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ORIGINAL RESEARCH PAPER

QUALITY OF CATFISH LUNCHEON SUPPLEMENTED WITH MARINE ALGAE (*ULVA LACTUCA* AND *NANNOCHOLOROPSIS OCULATA*) DURING FROZEN STORAGE

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Abstract

This study investigated the composition of marine algae *(Ulva lactuca and Nannochloropsis oculata)* and evaluated the effect of using their powders instead of wheat flour, as a good source of marine oil, fiber, chlorophyll, and gelling and thickening agents, on the quality of catfish luncheon product during three months of frozen storage. Addition of 10% of *U. lactuca* and. *Nannochloropsis oculata* powders to catfish luncheon decreased protein slightly (28.43 and 28.36%, respectively) while fat and caloric value were increased ($P \leq 0.05$) with the addition of *Nannochloropsis oculata* (43.3% and 575.82 Kcal/ 100g) and decreased with the *Ulva lactuca* (38.51% and 539.63 Kcal/ 100g, respectively). TBARS values were still low (<1 mg MDA/Kg) while TVB-N decreased slightly in both supplemented catfish luncheon (9.89 and 16.14 mg/100g) compared with control. Lightness, redness and yellowness values were decreased ($P \leq 0.05$) in both supplemented catfish luncheon. Addition of *Nannochloropsis oculata* decreased hardness, gumminess and chewiness in catfish luncheon while the addition of *Ulva lactuca* increased these parameters. In most sensory properties, both supplemented catfish luncheon with algae had higher scores than control throughout 3 months of storage at -10±2°C. New catfish luncheon products formulated with algae had good microbial properties under frozen storage.

Keywords: *Ulva lactuca*, *Nannochloropsis oculata*, catfish luncheon, caloric value, colour values, texture parameters

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Introduction

Luncheon roll is an important industrial meat product which is ready to eat with a distinctive taste and preferred by consumers of different ages. The uncured luncheon is prepared by emulsifying the minced meat with fat and non-meat binders besides with or without adding cereals then packed as roll before heating process (Goethals *et al*., 2020). The functional properties like water and fat binding, cooking yield and slicing ability are affected by the binder and cereal added in product formula (Farag, 2023). Luncheon meat composed of 60% moisture, 15% protein, 35% fat as the EOS (2023) for luncheon meat.

Recently, there has been a need to use healthy alternative ingredients in luncheon processing since the costs of meat production have increased and the processors tend to use less desirable meat cuts and trims beside the appearing of health hazards from saturated beef fat and chemical additives such as curing agents. Also, due to the increasing of awareness with health and nutrition prospects, consumers tend to prefer fish products more than meat products (Morales and Higuchi, 2018).

Egypt ranked first in fish farming production in Africa and catfish (*Clarias gariepinus*) production was 14,681 tons in 2019 but this species has low economic value due to its unpalatable taste (Mohamed *et al*., 2022). The inclusion of catfish flesh as alternative to meat in luncheon formula will raise the added value of the catfish and support the luncheon with fish protein.

Algae are a versatile and sustainable source and about 200 algae have been allowed to be consumed according to the European regulations (European Comission, 2022). Some macroalgal and microalgal were considered healthy foods due to important nutrients that are essential to the human body such as omega fatty acids, vitamins, fibers and minerals (Zhou *et al*., 2022). Globally, inserting algae biomass in food products is growing in relation to its functional benefits (Boukid and Castellari, 2022). Algae contain polysaccharides like alginate, ulvan and carrageenan which play a crucial role in gel forming and thickening texture of products (Ścieszka and Klewicka, 2019).

The use of fish and algae opens up new opportunities for better food manufacturing of fish-luncheon products, due to their chemical composition, nutritional value, and bioactive components with potential health benefits. The objectives of this study are to formulate new algae catfish luncheon products by substituting flour with *Ulva lactuca* (macroalgae) or *Nannocholoropsis oculata* (microalgae) and investigate their quality and acceptability throughout three months of frozen storage (-10° ±2°C) in comparison to a control product without algae.

Materials and methods

Materials

Powdered seaweed (*Ulva lactuca*) and green algae (*Nannocholoropsis oculata*) were prepared as described in our previous research (Metwaly *et al*., 2023). Twenty kilograms of catfish (*Clarias gariepinus*) was purchased from a local fish market in

Suez Governorate (Egypt). The average length and weight of catfish ranged from 43 to 45 cm and from 0.550 to 0.750 kg, respectively.

All the fresh algae and fish samples were transported on ice (1:1 sample to ice ratio) within 1h to the Fish Processing whose Unit belongs to the faculty of Fish Resources, Suez University, Egypt.

All chemicals (hydrochloric acid (HCl), sodium hydroxide (NaOH), boric acid, glacial acetic acid, methanol and chloroform) were purchased from Piochem Chemicals, Egypt. Potato Dextrose Agar and Plate Count Agar media were obtained from Oxoid, UK. All chemicals and media used in this study were analytical grade type.

Wheat flour (72%), black pepper powder, dried onion, dried garlic, paprika, common salt (NaCl), egg, corn oil, chicken bouillon, creamy cheese, low density polyethylene (LDPE) bags, and aluminum Foil (AL–F) were purchased from the local market in Suez government, Egypt.

Preparation of the catfish luncheon products

The catfish fillets were manually separated and immersed in a brine solution (5%) for 10 minutes. Then, the fillets (about 8 kg) were minced twice in an electric meat mincer (SH-C77, Sonai grinder) with a 3 mm holes plate. The minced catfish flesh 36%, garlic powder 0.7%, paprika 0.3%, onion powder 0.5%, black pepper powder 0.5%, chicken bouillon 3%, whole egg 26%, creamy cheese 10% and corn oil 13% were mixed by hand and ground twice through a 4 mm plate. Three catfish luncheon formulas were prepared based on adding wheat flour or substituted with one alga where: catfish luncheon formula containing 10% wheat flour is control (C), the catfish luncheon formula containing 10% *Nannochloropsis oculata* is (N) and the catfish luncheon containing 10% *Ulva lactuca* is (U). Each formula was packed in LDPE bags and wrapped in AL- F then immersed in boiling water for 1 h and cooled in an ice-water bath for 10 min. These thermally processed products were stored under vacuum in PE bags at -10±2°C for three months.

