International Journal of Food Science +Technology

Original article

The use of *Nannochloropsis* sp. as a source of omega-3 fatty acids in dry pasta: chemical, technological and sensory evaluation

Estefanía Rodríguez De Marco,[1] María Eugenia Steffolani,[1] Marcela Martínez[2] & Alberto E. León[1]*

1 Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba (UNC), Instituto de Ciencia y Tecnología de Alimentos Córdoba (ICYTAC), CONICET-UNC, Av. Filloy S/N, Ciudad Universitaria, X5000HUA, Córdoba, Argentina
2 Instituto Multidisciplinario de Biología Vegetal (IMBIV, CONICET), Cátedra de Química Orgánica and Instituto de Ciencia y Tecnología de los Alimentos (ICTA – Facultad de Ciencias Exactas Físicas y Naturales – Universidad Nacional de Córdoba), Av. Vélez Sarsfield 1666, X5016GCN, Córdoba, Argentina

(Received 23 June 2017; Accepted in revised form 27 August 2017)

Summary *Nannochloropsis* is a microalga characterised by having high amounts of eicosapentaenoic acid (EPA), a fatty acid known for its health benefits. The aim of this study was to elaborate dry pasta with a significant contribution of EPA using *Nannochloropsis* sp., without affecting the quality product and with good consumer acceptance. Technological quality was analysed in terms of cooking properties and texture profile. Cooked pasta was characterised through proximal composition, phenolic compound, fatty acid content and sensorial analysis. It was possible to replace up to 30% of wheat flour with microalgae without affecting the technological quality of pasta and with a significant contribution of EPA to the daily diet (0.237 g per 100 g pasta). The incorporation of 10% and 20% *Nannochloropsis* in pasta formulation allowed to decrease the n6:n3 ratio from 25:1 to 5:1 and 2:1, respectively. Therefore, the microalgae are an interesting ingredient to increase EPA consumption in products like pasta, while the sensory evaluation confirms the possibility towards a commercial approach.

Keywords Dried pasta, eicosapentaenoic acid, *Nannochloropsis* sp., omega-3.

Introduction

The therapeutic significance of the intake of omega-3 polyunsaturated fatty acid (n-3, PUFA) in the modulation and prevention of human diseases has been clearly reported by clinical, animal and epidemiological studies. These important health benefits are associated mainly with n-3 long-chain polyunsaturated fatty acid (LC-PUFA, 20 or more carbon atoms), particularly eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6). EPA plays an essential role in several functions of biological membranes and is the precursor of bioactive metabolites, such as eicosanoids and resolvins, which intervene in many physiological functions (Gill & Valivety, 1997). There is extensive evidence of EPA preventive effect on cardiovascular diseases (Gebauer *et al*., 2006; Siegel & Ermilov, 2012) as well as of EPA potential anti-inflammatory effect (Babcock *et al*., 2000; Calder, 2015).

As α-linolenic acid (n-3 PUFA), a precursor of n-3 LC-PUFA, is an essential fatty acid that cannot be synthesised by humans, it must be provided by the diet; the major dietary sources are vegetable oils such as canola, soya bean, chia, flax and walnut oil (Abedi & Sahari, 2014). Although α-linolenic acid is metabolized to EPA and DHA by enzymatic elongation and desaturation, conversion is slow and occurs only in particular tissues, and α-linolenic acid competes with linoleic acid (n-6 PUFA) for the same enzymes to produce arachidonic acid (n-6, AA, 20:4) (Trautwein, 2001). As a consequence, the intake of EPA and DHA directly from the diet is recommended.

The conventional source of n-3 LC-PUFA is fish and, because of this, the American Heart Association recommends eating fish at least twice a week to meet the daily suggested intake of n-3 LC-PUFA, which is about 0.250–2 g of EPA+DHA/day in the case of adults (Krauss *et al*., 2000; FAO 2010). However, Western population does not consume high amounts of fish and consequently, the intake of EPA and DHA is lower than the suggested value. Also, the high intake of vegetable seed oils (corn, sunflower, safflower, cottonseed and soya bean oils) as well as animal products, provide high amounts of n-6 fatty acids, turning the recommended n-6:n-3 ratio from 2-4:1 to about 20–30:1 (Simopoulos, 2008). This has led to a growing

*Correspondent: E-mail: aeleon@agro.unc.edu.ar

doi:10.1111/ijfs.13609

industry designed to enrich or fortify popular food products with n-3 LC-PUFA, in order to correct gross nutritional deficiencies in modern society.

Considering the increasing demand of n-3 LC-PUFA, the fact that fish oil is limited and that its use has several disadvantages as its taste, odour and expensive recovery, new sources of EPA and DHA are being explored. Microalgae are the most promising alternative because they are the main producers of EPA and because fish usually gets this fatty acid via bioaccumulation in the food chain (Wen & Chen, 2003). *Nannochloropsis* sp., *Eustigmatophyceae* microalgae, are known for their high lipid content with valuable amounts of EPA; however, the composition of microalgae depends on culture condition and growth phase at the time of harvest (Rebolloso-Fuentes *et al*., 2001). Also, high amounts of carotenoid and high antioxidant activity were reported (Royckebosch *et al*., 2014).

