Algal Research 79 (2024) 103447

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Printable formulations of protein and *Chlorella vulgaris* enriched vegetable puree for dysphagia diet

Larisa Giura [a,b,1,2], Leyre Urtasun [b,2], Diana Ansorena [a,*,1], Iciar Astiasaran [a,1], Anabela Raymundo [c]

[a] *Department of Nutrition, Food Science and Physiology, Faculty of Pharmacy and Nutrition, Universidad de Navarra, IDISNA – Instituto de Investigación Sanitaria de Navarra, Pamplona, Spain*
[b] *National Centre for Food Technology and Safety (CNTA), Spain*
[c] *LEAF Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidad de Lisboa, Tapada de Ajuda, 1349-017 Lisboa, Portugal*

ARTICLE INFO

Keywords:
Microalgae
Nutrition
Dysphagia foods
3D printing
Texture

ABSTRACT

Three *Chlorella vulgaris* biomasses, smooth *Chlorella*, honey *Chlorella* and white *Chlorella* were used at a percentage of 3 % in vegetable purees (10 % zucchini and 10 % carrots), enriched in protein (22 % lentil protein concentrate) and 0.8 % of xanthan gum. Their nutritional, *in vitro* digestibility, physicochemical, rheological and 3D-printability properties were determined. Sample with white *Chlorella* showed significantly higher antioxidant capacity than the control, with FRAP value of 100.62 and DPPH of 20.72 μmol Trolox/100 g puree. Purees containing *C. vulgaris* can be claimed to be a source of iron since they provide >15 % of the Nutrient Reference Value (NRV) for this essential mineral (2.39–2.36 mg/100 g). Total digestibility was high (90.9–92.6 %) for all the samples whereas the highest protein digestibility values were observed for purees with smooth *Chlorella* (46 %). Smooth *Chlorella* showed very noticeable color differences compared to control (ΔE values >20). All the purees showed a shear thinning behavior and a weak gel structure (tan δ 0.206–0.212), which made them suitable for dysphagia diet and also for being successfully printed in 3D.

1. Introduction

Foods for dysphagia diets have to be developed taking into account not only their suitability to be swallowed without problems but also their nutritional aspects. The elderly and persons with neurological and respiratory complications are prone to suffer from dysphagia resulting in considerable health and psychosocial consequences and reduced quality of life [1]. In a recent review about the use of texture-modified meals in dysphagia diets it is pointed out that the modification of food may affect the micronutrient content and that, currently, there is a limited number of studies focusing on micronutrient content of texture-modified meals [2].

Microalgae have been consumed in Asian countries from centuries and are considered healthy foods because of their high contents of vitamins, minerals, proteins and fibers as well as several other bioactive compounds [3–5]. The quality of microalgae proteins was found to be superior than of plant sources [6]. In the last few years, the microalgal industry has significantly grown worldwide, being microalgae used as nutraceuticals and as ingredients in food and feed applications [7]. Due to their high concentration of minerals, polyunsaturated fatty acids (PUFA), antioxidants, pigments, and amino acids, microalgae can be used as sustainable food ingredients to develop healthy and clean-label food products [8]. In this sense Demarco et al. [9] pointed out the positive impact on health of consuming traditional foods (cookies, snacks, bread, yogurt) with 5 % of algal biomass as a consequence of their content of bioactive compounds. *Chlorella* spp. is one of the most common green microalgae. Different meta-analysis have revealed that dietary supplementation with *Chlorella* can improve cholesterol, low-density lipoprotein cholesterol levels, systolic blood pressure, diastolic blood pressure, and fasting blood glucose levels, being all these

---

* Corresponding author.
E-mail addresses: lgiura@alumni.unav.es, lgiura@cnta.es (L. Giura), lurtasun@cnta.es (L. Urtasun), dansorena@unav.es (D. Ansorena), iastiasa@unav.es (I. Astiasaran).

[1] Postal address: C/Irunlarrea s/n, 31008 Pamplona, Spain.

[2] Postal address: NA 134, Km. 53, 31570 San Adrián, Navarre, Spain.

https://doi.org/10.1016/j.algal.2024.103447
Received 13 September 2023; Received in revised form 19 February 2024; Accepted 25 February 2024

Table 1
Nutritional composition of the algae biomasses (for 100 g of sample) used in the experiments ([a]).

Nutrients	Smooth *Chlorella*	Honey *Chlorella*	White *Chlorella*
Protein (g)	30	30.5	36.1
Carbohydrates (g)	34.8	40.8	48.8[b]
Fat (g)	8.1	11.5	7.6
Fiber (g)	18.8	12.9	–
Ash (g)	4.3	2	4.6
Moisture (g)	4	2.4	2.9
Omega 3 (g)	0.6	0.71	0.3
Vitamin B12 (μg)	15	25	55

[a] Information supplied by the company.

[b] In the case of White *Chlorella*, the dietary fiber was included in the carbohydrates.

Table 2
Nutritional composition of the control puree and purees with 3 % of smooth, honey and white *Chlorella*.

Samples	Protein %	Fat %	Carbohydrates %	Moisture %
Control	11.46 ± 0.92^{A}	3.25 ± 0.91^{A}	7.95 ± 2.19^{A}	72.98 ± 0.99^{A}
SC	11.62 ± 0.34^{A}	3.15 ± 0.071^{A}	7.38 ± 0.3^{A}	74.1 ± 0.71^{A}
HC	11.34 ± 0.09^{A}	3.3 ± 0.26^{A}	5.79 ± 0.47^{A}	74.56 ± 0.07^{A}
WC	11.92 ± 0.25^{A}	3.07 ± 0.31^{A}	5.58 ± 0.59^{A}	74.73 ± 0.16^{A}

Note: Data are presented as mean ± standard deviation of three independent experiments. Different capital letters, in the same column, indicate significant differences ($p \leq 0.05$) among the samples based on the *post hoc* Tukey test.
Smooth *Chlorella* (SC), Honey *Chlorella* (HC), White *Chlorella* (WC).

Table 3
Total phenolic compound and antioxidant capacity of the control puree and purees with 3 % of smooth, honey and white *Chlorella*.

Samples	FRAP (μmol Trolox/ 100 g puree)	DPPH (μmol Trolox/ 100 g puree)	TPC (mg GAE/100 g puree)
Control	82.37 ± 4.67^{A}	14.28 ± 2.22^{A}	25.67 ± 0.88^{AB}
SC	81.58 ± 3.83^{A}	14.79 ± 0.73^{A}	23.25 ± 2.05^{A}
HC	91.29 ± 6.8^{AB}	15.35 ± 1.14^{A}	23.99 ± 1.66^{A}
WC	100.62 ± 3.68^{B}	20.72 ± 1.37^{B}	28.66 ± 2.07^{B}

Note: Data are presented as mean ± standard deviation of three independent experiments. Different capital letters, in the same column, indicate significant differences ($p \leq 0.05$) among the samples based on the *post hoc* Tukey test.
Smooth *Chlorella* (SC), Honey *Chlorella* (HC), White *Chlorella* (WC).

beneficial effects linked to synergism between multiple nutrient and antioxidant compounds [10]. Another beneficial effect of *C. vulgaris* is its potential to promote muscle regeneration, which can be a good strategy to prevent sarcopenia in patients with dysphagia [11]. Although its composition can vary with many factors such as culture medium, pH level, salinity, temperature and light intensity/duration [12], it is rich in proteins (61.6 %), fat (12.5 %), carbohydrates (13.7 %), trace elements and vitamins [13,14]. *C. vulgaris* is used as an efficient supplement to enrich diets with micronutrients and bioactive compounds and has also been used to develop various food products such as couscous [15], pork frankfurters [8], snacks [16] or bread [17]. However, to our knowledge no studies have investigated to date the microalgae biomass incorporation in dysphagia-oriented foods.

Along with nutritional composition of dysphagia foods, sensory aspects are also very important. Foods for dysphagia must be safe to swallow, namely, with rheological and textural properties that make them difficult to aspiration and also visually appealing. 3D food printing is a developing technology showing an unprecedented capability to obtain a wide versatility and efficiency to develop new personalized nutrition and new eating experiences [18]. Comparing to the conventional use of molds, 3D printing can reach a higher level of perfection getting different appealing foods, saves time and effort, reduce the food wastage and have possibilities to produce personalized food with a proper amount of nutrients for specific needs [19–21]. Chachlioutaki et al. [22] using 3D printing to develop paediatric chocolate-based dosage forms found that this technology is an acceptable alternative method to mold casting. In the last years, 3D printing has been used by various researchers to develop visually appealing dysphagia friendly foods such as fresh vegetables, pork paste, beef paste and shiitake mushroom [21,23–26].

