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Effect of *Spirulina platensis* Biomass with High Polysaccharides Content on Quality Attributes of Common Carp (*Cyprinus carpio*) and Common Barbel (*Barbus barbus*) Fish Burgers

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Abstract: Lately, microalgae have been used as natural additives in fish-transformed products to improve their nutritional quality. In this research, the effects of adding *Spirulina platensis* at concentrations of 0.5, 1 and 1.5% *w/v* on both the texture and the sensory characteristics of canned burgers were studied. In fact, the addition of *Spirulina platensis* to fish burgers improves their nutritional composition. Compared to the results of the other fish burger treatments, the treatments that contain 1% of *Spirulina platensis* had better texture and sensory properties ($p < 0.05$). Besides, these treatments showed higher swelling ability as well as water and oil holding capacities, due to the important dietary fibers and polysaccharides contents found in *Spirulina platensis*. No mold or foodborne pathogens were detected in any of the canned burgers up to 8 months of storage at 4 °C. Furthermore, burgers prepared with *Spirulina* were distinguished by the lowest mean (a^* and b^*) values ($p < 0.05$), which shows that the yellow color gradually diminished towards a greenish color. Because of the presence of polysaccharides and pigments (chlorophylls, carotenoids and phycocyanin), *Spirulina platensis* considerably ameliorates the antioxidant activities of the newly prepared fish burgers. On the whole, we can conclude that *Spirulina platensis* can be used as a nutritious additive to produce new fish-based products with high alimentary qualities.

Keywords: Canned fish burgers; *Spirulina platensis*; texture; polysaccharides; carotenoids; antioxidant activities

1. Introduction

Compared to many other food types such as meat, poultry and dairy products, fish is highly nutritious, delicious and easily digested. It has an excellent nutritional value. It provides high quality proteins and a wide variety of vitamins such as vitamins A and D and minerals including phosphorus, magnesium, selenium and iodine [1]. Not only its protein is easily digestible but also it favorably complements the dietary protein provided by cereals and legumes that are typically consumed in many developing countries [2]. Currently, the consumption of fish-based products is gradually rising

due to increasing consumer concerns about health problems. In 2013, fish accounted for 6.7% of all protein consumed and for 17% of the equivalent global figure [2]. The overexploitation of most valuable fish stocks has contributed to an imbalance in supply and demand of fishery products in several countries [3]. As a result, the idea of exploiting freshwater fish species (Nile tilapia, common carp and common barbel) in intensive farming has appeared.

The common carp (*Cyprinus carpio*) and the common barbel (*Barbus barbus*), are two species of freshwater fish that belong to the family of *Cyprinidae* [4,5]. Their flesh is characterized by a beneficial nutritional composition of indispensable amino acids, high omega-3 and -6 polyunsaturated fatty acids contents, certain minerals, fat and water-soluble vitamins [6] and low levels of cholesterol and saturated fats [1]. The presence of many intramuscular bones in their flesh is the cause of their low consumption by Tunisians. Hence, it is very important to produce new fish-based products from these underused fish species in the field of fish processing through various transformation methods to make them useful and savory aliments for a human diet. In recent years, the consumer preferences have been considerably directed towards the consumption of ready-to-eat fish products, since there has been a rapid process of urbanization and a rapid increase in the working population in most countries. One of the most popular mince-based products is fish burger. It is highly consumed, principally due to the increase in the number of fast foods available worldwide, as well as its convenience and low price [7]. To the best of our knowledge, in the literature there aren't many studies concerning fish-based products and very little information about the potential use of flesh from common carp and common barbel in manufacturing fish burgers.

Despite their health benefits, fish burgers are not generally considered as a major source of bioactive compounds (e.g., polysaccharides, carotenoids, essential oils, antioxidants and flavors) [1]. During recent years, nutritionists have mentioned that the fortification of fish-based food products using functional ingredients and supplements is an optimal way to ameliorate the total food intake. Meanwhile, the fish burger has continued to draw the attention of consumers because it's both healthy and tasty. In the agri-food industries, divers' synthetic additives have been used for the sake of coloring, flavoring, fortifying and extending the shelf-life of the commercialized products [7]. Still, many recent scientific studies have shown that an excessive consumption of synthetic food supplements is linked to many health problems [8]. Thus, the need to use natural food fortifiers has pushed experts to prepare additives from natural resources that might be suitable in the production of fish-products [7].

Recently, microalgae have attracted attention in the food industry and the principal genera used for functional foods are *Chlorella*, *Dunaliella* and *Spirulina*. *Spirulina platensis* is a cyanobacterium with a special composition of nutritional and bioactive substances (e.g., proteins, vitamins, minerals, pigments and phenolic acids) and with a large range of medical applications [9]. This cyanobacterium contains a very potent naturally occurring antioxidant and free radical scavenging agents such as pigments (phycocyanin and Beta-carotene) [10] and polysaccharides [11], that are well-known products to protect against various diseases, such as renal failure [12], hypertension [13] and cancers [11,14]. Besides their antioxidant effects, pigments and polysaccharides of *Spirulina platensis* have been found to show antibacterial and antiviral activities [15,16].