Dry pasta is a popular wheat flour food item widely consumed worldwide. Linoleic acid (n-6) is the main fatty acid in wheat flour, containing a very small proportion of fatty acids of the n-3 series (Morrison, 1978). The replacement of wheat flour by microalgae in cereal products could increase n-3 LC-PUFA content and reduce the n-6:n-3 ratio. Prabhasankar *et al*. (2009) incorporated seaweed (*Undaria pinnatifida*) in pasta to develop a product with functional compounds, improving n-3 LC-PUFA content, enhancing amino acid profile and fucoxanthin and fucosterol contents, but the sensorial analysis was carried with semi-trained panellists ($n = 15$) and the acceptability expressed the preference of few panellists. Fradique *et al*. (2012) elaborated fresh pasta with two microalgae (*Isochrysis galbana* and *Diacronema vlkianum*) as PUFA sources, increasing EPA and DHA in the final product and sensorial evaluation, revealed a slight depreciative fish flavour for the pasta with 2 g per 100 g microalgal biomass contents. The production of dry pasta has the advantage that this product has a longer shelf life, although it is important to study whether the drying and cooking processes affect PUFAs due to their high susceptibility towards oxidation. Furthermore, the microalgae present high content of phenolic compounds, and these are responsible for the antioxidant properties (Hajimahmoodi *et al*., 2010). In previous works, it was possible to increase the antioxidant capacity of dry pasta through the incorporation of lyophilised tomato matrix or with durum wheat bran extracts without decreasing pasta quality (Pasqualone *et al*., 2015, 2016). Consumer's acceptance still remains a major obstacle in the food industry and sensory evaluation provides important information for the development and marketing of new products. Adams *et al*. (2007) proposed the application of CATA (Check-all-that-apply) questions as a simple method to gather information about consumers' perception. This method consists of a list of words or phrases from which consumers select all those they consider appropriate to describe and characterise food products.

The aim of this study was to characterise dry pasta elaborated with *Nannochloropsis* sp. and to determine the amount of microalgae biomass that could be incorporated in dry pasta to improve its nutritional quality through the addition of omega-3 LC-PUFAs and phenolic compounds, without affecting both the quality and the acceptability of the final product. This would mean a significant contribution to the recommended omega-3 LC-PUFA intake and thus to consumers' acceptance.

Materials and methods

Raw materials

Wheat flour (*Triticum aestivum*) was obtained commercially (Graciela Real, Córdoba, Argentina): moisture $12.9 \pm 0.1\%$; proteins $14.3 \pm 0.1\%$; ash $0.7 \pm 0.1\%$; lipids $0.9 \pm 0.2\%$; carbohydrates $71.2 \pm 0.3\%$. *Nannochloropsis* sp. biomass (moisture $4.0 \pm 0.1\%$, 90% particle size < 250 μm) was provided by Monzón Biotech SL (Spain).

Pasta processing

Pasta samples were elaborated from wheat flour replaced with increasing amounts of *Nannochloropsis* sp. biomass: 10, 20, 30 and 40 g per 100 g (N10, N20, N30 and N40, respectively). A control sample (CO) was made without microalgae biomass. Blends (50 g) were mixed with optimum amounts of distilled water (determined experimentally, about 30% flour basis) and salt (500 mg per 50 g of blend), with a hand mixer (Philips, 190W, Buenos Aires, Argentina) for 3 min at maximum speed. The dough was sheeted using a pasta maker (Pastalinda®, Buenos Aires, Argentina) and cut in strips of 2 mm wide and 2 mm thick. Then, the strips were dried at low temperature in two steps: 30 min predrying stage at 30 °C with humidity-controlled (35%), followed by a drying stage at 45 °C with controlled humidity (75%) during 17 h in a moisture-controlled cabinet (FAC, Fabricantes de Aparatos Científicos S.A., Quilmes, Argentina) (Rodríguez De Marco *et al*., 2014). The final moisture of dried pasta was 9.5 ± 0.5 g per 100 g. Three pasta samples of each formulation were prepared on different occasions, and all determinations were measured for each sample. After a week of storage in hermetically closed bags, dry pasta from each formulation was cooked, freeze-dried and stored at −18 °C. Proximal

composition, phenolic and fatty acid contents of freeze-dried samples were determined.

Optimum cooking time of pasta

Optimum cooking time of pasta was determined according to AACC 16-50 (2000). Pasta strands of 5 cm (4 g) were cooked in boiling water (200 mL) and analysed at 30-s intervals by compressing the strands between two glass slides until the inner white core of the pasta disappeared.

Microalga and pasta composition

Proximal analysis

Moisture, proteins, ash and lipids were determined according to AOAC (2006). All tests were performed in triplicate. Carbohydrates were obtained by difference.

Fatty acid content

The identification of fatty acids from microalgae oil and cooked pasta (obtained from cold extraction with hexane during 24 h) was carried out using gas chromatography coupled with a mass spectrometer (GS-MS). The hydrolysis of fatty acids was prepared with 0.10 g of oil and 5 mL KOH 0.5 N under reflux for 15 min. Then, fatty acid methyl esters (FAMEs) were prepared with 15 mL of NH_4Cl/H_2SO_4 in methanol, under reflux during 5 min. After cooling, FAMEs were extracted with n-hexane (3 × 20 mL). The organic phase was dried over anhydrous sodium sulphate, filtered, and the solvent was removed using a rotatory evaporator and the FAMEs dissolved in 0.5 mL of n-hexane. The analysis was performed using a gas chromatograph (Clarus 600, Perkin Elmer, Shelton, CT, USA). The separation was achieved using a capillary column Carbowax (60 m, 0.25 mm ID, 0.25 μm de particle, Perkin Elmer). Helium was used as the carrier gas; after holding at 180 °C for 5 min, temperature was ramped at 4 °C min^{-1} to 230 °C and maintained at 230 °C for 25 min with the injector at 250 °C. The split ratio was 1:100. Fatty acid methyl esters were identified by comparison with the retention time of individual standards (NIST MS Search 2.0.). The quantification was performed using the internal standard (11:0). The limit of detection was 1 mg per 100 g. All analytical determinations were performed in triplicate.