Proximate composition

Moisture, protein, ash and sodium chloride constituents (NaCl) were determined according to AOAC (2019). Fat content was determined following the procedure of Ali *et al*. (2017) using a solvent mixture (chloroform: methanol, 2:1 v/v). Carbohydrates were computed by difference. Caloric value of each sample was computed depending on its chemical composition using the following equation (Ershidat *et al*., 2024).

$$\text{Caloric value (kcal/100g)} = [(\text{P} \times 4) + (\text{L} \times 9) + (\text{C} \times 4)] \qquad (1)$$

were P=proteins, L=lipids, C= carbohydrates

Total Chlorophyll and carotenoid (Car) determination

Macro and microalgae total chlorophyll and carotenoid were determined using the Unico UV spectrophotometer by following the procedures in Metwaly *et al*. (2023). The Chl-*a*, Chl-*b* and Car $\mu g \cdot mL^{-1}$ calculated depending on the following equations:

$$\mathrm{Chl-a} = 10.3\ \mathrm{E}_{665} - 0.918\ \mathrm{E}_{650} \quad (2)$$

$$\mathrm{Chl-b} = 19.7\ \mathrm{E}_{650} - 4.87\ \mathrm{E}_{665} \quad (3)$$

$$\mathrm{Car} = 4.2\ \mathrm{E}_{452} - (0.0246\ \mathrm{Chl-a} + 0.426\ \mathrm{Chl-b}) \quad (4)$$

Total polyphenols determination

The total polyphenol content in macro- and microalgae was measured using UV-Vis spectrophotometry with the Folin-Ciocalteu reagent, according to Elsebaie and Essa (2018). Using the gallic acid calibration curve as a reference, the absorbance at 760 nm was calculated.

Thiobarbituric acid reactive substances (TBARS) determination

TBARS measurement was performed to evaluate the extent of lipid oxidation following the distillation method as described by Elsebaie *et al.* (2022). Thiobarbituric acid (TBARS) reagent reacted with malonaldhyde that was found in distilled samples and resulted in a colour compound after 35 min in a boiling water bath (Elsebaie *et al.*, 2022). The absorbance (O.D) of the formed pink colour was measured against a blank at 537 nm using a spectrophotometer (T60 UV-Visible Spectrophotometer). TBARS value was computed as follows:

$$\mathrm{TBARS\ (mg\ MAD/\ kg\ sample)} = 7.8 \times \mathrm{O.D} \quad (5)$$

Total volatile basic nitrogen (TVB-N) determination

TVB-N value was estimated by distillation method (Aly and Morsy, 2019). The distillate solutions were neutralized with HCl and the TVBN content of the samples was determined using the following equation:

$$\mathrm{mg\ of\ TVB\text{-}N/100g} = \frac{\mathrm{ml\ 0.1\ N\ HCl} \times 1.4 \times 100}{\mathrm{weight\ of\ sample}} \quad (6)$$

pH measurement

pH value was determined at 0, 1, 2 and 3 months of storage. pH value was determined on a homogenized sample (10 g) in distilled water (90 mL) using a calibrated pH meter (2100 Bench pH meter, OHAUS Instruments, USA) (AOAC, 2019).

Water holding capacity (WHC) and oil binding capacity (OBC)

Water holding capacity (WHC) and oil binding capacity (OBC) of catfish luncheon samples were measured according to Samard and Ryu (2019). In brief, 1.0 g of each sample was combined with 10 mL of distilled water or olive oil and vortexed for 30 sec. After standing at room temperature for 30 min, the mixture was then centrifuged for 30 min at 4000 rpm, and the surplus water or oil was decanted before the sample was weighed.

$$\mathrm{WHC\ or\ OBC\ (\%)} = \frac{\mathrm{Final\ weight\text{–}\ Initial\ weight}}{\mathrm{Initial\ weight}} \times 100 \quad (7)$$

Texture profile analysis

Texture profile analysis was carried out by a texture analyzer (CT3, Brookfield USA) (Essa and Elsebaie, 2022). Catfish luncheon samples (1cm height × 2cm diameter) were centered on the plate of texture analyzer. Two successive cycles of 30%

compression were applied axially. The test was conducted using the probe TA44, a trigger load of 5 g, a load cell of 10 kg, a test speed of 1 mm/s, a pretest speed of 2 mm/s, a posttest speed of 2 mm/s, and a distance of 5 mm. The textural criteria determined were hardness (N), adhesive force (N), cohesiveness, springiness (mm), Gumminess (N), and chewiness (mJ).

Color measurements

Color of catfish luncheon samples were performed using Ultra Scan VIS (Hunter Lab, USA) spectrophotometer in the complete CIE recommended spectral range. The samples were placed in the sample holder and scanned in the wavelength range between 360 and 780 nm and the reflectance spectra were collected. The colour measurements were collected in CIE-Lab system that describes colour coordinates L* value that represents lightness [ranges from 0 (black) to 100 (white)], a* value that represent redness (+a) and greenness (-a), and b* value hat represent blueness (+b) and yellowness (-b).

Microbiological analysis

Each representative sample (10 g) was diluted and homogenized for 2.5 min at 1400 rpm in 0.1% peptone water (90 mL) (DM185D, MAST, UK) and was considered as dilution 10^{-1} from which subsequent serial dilutions up to 10^{-6} were prepared. The total aerobic mesophilic bacteria and psychrophilic bacteria counts were determined on Plate Count Agar (Oxoid, UK) media using the spread plate method. The incubated plates at 35±2°C for 48 hours and at 7°C for 10 days in the JSGI-100T incubator were examined for total mesophilic bacterial counts and psychrophilic bacterial counts, respectively (APHA, 2015). Bacterial population counts were reported as log clone for unit (log CFU) per g of sample. This examination was done on catfish luncheon products at 0, 1, 2, and 3 months of storage at -10 ±2°C. Yeasts and moulds were determined on acidified potato dextrose agar (Oxoid, UK) media. Colonies on each sample were recorded after incubation at 25 ± 2˚C for 3-5 days and reported as log CFU per g of sample (APHA, 2015). This examination was done on catfish luncheon products at 0, 1, 2, and 3 months of storage.

