



## Effect of Spirulina Powder on Quality and Sensory Attributes of Catfish Burger



El-Sayed A. H. El-Shennawy<sup>a\*</sup>, Ahmed Z HassabAlla<sup>b</sup>, Abd El-Rahman M Sulieman<sup>c</sup> and Ahmed A El-Badwi<sup>d</sup>

<sup>a</sup> Food Science Department, Faculty of Agriculture, Zagazig University.

<sup>b</sup> Laboratory for Aquaculture Researches – Agric. Researches Center

<sup>c</sup> Food Science Department, Faculty of Agriculture, Zagazig University

<sup>d</sup> Food Science Department, Faculty of Agriculture, Zagazig University

### Abstract

In order to enhance the nutritional value of fish-transformed goods, microalgae have recently been added naturally. This study examined how adding *Spirulina platensis* powder (SSP) at concentrations of 1, 2, and 3% w/v affected the catfish burger's chemical, microbiological, and sensory qualities. The results showed that the SPP has high levels of DPPH inhibition % (92.6%), total phenolic content (850.8 mg/100g), and total flavonoid content (84.5 mg/100g). The chemical composition, texture, microbiological, phytochemicals, and sensory properties of fish burgers have been improved by adding SSP. The microbial load of the treatments was found to have decreased at the end of the storage period in comparison to the control, and the total volatile base nitrogen (TVBN) values for the treatments showed a high degree of stability in comparison to the control, reaching a value of 15.34 at month 6, which is significantly above the acceptable limit and indicates spoiling. When comparing all of the treatments to the control, the pH values were higher and the overall acidity was lower. Compared to the results of the other fish burger treatments, the treatments that contain 2 % of SSP had better sensory properties. In conclusion, extending the freshness of burgers by using *Spirulina platensis* as a natural preservative is a creative and sustainable method of food preservation. Utilising the potential of this green superfood helps the food sector become healthier and more ecologically conscious while also extending the shelf life of our products.

**Keywords:** Fish burgers, *Spirulina platensis*; polysaccharides; antioxidant, carotenoids

### Introduction

Fish has been crucial in helping developing nations address the issue of the sustainability of people's food and livelihoods. Over 400 million individuals in Asia and Africa get at least 50% of their animal protein from fish, while about 2.6 billion people worldwide get 20% of their animal protein from fish. However, emerging nations only supply 13% of the world's animal protein needs. Fish is one of the most important sources of animal protein in the tropics and has long been known to provide high-quality protein along with other necessary components [1]. The Egyptian fisheries, which comprise seas, numerous lakes, the Nile River, and fish farms spread throughout the country, cover more than 13 million acres, or around 150% of the country's arable land. Egypt presently produces 1 million 920 thousand tonnes of fish, of which 80% come from fish farming and 20% from catch fisheries, according to a 2020 report published by the Egyptian Fisheries Authority [2]. Primarily freshwater fish, catfish (*Clarias gariepinus*) are suited to small spaces and resilient to both disease and manipulation. It is generated in vast amounts around the freshwater lakes in Wadi El-Rayan Lake's initial pond, fish farming, and the Nile fisheries, especially Nasser's Lake. With a high concentration of unsaturated fatty acids, vitamins, proteins, and minerals, catfish is incredibly nutrient-dense. The availability, consistency, and health advantages of catfish (*Clarias gariepinus*) have led to a sharp rise in consumption in recent years [3]. African catfish aquaculture has transformed the fishery from an undesired to a desirable species by increasing output and gaining considerable importance in several African countries recently [4].

Fish is an important dietary source that has high biological value proteins, mineral salts, and unsaturated fatty acids (omega-3 and omega-6; [5]). According to Delfino et al. [6], these functional elements are crucial and support human health, particularly for the heart and brain. Because fish plays a significant role in human nutrition, it is vital to produce a variety of

\*Corresponding author e-mail: shenosameh8@gmail.com., (El-sayed A. El-shennawy).

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ready-to-eat items to increase diversity and boost consumption [7,8]. Because fish has a short shelf life and is easily spoiled by microbes, fish and its products are extremely perishable foods [5,9]. Under the right circumstances, procedures, handling, and storage can enhance the security and calibre of fisheries products [7,10]. To increase the shelf life of these perishable goods, nevertheless, further technologies or preservative chemicals are required [11]. Chemical additions known as preservatives can extend the shelf life, stability, and quality of edible items while lowering their microbial load and monetary losses [6,12]. However, it has been established that these chemical preservatives have been shown to be expensive [6,13]. Consequently, due to consumer knowledge, natural alternatives are used in the food business in place of chemical and synthetic additives [14].

A growing number of food companies are interested in using microalgae in functional foods; the three main genera used are *Chlorella*, *Dunaliella*, and *Spirulina*. *Spirulina platensis* is a cyanobacterium that has a unique composition of bioactive and nutritional substances (such as proteins, vitamins, minerals, pigments, and phenolic acids) and is used in many different medical applications [15]. This cyanobacterium is home to pigments (phycocyanin and beta-carotene) [16] and polysaccharides [17], which are well-known for their ability to fend off a variety of illnesses, including cancer [18], hypertension [19], and renal failure [20]. It has been discovered that *Spirulina platensis* pigments and polysaccharides exhibit antibacterial and antiviral properties in addition to their antioxidant benefits [21]. *Spirulina*'s positive health impacts are another reason why it's added to food goods. Its increased nutritional value is a result of its abundance of vital amino acids, vitamins (particularly B-complex vitamins and  $\beta$ -carotene), and minerals like calcium and iron [22]. *Spirulina* added to food can increase dietary intake, especially in areas where nutrient deficits and malnutrition are common. Additionally, it has been demonstrated that spirulina-enriched diets reduce blood sugar and cholesterol, which helps to improve metabolic and cardiovascular health [23]. *Spirulina*'s antioxidant qualities also make it a useful component of functional diets that try to lower oxidative stress [24]. Incorporating spirulina into common food items provides protection against chronic conditions such as cancer, arthritis, heart disease, and skin conditions [25]. Additionally, because of its antibacterial qualities, spirulina is now used in food preservation to extend the shelf life of perishable goods [26]. So, in the present study, determined the bioactive components and antioxidant traits of *Spirulina*. Then, the application of *Spirulina* was examined to improve the quality and shelf life of catfish burgers during freezing storage ( $-18^{\circ}\text{C}$ ).

## Materials and Methods:

### Materials

*Spirulina platensis* was procured from the Algal Biotechnology Unit at Egypt's National Research Centre in Giza, Egypt. The study employed sixty kilogrammes of live karmout catfish (*Clarias gariepinus*), with an average length of  $57 \pm 2$  cm and weight of  $1450 \pm 10$  g, obtained from the Institute of Fisheries Research in Al -Abbasa Sharkia Governorate, Egypt. The local market in Cairo, Egypt was the source of spices (black pepper, cumin powder, thyme powder, onion powder, and garlic powder), salt, and wheat flour. Analytical-grade solvents and chemicals were procured from Sigma Chemical Co. (St. Louis, MI, USA).

We prepared the BG-11 medium in accordance with Al-Rikabey and Al-Mayah [27]. The protocols outlined in Zimbro et al. [28] were used to create LB broth and LB agar, total count agar, baird parker agar, potato dextrose agar, and MacConkey agar.

Two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus* ATCC 13565) and one Gram-negative (*Escherichia coli*, O:157 ATCC 1659) bacteria were used as indicator microorganisms for antimicrobial activity. The strains mentioned below were all acquired from the Microbiological Resources Centre Cairo (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt, as living, growing cultures.

### Methods:

#### Preparation of cat fish burger (CFB):

Fish were directly shipped, chilled, to the Department of Food Science Laboratory Fisheries Research in the Governorate of Al-Abbasa, Sharkia, Egypt. The fish was carefully cleaned, gutted, and then hand-formed into fillets before being mechanically minced using a meat grinder with a 4 mm (coarse) blade. Before being used, minced fish was kept in a refrigerator at  $4^{\circ}\text{C}$ . The catfish burger product was made using the recipe found in (Table A) and as detailed by Kenawi and Abdelaal [2]. After thoroughly mixing all the ingredients, each part was divided into 50 g balls, which were then shaped into burger-like shapes with a 10 cm diameter and 0.5 cm thickness. The burgers were then placed in a polystyrene (foam) tray with LDPE sheets between them to keep them from sticking.

