2. Material and methods

2.1. Raw materials

Bread wheat flour (*Triticum aestivum*), without additives, was provided by Industrias Alimenticias Tiranti S.R.L. (Argentina): Moisture content 12.98 ± 0.09 g/100 g, Ash 0.54 ± 0.01 g/100 g, Protein 13.97 ± 0.03 g/100 g, Fat 0.9 ± 0.02 g/100 g and carbohydrates 71.21 ± 0.3 g/100 g. All the analyses were determined according to AACC International, 2000 in triplicate.

Spirulina Biomass was obtained commercially (*Spirulina platensis*, Spirulina Spirel, Cuba): Moisture content 12.15 ± 0.08 g/100 g, Ash 11.27 ± 0.04 g/100 g, Protein 67.82 ± 1.71 g/100 g, Fat 1.62 ± 0.03 g/100 g and carbohydrates 7.14 ± 2.00 g/100 g. All the analyses were determined according to AOAC International (1998) in triplicate.

2.2. Pasta processing

The pasta sample was prepared using wheat flour combined with an increasing amount of spirulina biomass: 5, 10 and 20 g/100 g (S5, S10 and S20, respectively). A sample without spirulina biomass was made as control (CO). Blends (50 g) were mixed, with optimum amo unts of distilled water (determined experimentally) and salt (500 mg/50 g of blend), with a hand mixer (Philips, 190W, Buenos Aires, Argentina) at maximum speed for 3 min. Then, the dough was sheeted using a pasta maker (Pastalinda®, Buenos Aires, Argentina). Pasta strips of 2 mm wide and 2 mm thick were dried at low temperature in two steps: 30 min pre-drying stage at 30 °C with humidity-controlled (35%), followed by a drying stage at 45 °C with controlled humidity (75%), for 17 h. The final moisture of dried pasta was 9.5 ± 0.5 g/100 g.

2.3. Technological qualities

2.3.1. Cooking quality parameters

Optimum cooking time (OCT) was determined according to AACC 16-50 (2000). Pasta (4 g), cut into pieces of 5 cm, was cooked in boiling water (200 mL) and analyzed at 30-s intervals by compressing the pasta between two glass slides until the inner white core of the pasta disappeared.

Swelling index of cooked pasta (g water/g dry pasta) was evaluated by drying pasta samples to constant weight at 105 °C, expressed as: [weight of cooked product (W1) − weight after drying (W2)]/weight after drying (W2) (Tudorică, Kuri, & Brennan, 2002).

Water absorption of drained pasta was determined as: [[weight of cooked pasta (W1) − weight of raw pasta (W3)]/weight of raw pasta (W3)] × 100 (Tudorică et al., 2002).

Cooking loss in the cooking water collected was determined by evaporation to constant weight in an air oven at 105 °C. The residue was weighed and reported as percentage of the original pasta sample (Tudorică et al., 2002).

All the tests were carried out in triplicate.

2.3.2. Texture analysis

Raw pasta fracturability was evaluated using a texture analyzer with HDP/3 PB probe (TA-XT2, Stable Micro System, Surrey, UK). A pasta strand of 5 cm long was compressed and fracturability (N) was determined as the maximum peak force until the pasta strand was fractured. The test was repeated on six samples.

Texture profile analysis (TPA) of cooked pasta was measured using a texture analyzer with a stainless steel rectangular probe HDP/PFS (TA-XT2, Stable Micro System, Surrey, UK). Pasta strand of 5 cm long were cooked. Three pieces of cooked pasta were placed perpendicularly to the probe so that they touched each other along their entire length. The samples were compressed twice at a rate of 0.50 mm/s and at a ratio of 70%. TPA values for firmness (N), stickiness (measures as negative force, N), cohesiveness, springiness and chewiness (N) were obtained. The test was repeated on five samples.

2.3.3. Confocal Laser Scanning Microscopy (CLSM)

The microstructure of cooked pasta was observed on the surface of a pasta strand, using an Olympus LEXT 3D Confocal Laser Microscope (Japan).

2.4. Nutritional qualities

2.4.1. Protein content and in vitro protein digestion

Nitrogen content in raw and cooked pasta was determined by Kjeldahl method according to AACC 46-13 (2000). The percentage of total protein was calculated as Nx6.25.

