Use of Spirulina to Enhance the Nutritional Value of Durum Wheat Spaghetti

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Abstract

In this study the enrichment of durum wheat semolina spaghetti with spirulina flour has been studied to create a new product balanced from both nutritional and sensory quality point of view. To the aim of the work, Spirulina Flour (SF) was added to the dough at two different concentrations (5% and 10%). Then, to improve pasta quality in terms of cooking characteristics and sensory properties, the Transglutaminase (TG) addition has been tested. The SF addition to dough provoked a worsening of the overall quality of spaghetti. On the other hand, as Compared To Sample Control (CTRL), the samples with SF recorded an increase in proteins and dietary fibres contents. The fortified samples also showed a noticeable decline of the in vitro starch digestibility value that increased as the amount of SF increased. The novel pasta formulation offers a broad spectrum of new food products specific for people who take care to the nutritional quality of diet.

Keywords: Arthtospira; Spirulina Flour; Functional Pasta; Pasta Quality

Introduction

Pasta is a traditional food product with origins dating back to the first century BC [1] where it was a staple food in people’s diets in many countries known at that time. Pasta products are well accepted by consumers for their sensory attributes, low cost, ease of preparation and transportation. Pasta is mainly used as an energy source due to its complex carbohydrate content, being regarded as a product with low glycaemic index [2]. These nutritional advantages, along with the appeal of pasta amongst consumers, have made this food product a potential vehicle for nutraceuticals such as vitamins or polyunsaturated fatty acids [3]. In recent years, different healthy ingredients have been used in the production of pasta to enhance its nutritional profile or to confer functional properties.

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 [4]. Their well-balanced chemical composition (good quality proteins, balanced fatty acid profiles, vitamins, antioxidants and minerals), superior to that of traditional foods and their interesting health-promoting effects can be applied in the formulation of novel food products [5].

Spirulina (Arthospira sp.), a filamentous blue-green microalga or cyanobacterium, has a history of human consumption in Mexico and in Africa, and has recently been stated in the expeditions in Chad and Niger [6]. Spirulina for human nutrition is mainly marketed as nutritional supplements in many countries [7]. However, the increase in global population and the prediction of insufficient protein supplement, as well as the increase in malnutrition, have directed to the search of alternative sources of protein and important bioactive functional products. Arthospira platensis could be useful in this regard [5,7]. Besides, it is recognized as a safe microalga and authorized to be put on the market according to EU food law (EC regulation 258/97). The food potential of spirulina as source of complete proteins (60-70% w/w), unsaturated fatty acids, group B vitamins and various minerals, has been reported [8]. Apart from chlorophyll, spirulina contains
phycocyanin, which is a blue pigment known to have an interesting antioxidant power [8,9]. In fact, various studies demonstrated the role of spirulina in the prediction of pathologies as cancer, cardio-vascular disease and premature ageing [7,10,11]. As a result, there are many applications of spirulina in human food: instantaneous noodles for children [12], beverages [13] and tablets [14]. Recently, studies have also been carried out on pasta with macro and microalgae. Prabhasankar [15] 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 [16] incorporated Chlorella vulgaris and Arthrospira maxima in fresh pasta, resulting in products with enhanced chemical compositions, without affecting the cooking quality. Also, Özyurt [17] evaluated the effect of the addition of Spirulina platensis on the nutritional and cooking quality of fresh and dried semolina pasta. De Marco [18] evaluates the effect of different levels of spirulina biomass incorporation on technological and nutritional quality of dried bread pasta. The incorporation of spirulina resulted in an increase of protein content; however, protein digestibility was reduced as the microalgae content increased. Pasta with spirulina exhibited high phenolic compounds content and antioxidant activity compared to control pasta.