Phenolic content

Sample preparation was carried out as described in a previous work (Rodríguez De Marco *et al.*, 2014). Freeze-dried biomass and cooked freeze-dried pasta samples were extracted with 2 mL of hexane for 30 min at 37 °C. The samples were centrifuged at 13 000 ***g*** for 10 min at 4 °C, and the supernatants were recovered. The extractions were repeated and the two supernatants combined. Then, the residues were extracted twice with ethyl acetate (2 mL each time) for 30 min at 37 °C and the supernatants were combined. Finally, the residues were extracted twice with water (2 mL each time) for 30 min at 37 °C and the supernatants were combined. The hexane and the ethyl acetate extract were recovered in a rotary evaporator, and the water extract was lyophilised. All pellets were redissolved in methanol, obtaining three different extracts: hexane, ethyl acetate and water extract. Total phenolic content (TPC) was determined by the Folin–Ciocalteu method, according to Singleton & Rossi (1965) with slight modifications. Extract (20 mL) was mixed with 90 mL of methanol, 1.68 mL of distilled water and 100 mL of Folin–Ciocalteu reagent, and stirred in a vortex for 20 s. Three hundred millilitres of Na_2CO_3 (20 g per 100 mL) was added, and tubes were left to stand for 2 h in the dark. Then, absorbance was read at 750 nm. TPC was calculated by linear regression using gallic acid (Riedel-de-Haën, China) as standard. Results are expressed in mg L^{-1} gallic acid equivalents (GAE). All samples were analysed in triplicate.

Cooking quality parameters

The swelling index of cooked pasta (g water per g dry pasta) was evaluated by drying cooked pasta samples to constant weight at 105 °C, expressed as: [weight of cooked product (W1) − weight after drying (W2)]/weight after drying (W2) (Tudorica *et al.*, 2002).

The water absorption of drained pasta was determined as: [[weight of cooked pasta (W1) − weight of raw pasta (W3)]/weight of raw pasta (W3)] × 100 (Tudorica *et al.*, 2002).

Cooking loss in cooking water was determined by evaporation to constant weight in an air oven at 105 °C. The residue was weighed and reported as percentage of the original pasta sample (Tudorica *et al.*, 2002).

All the tests were carried out in triplicate.

Texture analysis profile

Cooked pasta texture analysis profile was measured according to Martinez *et al.* (2007) with slight modifications. A texture analyser with a stainless steel circular probe of 25 cm diameter (Universal Testing Machine, Model 3342, Instron Inc., Norwood, MA, USA) was used. Pasta strands of 5 cm long were cooked, and three pieces of cooked pasta were placed perpendicularly to the probe so that each strand was in contact with the next. The samples, approximately 2 mm thick, were compressed at a rate of 0.83 mm s^{-1} and at a strain ratio of 60%. The probe was retracted and followed by a second compression cycle after 15 s.

TPA values for hardness (the peak force attained during the first compression), adhesiveness (negative area under the first compression, representing the work necessary to pull the compressing plunger away from the sample), cohesiveness (the ratio of area under the second peak to the area under the first peak), springiness (the rate at which a deformed sample went back to its undeformed condition after the deforming force is removed, calculated as the ratio of distance of the first half of the second peak to the distance of the first half of the first peak) and chewiness (the product of hardness, cohesiveness and springiness) were obtained through five measurements for each sample, on three sample prepared on different occasions, totalling 15 measurements. Texture profile analysis was performed in dry pasta after one week of storage.

Sensorial evaluation

A consumer test was conducted in order to evaluate the acceptance of pasta elaborated with microalga, and a CATA (check-all-that-applied) method was performed to describe each sample.

The samples evaluated were control, N10, N20 and N30; N40 was not analysed because it showed some differences in cooking properties and textural parameters from the control pasta. All samples were cooked at their optimal cooking time and served to the panellists in white plastic glass, in random order with three random numbers. Drinking water was provided for palate cleansing between each sample.

Consumers acceptance and check-all-that-applied questions (CATA)

An untrained panel of seventy-one individuals (forty-three female and twenty-eight male, ages ranging from 22 to 60 years old) tested cooked pastas. Consumers were asked to evaluate surface appearance, flavour, texture and global acceptance of each sample (attributes generally perceived by consumers) on a 9-point hedonic scale (1 = 'dislike extremely', 9 = 'like extremely'). Products were considered acceptable if their mean scores were above 5 (neither like nor dislike) (Bustos *et al.*, 2011). Then, a list of selected attributes (firmness, strange taste, slight fish taste, moderate fish taste, intense fish taste, unpleasant smell, pleasant smell, bad appearance, good appearance, ugly colour and nice colour) was presented to the consumer panel of pasta for them to check all attributes that applied (CATA) to a given sample. A count of selected attributes in each sample was carried out to describe pasta samples. The test and the ways to administer them were performed according to Varela & Ares (2012) and Valentin *et al.* (2012). Sensory analysis of dry pasta was conducted after one week of storage.

Statistical analysis

Results were expressed as the mean of replications ± SD. Analysis of variance (ANOVA) followed by Fisher's test using the InfoStat Statistical Software (Facultad de Ciencias Agropecuarias, UNC, Argentina) were performed for statistical analysis. Pearson's correlation coefficient was calculated as a measure of the relationships.

CATA data statistical analysis was carried out according to Meyners *et al.* (2013) using XLStat software version 2014.6.2 (Adinsoft, Paris, France). Chocran *Q* test was conducted to identify whether there were significant differences between samples for each attribute evaluated by CATA questions; a correspondence analysis (CA) was performed on the frequency table to obtain a bidimensional representation of the samples and descriptors.