In vitro protein digestion was measured in freeze-dried samples of cooked pasta, following Pasini, Simonato, Giannattasio, Peruffo, and Curioni (2001) description, with slight modifications. Samples (60 mg) were mixed with 0.02 mol equi/L HCl (pH 2.2) containing 0.05 mg/mL of pepsin (4520 U/mg, pepsin from porcine gastric mucosa, Sigma–Aldrich) and incubated at 37 °C for 30 min. Then, 1.15 mL of 1 mol/L boric acid, 0.5 mol equi/L NaOH and 5 mol equi/L HCl (pH 6.8) containing 0.25 mg/mL of pancreatin was added. The reaction was performed at 37 °C in a shaking water bath and was stopped after 120 min by the addition of 1 volume of 2 g/100 mL trichloroacetic acid (TCA). After standing for 1 h, the samples were centrifuged (8000 g, 10 min, 25 °C) and the pellet was analyzed for nitrogen content and reported as protein content after digestion. The digestibility was expressed as: [(total protein content − protein content after digestion) × 100/total protein content].

Protein availability refers to the quantity of protein digested in pasta. It was calculated over the protein content in cooked pasta and the protein digestibility as: [(protein digestibility × protein content in cooked pasta)/100].

The assays were analyzed in triplicate.

2.4.2. In vitro digestion of starch and estimated glycemic index

Starch analysis (total, soluble and resistant starch) was performed using a Megazyme Kit (K-RTAR 05/2008, Ireland), according to AACC 32-40 (2000).

In vitro digestion was performed using a multi-enzymatic method according to Bustos, Pérez, and León (2011). Samples of cooked pasta (4 g) were mixed with 20 mL sodium potassium phosphate buffer (PBS, pH 6.9) and disintegrated with a homogenizer. The pH was adjusted to 1.5 with HCl 8 mol/L and 5 mL of pepsin solution (115 U/mL, pepsin from porcine gastric mucosa, Sigma–Aldrich) were added to the sample, followed by incubation at 37 °C for 30 min. Then, the pH was readjusted to 6.9 with 10 g/100 mL NaOH and the sample brought to 49 mL with PBS and 1 mL of α-Amylase solution (110 U/mL, α-Amylase, Type VI-B from porcine pancreas, Sigma–Aldrich). The samples were incubated for 3 h at 37 °C. Every 30 min, for 3 h, aliquots of 1.5 mL were withdrawn to analyze the reduction of sugar content, using the 3.5-dinitrosalicylic acid (DNS) method. A standard curve, using maltose, was prepared. The maltose was converted into starch by multiplying the maltose value by 0.9 and the rate of starch digestion was expressed as the percentage of total starch hydrolyzed at different times (30, 60, 90, 120, 150 and 180 min). A non-linear model established by Goñi, García-Alonso, and Saura-Calixto (1997) was applied to describe the kinetics of starch hydrolysis. The first order equation has the following formula: $C = C_\infty (1 - e^{-kt})$, where C corresponds to the percentage of starch hydrolyzed at time t; C_∞ is the equilibrium percentage of starch hydrolyzed after 180 min; k is the kinetic constant and t is the time (min). Parameter estimation was carried out using ORIGIN PRO software, version 8. The area under the hydrolysis curve (AUC) was calculated and the hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample (fresh white bread). Expected GI was estimated using the model $GI = 39.71 + 0.549 \times HI$.

All tests were performed in triplicate.

2.4.3. Polyphenols content and antioxidant activity

The *sample preparation* was carried out according to Li et al. (2007) with slight modifications. Freeze-dried spirulina and cooked freeze-dried pasta samples were extracted with 2 mL of hexane for 30 min at 37 °C. The samples were centrifuged at 13,000 g for 10 min at 4 °C and the supernatants were recovered. The extractions were repeated and the two supernatants combined. Then, the residues were twice extracted with ethyl acetate (2 mL each time) for 30 min at 37 °C and the supernatants were combined. Finally, the residues were twice extracted with water (2 mL each time) for 30 min at 37 °C and the supernatants were combined. The hexane and the ethyl acetate extract were recovered in a rotary evaporator and the water extract was lyophilized. All pellets were re-dissolved in methanol, obtaining three different extracts: hexane, ethyl acetate and water extract.

Trolox equivalent antioxidant capacity (TEAC) assay was performed in accordance to Re et al. (1999) with subtle modifications by free radical scavenging activity against $ABTS^{+\bullet}$. To form the radical, ABTS (7 mmol/L) was mixed with potassium persulphate and left to stand for 12 h in the darkness. Working solution was prepared by dilution of radical solution with methanol until an absorbance of 0.70±0.02 at 734 nm was obtained. One hundred μL of extract was mixed with 3 mL of working solution and vortex for 10 s. A decrease of the absorbance was measured at 734 nm after 30 min reaction at 25 °C. A standard curve, using TROLOX (Sigma–Aldrich), was prepared and results were expressed as μmol/L TROLOX/100 g.