Besides the nutritional purpose, pasta quality may also be evaluated by its appearance, flavour and textural attributes. Partial substitution or addition of flour or functional ingredients could alter its textural and sensory profile. Among the compounds to improve textural properties, enzymes play a major role. Transglutaminase (TG, EC 2.3.2.13) catalyses an acyl-transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors) [19]. The covalent bond of ε-(γ-Glu)-Lys is formed when the ε-amino group of lysine residues acts as acyl acceptor and cross links with other proteins. The formation of homologous and heterologous polymers among different proteins (e.g. whey, soybean, rice, casein, a venin, etc.) results from the addition of transglutaminase. The enzyme increases the elasticity, the water-holding capability and other functional properties [20-22]. TG is widely utilized in the production of noodles and pasta in Japan [23].

Therefore, to the lack information about technological solution to improve pasta enriched with spirulina, the objective of this study was to prepare durum wheat spaghetti enriched with spirulina flour to balance the nutritional and sensory quality. To this aim, the study was organized in two steps: in the first one, Spirulina Flour (SF) was added to the dough at two different concentrations (5% and 10%). The second experimental step was aimed to improve with TG the quality of spaghetti enriched with spirulina flour at 10%, in terms of cooking characteristics and sensory properties.

Methods and Materials

Raw Material

The commercial semolina was purchased by Agostini mill (Montefiore dell’ Aso, Ascoli Piceno, Italy). The Arthrospira platensis flour (hereinafter called Spirulina flour-SF) was produced in closed photo bioreactors operated at the commercial plant of Archimede Ricerche Srl (Imperia, Italy). The Spirulina flour was analyzed for protein, carbohydrate, dietary fibre content (62.4%, 12.7% and 3.6% respectively). The plant is housed under a greenhouse, covers 1000 m2 and consists of four independent photo bioreactor modules each including ten 1-m high, 12.5-m long, 4-cm thick and 500 L in culture volume Green Wall Panel photo bioreactors of the first generation (GWP-I), positioned at a distance of 1.5 m from each other [24, 25]. The produced biomass was harvested by filtration using a vibrating screen equipped with a mesh sieve of 35 micron pore size and washed to remove culture medium residual salts, then freeze-dried, milled and stored at -20°C vacuum packed.

Spaghetti Preparation

Durum wheat semolina was mixed with water (30% w/w) in the rotary shaft mixer (Namad, Rome, Italy) at 25°C for 20 minutes to uniformly distribute water. In the first experimental phase, the spirulina flour was added to the wheat flour at two concentrations: 5% and 10% (w/w). Then, to the formulation 10% SF was added 0.6% Transglutaminase (TG) and mixed with water so as to obtain dough with 30% moisture content. The enzyme powder was previously dissolved in water in order to ensure its solubility. Spaghetti based only on durum wheat semolina were also manufactured and used as the reference sample (CTRL). In all the steps, dough was extruded with a 60VR extruder (Namad). Subsequently, the pasta was dried in a dryer (SG600; Namad). The process conditions were according to Padalino et al. [26].

Sensory analysis

Dry spaghetti samples were submitted to a panel of 15 trained tasters (seven men and eight women, aged between 28 and 45 years) in order to evaluate their sensory attributes. The panel lists were selected on the basis of their sensory skills (ability to accurately determine and communicate the sensory attributes as appearance, odour, flavour and texture of a product). The panelists were also trained in sensory vocabulary and identification of particular attributes by evaluating Durum wheat commercial spaghetti [27]. They were asked to indicate colour, homogeneity and resistance to breaking of dry uncooked spaghetti. Elasticity, firmness, bulkiness, adhesiveness, colour, odour and taste were evaluated on dry cooked spaghetti. To the aim, a nine-point scale, where 1
corresponded to “extremely unpleasant”, 9 to “extremely pleasant” and 5 to the threshold acceptability, was used to quantify each attribute. On the basis of the above-mentioned attributes, panellists were also asked to score the overall quality of both cooked and uncooked products using the same nine-point scale [26].