The principal objectives of this study are first to produce new canned fish burgers prepared from common carp and common barbel fortified with *Spirulina*, and second to assess the effects of *Spirulina* on the sensorial, microbiological, functional and textural characteristics of these products.

2. Material and Methods

2.1. Collection of Fish

Fish (common carp and common barbel) were caught from the reservoir of Sidi Salem in January 2017. Then, they were directly transferred in polyethylene bags to the pilot unit of fish processing of Tabarka (Governorate of Jendouba, Tunisia), where they were tested for their freshness and processed

(washed, beheaded, gutted and fileted). Next, the skinless fish filets were minced in a blender (Robot Coupe USA Inc., Ridgeland, MS, USA) and kept at $-20\text{ }^{\circ}\text{C}$ for 48 h.

2.2. Fish Burger Manufacture

Fish burgers were manufactured using a commercial burger maker (Model Hamburger MV NEW, Food Tech S.r.l.,-Bologna, Italy), after being thawed and chopped to a diameter of about 10 mm, mixed thoroughly with salt (2% *w/v*), starch (1% *w/v*), and different concentrations (0.5, 1 and 1.5%) of *Spirulina* powder (Bio Algues, Mahdia, Tunisia) and transformed into disc burgers that were 6 cm wide, 1.5 cm thick and that weighed 100 g. The obtained burgers were soaked in sunflower oil (20 mL) and individually placed in RO-100 cans (6.52 cm diameter, 3 cm height) using a seamer machine (Seamer Semiautomatica, MOD.AGM, S. Bologna, Italy). The canned burgers were then cooked at $120\text{ }^{\circ}\text{C}$ for 40 min using a retort. After cooling, cans were stored at $4\text{ }^{\circ}\text{C}$ in a refrigerator for further analysis (8 months). *Spirulina*-free burgers were used as controls.

2.3. Physicochemical Properties of Fish Burger

A pH meter (Metrohm-744 pH meter, Heirisau, Switzerland) was calibrated with standard buffer solutions and used to measure the pH of *Spirulina*-fortified and unfortified fish burgers. Their water activity (a_w) was determined using a Novasina Thermoconstanter SPRINT TH500 (Axair Ltd., Pfäffikon, Switzerland) at $25\text{ }^{\circ}\text{C}$. The equipment was previously calibrated using the calibration procedure of the equipment manufacturer, using the salts: MgCl_2 , NaCl , BaCl_2 and $\text{K}_2\text{Cr}_2\text{O}_7$ (Sigma-Aldrich, St. Louis, MO, USA). All measurements were carried out in triplicate.

The infra-red absorption spectra of all burger formulations were obtained using the Cary 630 FTIR spectrometer (Agilent Technologies., Santa Clara, CA, USA) as described by Ben Atitallah et al. [17]. The acquisition and the processing of spectra were carried out using the Agilent MicroLab PC software v.5.3 (Agilent Technologies Inc.).

Total solids (TS) and Ash contents were determined as described by Ben Atitallah et al. [17]. The content of protein was estimated using the classical Kjeldhal procedure of the AOAC International (method 981.10) with a nitrogen conversion factor of 6.25 [18]. Total lipids were extracted using the modified procedure of Bligh and Dyer [19] and measured as described by Fendri et al. [20]. The determination of total dietary fibers in burger samples was done according to the method described by Barkallah et al. [21]. Contents of chlorophylls and carotenoids were estimated as described by Lichtenthaler and Wellburn [22] and Kumar et al. [23]. The determination of c-phycocyanin was done according to the protocol described by Bennet and Bogorad [24].

2.4. Functional Properties of Fish Burger

The swelling capacity (SWC) of powdered fish burgers was determined as described by Kuniak and Marchessault [25]. The SWC was expressed as mL of swollen sample/g dry weight (mL/g dw). All measurements were carried out in duplicates.

The water holding capacity (WHC) of powdered fish burgers was determined as described by Okezie and Bello [26]. The sample was weighed immediately and the WHC of burger samples was expressed as g water/g dw. All analyses were done in duplicates.

The oil holding capacity (OHC) of powdered burgers was determined as described by Wong and Cheung [27]. The OHC of burger samples was expressed as g of oil held/g of sample (g/g dw).