Ferric reducing ability of plasma (FRAP) assay was carried out according to Benzie and Strain (1996) with a few modifications. Working solution was prepared mixing acetate buffer (pH = 3.6), TPTZ 10 mmol/L in 40 mmol/L HCl and 20 mmol/L $FeCl_3 \cdot 6H_20$ (10:1:1). One hundred μL of extract was mixed with 3 mL of working solution and vortex for 30 s. After 30 min reaction, absorbance was determined at 593 nm at 25 °C. A standard curve, using TROLOX (Sigma–Aldrich), was prepared and results were expressed as μmol/L TROLOX/100 g.

Total phenolic content (TPC) was determined by the Folin–Ciocalteu method, according to Singleton and Rossi (1965) with slight modifications. Extract (20 μL) was mixed with 90 μL of methanol, 1.68 mL of distilled water and 100 μL of Folin–Ciocalteu reagent, and stirred in a vortex for 20 s. Three hundred μL of Na_2CO_3 (20 g/100 mL) were added and tubes were left to stand for 2 h in the dark. Then, absorbance was read at 750 nm. TPC was calculated by linear regression using gallic acid (Riedel-de-Haën, China) as standard. Results are expressed in mg/L gallic acid equivalents (GAE).

All samples were analyzed in triplicate.

2.5. Statistical analysis

Results were expressed as the mean of replications ± SD. Analysis of variance (ANOVA) followed by Fisher's test using the InfoStat Statistical Software (Facultad de Ciencias Agropecuarias, UNC, Argentina) were performed for statistical analysis.

3. Results and discussion

3.1. Cooking properties

The cooking behavior of different pasta samples: optimum cooking time (OCT), swelling index, water absorption and cooking loss, are presented in Table 1. The addition of increasing amounts of spirulina in pasta formulation decreased OCT and increased cooking loss. However, all cooking loss values were less than 8%, which is the limit value considered desirable for spaghetti prepared from semolina proposed by Dick and Youngs (1988). The incorporation of spirulina, with 60 g of proteins / 100 g of biomass, could weaken the overall structure of pasta due to their proteins are not able to develop a gluten network, and steric hindrance interferes in network formation. Since the protein network limits the diffusion of water into the central zone of pasta during cooking (Fardet et al., 1998), weaker gluten-protein network facilitates water diffusion into it, reducing cooking time. Also, since gluten-protein network is responsible for retaining pasta physical integrity during cooking, a weaker structure leaches more solids from pasta samples into cooking water, increasing cooking residues (Khan, Yousif, Johnson, & Gamlath, 2013; Martinez et al., 2013). Fradique et al. (2010) used *Spirulina maxima* to enrich fresh extruded pasta made of *durum* semolina flour, finding that OCT of substituted pasta was similar to

Table 1
Cooking quality parameters.

Sample	OTC (min)	Swelling index	Water absorption (g/100 g dry pasta)	Cooking loss (g/100 g dry pasta)
CO	11	1.98 ± 0.02^a	148.70 ± 1.82^a	4.97 ± 0.03^a
S5	10	1.94 ± 0.04^a	142.79 ± 3.36^a	5.72 ± 0.25^b
S10	10	1.95 ± 0.05^a	145.45 ± 3.80^a	5.90 ± 0.15^b
S20	9	2.09 ± 0.08^b	158.47 ± 7.34^b	7.39 ± 0.19^c

S5, S10, and S20: pasta prepared with 5, 10 and 20 g of spirulina / 100 g of flour, respectively.
Co: control sample.
Results in the table represent the mean of triplicate measurements.
Within the same column, values with a different letter are significantly different ($P \leq 0.05$), according to Fisher test.

the control sample and no changes were observed in cooking loss. However, the pasta elaborate with durum semolina flour resulted in a stronger protein-gluten network and the biomass of *S. maxima* incorporated (0.5, 1 and 2 g/100 g of durum semolina wheat) was much lower than the content of spirulina used in the present work. Furthermore, Prabhasankar et al. (2009) reported similar effects to those obtained in our study on cooking loss due to the incorporation of seaweeds (*U. pinnatifida*) in extruded dried pasta.

Pasta prepared with 20% of microalgae showed a higher swelling index and higher water absorption than other samples assayed. Although the OCT was shorter, the lack of a continuous protein network causes high hydration of the starch material, increasing the weight of pasta (Zardetto & Rosa, 2009).

3.2. Structural properties

Texture parameters are shown in Table 2. Texture of raw pasta was determined in terms of fracturability, defined as the necessary force to fracture the pasta strand. Although a high degree of mechanical strength of the raw product is desirable in spaghetti in order to minimize breakage during handling (Hollinger, 1963), the addition of microalgae resulted in a significant decrease of fracturability. This result suggested that the incorporation of spirulina made raw pasta weaker.