**Determination of total phenol content (TPC), total flavonoid content (TFC), and antioxidant activity.****Preparation of the SSP and burger Extracts**

Three grammes of burger samples were homogenised in six millilitres of 80% methanol at 10,000 rpm for one minute using a T18 homogeniser (IKA, Wilmington, NC, USA) to create burger extracts. The mixtures were centrifuged at 10,000× g for 10 minutes, then filtered through a 0.45 µm membrane filter and stored at -20° for additional analysis.

SSP and 80% methanol were combined at a 1:2 (w/v) ratio to create the SSP extracts. After six hours of stirring on a magnetic stirrer (Fisher, 14-511-1A, Tuscaloosa, AL, USA), the mixtures were filtered through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA) to ensure sterilisation. A Labconco 8811 freeze-dryer (Labconco Corporation, Kansas, MO, USA) was used to freeze-dry the filtrates, and the extracts were kept at -20 °C for further examination.

To calculate the TPC of SSP and catfish burger samples, the sample (0.1 mL), distilled water (2.8 mL), and Folin-Ciocalteu reagent (0.1 mL) were combined and vortexed. The mixture was then stirred with a 2. mL 7.5% (w/w) sodium carbonate solution, and it was then incubated for 60 minutes at 25°C in the dark. Finally, absorbance was measured at 750 nm using a spectrophotometer (Jenway 6305, England). Gallic acid (GA) standard curve was established, and TPC was expressed for samples as milligrammes of GA equivalents per gramme (mg GAE/g) [29].

**Table A.** Catfish burger Formula

Ingredient	Control catfish burger (C)	Spirulina powder catfish burger		
		1.0% (T1)	2.0% (T2)	3.0% (T3)
Ground catfish Fillet	89.8	88.9	88.0	87.1
Spirulina powder	0.00	0.9	1.8	2.7
Wheat flour	3.5	3.5	3.5	3.5
Salt	2.5	2.5	2.5	2.5
Black pepper powder	1.0	1.0	1.0	1.0
Onion powder	1.0	1.0	1.0	1.0
Garlic powder	1.0	1.0	1.0	1.0
Cumin powder	1.0	1.0	1.0	1.0
Thyme powder	0.2	0.2	0.2	0.2

To find the TFC of SSP and catfish burger samples, a sample (0.5 mL) was combined with 1.5 mL of 100% ethanol, 0.1 mL of 0.1 M CH<sub>3</sub>COOK solution, 0.1 mL of 10% (w/v) AlCl<sub>3</sub> solution, and 2.8 mL of distilled water. The mixture was left for 30 minutes. Following that, the mixture was filtered, and a spectrophotometer was used to measure the absorbance at 415 nm. TFC was determined as milligrammes of quercetin equivalent (QE) per gramme (mg QE/g) of SSP and catfish burger samples using a standard curve of quercetin in ethanol [30].

To test the DPPH radical scavenging % of SSP and catfish burger samples using the 1-(2, 6-dimethylphenoxy)- 2-(3, 4-dimethoxyphenylethylamino) free radical scavenging method, 0.3 mL of sample was vigorously mixed with 2.7 mL of methanolic solution (0.004%). After 60 minutes of incubation at room temperature and darkness, the light absorbance was measured at 517 nm against a blank (without sample), and Equation (1) [31] was used to compute the DPPH radical scavenging percentage:

$$\text{DPPH radical scavenging (\%)} = \frac{A(\text{blank}) - A(\text{sample})}{A(\text{blank})} \times 100$$

**Physicochemical assessments**

Burger pH was evaluated by mixing 5 g of each sample with 45 mL of distilled water for 60 seconds, filtering the mixture, and then using a pH meter (Zenit, Germany) to measure the result at 23 °C. After measuring total volatile base nitrogen (TVB-N, mg N 100 g<sup>-1</sup>), 10 g of fish patties were put to a magnesium oxide-filled Kjeldahl flask, which was then mixed with distilled water before being connected to the system. TVB-N was measured in a 100 g fish burger as follows [32]: the fluid

was put and collected in an Erlenmayer flask, together with boric acid (2%) and methyl red indicator through 10 N H<sub>2</sub>SO<sub>4</sub>, which changes colour from green to light red.

$$TVB-N \text{ (mgN100 g}^{-1}\text{)} = 1.4 \times \text{used H}_2\text{SO}_4 \times 100 \times \text{amount of sample} / 1000 \text{ mg.}$$

According to Ben Atitallah *et al.* [33] description, the contents of Ash and Total Solids (TS) were ascertained. Using a nitrogen conversion factor of 6.25 and the traditional Kjeldhal approach of the AOAC International (method 981.10), the protein content was determined [34]. The method of Bligh and Dyer [35] was adapted to extract total lipids, and Fendri *et al.* [36] provided the measurement guidelines. The method outlined by Barkallah *et al.* [37] was used to determine the total dietary fibre content of the burger samples. According to the methods outlined by Kumar *et al.* [38] and Lichtenthaler and Wellburn [39], the contents of carotenoids and chlorophylls were estimated. The method outlined by Bennet and Bogorad [40] was followed in order to determine the amount of c-phycocyanin.

### **Oxidative index measurement**

To measure the peroxide value (PV), 40 g of fish burger was first combined with 100 mL of chloroform and filtered through Whatman filter paper No. 44. After that, a rotary evaporator (Heidolph, Germany) separated 25 mL of the filtered solution, and 5 g of the extracted fat was transferred into a 250 mL Erlenmeyer flask. Subsequently, 30 mL of distilled water with 1 mL of starch solution was added after 60 seconds, along with 37 mL of acetic acid–chloroform solution (2:3) and 1 mL of saturated potassium iodide. PV was also reported as milliequivalents per kilogramme (meq/kg) for samples; the released iodine was titrated with sodium thiosulphate solution (0.01 N) until yellow faded and a milky colour was generated [41].

Burgers were subjected to the following procedures: 1 g of each sample was transferred to a Falcon tube; 3 mL of perchloric acid (4%) and 2 mL of trichloroacetic acid (10%) were added; the tubes were then closed, stirred for 3 minutes, and centrifuged for 15 minutes at 4000 rpm to settle the suspended substances in the extracts; 1 mL of the supernatant was then transferred to a test tube; 1 mL of TBA reagent (0.02 M) was added; this resulted in a cooled solution that was measured at 530 nm against a blank (which contained all test materials except the sample). According to Maghami *et al.* [42], the TBA for burgers was expressed as milligrammes of malondialdehyde (MDA) per kilogramme (mg MDA/kg).

### **Textural profile analysis (TPA)**

Using a cylindrical probe with a flat end (7.5 cm diameter) and a 250 N cell load, a texture analyser (LLOYD, RS 232, America) was used to TPA fish burgers. The device's probe measured the compression speed at 1 mm/s after cube-shaped slices (2 cm × 2 cm) were cut from the centre of the burgers. The samples were additionally reduced to half of their original height. The textural parameters, including hardness (N), cohesiveness, chewiness (N), and elasticity (cm), were examined in the current study [10].

### **Functional Properties of Fish Burger**

Fish burger powder's swelling capacity (SWC) was calculated using the methodology outlined by Kuniak and Marchessault [43]. The SWC was given in millilitres of swollen sample per gramme of dry weight, or mL/g dw. Every measurement was done in duplicate. According to Okezie and Bello's [44] instructions, the powdered fish burgers' water holding capacity (WHC) was calculated. The burger samples' WHC was expressed as g water/g dw after the sample was promptly weighed. Every analysis was carried out twice. Burger powder's oil holding capacity (OHC) was calculated using the methodology outlined by Wong and Cheung [45]. Burger samples' OHC was reported as grammes of oil retained per kilogramme of sample (g/g dw).

