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Functional Foods Enriched with Marine Microalga *Nannochloropsis oculata* as a Source of ω-3 Fatty Acids

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Summary

The demand for functional food incorporated with ω-3 fatty acids is increasing over the years due to their added health benefits, such as reducing the risk of cardiovascular diseases, type II diabetes, ocular diseases, arthritis, *etc*. This study mainly aims to develop functional cookies and pasta enriched with ω-3 fatty acids. *Nannochloropsis oculata* was used because of its relatively high growth rate, high lipid content, resistance to mixing and contamination together with high nutritional values. The effect of the incorporation of *Nannochloropsis oculata* biomass on colour, firmness, fatty acid profile and sensory characteristics of cookies and pasta were evaluated. The colour values were found to be stable for two months of storage and the firmness increased with the addition of microalgal biomass. Omega-3 polyunsaturated fatty acid (PUFA) levels (eicosapentaenoic and docosahexaenoic acids) of 98 mg per 100 g and 63 mg per 100 g were observed in cookies and pasta, respectively, enriched with 1 % of *Nannochloropsis oculata* biomass. Sensory evaluation showed that the addition of up to 2 and 3 % of microalgal biomass was positively evaluated and accepted for cookies and pasta, respectively. This study confirms that the cookies and pasta enriched with *Nannochloropsis oculata* biomass might be used as a potential source of ω-3 fatty acids.

Key words: marine microalgae, food ingredients, *Nannochloropsis oculata*, PUFA and fatty acid profile

Introduction

Consumers are increasingly making wiser food choices based on the ability of food to provide health benefits, such as enhancing body functions or reducing the risk for certain diseases. Therefore, the role of functional foods has been gaining significant importance. Functional food products contain added, technologically developed ingredients with specific health benefits (*1*).

Cookies are ready-to-eat, cheap and convenient food products that are consumed by people of all age groups in many countries (*2*) and hold an important position in snack food (*3*). Cookies have been reported to be rich in fats and carbohydrates; hence, they can be referred to as energy-giving food as well as good sources of proteins and minerals (*4*).

Pasta has a good nutritional profile, providing a source of complex carbohydrates, and it has a good consumer value. It has relatively long shelf-life, if stored in proper conditions. It is easy to cook and can be used in variety of meals (*5*). Because of their acceptability among all age groups, longer shelf-life and better taste, cookies and pasta are considered as food bases for fortification with ω-3 fatty acids and nutritional improvement.

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs) such as eicosapentaenoic acid (EPA; C20:5) and docosa-

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hexaenoic acid (DHA; C22:6) are precursors in the synthesis of prostaglandins, leukotrienes, tromboxanes and resolvins, which bind to specific protein receptors and signal cellular physiological responses to inflammation, vasodilation, blood pressure, pain and fever, playing an important role in the prevention of cardiovascular diseases, type II diabetes, ocular diseases, arthritis and cystic fibrosis (*6,7*).

In 2004, the Food and Drug Administration (FDA) approved health claim for reduced risk of CHD (coronary heart disease) for foods containing ω-3 PUFAs, mainly EPA and DHA. This provided a marketing leverage for functional foods fortified with ω-3 PUFAs. Although EPA and DHA can be synthesized endogenously from α-linolenic acid (ALA; C18:3), this reaction in humans is very inefficient (*8*). Anderson and Ma (*9*) provided an up-to-date and comprehensive review of health benefits specific for ALA, EPA, and DHA. Omega-3 (ω-3) and omega-6 (ω-6) PUFAs are essential for most of the animals. EPA and DHA can be used in pharmaceuticals and in food supplements due to their biochemical function.

Currently, the major dietary sources of EPA and DHA are only from fish (*e.g.* salmon, mackerel, sardines), whereas plant sources such as canola, walnut, soya bean and flaxseed oil contain ALA (*10,11*). Declining fish stocks, fishy odour, seasonal variations in quality (*12*) and, in addition to this, the presence of pollutants such as heavy metals and polychlorinated biphenyls (PCBs) in fish (*13, 14*) may compromise the consumption of fish as the major source of EPA and DHA. Therefore, other dietary sources of EPA and DHA are being sought. Food enrichment is probably the best long-term solution to boost the intake of long chain ω-3 PUFAs (*15*). Fish accumulate PUFAs by feeding on microalgae, which are the primary producers of PUFAs in the trophic chain (*16,17*).

Nannochloropsis oculata is a marine eustigmatophyte currently cultivated in many aquaculture hatcheries as the basis of an artificial food chain. The concentrated suspensions and frozen biomass of this microalga are commercially available (*18*). Because of its high EPA content, this eustigmatophyte is considered as a potential EPA source (*19*).

Nannochloropsis oculata biomass is available throughout the year as it is renewable and produced at low cost. The aim of the work is to use this microalgal biomass as a new functional ingredient in cookies. The effect of microalgal biomass on colour, texture, sensory evaluation and fatty acid profile of cookies and pasta were analyzed.

