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Enhancing energy bars with microalgae: A study on nutritional, physicochemical and sensory properties

Fabio Fanari [a,*], Josep Comaposada [b], Fatma Boukid [a,c], Elia Climent [b], Anna Claret Coma [b], Luis Guerrero [b], Massimo Castellari [a]

[a] Food Safety and Functionality Program, Institute of Agrifood Research and Technology (IRTA), 17121 Monells, Spain
[b] Food Quality and Technology Program Institute of Agrifood Research and Technology (IRTA), 17121 Monells, Spain
[c] ClonBio Group Ltd, D02 XE61 Dublin, Ireland

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ABSTRACT

Energy bars are popular meal supplements due to their convenience and high nutritional content. Microalgae, such as Spirulina and *Chlorella Vulgaris*, are appealing food ingredients containing high-quality proteins and essential bioactive compounds. This study investigated the incorporation of these microalgae into a simple energy bar model at three levels of addition (0.0 %, 2.5% and 5.0%). Bars were characterized in terms of colour, water activity, moisture content, texture as well as nutritional and sensory profiles. Results showed that microalgae improved the protein and vitamin B12 content, and influenced color, flavor, and texture of the final product. Spirulina provided the most significant changes, increasing dark green colour, sea/fishy flavours and candies and grass tastes. *Chlorella* offered different colourways depending on the strain and brought to the sensory profile some umami/fishy notes that need to be taken into account in the formulation of commercial products.

1. Introduction

Nutrition bars can be defined as meal replacers and/or supplements since they are generally rich in calories and nutrients but at the same time compact, convenient and ready-to-eat packaged. Such bars can be classified into energy bars, food bars, protein bars, or sports bars depending on their nutritional values (Boukid et al., 2022a). Energy bars, in particular, are normally prepared using cereals as the main ingredient. For this reason, they are rich in carbohydrates and useful, for example, for athletes as a quick source of energy, easy to carry out and consume before the workout.

The global cereal bar market is expected to grow at a Compound Annual Growth Rate of 8.5% until 2026 (Mordor Intelligence, 2020). These kinds of snacks are gaining popularity since are considered an easy way to ingest nutrients throughout all day. As a consequence, the snack market has shifted from mainly conventional bars towards the formulation of functional and more innovative products (Klerks et al., 2022). In literature, it is possible to find several studies aiming to enrich bars with functional ingredients like soy products (Aramouni & Abu-Ghoush, 2011; Lobato et al., 2012), banana peel flour (Carvalho & Conti-Silva, 2018), pear apple and date fiber (Bchir et al., 2018), tempeh (Melo et al., 2020), pulse flours (Maia et al., 2021), brewery spent grains (Stelick et al., 2021), fish protein concentrate (Vitorino et al., 2020) and fish oil (Nielsen & Jacobsen, 2009), dairy proteins (Hogan et al., 2012), whey protein concentrate and bioactive ingredients (Szydłowska et al., 2020) and wine fermentation biomass (Borges et al., 2021).

In response to the increasing demand for healthy and nutritious food products, bars are often fortified using a wide range of ingredients rich in phytonutrients, known to confer physiological benefits to the consumer. This means that they can also potentially serve as functional foods (Rawat & Darappa, 2015).

One of the most appealing functional ingredients that could be interesting to integrate into the bars are microalgae single cells. In the latest years, there is an increasing interest in the use of microalgae in food formulation since they are sustainable and nutritious ingredients fitting a wide range of food applications (Boukid et al., 2022b). Currently, *Arthrospira platensis* (commercially known as Spirulina) and *Chlorella vulgaris* (*C. vulgaris)* are the most employed species as food

* Corresponding author.
E-mail addresses: fabio.fanari@irta.cat (F. Fanari), josep.comaposada@irta.cat (J. Comaposada), fboukid@clonbioeng.com (F. Boukid), anna.claret@irta.cat (A. Claret Coma), luis.guerrero@irta.cat (L. Guerrero), massimo.castellari@irta.cat (M. Castellari).

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Table 1
Formulation of the energy bars (quantities for one experimental batch).

Code	Description	Microalgae	Puffed rice	Glucose Syrup
C	Control	0.0 g	134.0 g	402.0 g
WC25 HC25 SC25 SP25	*C. vulgaris*"White" 2.5 % *C. vulgaris* "Honey" 2.5 % *C. vulgaris* "Smooth" 2.5 % Spirulina 2.5 %	13.5 g	120.5 g	402.0 g
WC50 HC50 SC50 SP50	*C. vulgaris*"White" 5.0 % *C. vulgaris* "Honey" 5.0 % *C. vulgaris* "Smooth" 5.0 % Spirulina 5.0 %	26.8 g	107.2 g	402.0 g

ingredients since they have a long history of use and are authorized for food application in US, EU and many other countries (European Parliament Council of the European Union, 2015). They contain high-quality proteins (up to 65% of their dry matter) and all the essential amino acids (Brown et al., 1997; Chronakis & Madsen, 2011). Polysaccharides produced by microalgae usually present a very complex structure that may confer biological activities against cancer (Caetano et al., 2022). In addition, they contain vitamins and phenolic compounds with antioxidant properties (Andrade et al., 2018; Vaz et al., 2016), macrominerals and microminerals (Fe, Zn, Mn, and Cu) (Christaki et al., 2011), pigments (Pangestuti & Kim, 2011), sterols and polyunsaturated fatty acids (Wells et al., 2017).