Chemical Determination

Dry spaghetti samples were ground to fine flour on a Tecator Cyclotec 1093 (International PBI, Hoganas, Sweden) laboratory mill (1-mm screen - 60 mesh). Protein content (% Nx5.7) was analyzed using the micro-Kjeldahl method according to AACC method 46-13 [28]. Total Dietary Fibre (TDF), Soluble Water Dietary Fibre (SDF) and Insoluble Water Dietary Fibre (IDF) contents were determined by means of the total dietary fibre kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on the method of Lee [29]. The Available Carbohydrates (ACH) was determined according to the method of McCleary [30] as described in the ACH assay kit (Megazyme). Three measurements for each spaghetti sample were performed.

In vitro digestion

The digestion was carried out as described by Padalino et al. [26]. Dry spaghetti samples (5 g) were cooked (5.0 x 1.0 cm lengths) in 50 mL of boiling water to the Optimal Cooking Time (OCT). The cooked spaghetti were tipped into a digestion vessel with 50 mL of distilled water and 5 mL maleate buffer (0.2 M pH 6.0, containing 0.15 g CaCl₂ and 0.1 g sodium azide per liter) and allowed to equilibrate in a block at 37°C (GFL 1092; GFL Gesellschaft für Labortechnik, Burgwedel, Germany) for 15 min. Digestion was started by adding 0.1 mL amyloglucosidase (A 7095; Sigma Aldrich, Milan, Italy) and 1 mL of 2 g per 100 g pancreatin (P7545; Sigma Aldrich) in quick succession and the vessels were stirred at 130 rpm. An amount (0.5 mL) of the digested samples was taken at 0, 20, 60 and 120 min for the released glucose analysis. The sample digested to 120 min was homogenized through an Ultra Turrax (Ika Werke, Staufen, Germany).

Analysis of digested starch

The digested samples were mixed with 2.0 mL of ethanol. After 1 h, the samples were centrifuged (2000 g, 2 min) (Biofuge fresco; Heraeus, Hanau, Germany). Finally, the reducing sugar concentration was measured calorimetrically (λ= 530 nm) using a Shimadzu UV-Vis spectrophotometer (model 1700; Shimadzu corporation, Kyoto, Japan). Glucose standards of 10 mg/mL were used. Amyloglucosidase (0.25 mL) (EAMGDF, 1 mL per 100 mL in sodium acetate buffer 0.1 M, pH 5.2; Megazyme International 205 Ireland Ltd., Wicklow, Ireland) was added to 0.05 mL of the supernatant and incubated at 20°C for 10 min. Afterwards, 0.75 mL DNS solution (10% 3,5-dinitrosalicylic acid, 16% NaOH and 30% Na-K tartrate – Sigma Aldrich) was added to the above solution, heated to 100°C for 15 min and then cooled at 15°C for 1 h. Subsequently, 4 mL of distilled water (15°C) were added to the solution. The results were plotted as glucose release (mg) per g of sample vs. time. The starch digestibility was calculated as the area under the curve (0-120 min) for the tested products, and expressed as the percentage of the corresponding area for white bread [26].

Cooking quality

The Optimal Cooking Time (OCT) was evaluated according to the AACC approved method 66-50. The cooking loss, i.e. the amount of solid substance lost into the cooking water, was determined according to the AACC approved method 66-50 [28]. The swelling index and the water absorption of cooked pasta (grams of water per gram of dry pasta) were determined according to the procedure described by Padalino et al. [26]. Moreover, cooked spaghetti samples were submitted to hardness (mean maximum force, N) and adhesiveness (mean negative area, Nmm) analysis by means of a Zwick/Roell model Z010 Texture Analyzer (Zwick Roell Italia S.r.l., Genova, Italia) equipped with a stainless steel cylinder probe (2 cm diameter), according to the procedure described by Padalino et al. [26]. Six measurements for each spaghetti sample were performed.

Statistical analysis

Experimental data were compared by one-way Analysis Of Variance (ANOVA). A Duncan’s multiple range test, with the option of homogeneous groups (P < 0.05), was carried out to determine significant differences between spaghetti samples. STATISTICA 7.1 for Windows (StatSoft, Inc, Tulsa, OK, USA) was used for this aim.