The aim of this work was to study the effect of the incorporation of three varieties of *C. vulgaris* on the nutritional and physicochemical characteristics of dysphagia vegetable purees with a high protein concentration.

2. Material and methods

2.1. Ingredients and puree preparation

The vegetables (carrots, zucchini) and the olive oil (Olisone Almazara) were purchased from Lidl supermarkets in Lisbon, Portugal. Lentil protein concentrate (LPC; protein content: 55 g/100 g) was bought from AGT Foods (Regina, SK, Canada).

The heterotrophic biomasses of *C. vulgaris*: Smooth *Chlorella* (light green color), Honey *Chlorella* (yellow color) and White *Chlorella* (white

Table 5
In vitro dry matter digestibility (IVDMD) and *in vitro* protein digestibility (IVPD) of the control puree and purees with 3 % of smooth, honey and white *Chlorella*.

Samples	IVDMD (%)	IVPD (%)
Control	90.9 ± 0.9^{A}	37.9 ± 4.5^{B}
SC	92.6 ± 1.4^{A}	45.7 ± 0.9^{C}
HC	91.8 ± 0.9^{A}	34.4 ± 1.6^{A}
WC	91.6 ± 0.5^{A}	38.3 ± 7.2^{B}

Note: Data are presented as mean ± standard deviation of three independent experiments. Different capital letters, in the same column, indicate significant differences ($p \leq 0.05$) among the samples based on the *post hoc* Tukey test.
Smooth *Chlorella* (SC), Honey *Chlorella* (HC), White *Chlorella* (WC).

Table 4
Mineral composition (mg/100 g) and the % of the nutrient reference value (NRV) of the control puree and purees with 3 % of smooth, honey and white *Chlorella*.

NRV (mg)	Control	% NRV	SC	% NRV	HC	% NRV	WC	% NRV
K (NRV = 2000)	407.42 ± 4.25^{C}	20	365.62 ± 2.79^{A}	18	380.13 ± 5.59^{B}	18	385.22 ± 2.71^{B}	19
Ca (NRV = 800)	16.17 ± 1.91^{A}	2	21.76 ± 2.78^{AB}	3	25.64 ± 2.52^{B}	3	22.85 ± 3.54^{AB}	3
Mg (NRV = 375)	46.92 ± 0.65^{B}	13	44.60 ± 0.20^{A}	12	44.80 ± 0.50^{A}	12	44.20 ± 0.20^{A}	12
P (NRV = 700)	195.43 ± 4.85^{A}	28	199.52 ± 0.79^{A}	29	211.75 ± 11.40^{A}	30	211.30 ± 12.32^{A}	30
Fe (NRV = 14)	2.00 ± 0.02^{A}	14	2.36 ± 0.08^{B}	17	2.29 ± 0.01^{B}	16	2.31 ± 0.04^{B}	17
Cu (NRV = 1)	0.57 ± 0.01^{AB}	57	0.55 ± 0.02^{AB}	55	0.54 ± 0.01^{A}	54	0.57 ± 0.01^{B}	57
Zn (NRV = 10)	1.55 ± 0.03^{A}	17	1.96 ± 0.01^{B}	20	1.97 ± 0.03^{B}	20	1.92 ± 0.01^{B}	19
Mn (NRV = 2.7)	0.55 ± 0.01^{A}	28	0.61 ± 0.01^{C}	31	0.63 ± 0.01^{D}	32	0.59 ± 0.01^{B}	29

Note: Data are presented as mean ± standard deviation of three independent experiments. Different capital letters, in the same row, indicate significant differences ($p \leq 0.05$) among the samples based on the *post hoc* Tukey test.
Smooth *Chlorella* (SC), Honey *Chlorella* (HC), White *Chlorella* (WC).

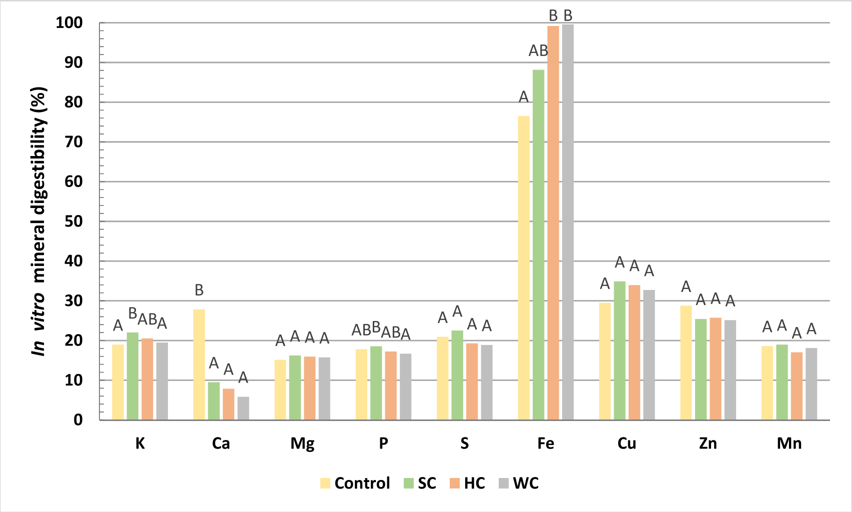

Fig. 1. *In vitro* mineral digestibility (%) of the control puree and purees with 3 % of smooth, honey and white *Chlorella*.
Note: Different capital letters, within each mineral, indicate significant differences ($p \leq 0.05$) among the samples based on the *post hoc* Tukey test.
Smooth *Chlorella* (SC), Honey *Chlorella* (HC), White *Chlorella* (WC).

Table 6
Color properties of the control puree and purees with 3 % of smooth, honey and white *Chlorella*.

Samples	L*	a*	b*	ΔE
Control	72.54 ± 0.44^{D}	-1.29 ± 0.18^{A}	40.17 ± 1.14^{A}	
SC	53.58 ± 0.47^{A}	-11.26 ± 0.05^{D}	39.66 ± 0.32^{A}	21.43
HC	68.39 ± 0.99^{B}	-2.74 ± 0.28^{c}	39.26 ± 3.36^{A}	4.49
WC	70.62 ± 0.05^{C}	-1.95 ± 0.04^{B}	36.98 ± 0.42^{A}	3.78

Note: Data are presented as mean ± standard deviation of three independent experiments. Different capital letters, in the same column, indicate significant differences ($p \leq 0.05$) among the samples based on the *post hoc* Tukey test.
Smooth *Chlorella* (SC), Honey *Chlorella* (HC), White *Chlorella* (WC).

color) were purchased from Allmicroalgae Natural Products (Pataias, Portugal). The heterotrophic culture was produced using glucose as the organic carbon source (C:N ratio of 6.7:1) in a bench-top fermenter in semi-continuous mode (New Brunswick BioFlo® CelliGen®115; Eppendorf AG, Hamburg, Germany). To create chlorophyll-deficient *C. vulgaris* mutants with various pigmentations, chemically random mutagenesis was produced [27]. Then the biomasses were collected, centrifuged and spray dried in order to obtain a powder. The nutritional composition (provided by the seller) of the three types of microalgae used in this study is shown in Table 1.

The control formulation was prepared with 21.8 % LPC, 10.0 % of fresh zucchini, 10.0 % of fresh peeled carrots, 54.9 % water, 2.5 % extra virgin olive oil and 0.8 % xanthan gum (XG). For purees with microalgae, a 3 % of each type was added as a replacement from LPC, while maintaining the proportions for the rest of ingredients.

Vegetables were put in a pot with water in a proportion of (1:2 w/v) and boiled for 30 min. The cooking water was separated and stored. The cooked vegetables were transformed in a homogeneous paste by grinding for 5 min at speed 7 with a Thermomix® (TM31, Vorwerk-Bimbi®, Portugal). The cooking water was mixed with the LPC (and the microalgae, if it were the case) with the Thermomix at speed 7 for 5 min. Then the vegetable paste, the extra virgin olive oil and the XG were incorporated and mixed again for 5 min at speed 7. The experiment (preparation and cooking of the different formulations) was carried out in three different days in order to obtain three different purees' batches to guarantee the representativity of the obtained results.