2.5. Sensory Evaluation

The attributes of fish burgers were evaluated by 20 female and 10 male panelists aged from 20 to 45 years. The tasting panel included researchers and employees in the pilot unit of fish processing of Tabarka. Burger samples were distributed on white polystyrene plates and shown to the panelists with three digit codes in a random order. Experiments were done in a sensory evaluation room equipped with white light and controlled ventilation; water was served for the purpose of cleaning the mouth

between samples. Panel members evaluated the burgers for taste, body and texture, color, appearance and odor based on a traditional 5 point-hedonic scale (1 = very bad, 2 = bad, 3 = neither bad nor good, 4 = good and 5 = very good) for each parameter [17]. A score of 4 will be considered the threshold for acceptance of the fish burger.

2.6. Color Analysis

The color evaluation of unfortified and *Spirulina*-fortified fish burgers was done using a spectro-colorimeter (Konica Minolta, Chroma Meter, CR400, Japan), calibrated with white and black ceramic plates. An average color value was measured by taking measurements from different points (Two on the top and three on the bottom) of the same sample, and the L*a*b* color space values were noted [17].

2.7. Textural Analysis

All instrumental texture analyses were carried out on fish burger formulations stored at 4 °C for 24 h, using a professional texture analyzer with blade probe (Texture Analyser, TA Plus, LLOYD instruments, England). Texture parameters were determined and interpreted as described by Bourne [28].

2.8. Antioxidant Properties Evaluation Using DPPH and FRAP Methods

The free radical scavenging activity of fish burger samples was estimated using the DPPH (1,1-diphenyl-2-picryl-hydrazil) method of Bersuder et al. [29]. The ferric reducing antioxidant power (FRAP) of fish burgers was determined as described by Yildirim et al. [30]. All antioxidant reactions were done in triplicates.

2.9. Microbiological Analyses

The microbiological analyses of fish burgers were done throughout the storage period (8 months). The tests for *Escherichia coli*, Enterobacteriaceae, mold, yeast, coliforms and foodborne pathogens (*Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*) were done using the standard microbiological methods for the analysis of ready-to-eat foods [31,32]. After enrichment, burger samples were plated onto polymyxin—acriflavine—lithium chloride—ceftazidime—aesculin—mannitol (PALCAM) agar (Conda, Madrid, Spain), Baird Parker agar (Oxoid Ltd., Basingstoke, UK) and Salmonella-Shigella (SS) agar (Conda) for the detection of *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella-Shigella*, respectively. Three presumptive colonies from each plate were selected for biochemical characterization [30]. Biochemical tests used to confirm *Staphylococcus aureus* were the coagulase test, catalase test, indole production, methyl red test, Voges-Proskauer reaction, urease production, citrate utilization and sugar fermentation. *Listeria monocytogenes*, *Salmonella* and *Shigella* colonies were biochemically characterized using API *Listeria* and API 20E test kits (BioMerieux Inc., Lyon, France), respectively. The burgers were also tested for the presence of coliforms and *Escherichia coli* using the most probable number (MPN) method [31]. Mold and yeasts were detected and enumerated by diluting 1 g of burger sample in 9 mL of 0.1% peptone water (Conda), followed by two min of homogenization at 2000 rpm using the hand-held homogenizer. Subsequently, serial dilutions were made using 0.1% peptone water and then samples were plated, using potato dextrose agar (Conda) acidified to pH 3.5 with 10% tartaric acid solution. All plates were then incubated at 25 °C and colonies were counted after 3 days. The results were expressed as CFU/g [17].

2.10. Statistical Analysis

Analytical determinations were done at least in duplicates and obtained values were expressed as the mean and standard deviation (SD). Comparison of means of values among different treatments was

effectuated using a One-way ANOVA test followed by using post hoc Duncan's Multiple Range Tests (DMRT) (p values < 0.05 were considered to be statistically significant). IBM SPSS software package version 19.0 (IBM Corp., USA) was used for this purpose.

3. Results and Discussion

3.1. Preliminary Results

The taste, after-taste, odor, color, texture and overall acceptability of the canned fish burgers were evaluated. Results are shown in Table 1. In general, the hedonic results showed better scores for carp and barbel burgers enriched with 0.5 and 1% (w/v) of *Spirulina*. Statistically, formulations with the highest *Spirulina* concentration (1.5%) possessed inferior sensory acceptability for the majority of the organoleptic characteristics compared to formulations with the lower *Spirulina* concentrations ($p < 0.05$) and controls ($p < 0.05$) (Table 1). The inappropriate taste caused by the supplementation of high concentration of *Spirulina* is related to the obtained compounds from the lipid oxidation and to the minerals that not only operate as pro-oxidant molecules but also might produce undesirable metallic off-flavors [33]. Yet, taste scores of carp and barbel burgers formulated with 0.5 and 1% of *Spirulina* were similar to control fish burgers ($p > 0.05$) (Table 1). The microalga addition modified the color of carp and barbel burgers from yellowish white to green, depending on the added concentration of microalga. This characteristic was considered by panelists as an inappropriate sensory feature (color appearance) ($p < 0.05$). The decrease in color pleasantness might be explained by the fact that batches rich in *Spirulina* showed an increase in the green color as the amount of chlorophylls increased, which results in a color very different from that expected in a control fish burger. In the case of mouth-texture, some panel members declared that the burger texture was ameliorated by increasing the percentage of *Spirulina*; however, these differences were significant ($p < 0.05$) compared to controls only for carp formulations (Table 1).