Results and Discussion

Sensory quality of pasta enriched with spirulina

Sensory data of dry spaghetti samples (uncooked and cooked) are presented in the (Table 1).
The results showed that the spirulina addition significantly influenced the overall quality of pasta. Results on the uncooked spaghetti showed that sample enriched with SF have the smallest overall quality due to a low break resistance as compared to the other investigated samples. Concerning the color, there were no statistically significant differences between samples. Pasta added with spirulina are visually very attractive, presenting pleasant bluish-green coloration [16]. Traditionally, semolina pastas exhibit light yellow coloration, derived from β-carotene. In the last few years, several colored pasta products have been produced using vegetable ingredients, such as spinach, tomato, carrot and beetroot. Achieving appealing and stable colorations is an important innovation for these types of product. Microalgae can be a sustainable alternative as colouring agents, resulting in products similar to vegetable-based pastas and present nutritional advantages over synthetic colourings. Table 1 shows that sample of cooked spaghetti containing 10% SF had the lowest overall quality score. The main discriminating attributes for the spaghetti quality were firmness, bulkiness and adhesiveness. Firmness score of pasta containing spirulina appeared to be significantly lower than that of the CTRL sample. Low adhesiveness and bulkiness scores were also observed in the sample 10% SF as compared to control sample. In fact, the stickiness is related to the amount of amylase leached from the gelatinized starch granule and the increase of amylase concentration on the pasta surface. Most probably, the presence of spirulina flour interfering with the gluten network formation promotes the formation of a weak network. In fact, gluten molecules could not be completely hydrated due to competition with spirulina flour. Moreover, spirulina flour could prevent gluten network formation due to steric hindrance interferes [18]. The incomplete starch and gluten hydration could be responsible for the increase of brittleness of spaghetti sample (lower firmness attribute). Whereas a weak gluten network could facilitate the diffusion of amylase toward the spaghetti surface. In fact, the stickiness is related to the amount of amylase leached from the gelatinized starch granule [31].

Regarding the color, the cooked spaghetti enriched with spirulina flour also showed a pleasant bluish-green colour without differences among the samples. The addition of spirulina did not affect pasta odor and taste.

### Chemical quality of pasta enriched with spirulina

The chemical composition of samples investigated in this work is shown in (Table 2).

| Sample | Protein (%) | IDF (%) | SDF (%) | TDF (%) | ACH (g/100g) | Starch Digestibility (%)
|--------|-------------|---------|---------|---------|-------------|-------------------
| CTRL   | 10.38±0.01a | 2.23±0.04a | 1.61±0.28a | 3.84±0.02b | 81±0.58a | 79±0.05a |
| 5%SF   | 12.96±0.08b | 1.31±0.12b | 2.98±0.00a | 4.29±0.12a | 78±0.20b | 68±0.01b |
| 10%SF  | 15.10±0.09b | 1.21±0.04b | 3.20±0.00a | 4.41±0.04a | 73±1.00a | 58±0.04a |

TDF, total dietary fibre; SDF, soluble water dietary fibre; IDF, insoluble water dietary fibre; ACH, available carbohydrates; SD, starch digestibility.

As expected, the incorporation of spirulina flour caused a noticeable increase in protein content compared to the CTRL sample. In fact, protein content increased from 10.38% for CTRL sample to 15.10% for 10% SF sample. This blue-green alga is typically characterized by high protein content (63 g/100 g of dry weight), which is one of the main reasons to consider it as an unconventional source of proteins [32]. Indeed, as can see from Table 2, the 10% SF sample recorded a higher protein content than that 5%SF sample. An increase in dietary fibres for samples supplemented with spirulina flour was also observed. It is plausible, that the addition of spirulina flour provides a great amount of functional compounds, such as dietary fibres [33].