2.2. *General proximate composition*

For the determination of the protein content 0.1 g of each sample (purees or micellar phase in digested samples) was weighted and measured by Dumas (VELP Scientific NDA 702 DUMAS Nitrogen Analyzer—TCD detector, Usmate, Italy), using a protein-to-nitrogen conversion factor of 6.25. The fat residue was determined gravimetrically, after rotary evaporation of 0.1 g of sample followed by an evaporation with N_2 gas [28]. Moisture was determined according to AOAC [29]. The carbohydrate content was calculated by difference. Analyses were done in triplicate.

2.3. *Minerals determination*

The mineral profile was evaluated by inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 7000 series, Thermo Scientific, Waltham, MA, USA). For the measurements, 0.5 g of each sample was mixed with 12 mL of HCl and 4 mL of HNO_3 were added. Samples were placed into a digestor for 1.5 h at 105 °C. After that, the samples were transferred to a volumetric flasks and deionized water was added until 50 mL and then measured. Results were expressed in mg/100 g for each mineral. Mineral determination was done in triplicate.

2.4. *Total phenolic compounds and antioxidant capacity*

To determinate the total phenolic compounds (TPC) and antioxidant capacity, samples were extracted with methanol (1:10 w/v) and stirred for 60 min at room temperature, using a Reax 2 w/o adapter (Heidolph, Schwabach, Germany). Then, the mixture was centrifuged at 12.000 rpm at 20 °C, for 10 min (Sigma 1-14 Microfuge, St Louis, MO, USA). The supernatant was used for the analysis.

For the quantification of the phenolic compounds, the Folin-Ciocalteu assay was performed [30]. A quantity of 150 μL of each extract was mixed with 140 μL of Folin-Ciocalteu reagent and 2.4 mL of distilled water and homogenized in a vortex. After 3 min of reaction, 300 μL Na_2CO_3 (1 M) was added and vortexed for 15 s. The tubes were left to stand for 30 min at 40 °C for color development. The absorbance was then measured at 725 nm (Agilent Technologies, Cary Series UV–Vis Spectrophotometer, Santa Clara, CA, USA). Results were expressed as mg gallic acid equivalents (GAE) per 100 g of sample.

The antioxidant capacity was determined by two methods: DPPH and FRAP. The DPPH method [30], based on the capture of the DPPH radical

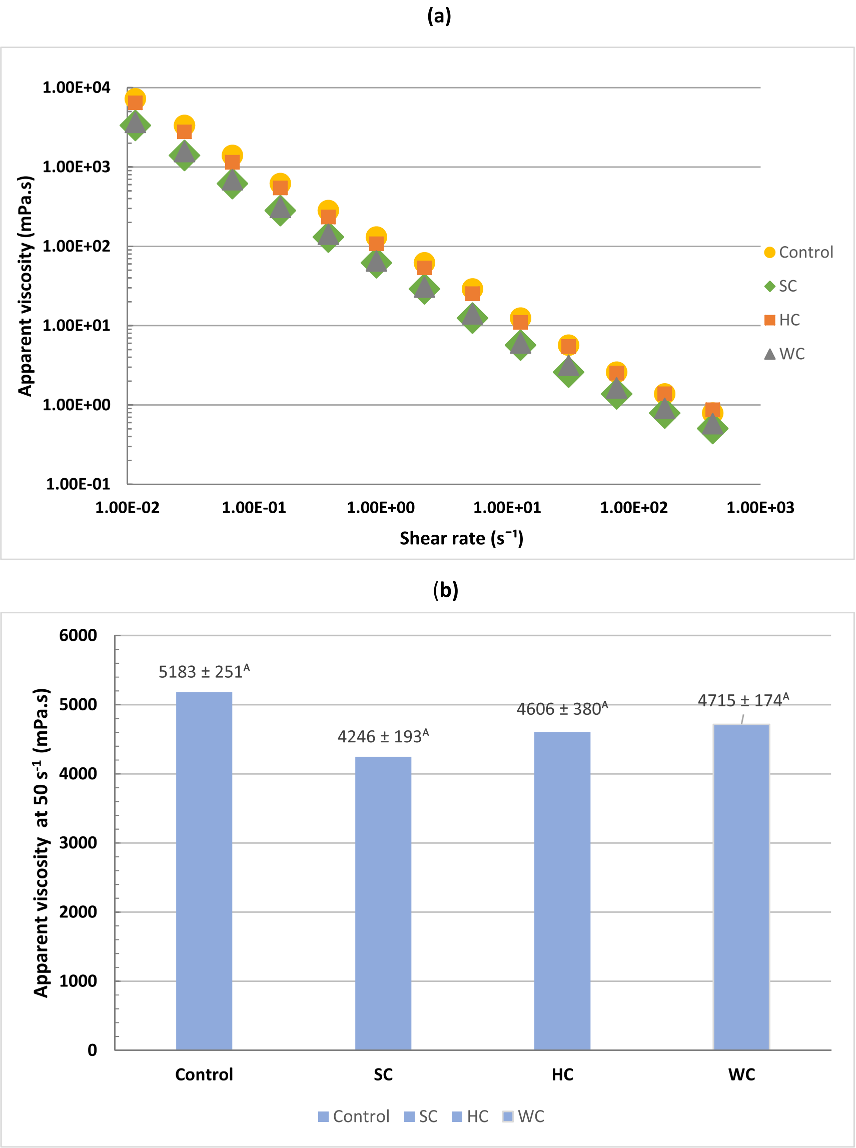

Fig. 2. Apparent viscosity curves (a) and apparent viscosity at 50 s^{-1} (b) of the control puree and purees with 3 % of smooth, honey and white *Chlorella*.
Note: Data are presented as mean ± standard deviation of three independent experiments. Different capital letters indicate significant differences ($p \leq 0.05$) among the samples based on the *post hoc* Tukey test. Smooth *Chlorella* (SC), Honey *Chlorella* (HC), White *Chlorella* (WC).

(2,2-diphenyl-1-picryl-hydrazyl) by the antioxidants, producing a decrease in absorbance at 515 nm. For this, 0.1 mL of each extract was added to 3.9 mL of DPPH radical and mixed in the vortex. The tubes were kept in darkness at room temperature for 40 min and the absorbance was read at 515 nm using methanol as a blank.

The FRAP (Ferric Reducing Antioxidant Power) method was also performed [31]. Each sample was prepared by adding 90 μL of extract, 270 μL of distilled water and 2.7 mL of FRAP reagent. The mixture was homogenized in a vortex and kept in a water bath at 37 °C for 30 min. Absorbance was read at 595 nm and water was used as a blank.

For both methods, the results were expressed as mg of Trolox equivalents (TE) per 100 g of sample (purees formulations). All the determinations were done in triplicate.

2.5. In vitro digestion: INFOGEST static model

The *in vitro* digestion of the purees was carried out following the INFOGEST protocol [32]. Each puree sample was mixed with simulated salivary fluid (SSF) containing amylase (300 U/mL) and incubated while mixing (2 min, 37 °C and pH 7). After that, the oral bolus was mixed with simulated gastric fluid (SGF) containing gastric enzyme (Pepsine 2000 U/mL) and incubated (2 h, 37 °C and pH 3). For the intestinal phase, simulated intestinal fluid (SIF) containing pancreatin (100 U/mL) and bile salts (20 mM) was added and incubated (2 h, 37 °C and pH 3). Finally, the intestinal phase was stopped using the protease inhibitor 4-(2 aminoethyl) benzensulfonylfluoride (AEBSF, trademark Pefabloc®, 500 mmol/L, Roche, Basel, Switzerland). All the analyses were made in triplicate and a reagent blank was also analyzed. After the digestion blocking, samples were centrifuged (18,000 ×g at 4 °C), the micellar fraction (supernatant) was collected and stored in the freezer until analysis and the pellet (residue) was dried at 80 °C for 6 h, and then at 45 °C, until constant weight following the method used elsewhere [15]. Analyses were done in triplicate.

2.6. In vitro digestibility (%)

The *in vitro* dry matter digestibility (IVDMD %), the *in vitro* protein digestibility (IVPD %) and the *in vitro* minerals digestibility (%) were assessed.

The IVDMD was calculated from the difference between the initial

Table 7

Viscoelastic parameters obtained from amplitude and frequency sweep tests of the control puree and purees with 3 % of smooth, honey and white *Chlorella*.