Table 1. Mean scores of tasting panelists ($n = 30$) for sensory properties of control and fortified burgers with different percentage (0.5, 1 and 1.5%) of *Spirulina*.

Sensory Properties	Fish Burger Types	Formulations			
		Control burger	0.5% <i>Spirulina</i> fish burger	1% <i>Spirulina</i> fish burger	1.5% <i>Spirulina</i> fish burger
Taste	CB	4.42 ± 0.05	4.48 ± 0.145	4.26 ± 0.179	3.98 ± 0.034 ^{b f g}
	BB	4.12 ± 0.53	4.03 ± 0.42	3.98 ± 0.296	3.02 ± 0.203 ^{c e h}
After-taste	CB	4.35 ± 0.296	4.13 ± 0.014	4.11 ± 0.015	3.99 ± 0.020 ^a
	BB	4.08 ± 0.251	4.10 ± 0.428	4.07 ± 0.381	3.39 ± 0.307 ^{a d g}
Odor	CB	3.99 ± 0.021	4.03 ± 0.024	4.04 ± 0.050	4.02 ± 0.017
	BB	4.01 ± 0.283	4.00 ± 0.261	4.01 ± 0.312	4.05 ± 0.311
Color	CB	4.26 ± 0.08	4.29 ± 0.06	4.15 ± 0.07	3.91 ± 0.08 ^{c f h}
	BB	4.01 ± 0.432	4.02 ± 0.419	4.02 ± 0.521	3.88 ± 0.489
Mouth-texture	CB	4.03 ± 0.090	4.09 ± 0.060	4.24 ± 0.050 ^{b d}	4.17 ± 0.022 ^a
	BB	3.98 ± 0.123	4.09 ± 0.327	4.02 ± 0.222	4.01 ± 0.284
Overall-acceptability	CB	4.12 ± 0.032	4.12 ± 0.020	4.13 ± 0.034	4.03 ± 0.023 ^{b e h}
	BB	4.12 ± 0.156	4.11 ± 0.238	4.08 ± 0.145	3.80 ± 0.05 ^{a d g}

CB: carp burgers; BB: barbel burgers; Control vs. fortified burgers (0.5%, 1% and 1.5%): ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$; 0.5% *Spirulina* burgers vs. 1 and 1.5% *Spirulina* burgers: ^d $p < 0.05$; ^e $p < 0.01$; ^f $p < 0.001$; 1% *Spirulina* burgers vs. 1.5% *Spirulina* burgers: ^g $p < 0.05$; ^h $p < 0.01$.

Overall, treatments with 1.5% of *Spirulina* minimized the sensorial quality of fish burgers, especially from the point of view of taste and after taste. According to these results, it seems that 0.5% *Spirulina*

concentration is the threshold level which has no significant effects on the sensory quality of the fish products; however, panel members were still satisfied with fish burgers containing 1% added *Spirulina*. Thus, carp and barbel burgers fortified with 0.5 and 1% of *Spirulina* were chosen for further analyses to assess their physicochemical properties.

The *Spirulina* incorporation into fish burger composition had no significant effect on pH values (Table 2). Furthermore, the addition of *Spirulina* (for any concentration) did not modify ($p > 0.05$) the protein and fat contents (Table 2) for the two types of burgers. These contents were confirmed qualitatively with FTIR analysis (Figure 1A,B). The strong absorption in the region of 3200 cm^{-1} corresponds to the hydroxyl (O-H) stretching vibration of proteins and residual water. The strong absorption in the region of 3000 and 2800 cm^{-1} corresponds to the stretching of NH_2 groups, which reflects the high protein content in burger samples. The protein spectrum was characterized by the presence of absorption peaks at 1624 and 1522 cm^{-1} attributed to the NH and C=O groups of amides I ($1590\text{--}1650\text{ cm}^{-1}$) and the stretching of NH groups and asymmetric N=O groups of amide II ($1500\text{--}1560\text{ cm}^{-1}$) [34]. The lipid spectrum was characterized by the presence of absorption peaks at 2922 , 2852 and 1742 cm^{-1} attributed to the stretching of $\text{Vas}(\text{CH}_2)$, $\text{Vs}(\text{CH}_2)$ and $\text{V}(\text{C}=\text{O})$, respectively, while the absorption peak at 1377 cm^{-1} mainly showed the C-O stretching vibrations of carboxylic acids [34]. The absorption peaks found between 950 and 1200 cm^{-1} represent carbohydrates [17]. Compared to control, *Spirulina*-fortified samples did not show a significant appearance of specific absorption peaks for the two types of fish burger (Figure 1A,B).