Sensory properties

The organoleptic quality of catfish luncheon products was assessed by twenty qualified panelists who served as representatives of graduate students and staff members at fish processing technology department, Faculty of fish resources, Suez University, Egypt.

Each sample′s appearance, taste, odour, flavour, colour, texture and overall acceptability were assessed using a 9-point hedonic scale, whereas 9 representing extremely like, 8 representing very much like, 7 representing moderately like, 6 representing slightly like, 5 representing neither like nor dislike, 4 representing slightly dislike, 3 representing moderately dislike, 2 representing very much dislike and 1 representing extremely dislike (Ali *et al.*, 2017).

Statistical analysis

The variation in values was estimated by ANOVA one-way test analysis, Duncan´s multiple range test and mean ± standard deviation were performed to estimate the amount of variation of a set of values, using IBM SPSS Statistics version 22 (SPSS, 2014). All data represent the mean of three replicate experiments (n = 3).

Results and discussion

Chemical constituents of Marine algae and wheat flour

Chemical composition of wheat flour and both dried seaweed (*Ulva lactuca*) and microalgae (*Nannochloropsis oculata*) are presented in Table (1). The moisture contents of wheat flour and *Ulva lactuca* were similar and higher (13.62 and 13.25 %, respectively) than *Nannochloropsis oculata* (3.5 %) which may be related to the content and nature of polysaccharides that hold more water. Protein, oil and fiber contents on dry weight basis were significantly higher (P<0.05) in *Nannochloropsis oculata* (32.19%, 10.29, and 11.91%, respectively) than in *Ulva lactuca* and white flour (20.44 and 11.93%, 0.65 and 1.27%, and 8.61 and 0.23%; respectively). White flour has the highest content of carbohydrate (85.53%) while ranged between 39.42 and 26.2% in *Ulva lactuca* and *Nannochloropsis oculata*, respectively.

Table1. Chemical constituents and caloric values in wheat flour and dried marine algae (*Nannochloropsis oculata and Ulva lactuca*).

Parameters		Wheat flour	Marine algae	
			Nannochloropsis oculata	***Ulva lactuca***
Moisture	%	13.62 ± 0.13^{a}	3.50 ± 0.10^{b}	13.25 ± 0.24^{a}
Protein	% on dry weight basis	11.93 ± 0.10^{c}	32.19 ± 0.20^{a}	20.44 ± 0.15^{b}
Fat		1.27 ± 0.27^{b}	10.29 ± 0.23^{a}	0.65 ± 0.02^{c}
Ash		1.04 ± 0.22^{c}	19.41 ± 1.02^{b}	30.88 ± 0.39^{a}
Crude fiber		0.23 ± 0.07^{c}	11.91 ± 0.09^{a}	8.61 ± 0.12^{b}
Carbohydrates		85.53 ± 0.31^{a}	26.20 ± 0.66^{c}	39.42 ± 0.15^{b}
Caloric value	Kcal/100g	401.27	326.17	245.29
Total chlorophyll	µg/mL	ND	1.18 ± 0.16^{b}	5.97 ± 0.07^{a}
Total carotenoid	µg/mL	ND	0.34 ± 0.04^{b}	3.34 ± 0.02^{a}
Total polyphenols	mgGAE/g	ND	2.08±0.07	2.87±0.05

[a,b,c] Letters in the same row between white flour, *Nannochloropsis oculata and Ulva lactuca* denote significant difference (P<0.05; ANOVA). ND: Not determined.

The composition of wheat flour in this study is agreed with the results of Lin *et al*. (2019). The results of *Ulva lactuca* in this study were consistent with those reported by Yaich *et al*. (2011) for the moisture content (14.94%) while differed for protein and oil (8.46% and 7.87%; respectively). Salehi *et al*. (2019) stated that green seaweeds had higher protein content than brown seaweeds. According to Aguilar-Ruiz *et al*. (2022), proteins (10.82 and 34.53%), lipids (22.87 and 28.66%) and carbohydrates (22.75 and 30.41%) of *Nannochloropsis oculata* depend on the conditions and phases of growth. The findings in the present study were in agreement with those reported by Aguilar-Ruiz *et al*. (2022) regarding the contents of protein and carbohydrate but they were lower in oil content.

The *Ulva lactuca* contained 8.6% crude fiber which was higher than the one found by Amin (2019) (6.03%). On the other hand, crude fiber was 11.91% in *Nannochloropsis oculata* which was lower than the one found by Molino *et al.* (2018) (17.7%). This discrepancy could be attributed to differences in the environmental conditions under which the algae grew. These fibers could improve human health by controlling the digestive tract, blood sugar and cholesterol levels, and prevention of serious diseases like diabetes, obesity and cancer (Catarino *et al*., 2018).

The total phytochemical chlorophyll, carotenoid and polyphenols in *Ulva lactuca* (5.97 µg/mL, 3.34 µg/mL and 2.87mgGAE/g, respectively) were more than in *Nannochloropsis oculata* (1.18 µg/mL, 0.34 µg/mL and 2.08 mgGAE/g, respectively) which may be due to the differences in light source (nature or artificial) during growth and genotype. Chlorophyll, carotenoid and polyphenols play an important role in preventing oxidation, microbial growth, and mutagens (Zanella and Vianello, 2020).

Caloric value appeared to be the highest in the wheat flour (401.27 Kcal/ 100g), however the *Nannochloropsis oculata* was higher than of *Ulva lactuca* (326.17 and 245.29 Kcal/ 100g) due to the variation in the main components (Table 1). From the results, both marine algae (*Nannochloropsis oculata* and *Ulva lactuca*) are sustainable sources of dietary protein, lipids, balanced pigments and fibers, compared to white flour.