Materials and Methods

Materials

Fine wheat flour, sugar, butter, baking powder, chilli powder, ginger, garlic, fennel and salt were used as raw materials. All ingredients were purchased from Spencer's Daily supermarket, Chennai, India.

Microalgal cultivation

Nannochloropsis oculata used in the study was obtained from Central Institute of Brackishwater Aquaculture, Muttukadu, Chennai, India. The microalga was grown in a 20-litre bubble column glass photobioreactor by bubbling air and passing 2 % CO_2. Conway medium was used for culturing the microalga and was kept under illuminated fluorescent lamps (TLM 40W Philips, Amsterdam, The Netherlands) at an irradiance level of 175–190 μmol/(m^2·s) with photoperiod of 12:12 h (light/dark). The culture was continuously stirred with filtered air and kept at (24±1) °C until the cell growth reached early stationary phase. Culture salinity was maintained at 25 g/L. The cultured microalgal biomass was concentrated by centrifugation (2500×*g*, 10 min) and then freeze dried. The growth was monitored by counting the cells with the help of Neubauer haemocytometer.

Preparation of sweet cookies

Cookies were prepared using (in %): flour 44, sugar 26, butter 29.5 and baking powder 0.5. The freeze-dried microalgal biomass at mass fractions of 1.0, 2.0 and 3.0 % were added to the cookies. Control cookies without microalgae were also prepared for comparative study.

Initially, weighed amounts of sugar and butter were mixed well. Then wheat flour was added and mixed to obtain dough. The cookies were shaped and baked in an oven for 10–15 min at 120 °C. Later the cookies were stored in zip lock pouches at (27±2) °C without light for two months.

Preparation of spicy cookies

Cookies were prepared using (in %): flour 44, sugar 17, butter 30, chilli powder 1, garlic 2, ginger 1, fennel 1 and salt 1. Mass fractions of freeze-dried microalgal biomass of 1.0, 2.0 and 3.0 % were added to the cookies. Spicy cookies without microalgae were also prepared for comparison. The prepared cookies were stored in zip lock pouches at (27±2) °C without light for two months.

Pasta production

Fresh pasta was produced from whole wheat flour and maida flour in the ratio of 3:1, mixed with water in the ratio of 2:1. After that, 2 % of salt were added along with freeze-dried microalgal biomass at 0.5, 1.0, 2.0 and 3.0 % mass fractions. The mixture was extruded in the shape of rotini or corkscrew pasta with a bench top pasta maker (Model DOLLY, La Monferrina s.r.l, Moncalieri, TO, Italy). The pasta was dried at 65 °C, until the final moisture content reached less than 5 %. Pasta without microalgal biomass (control) was also prepared for comparison. Cooking parameters such as optimal cooking time, cooking loss, water absorption and moisture content were determined (5 replications) in accordance with Brennan *et al.* (*20*).

AOAC and internationally recognized good manufacturing practices (GMP) were followed to ensure the quality and food safety. GMP covers all aspects of manufacturing including standard operating procedures, equipment maintenance and handling of materials.

Colour measurement

The cookie and pasta colour was measured instrumentally using a Minolta CR-300 (Tokyo, Japan) tri-stimulus colorimeter (8 mm ø contact area). The results were expressed in terms of L^*: lightness (from 0 (black) to 100 (white)), a^*: greenness/redness (from $-a^*$ (green) to $+a^*$ (red)) and b^*: blueness/yellowness (from $-b^*$ (blue) to $+b^*$ (yellow)), as per CIELab system. The measurements were observed under constant lighting conditions, at 28 °C, using a white control (L^*=98.76, a^*=–0.04, b^*=2.01).

Texture analysis

Cookie and pasta texture was measured objectively (after 1 week of stabilization) by using a texturometer (TA.XT-Plus Stable Micro Systems, Surrey, UK) in penetration mode with a cylinder of 2 mm diameter probe, plunged 2 mm into cookies at 2 mm/s. Pasta firmness was assessed by a HDP/BSG blade set with guillotine and it was measured by cutting force required to cut raw and cooked pasta strips. The resistance to penetration was measured by the maximum force (in N) shown on the texturogram peak which corresponds to the firmness value.

Proximate analysis

The proximate chemical composition of freeze-dried microalgal biomass, cookies and pasta (raw and cooked) was measured on dry mass basis by drying in an oven at 105 °C until constant mass. Total ash content was measured by incineration at 550 °C in a muffle furnace for 12 h after controlled (slow) carbonization in a heating plate. The crude protein (N×6.25) was measured by the Kjeldahl method after acid digestion. The fat content was measured by Soxhlet extraction with petroleum ether and the moisture content was measured by oven dry method. All the analyses were done in triplicates according to the methods of AOAC (*21*).