Texture of cooked pasta is generally recognized as its most important overall quality aspect. Protein content and quality are considered the most important of all the wheat grain components that affect cooking and texture quality. As gluten-protein content increases in wheat flour, cooked pasta becomes firmer and less sticky (Marchylo et al., 2004). However, the incorporation of proteins from other sources, such as spirulina biomass, could or could not improve cooking pasta behavior and pasta texture. Fradique et al. (2010) did not find any trend in firmness values of cooked pasta enriched with *S. maxima* biomass. In the present study, replacing wheat flour with spirulina resulted in an increase of firmness, cohesiveness and chewiness. Only the pasta sample elaborated with 20 g of spirulina / 100 g flour showed higher value of stickiness in comparison with control samples. The stickiness is related to the amount of amylose leached from the gelatinized starch granule and the enrichment of amylopectin on the pasta surface. As gluten was diluted by the substitution of wheat flour for spirulina biomass, the protein network became weaker, facilitating the leak of amylose into cooking water. Elasticity did not show any tendency, regardless of the different percentages of substitution.

The texture parameters of cooked pasta suggested that the structure was not largely affected by the incorporation of microalgae, being only modified in the presence of high biomass content.

The surface condition of pasta after being cooked is known to be related to the loss of matter into the cooking water and to the tendency to stickiness (Doxastakis et al., 2007). Cooked pasta was examined at the surface of the strand with a Confocal Scanning Laser Microscopy. Fig. 1 shows the structural changes of the surface of cooked pasta as microalgae biomass was increasing, which appears to be quite heterogeneous and less uniform when spirulina was incorporated into the pasta formulation. This is in agreement with the higher cooking loss and stickiness found in pasta enriched with high contents of spirulina.

3.3. Nutritional properties

3.3.1. Protein content and in vitro digestibility

Table 3 reports the values obtained for protein content in raw and cooked pasta, *in vitro* protein digestibility in cooked pasta and the proteins availability after digestion.

The incorporation of spirulina powder resulted in considerable improvements of the protein content in the product. No significant differences were found between raw and cooked pasta, indicating that proteins did not leach into cooking water. The enhancement of protein content in pasta results from the high concentration of these macromolecules in microalgae biomass. Unlike the majority of algae, spirulina as cyanobacteria has high digestibility due to the lack of cellulose in its cell wall. This facilitates its use for human consumption (Tomaselli, 2004). However, in this study, the percentage of *in vitro* protein digestibility of pasta enrichment with spirulina was reduced as a result of the increase in the level of the microalgae biomass. Little information is available about the *in vitro* digestibility of spirulina proteins. Previous studies suggested that *in vitro* digestibility of algal protein depends on the contents of antinutritional factors, which are either phenolic molecules or polysaccharides (Fleurence, 1999). Oxidized phenolic compounds may react with proteins and form insoluble complexes, inhibiting the activity of proteolytic enzymes and interfering with the utilization of proteins (Hurrell & Finot, 1985; Shahidi & Naczk, 1995). Horie, Sugase, and Horie (1995) showed the inhibitory action of soluble fiber – a group of polysaccharides – on *in vitro* pepsin activity and their negative effects on protein digestibility of brown algae.

As a consequence of low digestibility, the proteins digested increased slightly with the incorporation of microalgae in pasta formulation.

3.3.2. Phenolic compounds and antioxidant activity

Algae are an important source of numerous bioactive metabolites such as phenolic compounds, which are present in most algal groups. The most commonly phenolic component found in cyanobacteria are mycosporine-like amino acid (MAAs) and phenolic pigments. These molecules have been reported to possess antioxidant activity (Stengel, Connan, & Popper, 2011). Of the three extracts of spirulina obtained, only aqueous extracts exhibited high phenolic compound content and antioxidant activity (Table 4). Both parameters increased in pasta substituted with spirulina with the increase of microalgae content in the formulation. The total phenolic content of cooked pasta was slightly higher than the theoretical values calculated from the raw materials composition (0.43 mg GAE/g; 0.62 mg GAE/g; 1.01 mg GAE/g; S5, S10 and S20,

Table 2
Texture analysis.