### **Microbiological Analyses**

#### **Preparation of Samples for Microbiological Examinations**

Using a heated spatula, the samples were first cauterised. Then, using a sterilised scalpel and forceps, the cauterised portions were removed. A sterile blender flask holding 90 mL of 1% sterile peptone water was aseptically filled with 10 g of each sample, which were then homogenised for 2.5 minutes at 14,000 rpm. To accomplish homogenisation, the mixture was allowed to stand at room temperature for 15 minutes. After shaking the flask thoroughly, 1 mL of the contents was transferred into a different sterile tube that held 1 g of sterile peptone water (0.1%). From this tube, ten-fold serial dilutions up to 10<sup>−7</sup> were made [46].

#### **Determination of Aerobic Plate Count**

A sterile Petri dish was aseptically filled with 1 mL of the previously made dilution. Next, around 10 mL of standard plate count agar—which had been melted and tempered at 45 °C—was added and thoroughly mixed in a horizontal orientation.

Following solidification, both control and inoculation plates were incubated for 48 hours at 37°C while inverted. Colony-forming units per gramme (CFUs/g) of the sample was the measurement and expression of the number of colonies that grew.

#### **Determination of *Escherichia coli* (E. coli) Counts**

One millilitre aliquot from each of the previously made sterile dilutions was added to two sets of Petri dishes that had already been inoculated with ten millilitres of sterile MacConkey agar. Following solidification, both control and inoculation plates were incubated for 48 hours at 37 °C while inverted. Biochemical assays were used to confirm the identification of *E. coli* colonies, which were based on their distinctive shape. The results, which represented possible contamination and the efficacy of sanitation, were given as CFUs/g of the material.

#### **Determination of Yeast and Mold Count**

The amount of mould and yeast, which can contaminate food and lower its quality, is measured by the yeast and mould count. Ten millilitres of sterile potato dextrose agar medium were used to inoculate duplicate sets of Petri dishes with previously produced dilutions in 1 millilitre aliquots. Following solidification, both infected and control plates were incubated for 72 hours at 25 °C in an inverted posture. The number of yeast and mould colonies per gramme of sample was calculated and reported. This tally aided in determining the product's expiration date and any possible spoiling problems.

#### **Determination of *Staphylococcus Aureus* Count**

Baird-Parker agar was plated with the diluted dilution. For 24 to 48 hours, plates were incubated at 35 °C. Every dilution was made with a duplicate plate. Coagulase tests were used to confirm and count *Staphylococcus* colonies, which are frequently distinguished by their clear zones and black or grey appearance. Food poisoning risk due to *staphylococcus* bacteria was indicated by the results, which were expressed as CFUs/g of the sample.

#### **Sensory evaluation:**

Ten male and twenty female panellists, ranging in age from 20 to 45 year, assessed the qualities of fish burgers. Researchers and staff from Zagazig University's Faculty of Agriculture's Food Science Department participated in the tasting panel. The core temperature of the samples was 72°C, and the burgers were cooked on a hot plate at 150°C. Samples of hamburgers were given out on white polystyrene plates, and the panellists were shown them in a random order using three-digit codes. Water was provided to clean the mouth in between samples, and the experiments were conducted in a sensory evaluation room with controlled airflow and white light. Panel members evaluated the burgers using a nine-point hedonic scale for colour, taste, odour, and texture; a score of 9 indicated that the item was highly liked, and a score of 1 indicated that it was greatly disliked [47].

#### **Statistical analysis of data**

Every experiment was conducted in triplicate, and SPSS (version 22) software (IBM, Armonk, NY, USA) was used to analyse the data. Duncan's tests ( $p \leq 0.05$ ) were used to compare mean value differences in order to establish the significant threshold

### **Results and discussion**

#### **Nutritional composition of *S. platensis* powder**

Table 1 displays the findings of SSP's proximal composition. SSP had a moisture content of 5.8%; it is an excellent source of highly accessible protein (68.80%), fat (1.40%), crude fibre (10.30%), and ash (4.10%). High levels of protein (65–70%) were also found by other researchers in SSP [48]. Additional researchers found that SSP had a protein content of 65-71%, which was higher than that of soybeans and more easily digested because it didn't include antinutrients such polyphenols and phytic acid, which are known to inhibit the proteolytic enzymes [49].

There is a strong correlation between flavonoids and phenolic contents. Moreover, DPPH free radical scavenging and flavonoids are associated ( $p < 0.05$ ). According to Table 1's results, TFC, TFC, and DPPH% of SSP were 750.8 mg/100g, 84.5 mg/100g, and 92.6%, respectively. These findings are consistent with those of Rahim et al. [50], who discovered that the amounts of TPC, TFC, of SSP were, respectively, 700.0 mg/100g and 100.04 mg/100g.

According to Table 1's findings, the contents of SSP in terms of total carotenoids, phycocyanins, and chlorophyll were 1320, 1780, and 174 mg/100g, respectively. Compared to earlier research, SSP has higher quantities of phycocyanins and chlorophyll [51,52]. However, it has a low concentration of carotenoids. A number of variables, including pH, temperature, salinity, and light, can affect the pigment levels in SSP [53].

**Table 1.** Comparison of the nutritional composition of *S. platensis*

Components	<i>S. platensis</i> powder
<b>Proximate composition (on dry matter basis)</b>	
Moisture	5.5±0.14
Protein	68.80±1.6
Fat	1.40±0.08
Ash	4.10±0.11
Crude Fiber	10.3±0.24
<b>Phytochemical and phytopigments properties</b>	
TPC mg/100g	750.8±2.5
TFC mg/100 g	84.5±1.8
DPPH inhibition (%)	92.6±1.3
Chlorophylls (mg/100g)	1320±4.2
Phycocyanin (mg/100g)	1780±3.8
Carotenoids (mg/100g)	174±1.6

Means in the same row that are denoted by several little letters differ significantly ( $p \leq 0.05$ ).

### Effect of Spirulina powder and frozen storage at $-18 \pm 1^\circ\text{C}$ for 6 months on proximate composition of fish burger

The results of the analysis of the crude fibre, ash content, protein content, ether extract, and moisture content are displayed in Table 2. All fish burger treatments showed a range of 6764% to 6880% in terms of moisture content. At the beginning, the fish burger samples' protein levels ranged from 14.35 percent to 17.94%. The protein content decreased by 17.94%, 17.60%, 17.28%, and 17.0 % for the control group and by 1%, 2%, and 3% for the SSP additions, respectively, as a result of the addition. This decrease is explained by the low concentration used from SSP. At the first time point, the fish burger samples' ether extract contents varied from 4.30% to 4.70%. The burger samples' ash contents ranged from 2.72% to 3.33%. The samples containing SSP had the highest ash percentage, and the addition rate was correlated with an increasing trend in these samples. This increase could be explained by SSP's higher mineral content.

**Table 2:** Effect of Spirulina powder and frozen storage at  $-18 \pm 1^\circ\text{C}$  for 6 months on proximate composition of catfish burger