Fatty acid profiling

Fatty acid methyl esters (FAMEs) were prepared as per Cohen *et al.* (*22*). Preparation of FAMEs was carried out using freeze-dried material and 5 mL of the acetylchloride methanol mixture (1:19, by volume). Esterification was carried out at 80 °C for 1 h. After cooling, 1 mL of water and 2 mL of *n*-heptane were added to the mixture under stirring and centrifuged at 2500×*g* for 10 min. The organic phase was collected, filtered and dried over anhydrous sodium sulphate. Solvent was removed under nitrogen and the FAMEs were dissolved in 0.1 mL of *n*-heptane. The FAMEs were analyzed with a Shimadzu GC-15A chromatograph (Shimadzu Corporation, Kyoto, Japan), equipped with flame ionization detector operating under the following conditions: column 2.4 m×0.3 cm S-S packed with 15 % DEGS, with a column temperature of 180 °C, injection temperature of 220 °C, detector temperature of 230 °C and carrier gas nitrogen at 15 mL/min. The peaks were identified by comparing the retention time with authentic standards and represented in relative percentage. The quantification was done by using the internal standard (21:0). All the analytical determinations were done in duplicate, after preparation and 2 months of storage. Limit of detection (LOD) (in μg/mL) for each fatty acid was: C14:0 0.2, C16:0 0.9, C18:0 0.2, C16:1 0.1, C18:1 0.6, C18:2 0.2, C18:3 0.1, C20:4 0.8, C20:4 0.8, C20:5 1.8 and C22:6 2.0.

Sensory evaluation

Sensory evaluation of fresh pasta and cookies containing microalgal biomass was carried out using freshly prepared products and the products stored for 30 days at (37±2) °C to evaluate the impact of microalgal biomass incorporation on the sensorial characteristics. The sensory analysis was performed by 54 untrained panellists, 22 males and 32 females aged from 19 to 48, who were recruited among students, staff and professors of the Faculty of Food Technology, Anna University, Chennai, India. Sensory attributes such as colour, odour, flavour, texture and overall acceptability were investigated on a nine-point hedonic scale (1=disliked extremely, 9= liked extremely) (*23*). Each fresh pasta formulation was cooked and served immediately in white plastic plates. Samples were served in random order to the panellists.

Statistical analysis

Each experiment was carried out in duplicate and measurements were performed in triplicates. All the data were subjected to Scheffe's post hoc test in ANOVA, at 0.05 probability level, using STATISTICA program, v. 6.0 (StatSoft Inc., Tulsa, OK, USA).

Results and Discussion

Proximate analysis of cookies, pasta (raw and cooked) and freeze-dried microalgae was carried out. Their relative composition is presented in Table 1.

Quality and stability of the product were determined by its moisture content. Moisture content of raw (fresh) pasta was in the range of 31–33 % and of cooked pasta 61–72 %. This was in accordance with the results obtained by Fradique *et al.* (*24*). Moisture content of fresh pasta was brought below 5 % by drying and it was used for further analysis. Fat content of (1.2±0.1) and (2.0±0.1) g per 100 g was observed in cooked and raw pasta, respectively. Protein content of (15.5±0.3) and (7.0±0.2) g per 100 g was observed in raw and cooked pasta, respectively. Cooking process resulted in the loss of fat content around 0.8 % in pasta (*24*).

Optimal cooking time depends primarily on the rates of water penetration and starch gelatinization. For pasta it was 4–5 min with cooking loss in the range of 4.2–5.8 %. High cooking loss of 10 % was previously reported for pasta with incorporated seaweed (*25*). Water absorption of 88–98 % was observed along with swelling index in the range of 1.6–1.8 g of water per g of dry mass. The observed experimental values correlated well with the predicted results stated by Fradique *et al.* (*26*).

Protein content of (36.0±0.1) g per 100 g and fat content of (22.1±0.3) g per 100 g of *Nannochloropsis oculata* were observed. Protein content of (4.6±0.1) g per 100 g and fat content of (18.5±0.3) g per 100 g of cookies were measured. Moisture content of cookies (sweet and spicy) was found to be in the range of 5.5–6.0 %.

Sweet cookies, spicy cookies (with ginger, garlic and chilli as main ingredients) and pasta were prepared with freeze-dried microalgal biomass fraction ranging from 1 to 3 %.

Table 1. Proximate composition of cookies, pasta and freeze-dried *Nannochloropsis oculata*

Parameter	*Nannochloropsis*	Cookies*	Raw pasta*	Cooked pasta*	Cookies**	Cooked pasta**
Dry mass/(g/100 g)	$(97.0\pm0.2)^{b}$	$(95.0\pm0.3)^{b}$	$(32.0\pm0.6)^{a}$	$(29.0\pm0.2)^{a}$	96.0±0.2	29.0±0.3
Total ash/(g/100 g)	$(22.0\pm0.1)^{b}$	$(2.9\pm0.2)^{a}$	$(2.0\pm0.1)^{a}$	$(1.2\pm0.08)^{a}$	3.2±0.1	1.3±0.1
Crude protein/(g/100 g)	$(36.0\pm0.1)^{d}$	$(4.6\pm0.1)^{a}$	$(15.5\pm0.3)^{c}$	$(7.0\pm0.2)^{b}$	4.9±0.1	7.3±0.1
Crude fat/(g/100 g)	$(22.1\pm0.3)^{b}$	$(18.5\pm0.2)^{b}$	$(2.0\pm0.1)^{a}$	$(1.2\pm0.1)^{a}$	18.9±0.2	1.4±0.1
Crude carbohydrates/(g/100 g)	18^{a}	67^{c}	17^{a}	32^{b}	67	32
Moisture content/%	2–3	5.5–6.0	31–33	61–72	4.5– 5.5	61–72

