Effect of microalgae incorporation on the physicochemical, nutritional, and sensorial properties of an innovative broccoli soup

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Abstract

The aim of this paper was to develop a broccoli soup enriched in *Spirulina* sp., *Chlorella* sp., or *Tetraselmis* sp., at concentrations ranging from 0.5 to 2.0% (w/v), and to assess the effect of microalgae incorporation on their quality and acceptance. Incorporation of freeze-dried microalgae biomass into the broccoli soup resulted in lower L^* values, especially after incorporation of *Spirulina* sp. and *Chlorella* sp. Microalgae incorporation also led to an increased content of polyphenols and to a higher antioxidant capacity. Microalgae-containing soups showed a higher amount of bioaccessible polyphenols, calculated after a simulated gastrointestinal digestion (ranging between 32.9 ± 1.1 and 45.6 ± 0.5 mg/100 mL). The acceptability index of soups formulated using lower microalgae concentrations was over 70% suggesting that the soups would be well accepted. Indeed, the purchase intention of the soups containing microalgae at 0.5% (w/v) ranged between 3.4 and 4.1 (assessed using a 5-point hedonic scale).

Keywords: Functional foods, *Spirulina* sp., *Chlorella* sp., *Tetraselmis* sp., novel ingredients

1. Introduction

Microalgae biotechnology has increased exponentially over the last decade (Garrido-Cardenas, Manzano-Agugliaro, Acien-Fernandez, & Molina-Grima, 2018). This promising natural resource is being used (or studied) as animal or aquatic feed, in wastewater treatment or bioremediation applications, as a bio-fertiliser, and as a raw material for the generation of biofuels and high-value products such as pigments or bioactive compounds, among other applications (Rizwan, Mujtaba, Memon, Lee, & Rashid, 2018). Microalgae are, however, mainly used for human consumption as they contain a large number of valuable compounds that can supplement the nutritional energy needs of the currently expanding population (Vaz, Moreira, Morais, & Costa, 2016). However, despite the high content of macro- and micro-nutrients found in microalgae, only a limited number of products containing microalgae have been launched into the market.

Several scientific publications, recently reviewed by Caporgno and Mathys (2018), evaluated the potential of microalgae and microalgae-derived compounds for being used as novel ingredients with either functional or technofunctional properties into dairy products, pasta, or baked goods such as cookies or bread. The majority of these studies attempted (and achieved) to improve the nutritional properties of foods by incorporating microalgae biomass into their recipes (Gouveia et al., 2008; Rodríguez De Marco, Steffolani, Martínez, & León, 2014). However, only a limited number of studies evaluated the flavour and the acceptance of these products after a sensorial analysis. In most of the studies where a sensorial analysis was carried out, the authors reported relatively high acceptability scores and that higher microalgae concentrations resulted in reduced overall acceptance (Caporgno & Mathys, 2018). One reason could be that dairy products and baked goods are not naturally green, and the green colour of microalgae can adversely affect consumers' perception about taste and quality (Becker, 2007). The acceptance of foods is also determined by health concerns and consumers' familiarity with

the food ingredients, among other issues (Sandmann et al., 2015). Up the best of our knowledge, there are no studies evaluating the effect of microalgae incorporation into vegetable soups. Moreover, most of the studies carried out to date assessed the effect of incorporating *Spirulina* or *Chlorella* into foods. This makes sense, as these two strains are accepted for human consumption and are currently the most cultivated strains (Garrido-Cardenas, Manzano-Agugliaro, Acien-Fernandez, & Molina-Grima, 2018). However, there are thousands of microalgae strains available in culture collections worldwide and some of these have finally achieved commercial-scale success. For example, Fitoplancton Marino S.L. (Cadiz, Spain) recently launched the product *Plancton Marino Veta la Palma*, which is a freeze-dried *Tetraselmis chuii* product that has been authorized by the European Food Safety Authority (EFSA) to be marketed as a novel food in accordance with Article 3(1) of Regulation (EC) No 258/97 (AESAN, 2014).

The consumption of these underutilised strains would not only open novel commercial opportunities to processors but also potentially promote health. Therefore, the aim of the current manuscript was to develop a novel food product enriched with *Spirulina* sp., *Chlorella* sp., *and Tetraselmis* sp. biomass, all of them authorised for human consumption in the EU (Acién Fernández, Fernández Sevilla, & Molina Grima, 2018). The food matrix in which the microalgae biomass was incorporated was broccoli soup, which is naturally green and generally associated with positive health outcomes. The current paper also aimed at evaluating the effect of microalgae incorporation on the overall quality and acceptance of the end product. Parameters evaluated included colour, texture, antioxidant activity, total phenolic content (TPC), and a visual and sensorial analysis that involved assessment of flavour, texture, overall acceptance, and purchase intention.

2. Materials and methods

2.1 Preparation of the microalgae-containing broccoli soup

Soups were made according to the formulations listed in Table 1 at the pilot plant facilities of IRTA Fruitcentre (Lleida, Spain). Briefly, broccoli florets were cut off from their stalks and boiled at a broccoli:boiling water ratio of 1:1.5 (w/v) for 7 min. Once cooked, the broccolis were sieved and the boiling water was collected. Boiled broccolis, the recovered boiling water, and olive oil at the concentrations listed in Table 1 were pulled together and homogenized using a TF410/450W industrial blender (Irimar, Valencia, Spain) operating at maximum speed for 4 min. Then, the salt and the microalgal biomass were incorporated, resuspended in water at a concentration calculated to keep the water content of the different soups constant. All the ingredients were further homogenised for 1 min and boiled for a further 2 min period. Microalgae concentrations were selected based on preliminary experiments carried out to establish the maximum microalgae inclusion level that did not negatively affect the organoleptic properties of the soups – Higher microalgae concentrations resulted in unacceptable overall acceptability scores (data not shown). Soups were immediately chilled to 4 °C using an ABT 101L blast chiller (Infrico, Barcelona, Spain). Each soup was divided into two lots. The first one was used for visual, sensorial, and physicochemical analyses and was stored at 4 °C until further analysis (approximately 24 h). The second lot was used for determination of antioxidant capacity and total phenolic content (TPC) and was stored at -20 °C.