Algae are considered as sustainable and nutritious source of proteins, lipids, minerals, fibers, vitamins and pigments (Mendes *et al*., 2022; Metwaly *et al.,* 2023). Since algae have a dark green colour, umami flavour from glutamic acid, and volatile substances such as sulfur compounds that alter the taste and odour, their use as an ingredient in food products, especially fish products, requires compatibility (Mendes *et al*., 2022). Along with the necessity for palatability, the qualities of algae are two more concerns that must be addressed (Cavallo *et al*., 2021). Knowledge about the composition and nutritional value of both dried *Ulva lactuca* (macroalgae), and *Nannochloropsis oculata* (microalgae) is required before including them as a substitute for flour in the catfish luncheon formula.

Quality evaluation of supplemented catfish luncheon products

Good nutritional and quality characteristics of both *Nannochloropsis oculata* and *Ulva lactuca* drew attention to their benefits as food or to support human food as in Southeast Asian countries and in Western countries (Mendes *et al.*, 2022). In this study, catfish luncheon products which were supplemented with algae instead of flour were investigated for their chemical composition, microbial, physical and sensory quality during frozen storage time as follows:

Chemical composition estimated on dry weight of minced catfish, which is a part of luncheon formula, was 62.33% protein, 26.23% fat, 6.98% ash and 4.46% carbohydrate (Table 2). In the same context, the chemical constituents of catfish luncheon formula products which are composed of emulsion of protein and fat are shown in Table (2). The control (catfish luncheon formula without adding any algae) had the highest protein (29.71%), carbohydrate (22.03%) and fiber (2.75%) contents and had the lowest moisture (42.66%) and ash (5.35%) contents. By adding 10% dry *Nannochloropsis oculata* or *Ulva lactuca* instead of flour, the moisture contents increased significantly to 44.12% and 48.58%, respectively while protein and carbohydrate slightly decreased to 28.36 and 18.17%, and 28.43 and 19.83%, respectively. These decreases in protein and carbohydrates may be related to leakage soluble parts of protein and carbohydrates of algae origin during cooking process of catfish luncheon in boiling water. Although lipid in fish luncheon formula supplemented with 10% dry *Ulva lactuca* was low (38.51%); it was high when adding 10% dry *Nannochloropsis oculata* (43.30%) so the fish luncheon formula supplemented with 10% dry *Nannochloropsis oculata* had the highest nutritional value (575.82 Kcal/100g). The chemical composition of luncheon was affected by the change in the composition of algae added to the formula.

Table 2. Chemical constituents of minced catfish (*Clarias gariepinus*) and catfish luncheon (control and supplemented) products (on dry weight basis).

Constituents	**Minced catfish**	**Luncheon type**		
		C	***N***	***U***
Moisture (%)	69.92±0.32 ^{a}	42.66±0.26^{d}	44.12±0.35^{c}	48.58±0.28^{b}
Protein **(%)**	62.33±0.27^{a}	29.71±0.32^{b}	28.43±0.12^{c}	28.36±0.35^{d}
Fat **(%)**	26.23±0.12^{d}	40.16±0.36^{b}	43.30±0.24^{a}	38.51±0.5^{c}
Ash **(%)**	6.98±0.18^{c}	5.35±0.32^{d}	8.39±0.12^{b}	11.79±0.29^{a}
Crude fiber **(%)**	ND	2.75±0.08^{a}	1.78±0.12^{b}	1.44±0.09^{c}
Carbohydrates **(%)**	4.46±0.12^{d}	22.03±1.27^{a}	18.1±0.31^{c}	19.9±0.2^{b}
Caloric value (Kcal/100g)	ND	568.40	575.82	539.63

C: control formula contains 10% wheat flour; N: supplemented formula contains 10% *Nannochloropsis oculata*; U: supplemented formula contains 10% *Ulva lactuca*.

$^{a, b, c,}$ Letters denote significant difference ($P<0.05$; ANOVA) in the same row between different products. ND: Not determined

Physicochemical changes during storage period

Changes in some physicochemical properties of supplemented catfish luncheon products were examined during three months of frozen storage at -10°± 2°C (Figure 1). At zero time, the control product had the lowest pH (6.35) compared to the samples supplemented with 10% dry *Ulva lactuca* or *Nannochloropsis oculata* (6.47 and 6.63, respectively). After the first month of frozen storage the pH slightly increased which might be related to the little quantity of amines groups formed, then pH started to slightly again. This decrease might be due to the conversion of muscle glycogen to lactic acid. The pH of all catfish luncheon samples with or without algae ranged from 6.26 to 6.56 after three months of frozen storage (Figure 1A).