Table 2. Mean values for physicochemical characteristics of control and *Spirulina*-fortified burgers at one week after production.

Physicochemical Characteristics	Fish Burger Types	Burger Formulations		
		Control burger	0.5% <i>Spirulina</i> fish burger	1% <i>Spirulina</i> fish burger
pH	CB	7.21 ± 0.07	7.16 ± 0.01	7.17 ± 0.01
	BB	7.14 ± 0.02	7.1 ± 0.015	7.07 ± 0.025
Water activity (a_w)	CB	0.982 ± 0.000	0.979 ± 0.000 ^a	0.978 ± 0.000 ^c
	BB	0.982 ± 0.001	0.974 ± 0.000 ^c	0.969 ± 0.001 ^{cf}
Moisture (% FW)	CB	77.03 ± 0.055	76.55 ± 0.053	76.36 ± 0.078
	BB	77.57 ± 0.32	77.17 ± 0.072 ^a	77.00 ± 0.046 ^b
Total solids (% FW)	CB	22.96 ± 0.055	23.44 ± 0.053 ^c	23.63 ± 0.078 ^{ce}
	BB	22.43 ± 0.32	22.82 ± 0.072	23 ± 0.046 ^a
Protein (% DW)	CB	79.66 ± 0.119	79.50 ± 0.019	79.20 ± 0.096
	BB	78.24 ± 0.125	78.21 ± 0.070	78.02 ± 0.012 ^a
Fat (% DW)	CB	7.79 ± 0.15	7.67 ± 0.04	7.46 ± 0.068
	BB	8.33 ± 0.085	8.25 ± 0.095	8.24 ± 0.010
Ash (% DW)	CB	11.12 ± 0.105	11.18 ± 0.013	11.69 ± 0.046 ^{cf}
	BB	11.53 ± 0.03	11.56 ± 0.315	11.84 ± 0.155 ^{cf}
Total dietary fiber (g/100 g DW) *	CB	0.32 ± 0.024	0.5 ± 0.075 ^c	0.76 ± 0.024 ^{cf}
	BB	0.203 ± 0.000	0.308 ± 0.016 ^a	0.817 ± 0.158 ^{cf}

CB: carp burger; BB: barbel burger; Control vs. fortified burgers (0.5%, 1% and 1.5%): ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$; 0.5% *Spirulina* burgers vs. 1 and 1.5% *Spirulina* burgers: ^d $p < 0.05$; ^e $p < 0.01$; ^f $p < 0.001$; The percentage is calculated relative to a dry weight basis (DW) for lipid, protein, fat and ash whereas the moisture content and total solids are expressed with respect to a fresh weight basis (FW).

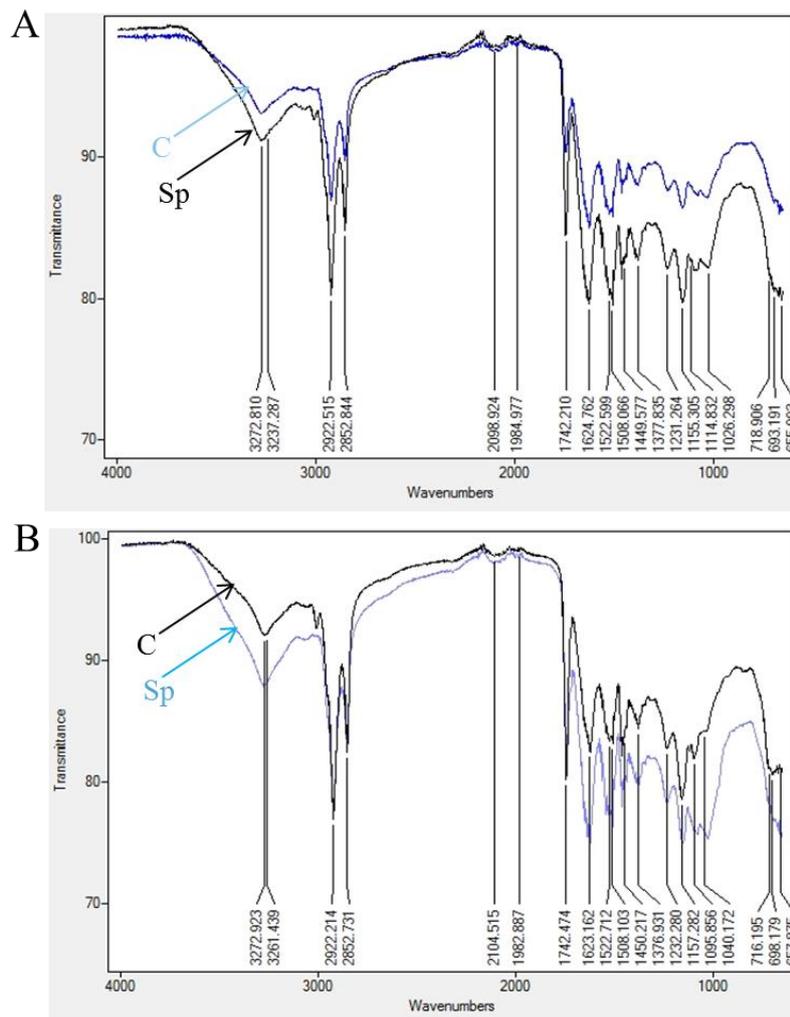


Figure 1. FTIR spectra of control (c) and *Spirulina*-fortified burgers made from barbel (A) and carp (B).