Components (%)	Storage period (month)	Treatments			
		C	T1	T2	T3
Moisture	0	68.80±0.54 <sup>Aa</sup>	68.35±0.50 <sup>ABa</sup>	68.02±0.46 <sup>ABb</sup>	67.64±0.44 <sup>Ba</sup>
	3	66.60±0.50 <sup>Ab</sup>	66.12±0.35 <sup>ABc</sup>	65.84±0.40 <sup>Bb</sup>	65.50±0.38 <sup>Bc</sup>
	6	65.90±0.55 <sup>Ac</sup>	65.44±0.43 <sup>ABd</sup>	65.12±0.46 <sup>ABd</sup>	64.90±0.52 <sup>Bd</sup>
Crude protein	0	17.94±0.42 <sup>Aa</sup>	17.60±0.64 <sup>Aa</sup>	17.28±0.55 <sup>Aa</sup>	17.00±0.60 <sup>ABa</sup>
	3	16.76±0.50 <sup>Ab</sup>	16.10±0.62 <sup>Ba</sup>	15.80±0.48 <sup>BCa</sup>	15.40±0.71 <sup>Ca</sup>
	6	15.85±0.58 <sup>A</sup>	15.06±0.66 <sup>Bb</sup>	14.72±0.42 <sup>Cb</sup>	14.35±0.55 <sup>CDa</sup>
Crude ether extract	0	4.70±0.12 <sup>Ab</sup>	4.50±0.11 <sup>Bc</sup>	4.40±0.06 <sup>BCb</sup>	4.30±0.09 <sup>Cc</sup>
	3	5.60±0.14 <sup>Aa</sup>	5.40±0.17 <sup>ABa</sup>	5.30±0.14 <sup>Bb</sup>	5.10±0.13 <sup>Cb</sup>
	6	5.65±0.12 <sup>Aa</sup>	5.45±0.16 <sup>Ba</sup>	5.35±0.18 <sup>BCa</sup>	5.15±0.14 <sup>Ca</sup>
Ash	0	4.22±0.09 <sup>Bc</sup>	4.36±0.08 <sup>ABd</sup>	4.48±0.06 <sup>ABb</sup>	4.62±0.08 <sup>Ab</sup>
	3	5.25±0.13 <sup>Bb</sup>	5.40±0.09 <sup>ABc</sup>	5.52±0.12 <sup>ABb</sup>	5.66±0.11 <sup>Ab</sup>
	6	5.33±0.12 <sup>Ba</sup>	5.48±0.07 <sup>ABb</sup>	5.63±0.4 <sup>ABa</sup>	5.72±0.16 <sup>Aa</sup>
Crude Fiber	0	0.35±0.02 <sup>Dc</sup>	0.48±0.01 <sup>Cc</sup>	0.72±0.05 <sup>Bc</sup>	1.06±0.08 <sup>Ac</sup>
	3	0.44±0.06 <sup>Db</sup>	0.65±0.03 <sup>Cb</sup>	0.96±0.04 <sup>Bb</sup>	1.22±0.06 <sup>Ab</sup>
	6	0.50±0.04 <sup>Da</sup>	0.72±0.06 <sup>Ca</sup>	1.02±0.03 <sup>Ba</sup>	1.30±0.05 <sup>Aa</sup>

\* \* The values are the mean ± SD of three calculations. Significant differences ( $p < 0.05$ ) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ( $P \leq 0.05$ ) in the values (means ±SD). C: Catfish burger, T1: Catfish burger fortified with 1% Spirulina powder, T2: Catfish burger fortified with 2% Spirulina powder, T3: Catfish burger fortified with 3% Spirulina powder

It's interesting to note that all treatments showed larger percentages of ash than the control, suggesting that the additions contained more minerals [50]. At the first time point, the fish burger samples' crude fibre content varied from 0.35% to 1.06%. The samples containing SSP had the largest percentage of crude fibre, and the addition rate was correlated with an increasing trend in these samples. When the fish burger samples were fortified with SSP, their levels of ash, crude fibre, and protein increased. These levels also increased when the quantity of SSP in the fish burger samples increased. Adding SSP to fish products raises their ash, crude fibre, and protein concentrations because it offers a wealth of minerals, fibre, and protein.

Additionally, freezing storage durations at  $-18\pm1^{\circ}\text{C}$  for six months had a substantial ( $p<0.05$ ) impact on the proximate composition of fish burgers. Increasing the frozen storage periods of fish burgers resulted in a significant ( $p < 0.05$ ) drop in both moisture and protein contents [54], the reason for this drop in moisture content during storage could be partly attributed to evaporation through the polyethylene bags used for fish burger packaging, as well as drip loss. Protein hydrolysis by naturally occurring fish enzymes and bacterial enzymes that are generated, along with the loss of water-soluble protein with segregated drip, could be the cause of the decline in protein content during storage [55, 56]. Conversely, longer frozen storage times resulted in a significant ( $p\leq0.05$ ) increase in the crude fat, total ash, and fibre contents of fish burgers. These increases could be explained by the decrease in moisture and protein during frozen storage times. These findings were consistent with those of [57,58], who observed that the ash, protein, and fibre contents of fish burgers made with algae *p. nanoliposome* powder or *Spirulina platensis* powder were significantly ( $p\leq0.05$ ) higher than those of the control group.

### **Effect of Spirulina powder as well as frozen storage at $-18\pm1^{\circ}\text{C}$ up to 6 months on chemical quality characteristics and pH value fish burgers**

#### **The TVB-N levels of burgers**

TVB-N levels were measured at the start of the shelf life as follows: 9.88 (mg N 100 g<sup>-1</sup>) for the control group and close to 9.82 (mg N 100 g<sup>-1</sup>) for the treated burgers using SSP. These levels were elevated significantly 15.34 (mg N 100 g<sup>-1</sup>) for the control group at frozen storage at  $18\pm1^{\circ}\text{C}$  up to 6 months. The TVB-N levels were significantly lower ( $p < .05$ ) in treated samples compared to controls during frozen storage, with a clear reduction correlating with increased concentrations. The TVB-N did not exceed the acceptability limit of 35 (mg N 100 g<sup>-1</sup>) for each group; the quality of fish and its products would be "high" with values up to 25 (mg N 100 g<sup>-1</sup>) for all present samples in this range [59]. Conversely, burgers treated with SSP [60] and *C. vulgaris* powder showed decreased TVB-N levels. This is consistent with the impact of SSP application as an antibacterial agent on the population of bacteria [60].

#### **The TBA index of burgers**

TBA gauges the production of MDAs in particular, which are secondary products of fat oxidation [8]. These elements result from the hydroperoxides that break down during the initial stage of fat oxidation and react with the TBA reagent to form a colour complex [61]. The maximum TBA for fish and its products is 5 mg MDA/kg because they are highly susceptible to oxidative spoiling due to the high levels of polyunsaturated fatty acids (PUFAs) in fish [62]. Table 3 shows the variations in TBA of fish burgers during frozen storage; at the start of storage, these values for samples were almost identical to 0.54 mg MDA/kg. Because of the increasing levels of secondary products and fat oxidation, TBA increased significantly over time for the samples ( $p < .05$ ). The burger without any additive (control) had the highest TBA (0.95 mg MDA/kg), as predicted, while the sample treated with SSP had the lowest TBA (0.68 mg MDA/kg) during the final month of storage, with a distinct decrease associated with higher concentrations. Although other bioactive components such polysaccharides, carotenoids, proteins, peptides, and pigments in some plants can also be implicated in neutralising free radicals, phenolic contents are the primary agents in suppressing free radicals [63]. Consistent with the current findings, [60] observed that SSP's antioxidant action helped postpone the oxidative deterioration of fish burgers.

#### **The PV index of burgers**

Peroxide is an indicator that represents principal products coming from oxidation, and hydroperoxides are odourless compounds that are the primary oxidation products of fats and oils [63]. When PV was less than 2 meq/kg for food—which shouldn't be more than 5 meq/kg—it was generally seen as a good feature [64]. Fish burger PV evaluation findings are shown in Table 3, where PV was 0.64 meq/kg at the start of storage. Due to fat oxidation and hydroperoxide formation, the PV of all the burger samples increased significantly with time; the control sample had the greatest PV because it had no preservative additive ( $p < .05$ ). Because SSP has a greater phenolic content in fish burgers, it was able to significantly lower the rate of hydroperoxide generation. Additionally, treated samples showed a stronger antioxidant capacity than untreated ones. At the conclusion of storage, samples treated with 3% SSP had the lowest level of PV (1.25 meq/kg), while control had the highest

level (2.64 meq/kg). For the six months that the catfish burgers were on the shelf, the SSP demonstrated protection against lipid oxidation caused by primary and secondary manufacturers. In fact, polyphenols have the ability to stop this cycle of fat oxidation and inhibit free radicals, particularly peroxy radicals, which are among the most reactive reactants [64]. As a result, fewer hydroperoxides are produced. Prior studies have validated SSP's antioxidant capacity [65]. According to reports, the amount of hydroperoxide formed in fish burgers with SSP significantly decreased [60].

### The pH index of burgers

Table 3 presents the results of a systematic investigation of the changes in pH values of the burger samples that were stored at  $-18^{\circ}\text{C}$  for 0, 3, and 6 months. When comparing the treated samples to the control sample, Table 3's data shows an increasing trend in pH values over time, however the control sample shows a fall in the sixth month. The effect that SSP clearly has on pH levels is especially remarkable; Table 4 shows a definite escalation that corresponds with higher concentrations. This observed influence suggests that the additives—in particular, SSP—generated a strong antibacterial effect that decreased the activity of microorganisms. The pH then rose as a result of this. The microbial activity and pH relationship is consistent with findings, [66] which show that the abundance of microbes and pH values are inversely correlated. Moreover, the pH of SSP may be slightly alkaline depending on its polyphenol, sugar, mineral, and flavonoid contents; thus, when applied to a fish product, the pH can be affected. This is consistent with Barkallah *et al.* [60], who found that adding SSP to fish burger raises the pH level. The current study emphasises the role of additives, especially SSP, in modulating pH dynamics and influencing the microbial activity in the stored burger samples.