Results and discussion

Microalgae characterisation

Table 1 shows the physicochemical composition of *Nannochloropsis* sp. biomass, which contains high protein, lipid and ash contents. The major fatty acids present in the microalgae were as follows: palmitic acid, EPA and palmitoleic acid. This trend was observed with slight variations on several species of the genus *Nannochloropsis* (Volkman *et al.*, 1993; Pieber *et al.*, 2012). The differences may be explained in terms of the natural diversity in biological samples as well as growth conditions and state. The main objective to use this microalga as food or functional ingredients is the high content of EPA fatty acid, known for its health effects. Also, arachidonic acid (AA, 20:4, omega-6) is present in microalga composition. Like EPA, AA is a precursor of eicosanoid compound, but the eicosanoids from both fatty acids are structurally and functionally different and sometimes present even antagonistic effects, so a balanced uptake EPA/AA can prevent eicosanoid dysfunctions (Wen & Chen, 2003).

Considering phenolic content of the biomass, only the water extract presented a high phenolic compound content. The hexane and ethyl acetate fraction did not show phenolic content, and this can be ascribed to the overall polar nature of phenolic compounds. This result is in agreement with Hajimahmoodi *et al.* (2010), who also found, in some strains of microalgae, the highest phenolic content in the water fraction, although these authors performed the extraction at 80 °C, while zero or low phenolic content in hexane and ethyl acetate fraction were reported. However, it is also important to consider that the phenolic compounds composition strongly depends on the

Table 1 Microalgae and cooked pasta characterisation

	***Nannochloropsis* sp.**	**CO**	**N10**	**N20**	**N30**	**N40**
Protein[†]	55.3 ± 0.2	15.3 ± 0.1[a]	19.1 ± 1.1[b]	24.5 ± 0.8[c]	28.1 ± 0.4[d]	33.5 ± 2.1[e]
Lipid[†]	8.9 ± 0.2	0.6 ± 0.1[a]	1.3 ± 0.1[c]	2.2 ± 0.1[d]	3.0 ± 0.1[e]	3.6 ± 0.1[f]
Carbohydrates[†]	27.2 ± 0.5	83.5 ± 0.1[e]	78.7 ± 1.3[d]	71.8 ± 0.8[c]	66.7 ± 0.3[b]	60.2 ± 2.2[a]
Ash[†]	8.6 ± 0.1	0.6 ± 0.1[a]	0.9 ± 0.5[b]	1.5 ± 0.1[c]	2.0 ± 0.8[d]	2.6 ± 0.6[e]
Phenolic content[‡]	5.1 ± 0.1	0.2 ± 0.1[a]	0.7 ± 0.1[b]	1.1 ± 0.1[c]	1.5 ± 0.1[d]	2.0 ± 0.1[e]
Fatty acid profile[§]						
14:00	118.9 ± 1.2	nd[a]	8.0 ± 0.2[b]	22.2 ± 1.4[c]	36.0 ± 0.8[d]	44.5 ± 0.8[e]
14:01	54.1 ± 1.5	nd[a]	1.5 ± 0.0[b]	3.5 ± 0.2[c]	5.8 ± 0.1[d]	6.0 ± 0.2[d]
16:00	1064.5 ± 38.4	42.2 ± 0.0[a]	84.3 ± 0.8[b]	222.7 ± 13.8[c]	336.2 ± 4.4[d]	424.3 ± 7.8[e]
16:01	587.9 ± 33.9	nd[a]	108.1 ± 2.5[b]	202.9 ± 14.2[c]	257.8 ± 1.2[d]	298.3 ± 4.7[e]
18:01	115.3 ± 17.7	38.2 ± 0.2[a]	71.4 ± 0.7[b]	105.5 ± 5.8[c]	116.5 ± 0.4[d]	121.9 ± 1.1[d]
18:02	161.3 ± 4.4	132.3 ± 2.8[a]	205.2 ± 2.0[b]	235.8 ± 2.3[c]	251.8 ± 3.0[c]	282.2 ± 22.8[d]
18:03	16.2 ± 4.4	5.3 ± 0.1[a]	5.9 ± 0.6[a]	13.4 ± 1.5[b]	14.2 ± 0.9[b]	14.8 ± 1.7[b]
20:01	nd	2.3 ± 0.0[b]	nd[a]	nd[a]	nd[a]	nd[a]
20:04	100.8 ± 9.2	nd[a]	10.2 ± 5.0[ab]	18.1 ± 2.9[bc]	20.7 ± 6.0[c]	41.1 ± 0.1[d]
20:05	1017.2 ± 80.4	nd[a]	39.2 ± 1.5[b]	127.7 ± 6.8[c]	236.7 ± 3.9[d]	309.8 ± 22.0[e]
n6	262.1	132.2	215.4	253.9	272.5	323.3
n3	1033.4	5.3	45.0	141.1	250.9	324.6
n6:n3	0.25:1	25:1	5:1	2:1	1:1	1:1

CO, control sample; nd, <Limit of detection (LOD: 1 mg per 100 g).

N10, N20, N30 and N40: pasta prepared with 10, 20, 30 and 40 g per 100 g (w/w) of *Nannochloropsis* sp., respectively.

Results in the table represent the mean of triplicate measurements.

Within the same row, values with a different letter are significantly different ($P \leq 0.05$), according to Fisher test.

[†]g per 100 g of dry product.

[‡]mg GAE per g of dry product.

[§]mg per 100 g of dry product.

microalgae growing conditions (nutrients availability, temperature, pH).