2.2 Viscosity and water- and oil-holding capacities

WHC and OHC were determined following the methodology described by Lafarga, Álvarez, Bobo, and Aguiló-Aguayo (2018) and using a Sigma 3–18 KS centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). Determinations were carried out in

triplicate for each microalgae specie and results were expressed either as g of water or sunflower oil per g or freeze-dried microalgae. Viscosity was measured using a ST-2020 L rotary viscometer (JP Selecta, Barcelona, Spain) at 40 ± 1 ºC and in duplicate. Results were expressed as Pa·s.

2.3 Colour

Colour recordings of the soups were taken in triplicate using a Minolta CR-200 colorimeter (Minolta INC, Tokyo, Japan) as described by Lafarga et al. (2018). Chroma (*Ch*) and difference from the control (δE) values were calculated using the following equations:

$$Ch = \sqrt{a^{*2} + b^{*2}}$$

$$\delta E = \sqrt{(L^*_{CK} - L^*_i)^2 + (a^*_{CK} - a^*_i)^2 + (b^*_{CK} - b^*_i)^2}$$

where $\boldsymbol{L^*_{CK}}$, $\boldsymbol{a^*_{CK}}$, and $\boldsymbol{b^*_{CK}}$ are the colour parameters of the control soup and $\boldsymbol{L^*_i}$, $\boldsymbol{a^*_i}$, and $\boldsymbol{b^*_i}$ the colour parameters of each broccoli-containing soup.

2.4 Determination of total phenolic content

The TPC was determined by the Folin-Ciocalteu method as described by Lafarga et al. (2019). Briefly, for the extraction of polyphenols, the soups were homogenised with methanol 70% (v/v) at a sample:methanol ratio of 1:4 (w/v) at room temperature. Samples were homogenised using a T-25 ULTRA-TURRAX® homogeniser (IKA, Staufen, Germany) operating at 14,000 rpm for 30 s. Immediately after homogenisation, samples were placed on a stirrer at room temperature for 2 h and centrifuged using a Sigma-3-18 KS centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) operating at 12,000 rpm for 20 min. TPC was determined in triplicate using a GENESYS™ 10S-UV Vis spectrophotometer

(Thermo Fisher Scientific, MA, USA). Results were expressed as mg of gallic acid equivalents per 100 mL of soup. Standard curves were prepared daily.

2.5 Assessment of antioxidant activity

Antioxidant activity was determined using both the ferric ion reducing antioxidant power (FRAP) and the DPPH scavenging activity assays as described by Lafarga et al. (2019). Antioxidant activity was determined in triplicate using the same extracts used for determination of TPC and results were expressed as mg of ascorbic acid equivalents per 100 mL. Standard curves were prepared daily.

2.6 Simulated gastrointestinal digestion

A simulated gastrointestinal digestion of the control and microalgae-containing soups was done following the method of Minekus et al. (2014). A blank was prepared using only distilled water instead of the sample and following the same procedure. Determinations of TPC and antioxidant activity were performed after the intestinal phase as described in previous sections.

2.7 Sensorial and visual analysis

Both visual and sensorial analysis were undertaken approximately 24 h after the soups were made with 30 semi-trained consumers (17 women, 13 men, age 20-50 years) recruited from IRTA Fruitcentre (Lleida, Spain) who would be willing to buy the product. Consumers were considered as semi-trained as all of them were familiar with the quality attributes of vegetable soups and were capable of discriminating differences and communicating their reactions, though they were not formally trained. Sensory evaluation was conducted in a sensory laboratory with separate booths. Briefly, approximately 30 mL of the soups were place in white polystyrene glasses labelled with random codes and presented to the panellists in a randomised order. Each panellists assessed a maximum of five samples per day and all the panellists

assessed all of the samples. A 60-s time laps was employed between each sensory palate to reduce sensory fatigue. Panellists assessed the samples and were asked to indicate his or her opinion on the flavour and the overall acceptance of the soups using a nine-point hedonic scale (from 1: dislike extremely to 9: like extremely) as described by Amaral et al., 2018 and Souza et al., 2019. The acceptability index was calculated using the following equation:

$$Acceptability\ index\ (\%) = \frac{X}{9} \cdot 100$$

where X is the mean of the scores obtained for overall acceptance.

To assess flavour and texture, soups were given to the panellists with the green lights of the sensory booths on to mask the soups colour. Purchase intention was assessed using a five-point hedonic scale which ranged from 1: certainly would not buy to 5: certainly would buy (Lucas, Morais, Santos, & Costa, 2018). Finally, for assessment of visual appearance, 300 mL of the soups were put into 300 mL glass bottles (commercially used for storing soups and sauces), simulating the final presentation of the product. Each panellist assessed all the samples and was asked to indicate his or her opinion on the overall visual appearance of the soups, focusing on their colour and texture and using a nine-point hedonic scale (from 1: dislike extremely to 9: like extremely).

2.8 Statistical analysis

Results are expressed as mean ± standard deviation (S.D.). Difference between samples were analysed using analysis of variance (ANOVA) with JMP 13 (SAS Institute Inc., Cary, USA). Where significant differences were present, a Tukey pairwise comparison of the means was conducted to identify where the sample differences occurred. The criterion for statistical significance was $p<0.05$. To identify relationships between parameters, bivariate Pearson's correlation analysis was carried out.