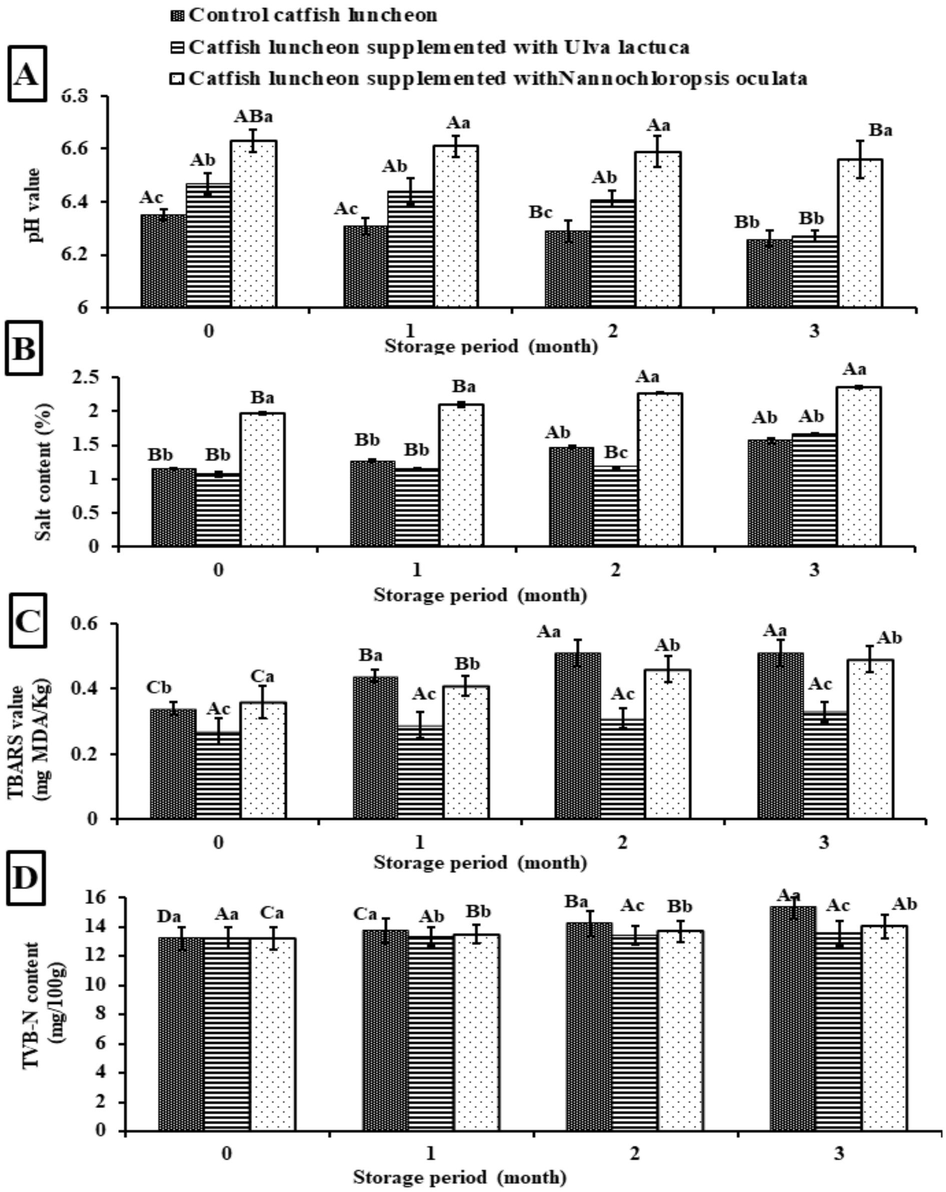

Figure 1. Changes in pH value (A), salt content (B), TBARS values (C) and TVB-N values (D) of supplemented catfish luncheon products during frozen storage (-10°C±2°C) for three months. Different superscripts of (a–c) lowercase letters indicate significant differences at $p < 0.05$ between treatments for each storage time. Different superscripts of (A-C) uppercase letters indicate significant differences at $p < 0.05$ for each sample over the storage period.

Our findings are consistent with the results of Ali *et al*. (2017) where the pH values of canned tilapia fish luncheon were gradually increased during six months of storage at room temperature (25–35 °C) and ranged from 6.02 to 7.34. In other study, Farag (2023) found that pH values of all red tuna fish luncheon samples ranged from

5.76 to 5.82 due to the formation of lactic acid in fish muscle by respiration anaerobically. The salt contents of control and supplemented catfish luncheon samples increased during the three months of frozen storage (Figure 1B). Catfish luncheon supplemented with 10% dry *Ulva lactuca* appeared to have the lowest salt content while samples supplemented with *Nannochloropsis oculata* appeared to have the highest salt content. This variation in salt content may be related to the variation in mineral contents between the two algae. There was a significant increase in salt content with extending the period of frozen storage from 0 to 3 months. The increase in salt content throughout the storage period may be related to the fact that moisture content decreases with the extension of the frozen storage period. Salt contents in catfish luncheon with *Ulva lactuca* or *Nannochloropsis oculata* ranged from 1.07 to 1.66% and from 1.97 to 2.1%; respectively by the end of frozen storage.

TBARS measurements have been utilized to detect secondary lipid oxidation products, particularly aldehydes, as well as hydroperoxide breakdown products (Prabha and Manjulatha, 2023). Changes in TBARS values in the control and supplemented catfish luncheon during frozen storage at -10°C for three months are shown in Figure 1C. The TBARS values of all investigated luncheon samples at zero time ranged between 0.27 and 0.36 mg MDA/kg sample, where the luncheon containing *Nannochloropsis oculata* was the highest. The high readings of TBARS at zero time are due to the cooking process during lunchtime preparation, which releases iron from the iron carrier proteins. Iron and oxygen combined speed up the oxidation process, which raises TBARS levels (Farag, 2023).

Luncheon containing *Nannochloropsis oculata* has the highest TBARS value at zero time; this may be due to its highest content of oil compared to the other investigated samples. On the other hand, luncheon containing *Ulva lactuca* has the lowest TBARS value at zero time because it has the lowest content of oil and the highest content of natural antioxidant compounds (total chlorophyll, total carotenoid, and total polyphenols) compared to the other investigated samples. All lunchtime samples under investigation showed an insignificant rise in TBARS readings after frozen storage. One possible explanation for the rise in TBARS readings was the fish muscle denaturation over the prolonged storage period. Lipid oxidation may proceed more quickly if denatured muscle cells release catalysts. Additionally, during the freezing process, ice crystals may develop, rupturing cells and emitting pro-oxidants, particularly free iron, which aid in the oxidation of lipids (Sriket and La-ongnual, 2018).

By the end of the storage period, control luncheon has the highest TBARS value (0.51 mg MDA/kg) with an increment rate of 50%, followed by luncheon containing *Nannochloropsis oculata* (TBARS value 0.49 mg MDA/kg with an increment rate of 36.11%) and luncheon containing *Ulva lactuca* (TBARS value 0.33 mg MDA/kg with an increment rate of 22.22%) (Figure 1C). This may be due to the fact that *Ulva lactuca* has the highest content of natural antioxidant compounds (total chlorophyll, total carotenoid, and total polyphenols), followed by *Nannochloropsis oculata*. At the end of the frozen storage period, TBARS values of all investigated samples (ranging from 0.33 to 0.51 mg MDA/kg) were still lower than the safe limit (0.9 mg MDA/kg) as reported by EOS (2023). Our results are consistent with Ali *et al*.