*Without the addition of *Nannochloropsis*, **with the addition of 1 % of freeze-dried *Nannochloropsis*
Data are reported as mean values±standard deviations of triplicate measurements
Different letters in the same row represent significant differences ($p<0.05$)

Colour values of cookies and pasta

The colour of food surface is the first quality parameter evaluated by consumers. It plays a major role in the acceptability of a food product as an indicator of quality, freshness, conservation state, flavour expectation and commercial value. With the addition of the increasing range of microalgal biomass (1–3 %) the lightness (L^*) values decreased significantly ($p<0.05$) from 75 to 45, which leads to the formation of darker cookies (Fig. 1). Similar results were reported in previous studies carried out with the incorporation of flaxseeds in chikkis (*27*) and with cookies with incorporated microalgal biomass (*28*). Above 1 % addition of microalgal biomass to cookies significantly ($p<0.05$) increased the green colour, which results in negative a^* values (–2 to –5) (Fig. 2), while the yellow tonality (b^*) significantly ($p<0.05$) decreased (Fig. 3). Dominant yellow chromaticity (positive b^*) with lower a^* values was observed in control cookies. Cookie colour parameters, L^*, a^* and b^*, remained very stable ($p<0.05$) during storage for 2 months, as observed by Gouveia *et al.* (*28*).

A decrease in lightness value (L^*) was observed with the addition of microalgal biomass (L^*=58 to 42) compared to control pasta (L^*=68). Furthermore, the addition of microalgal biomass to pasta resulted in products with negative a^* (a^*=3 to –10) and positive b^* values (b^*=15 to 23). This was in accordance with the results achieved for pasta incorporated with *Chlorella* and *Spirulina* biomass (*24*).

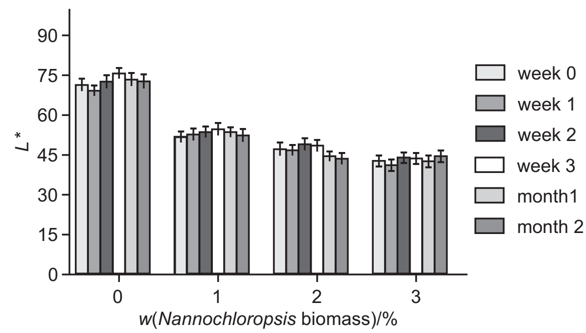

Fig. 1. The L^* values for cookies at different time intervals

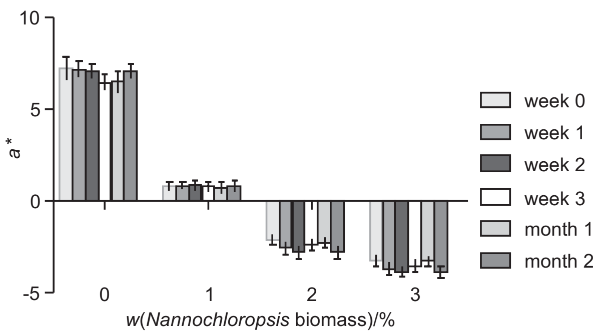

Fig. 2. The a^* values for cookies at different time intervals

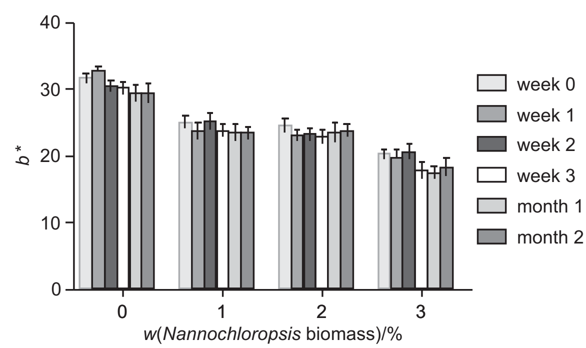

Fig. 3. The b^* values for cookies at different time intervals

Texture of cookies and pasta

The firmness values of cookies ranged from 20–35 N, which increased with the addition of microalgal biomass (Fig. 4). This may be due to the presence of microalgal proteins and carbohydrates, which play a significant role in water absorption process, resulting in more compact structure (*29*). Firmness of control cookies decreased significantly ($p<0.05$) during the 8th week, due to the absorption of moisture (Fig. 4).