**Table 3:** Chemical quality characteristics and pH value of Spirulina-fortified catfish burgers during frozen storage at  $-18 \pm 1^{\circ}\text{C}$  for 6 months

Components (%)	Storage period (month)	Treatments			
		C	T1	T2	T3
TVB-N (mg/100g)	0	9.88 $\pm$ 0.24 <sup>Ac</sup>	9.82 $\pm$ 0.66 <sup>Ac</sup>	9.80 $\pm$ 0.46 <sup>Ac</sup>	9.82 $\pm$ 0.44 <sup>Ac</sup>
	3	12.60 $\pm$ 0.90 <sup>Ab</sup>	11.20 $\pm$ 0.42 <sup>Bb</sup>	10.80 $\pm$ 0.45 <sup>Cb</sup>	10.20 $\pm$ 0.36 <sup>Db</sup>
	6	15.34 $\pm$ 0.78 <sup>Aa</sup>	13.50 $\pm$ 0.56 <sup>Ba</sup>	12.20 $\pm$ 0.60 <sup>Ca</sup>	11.70 $\pm$ 0.48 <sup>Da</sup>
TBA (mg malonaldehyde/kg)	0	0.54 $\pm$ 0.02 <sup>Ac</sup>	0.52 $\pm$ 0.04 <sup>Ac</sup>	0.54 $\pm$ 0.03 <sup>Ac</sup>	0.53 $\pm$ 0.04 <sup>Ac</sup>
	3	0.86 $\pm$ 0.04 <sup>Ab</sup>	0.74 $\pm$ 0.02 <sup>Bb</sup>	0.68 $\pm$ 0.02 <sup>Cb</sup>	0.60 $\pm$ 0.03 <sup>Db</sup>
	6	0.95 $\pm$ 0.02 <sup>Aa</sup>	0.82 $\pm$ 0.04 <sup>Ba</sup>	0.76 $\pm$ 0.03 <sup>Ca</sup>	0.68 $\pm$ 0.02 <sup>Da</sup>
peroxide value meq/kg	0	0.64 $\pm$ 0.02 <sup>Ac</sup>	0.62 $\pm$ 0.04 <sup>Ac</sup>	0.64 $\pm$ 0.06 <sup>Ac</sup>	0.63 $\pm$ 0.04 <sup>Ac</sup>
	3	1.90 $\pm$ 0.09 <sup>Ab</sup>	1.56 $\pm$ 0.12 <sup>Bb</sup>	1.22 $\pm$ 0.04 <sup>Cb</sup>	1.02 $\pm$ 0.11 <sup>Db</sup>
	6	2.64 $\pm$ 0.11 <sup>Aa</sup>	1.90 $\pm$ 0.08 <sup>Ba</sup>	1.64 $\pm$ 0.06 <sup>Ca</sup>	1.25 $\pm$ 0.07 <sup>Da</sup>
pH	0	6.33 $\pm$ 0.02 <sup>D</sup>	6.40 $\pm$ 0.05 <sup>Cc</sup>	6.48 $\pm$ 0.04 <sup>Bc</sup>	6.55 $\pm$ 0.02 <sup>Ac</sup>
	3	6.14 $\pm$ 0.05 <sup>Db</sup>	6.50 $\pm$ 0.02 <sup>Ca</sup>	6.54 $\pm$ 0.03 <sup>Bb</sup>	6.64 $\pm$ 0.04 <sup>Ab</sup>
	6	6.02 $\pm$ 0.04 <sup>Da</sup>	6.55 $\pm$ 0.03 <sup>Ca</sup>	6.62 $\pm$ 0.04 <sup>Ba</sup>	6.75 $\pm$ 0.02 <sup>Aa</sup>

\*\* The values are the mean  $\pm$  SD of three calculations. Significant differences ( $p < 0.05$ ) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ( $P \leq 0.05$ ) in the values (means  $\pm$  SD). C: Catfish burger, T1: Catfish burger fortified with 1% Spirulina powder, T2: Catfish burger fortified with 2% Spirulina powder, T3: Catfish burger fortified with 3% Spirulina powder.

### Functional Properties of Fish Burgers

Table 4 displays the functional attributes of the burgers with and without Spirulina. The WHC values of Spirulina-fortified burgers increased considerably ( $p < 0.001$ ) at the start of storage compared to controls, going from 2.22 for the control to 2.64, 2.82, and 3.16 for the 1, 2, and 3% Spirulina-fortified burger formulations, respectively (Table 4). Most likely, the addition of spirulina fibres was the cause of this. The high water content of SSP suggests that it could be utilised as a useful natural element in food treatments to alter the texture and viscosity, lessen food product dehydration during storage, and reduce energy value. Additionally, Table 4 shows that there were significant changes ( $p < 0.001$ ) in the OHC levels for the two varieties of burgers supplemented with spirulina. These OHC levels may be particularly relevant, particularly in relation to the binding of fat during the industrial manufacturing and storage of food. The high capabilities of the microalgae fibres to bind water and oil may be the cause of this [67]. Furthermore, the burgers with 3% Spirulina fortification had the highest



SWC values (4.04 mL/g) ( $p < 0.001$ ). At the start of storage, the control burgers' swelling capacity (3.07 mL/g) was significantly lower (Table 4). All fish burgers' WHC, OHC, and SWC values dropped while they were frozen. Furthermore, the structural characteristics of each material following the addition of spirulinafibres may account for the discrepancy in the functional capacities of the control and 1% SSP formulations. The findings align with earlier research conducted by Barkallah et al. [60].

**Table 4:** Functional parameters of Spirulina-fortified catfish burgers during frozen storage at  $-18 \pm 1$  °C for 6 months

Components (%)	Storage period (month)	Treatments			
		C	T1	T2	T3
SWC (mL/g DW)	0	3.07 $\pm$ 0.12 <sup>Da</sup>	3.48 $\pm$ 0.09 <sup>Ca</sup>	3.82 $\pm$ 0.16 <sup>Ba</sup>	4.04 $\pm$ 0.14 <sup>Aa</sup>
	3	2.85 $\pm$ 0.11 <sup>Db</sup>	3.04 $\pm$ 0.08 <sup>Cb</sup>	3.50 $\pm$ 0.14 <sup>Bb</sup>	3.74 $\pm$ 0.12 <sup>Ab</sup>
	6	2.60 $\pm$ 0.14 <sup>Dc</sup>	2.85 $\pm$ 0.11 <sup>Cc</sup>	3.12 $\pm$ 0.12 <sup>Bc</sup>	3.35 $\pm$ 0.09 <sup>Ac</sup>
OHC (g/g DW)	0	0.88 $\pm$ 0.04 <sup>Da</sup>	1.02 $\pm$ 0.05 <sup>Ca</sup>	1.14 $\pm$ 0.03 <sup>Ba</sup>	1.30 $\pm$ 0.05 <sup>Aa</sup>
	3	0.76 $\pm$ 0.02 <sup>Db</sup>	0.94 $\pm$ 0.04 <sup>Cb</sup>	1.02 $\pm$ 0.02 <sup>Bb</sup>	1.16 $\pm$ 0.02 <sup>Ab</sup>
	6	0.62 $\pm$ 0.05 <sup>Dc</sup>	0.85 $\pm$ 0.06 <sup>Cc</sup>	0.95 $\pm$ 0.06 <sup>Bc</sup>	1.03 $\pm$ 0.04 <sup>Ac</sup>
WHC (g/g DW)	0	2.22 $\pm$ 0.11 <sup>Da</sup>	2.64 $\pm$ 0.09 <sup>Ca</sup>	2.82 $\pm$ 0.16 <sup>Ba</sup>	3.16 $\pm$ 0.06 <sup>Aa</sup>
	3	2.04 $\pm$ 0.12 <sup>Db</sup>	2.46 $\pm$ 0.12 <sup>Cb</sup>	2.74 $\pm$ 0.12 <sup>Bb</sup>	2.95 $\pm$ 0.11 <sup>Ab</sup>
	6	1.92 $\pm$ 0.11 <sup>Dc</sup>	2.33 $\pm$ 0.09 <sup>Cc</sup>	2.62 $\pm$ 0.08 <sup>Bc</sup>	2.86 $\pm$ 0.12 <sup>Ac</sup>