(2017), who found that the TBARS values of canned tilapia fish luncheon ranged between 0.44 and 1.97 mg MDA/kg sample. Also, Farag (2023) found that the TBARS values of all red tuna fish luncheon samples ranged from 0.77 to 3.0 mg MDA/kg sample according to the ingredients added to the basic formula.

TVB-N is a good indicator of deterioration in fresh and weakly preserved aquatic foods (Farag, 2023). It is recognized as a by-product of endogenous enzyme activity and bacterial deterioration; its concentration is frequently utilized as a gauge to evaluate the shelf life and maintain the quality of products (Wang et al., 2022). Changes in TVBN values in the control and supplemented catfish luncheon during frozen storage at -10°C for three months are shown in Figure 1D. The TVBN values of all investigated luncheon samples at zero time ranged between 13.19 and 13.23 mg N/100g of sample, where the luncheon containing *Ulva lactuca* has the lowest TVBN value at zero time because *Ulva lactuca* has the highest content of bioactive compounds (total chlorophyll, total carotenoid, and total polyphenols) with a protective impact against microbes, which accelerates protein breakdown to volatile nitrogen. All lunchtime samples under investigation showed a significant ($p < 0.05$) rise in TVBN values after frozen storage. One possible explanation for the rise in TVBN readings was the protein decomposition into molecules with a low molecular weight (such as peptones, peptides, and amino acids) throughout the frozen storage period.

By the end of the storage period, control luncheon has the highest TVBN value (15.32 mg N/100g) with an increment rate of 16.15%, followed by luncheon containing *Nannochloropsis oculata* (TVBN value 14.01 mg N/100g with an increment rate of 5.98%) and luncheon containing *Ulva lactuca* (TVBN value 13.56 mg N/100g with an increment rate of 2.49%) (Figure 1D). This may be due to the fact that *Ulva lactuca* has a higher content of bioactive compounds with antimicrobial activity than *Nannochloropsis oculata (*Silva *et al., 2020).* At the end of the frozen storage period, TVBN values of all investigated samples ranged from 13.56 to 15.32 mg N/100 g. At the end of the frozen storage period, luncheon containing algae (*Ulva lactuca or Nannochloropsis oculata*) TVBN values were still lower than the safe limit (15 mg N/100 g) as reported by EOS (2023), while the opposite was found in the case of the control luncheon.

Our results are consistent with Ali *et al*. (2017), who found that the TVB-N values of control tilapia fish luncheon increased from 12.55 mg N/100 g at zero time to 23.81 mg N/100 g after 6 months of storage at room temperature (25–35 °C).

The previous physicochemical parameters indicated that adding 10% of algae powder as alternative to wheat flour in the fish luncheon formula did not affect negatively on the protein and lipid. Thus, the algae can be considered as a good additive that could preserve the quality of this product under frozen storage.

Color and texture values and functional properties

The color profile of control (C) and supplemented (N and U) catfish luncheon was measured (Table 3). The lightness (L*) value of control sample (C) (64.48) was decreased after algae powder replacement especially in N sample (47.14) which was darker than in U sample (52.05) (Table 4). These values were less bright but close to the values (38.42 - 53.05) of canned tilapia fish luncheon (Ali *et al*., 2017). Also, the redness (a*) value of catfish luncheon (4.47) was decreased in supplemented samples especially in N sample (-2.32) than in U sample (-2.12) which means a clear appearance of green color after algae replacement. In supplemented samples, a* values differed than what Ali *et al*. (2017) reported for canned tilapia fish luncheon (2.54 - 3.24). In the same context, the yellowness (b*) value of C sample (23.47) was higher than in U sample (19.66) and N sample (15.24). The b* values in both supplemented samples (N and U) resembled to b* values of canned tilapia fish luncheon (14.63- 18.32) (Ali *et al*., 2017). Cooking process of supplemented catfish luncheon (N and U) may damage the chlorophyll pigments and induce changing in moisture contents which consequently effected on colour parameters especially U sample. The degradation of the green chlorophyll pigments in U sample resulted in accumulation of brown compounds (pheophorbides and pyropheophorbide) which affected the final colour of the product (BI= 42.94). The intensity of the degradation process was lower in the case of N sample (BI= 34.12). Also, chroma values decreased as affected by cooking treatment and its ability to degrade the pigment especially in N sample while hue values in algae samples were higher than control and related to the chlorophyll pigment.

Table 3. Color, texture and functional properties of control and supplemented catfish luncheon products.

Parameters	Fish luncheon		
	C	N	U
L*	64.48± 0.36^{a}	47.14±0.98^{c}	52.05±1.02^{b}
a*	4.47±0.06^{a}	-2.32±0.06^{c}	-2.12±0.01^{b}
b*	23.47±0.15^{a}	15.24± 0.80^{c}	19.66± 0.03^{b}
Browning index (BI)	49.41	34.12	42.94
Hue value	79.22	98.66	96.15
Chroma value	23.89	15.42	19.77
Hardness (N)	7.33±0.11^{b}	5.70±0.04^{c}	10.04±0.10^{a}
Adhesive Force(N)	0.07±0.01^{a}	0.08±0.01^{a}	0.04±0.02^{a}
Cohesiveness	0.80±0.02^{a}	0.78±0.07^{a}	0.73±0.01^{a}
Springiness(mm)	2.67±0.12^{a}	2.56±0.04^{a}	2.57±0.06^{a}
Gumminess(N)	5.86±0.15^{b}	4.45±0.90^{c}	7.33±0.12^{a}
Chewiness(mJ)	15.64± 0.04^{b}	11.39± 0.07^{c}	18.84±0.40^{a}
WHC	51.00±0.36^{c}	69.7±0.10^{a}	60.75±0.25^{b}
OBC	90.00±0.80^{c}	110±0.90^{b}	124± 0.70^{a}

C: control formula contains 10% flour; N: supplemented formula contains 10% *Nannochloropsis oculata*; U: supplemented formula contains 10% *Ulva lactuca*. Water holding capacity (WHC) and oil binding capacity (OBC).

$^{a, b, c,}$ Letters denote significant difference (P<0.05; ANOVA) in the same raw between different products.