The addition of microalgal biomass resulted in a significant ($p<0.05$) increase in the raw pasta firmness (2.9–4 N), when compared with control (1.8 N) (Figs. 5 and 6). Similar results were achieved by Bahnassey and Khan (*30*) of the fortification of pasta with legume flour (navy bean, pinto bean, lentil, green pea). Fradique *et al.* (*24*) found a similar behaviour when *Chlorella* and *Spirulina* biomass were added to pasta. Increased hardness and chewiness were observed in texture profile analysis of cheese fortified with vegetable and animal sources of ω-3 fatty acids (*31*).

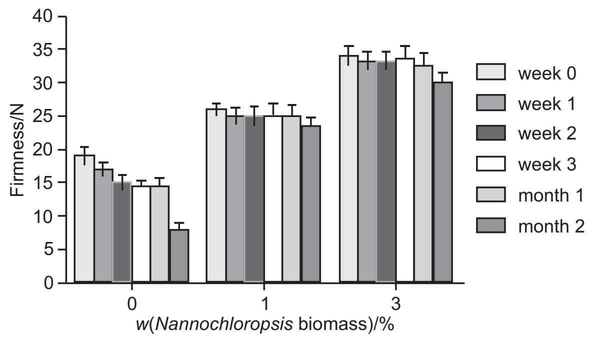

Fig. 4. Texture analysis of cookies

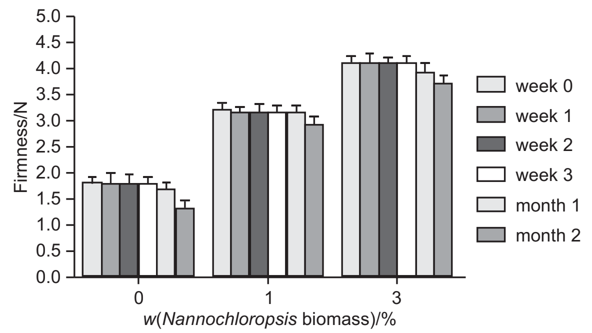

Fig. 5. Texture analysis of raw pasta

No significant differences ($p<0.05$) were observed for firmness values of cooked pasta enriched with microalgal biomass (1.0–3.0 %) (Fig. 6).

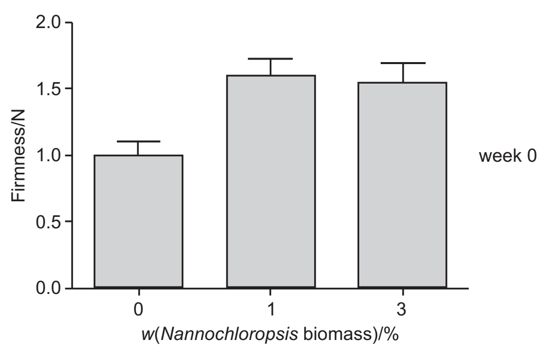

Fig. 6. Texture analysis of cooked pasta

Fatty acid profile

Fatty acid profile of freeze-dried *Nannochloropsis oculata* biomass consisted of 42 % of monounsaturated fatty acids (MUFAs), followed by 34 % of saturated fatty acids (SFAs) and 24 of % polyunsaturated fatty acids (PUFAs). The major fatty acids present in cookies were: palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic acids (18:2) (Table 2).

In control cookies, neither EPA nor DHA were detected. Cookies enriched with 1 % of *Nannochloropsis oculata* biomass had ω-3 PUFA levels (EPA+DHA) of 98 mg per 100 g. Cookies enriched with 2 and 3 % of *Nannochloropsis oculata* biomass had ω-3 PUFA levels (EPA+DHA) of 197 and 298 mg per 100 g, respectively. No significant differences ($p<0.05$) in ω-3 PUFA levels were observed even after two months of storage at (27±2) °C (Fig. 7). One portion (28 g) of cookies enriched with 1 % of microalgal biomass can provide 26 mg of ω-3 long-chain PUFA (LC-PUFA; EPA and DHA). Gouveia *et al.* (*32*) reported almost similar level of ω-3 fatty acids in biscuits enriched with *Isochrysis galbana* biomass.

Table 2. Fatty acid profile of cookies and freeze-dried *Nannochloropsis oculata*

	w(fatty acid)/%	Cookies	*Nannochloropsis*
SFA	Myristic acid (C14:0)	1.3	4.6
	Palmitic acid (C16:0)	45.2	26.5
	Stearic acid (C18:0)	5.5	2.3
MUFA	Oleic acid (C18:1)	35.8	10.3
	Palmitoleic acid (C16:1)	0.5	31.8
PUFA	Linoleic acid (C18:2)	11.5	1.5
	Arachidonic acid (C20:4)	–	2.3
	Eicosapentaenoic acid (EPA)(C20:5)	–	18.5
	Docosahexaenoic acid (DHA) (C22:6)	–	1.0
Others			0.5