Samples	Stress sweep tests			Frequency sweep tests (1 Hz)		
	yield strain_{LVR} (%)	yield stress_{LVR} (Pa)	Flow point (Pa)	G′ (Pa)	G″ (Pa)	tan(δ)
Control	2.51 ± 0.16^{B}	13.68 ± 0.69^{C}	191.37 ± 36.2^{C}	900.7 ± 44.2^{AB}	191 ± 11.6^{AB}	0.212 ± 0.003^{A}
SC	1.23 ± 0.16^{A}	7.12 ± 0.69^{A}	136.63 ± 7.8^{A}	1034.1 ± 106.8^{B}	212.8 ± 22.7^{B}	0.206 ± 0.003^{A}
HC	2.34 ± 0.29^{B}	11.38 ± 2.14^{B}	143.27 ± 19.75^{A}	967.7 ± 108^{AB}	201.6 ± 22.9^{AB}	0.208 ± 0.002^{A}
WC	1.58 ± 0.31^{A}	8.81 ± 1.62^{A}	176.00 ± 48.08^{B}	771.1 ± 21.9^{A}	162.9 ± 5.2^{A}	0.211 ± 0.004^{A}

Note: Data are presented as mean ± standard deviation of three independent experiments. Different capital letters, in the same column, indicate significant differences ($p \leq 0.05$) among the samples based on the *post hoc* Tukey test. Smooth *Chlorella* (SC), Honey *Chlorella* (HC), White *Chlorella* (WC).

Table 8

Textural properties of the control vegetable puree and the samples with 3 % of smooth, honey and white *Chlorella* obtained through a back extrusion test.

Samples	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Index of viscosity (N.s)
Control	4 ± 0.18^{A}	69.46 ± 0.79^{A}	6.48 ± 0.17^{A}	8.52 ± 0.87^{A}
SC	3.85 ± 0.29^{AB}	63.05 ± 0.74^{B}	5.69 ± 0.11^{B}	7.72 ± 0.3^{A}
HC	3.65 ± 0.05^{AB}	63.73 ± 1.26^{B}	5.66 ± 0.17^{B}	7.88 ± 0.77^{A}
WC	3.52 ± 2.07^{B}	61.92 ± 2.07^{B}	5.51 ± 0.06^{B}	7.86 ± 0.2^{A}

Note: Data are presented as mean ± standard deviation of three independent experiments. Different capital letters, in the same column, indicate significant differences ($p \leq 0.05$) among the samples based on the *post hoc* Tukey test. Smooth *Chlorella* (SC), Honey *Chlorella* (HC), White *Chlorella* (WC).

sample and the undigested biomass (pellet) after correction (for the blank assay) divided by the initial biomass and multiplied by 100 [15,33].

The protein and minerals from the micellar fraction were analyzed as described in Sections 2.2 and 2.3, respectively. The results of the protein and mineral content were referred to initial sample after correction for the blank assay. Then the *in vitro* protein digestibility (IVPD) and *in vitro* mineral digestibility were calculated from the difference between the initial sample and the undigested biomass divided by the initial biomass and multiplied by 100 [15].

2.7. Color analysis

The color of the samples was evaluated with a CR400 Chromameter (Minolta, Japan) and evaluated according to CIELAB system color parameters (L*, a* and b*), where L* indicates brightness, a* indicates the degree of redness or greenness and b* indicates the degree of yellowness or blueness.

The absolute color difference between the control and the microalgae purees was also calculated as: $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$ and classified as follows: 0–0.5, not noticeable difference; 0.5–1.5, slightly noticeable difference; 1.5–3.0, noticeable difference; 3.0–6.0, well visible difference; 6.0–12.0, great noticeable difference [34]. The measurements were made in triplicate.

2.8. Rheological characterization

The rheological properties of the purees were measured at 40 °C in a controlled-stress rheometer (Haake Mars III—Thermo Scientific, Karlsruhe, Germany), equipped with a UTC-Peltier temperature system. A serrated parallel plate-plate geometry (PP20) was used with a gap of 1.2 mm. All the samples were measured in triplicate.

The steady shear viscosity as a function of shear rate was measured at shear rates that ranged from 0.01 to 400 s^{-1} and the apparent viscosity at 50 s^{-1} shear rate was recorded.

A stress sweep test was performed from 0.0002 to 1000 Pa, and at a frequency of 1 Hz in order to determine the linear viscoelastic region (LVR) of each sample. The value at which the G′ value deviated 5 % from the plateau value (according to the standards ISO 6721–10 and EN/DIN EN 14770) was taken as the LVR limit. Then the yield stress_{LVR} (Pa) the corresponding yield strain_{LVR} (%), and the flow point (Pa) were recorded.

A frequency sweep test was also carried out from 0.1 to 10 Hz and 1 Pa. Then the storage modulus (G′, Pa), loss modulus (G″, Pa) and tan δ (G″/G′) were obtained. The G′, G″ and tan δ at a frequency of 1 Hz were selected to compare among the samples.

2.9. Texture analysis

The purees texture was evaluated through a texturometer, TA-XTplus (Stable MicroSystems, Surrey, UK), equipped with a 5 kg load cell. A back extrusion test was carried out at 40 °C in triplicate for each sample. The test was run following the settings used by Giura et al. [35]. The measurements were made in triplicate.

2.10. SEM microscopy

The microstructural characteristics of all formulations were characterized with a scanning electron microscopy (TM 3030 Plus, Hitachi, Tokyo, Japan). Samples were placed into the SEM chamber and observed 10 kV with magnitudes from 50× to 500×.

2.11. 3D printing and shape fidelity of the purees

Printing was performed using a Foodini 3D printer (Natural Machines Inc., Barcelona, Spain). Printing settings were as follows: nozzle diameter was 4.0 mm, the printing speed was 12,000 mm/min, the line thickness was 3.4 mm, and the distance between layers: 3.4 mm.

The shape fidelity of the printed purees was determined by comparing the dimensions of the sliced 3D model (2.55 cm × 7.21 cm × 7.21 cm [x-y-z]) provided by the Foodini software and the dimensions of the printed purees, measured with a digital caliper (powerfix). The variables analyzed were the height (x-axis), upper diameter (y-axis) and lower diameter (z-axis). The shape fidelity of each variable was calculated following the equation used by Vieira et al. [37].

$$\text{Shape fidelity } (\%) = \frac{\text{Dimension of the printed purees}}{\text{Theoretical dimension of the 3D model}} * 100$$

The dimensions of the used model were considered as 100 % shape fidelity.

2.12. Statistical analyses

STATA 15 (Stata Corp LLC, TX, USA) was used to perform the statistical analyses. To evaluate statistically significant differences ($p \leq 0.05$) among all the samples, one-way analysis of variance (ANOVA) was performed followed by a *post hoc* test Tukey to detect statistically significant differences between the samples. The results were expressed as mean ± standard deviation.

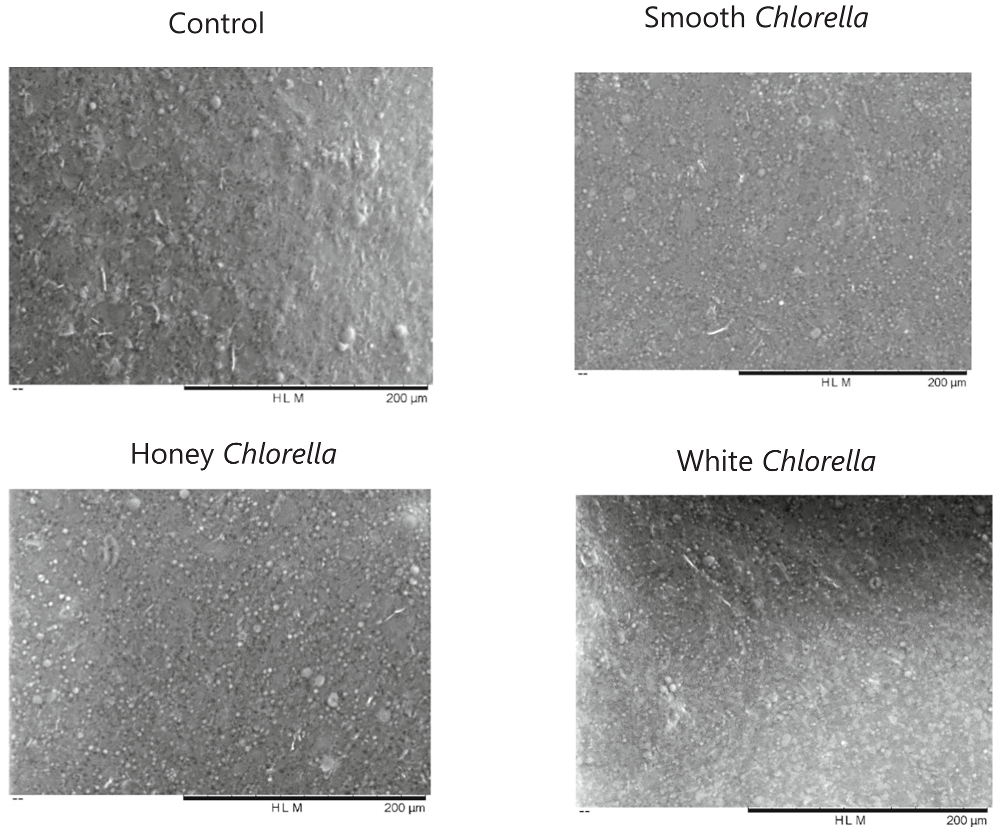

Fig. 3. SEM Microscopy of the control vegetable puree and the samples with 3 % of smooth, honey and white *Chlorella*.