Nevertheless, total solids (TS), total dietary fibers and ash contents were found to be the highest in carp and barbel burgers fortified with *Spirulina* ($p < 0.05$) (Table 2). For carp formulations, TS of the control and fortified burgers with 0.5 and 1% of *Spirulina* were 22.96, 23.44 and 23.63%, respectively. There was a significant difference in terms of TS between these 3 formulations ($p < 0.001$) (Table 2). This increase was not significant between barbel formulations. Similarly, the dietary fiber values of all *Spirulina*-fortified burgers were significantly ($p < 0.01$) increased when compared with that of control burgers. This was probably due to the high dietary fiber content of *Spirulina*. For the two types of fish burger, samples treated with 0.5% of *Spirulina* significantly showed ($p < 0.001$) lower values for dietary fibers in comparison to samples treated with 1% of *Spirulina*. The high dietary fiber content of fortified burgers might have played an important role in a reduced level of water activity (a_w) compared with the control burgers [35]. In addition, *Spirulina* increased the Ash content of carp and barbel burgers depending on microalga concentration (Table 2). These findings are consistent with previous results that indicated the richness of *Spirulina* in minerals [21]. Variations in these physicochemical parameters, especially regarding the contents of dietary fibers, may affect certain other functional parameters such as water and oil holding capacities. According to the obtained results, carp and barbel burgers supplemented with 1% of *Spirulina* were chosen as the best final products and selected for further analyses to evaluate their textural, functional, microbiological and antioxidant properties.

3.2. Textural Parameters

Actually, textural amendment is one of the most commonly used modes in the evaluation and treatment of swallowing difficulties (dysphagia). It is an essential criterion used to define the organoleptic quality of food products. Table 3 shows the effect of adding 1% of *Spirulina* on the textural properties of fish burgers.

Table 3. Parameters obtained using texture analyzer for control and fortified burgers with different concentrations of *Spirulina*.

Textural Parameters	Fish Burger Types	Burger Formulations	
		Control burger	1% <i>Spirulina</i> fish burger
Hardness (N)	CB	8.35	13.53 ^c
	BB	6.69	7.39 ^a
Elasticity (cm)	CB	0.36	0.38
	BB	0.56	0.85 ^c
Chewiness (N.cm)	CB	1.11	2.21 ^c
	BB	0.88	2.40 ^c
Gumminess (N)	CB	3.09	5.84 ^c
	BB	1.58	2.83 ^c
Cohesion	CB	0.371	0.432 ^a
	BB	0.239	0.383 ^c

CB: carp burgers; BB: barbel burgers; Control vs. 1% *Spirulina*-fortified burgers: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$.

Compared to control burgers made from carp and barbel, the addition of 1% *Spirulina* led to an increase in hardness from 8.35 to 13.53 N ($p < 0.001$) and from 6.69 to 7.39 N ($p < 0.05$), respectively. This could presumably be attributed to the composition differences between unfortified and *Spirulina*-fortified burgers, resulting in different protein/fat/water ratios, which are determining elements in the resulting gel consistency [34]. These results are in accordance with reports by Cofrades et al. [36] and Fernandez-Martin et al. [37] in burger patties with added seaweeds. However, Dolea et al. [38] suggested that the hardness was not modified by the addition of thyme and oregano essential oils in salmon and seaweed burgers. Furthermore, fibers and polysaccharides of *Spirulina* can also play an important role in modifying the hardness of these processed products.

Depending on the amount and type of fibers, controversial results have been reported on the hardness of food products. Thus, both hardening and softening have been observed when fibers were added to various cooked food products [39,40]. Chewiness was increased significantly ($p < 0.001$) when the microalga was added (Table 3). These results are in good agreement with the experimental data obtained by Jiménez Muñoz et al. [41]. Cohesiveness values increased when *Spirulina* was added and statistically significant differences were found between the control and 1% *Spirulina*-fortified samples for the two types of fish burger. Cohesiveness results of carp and barbel burgers formulated with 1% *Spirulina* were comparable with the values reported by Kumarathunge et al. [42] for catla burgers formulated with 0.5% (*w/w*) *Ulva lactuca*. In industry, the mentioned texture results might be considered satisfactory because *Spirulina* did not mischaracterize the textural characteristics that were already known and accepted by the panelists.