Others=unknown fatty acids
SFA, MUFA and PUFA=saturated, mono- and polyunsaturated fatty acids, respectively

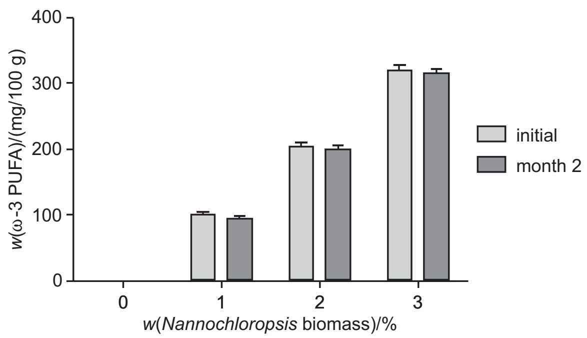

Fig. 7. Omega-3 PUFA levels found in cookies with 0 (control), 1, 2 and 3 % microalgal biomass, initially and after 2 months of storage

Omega-3 fatty acids did not decompose during the preparation of cookies; microalgal cells resisted thermal treatment by encapsulating the fatty acid molecules and thus protecting them from oxidation (*32*).

As observed in Table 3, pasta fatty acid profile consists mainly of 54 % PUFAs (with the absence of EPA and DHA), 27 % SFAs and 17 % MUFAs. Increase in EPA and DHA level was achieved in raw pasta with 1–3 % of microalgal biomass.

International Society for the Study of Fatty Acids and Lipids recommends an intake of at least 500 mg/day of EPA and DHA (*33*). The World Health Organization and North Atlantic Treaty Organization have made formal, population-based recommendations: daily recommended amounts of EPA+DHA range from 300 to 500 mg/day, and of ALA from 800 to 1100 mg/day (*10*).

Table 3. Fatty acid profile of pasta expressed as average value of the percent of total fatty acids with and without freeze-dried *Nannochloropsis* biomass

Fatty acid	Control		*w*(*Nannochloropsis* biomass in CP)/%					
			Initial			After 2 months		
	Raw pasta (RP)	Cooked pasta (CP)	0.5	1.0	3.0	0.5	1.0	3.0
14:0	0.37[a]	0.39[a]	0.413[a]	1.12[b]	1.98[c]	0.42[a]	1.14[b]	2.03[c]
16:0	22.38[a]	21.78[a]	21.91[a]	20.42[a]	19.52[a]	21.93[a]	20.51[a]	19.68[a]
18:0	2.27[b]	2.12[ab]	2.28[b]	2.03[a]	1.99[a]	2.34[b]	2.23[b]	2.14[ab]
Other SFA	0.22	0.17	0.45	0.60	0.92	0.43	0.64	0.85
ΣSFA	25.24	23.46	25.053	23.58	23.46	25.12	24.52	24.7
16:1	0.21[a]	0.22[a]	2.384[b]	2.331[b]	2.285[b]	2.435[b]	2.402[b]	2.358[b]
18:1	15.41[c]	14.87[b]	15.615[c]	14.231[a]	13.953[a]	15.71[c]	14.32[a]	13.96[a]
Other MUFA	1.52	1.32	1.27	0.57	0.63	1.23	0.59	0.64
ΣMUFA	17.14	16.41	19.269	17.132	16.868	19.375	17.312	16.958
18:2	49.31[b]	50.48[c]	48.195[b]	50.387[d]	46.59[a]	47.97[b]	50.183[d]	46.154[a]
18:3	3.54[a]	4.19[b]	4.09[b]	3.42[a]	3.45[a]	3.98[b]	3.35[a]	3.32[a]
20:4	–	–	0.33[a]	0.47[b]	0.70[c]	0.30[a]	0.43[b]	0.66[c]
20:5	–	–	2.26[b]	4.37[c]	8.25[d]	1.98[b]	4.14[c]	8.03[d]
22:6	–	–	0.15[b]	0.30[c]	0.53[d]	0.12[b]	0.25[c]	0.47[d]
Other PUFA	1.20	1.12	0.085	0.128	0.67	0.085	0.128	0.67
ΣPUFA	54.05	55.79	55.11	59.075	60.19	54.43	58.48	59.30

Different letters in the same row represent significant differences ($p<0.05$)
SFA, MUFA and PUFA=saturated, mono- and polyunsaturated fatty acids, respectively

Pasta with 0.5 % of *Nannochloropsis oculata* biomass had ω-3 PUFA levels (EPA+DHA) of 31 mg per 100 g, while pasta with 1 and 3 % of *Nannochloropsis oculata* biomass had ω-3 PUFA levels (EPA+DHA) of 63 and 190 mg per 100 g, respectively. One portion (28 g) of pasta enriched with 1 and 3 % of microalgal biomass can provide 16 and 43 mg of ω-3 LC-PUFA (EPA and DHA), respectively. For comparison, some of the reported values of ω-3 LC-PUFA in instant fortified foods are between 4 and 32.5 mg of ω-3 LC-PUFA per portion; the range of products is quite broad, ranging from tomato soup, jelly and milk to infant foods (*34*). Successful incorporation of *Isochrysis galbana* and *Diacronema vlkianum* biomass as a source of ω-3 fatty acids in pasta was reported by Fradique *et al.* (*26*). EPA and DHA content of 55.5 and 80.8 mg per 100 g of dry mass was observed in functional spaghetti enriched with ROPUFA powder, which was lower when compared with the present study (*35*). These results confirmed the importance of microalgal incorporation in traditional foods to enrich the nutritional value with bioactive molecules. Only minor variations in fatty acid profiles were observed after two months of storage (shown in Table 3).