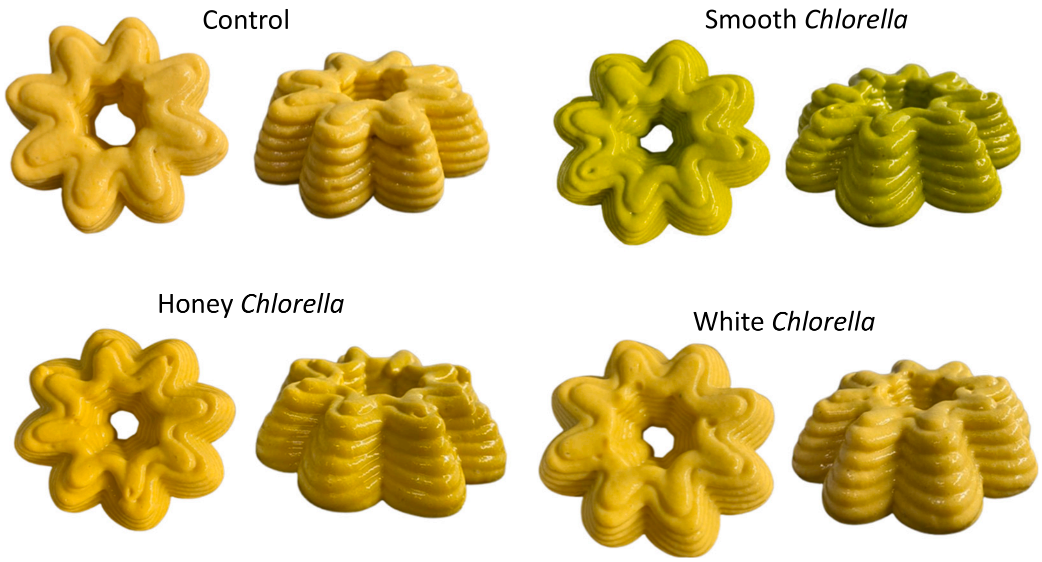

Fig. 4. 3D-printed samples of the control puree and purees with 3 % of smooth, honey and white *Chlorella*.

3. Results and discussion

3.1. Nutritional aspects

The substitution of lentil protein concentrates by a 3 % of *C. vulgaris* did not affect the general nutrient composition of the vegetable puree. No significant differences ($p > 0.05$) were found in any of the macronutrients between control samples and formulations with *C. vulgaris* (Table 2). The addition of the microalgae improved, in some cases, the antioxidant capacity (Table 3). White *C. vulgaris* showed significantly higher antioxidant capacity than control samples for DPPH and FRAP but not for TPC. The highest antioxidant capacity of the samples with white *Chlorella* could be attributed to its high amount of phytoene [27], which is considered a colorless carotenoid with high antioxidant effect [38]. However, the addition of smooth and honey varieties did not affect any of the analyzed parameters. In a previous study by Batista et al. [7] higher levels of phenolic compounds and antioxidant capacity (FRAP) were observed in cookies containing 2 % of *C. vulgaris* compared to the

Table 9
Shape fidelity of the control puree and purees with 3 % of smooth, honey and white *Chlorella*.

Samples	Dimensions (cm) and Shape fidelity (%)		
	Height (cm)	Upper diameter (cm)	Lower diameter (cm)
Model	2.55 (100 %)	7.21 (100 %)	7.21 (100 %)
Control	5.1 (200 %)	7.21 (100 %)	7.6 (105 %)
SC	4.75 (186 %)	6.9 (96 %)	7.9 (110 %)
HC	4.5 (176 %)	6.8 (94 %)	8.1 (112 %)
WC	4.65 (182 %)	7.05 (99 %)	7.8 (108 %)

Note: Data are presented as mean ± standard deviation of three independent experiments. For each parameter different capital letters indicate significant differences ($p \leq 0.05$) among the samples based on the *post hoc* Tukey test.
Smooth *Chlorella* (SC), Honey *Chlorella* (HC), White *Chlorella* (WC).

control formulation. However, those values were lower than the ones observed when incorporating other microalgae such as *A. platensis*, *T. suecica*, and *P. tricornutum*. Nonetheless, in a study conducted by Batista et al. [39] on crackers, no increases in DPPH activity with the same algae and concentrations were observed. However, the authors noticed increases in total phenolic compound levels when the concentration of *C. vulgaris* was 6 %. In other type of bakery product ("crostini"), Niccolai et al. [5] evaluated the influence of the addition (2 %, 6 % and 10 %) of a microalga (spirulina) and reported increases in phenolic compounds of up to 2 %, whereas in the case of DPPH the increases were only significant with the addition of 6 % of microalgae. These results show that the type of microalgae, its concentration and the composition of the food matrix affect the total antioxidant capacity in the final reformulated products.

Mineral contents, their nutrient reference value (NRV) [40,41] and the percentage supplied by each type of formulation are included in Table 4. The addition of *C. vulgaris* caused statistically significant increases in the amount of Fe, Zn and Mn, for the three varieties and in the amount of Ca only for the honey variety. However, all the formulations, including the control, presented a good mineral composition, being considered a source of K, P, Cu, Zn, Mn as they supplied >15 % of the NRV according to the cited regulation. Regarding Fe, all samples including controls had rather high values, which could be explained by the use of lentil protein, known for its elevated iron content. Moreover, in the case of formulations containing *C. vulgaris* the content was slightly higher, making it possible to be claimed as "source of iron" since they provide >15 % of the NRV for this essential mineral (2.39–2.36 mg/100 g). Iron contributes to a good function of the esophagus and can help to reduce the risk of Plummer–Vinson syndrome, which is characterized by a triad of symptoms: dysphagia, iron deficiency anemia and esophageal web [42]. Therefore, the increases in minerals reached with the addition of a 3 % of *Chlorella* can be considered interesting from the nutritional point of view for patients with dysphagia.

3.2. *In vitro* digestion

The nutritional assessment was completed applying an *in vitro* digestion process to the developed formulations. Total dry matter *in vitro* digestibility, protein digestibility and minerals digestibility were examined.

The *in vitro* dry matter digestibility (IVDMD %) was examined in order to assess the differences in total digestibility among formulations. The IVDMD remained similar across samples, irrespective of microalgae addition (Table 5). Khemiri et al. [15], reported similar findings, with no observed differences in the total digestibility of couscous when supplemented with microalgae compared to the control samples. Regarding the protein digestibility (IVPD %) very different results were found depending on the *Chlorella* variety, with ranges between 34 and 46 % for the microalgae formulations and 38 % for the control. These results are very similar to those obtained by Batista et al. [39], when analyzing *C. vulgaris*-enriched crackers (42 % IVPD). Honey *Chlorella* showed the lowest protein digestibility, smooth *Chlorella* the highest and, in the case of white chlorella, no differences were observed *versus* the control samples. *In vitro* protein digestibility depends on the contents of phenolic compounds, polysaccharides, and dietary fibers [43]. The existing differences in the composition of our samples could explain the differences in protein digestibility values. Moreover, the different configuration and composition of the proteins from the different varieties of *Chlorella* could be the reason for these results.