Pasta composition

The substitution of wheat flour for *Nannochloropsis* sp. biomass in the formulation of pasta significantly increased protein, lipids and ash contents, proportionally according to microalgae biomass content (Table 1). When the experimental values with the expected theoretical values for each formulation were compared, ash content was less than expected in pasta with 40% of *Nannochloropsis* sp., probably due to mineral loss during cooking. Also, experimental proteins in cooked pasta were higher than expected, probably due to the effect of concentration as a consequence of material loss in water during cooking. As in the case of microalga biomass, only aqueous extract showed the presence of phenolic compounds, and the content of this compound significantly increases with the highest proportion of microalga. These experimental values were slightly lower than the theoretical values obtained from each formulation; these results were expected, considering the effects of the drying and cooking processes on pasta. Prabhasankar *et al*. (2009) found a smaller content of phenolic compounds in cooked pasta elaborated with seaweed (*Undaria pinnatifida*) than in raw pasta, and a high content in gruel, indicating a leaching of phenolic compounds into the cooking medium. However, the amount of phenolic compounds found in cooked pasta with *Nannochloropsis* sp. in the present study is relevant, and specific investigations on phenolic compounds would be interesting to determine their antioxidant activity.

Also, Table 1 shows the amount (mg per 100 g dry basis) of fatty acids present in control and microalgae cooked pasta. The major fatty acid present in control pasta was linoleic acid (18:2) followed by palmitic and oleic acid, in a smaller proportion. Omega-3 fatty acids were in a very low amount, the ratio of n-6:n-3 being too high (25:1). This predominance of n-6 linoleic acid in grain cereal is a major contribution to the imbalanced n-6:n-3 ratio consumption in Western diet (Fradique *et al*., 2012). Increasing the percentage of microalgae in pasta formulation significantly enhanced EPA fatty acid content, so the use of *Nannochloropsis* sp. as an ingredient in pasta formulation allowed us to elaborate pasta with EPA, a fatty acid absent from control sample. In turn, pasta elaborated

with microalga presented an important proportion of this LC-PUFAs in relation to other fatty acids. Even in pasta elaborated with the minimum percentage of substitution (N10), it was possible to obtain an important reduction of the ratio n-6:n-3 (5:1) in comparison with control pasta. The recommendations of EPA + DHA are 0.250–2 g day^{-1}, so the consumption of a N40 pasta dish (about 100 g of pasta – dry basis) provides 0.309 g of EPA, which represent almost 100% of the minimum daily value recommended, and therefore, the EPA minimum requirement would be achieved. However, the other formulations also provide a significant EPA contribution. Similar results where microalgae (*Isochrysis galbana* and *Diacronema vlkianum*) is used to enhance EPA and DHA in pasta products were reported by Fradique *et al.* (2012), although these authors worked with fresh pasta whose shelf life is minor. Comparing experimental values with theoretical values from each formulation, experimental EPA was significantly lower. Polyunsaturated fatty acids are easily oxidised and both the drying and the cooking processes affect their stability; however, a significant amount of EPA remained stable in the final product. Future studies about the effects of the drying process, storage conditions and cooking process of pasta on EPA content are necessary.

Technological quality of cooked pasta

To analyse the effect of the incorporation of *Nannochloropsis* sp. in the structure and the quality of cooked pasta, cooking quality parameters and texture analysis profile were evaluated.

The values obtained from cooking quality parameters are presented in Table 2. The incorporation of nonwheat ingredients in pasta formulation could interfere with gluten protein network development and could affect their integrity. When the network is discontinuous and not strong or elastic enough, starch swells and gelatinizes before protein coagulation, reducing the optimal cooking time of the pasta sample; amylose is lost mainly into the cooking water increasing cooking loss; while amylopectin concentrates on the pasta surface increasing stickiness, which results in a poor texture pasta (De Noni & Pagani, 2010). Only pasta elaborated with 40% of *Nannochloropsis* sp. presented a significantly higher cooking loss than control pasta, although the value was less than 8%, the limit value for semolina pasta (Dick & Youngs, 1988); this indicates that pasta structure was not largely affected by microalga incorporation. Cooking loss is the main parameter that affects consumer acceptance, which is why it is considered a cooked pasta quality indicator, so pasta elaborated with microalgae would be considered of good quality.

Water absorption closely depends on cooking time; however, a decrease in water absorption was observed in pasta elaborated with microalga, although no differences were detected in OCT. Also, water absorption and swelling index are related to starch content and gelatinization process. These parameters significantly decreased as microalgae content was increased in the formulation, probably as a consequence of a lower amount of wheat flour used in formulations. Considering that the swelling index is the relation between the weight of cooked pasta and the weight of cooked pasta that was dried at constant weight, the lower value of water absorption could explain the lower swelling index.