In particular, the high vitamin B12 content of microalgae ingredients is very interesting for the formulation of functional foods and supplements. This vitamin is known as an essential nutrient, especially for vegan and vegetarian people since it is not synthesized by plants. Its deficiency could increase the risk of a range of neuro, vascular, immune, and inflammatory disorders (Niklewicz et al., 2023). Microalgae ingredients contain high levels of vitamin B12 although the origin of this compound is still an open debate. It is known that Spirulina is able to synthesize B12, but not in the active form while *C. vulgaris* does not synthesize at all this compound (Helliwell et al., 2016; Tanioka et al., 2010). Despite this, there are several studies that demonstrate the presence of active B12 in the dried biomass of these microalgae (Edelmann et al., 2019; Watanabe et al., 2013). The reason, as reported in the literature, could be that this B12 originates from a symbiotic relationship with B12-producing bacteria and the microalgae, and these latter ones absorb it from the culture medium (Bito et al., 2016; Croft et al., 2005).

In terms of food application Spirulina and *C. vulgaris* have been used in different products such as pasta and baked goods (Batista et al., 2017; Koli et al., 2022; Qazi et al., 2022), milk-based products (Barkallah et al., 2017; Winarni Agustini et al., 2016), snacks (da Silva et al., 2021), and soups (Boukid et al., 2021; Lafarga et al., 2019). Anyway, it should be underlined that very few preliminary studies focused on the use of microalgae to reformulate nutrition bars. Kumar et al. (Kumar et al., 2018) showed that the inclusion of Spirulina in percentages higher than 3.75% significantly decreased the sensory scores of the bars in terms of taste, colour, texture and appearance. However, the panelists give an acceptability score between 7 and 8 for all the formulations tested (the control bar without Spirulina average acceptability score was 8.12). On the other side, Lucas et al. (Lucas et al., 2020) after carrying out a consumer test with children, stated that no significant differences were founded in appearance, taste and flavor among the control bar and those prepared with Spirulina.

Thus, more research is necessary to successfully include microalgal ingredients in energetic bars in order to improve their nutritional characteristics maintaining the sensory properties of the product under control.

The aim of the work was to investigate the effects of the incorporation of single-cell ingredients, with very different compositional and sensory traits, on the nutritional (carbohydrates, sugars, protein, fats and vitamin B12 content), physicochemical and sensory characteristics of an energy bar model prepared with puffed rice and glucose syrup. To the best of our knowledge, single cell ingredients derived from green/white/yellow strains of *C. vulgaris* have been studied for the first time as functional ingredients in energy bars production. Moreover, the complete characterization of the sensory profile of energy bars enriched with Spirulina is also something that cannot be found in the literature.

The full factorial experimental design considered a simple energy bar model formulated with puffed rice and glucose syrup, and two main independent factors, i.e., *i)* four microalgal single-cell ingredients from Spirulina and *C. vulgaris* with green, white and yellow colours and ii) three level of addition of the microalgal single-cell ingredients (0.0 %, 2.5 % and 5.0 %).

The independent and combined effect of the two factors on the quality of the final products was assessed considering nutritional values, physicochemical properties (colour, a_w, moisture content), textural properties and sensory test.

2. Materials and methods

2.1. Materials

Puffed rice was bought in bulk in an online shop (https://granel.cat/) while glucose syrup (44° DE, 85° Bx) was purchased from Dekora Innova SAU (Alicante, Spain). They were both stored at room temperature in a dry warehouse. The four microalgal ingredients used, provided by Allmicroalgae (Allmicroalgae - Natural Products, SA - Pataias, Portugal), were stored at 4 °C in opaque and vacuum packaging. These ingredients were specifically: i) *C. vulgaris* "Honey" (HC, yellow colour), ii) *C. vulgaris* "Smooth" (SC, pale green colour), iii) *C. vulgaris* "White" (WC, pale yellow colour) and iv) Spirulina (SP, intense green colour) (Fig. A1). They consisted of commercial single cell ingredients in powder form obtained from microalgae produced in close photobioreactors.

The main compositional parameters of the ingredients, obtained from their technical sheet, are reported in Table A1.

2.2. Samples formulation

Energy bars were prepared according to nine different formulations, including the control recipe (without microalgae ingredients) and two formulations with two levels of addition (2.5 % and 5.0 %) for each of the four microalgae ingredients (see Table 1). The basic ingredients of the bar were puffed rice and glucose syrup. The choice of this formulation was taken based on the will to formulate a model bar that can be representative of the category. Puffed rice was chosen as it is a gluten-free source of carbohydrates while glucose syrup is commonly used as an aggregator element in the bars and also for its capacity to offer quick absorbable energy. The amount of microalgae powder to be incorporated was calculated on the basis of the total weight of the ingredients. When microalgae were added, the ingredients total weight was maintained constant by varying the puffed rice amount, while the glucose amount was not changed. The level of inclusion investigated was chosen according to the studies reported in the literature for these products, which does not exceed 5% due to the fact that consumers usually do not appreciate products with a level of inclusion higher than 5% (Castillejo et al., 2018; Kumar et al., 2018; Lafarga, 2019; Lucas et al., 2020; Paternina et al., 2022; Robertson et al., 2016).