\*\* The values are the mean  $\pm$  SD of three calculations. Significant differences ( $p < 0.05$ ) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ( $P \leq 0.05$ ) in the values (means  $\pm$ SD). C: Catfish burger, T1: Catfish burger fortified with 1% Spirulina powder, T2: Catfish burger fortified with 2% Spirulina powder, T3: Catfish burger fortified with 3% Spirulina powder

#### Texture analyzer of burgers

In fact, one of the most popular approaches for diagnosing and treating swallowing problems is textural modification. It is a crucial factor that determines the organoleptic quality of food items. The impact of 1, 2, and 3% SSP addition on the textural characteristics of fish burgers is displayed in Table 5. At the start of storage, the hardness of the burgers increased from 7.80 to 11.30, 13.05, and 15.12 N ( $p < 0.001$ ) when 1, 2, and 3% SSP was added, in comparison to the control burgers. This is most likely due to the compositional variations between unfortified and burgers fortified with spirulina, which provide distinct protein/fat/water ratios and ultimately determine the gel consistency [68]. These findings are consistent with studies on burgers with additional SSP or seaweeds [60,69]. Moreover, SSP fibres and polysaccharides can have a significant impact on how hard these processed products are made. Controversial findings about the hardness of food products have been reported, depending on the type and quantity of fibres. Thus, adding fibres to different cooked food products has been shown to cause both hardening and softening [70]. The addition of microalga resulted in a significant ( $p < 0.001$ ) increase in chewiness (Table 5). These outcomes compare favourably to the experimental data that [60] collected. When SSP was added, cohesiveness values rose, and there were statistically significant differences between the samples that were fortified with Spirulina and the control group.

**Table 5:** Texture analyzer for Spirulina-fortified catfish burgers during frozen storage at  $-18 \pm 1$  °C for 6 months

Texture profile	Storage period (month)	Treatments			
		C	T1	T2	T3
Hardness (N)	0	7.80 $\pm$ 0.14 <sup>Dc</sup>	11.30 $\pm$ 0.20 <sup>Cc</sup>	13.05 $\pm$ 0.16 <sup>Bc</sup>	15.12 $\pm$ 0.14 <sup>Ac</sup>
	3	8.04 $\pm$ 0.24 <sup>Db</sup>	11.90 $\pm$ 0.16 <sup>Cb</sup>	13.95 $\pm$ 0.24 <sup>Bb</sup>	16.04 $\pm$ 0.33 <sup>Ab</sup>
	6	8.66 $\pm$ 0.12 <sup>Da</sup>	12.40 $\pm$ 0.22 <sup>Ca</sup>	14.66 $\pm$ 0.32 <sup>Ba</sup>	16.88 $\pm$ 0.22 <sup>Aa</sup>
Elasticity (cm)	0	0.44 $\pm$ 0.02 <sup>Da</sup>	0.62 $\pm$ 0.05 <sup>Ca</sup>	0.84 $\pm$ 0.03 <sup>Ba</sup>	0.92 $\pm$ 0.03 <sup>Aa</sup>
	3	0.52 $\pm$ 0.04 <sup>Db</sup>	0.75 $\pm$ 0.08 <sup>Cb</sup>	0.96 $\pm$ 0.04 <sup>Bb</sup>	1.02 $\pm$ 0.07 <sup>Ab</sup>
	6	0.60 $\pm$ 0.06 <sup>Da</sup>	0.84 $\pm$ 0.05 <sup>Ca</sup>	1.04 $\pm$ 0.06 <sup>Ba</sup>	1.16 $\pm$ 0.05 <sup>Aa</sup>
Chewiness	0	0.95 $\pm$ 0.08 <sup>Da</sup>	2.33 $\pm$ 0.12 <sup>Ca</sup>	3.80 $\pm$ 0.16 <sup>Ba</sup>	4.92 $\pm$ 0.18 <sup>Aa</sup>

(N.cm)	3	1.02±0.14 <sup>Db</sup>	2.64±0.18 <sup>Cb</sup>	3.96±0.14 <sup>Bb</sup>	5.08±0.16 <sup>Ab</sup>
	6	1.18±0.18 <sup>Da</sup>	2.98±0.16 <sup>Ca</sup>	4.24±0.20 <sup>Ba</sup>	5.42±0.14 <sup>Aa</sup>
Gumminess (N)	0	1.72±0.15 <sup>Dc</sup>	4.46±0.18 <sup>Cc</sup>	6.38±0.24 <sup>Bc</sup>	7.60±0.33 <sup>Ac</sup>
	3	1.88±0.14 <sup>Db</sup>	4.70±0.24 <sup>Cb</sup>	6.68±0.33 <sup>Bb</sup>	7.92±0.24 <sup>Ab</sup>
	6	1.96±0.32 <sup>Da</sup>	4.92±0.36 <sup>Ca</sup>	6.84±0.25 <sup>Ba</sup>	8.04±0.32 <sup>Aa</sup>
Cohesion	0	0.320±0.001 <sup>Da</sup>	0.418±0.002 <sup>Ca</sup>	0.492±0.001 <sup>Ba</sup>	0.576±0.004 <sup>Aa</sup>
	3	0.380±0.003 <sup>Db</sup>	0.440±0.005 <sup>Cb</sup>	0.536±0.002 <sup>Bb</sup>	0.590±0.003 <sup>Ab</sup>
	6	0.450±0.002 <sup>Da</sup>	0.485±0.004 <sup>Ca</sup>	0.568±0.003 <sup>Ba</sup>	0.622±0.002 <sup>Aa</sup>

\* The values are the mean ± SD of three calculations. Significant differences ( $p < 0.05$ ) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ( $P \leq 0.05$ ) in the values (means ±SD). C: Catfish burger, T1: Catfish burger fortified with 1% Spirulina powder, T2: Catfish burger fortified with 2% Spirulina powder, T3: Catfish burger fortified with 3% Spirulina powder

The cohesiveness results of burgers made with spirulina were similar to those of fish burgers made with 1% (w/w) SSP [60]. All of the fish burgers' textural amendment values rose while they were frozen. Because SSP did not mischaracterise the textural features that the panellists already knew and acknowledged, the aforementioned texture results might be deemed satisfactory in the industry [60].

### Antioxidant Properties of Fish Burgers

Due to their effectiveness and safety in treating a variety of human ailments, a large portion of enhanced meals now contain bioactive compounds, which are often obtained from plants, seaweeds, and microalgae. Actually, the abundance of both enzymatic and non-enzymatic free radical scavengers in microalgae was what gave rise to their antioxidant activity. Therefore, two distinct techniques were employed to quantify the contribution of SSP to the burger antioxidant capacities: the TPC and the DPPH free radical-scavenging assays. Table 6 provides an overview of the outcomes of different techniques. The burgers with SSP added had considerably higher antioxidant activity ( $p < 0.05$ ), as well as higher amounts of chlorophyll ( $p < 0.05$ ), carotenoid ( $p < 0.05$ ), and phycocyanin ( $p < 0.05$ ) at the start of storage when compared to the control burgers (Table 6). Moreover, it was evident that antioxidant activity and pigment contents were positively correlated. Burgers with 1, 2, and 3% SSP in the sample had DPPH scavenging activities that were substantially higher (60.50, 76.20, and 84.00%, respectively) than control burgers (45.80%) ( $p < 0.05$ ) (Table 6). Additionally, a similar pattern was observed for TPC. Specifically, the TPC for fish burgers that were unfortified and fortified with 1, 2, and 3% SSP were 8.20 and 14.50, 21.20 and 27.60 mg/100g ( $p < 0.05$ ), respectively. All fish burgers' antioxidant activity and pigment level dropped while they were frozen. In this sense, the immulina polysaccharides from Spirulina improve the enzymatic activity of the cell nucleus and synthesis of DNA repair, in addition to being a beneficial species for the immune system [71]. These results also showed that fish burger processing did not have a negative effect on the antioxidant components. The rise in scavenging of free radicals may be attributed to the increase in carotenoids [72], chlorophylls [73], phycocyanin [66], and polysaccharides [74] contents. The results of burgers formulated with SSP were comparable with the values reported by Barkallah *et al.* [60] for fish burgers formulated with 1% (w/w) SSP.