Cooked pasta texture is generally recognised as its most important overall quality aspect. To a certain extent, hardness, chewiness and elasticity can be considered positive attributes, because firm pasta is valued by the consumer; such higher sample values indicate better pasta quality. In contrast, stickiness can be

Table 2 Pasta cooking quality parameters

Sample	CO	N10	N20	N30	N40
Optimum cooking time (min)	7:30	6:30	6:30	7:30	7:00
Swelling index	0.83 ± 0.02^{e}	0.53 ± 0.01^{d}	0.37 ± 0.01^{c}	0.33 ± 0.01^{b}	0.26 ± 0.00^{a}
Water absorption (g per 100 g pasta)	154.6 ± 1.2^{c}	128.4 ± 2.8^{b}	114.9 ± 3.4^{a}	110.9 ± 0.9^{a}	105.6 ± 6.8^{a}
Cooking loss (g per 100 g pasta)	6.3 ± 0.1^{ab}	6.1 ± 0.1^{a}	6.1 ± 0.3^{a}	6.8 ± 0.2^{bc}	7.2 ± 0.2^{c}
Firmness (N)	7.05 ± 0.68^{a}	15.58 ± 1.47^{b}	19.35 ± 2.06^{c}	26.05 ± 1.50^{d}	38.11 ± 1.60^{e}
Stickiness (10^{-4} J)	2.99 ± 0.29^{a}	3.99 ± 0.34^{b}	4.47 ± 0.69^{b}	5.13 ± 0.77^{c}	7.18 ± 1.30^{d}
Elasticity	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	1.0 ± 0.1^{a}	1.0 ± 0.1^{a}
Cohesiveness	0.76 ± 0.02^{b}	0.67 ± 0.03^{a}	0.68 ± 0.02^{a}	0.65 ± 0.03^{a}	0.66 ± 0.02^{a}
Chewiness	5.04 ± 0.38^{a}	10.42 ± 0.94^{b}	12.96 ± 1.30^{c}	17.28 ± 0.86^{c}	24.98 ± 1.11^{e}

CO, control sample.

N10, N20, N30 and N40: pasta prepared with 10, 20, 30 and 40 g per 100 g (w/w) of *Nannochloropsis* sp., respectively.

Results in the table represent the mean of triplicate measurements.

Within the same row, values with a different letter are significantly different ($P \leq 0.05$), according to Fisher test.

Table 3 Consumer's acceptance

Sample	CO	N10	N20	N30
Surface appearance	7.1^{c}	6.1^{b}	5.2^{a}	4.9^{a}
Flavour	7.0^{d}	5.6^{c}	4.8^{b}	4.2^{a}
Texture	7.2^{c}	6.7^{c}	5.3^{b}	4.45^{a}
Global acceptance	7.2^{d}	6.1^{c}	4.9^{b}	4.1^{a}

CO, control sample.
N10, N20 and N30: pasta prepared with 10, 20 and 30 g per 100 g (w/w) of *Nannochloropsis* sp., respectively.
Within the same row, values with a different letter are significantly different ($P \leq 0.05$), according to Fisher test.

considered a negative attribute and a lower sample value indicates better pasta quality (Martinez *et al.*, 2007). Texture parameters are shown in Table 3. Hardness and chewiness increased with the microalga content, probably due to the high protein content in microalga composition. Also, stickiness increased with the microalga content and elasticity was not affected when microalgae was used as an ingredient in pasta. Cohesiveness decreased significantly with microalgae incorporation, but the amount of *Nannochloropsis* sp. in pasta did not affect this parameter. Texture parameters of cooked pasta suggested that the structure was not largely affected by the incorporation of microalgae and that it was only modified in the presence of high biomass content, except N40 sample which showed significant different values in all technological parameters, showing a poorer quality than control sample.

Sensory analysis

Table 3 shows the average of each attribute evaluated by consumer panellist. Surface appearance significantly decreased with microalga incorporation; however, N10 and N20 obtained values above 5. Global acceptance of N10 was lower than control, but it was classified positively as 'like slightly'. Also, the average of flavour significantly decreased while *Nannochloropsis* sp. increased in the formulation and only N10 obtain a value above 5. Consumers did not find significant differences between CO and N10 in texture, which were considered as 'like moderately'; however, the high amount of microalgae decreased the average of these attributes.

Figure 1 presents the results obtained from CATA methods as the frequency used for each attribute to describe different samples. The consumers select all word or phrases which they considered appropriate to describe microalgae pastas. The sample with lower