3.3. Functional Properties of Fish Burgers

The functional characteristics of control and *Spirulina*-fortified burgers are shown in Table 4.

Table 4. Functional parameters of control and *Spirulina*-fortified fish burgers.

Functional Characteristics	Fish Burger Types	Burger Formulations	
		Control burger	1% <i>Spirulina</i> fish burger
SWC (mL/g DW) *	CB	3.13 ± 0.115	3.56 ± 0.110 ^a
	BB	2.99 ± 0.03	3.43 ± 0.02 ^a
OHC (g/g DW) *	CB	0.85 ± 0.07	0.90 ± 0.017 ^a
	BB	0.86 ± 0.014	1.06 ± 0.056 ^a
WHC (g/g DW) *	CB	2.31 ± 0.135	2.78 ± 0.162 ^a
	BB	2.12 ± 0.042	2.52 ± 0.042 ^a

CB: carp burgers and BB: Barbel burgers. * Results are expressed as means ± SD (n = 3). Control burgers vs. *Spirulina*-fortified burgers: ^a $p < 0.001$.

Compared to controls, the WHC values of 1% *Spirulina*-fortified burgers increased significantly ($p < 0.001$) from 2.31 to 2.78 and 2.12 to 2.52 for carp and barbel formulations, respectively (Table 4). This was probably due to the addition of *Spirulina* fibers. The great WHC of *Spirulina* proposes that it could be used as a functional natural ingredient in treatments of food to modify viscosity and texture, reduce dehydration during storage, and minimize energetic value of food products. Furthermore, significant differences ($p < 0.001$) of OHC values were also noted for the two types of *Spirulina*-fortified burgers (Table 4). These OHC values could be of particular interest, especially for the binding of fat during food industrial production and preservation. This might be attributed to the high water binding and oil binding capacities of the microalgae fibers [40,43]. Added to that, the highest values of swelling capacity were obtained by the *Spirulina*-fortified burgers made from carp (3.56 mL/g) ($p < 0.001$) and barbel (3.43 mL/g) ($p < 0.001$). The control burgers showed significant inferior values of swelling capacity for carp (3.13 mL/g) and barbel (2.99 mL/g) (Table 4). Furthermore, the difference between the functional capacities of control and 1% *Spirulina* formulations could be explained by the structural properties of each material after the addition of *Spirulina* fibers. These results are in accordance with previous studies [37,44,45].

3.4. Microbiological Quality of Fish Burgers

The burger production process entails a lot of handling of raw food, and therefore hygiene in these food products is a concern for public health given their association with enteric diseases [39]. In our experiment, no mold, Enterobacteriaceae, yeast, coliform bacteria or foodborne pathogens (*Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *Bacillus cereus* and *Campylobacter* spp.) were detected in any of the control and *Spirulina*-fortified burgers up to 8 months of storage at 4 °C. The absence of these pathogens indicates that the carp and barbel burgers were both clean and safe even after an extended storage period. These data prove that the processing of the final fish products was executed under perfect hygienic-sanitary conditions.

3.5. Color Measurement of Burger Samples

Because of the important influence of food colors on the consumers acceptance, the surface color of carp and barbel burgers have been interpreted into values of color indices (L^* , a^* , b^*) that can explain the difference between different treatments using a standard illuminant light source (Table 5).

Table 5. Color analysis of control burgers and *Spirulina*-fortified burgers.

Color Parameters	Fish Burger Types	Burger Formulations	
		Control burger	1% <i>Spirulina</i> fish burger
L*	CB	46.75 ± 0.335	40.23 ± 0.961 ^a
	BB	40.6 ± 0.07	33.27 ± 0.530 ^a
a*	CB	10.65 ± 0.22	6.23 ± 0.084 ^a
	BB	8.18 ± 0.084	5.2 ± 0.098 ^a
b*	CB	11.39 ± 0.715	7.77 ± 0.692 ^a
	BB	18.11 ± 0.664	5.73 ± 0.001 ^a

CB: carp burgers; BB: barbel burgers; Control burgers *vs* *Spirulina*-fortified burgers: ^a $p < 0.001$.

The L* value measures the brightness of food samples, ranging from 0 (black) to 100 (white) [40]. In cooked burgers, lightness (L*) decreased when *Spirulina* was added. Statistically significant differences ($p < 0.001$) were found between the 1% *Spirulina*-fortified samples and the control samples and this is due to the darker colors obtained from *Spirulina*. We speculate that reducing sugars derived from the hydrolysis of *Spirulina* polysaccharides during the burgers production may elicit the Maillard reaction to form brown melanoidins [46]. Further, this decrease could be attributed to differences in composition, resulting in different amounts of pigments (chlorophylls and phycocyanin) and water, which is a determining element in the resulting color product [40]. These results indicate that *Spirulina* has no tendency to lighten the burger's color. Indeed, these results are consistent with those obtained by Senthil et al. [47]. Moreover, carp and barbel burgers fortified with 1% of *Spirulina* powder were characterized by significantly low a* and b* values ($p < 0.001$), which shows that the yellow color diminished to a greenish color (Table 5). This could be mainly explained by the fact that *Spirulina* is rich in chlorophylls. These data could be useful in predicting the effect of *Spirulina* on the conventional color of the products where it will be used. In addition, we can argue that *Spirulina* pigments could also be useful as natural dyes in fishery products.