Mineral digestibility was also determined (Fig. 1). In general, the digestibility of the minerals was between 5 and 35 % and very similar across samples. In the case of Fe, higher values were found (76–99 %) for all samples including the control. Although these results could be related to the high amounts of Fe which have been commented before, they are much higher than expected according to current literature. The most significant differences between the control and microalgae formulations were observed in Ca levels, indicating that samples containing *C. vulgaris* exhibited lower digestibility values compared to the control. It is not easy to explain these differences in minerals digestibility, which depends on the potential interactions between the minerals and other compounds present in the ingredients of the formulations. Other authors had also found different behaviors in mineral bioaccessibility when analyzing fruit and vegetable purees [44], with the highest values for Mg, Mn and Zn, followed by Fe and Cu. In a study conducted by Uribe-Wandurraga et al. [45], which involved incorporating *Spirulina* and *Chlorella* into cookie formulations, it was found that only samples containing *Spirulina* showed an increase in Ca bioaccessibility compared to the control samples.

3.3. Physicochemical properties

With the addition of algae biomass, the purees exhibited changes in color, compared to the control (Table 6). As expected, the sample containing smooth *Chlorella* was the most different from the control, showing the lowest lightness and the highest greenness. Samples with honey and white *Chlorella* were the most similar in color to the control ones (see Fig. 4). According to visual appreciation, ΔE showed "very noticeable" differences between control and smooth *Chlorella* samples ($\Delta E > 20$), whereas the values for ΔE in the case of honey and white *Chlorella* formulations were much lower (3.7–4.5) corresponding to the classification of "well visible" color changes in relation to control. Khemiri et al. [15] determined the color of a couscous with the same *Chlorella* varieties and found similar results for L*, with the lowest values for formulations with the smooth variety, but no differences between control and the formulations with the three varieties in the case of a*. The obtained results gave rise to conclude that the use of 3 % smooth chlorella in food formulations designed for dysphagia patients could be a useful solution to increase the variety of colors in the diet without losing nutritional value.

Rheological and textural properties were less affected by algae addition as compared to color. Every puree formulation, including control, showed a shear thinning behavior (Fig. 2a), with a clear decrease of their apparent viscosities as the shear rate increases. The shear rate of 50 s^{-1} is taken as the standard of the oral shear rate during swallowing, although there is some controversy about its suitability [46]. In this work, every formulation presented similar values of viscosities at 50 s^{-1} shear rate, with no significant differences across formulations, being in an adequate range for a dysphagia food (>1750 mPa.s for pudding like texture), according to NDD (Fig. 2b).

From the amplitude sweeps test, the yield strain$_{LVR}$, yield stress$_{LVR}$, and flow point were determined (Table 7). Samples with smooth and white *Chlorella* showed significantly lower values for yield strain$_{LVR}$ and yield stress$_{LVR}$ than the control sample, which implies lower deformability capacity and weaker structural stability [35,36,47]. Samples with honey *Chlorella* showed lower values than control only for yield stress$_{LVR}$. The flow point was in the range between 137 and 191 Pa for all the microalgae formulations, showing lower values than the control. Anyway, it has to be pointed out that differences, although statistically significant, should not be considered as relevant, due to the similar amount of protein and hydrocolloid (XG) in all the samples.

Storage modulus (G′), loss modulus (G″) and tan δ were obtained from the frequency sweep tests and the values of 1 Hz were recorded (Table 7). The values of tan δ did not show significant differences across samples and were in the range of 0.206–0.212, indicating a weak gel-like structure, which means that all the formulations are in a suitable range for dysphagia [48]. This behavior has been observed by many authors, when developing dysphagia foods [35,36,49–53]. The lack of differences in G′, G″ and tan δ among control samples and formulations with microalgae indicate similar viscoelastic properties in all of them. The substitution of the small percentage (3 %) of lentil protein with microalgae probably contributed to the similar viscoelastic behaviors among the samples.

The incorporation of the microalgae to the puree formulations gave rise to slight decreases in firmness and cohesiveness, and especially in consistency (Table 8). These differences were more evident in textural properties as compared to rheological ones, probably due to the different types of applied stresses or strains when performing the different tests. Very different behaviors are obtained in the textural properties in studies related to microalgae incorporation in different formulations. Letras et al. [54], using *C. vulgaris* and *Spirulina* at different biomass levels (5–30 %) in gluten free cereals snacks, found different effects in firmness, adhesiveness and cohesiveness depending on the algae concentration. Khemiri et al. [15], using different varieties of microalgae (6 %) in a couscous formulation found a decrease in firmness.

Scanning electron microscopy highlighted once again that no structural differences were observed among formulations. Formulations with microalgae exhibited the same structure as the control one, showing a highly homogenous product (Fig. 3). This achievement is relevant, since the microalgae enriched purees presented a similar physical behavior to the control. On the contrary, differences in the microstructure compared to control samples were found when just a 2 % of microalgae were incorporated in crackers, suggesting an influence over the gluten matrix formation and/or starch gelatinization mechanisms [39].

3.4. 3D-Printing

The printability of a food matrix can be evaluated by its syneresis, shape fidelity and structural integrity [21]. No syneresis problems were found in any of the used formulations, including the control. Fig. 4 shows the results of the 3D-printing for the four formulations. The degree of deviation of the purees from the model used in the 3D printing was evaluated through the shape fidelity (Table 9). The most notable deviations from the model corresponded to the height, which increased between 176 and 200 %. This effect was probably due to the creamy structure of the formulations that lead to a thicker outcome from the equipment. Also, it has to be noted that every formulation showed a deviation (99–110 %) of the diameter in the base (lower diameter) which indicate a certain break of the structure as a consequence of the weight of the ink (vegetable puree).

In relation to the rheological measurements, the shear thinning behavior displayed by the four formulations anticipated their capacity to be printable [55,56]. Foods with strong shear-thinning behavior present less slimy feeling during chewing, which can provide a pleasurable texture for people with dysphagia Liu et al. [25].

The yield stress is also a parameter to be taken into account for the printability of the food matrices. It should be optimized to provide printed foods with enough mechanical stability without adversely compromising the extrusion process [21]. The values of the yield strain and yield stress of the four purees formulations seemed to be quite high comparing to those of other types of pudding-like foods designed for dysphagia [51,53] which can indicate higher deformability resistance and better stability in our samples.

These results revealed that 3D printing of dysphagia-oriented purees can bring an added sensory value to the formulations without significantly modifying their rheological properties. Moreover, it has to be noted that all the developed products were tasted by laboratory staff in order to guarantee that the sensory properties (taste and aroma) of every developed product were adequate. Similarly, a previous study revealed that, when compared to the use of molded carrots, 3D printing did not affect the taste properties of pureed carrots produced with gelatin, guar gum and xanthan gum [57].

4. Conclusions

Vegetable purees with lentil protein and xanthan gum designed for people with dysphagia were fortified with 3 % of three different varieties of *C. vulgaris*. This small degree of substitution was selected in order to avoid undesirable sensory attributes. However, it was a clear limitation of the experimental design because it implied that no important changes would be found in the composition of the final products. The results demonstrated that some nutritional aspects of the formulations were improved. The fortification improved the antioxidant capacity, especially for white *Chlorella*. Also increases in the mineral content were observed, particularly in the case of Fe. The digestibility measured by an *In vitro* model resulted in high values in every formulation. Regarding color, the addition of smooth *Chlorella* resulted in green purees with an innovative appearance. The incorporation of microalgae had a minimal influence on the apparent viscosity, viscoelastic and textural characteristics of the purees, ensuring they remained within an optimal range for individuals with dysphagia. All the purees could be successfully printed in 3D. These results revealed that dysphagia-oriented microalgae enriched purees, even added in small concentrations, can bring an added nutritional value to the formulations without significantly modifying their rheological properties. Future research to assess the sensory acceptability of these formulations from people affected by dysphagia should be carried out.

Funding

This research was funded by Gobierno de Navarra, DEGLUSEN project (PhD grant: Ayudas para la contratación de doctorandos y doctorandas por empresas, centros de investigación y centros tecnológicos: Doctorados industriales 2020) and FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04129/2020 of LEAF-Linking Landscape, Environment, Agriculture and Food, Research Unit.

CRediT authorship contribution statement

Larisa Giura: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Leyre Urtasun:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition. **Diana Ansorena:** Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation, Funding acquisition, Conceptualization. **Iciar Astiasaran:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Anabela Raymundo:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank the collaboration of Cristiana Maria dos Santos Duarte for the SEM Microscopy measurements and Joana Sales, Sheyma Khemiri and Sonia Oliveira for their assistance in experimental work.