3. Results and discussion

3.1 Functional and antioxidant properties of the microalgal biomass

Colour attributes of the dried microalgal biomass were measured. The *L** value was 32.58 ± 0.61, 35.06 ± 0.21, and 40.16 ± 0.12 for *Spirulina* sp., *Chlorella* sp., and *Tetraselmis* sp., respectively. In addition, *a** values were measured as -1.66 ± 0.15, -3.38 ± 0.40, and -7.81 ± 0.12 for *Spirulina* sp., *Chlorella* sp., and *Tetraselmis* sp., respectively. Overall, *Tetraselmis* showed the highest *L** and the lowest *a** values ($p<0.05$), suggesting a lighter and greener hue when compared to powdered *Spirulina* sp. and *Chlorella* sp.

The WHC of the dried microalgal biomass was 1.96 ± 0.05, 1.48 ± 0.07, and 0.99 ± 0.20 g/g for *Spirulina* sp., *Chlorella* sp., and *Tetraselmis* sp., respectively. *Tetraselmis* sp. showed the lowest WHC values ($p<0.05$), probably caused by the higher salt content of the biomass which was dissolved in water during the assay. In addition, the OHC of *Spirulina* sp., *Chlorella* sp., and *Tetraselmis* sp. was 1.64 ± 0.10, 1.47 ± 0.23, and 1.23 ± 0.15, respectively. Similar WHC and OHC values were previously reported for proteins extracted from pulses (Lafarga, Álvarez, et al., 2018) or microalgae (Waghmare, Salve, LeBlanc, & Arya, 2016). Interactions of food ingredients with water and oil are of key importance in the food industry because these will affect flavour and texture of foods (Kumar, Ganesan, Selvaraj, & Rao, 2014). High WHC values are desirable in viscous foods such as vegetable soups, sauces, or custards to provide thickening and viscosity. Microalgal cells are grown in suspension, and are therefore not water soluble. Because of this, and based on their WHC and OHC values, microalgae can be used to provide not only colour and flavour to foods but also thickening and viscosity.

The antioxidant capacity of the powdered microalgae was also determined. When assessed using the FRAP assay, the antioxidant capacity of *Spirulina* sp., *Chlorella* sp., and *Tetraselmis* sp. was 356.23 ± 16.88, 224.11 ± 9.65, and 308.67 ± 22.58 mg/100 g of dry weight, respectively. Moreover, when assessed using the DPPH assay, the antioxidant capacity of

194 selected microalgae was 304.66 ± 9.54, 195.23 ± 3.97, and 254.33 ± 11.97 mg/100 g of dry
195 weight, respectively. Similar antioxidant capacity values were reported previously (Goiris et
196 al., 2012; Li et al., 2007).

197 **3.2 Physicochemical attributes**

198 Colour attributes of the soups were significantly affected by microalgae concentration
199 (p<0.001), specie (p<0.001), and the interaction of both factors (p<0.001). Incorporation of
200 freeze-dried microalgae biomass into the broccoli soup resulted in lower L^* values, which
201 means that microalgae-containing soups presented a darker colour (Figure 1). As expected,
202 higher microalgae concentration resulted in lower L^* values. A negative correlation was
203 observed between L^* values and the concentration of *Spirulina* sp. (r^2=0.9600; p<0.001),
204 *Chlorella* sp. (r^2=0.7847; p<0.05), and *Tetraselmis* sp. (r^2=0.9872; p<0.001). The a^* parameter
205 was for all the studied samples negative, which denotes a greenish colour. *Tetraselmis* sp.
206 incorporation into the broccoli soup resulted in lower a^* values when compared to the control
207 and to soups formulated using *Chlorella* sp. or *Spirulina* sp. (p<0.05). This is clear in Figure
208 1, where T1-T4 show a more distinctive green colour. Indeed, positive correlations were
209 observed between a^* values and *Spirulina* sp. (r^2=0.9875; p<0.001), *Chlorella* sp. (r^2=0.8244;
210 p<0.001), and *Tetraselmis* sp. (r^2=0.9399; p<0.001) concentration. Both, microalgae
211 concentration and specie had an effect on *Ch* (p<0.001), as well as the interaction of both
212 factors (p<0.001). Higher microalgae concentration reduced *Ch* values, which suggest a loss
213 of colour intensity after incorporation of microalgae into the soup. The observed loss in colour
214 intensity was higher after incorporation of *Spirulina* sp. or *Chlorella* sp. when compared to
215 *Tetraselmis* sp. Indeed, no differences were observed in the *Ch* values of samples CK and T1.
216 Moreover, in the current study, δE was higher than three for all the formulated soups (data not
217 shown), with those made using *Spirulina* sp. having significantly higher δE values (p<0.05).
218 This suggests that the colour differences between the control and the microalgae-containing

soups were visible to the human eye, especially for soups prepared using *Spirulina* sp. (Figure 1). Furthermore, microalgae incorporation into the broccoli soup led to increased viscosity. A positive correlation was observed between microalgae concentration and viscosity (r^2=0.8760; p<0.05).