Sample	Fracturability (N)*	Firmness (N)**	Stickiness ($x-1$, N)**	Cohesiveness**	Springiness**	Chewiness (N)**
CO	5.35 ± 0.40^{a}	38.3 ± 1.7^{a}	1.08 ± 0.01^{b}	0.74 ± 0.01^{a}	0.96 ± 0.01^{ab}	27.4 ± 1.6^{a}
S5	4.94 ± 0.46^{b}	47.8 ± 1.2^{b}	1.08 ± 0.04^{b}	0.74 ± 0.01^{a}	0.97 ± 0.01^{b}	33.4 ± 1.6^{b}
S10	4.57 ± 0.33^{b}	52.1 ± 2.8^{c}	1.24 ± 0.04^{ab}	0.80 ± 0.04^{b}	0.96 ± 0.01^{ab}	39.9 ± 0.3^{c}
S20	3.08 ± 0.18^{c}	56.6 ± 2.9^{d}	1.32 ± 0.08^{a}	0.80 ± 0.01^{b}	0.96 ± 0.01^{a}	42.9 ± 1.6^{d}

S5, S10, and S20: pasta prepared with 5, 10 and 20 g of spirulina / 100 g of flour, respectively.
Co: control sample.
*Was determined in raw pasta, repeated on six samples; **were determined in cooked pasta, repeated on five samples.
Within the same column, values with a different letter are significantly different ($P \leq 0.05$), according to Fisher test.

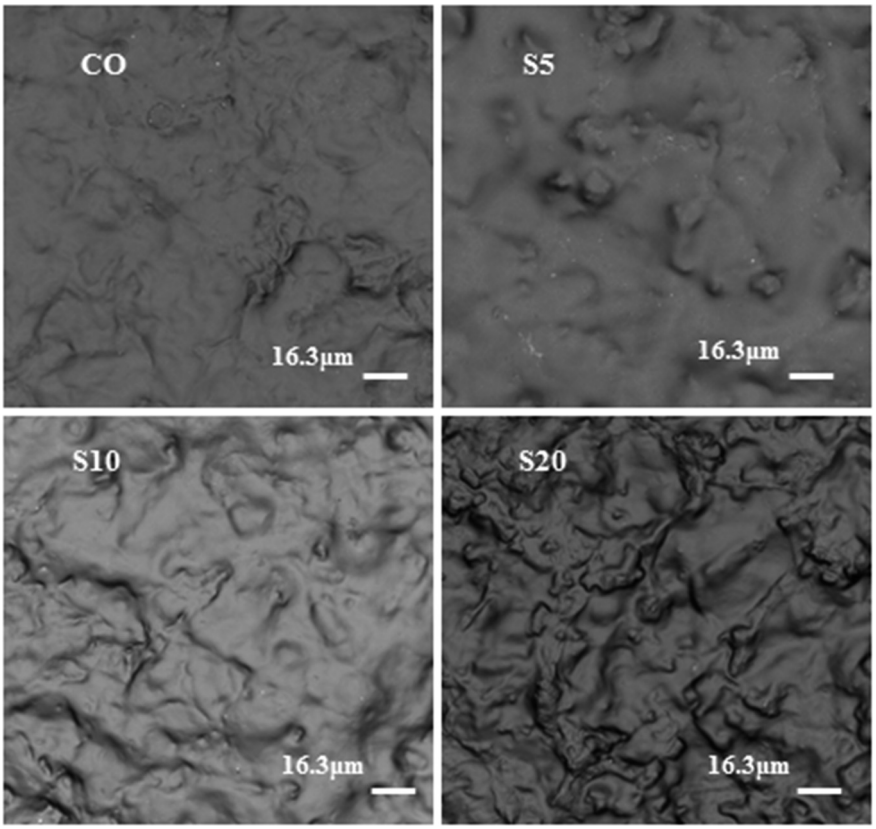

Fig. 1. Surface of cooked pasta by Confocal Laser Microscopy. CO: control sample. S5, S10, and S20: pasta prepared with 5, 10 and 20 g of spirulina / 100 g of flour, respectively.

Table 3
Protein content (PC), *in vitro* protein digestion (PD) and protein availability (PA).

Sample	PC in raw pasta (g/100 g dry pasta)	PC in cooked pasta (g/100 g dry pasta)	PD (%)	PA (g/100 g dry pasta)
CO	12.91 ± 0.03^{a}	13.09 ± 0.23^{a}	80.88 ± 0.36^{a}	10.30 ± 0.15^{a}
S5	15.43 ± 0.12^{b}	15.73 ± 0.22^{b}	71.04 ± 0.60^{b}	10.91 ± 0.06^{b}
S10	18.02 ± 0.13^{c}	18.46 ± 0.29^{c}	62.15 ± 0.58^{c}	11.19 ± 0.10^{c}
S20	23.49 ± 0.52^{d}	23.74 ± 0.18^{d}	55.45 ± 1.27^{d}	12.90 ± 0.01^{d}

S5, S10, and S20: pasta prepared with 5, 10 and 20 g of spirulina / 100 g of flour, respectively.
Co: control sample.
Results in the table represent the mean of triplicate measurements.
Within the same column, values with a different letter are significantly different ($P \leq 0.05$), according to Fisher test.