lar, the soluble dietary fibre (SDF) content of the 10% SF sample was higher (3.20%) when compared to that of CTRL sample and 5% SF sample (1.61% and 2.98% respectively). Otherwise, IDF decreased adding spirulina flour. On the contrary, the samples enriched with spirulina flour had the lowest Available Carbohydrate (ACH) content (78 g per 100 g for 5%SF sample and 73 g per 100 g for 10%SF sample) when compared to the CTRL (81 g per 100 g) sample. These results could be due to the fact that the rise in dietary fibres caused a drop in carbohydrates responsible for the increase of the glycaemic response. In fact, the dietary fibres are actually defined as ‘edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine’ [34]. Table 2 also shows that the incorporation of spirulina flour influenced the in vitro starch digestibility (SD). In fact, the 5% and 10% samples have a lower starch digestibility value (68 and 58 respectively) as compared to the CTRL sample (79). It is possible that these results could be due to the high protein content in the samples enriched with spirulina flour. As a fact, the addition of proteins could limit the accessibility of γ-amylase to starch, because proteins encapsulate starch granules, reducing starch digestibility and glycemic index [35]. The glycemic index is an important indicator of starch digestibility that widely varies with the nature of the food [36-38].

Therefore, enrichment of semolina with nutritionally rich spirulina flour could enhance the nutritional quality of the product. To sum up, the results obtained in the first experimental step highlighted that spirulina flour at 10% is the best solution among those investigated as spirulina flour increased both protein and dietary fibres content and at the same time reduced the ACH and starch digestibility values, the latter correlated with the glycemic index. However, the same sample has the lowest sensory quality among the samples investigated in this work. In order to balance nutritional and sensory quality of pasta, a second experimental step was focused on the improvement of the sensory quality.

### Optimization of pasta enriched with spirulina flour (10%)

In order to improve the sensory quality of 10% SF sample, transglutaminase (TG) was also added to the dough. As can be seen from (Table 3),

| Uncooked Spaghetti | Cooked Spaghetti |
|-------------------|-----------------|
| Color             | Color           |
| Break to Resistance | Odor            |
| Overall Quality   | Taste           |
| Elasticity        | Overall Quality |
| Firmness          | Color           |
| Bulkiness         | Odor            |
| Adhesiveness      | Taste           |

The addition of TG caused a significant improvement of the overall quality of uncooked and cooked enriched spaghetti samples. Specifically, the uncooked sample 10% SF-TG showed higher break resistance score with respect to the spaghetti sample 10% SF. The cooked 10% SF-TG spaghetti sample exhibited significantly higher sensory scores for elasticity and firmness when compared to the sample without transglutaminase. These results are in agreement with Yeoh [39], who found that the TG treatment increased hardness and elasticity of wheat flour noodles by forming the covalent cross-linking among the gluten proteins. Most probably, TG cross-linking gave more strength to the gluten network, thus enhancing the tensile strength and elasticity of the cooked pasta, making it more resistant to cooking-induced changes in texture [40]. Besides, the sample 10% SF-TG showed a significant decline of both adhesiveness and bulkiness (higher adhesiveness and bulkiness attribute values) as compared to sample 10% SF. These results are consistent with data reported in the literature, where it was found that TG treatment of pasta prevented deterioration of texture after cooking and increased hardness and elasticity and decreased stickiness of cooked noodles [41]. Regarding the color, odor and taste, no significant differences among samples were observed. The effect of TG on cooking quality of spaghetti is given in (Table 4).

![Table 3: Sensory characteristics of uncooked and cooked dry spaghetti samples studied in the 2° step.](https://example.com/table3)

| OCT (min) | Cooking Loss (%) | Swelling Index (g water per g dry spaghetti) | Water Absorption (%) | Adhesiveness (Nmm) | Hardness (N) |
|-----------|------------------|---------------------------------------------|----------------------|-------------------|--------------|
| CTRL      | 10.30            | 4.89±0.16c                                  | 1.46±0.03c           | 125±0.16a         | 0.60±0.12a   | 6.70±0.32c   |
| 10%SF     | 6.00             | 6.11±0.40b                                  | 1.61±0.00b           | 132±0.61a         | 0.72±0.13a   | 7.96±0.23c   |
| 10%SF/TG  | 7.00             | 4.92±0.24b                                  | 1.45±0.07b           | 122±0.82b         | 0.58±0.11b   | 8.15±0.41b   |