3.3 Total phenolic content and antioxidant activity

The effect of microalgae inclusion on the TPC and antioxidant activity of the soups is shown in Figure 2. Although cruciferous vegetables are specially known for their content in glucosinolates, these vegetables are also rich sources of polyphenols (Lafarga, Bobo, Viñas, Collazo, & Aguiló-Aguayo, 2018; Lafarga, Viñas, Bobo, Simó, & Aguiló-Aguayo, 2018). The TPC of the control soup was calculated as 30.02 ± mg/100 mL. Incorporation of microalgae into the broccoli soup resulted in increased TPC (p<0.05) – an increase was also observed after incorporation of *Tetraselmis* sp. at a concentration of 0.5% (w/v) but it was not statistically significant. In the current study, higher microalgae concentrations resulted in increased TPC (p<0.05). Several recent studies demonstrated that microalgae contain important amounts of polyphenols, which contribute significantly to their antioxidant capacity (Custódio et al., 2012; Goiris et al., 2012; Hajimahmoodi et al., 2010). No major differences were observed in the TPC of soups formulated using either *Chlorella* sp. or *Tetraselmis* sp. (Figure 2). This does not mean that *Spirulina* sp. contain higher concentrations of polyphenols, as their content depends largely on several factors including the cultivation conditions: nutrient limitation resulted in decreased TPC previously (Goiris et al., 2015). Results obtained for antioxidant activity correlate well with those obtained for TPC. Both DPPH and FRAP values were affected by microalgae incorporation (p<0.05). The observed increase in antioxidant activity after incorporation of microalgae into the soup recipe was more evident when assessed using the DPPH assay. C1 and T1 showed a lower antioxidant capacity when compared to the control soup and when assessed using the FRAP assay (p<0.05). Although these differences were

relatively small, they can be attributed to a dilution of the broccoli polyphenols of the control soup after incorporation of the microalgae and the broccoli boiling water. Also, the antioxidant capacity of *Chlorella* sp. and *Tetraselmis* sp. was lower than that of *Spirulina* sp. when assessed using both the FRAP and DPPH assays ($p<0.05$).

The known "French paradox" ignited the interest of food processors and scientists on plant polyphenols. However, in order to exert a health effect *in vivo*, food polyphenols must first be bioavailable. Bioaccessibility is one of the main factors limiting bioavailability and has been defined as the release of compounds from their natural food matrix to be available for intestinal absorption (Stahl et al., 2002). Figure 2 shows the amount of bioaccessible polyphenols after a simulated gastrointestinal digestion and the antioxidant capacity of the enzymatic digestive extracts. Overall, the TPC after the intestinal phase of digestion was higher for all the studied samples when compared to the initial stage – after a methanol:water extraction ($p<0.05$). Similar results were previously reported for cereals (Pérez-Jiménez & Saura-Calixto, 2005), pulses (Lafarga, Villaró, Bobo, Simó, & Aguiló-Aguayo, 2019), and fruit (Chen et al., 2014). Higher microalgae concentrations resulted in higher amounts of bioaccessible polyphenols ($p<0.05$). It is thought that free and some conjugated phenolic compounds are available for absorption in the human small and large intestines. However, those bound covalently to large polysaccharides may be absorbed after being released from cells by digestive enzymes or microorganisms in the intestinal lumen (Wang, He, & Chen, 2014). It is possible that the strong pH variations suffered during the *in vitro* digestion, together with the activity of α-amylase, pepsin, and pancreatin (the pancreatin utilized contained enzymatic components including trypsin, lipase, ribonuclease, and proteases which allowed hydrolysing proteins, carbohydrates, and fats) facilitated the release of polyphenols from the interior of the plant or microalgae cells. The longer extraction time could also partially explain these findings. One of the main problems associated with the utilisation of microalgae as raw material for the isolation of

healthy compounds is the limited (mild) technological options and the high costs associated with the cell wall disruption and extraction steps. Results obtained herein could also promote the consumption of the whole microalgal biomass as they suggest that healthy compounds such as polyphenols can be bioavailable without a cell wall disruption step as they can be released during cooking and digestion. However, further studies are needed in order to prove this hypothesis. As mentioned previously, the methanol:water extracts of the soups formulated using *Spirulina* sp. showed a higher TPC ($p<0.05$). That difference in TPC for soups containing *Spirulina* sp. was not observed in the enzymatic digestive extracts obtained after a simulated digestion. Indeed, the content of bioaccessible polyphenols was not affected by microalgae specie. Results obtained for antioxidant capacity after a simulated gastrointestinal digestion compare well with those observed for TPC. As expected, the antioxidant capacity of the enzymatic digestive extracts after the intestinal phase of digestion was higher for all the studied samples when compared to the initial stage ($p<0.05$). This is probably caused by a higher content of polyphenols when compared to the extracts obtained after an extraction using methanol:water. Another reason could be that microalgae are protein-rich foods, and these proteins could have been hydrolysed during the simulated gastrointestinal digestion leading to the release of bioactive peptides with antioxidant activity (Ejike et al., 2017). Results reported in the current paper are consistent with previous studies that suggested that extractions using methanol or other organic solvents could be underestimating the actual antioxidant capacity of foods (Lafarga et al., 2019; Pérez-Jiménez & Saura-Calixto, 2005).

3.4 Visual and sensorial analysis

The analysis of variance revealed that the visual acceptance of the soups was significantly affected by microalgae concentration ($p<0.001$), specie ($p<0.001$), and the interaction of both factors ($p<0.001$). Visual and sensorial acceptance scores are listed in **Table 3**. Higher microalgae concentration resulted in reduced overall visual acceptance of the soups. However,

no differences were observed between the overall visual acceptance scores of the control soup (CK) and formulations T1, T2, T3, C1, and C2. Visual scores also depended on the specie incorporated into the broccoli soup formulation (p<0.001). As mentioned previously, colour attributes were significantly affected after incorporation of microalgae into the soup formulations (Table 2; p<0.05). It is widely accepted that colour of foods has a striking effect on consumers' expectations (Spence, 2018). A positive correlation was observed between the L^* value of the soups and visual acceptance (r^2=0.6304; p<0.05) suggesting that the panellists preferred soups with lighter colours. In addition, visual acceptance was negatively correlated with a^* values (r^2=0.7338; p<0.05) which means that the greener the colour of the soup, the higher the visual acceptance score. Soups formulated using *Spirulina* sp, obtained the lowest visual acceptability scores, which ranged from 4.6 ± 0.3 to 6.0 ± 0.3 for S4 and S1, respectively. In turn, incorporation of *Tetraselmis* sp, at concentrations ranging from 0.5 to 1.5% (w/v) into the broccoli soups did not significantly affect its visual appearance: no differences were observed between the visual appearance scores of T1, T2, or T3 and the control (CK). This could be caused by the greener and lighter hue of the soups formulated using *Tetraselmis* sp. when compared to *Spirulina* sp. (Figure 1).