Table 4
Total phenolic content (TPC), trolox equivalent antioxidant capacity (TEAC) and ferric reducing ability of plasma (FRAP) in samples.

Sample	TPC (mg GAE/g)	FRAP (μMoles Trolox/100 g)	TEAC (μMoles Trolox/100 g)
Spirulina	4.08 ± 0.02^{e}	16.27 ± 0.12^{e}	25.45 ± 0.16^{e}
CO	0.24 ± 0.01^{a}	1.16 ± 0.06^{a}	0.59 ± 0.08^{a}
S5	0.55 ± 0.01^{b}	1.93 ± 0.10^{b}	1.79 ± 0.12^{b}
S10	0.76 ± 0.02^{c}	2.87 ± 0.04^{c}	5.04 ± 0.06^{c}
S20	1.05 ± 0.01^{d}	4.24 ± 0.27^{d}	8.42 ± 0.27^{d}

S5, S10, and S20: pasta prepared with 5, 10 and 20 g of spirulina / 100 g of flour, respectively.
Co: control sample.
Results in the table represent the mean of triplicate measurements.
Within the same column, values with a different letter are significantly different ($P \leq 0.05$), according to Fisher test.

respectively). This could be explained in terms of a concentration effect resulting from the material loss in water cooking. However, Kahn et al. (2013) found a significant decrease in total phenolic content in cooked sorghum enriched pasta compared to raw formulation, as observed by Prabhasankar et al. (2009) in seaweed enriched pasta. Both agreed that during cooking, phenolic compounds leach into the cooking medium and degrade due to thermal treatment. Possibly, in our case, the structure of pasta with spirulina was able to retain these compounds upon cooking.

These results indicate that it is possible to enhance the nutritional profile of pasta products with spirulina, since the biological activity is not lost during pasta elaboration and cooking process.

3.3.3. *Starch digestibility and glycemic index estimation*

Pasta has a low glycemic index due to its compact structure, in which protein network controls starch degradation and limits starch granules to the action of α-amylase enzyme (Fardet et al.,

Table 5
Total Starch (TS), Soluble Starch (SS) and Resistant Starch (RS) in pasta samples.

Sample	TS (g/100 g of dry pasta)	SS (g/100 g of dry pasta)	RS (g/100 g of dry pasta)
CO	72.01 ± 0.10^{d}	71.22 ± 0.08^{d}	0.79 ± 0.02^{c}
S5	68.11 ± 0.43^{c}	67.59 ± 0.43^{c}	0.52 ± 0.01^{b}
S10	65.11 ± 0.74^{b}	64.53 ± 0.79^{b}	0.59 ± 0.05^{b}
S20	57.70 ± 0.38^{a}	57.44 ± 0.37^{a}	0.26 ± 0.01^{a}

S5, S10, and S20: pasta prepared with 5, 10 and 20 g of spirulina / 100 g of flour, respectively.
Co: control sample.
Results in the table represent the mean of triplicate measurements.
Within the same column, values with a different letter are significantly different ($P \leq 0.05$), according to Fisher test.

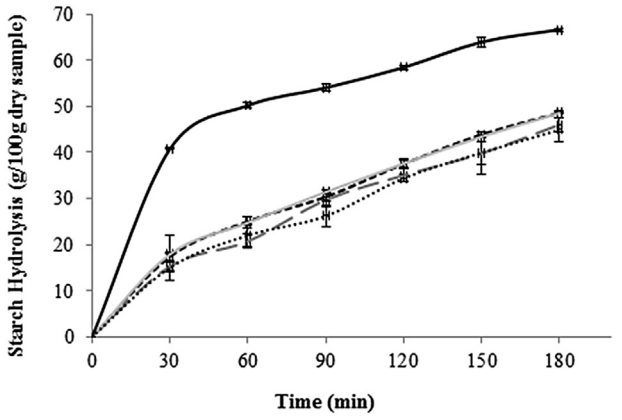

Fig. 2. Starch hydrolysis. CO: control sample (– –). S5 (— ·), S10 (——), and S20 (·····): pasta prepared with 5, 10 and 20 g of spirulina / 100 g of flour, respectively. Bread: Reference sample (——).

1999). The incorporation of functional ingredients in pasta formulation could affect the arrangement of components in pasta structure modifying the integrity of the protein network. A disruption of the protein starch matrix could become more accessible and susceptible to enzyme degradation, increasing starch digestibility and glycemic index. Also, the addition of proteins could limit the accessibility of α-amylase to starch because proteins encapsulate starch granules, reducing starch digestibility and glycemic index (Bustos et al., 2011; Colonna et al., 1990; Fardet et al., 1998). To evaluate the expected glycemic index, total starch, soluble starch and resistant starch were determined (Table 5). All fractions of starch decreased with the incorporation of microalgae in pasta formulation. Nonetheless, no significant differences were found between experimental and theoretical values calculated from the raw material formulations. These results were to be expected considering that the amount of wheat flour was lower, while the spirulina content increased. The starch hydrolysis of pasta samples is shown in Fig. 2. There was no significant difference in the hydrolyzed starch among the samples every time they were assayed.