Sensorial characteristics of cookies and pasta

It is necessary to maintain typical sensorial characteristics of a product in order to meet commercial success in the development of functional foods. The main sensory characteristics (colour, odour, flavour, texture and ccthe overall acceptability) of products were determined by an untrained panel. Control cookies were preferred over those with incorporated microalgae. However, cookies with 1 and 2 % of microalgal biomass were positively classified and appreciated. The panel identified a fishy taste at the higher fraction of microalgae (3 %) in cookies, which might lead to a weak global appreciation (Tables 4 and 5).

Sensory evaluation of cookies revealed that there are significant differences ($p<0.05$) in the mean scores of sensory attributes like taste, colour, flavour and overall acceptability among cookies with various fractions of microalgal biomass. Quality scores obtained from the evaluation of colour, flavour and texture of the cookies were used for the determination of overall acceptability. It was found to be higher for pasta when compared to cookies. The decrease in the overall acceptability was due to the decrease in colour, flavour, taste and texture scores.

The panellists preferred control pasta; however, pasta prepared with 3 % of microalgae was positively classified (Table 6). The pasta prepared with a microalgal biomass exceeding 3.0 g per 100 g of dry mass was least appreciated in terms of odour and texture.

Incorporation of up to 20 % of edible seaweed wakame (*Undaria pinnatifida*) resulted in improved amino acid and fatty acid profiles, increase of antioxidant activity, and higher content of fucoxanthin and fucosterol in seaweed pasta, and received sensorial acceptance (*25*). Sensory evaluation of fortified spaghetti was performed by Iafelice *et al.* (*36*) using different sources of ω-3 fatty acids; an unpleasant flavour and aftertaste were detected with higher additions of ω-3 fatty acids. Sensory evaluation studies showed that up to 2 % of microalgal incorporation in pasta was found to be acceptable (*24*).

Table 4. Sensory analysis of sweet cookies with freeze-dried *Nannochloropsis oculata*

w(microalgal biomass)/%	Colour	Odour	Texture	Flavour	Overall acceptability
Initial					
Control	$(7.5\pm0.2)^{b}$	$(7.7\pm0.2)^{b}$	$(7.1\pm0.2)^{a}$	$(7.4\pm0.1)^{ab}$	$(7.0\pm0.2)^{a}$
1.0	$(6.0\pm0.4)^{ab}$	$(5.4\pm0.2)^{a}$	$(6.6\pm0.3)^{b}$	$(6.0\pm0.2)^{ab}$	$(6.0\pm0.2)^{ab}$
2.0	$(4.9\pm0.1)^{a}$	$(4.8\pm0.3)^{a}$	$(5.1\pm0.09)^{a}$	$(4.7\pm0.4)^{a}$	$(4.8\pm0.2)^{a}$
3.0	$(3.0\pm0.1)^{a}$	$(3.0\pm0.07)^{a}$	$(4.1\pm0.2)^{b}$	$(3.4\pm0.3)^{a}$	$(3.3\pm0.3)^{a}$
After 30 days					
Control	$(7.3\pm0.1)^{b}$	$(7.4\pm0.6)^{b}$	$(6.6\pm0.1)^{a}$	$(7.0\pm0.2)^{ab}$	$(6.8\pm0.3)^{a}$
1.0	$(5.8\pm0.2)^{a}$	$(5.6\pm0.4)^{a}$	$(6.0\pm0.3)^{b}$	$(5.9\pm0.2)^{a}$	$(5.8\pm0.2)^{a}$
2.0	$(4.6\pm0.1)^{a}$	$(4.6\pm0.3)^{a}$	$(4.5\pm0.2)^{a}$	$(4.4\pm0.3)^{a}$	$(4.5\pm0.1)^{a}$
3.0	$(3.1\pm0.2)^{a}$	$(3.0\pm0.1)^{a}$	$(3.7\pm0.1)^{b}$	$(3.1\pm0.2)^{a}$	$(3.2\pm0.1)^{a}$