Colour of foods can affect consumers' flavour perception (Spence, 2018). Previous studies even demonstrated that colour of soups can modulate satiety and thermal sensation! (Suzuki et al., 2017). Therefore, in the current study, the flavour of foods was assessed under green lighting. Flavour scores are listed in Table 3. Flavour scores were significantly affected by microalgae concentration (p<0.001), specie (p<0.001), and the interaction of both factors (p<0.001). No differences were observed between the flavour of the control soup and formulations S1, T1, and T2, which suggest that incorporation of *Spirulina* sp. and *Tetraselmis* sp. at low concentrations do not negatively affect flavour. However, higher microalgae concentrations resulted in decreased flavour scores. Incorporation of *Chlorella* sp. into broccoli

soup, even at the lowest concentration studied in the current study, which was 0.5% (w/v), resulted in a negative effect on flavour (p<0.05). Chacón-Lee and González-Mariño (2010) reviewed the opportunities and challenges of microalgae utilisation in the food industry and suggested that incorporation of exotic-flavoured ingredients, such as Asian or Indian spices, together with the microalgae biomass would facilitate their incorporation into foods, especially for Western consumers. Other studies utilised sugar and butter to mask the flavour of microalgae in biscuits and obtained high flavour scores (Singh, Singh, Jha, Rasane, & Gautam, 2015). This is important as unlike Japanese consumers, who consider functional foods a distinct food where the importance of their health benefits exceeds the importance of their sensory attributes, Western consumers do not seem to be willing to compromise taste for health (Grasso, Brunton, Lyng, Lalor, & Monahan, 2014).

Results listed in Table 3 suggest that incorporation of microalgae did not significantly affect the texture of the soups, expect for a significantly lower texture score for S4 (p<0.05). Finally, the acceptability index of the soups ranged between 56.7-80.0, 47.8-73.3, and 58.9-82.2% for soups containing *Spirulina* sp., *Chlorella* sp., and *Tetraselimis* sp., respectively. For all the studied soups, the acceptability index was lower than that of the broccoli-only soup, which was calculated as 91.1% (p<0.05). The acceptability of the soups containing microalgae at lower concentrations was comparable to that of other products formulated using microalgae previously. Indeed, Lucas, Morais, Santos, & Costa (2018), recently reported an acceptability index of 82.2% for extruded snacks enriched in *Spirulina*. For a product to be accepted in terms of sensorial characteristics, it is necessary to achieve an acceptability index greater than 70% (Lucas et al., 2018). We can therefore expect that the manufactured microalgae-containing soups would have a good acceptance if commercialised. Indeed, when calculated as a percentage, the purchase intention of S1, C1, and T1 was 72.0, 68.0, and 82.0%, respectively. The purchase intention was significantly lower for soups containing microalgae at higher

concentrations as S4, C4, and T4 had a purchase intention ranging between 1 (certainly would not buy) and 2 (probably would not buy).

4. Conclusions

Results suggest that when incorporated at concentrations ranging from 0.5 to 1.0% (w/v), microalgae can be used as an innovative ingredient in the manufacture of microalgae-enriched broccoli soup. However, when microalgae biomass was incorporated into the broccoli soup at higher concentrations, flavour and overall acceptability scores were low, especially for soups formulated using *Chlorella* sp. Sensorial analysis also suggested that consumers preferred soups with a lighter and greener hue. Results suggested that when formulated correctly, microalgae-containing foods show good consumer acceptance and this would allow increasing the utilisation of this valuable and underused ingredient. Moreover, microalgae-containing soups had higher phenolic content and antioxidant activity when compared to the control broccoli soups. The amount of bioaccessible polyphenols as well as the antioxidant capacity of the digestive enzymatic extracts was also higher in the microalgae-containing soups when compared to the controls suggesting healthier products. Results reported herein would open novel commercial opportunities for the utilization of microalgae as an ingredient in vegetables soups allowing not only to differentiate by using a "trendy" ingredient but also to promote health. Further studies will assess which compounds were responsible for the observed increase in antioxidant activity as well as the effect of thermal processing and/or high pressure processing on the health-promoting compounds found in the soup.

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482 **Table 1. Composition of the broccoli soups containing microalgae**

	Boiled broccoli (g)	Broccoli boiling water (g)*	Olive oil (g)	Salt (g)**	Freeze-dried microalgae (g)
CK: *Control*	47,8	44,7	7	0,50	0,0
S1: *Spirulina* sp. 0.5% (w/w)	47,2	44,8	7	0,50	0,5
S2: *Spirulina* sp. 1.0% (w/w)	46,6	44,9	7	0,50	1,0
S3: *Spirulina* sp. 1.5% (w/w)	46,0	45,0	7	0,50	1,5
S4: *Spirulina* sp. 2.0% (w/w)	45,4	45,1	7	0,50	2,0
C1: *Chlorella* sp. 0.5% (w/w)	47,2	44,8	7	0,50	0,5
C2: *Chlorella* sp. 1.0% (w/w)	46,6	44,9	7	0,50	1,0
C3: *Chlorella* sp. 1.5% (w/w)	46,0	45,0	7	0,50	1,5
C4: *Chlorella* sp. 2.0% (w/w)	45,4	45,1	7	0,50	2,0
T1: *Tetraselmis* sp. 0.5% (w/w)	47,2	44,8	7	0,38	0,5
T2: *Tetraselmis* sp. 1.0% (w/w)	46,6	44,9	7	0,35	1,0
T3: *Tetraselmis* sp. 1.5% (w/w)	46,0	45,0	7	0,33	1,5
T4: *Tetraselmis* sp. 2.0% (w/w)	45,4	45,1	7	0,30	2,0