Despite the fact that the structure of pasta was slightly affected by the incorporation of microalgae, it was possible to keep the glycemic index in the pasta with spirulina similar to the control and for all the samples the glycemic index estimated was considered to be low (CO 75^{b}; S5 72^{ab}; S10 75^{b}; S20 72^{a}), according to the classification proposed by Schakel, Schauer, Himes, Harnack, and Van Heel (2008).

4. Conclusions

Spirulina represents a source of important natural compounds for human nutrition. Even though their incorporation in pasta formulation did not enhance the content of digested protein as we expected due to the low digestibility found, the cyanobacteria has numerous bioactive compounds, such as phenolic compounds, that could enhance the nutritional profile of the product. Also, pasta with microalgae, at all the levels of incorporation, exhibited higher antioxidant activity than in the control sample. The structure of pasta was not largely affected by the addition of spirulina, and the changes made did not modify the estimated glycemic index. Although the texture profile did not show major differences from the control sample, additional studies are now necessary to evaluate consumer's acceptance.

References

AACC International. (2000). *Approved methods of analysis* (10th ed.). St. Paul, MN, USA: American Association of Cereal Chemists International.

Anitha, L., & Chandralekh, K. (2010). Effect of supplementation of spirulina in blood glucose, glycosylated hemoglobin and lipid profile of male noninsulin dependent diabetics. *Asian Journal Experimental Biological Science, 1*, 36–46.

AOAC International. (1998), 4th revision (16th ed.). *Offical methods of analysis of AOAC International* (Vol. II) (Maryland. USA).

Belay, A., Ota, Y., Miyakawa, K., & Shimamatsu, H. (1993). Current knowledge on potential health benefits of *Spirulina*. *Journal of Applied Phycology, 5*, 235–241.

Benzie, I., & Strain, J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Bruneel, C., Pareyt, B., Brijs, K., & Delcour, J. (2010). The impact of the protein network on the pasting and cooking properties of dry pasta products. *Food Chemistry, 120*, 371–378.

Bustos, M. C., Pérez, G. T., & León, A. E. (2011). Sensory and nutritional attributes of fiber enriched pasta. *Food Science and Technology, 44*, 1429–1434.

Chillo, S., Laverse, J., Falcone, P., & Del Nobile, M. (2008). Quality of spaghetti in base amaranthus whole meal flour added with quinoa, broad bean and chick pea. *Journal of Food Engineering, 85*, 101–107.

Colonna, P., Barry, J., Cloarec, D., Bornet, F., Gouilloud, S., & Galmiche, J. (1990). Enzymic susceptibility of starch from pasta. *Journal of Cereal Science, 11*, 59–70.

Dick, J., & Youngs, V. (1988). Evaluation of durum wheat, semolina and pasta in the United States. In G. Fabriani, & C. Lintas (Eds.), *Durum wheat chemistry and technology* (pp. 237–248). St. Paul, MN: American Association of Cereal Chemists.

Doxastakis, G., Papageorgiou, M., Mandalou, D., Irakli, M., Papalamprou, E., & D'Agostina, A. (2007). Technological properties and non-enzymatic browning of white lupin protein enriched spaghetti. *Food Chemistry, 101*, 57–64.

Fardet, A., Abecassis, J., Hoebler, C., Baldwin, P. M., Buléon, A., & Bérot, S. (1999). Influence of technological modifications of the protein network from pasta on in vitro starch degradation. *Journal of Cereal Science, 30*, 133–145.

Fardet, A., Hoebler, C., Baldwin, P., Bouchet, B., Gallant, D., & Barry, J. (1998). Involvement of the protein network in the in vitro degradation of starch from spaghetti and lasagna: a microscopic and enzymic study. *Journal of Cereal Science, 27*, 133–145.

Fleurence, J. (1999). Seaweeds proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science and Technology, 10*, 25–28.

Fradique, M., Batista, A., Nunes, M., Gouveia, L., Bandarra, N., & Raymundo, A. (2010). Incorporation of *Chlorella vulgaris* and *Spirulina maxima* biomass on pasta products. Part 1: preparation and evaluation. *Journal of Science Food Agriculture, 90*, 1656–1664.

Goñi, I., García-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*, 427–437.