**Table 6:** Antioxidant activities and pigments content of Spirulina-fortified catfish burgers during frozen storage at  $-18 \pm 1$  °C for 6 months

Components (%)	Storage period (month)	Treatments			
		C	T1	T2	T3
Chlorophylls (mg/100g DW)	0	0.00	21.70±1.2 <sup>Ca</sup>	38.40±1.56 <sup>Ba</sup>	55.50±1.7 <sup>Aa</sup>
	3	0.00	17.50±1.4 <sup>Cb</sup>	34.20±1.3 <sup>Bb</sup>	50.40±1.52 <sup>Ab</sup>
	6	0.00	13.20±1.1 <sup>Cc</sup>	30.70±1.22 <sup>Bc</sup>	46.30±1.43 <sup>Ac</sup>
Phycocyanin (mg/100g DW)	0	0.00	0.442±0.002 <sup>Ca</sup>	0.820±0.003 <sup>Ba</sup>	1.08±0.002 <sup>Aa</sup>
	3	0.00	0.396±0.004 <sup>Cb</sup>	0.754±0.003 <sup>Bb</sup>	0.960±0.004 <sup>Ab</sup>
	6	0.00	0.322±0.003 <sup>Cc</sup>	0.712±0.002 <sup>Bc</sup>	0.890±0.005 <sup>Ac</sup>

<b>Carotenoids (mg/100g DW)</b>	<b>0</b>	0.00	13.60±0.55 <sup>Ca</sup>	24.50±1.02 <sup>Ba</sup>	32.90±1.14 <sup>Aa</sup>
	<b>3</b>	0.00	10.40±0.42 <sup>Cb</sup>	19.50±0.77 <sup>Bb</sup>	27.90±0.95 <sup>Ab</sup>
	<b>6</b>	0.00	7.20±0.65 <sup>Cc</sup>	14.80±0.85 <sup>Bc</sup>	23.60±0.80 <sup>Ac</sup>
<b>TPC mg/100g</b>	<b>0</b>	8.20±0.22 <sup>Da</sup>	14.50±1.36 <sup>Ca</sup>	21.20±1.44 <sup>Ba</sup>	27.60±1.80 <sup>Aa</sup>
	<b>3</b>	5.80±0.84 <sup>Db</sup>	10.40±0.90 <sup>Cb</sup>	18.60±1.02 <sup>Bb</sup>	23.80±1.22 <sup>Ab</sup>
	<b>6</b>	2.90±0.92 <sup>Dc</sup>	8.20±0.75 <sup>Cc</sup>	14.50±1.14 <sup>Bc</sup>	18.30±1.12 <sup>Ac</sup>
<b>Scavenging activity (%) *</b>	<b>0</b>	45.80±2.4 <sup>Da</sup>	60.50±1.9 <sup>Ca</sup>	76.20±3.2 <sup>Ba</sup>	84.00±2.5 <sup>Aa</sup>
	<b>3</b>	40.30±1.8 <sup>Db</sup>	55.20±2.7 <sup>Cb</sup>	70.50±1.9 <sup>Bb</sup>	78.70±2.6 <sup>Ab</sup>
	<b>6</b>	33.50±2.2 <sup>Dc</sup>	41.40±2.6 <sup>Cc</sup>	64.30±2.0 <sup>Bc</sup>	72.60±2.4 <sup>Ac</sup>

\* The values are the mean ± SD of three calculations. Significant differences ( $p < 0.05$ ) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ( $P \leq 0.05$ ) in the values (means ±SD). C: Catfish burger, T1: Catfish burger fortified with 1% Spirulina powder, T2: Catfish burger fortified with 2% Spirulina powder, T3: Catfish burger fortified with 3% Spirulina powder

### Microbiological Quality Standards for Spirulina-fortified catfish burgers

Meat and fish products are prone to contamination and microbial growth, which can result in spoilage and the potential for foodborne disease transmission. Table 7 details the effects of different SSP concentrations (0%, 1%, 2% and 3%) on total bacterial, total yeast and mould, total coliform group, and total staph counts in fish burgers over 0, 3, and 6 months at  $-18^{\circ}\text{C}$ .

For the corresponding storage times, the logarithmic values of total bacteria for the 1% SSP treatment were 5.05, 3.84, and 2.72. The 2% SSP treatments showed values of 5.06, 3.30, and 2.60 in accordance. The logarithmic values for the 3% SSP treatments were 5.08, 2.80, and 2.48. By comparison, the control group displayed values of 5.12, 4.92, and 4.40. The addition of SSP significantly decreased ( $P \leq 0.05$ ) the logarithmic total bacteria count.

**Table 7:** Change in microbial counts (log<sub>10</sub> cfu/g) of Spirulina-fortified catfish burgers during frozen storage for 6 months

<b>Components (%)</b>	<b>Storage period (month)</b>	<b>Treatments</b>			
		<b>C</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>
<b>Total bacterial count (log<sub>10</sub> cfu/g)</b>	<b>0</b>	5.12±0.20 <sup>Aa</sup>	5.05±0.12 <sup>Aa</sup>	5.06±0.08 <sup>Aa</sup>	5.08±0.09 <sup>Aa</sup>
	<b>3</b>	4.92±0.24 <sup>Ab</sup>	3.84±0.40 <sup>Ba</sup>	3.30±0.32 <sup>Ca</sup>	2.80±0.25 <sup>Da</sup>
	<b>6</b>	3.45±0.30 <sup>Ac</sup>	2.72±0.26 <sup>Bb</sup>	2.60±0.33 <sup>Cb</sup>	2.48±0.22 <sup>Db</sup>
<b>Total yeast and mould count (log<sub>10</sub> cfu/g)</b>	<b>0</b>	3.85±0.26 <sup>Aa</sup>	3.86±0.28 <sup>Aa</sup>	3.85±0.30 <sup>Aa</sup>	3.86±0.24 <sup>Aa</sup>
	<b>3</b>	3.12±0.12 <sup>Ab</sup>	2.70±0.11 <sup>Ba</sup>	2.60±0.20 <sup>Ca</sup>	2.54±0.16 <sup>Da</sup>
	<b>6</b>	2.88±0.15 <sup>Ac</sup>	1.20±0.02 <sup>Bb</sup>	1.14±0.07 <sup>Cb</sup>	1.02±0.14 <sup>Db</sup>
<b>Total Coliform group count (log<sub>10</sub> cfu/g)</b>	<b>0</b>	4.02±0.14 <sup>Aa</sup>	4.14±0.15 <sup>Aa</sup>	4.12±0.07 <sup>Aa</sup>	4.11±0.16 <sup>Aa</sup>
	<b>3</b>	3.74±0.11 <sup>Ab</sup>	3.42±0.14 <sup>Ba</sup>	3.30±0.16 <sup>Ca</sup>	3.21±0.19 <sup>Da</sup>
	<b>6</b>	3.22±0.18 <sup>Ac</sup>	2.84±0.12 <sup>Bb</sup>	2.50±0.11 <sup>Cb</sup>	2.38±0.12 <sup>Db</sup>
<b>Total Staphylococcus count (log<sub>10</sub> cfu/g)</b>	<b>0</b>	4.40±0.12 <sup>Aa</sup>	4.36±0.11 <sup>Aa</sup>	4.38±0.14 <sup>Aa</sup>	4.36±0.08 <sup>Aa</sup>
	<b>3</b>	4.12±0.20 <sup>Ab</sup>	3.70±0.32 <sup>Ba</sup>	3.50±0.24 <sup>Ca</sup>	3.25±0.15 <sup>Da</sup>
	<b>6</b>	3.40±0.17 <sup>Ac</sup>	1.60±0.15 <sup>Bb</sup>	1.48±0.11 <sup>Cb</sup>	1.36±0.18 <sup>Db</sup>

\* The values are the mean ± SD of three calculations. Significant differences ( $p < 0.05$ ) exist between means with different superscripts in the same row A–D and the same column a–c. Different superscript letters indicate statistically significant differences ( $P \leq 0.05$ ) in the values (means ±SD). C: Catfish burger, T1: Catfish burger fortified with 1% Spirulina powder, T2: Catfish burger fortified with 2% Spirulina powder, T3: Catfish burger fortified with 3% Spirulina powder.