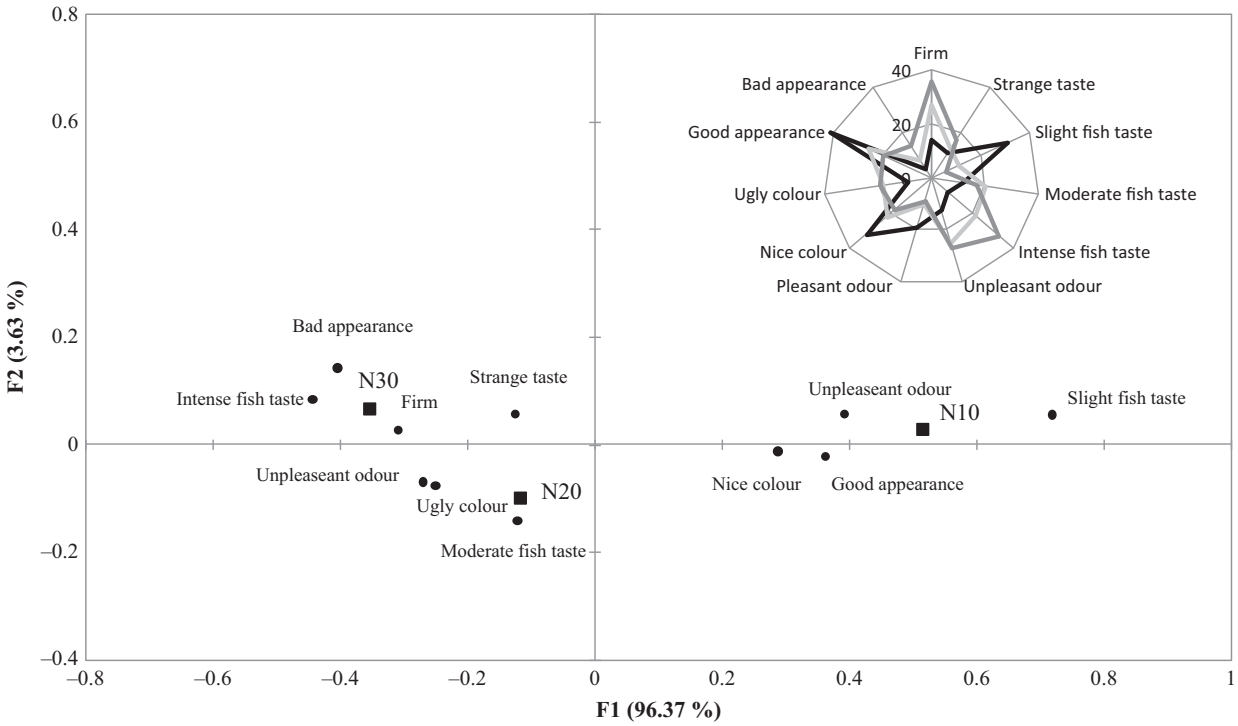

Figure 1 Bidimensional representation of the samples and descriptors obtained from check-all-that-apply method. N10, N20 and N30: pasta prepared with 10, 20 and 30 g per 100 g (w/w) of *Nannochloropsis* sp., respectively. Radial graph of the frequency of use of each descriptor for each sample: N10 (black), N20 (light grey) and N30 (grey), is shown in the upper right of representation.

microalga content (N10) was defined as pasta with good appearance, nice colour, slight fish taste and less firm. N20 was defined as having an unpleasant odour and a moderate-to-intense fish taste; the positive response to good appearance decreased and to firmness increased. Sample N30 was the firmest and it was related to intense fish taste, strange taste, unpleasant odour and ugly colour. There was a significant difference between the frequency of citation of each attribute, using the Cochran Q test ($P < 0.05$ in all cases), which suggests that this methodology was effective in detecting the sensorial difference between microalga pasta samples. This conclusion has already been reported in other food matrixes, such as milk desserts, yogurt and strawberries, among others (Ares *et al.*, 2010; Bruzzone *et al.*, 2011; Ares & Jaeger, 2013; Cruz *et al.*, 2013). Considering that CATA technique is not recommended for evaluating very similar samples (Varela & Ares, 2012) and that the samples evaluated have shown significant differences among them, it was possible to achieve the correct characterisation of all the pasta studied. Moreover, the descriptors used to define different pasta formulations agreed with the results obtained with the other two sensorial methodologies analysed.

Conclusions

It was possible to replace up to 30% of wheat flour with microalgae *Nannochloropsis* sp. with a significant contribution of EPA for the daily diet, without affecting pasta structure. The incorporation of *Nannochloropsis* sp. also enhances the content of phenolic compounds which would exhibit a high antioxidant activity. According to consumer's acceptance, the sample with 10% of *Nannochloropsis* sp. was the best qualified and no important differences were found with the control sample. Even though N20 was not well accepted by consumers (slightly unpleasant), the presentation of the dish in normal conditions (sauce and cheese) could improve consumer perception. Furthermore, the incorporation of 10% and 20% *Nannochloropsis* in the pasta formulation allowed to obtain lower n6:n3 ratio (5:1 and 2:1, respectively) in the enriched product as compared with control pasta (25:1). The use of *Nannochloropsis* sp. as an ingredient is an interesting way to increase EPA consumption and to reduce n-6:n-3 ratio in products like pasta, while the sensory evaluation confirms the possibility towards a commercial approach.

Acknowledgments

Gabriela Diaz Cortez did the language quality check of this manuscript.

References

AACC International (2000). *Approved Methods of Analysis*, 10th edn. St. Paul, MN: American Association of Cereal Chemists International.

Abedi, E. & Sahari, M.A. (2014). Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Science & Nutrition*, **2**, 443–463.

Adams, J., Williams, A., Lancaster, B. & Foley, M. (2007). Advantages and uses of check-all-that-apply response compared to traditional scaling of attributes for salty snacks. 7th Pangborn Sensory Science Symposium. Minneapolis, USA, 12–16 August.

AOAC (2006). *Official Methods of Analysis*, 18th edn. Rockville, MD: Association of the Official Analytical Chemists.

Ares, G. & Jaeger, S. (2013). Check-all-that-apply questions: influence of attribute order on sensory product characterization. *Food and Quality Preference*, **28**, 141–153.

Ares, G., Deliza, R., Barreiro, C., Giménez, A. & Gámbaro, A. (2010). Comparison of two sensory profiling techniques based on consumer perception. *Food Quality and Preference*, **21**, 417–426.

Babcock, T., Helton, W.S. & Espat, N.J. (2000). Eicosapentaenoic acid (EPA): an antiinflammatory N-3 fat with potential clinical applications. *Nutrition*, **16**, 1116–1118.

Bruzzone, F., Ares, G. & Giménez, A. (2011). Consumers' texture perception of milk desserts. Ii – comparison with trained assessors' data. *Journal of Texture Studies*, **43**, 214–226.

Bustos, M.C., Pérez, G.T. & León, A.E. (2011). Sensory and nutritional attributes of fibre-enriched pasta. *LWT – Food Science and Technology*, **44**, 1429–1434.

Calder, P. (2015). Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. *Biochimica et Biophysica Acta*, **1851**, 469–484.

Cruz, A.G., Cadena, R.S., Castro, W.F. *et al.* (2013). Consumer perception of probiotic yogurt: performance of check all that apply (CATA), projective mapping, sorting and intensity scale. *Food Research International*, **54**, 601–610.