"The authors report no commercial or proprietary interest in any product or concept discussed in this article."

References

[1] M.C. Rivelsrud, L. Hartelius, L. Bergström, M. Løvstad, R. Speyer, Prevalence of oropharyngeal dysphagia in adults in different healthcare settings: a systematic review and meta-analyses, in: Dysphagia vol. 38, Springer, US, 2023, https://doi.org/10.1007/s00455-022-10465-x.

[2] B.G. Rodd, A.A. Tas, K.D.A. Taylor, Dysphagia, texture modification, the elderly and micronutrient deficiency: a review, Crit. Rev. Food Sci. Nutr. 62 (26) (2022) 7354–7369, https://doi.org/10.1080/10408398.2021.1913571.

[3] F.J. Barba, Microalgae and seaweeds for food applications: challenges and perspectives, Food Res. Int. 99 (2017) 969–970, https://doi.org/10.1016/j.foodres.2016.12.022.

[4] J. Matos, C. Cardoso, N.M. Bandarra, C. Afonso, Microalgae as healthy ingredients for functional food: a review, Food Funct. 8 (8) (2017) 2672–2685, https://doi.org/10.1039/c7fo00409e.

[5] A. Niccolai, M. Venturi, V. Galli, N. Pini, L. Rodolfi, N. Biondi, M.R. Tredici, Development of new microalgae-based sourdough "crostini": functional effects of *Arthrospira platensis* (spirulina) addition, Sci. Rep. 9 (1) (2019) 1–12, https://doi.org/10.1038/s41598-019-55840-1.

[6] A. Mendes, T.L. Da Silva, A. Reis, DHA concentration and purification from the marine heterotrophic microalga *Crypthecodinium cohnii* CCMP 316 by winterization and urea complexation, Food Technol. Biotechnol. 45 (1) (2007) 38–44.

[7] A.P. Batista, A. Niccolai, P. Fradinho, S. Fragoso, I. Bursic, L. Rodolfi, A. Raymundo, Microalgae biomass as an alternative ingredient in cookies: sensory, physical and chemical properties, antioxidant activity and in vitro digestibility, Algal Res. 26 (June) (2017) 161–171, https://doi.org/10.1016/j.algal.2017.07.017.

[8] M. Bošković Cabrol, M. Glišić, M. Baltić, D. Jovanović, Č. Silađi, S. Simunović, A. Raymundo, White and honey *Chlorella vulgaris*: sustainable ingredients with the potential to improve nutritional value of pork frankfurters without compromising quality, Meat Sci. 198 (January) (2023), https://doi.org/10.1016/j.meatsci.2023.109123.

[9] M. Demarco, J. Oliveira de Moraes, Â.P. Matos, R.B. Derner, F. de Farias Neves, G. Tribuzi, Digestibility, bioaccessibility and bioactivity of compounds from algae, Trends Food Sci. Technol. 121 (January) (2022) 114–128, https://doi.org/10.1016/j.tifs.2022.02.004.

[10] T. Bito, E. Okumura, M. Fujishima, F. Watanabe, Potential of chlorella as a dietary supplement to promote human health, Nutrients 12 (9) (2020) 1–21, https://doi.org/10.3390/nu12092524.

[11] N.Z. Azlan, Y.A. Mohd Yusof, E. Alias, S. Makpol, *Chlorella vulgaris* improves the regenerative capacity of young and senescent myoblasts and promotes muscle regeneration, Oxidative Med. Cell. Longev. 2019 (2019), https://doi.org/10.1155/2019/3520789.

[12] R. Sharma, Effects of culture conditions on growth and biochemical profile of *Chlorella vulgaris*, J. Plant Pathol. Microbiol. 03 (05) (2012), https://doi.org/10.4172/2157-7471.1000131.

[13] V. Blas-Valdivia, R. Ortiz-Butrón, M. Pineda-Reynoso, A. Hernández-Garcia, E. Cano-Europa, *Chlorella vulgaris* administration prevents HgCl2-caused oxidative stress and cellular damage in the kidney, J. Appl. Phycol. 23 (1) (2011) 53–58, https://doi.org/10.1007/s10811-010-9534-6.

[14] I. Rodriguez-Garcia, J.L. Guil-Guerrero, Evaluation of the antioxidant activity of three microalgal species for use as dietary supplements and in the preservation of foods, Food Chem. 108 (3) (2008) 1023–1026, https://doi.org/10.1016/j.foodchem.2007.11.059.

[15] S. Khemiri, M.C. Nunes, R.J.B. Bessa, S.P. Alves, I. Smaali, A. Raymundo, Technological feasibility of couscous-algae-supplemented formulae: process description, nutritional properties and in vitro digestibility, Foods 10 (12) (2021), https://doi.org/10.3390/foods10123159.

[16] S. Oliveira, I. Sousa, A. Raymundo, Printability evaluation of *Chlorella vulgaris* snacks, Algal Res. 68 (November 2021) (2022) 102879, https://doi.org/10.1016/j.algal.2022.102879.

[17] M. WaqasQazi, I.G. de Sousa, M.C. Nunes, A. Raymundo, Improving the nutritional, structural, and sensory properties of gluten-free bread with different species of microalgae, Foods 11 (3) (2022), https://doi.org/10.3390/foods11030397.

[18] J. Sun, Z. Peng, W. Zhou, J.Y.H. Fuh, G.S. Hong, A. Chiu, A review on 3D printing for customized food fabrication, Procedia Manuf. 1 (2015) 308–319, https://doi.org/10.1016/j.promfg.2015.09.057.

[19] M. Gunjal, P. Rasane, J. Singh, S. Kaur, J. Kaur, Three-dimensional (3D) food printing: methods, processing and nutritional aspects, in: K. Sandhu, S. Singh (Eds.), Food Printing: 3D Printing in Food Industry, Springer Singapore, Singapore, 2022, pp. 65–80, https://doi.org/10.1007/978-981-16-8121-9_5.

[20] H. Jiang, L. Zheng, Y. Zou, Z. Tong, S. Han, S. Wang, 3D food printing: main components selection by considering rheological properties, Crit. Rev. Food Sci. Nutr. 59 (14) (2019) 2335–2347, https://doi.org/10.1080/10408398.2018.1514363.

[21] A. Pant, A.Y. Lee, R. Karyappa, C.P. Lee, J. An, M. Hashimoto, Y. Zhang, 3D food printing of fresh vegetables using food hydrocolloids for dysphagic patients, Food Hydrocoll. 114 (October 2020) (2021) 106546, https://doi.org/10.1016/j.foodhyd.2020.106546.

[22] K. Chachlioutaki, C. Karavasili, E.E. Mavrokefalou, C.I. Gioumouxouzis, C. Ritzoulis, D.G. Fatouros, Quality control evaluation of paediatric chocolate-based dosage forms: 3D printing vs mold-casting method, Int. J. Pharm. 624 (July) (2022) 121991, https://doi.org/10.1016/j.ijpharm.2022.121991.

[23] A. Dick, B. Bhandari, X. Dong, S. Prakash, Feasibility study of hydrocolloid incorporated 3D printed pork as dysphagia food, Food Hydrocoll. 107 (December 2019) (2020) 105940, https://doi.org/10.1016/j.foodhyd.2020.105940.

[24] A. Dick, B. Bhandari, S. Prakash, Printability and textural assessment of modified-texture cooked beef pastes for dysphagia patients, Future Foods 3 (December 2020) (2021) 100006, https://doi.org/10.1016/j.fufo.2020.100006.

[25] Z. Liu, B. Bhandari, C. Guo, W. Zheng, S. Cao, H. Lu, H. Li, 3D printing of shiitake mushroom incorporated with gums as dysphagia diet, Foods 10 (9) (2021) 1–20, https://doi.org/10.3390/foods10092189.

[26] Z. Liu, X. Xing, H. Mo, D. Xu, L. Hu, H. Li, B. Chitrakar, 3D printed dysphagia diet designed from *Hypsizygus marmoreus* by-products with various polysaccharides, J. Food Eng. 343 (September 2022) (2023) 111395, https://doi.org/10.1016/j.jfoodeng.2022.111395.

[27] L. Schüler, E. Greque de Morais, M. Trovão, A. Machado, B. Carvalho, M. Carneiro, J. Varela, Isolation and characterization of novel *Chlorella vulgaris* mutants with low chlorophyll and improved protein contents for food applications, Front. Bioeng. Biotechnol. 8 (May) (2020) 1–10, https://doi.org/10.3389/fbioe.2020.00469.