Habib, M., Parvin, M., Huntington, T., & Hasan, M. (2008). *A review on culture, production, and use of Spirulina as food for humans and feeds for domestic animals and fish*. FAO Fisheries and Aquaculture Circular. No: 1034.

Hirahashi, T., Matsumoto, M., Hazeki, K., Saeki, Y., Ui, M., & Seya, T. (2002). Activation of the human innate immune system by *Spirulina*: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*. *International Immunopharmacology, 2*, 423–434.

Hollinger, A. (1963). Improved method for testing macaroni products. *Cereal Chemistry, 40*, 231–240.

Horie, Y., Sugase, K., & Horie, K. (1995). Physiological differences of soluble and insoluble dietary fubre fractions of brown algae and mushrooms in pepsin activity in vitro and protein digestibility. *Asian Pacific Journal of Clinical Nutrition, 4*, 251–255.

Hurrell, R., & Finot, P. (1985). Effect of food processing on protein digestibility and amino acids availability. In J. W. Finely, & D. T. Hopkins (Eds.), *Digestibility and amino acids availability in cereals and oilseeds* (pp. 233–246). St Paul, MN: American Association of Cereals Chemists.

Khan, I., Yousif, A., Johnson, S., & Gamlath, S. (2013). Effect of sorghum flour addition on resistant starch content, phenolic profile and antioxidant capacity of durum wheat pasta. *Food Research International, 54*, 578–586.

Li, H., Cheng, K., Wong, C., Fan, K., Chen, F., & Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry, 102*, 771–776.

Mao, T., Van de Water, J., & Gershwin, M. (2005). Effects of a *Spirulina*-based dietary supplement on cytokine production from allergic rhinitis patients. *Journal of Medicinal Food, 8*, 27–30.

Marchylo, B., Dexter, J., & Malcolmson, L. (2004). Improving the texture of pasta. In D. Kilcast (Ed.), *Solid foods: Vol. 2. Texture in food*. New York: Woodhead Publishing Ltd and CRC Press LLC.

Martínez, C., Ribotta, P., Añón, M. C.-, & León, A. E. (2013). Effect of amaranth flour (*Amaranthus mantegazzianus*) on the technological and sensory quality of bread wheat pasta. *Food Science and Technology International*. http://dx.doi.org/10.1177/1082013213476072.

Miranda, M., Cintra, R., Barros, S., & Mancini-Filho, J. (1998). Antioxidant activity of the microalga *Spirulina maxima*. *Brazilian Journal of Medical and Biological Research, 31*, 1075–1079.

Narmadha, T., Sivakami, V., Ravikumar, M., & Mukeshkumar, D. (2012). Effect of *Spirulina* on lipid profile of hyperlipidemics. *World Journal of Science and Technology, 2*, 19–22.

Pasini, G., Simonato, B., Giannattasio, M., Peruffo, A., & Curioni, A. (2001). Modifications of wheat flour proteins during in vitro digestion of bread dough, crumb and crust: an electrophoretic and immunological study. *Journal of Agricultural and Food Chemistry, 49*, 2254–2261.

Patel, A., Mishra, S., & Glosh, P. (2006). Antioxidant potential of C-phycocyanin isolated from cyanobacterial species *Lyngbya phormidium* and *Spirulina* sp. *Indian Journal of Biochemistry and Biophysics, 43*, 25–31.

Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Stephen, N., & Gowda, L. (2009). Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: chemical, functional and structural evaluation. *Food Chemistry, 115*, 501–508.

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

Ramamoorthy, A., & Premakumari, S. (1996). Effect of supplementation of *Spirulina* on hypercholesterolemic patients. *Journal of Food Science Technology, 33*, 124–128.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, A., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Schakel, S., Schauer, R., Himes, J., Harnack, L., & Van Heel, N. (2008). Development of a glycemic index database for dietary assessment. *Journal of Food Composition and Analysis, 21*, 50–55.

Shahidi, F., & Naczk, M. (1995). *Food phenolics: Sources, chemistry, effects and applications*. Switzerland, Basel: Technomic Publishing Company.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–153.

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

Stengel, D., Connan, S., & Popper, Z. (2011). Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. *Biotechnology Advances, 29*, 483–501.

Tomaselli, L. (2004). The microalgal cell. In A. Richmond (Ed.), *Handbook of microalgal culture: Biotechnology and applied phycology* (pp. 3–19). Blackwell Publishing Ltd.

Tudorica, C., Kuri, V., & Brennan, C. (2002). Nutritional and physicochemical characteristics of dietary fiber enriched pasta. *Journal of Agricultural and Food Chemistry, 50*, 347–356.

Zardetto, S., & Rosa, M. (2009). Effect of extrusion process on properties of cooked, fresh egg pasta. *Journal of Food Engineering, 92*, 70–77.