3.6. Antioxidant Properties of Fish Burgers

Currently, much recent interest has been given to the considerable part of the enriched foods with bioactive substances, usually derived from plants, seaweeds and microalgae thanks to their safety and efficiency in the cure of different human disorders. In fact, the antioxidant activity of microalgae resulted from their abundance in enzymatic and non-enzymatic free radical scavengers. Thus, to estimate the input of *Spirulina* to the burger antioxidant capacities, two different methods were used: the FRAP and the DPPH free radical-scavenging assays. The results of these methods are summarized in Table 6.

Compared to the control burgers, the addition of *Spirulina* in burgers increased significantly their antioxidant activities ($p < 0.001$) as well as their chlorophyll ($p < 0.001$), carotenoid ($p < 0.001$) and phycocyanin ($p < 0.001$) contents (Table 6). Furthermore, a positive correlation between antioxidant activity and pigment contents was clearly noticed. At a sample concentration of 10 mg/ml, carp and barbel burgers containing 1% of *Spirulina* showed significantly higher DPPH scavenging activities (86.76 and 94.09%, respectively) than control burgers (56.28 and 40.09%) ($p < 0.001$) (Table 6). Furthermore, a comparable trend was also noticed for FRAP. In fact, the ferric reducing activities for unfortified and fortified carp burgers with 1% of *Spirulina*, were equal to 0.31 and 0.651 ($p < 0.001$) respectively, at a sample concentration of 10 mg/ml (Table 6). Similarly, significant differences ($p < 0.001$) were also found for barbel formulations between 1% *Spirulina*-fortified samples and controls (Table 6). The rise in scavenging of free radicals may be attributed to the increase in the carotenoids [48,49], chlorophylls [50], phycocyanin [51,52] and polysaccharides [45] contents, which had an important role in the protection of the body's cells from oxidative stress and many other diseases. In this sense,

the immulina polysaccharides from *Spirulina* improve the enzymatic activity of the cell nucleus and synthesis of DNA repair, besides being a beneficial species for the immune system [53,54]. Further, these polysaccharides were showed to have high scavenging activities against superoxide and hydroxyl radicals and moderate reductive power in a concentration-dependent manner [11,46,55]. These results also showed that fish burger processing did not have a negative effect on the antioxidant components.

Table 6. Pigments content and antioxidant activities of control and 1% *Spirulina*-fortified burgers.

Color Parameters	Fish Burger Types	Burger Formulations	
		Control burger	1% <i>Spirulina</i> fish burger
Chlorophylls (mg/100g DW)	CB	0	23.93 ± 1.675 ^a
	BB	0	20.05 ± 1.97 ^a
Phycocyanin (mg/100g DW)	CB	0	0.431 ± 0.033 ^a
	BB	0	0.446 ± 0.199 ^a
Carotenoids (mg/100g DW)	CB	0	12.39 ± 0.657 ^a
	BB	0	14.06 ± 0.848 ^a
Scavenging activity (%) *	CB	56.28	86.76 ^a
	BB	40.09	94.09 ^a
Reducing power *	CB	0.315	0.615 ^a
	BB	0.410	0.584 ^a

CB: carp burgers; BB: barbel burgers; Control burgers *vs.* *Spirulina*-fortified burgers: ^a $p < 0.001$; * The scavenging activity of DPPH free radicals (%) and the reducing power (absorbance at 700 nm) were determined at a samples concentration of 10 mg/ml.

4. Conclusions

The addition of *Spirulina* seems to be a promising technological alternative for producing fish products, such as burgers made from carp and barbel, because the natural and healthy image of these products, by the consumers, could be improved. Besides its role as a nutritional component, *Spirulina* serves as suitable source of beneficial natural antioxidant and coloring agents. Furthermore, *Spirulina*, rich in dietary fibers, plays a central role in the textural maintenance of the final fish products by improving their functional properties (water and oil holding capacities). According to these results, the addition of 1% of *Spirulina* in fish burger canning can be recommended since *Spirulina* is as a natural source of bioactive compounds noting (chlorophylls, carotenoids and phycocyanin). Therefore, the evaluated microalga might be an effective alternative to fortification with synthetic chemical supplements that can cause undesirable effects for consumers. The reasonable selection of *Spirulina* as a fortifier agent in fish-based products seems to be substantial and it provides food that is both satisfying to the consumer and beneficial to their health.

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