**Table 4: Cooking quality of dry spaghetti studied in the 2° step**

* a-cMean in the same column followed by different superscript letters differ significantly (P < 0.05).
Optimal cooking time depends primarily on the rates of water penetration and starch gelatinisation. Pasta samples added with spirulina flour have an optimal cooking time lower than CTRL sample. As reported beforehand, the incorporation of spirulina might have weakened the overall pasta structure due to incomplete starch and gluten hydration, and steric hindrance interferes in network formation. Since the protein network limits the diffusion of water into the central zone of pasta during cooking [42], weaker gluten-protein network facilitates water diffusion into it, reducing the 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. In fact, the sample 10% SF showed the highest cooking loss value, whereas significantly lower cooking loss value was observed in the TG supplemented spaghetti sample. This can be explained by the formation of covalent cross-links catalyzed by TG, which strengthening the gluten network reducing the amounts of solids released during cooking. The sample 10% SF presented higher swelling index and water absorption values as compared to the other samples studied (Table 4). This result could be due to the ability of the microalgae to absorb water and retain it in the protein-starch net. The addition of the TG caused a significant decline on water absorption and swelling index values as compared to sample 10% SF. Most probably, this is due to the fact that TG cross-linking yields a stronger and tighter protein network between the starch granules, thus helping to limit excessive water uptake during cooking.

Cooking quality is also related to the ability of spaghetti to maintain textural properties during cooking. Mean values for adhesiveness and hardness show significant differences among the investigated samples (Table 4). The addition of spirulina resulted in a rise of adhesiveness and hardness values as compared with the CTRL sample. The addition of TG to the sample 10% SF resulted into a significant reduction of adhesiveness value, and into a non statistically significant variation of hardness. In fact, the adhesiveness value changed from 0.72 Nmm for the sample 10% SF to 0.58 Nmm for sample 10% SF-TG. These results are consistent with those obtained by the sensory analysis.

**Conclusion**

In this work, the impact of spirulina flour addition on chemical composition, cooking and sensory quality of durum wheat spaghetti was evaluated by replacing 5 and 10% of durum wheat semolina with spirulina flour. The incorporation of spirulina increased the protein and the dietary fibres contents in spaghetti samples as compared to CTRL sample. The samples enriched with SF showed a noticeable decline in the starch digestibility value, with the increase in SF amount. Specifically, the 10% SF sample showed a lower in vitro starch digestibility (58) compared to the CTRL sample (79). Regarding the sensory characteristics, the addition of spirulina determined a decline of pasta overall quality. The main discriminating attributes for the spaghetti quality were firmness, bulkiness and adhesiveness. In the second experimental step, the use of TG improved the pasta quality, in terms of cooking characteristics and sensory properties. Specifically, the 10% SF-TG exhibited significantly higher sensory scores for elasticity and firmness when compared to the sample without TG. Moreover, TG treatment of spaghetti prevented deterioration of texture after cooking and increased hardness and decreased adhesiveness values, respect to the sample without TG.

To sum up, the partial substitution of durum wheat semolina with spirulina resulted an interesting way to increase the nutritional composition of pasta (protein and fiber contents). The novel formulation offers a broader spectrum of products for people wishing to improve the nutritional quality of diet and/or to benefit specific nutritional target such as elderly, athletes and vegans. In less prosperous countries, the exceptional protein content of spirulina could provide a balanced diet and reduce malnutrition, especially in children. One of the main critical points that still hamper a larger use of microalgae as functional ingredients of traditional foods is the high algal biomass production cost (not less than 64-5 /kg). To make microalgal biomass economically competitive and sustainable, the cost of the culture system, as well as the operational costs, must be significantly reduced. Although most of the microalgae commercial production is currently conducted in open ponds, the use of more controlled systems that allow to comply with good manufacturing practices and to ensure safety and good nutritional quality is recommended for applications in the food sector.

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