483 * The amount of water used in different formulations varied to achieve a comparable water content in each formulation: 87.7 ± 0.8%

484 **Salt content of soups formulated using *Tetraselmis* sp. had to be re-adjusted because of the salty taste of the microalgal biomass.
485

Table 2. Physicochemical properties of the manufactured microalgae-containing soups

Sample	*L**	*a**	*b**	*Ch*	pH	Viscosity (Pa.s)
CK	61,6 ± 0,6 ^{A}	-12,7 ± 0,2 ^{I}	25,5 ± 0,4 ^{A}	28,5 ± 0,4 ^{A}	6.73 ± 0.04 ^{E}	2,93 ± 0,06 ^{F}
S1	44,4 ± 0,5 ^{C}	-10,7 ± 0,1 ^{F}	7,6 ± 0,1 ^{G}	13,1 ± 0,0 ^{G}	6.52 ± 0.01 ^{G}	3,07 ± 0,04 ^{E}
S2	39,0 ± 0,1 ^{F}	-8,0 ± 0,1 ^{D}	3,4 ± 0,1 ^{H}	8,7 ± 0,1 ^{H}	6.46 ± 0.01 ^{H}	3,15 ± 0,06 ^{DE}
S3	36,5 ± 0,1 ^{G}	-6,4 ± 0,1 ^{B}	1,5 ± 0,2 ^{I}	6,6 ± 0,1 ^{I}	6.63 ± 0.01 ^{F}	3,24 ± 0,08 ^{C}
S4	33,9 ± 0,2 ^{H}	-4,5 ± 0,2 ^{A}	0,5 ± 0,2 ^{J}	4,6 ± 0,3 ^{J}	6.37 ± 0.03 ^{I}	3,33 ± 0,16 ^{AB}
C1	47,7 ± 0,2 ^{B}	-10,9 ± 0,1 ^{F}	18,4 ± 0,3 ^{D}	21,4 ± 0,3 ^{D}	6.73 ± 0.03 ^{E}	3,02 ± 0,05 ^{EF}
C2	42,9 ± 0,1 ^{D}	-9,7 ± 0,2 ^{E}	15,4 ± 0,4 ^{E}	18,2 ± 0,5 ^{E}	6.63 ± 0.04 ^{F}	3,24 ± 0,06 ^{C}
C3	37,6 ± 0,3 ^{G}	-7,7 ± 0,1 ^{C}	12,0 ± 0,2 ^{F}	14,3 ± 0,2 ^{F}	6.63 ± 0.01 ^{F}	3,38 ± 0,03 ^{B}
C4	39,2 ± 0,2 ^{F}	-8,1 ± 0,2 ^{D}	12,6 ± 0,4 ^{F}	15,0 ± 0,4 ^{F}	6.56 ± 0.05 ^{FG}	3,56 ± 0,06 ^{A}
T1	48,9 ± 0,9 ^{B}	-15,8 ± 0,3 ^{H}	23,0 ± 0,4 ^{B}	27,8 ± 0,6 ^{A}	6.83 ± 0.04 ^{D}	3,03 ± 0,03 ^{EF}
T2	43,7 ± 0,6 ^{CD}	-15,3 ± 0,1 ^{H}	20,4 ± 0,2 ^{C}	25,5 ± 0,2 ^{B}	7.07 ± 0.02 ^{C}	3,15 ± 0,01 ^{D}
T3	40,6 ± 0,1 ^{E}	-14,4 ± 0,3 ^{G}	18,1 ± 0,1 ^{D}	23,2 ± 0,2 ^{C}	7.13 ± 0.02 ^{B}	3,25 ± 0,07 ^{C}
T4	37,0 ± 1,1 ^{G}	-12,7 ± 0,0 ^{I}	15,7 ± 0,1 ^{E}	20,1 ± 0,1 ^{D}	7.20 ± 0.03 ^{A}	3,29 ± 0,01 ^{BC}

Composition of the soups is listed in Table 1. Values represent the mean of three independent measurements ± S.D. Different letters in the same column indicate significant differences. The criterion for statistical significance was $p<0.05$.