De Noni, I. & Pagani, M.A. (2010). Cooking properties and heat damage of dried pasta as influenced by raw material characteristics and processing conditions. *Critical Reviews in Food Science and Nutrition*, **50**, 465–472.

Dick, J.W. & Youngs, V.L. (1988). Evaluation of durum wheat, semolina, and pasta in the United States. In: *Durum: Chemistry and Technology* (edited by G. Fabriani & C. Lintas). Pp. 237–248. St. Paul, MN: American Association of Cereal Chemists.

Food and Agriculture Organization of the United Nations (2010). Fats and fatty acids in human nutrition: Report of an expert consultation. FAO Food and Nutrition Paper 91. Rome.

Fradique, M., Batista, A.P., Nunes, M.C., Gouveia, L., Bandarra, N.M. & Raymundo, A. (2012). *Isochrysis galbana* and *Diacronema vlkianum* biomass incorporation in pasta products as PUFA's source. *LWT – Food Science and Technology*, **51**, 312–319.

Gebauer, S.K., Psota, T.L., Harris, W.S. & Kris-Etherton, P.M. (2006). N-3 Fatty acid dietary recommendation and food sources to achieve essentiality and cardiovascular benefits. *American Journal of Clinical Nutrition*, **83**, 1526–1535.

Gill, I. & Valivety, R. (1997). Polyunsaturated fatty acids: part 1. Occurrence, biological activities and application. *Trends in Biotechnology*, **15**, 401–440.

Hajimahmoodi, M., Faramarzi, M.A., Mohammadi, N., Soltani, N., Oveisi, M.R. & Nafissi-Varcheh, N. (2010). Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. *Journal of Applied Phycology*, **22**, 43–50.

Krauss, R.M., Eckel, R.H., Howard, B. *et al.* (2000). AHA Dietary Guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation*, **102**, 2284–2299.

Martinez, C.S., Ribotta, P.D., León, A.E. & Anón, M.C. (2007). Physical, sensory and chemical evaluation of cooked spaghetti. *Journal of Texture Studies*, **38**, 666–683.

Meyners, M., Castura, J.C. & Carr, T.B. (2013). Existing and new approaches for the analysis of CATA data. *Food Quality and Preference*, **30**, 309–319.

Morrison, W.R. (1978). Cereal lipids. In: *Advances in Cereal Science and Technology* (edited by Y. Pomeranz). Pp. 221–348. St. Paul, MN: American Association of Cereal Chemistry.

Pasqualone, A., Delvecchio, L.N., Gambacorta, G. *et al.* (2015). Effect of supplementation with wheat bran aqueous extracts obtained by ultrasound-assisted technologies on the sensory properties and the antioxidant activity of dry pasta. *Natural Product Communications*, **10**, 1739–1742.

Pasqualone, A., Gambacorta, G., Summo, C. *et al.* (2016). Functional, textural and sensory properties of dry pasta supplemented with lyophilized tomato matrix or with durum wheat bran extracts produced by supercritical carbon dioxide or ultrasound. *Food Chemistry*, **213**, 545–553.

Pieber, S., Schober, S. & Mittelbach, M. (2012). Pressurized fluid extraction of polyunsaturated fatty acids from the microalga *Nannochloropsis oculata*. *Biomass and Bioenergy*, **47**, 474–482.

Prabhasankar, P., Ganesan, P., Bhaskar, N. *et al.* (2009). Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: chemical, functional and structural evaluation. *Food Chemistry*, **115**, 501–508.

Rebolloso-Fuentes, M.M., Navarro-Pérez, A., García-Camacho, A., Ramos-Miras, J.J. & Guil-Guerrero, J.J. (2001). Biomass nutrient profiles of the microalga nannochloropsis. *Journal of Agriculture and Food Chemistry*, **49**, 2966–2972.

Rodríguez De Marco, E., Steffolani, M.E., Martínez, C.S. & León, A.E. (2014). Effects of spirulina biomass on the technological and nutritional quality of bread wheat pasta. *LWT – Food Science and Technology*, **58**, 102–108.

Royckebosch, E., Bruneel, C., Termote-Verhalle, R., Goiris, K., Muylaert, K. & Foubert, I. (2014). Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. *Food Chemistry*, **160**, 393–400.

Siegel, G. & Ermilov, E. (2012). Omega-3 fatty acids: benefits for cardio-cerebro-vascular diseases. *Atherosclerosis*, **225**, 291–295.

Simopoulos, A.P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine*, **233**, 674–688.

Singleton, V.L. & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144–153.

Trautwein, E.A. (2001). n–3 Fatty acids–physiological and technical aspects for their use in food. *European Journal of Lipid Science and Technology*, **103**, 45–55.

Tudorica, C.M., Kuri, V. & Brennan, C.S. (2002). Nutritional and physicochemical characteristics of dietary fiber enriched pasta. *Journal of Agricultural and Food Chemistry*, **50**, 347–356.

Valentin, D., Chollet, S., Lelievre, M. & Abdi, H. (2012). Quick and dirty still pretty good: a review of new descriptive methods in food science. *International Journal of Food Science and Technology*, **47**, 1563–1578.

Varela, P. & Ares, G. (2012). Sensory profiling, the blurred line between sensory and consumer science. A review of novel methods for product characterization. *Food Research International*, **48**, 893–908.

Volkman, J.K., Brown, M.R., Dunstan, G.A. & Jeffrey, S.W. (1993). The biochemical composition of marine microalgae from the class *Eustigmatophyceae*. *Journal of Phycology*, **29**, 69–78.

Wen, Z.H. & Chen, F. (2003). Heterotrophic production of eicosapentanoic acid by microalgae. *Biotechnology Advances*, **21**, 273–294.