Table 5. Sensory analysis of spicy cookies with freeze-dried *Nannochloropsis oculata*

w(microalgal biomass)/%	Colour	Odour	Texture	Flavour	Overall acceptability
Initial					
Control	$(7.5\pm0.2)^{ab}$	$(7.7\pm0.2)^{b}$	$(7.1\pm0.2)^{a}$	$(7.4\pm0.1)^{ab}$	$(7.0\pm0.2)^{a}$
1.0	$(6.3\pm0.2)^{a}$	$(6.2\pm0.2)^{a}$	$(6.8\pm0.2)^{b}$	$(6.4\pm0.2)^{a}$	$(6.5\pm0.3)^{a}$
2.0	$(5.1\pm0.2)^{a}$	$(4.9\pm0.4)^{a}$	$(5.3\pm0.1)^{b}$	$(5.0\pm0.3)^{a}$	$(5.1\pm0.1)^{a}$
3.0	$(3.4\pm0.06)^{a}$	$(3.4\pm0.3)^{a}$	$(3.9\pm0.09)^{b}$	$(3.8\pm0.1)^{b}$	$(3.5\pm0.2)^{a}$
After 30 days					
Control	$(7.3\pm0.1)^{b}$	$(7.4\pm0.6)^{b}$	$(6.6\pm0.1)^{a}$	$(7.0\pm0.2)^{a}$	$(6.8\pm0.3)^{a}$
1.0	$(6.0\pm0.2)^{b}$	$(5.7\pm0.2)^{a}$	$(6.3\pm0.2)^{c}$	$(6.0\pm0.2)^{b}$	$(6.1\pm0.2)^{b}$
2.0	$(4.8\pm0.1)^{a}$	$(4.6\pm0.2)^{a}$	$(4.9\pm0.3)^{b}$	$(4.7\pm0.3)^{a}$	$(4.8\pm0.09)^{a}$
3.0	$(3.2\pm0.2)^{a}$	$(3.5\pm0.1)^{ab}$	$(3.7\pm0.2)^{b}$	$(3.6\pm0.2)^{b}$	$(3.5\pm0.2)^{ab}$

Table 6. Sensory analysis of pasta with freeze-dried *Nannochloropsis oculata*

w(microalgal biomass)/%	Colour	Odour	Texture	Flavour	Overall acceptability
Initial					
Control	$(8.2\pm0.2)^{b}$	$(8.0\pm0.2)^{b}$	$(7.9\pm0.2)^{a}$	$(7.8\pm0.2)^{a}$	$(7.9\pm0.3)^{a}$
1.0	$(7.1\pm0.3)^{a}$	$(7.0\pm0.3)^{a}$	$(7.1\pm0.2)^{a}$	$(6.9\pm0.3)^{a}$	$(6.9\pm0.2)^{a}$
2.0	$(5.9\pm0.2)^{ab}$	$(6.0\pm0.1)^{b}$	$(6.1\pm0.1)^{b}$	$(5.5\pm0.3)^{a}$	$(5.8\pm0.2)^{ab}$
3.0	$(4.3\pm0.2)^{a}$	$(4.8\pm0.1)^{b}$	$(5.0\pm0.3)^{b}$	$(4.6\pm0.3)^{ab}$	$(4.7\pm0.3)^{ab}$
After 30 days					
Control	$(8.0\pm0.2)^{b}$	$(7.8\pm0.3)^{a}$	$(7.7\pm0.2)^{a}$	$(7.6\pm0.3)^{a}$	$(7.8\pm0.3)^{a}$
1.0	$(7.0\pm0.2)^{b}$	$(6.6\pm0.3)^{ab}$	$(6.5\pm0.3)^{ab}$	$(6.1\pm0.4)^{a}$	$(6.4\pm0.2)^{ab}$
2.0	$(5.5\pm0.2)^{b}$	$(5.2\pm0.3)^{a}$	$(5.4\pm0.2)^{b}$	$(5.0\pm0.2)^{a}$	$(5.2\pm0.3)^{a}$
3.0	$(4.0\pm0.2)^{a}$	$(4.9\pm0.1)^{b}$	$(4.4\pm0.3)^{ab}$	$(4.3\pm0.2)^{a}$	$(4.5\pm0.3)^{ab}$

Data are reported as mean values±standard deviations of triplicate measurements
Different letters in the same row represent significant differences ($p<0.05$)

Conclusions

In the present study, freeze-dried *Nannochloropsis oculata* biomass was utilized as a functional ingredient in cookies and pasta for the enrichment with ω-3 fatty acids EPA and DHA. Colour values were found to be stable for a storage period of two months. The addition of microalgal biomass increased the firmness value. Sensory acceptance level of microalgal biomass incorporation was found to be 2 % for cookies and 3 % for pasta. Omega-3 PUFA levels (EPA+DHA) of 197 mg per 100 g of cookies enriched with 2 % of *Nannochloropsis oculata* and 190 mg per 100 g of pasta incorporated with 3 % of *Nannochloropsis oculata* biomass were observed. Omega-3 fatty acid profiles were found to be stable for two months of storage and no significant losses were observed throughout this period. Functional cookies and pasta enhanced with PUFA offer a niche in food market.

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