Table 3. Visual and sensorial analysis of the microalgae-enriched broccoli soups

Sample	Visual appearance score	Flavour score*	Texture score	Overall acceptance score	Acceptability index (%)	Purchase intention**
CK	7.8 ± 0.2^{A}	8.2 ± 0.1^{D}	7.9 ± 0.2^{ABD}	8.2 ± 0.1^{B}	91.1	4.6 ± 0.1^{E}
S1	6.0 ± 0.3^{BCDE}	7.5 ± 0.3^{BD}	8.2 ± 0.1^{A}	7.2 ± 0.2^{ABF}	80.0	3.6 ± 0.1^{AB}
S2	5.6 ± 0.3^{CDE}	6.6 ± 0.2^{ABE}	8.1 ± 0.2^{AD}	6.7 ± 0.2^{ABEF}	74.4	3.2 ± 0.2^{ABF}
S3	5.0 ± 0.3^{DE}	5.9 ± 0.3^{ACEF}	7.8 ± 0.2^{ABD}	5.7 ± 0.3^{ACDE}	63.3	2.9 ± 0.2^{ACF}
S4	4.6 ± 0.3^{E}	5.4 ± 0.2^{ACF}	6.6 ± 0.2^{C}	5.1 ± 0.3^{CDE}	56.7	1.7 ± 0.1^{D}
C1	7.2 ± 0.3^{AB}	6.6 ± 0.3^{ABE}	8.2 ± 0.1^{A}	6.6 ± 0.3^{ABEF}	73.3	3.4 ± 0.2^{ABF}
C2	6.6 ± 0.3^{ABC}	5.8 ± 0.2^{ACEF}	7.9 ± 0.1^{ABD}	6.1 ± 0.2^{ACEF}	67.8	2.8 ± 0.2^{ACF}
C3	6.3 ± 0.2^{BCD}	5.0 ± 0.4^{CF}	7.3 ± 0.3^{ABCD}	4.9 ± 0.5^{CD}	54.4	2.1 ± 0.2^{CD}
C4	6.0 ± 0.3^{BCDE}	4.6 ± 0.3^{C}	6.9 ± 0.2^{BC}	4.3 ± 0.4^{D}	47.8	1.5 ± 0.1^{D}
T1	7.8 ± 0.2^{A}	7.3 ± 0.2^{BD}	8.1 ± 0.1^{AD}	7.4 ± 0.2^{BF}	82.2	4.1 ± 0.1^{BE}
T2	7.2 ± 0.2^{AB}	6.9 ± 0.2^{BDE}	8.0 ± 0.1^{ABD}	7.0 ± 0.2^{ABF}	77.8	3.5 ± 0.2^{AB}
T3	7.1 ± 0.2^{AB}	6.2 ± 0.2^{ABEF}	7.1 ± 0.2^{BCD}	6.1 ± 0.2^{ACEF}	67.8	2.4 ± 0.2^{CDF}
T4	6.1 ± 0.2^{BCD}	6.1 ± 0.2^{ABEF}	6.9 ± 0.2^{BC}	5.3 ± 0.4^{CDE}	58.9	2.0 ± 0.2^{CD}

Composition of the soups is listed in Table 1. * Flavour was assessed under green lighting conditions to mask the colour of the soups. ** Purchase intention was assessed using a 5-point hedonic scale. Values represent mean ± SEM (n=30). Different letters in the same column indicate significant differences between samples. The criterion for statistical significance was $p<0.05$.

493 **Figure legends**

494 **Figure 1. Visual appearance of the soups**

495 CK: Control broccoli soup. S1-S4: Broccoli soups enriched in *Spirulina* sp. at concentrations
496 ranging from 0.5 to 2.0% (w/w). C1-C4: Broccoli soups enriched in *Chlorella* sp. at
497 concentrations ranging from 0.5 to 2.0% (w/w). T1-T4: Broccoli soups enriched in *Tetraselmis*
498 sp. at concentrations ranging from 0.5 to 2.0% (w/w).

499

500 **Figure 2. (A) Total phenolic content and antioxidant activity assessed using the (B) FRAP**
501 **and (C) DPPH· assays of the control and microalgae-containing broccoli soups**

502 Values represent mean values ± S.D. of three independent determinations. Different capital
503 letters indicate differences in either TPC or antioxidant activity of extracts obtained after a
504 methanol:water extraction. Different lower case letters indicate significant differences between
505 TPC or antioxidant activities of enzymatic digestive extracts obtained after a simulated
506 gastrointestinal digestion. The criterion for statistical significance was $p<0.05$. (Legend: ■ after
507 methanol:water extraction and ■ after *in vitro* gastrointestinal digestion).