The texture profile of ready to eat control (C) and supplemented (N and U) catfish luncheon samples was shown in Table (3). Hardness, gumminess and chewiness values of C sample were higher (7.33 N, 5.91 N and 15.8 mJ; respectively) than the values of N sample (5.7 N, 4.44 N and 11.4 mJ; respectively) while they were lower than values of U sample (10.04 N, 7.32 N and 18.8 mJ; respectively). On the other hand, adhesive force, cohesiveness and springiness were slightly decreased in supplemented samples (N and U) than control sample values (0.07 N, 0.8 and 2.67 mm; respectively) but N sample had the lowest adhesive force and springiness. The content and composition of polysaccharides especially starch (Moiraghi *et al*., 2019) in white flour; cellulose and ulvan (Kidgell *et al*., 2019) in *Ulva lactuca* and cellulose and (β1→3)-glucans (Pandeirada *et al*., 2019) in *Nannochloropsis oculata* beside proteins are responsible for gelling formation and viscosity enhancement during cooking process. Ali *et al*. (2017) reported that canned tilapia fish luncheon had firmness, cohesiveness and springiness values that ranged from 4.55 to 6.18 N, 0.68 to 1.12 and 0.81 to 1.18 mm, respectively.

Water holding capacity (WHC) and oil binding capacity (OBC) are important functional properties to reduce syneresis, to improve the texture and viscosity and to stabilize the formulated food emulsion. As mentioned in Table 3, WHC and OBC increased in N sample by 36.67% and 22.22%, respectively and in U sample by 19.12 and 37.78%, respectively than in control (C) sample. The increase in OBC in luncheon supplemented with algae might be due to hydrophobic nature of algae protein. These results suggest that the algae components affected the thermal treatment during fish luncheon processing. In particular, the oil absorption ability of the product was improved, as well as the emulsify ability.

Microbiological changes during storage period

Mesophilic and psychrophilic aerobic bacteria, in addition to molds and yeasts were counted in control (C) and supplemented (N and U) catfish luncheon products during storage period for three months at -10 ±2°C (Table 4).

Initial total aerobic mesophilic bacteria count of control (C) and supplemented (N and U) catfish luncheon samples ranged between 2.87 and 3.43 log CFU/g, which was higher than those reported for chicken luncheon containing lentil powder by Aly and Morsy (2019), and for red tuna luncheon by Farag (2023). The ingredients of the luncheon such as wheat flour etc.; could have contributed to the high amounts of bacteria since the ingredients were not sterilized. Throughout the frozen storage, initial microbial load increased sharply ($p < 0.05$) to the value of 5.32 log CFU/g in control luncheon, 4.18 log CFU/g in luncheon supplemented with *Nannochloropsis oculata* and 3.49 log CFU/g in luncheon supplemented with *Ulva lactuca* (Table 4). Total aerobic mesophilic bacteria count of catfish luncheon did not exceed the microbiological limit (<6 log CFU/g) recommended by EOS (2023) during storage period for all samples. Significant differences ($P < 0.05$) were observed between luncheon groups at the same time of storage.

Psychrophilic bacteria are crucial because they produce various metabolic chemicals such aldehydes, ketones, biogenic amines, and volatile sulphides, which are responsible for most alterations in flavour, texture, and odor (Ragab et al., 2020).

Initial psychrophile bacteria in all investigated catfish luncheon ranged from 3.49 log CFU/g for luncheon supplemented with *Nannochloropsis oculata* to 3.79 log CFU/g for control. Throughout the freezing storage there was a significant ($p < 0.05$) increase in psychrotrophic bacteria count. The psychrotrophic bacteria count at the end of frozen storage period reached to 5.30 log CFU/g in control luncheon, 4.37 log CFU/g in luncheon supplemented with *Nannochloropsis oculata* and 4.01 log CFU/g in luncheon supplemented with *Ulva lactuca*.

Table 4. Microbiological load (CFU/g) of control and supplemented catfish luncheon products during frozen storage (-10 ±2°C) for three months.

Samples	Storage period (month)	Microbial count		
		Total aerobic nesophilic bacteria (log CFU/g)	Psychrophilic Bacteria (log CFU/g)	Molds and Yeasts (log CFU/g)
C	0	2.95±0.01 ^{d}	3.79±0.23^{c}	ND
	1	3.87±0.76^{c}	4.19±0.42^{b}	ND
	2	4.36±0.55^{b}	4.46±1.96^{b}	1.30±1^{b}
	3	5.32±1.07^{a}	5.30±0.60 ^{a}	1.60±1^{a}
N	0	3.43±0.06^{c}	3.49±0.78 ^{a}	ND
	1	3.75±1.29^{a}	3.86±0.19 ^{a}	ND
	2	4.10±0.02^{b}	4.11±0.08 ^{a}	ND
	3	4.18±0.02^{b}	4.37±0.16 ^{a}	ND
U	0	2.87±0.76^{c}	3.52±0.06^{c}	ND
	1	3.12±1.53^{a}	3.77±1.53^{a}	ND
	2	3.25±0.13^{b}	3.95±0.06^{b}	ND
	3	3.49±0.06^{b}	4.01±0.25^{b}	ND

C: control formula contains 10% wheat flour; N: supplemented formula contains 10% *Nannochloropsis oculata*; U: supplemented formula contains 10% *Ulva lactuca*.

$^{a, b, c}$ Letters denote significant difference (P<0.05 ANOVA) in the same column for each product. ND: means not detected.

Generally, the increment rate in total aerobic mesophilic and psychrotrophic bacteria in control luncheon (80.33% and 39.84%, respectively) were higher than in luncheon supplemented with *Nannochloropsis oculata* (21.87% and 25.21%, respectively) and in luncheon supplemented with *Ulva lactuca* (21.60% and 13.92%, respectively). These results could be due to the antimicrobial effect of algae powder. Also, the variation between luncheons supplemented with *Nannochloropsis oculata* or *Ulva lactuca* may be due to the high content of bioactive components in *Ulva lactuca* compared to *Nannochloropsis oculata*.