[28] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (8) (1959) 911–917.

[29] A.O.A.C, Analytical Methods 1. Moisture Content (AOAC, 1999) Method 3. Biuret Method (Robinson and Hodgen, 1940) Reagents, 2001, pp. 124–130.

[30] M. Bunzel, R.R. Schendel, in: S.S. Nielsen (Ed.), Determination of (Total) Phenolics and Antioxidant Capacity in Food and Ingredients BT-Food Analysis, Springer International Publishing, Cham, 2017, pp. 455–468, https://doi.org/10.1007/978-3-319-45776-5_25.

[31] Iris F.F. Benzie, J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Anal. Biochem. 239 (1996) 70–76.

[32] A. Brodkorb, L. Egger, M. Alminger, P. Alvito, R. Assunção, S. Ballance, I. Recio, INFOGEST static in vitro simulation of gastrointestinal food digestion, Nat. Protoc. 14 (4) (2019) 991–1014, https://doi.org/10.1038/s41596-018-0119-1.

[33] I.J. Vervaeke, N.A. Dierick, D.I. Demeyer, J.A. Decuypere, Approach to the energetic importance of fibre digestion in pigs. I. Importance of fermentation in the overall energy supply, Anim. Feed Sci. Technol. 23 (1–3) (1989) 141–167, https://doi.org/10.1016/0377-8401(89)90095-3.

[34] Z. Cserhalmi, Á. Sass-Kiss, M. Tóth-Markus, N. Lechner, Study of pulsed electric field treated citrus juices, Innov. Food Sci. Emerg. Technol. 7 (1–2) (2006) 49–54, https://doi.org/10.1016/j.ifset.2005.07.001.

[35] L. Giura, L. Urtasun, D. Ansorena, I. Astiasaran, Comparison between the use of hydrocolloids (xanthan gum) and high-pressure processing to obtain a texture-modified puree for dysphagia, Food Res. Int. 170 (May) (2023) 112975, https://doi.org/10.1016/j.foodres.2023.112975.

[36] L. Giura, L. Urtasun, I. Astiasaran, D. Ansorena, Application of HPP for the development of a dessert elaborated with casein and cocoa for dysphagia diet, Foods 12 (4) (2023) 882, https://doi.org/10.3390/foods12040882.

[37] M.V. Vieira, S.M. Oliveira, I.R. Amado, L.H. Fasolin, A.A. Vicente, L.M. Pastrana, P. Fuciños, 3D printed functional cookies fortified with *Arthrospira platensis*: evaluation of its antioxidant potential and physical-chemical characterization, Food Hydrocoll. 107 (March) (2020), https://doi.org/10.1016/j.foodhyd.2020.105893.

[38] A.J. Meléndez-Martínez, P. Mapelli-Brahm, C.M. Stinco, The colourless carotenoids phytoene and phytofluene: from dietary sources to their usefulness for the functional foods and nutricosmetics industries, J. Food Compos. Anal. 67 (February 2017) (2018) 91–103, https://doi.org/10.1016/j.jfca.2018.01.002.

[39] A.P. Batista, A. Niccolai, I. Bursic, I. Sousa, A. Raymundo, L. Rodolfi, M.R. Tredici, Microalgae as functional ingredients in savory food products: application to wheat crackers, Foods 8 (12) (2019), https://doi.org/10.3390/foods8120611.

[40] Regulation (EC) No 1924/2006. (n.d.). The Regulation (EC) N. 1924/2006 of the European Parliament and of the Council of 20 December 2006 on Nutrition and Health Claims Made on Foods. Progress in Nutrition.

[41] Regulation (EU) No 1169/2011, Celex, in: Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011, 2011, https://doi.org/10.1075/ttwia.27.04ker.

[42] A. Goel, S.S. Bakshi, N. Soni, N. Chhavi, Iron deficiency anemia and Plummer-vinson syndrome: current insights, J. Blood Med. 8 (2017) 175–184, https://doi.org/10.2147/JBM.S127801.

[43] G.A. Annor, C. Tyl, M. Marcone, S. Ragaee, A. Marti, Why do millets have slower starch and protein digestibility than other cereals? Trends Food Sci. Technol. 66 (2017) 73–83, https://doi.org/10.1016/j.tifs.2017.05.012.

[44] E. do Nascimento da Silva, L.O. de Farias, S. Cadore, The total concentration and bioaccessible fraction of nutrients in purées, instant cereals and infant formulas by ICP OES: a study of dietary recommended intakes and the importance of using a

standardized in vitro digestion method, J. Food Compos. Anal. 68 (June 2017) (2018) 65–72, https://doi.org/10.1016/j.jfca.2017.06.007.

[45] Z.N. Uribe-Wandurraga, M. Igual, P. García-Segovia, J. Martínez-Monzó, In vitro bioaccessibility of minerals from microalgae-enriched cookies, Food Funct. 11 (3) (2020) 2186–2194, https://doi.org/10.1039/c9fo02603g.

[46] J.J.X. Ong, C.M. Steele, L.M. Duizer, Challenges to assumptions regarding oral shear rate during oral processing and swallowing based on sensory testing with thickened liquids, Food Hydrocoll. 84 (May) (2018) 173–180, https://doi.org/10.1016/j.foodhyd.2018.05.043.

[47] T. Mezger, For Users of Rotational and Oscillatory Rheometers, Vincentz Network GmbH & Co KG, Hannover, Germany, 2020, https://doi.org/10.1515/9783748603702.

[48] L. Giura, L. Urtasun, A. Belarra, D. Ansorena, I. Astiasarán, Exploring tools for designing dysphagia-friendly foods: a review, Foods 10 (6) (2021) 1–16, https://doi.org/10.3390/foods10061334.

[49] M. Sharma, E. Kristo, M. Corredig, L. Duizer, Effect of hydrocolloid type on texture of pureed carrots: rheological and sensory measures, Food Hydrocoll. 63 (2017) 478–487, https://doi.org/10.1016/j.foodhyd.2016.09.040.

[50] K. Suebsaen, B. Suksatit, N. Kanha, T. Laokuldilok, Instrumental characterization of banana dessert gels for the elderly with dysphagia, Food Biosci. 32 (January) (2019) 100477, https://doi.org/10.1016/j.fbio.2019.100477.

[51] P. Talens, M.L. Castells, S. Verdú, J.M. Barat, R. Grau, Flow, viscoelastic and masticatory properties of tailor made thickened pea cream for people with swallowing problems, J. Food Eng. 292 (May 2020) (2021) 110265, https://doi.org/10.1016/j.jfoodeng.2020.110265.

[52] L. Giura, L. Urtasun, D. Ansorena, I. Astiasarán, Effect of freezing on the rheological characteristics of protein enriched vegetable puree containing different hydrocolloids for dysphagia diets, Lwt 169 (September) (2022) 114029, https://doi.org/10.1016/j.lwt.2022.114029.

[53] S. Ribes, R. Grau, P. Talens, Use of chia seed mucilage as a texturing agent: effect on instrumental and sensory properties of texture-modified soups, Food Hydrocoll. 123 (August 2021) (2022) 107171, https://doi.org/10.1016/j.foodhyd.2021.107171.

[54] P. Letras, S. Oliveira, J. Varela, M.C. Nunes, A. Raymundo, 3D printed gluten-free cereal snack with incorporation of Spirulina (*Arthrospira platensis*) and/or *Chlorella vulgaris*, Algal Res. 68 (March) (2022) 102863, https://doi.org/10.1016/j.algal.2022.102863.

[55] L. Liu, Y. Meng, X. Dai, K. Chen, Y. Zhu, 3D printing complex egg white protein objects: properties and optimization, Food Bioprocess Technol. 12 (2) (2019) 267–279, https://doi.org/10.1007/s11947-018-2209-z.

[56] Z. Liu, B. Bhandari, S. Prakash, S. Mantihal, M. Zhang, Linking rheology and printability of a multicomponent gel system of carrageenan-xanthan-starch in extrusion based additive manufacturing, Food Hydrocoll. 87 (June 2018) (2019) 413–424, https://doi.org/10.1016/j.foodhyd.2018.08.026.

[57] H. Strother, R. Moss, M.B. McSweeney, Comparison of 3D printed and molded carrots produced with gelatin, guar gum and xanthan gum, J. Texture Stud. 51 (6) (2020) 852–860, https://doi.org/10.1111/jtxs.12545.