508 **Figure 1**

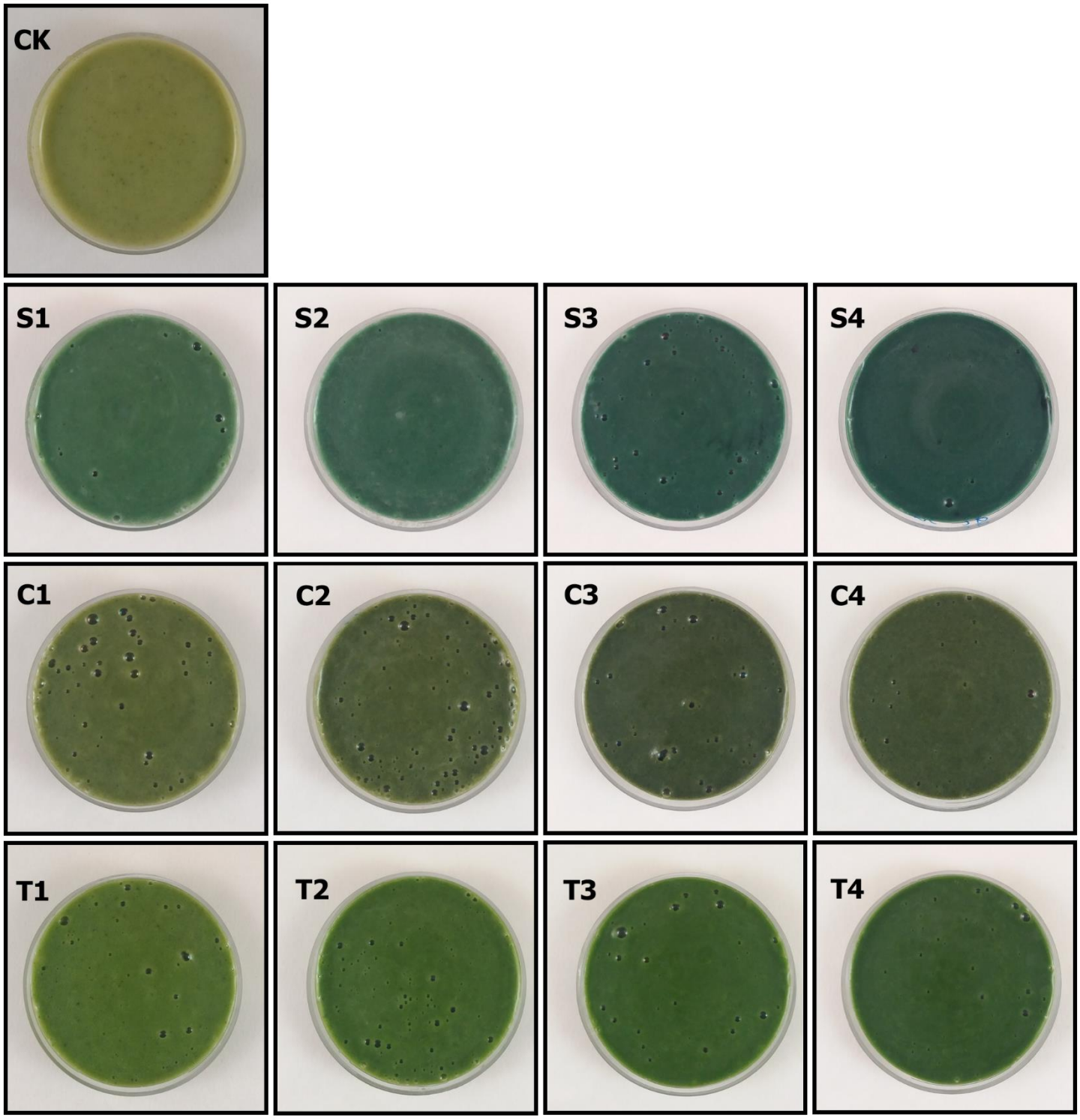
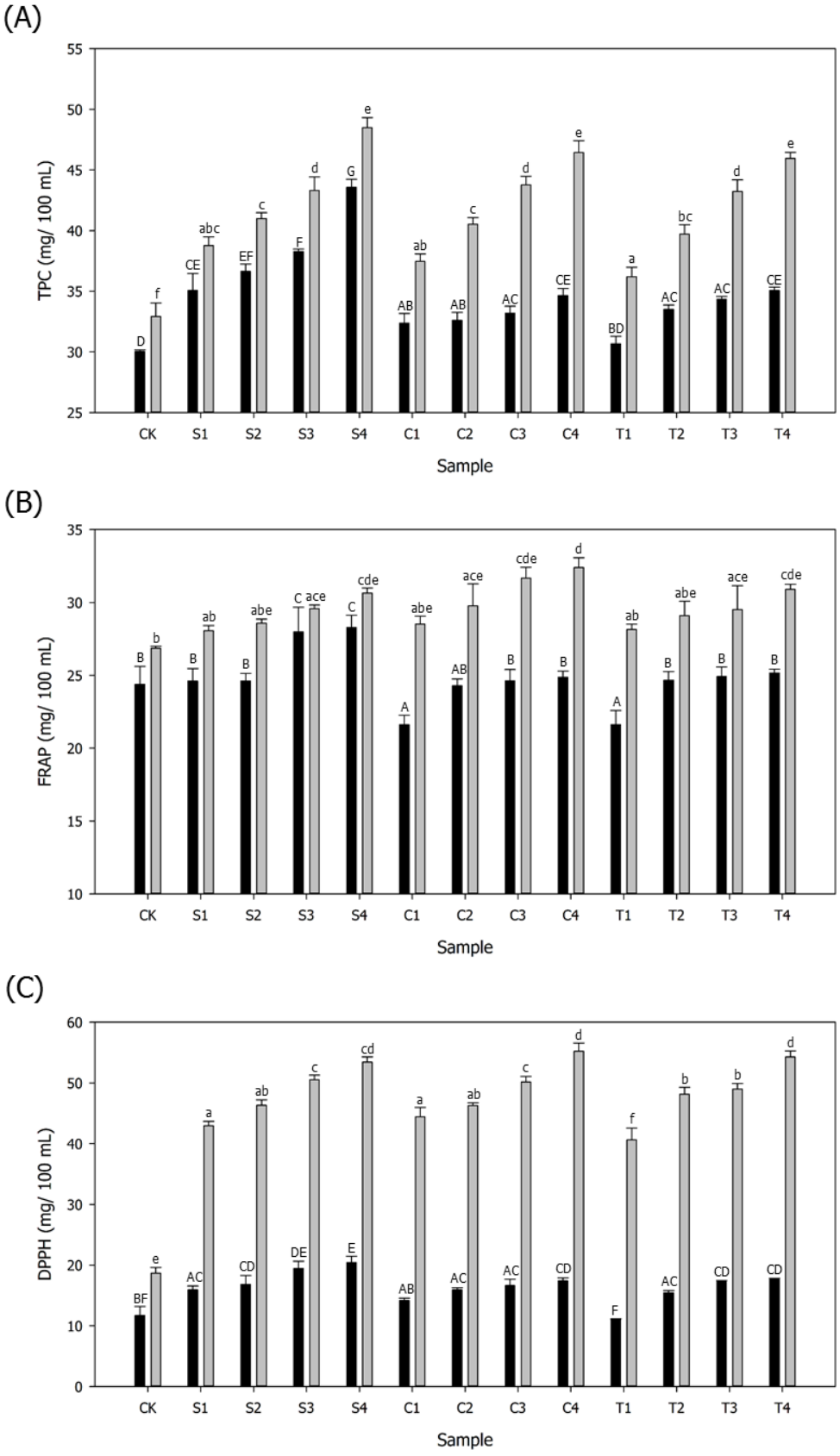


Figure 2


(A)
TPC (mg/ 100 mL)
Sample
CK S1 S2 S3 S4 C1 C2 C3 C4 T1 T2 T3 T4
(B)
FRAP (mg/ 100 mL)
Sample
CK S1 S2 S3 S4 C1 C2 C3 C4 T1 T2 T3 T4
(C)
DPPH (mg/ 100 mL)
Sample
CK S1 S2 S3 S4 C1 C2 C3 C4 T1 T2 T3 T4