According to data presented in Table 4, yeast and mold growth did not show in catfish luncheon supplemented with algae during the frozen storage period but it showed in control sample in the second month of storage (1.30 log CFU/g) and increased to 1.60 log CFU/g the third month.

Sensory evaluation

The changes in sensory properties in control (C) and supplemented (N and U) catfish luncheon products were monitored for three months during frozen storage at -10 ±2°C.

Table 5. Sensory properties of control and supplemented catfish luncheon products during storage at frozen temperature (-10 ± 2°C) for 3 months.

Sensory properties	Samples	Storage Time (months)			
		0	1	2	3
Appearance	**C**	$8.0±0.6^{a}$	$7.10 ±0.70^{ab}$	$6.87±0.15^{baBA}$	$6.47±0.59^{bBA}$
	N	$7.30±0.5^{a}$	$7.27±0.45^{ab}$	$6.63±0.32^{cbB}$	$6.23±0.25^{cB}$
	U	$7.77±0.25^{a}$	$7.54±0.33^{ab}$	$7.23±0.23^{cbA}$	$7.05±0.15^{cA}$
Color	**C**	$7.80±1.02^{a}$	$7.67±1.50^{a}$	$6.42 ±1.06^{abB}$	$5.35 ± 0.61^{bB}$
	N	$8.33±0.58^{a}$	$7.57±0.25^{b}$	$7.33±0.41^{bA}$	$7.07±0.25^{bA}$
	U	$7.67±0.58^{a}$	$7.53±0.25^{a}$	$7.25±0.15^{abA}$	$6.87±0.15^{bA}$
Odor	**C**	$7.53±0.15^{a}$	$7.20±0.35^{ba}$	$7.07±1.11^{b}$	$6.60±0.20^{c}$
	N	$7.47±0.15^{s}$	$7.27±0.25^{s}$	$7.15±0.15^{s}$	$6.4±0.20^{b}$
	U	$8.10±0.66^{a}$	$7.33±0.25^{b}$	$7.11±0.10^{b}$	$6.82±0.36^{b}$
Taste	**C**	$7.97±0.60^{a}$	$7.57±0.93^{ab}$	$6.43±0.51^{bc}$	$5.17±0.87^{cB}$
	N	$7.43±0.41^{a}$	$7.30±0.20^{ab}$	$7.0±0.44^{ba}$	$6.57±0.5^{bA}$
	U	$7.93±0.41^{a}$	$7.42±0.45^{ab}$	$7.12±0.12^{bc}$	$6.45±0.39^{cA}$
Texture	**C**	$7.33±0.58^{a}$	$7.10±0.20^{a}$	$6.97±0.59^{a}$	$5.93±0.47^{b}$
	N	$7.80±0.26^{a}$	$7.27±0.55^{ab}$	$6.57±0.31^{cb}$	$6.43±0.33^{c}$
	U	$7.57±0.41^{a}$	$7.31±0.36^{a}$	$6.97±1.07^{ab}$	$6.39±0.44^{b}$
Overall acceptability	**C**	$7.23±0.68^{a}$	$6.93±0.15^{aB}$	$6.52±0.35^{ab}$	$5.87±0.21^{bB}$
	N	$7.30±0.50^{a}$	$7.13±0.15^{abBA}$	$6.70±0.20^{cb}$	$6.37±0.06^{cAB}$
	U	$7.80±0.72^{a}$	$7.57±0.42^{abA}$	$6.99±0.18^{ba}$	$6.72±0.51^{bA}$

C: control formula contains 10% wheat flour; N: supplemented formula contains 10% *Nannochloropsis oculata*; U: supplemented formula contains 10% *Ulva lactuca*.

$^{a, b, c}$ Letters denote significant difference (P<0.05 ANOVA) in the same raw for each product. $^{A, B}$ Letters denote significant difference (P<0.05 ANOVA) in the same column between different products following each property.

As illustrated in Table (5), supplemented sample (N) had the highest scores for colour (8.33) and texture (7.8) while supplemented sample (U) had the highest scores for taste (7.93), odor (8.1) and overall acceptability (7.8) in comparison with control sample. All catfish luncheon samples had sensory scores before storage ranged between like very much to like moderately. Better sensory properties of both samples N and U can be explained by the cooking treatment during luncheon process which may improve umami flavour and stronger sea smell related to both *Nannochloropsis oculata* and *Ulva lactuca*. During frozen storage sensory properties scores reduced significantly (p≤0.05) especially in control sample which becomes between like slightly and not like nor dislike (5.17- 6.6). Also, both sample N and U had organoleptic scores between like moderately (7.07) and like slightly (6.23) which is better than C sample at the end of 3rd month of storage. Significant differences in appearance, colour, taste and overall acceptability were showed between all treatments after three months of storage. These results agreed with Ali *et al*. (2017) who found that scores of organoleptic properties of control canned tilapia luncheon at the beginning was between like moderately and like very much (7.11–8.23) while acceptability reduced after 4 months of storage at 30±5°C to like slightly and like moderately (6.84–7.64).

Conclusions

The important nutritional components make both types of *Nannochloropsis oculata* (microalgae) and *Ulva lactuca* (seaweed) distinct sources of caloric value, fiber, oil and protein, in addition to the presence of carotenoid and chlorophyll pigments (nature bioactive substances). Replacing wheat flour by algae powder (*Nannochloropsis oculata* or *Ulva lactuca*) to supplement new catfish luncheon products caused an improvement in chemical constituents and caloric value, in addition, improving the WHC and OBC. Colour parameters showed a decrease in lightness, redness and yellowness in supplemented catfish luncheon and the green colour appeared especially with sample supplemented with *Nannochloropsis oculata*. The sample supplemented with *Nannochloropsis oculata* showed a decrease in all texture parameters including hardness, gumminess and chewiness, while sample supplemented with *Ulva lactuca* increased them. The microbiological and sensory evaluation showed that samples supplemented with *Nannochloropsis oculata* or *Ulva lactuca* had better quality and sensory properties than control sample that contained wheat flour. Additionally, more research about the economic feasibility of scaling up production of algae-supplemented fish luncheon would be beneficial.

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