The findings, which are presented in Table 8, indicate that the addition of SSP significantly decreased the logarithmic yeast and mould count, outperforming the control group for the whole storage period. The logarithmic values of the total yeast and mould count for various time intervals (0, 3, and 6 months) and for various SSP concentrations (1%, 2%, and 3%). In particular, the logarithmic values for the 1% *C. vulgaris* treatments were 3.86, 2.70, and 1.20 for the corresponding storage times. Likewise, the treatments with 2% SSP showed values of 3.85, 2.60, and 1.14. The logarithmic values for the 3% SSP treatments were 3.86, 2.54, and 1.02. For the 1% SSP treatments, the logarithmic values of the total coliform group count were 4.14, 3.42, and 2.84 for the corresponding storage times. The 2% SSP treatments showed values of 4.12, 3.30, and 2.50 in simultaneously. The logarithmic values for the 3% SSP treatments were 4.11, 3.21, and 2.38. By contrast, the control group

displayed scores of 4.02, 3.74, and 3.22, in that order. Over the course of the corresponding storage periods, the logarithmic values of the total staph count for the 1% SSP treatments were found to be 4.36, 3.70, and 1.60. The 2% SSP treatment showed values of 4.38, 3.50, and 1.48 in accordance. The logarithmic values for the 3% SSP treatments were 4.36, 3.25, and 1.36. The control group, on the other hand, had values of 4.40, 4.12, and 3.40, respectively. The results clearly show that over the frozen burger's storage period, the logarithms of the total bacterial, total yeast and mould, total coliform group, and total staph counts all reduced significantly ( $P \leq 0.05$ ) as the SSP concentration increased. By the end of the storage period, the SSP treatments showed a significantly larger drop in the logarithm of the total bacterial count, total yeast and mould count, total coliform group count, and total staph count than the control. Thus, the use of SSP can be assumed to have the capacity to extend the shelf life of hamburgers. The findings align with earlier research conducted by [60, 66].

### Sensory evaluation results

Consumer acceptance of food products is significantly influenced by sensory characteristics [12]. Overall, unfavourable changes resulted in microbial growth, fat oxidation, and protein structure degradation during storage, and consumer acceptance of the product was decreased [41]. These changes included colour, taste, odour, and texture, and they were linked to a reduction in the product's shelf life [30]. The sensory characteristics results for fish burgers are shown in Table 8. The fish burgers' flavour, aroma, colour, and texture were assessed. Table 8 displays the results. The hedonic results generally indicated that catfish burgers enhanced with 1 and 2% (w/v) of SSP received higher scores. In comparison to formulations with lower SSP concentrations ( $p < 0.05$ ) and controls ( $p < 0.05$ ), formulations with the highest SSP concentration (3%) statistically had worse sensory acceptance for most organoleptic parameters (Table 8).

**Table 8:** Sensory evaluation of supplemented catfish burger during frozen storage (at  $-18^\circ\text{C}$  for 6 months).

Components (%)	Storage period (month)	Treatments			
		C	T1	T2	T3
Color	0	8.50±0.22 <sup>Aa</sup>	8.40±0.30 <sup>ABa</sup>	8.00±0.26 <sup>Ba</sup>	6.80±0.17 <sup>Ca</sup>
	3	7.50±0.14 <sup>Ab</sup>	7.10±0.18 <sup>Bb</sup>	6.80±0.20 <sup>Cb</sup>	6.60±0.15 <sup>Da</sup>
	6	7.00±0.14 <sup>Ac</sup>	6.70±0.16 <sup>Bc</sup>	6.50±0.18 <sup>Cc</sup>	6.40±0.22 <sup>Cc</sup>
Taste	0	7.50±0.24 <sup>Aa</sup>	7.30±0.22 <sup>ABa</sup>	7.20±0.23 <sup>ABa</sup>	6.70±0.22 <sup>Ba</sup>
	3	7.30±0.20 <sup>Ab</sup>	7.10±0.18 <sup>ABb</sup>	7.0±0.30 <sup>ABb</sup>	6.60±0.28 <sup>Bb</sup>
	6	7.10±0.22 <sup>Ac</sup>	6.80±0.27 <sup>ABc</sup>	6.70±0.25 <sup>ABc</sup>	6.55±0.34 <sup>Bc</sup>
Odor	0	8.00±0.30 <sup>BCa</sup>	8.20±0.25 <sup>B</sup>	8.50±0.22 <sup>ABa</sup>	8.60±0.24 <sup>Aa</sup>
	3	7.70±0.24 <sup>Ba</sup>	8.0±0.32 <sup>ABc</sup>	8.10±0.22 <sup>ABb</sup>	8.30±0.25 <sup>Ab</sup>
	6	7.40±0.25 <sup>Bb</sup>	7.70±0.23 <sup>ABd</sup>	7.80±0.24 <sup>ABd</sup>	8.0±0.32 <sup>Ac</sup>
Texture	0	7.50±0.22 <sup>Ba</sup>	7.70±0.36 <sup>ABa</sup>	7.80±0.24 <sup>ABa</sup>	8.00±0.20 <sup>Aa</sup>
	3	7.20±0.33 <sup>Bb</sup>	7.40±0.20 <sup>ABb</sup>	7.50±0.32 <sup>ABb</sup>	7.70±0.33 <sup>Ab</sup>
	6	7.0±0.20 <sup>Bc</sup>	7.10±0.22 <sup>ABc</sup>	7.30±0.25 <sup>ABc</sup>	7.50±0.22 <sup>Ac</sup>

\* Values (means ±SD) with different superscript letters are statistically significantly different ( $P \leq 0.05$ ). C: Catfish burger, T1: Catfish burger fortified with 1% Spirulina powder, T2: Catfish burger fortified with 2% Spirulina powder, T3: Catfish burger fortified with 3% Spirulina powder

The chemicals that result from lipid oxidation and the minerals that not only function as pro-oxidant molecules but may also give off undesired metallic off-flavors are linked to the incorrect taste brought on by high concentrations of SSP supplementation [75]. However, taste ratings for catfish burgers made with 1% and 2% SSP were comparable to those of fish burgers without SSP ( $p > 0.05$ ). Depending on the amount of microalga used, the colour of the catfish burgers changed from yellowish white to green. Panellists regarded this attribute (colour appearance) as an unacceptable sensory aspect ( $p < 0.05$ ). The fact that batches rich in SSP had an increase in the green colour as the number of chlorophylls increased—a colour that differs greatly from that of a control fish burger—might help to explain the decrease in colour pleasantness. Regarding texture, some panellists stated that adding more SSP improved the burger's texture; nevertheless, these differences were only statistically significant ( $p < 0.05$ ) when compared to the controls for catfish formulations. Overall, the sensory quality of fish burgers was reduced by treatments containing 3% SSP, particularly when it came to taste and colour. These findings suggest that 1% SSP concentration is the cutoff point at which the sensory quality of fish items is not significantly affected; panellists were nevertheless content with fish burgers that had 2% more SSP. The findings clearly show that as the frozen storage period increased, all fish burgers' sensory qualities declined.

## Conclusions

Spirulina appears to be a promising technological substitute for making fish products, like catfish burgers, since it could enhance the perception of these goods among customers as being natural and healthful. In addition to being an essential part of diets, spirulina is a good source of natural colouring and antioxidants. Additionally, spirulina, which is high in dietary fibre, enhances the functional qualities of finished fish products, such as their ability to hold onto water and oil, and is crucial for maintaining their texture. The results of the current study indicate that adding 2% spirulina to catfish burgers improved the chemical composition, texture, microbiological, phytochemicals, and sensory properties of the resulting burgers during frozen storage up to 6 months. Limitations of the study include the need for further sensory experiments or shelf life studies under different storage conditions. Future research directions, such as optimizing the concentration of spirulina powder for various fish formulations, should be suggested.

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