To prepare the bars, first, the glucose syrup was pre-heated in a water bath at 40 °C, then weighed in a glass bowl suitable for microwaving and heated at 900 W for two minutes in a domestic microwave oven Panasonic model NN-T251W (Panasonic co., Kadoma, Japan), reaching a temperature around 85 °C. After the heating process, microalgae powder

Table 2
Nutritional values of the energy bar formulated with different levels of microalgae inclusion (100 g of product).

Sample	Energy [kcal]	Carbohydrates [g]	Sugars [g]	Proteins [g]	Fats [g]	Vitamin B12 [μg]	*Vitamin B12 %RV
Control	340.5	82.1	24.0	1.6	0.5	0.0	0.0%
WC25	339.8	80.8	23.5	2.2	0.6	1.4	35.0%
HC25	345.1	81.5	23.5	2.2	0.7	0.6	15.0%
SC25	340.3	80.9	23.7	2.2	0.6	0.4	10.0%
SP25	340.2	80.3	23.5	3.0	0.6	4.7	119.0%
WC50	339.1	79.4	23.5	2.8	0.8	2.7	67.5%
HC50	349.7	80.9	23.1	2.9	0.9	1.2	30.0%
SC50	349.2	81.6	24.0	3.0	0.8	0.7	17.5%
SP50	339.9	78.5	22.9	4.5	0.7	9.5	237.0%

* *Percentage of daily estimated average requirement for Vitamin B12 (adults = 4 μg/day,* European Food Safety Authority, 2015*).*

(if any) was added and mixed manually for 15 s with a spatula. Finally, the puffed rice was included, and the ingredients were manually mixed for 1 min again using a spatula. The mixture, with a temperature of around 78 °C, was immediately poured into a stainless-steel mold (270 × 220 × 12 mm, w*l*h), covered with edible oven paper. At this point, the mixture in the mold was pressed with a wooden roll in order to obtain a flat cake. This cake was stored at 4 °C for 2 h and then manually cut into small bars (30 × 80 × 12 mm, w*l*h). Three independent batches were produced and characterized for each of the nine formulations.

2.3. Energy bars characterization

2.3.1. Physicochemical analysis

Moisture content (M%) was assessed by drying the samples in a forced-air oven (J.P. Selecta, Abrera, Spain) at 105 °C to a constant weight, for 24 h (AACC, 2005). Water activity (a_w) was measured at 25 °C using an Aqualab 4TE (Decagon Devices Inc., Pullman, WA, USA) (ISO 18787, 2017). Both analyses were carried out in replicate on 3 different batches for each treatment.

Colour was evaluated with a CR-600d D65 colorimeter (Minolta Co., Osaka, Japan) by measuring CIE L*a*b parameters (Luo, 2015) in three points of ten bars for each formulation and replicate. Total colour difference (ΔE) from the control sample was calculated using the CIE76 formula (Equation (1), based on the Euclidian distances between colours in CIELab space):

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (1)$$

where L, a, and b are the CIELab parameters of a given sample bar, and L_0, a_0 and b_0 are the CIELab parameters of the control sample.

2.3.2. Texture profile analysis

The instrumental texture of the bars was evaluated using a texture analyser TA.HD plus (Stable Microsystems, Surrey, UK) equipped with a 250 kg load cell. A TPA (Texture Profile Analysis) was carried out at a speed of 2 mm/s and deformation of 75% following Bourne (Bourne, 1978). Samples were further cut into squares of 2 × 2 cm and kept for 2 h at 20 °C and relative humidity of 45–50% to standardize the water absorption before the test. 10 different pieces for 3 different batches of all the formulations were evaluated. The textural attributes namely, hardness, adhesiveness, cohesiveness, springiness, chewiness and resilience were calculated following Szczesniak (Szczesniak, 2002).

2.3.3. Sensory evaluation

The sensory analysis of the energy bars was carried out by a trained panel of 8 tasters. All of them had more than 2 years of experience in descriptive analysis of different foods. Initially, an open discussion session was held to select by consensus the sensory attributes to be assessed. A total of 7 odour attributes (Global intensity, Cereal, Glucose syrup, Wet dog, Amines, Dehydrated prawn, Dry Grass) and 9 taste attributes (Global intensity, Puffed rice, Sweet, Refreshing, Candies, Prawn, Amines, Dry grass, Green grass) were selected. Neither visual nor textural attributes were included as both were determined instrumentally to simplify the tasters' work. Two additional training sessions were held to unify the use of the scale among tasters.

The evaluation of the samples was carried out using an incomplete design, in which for each tasting session, 4 different treatments and the control sample were evaluated. A total of 12 sessions were performed, 4 for each of the 3 production batches. In the end, all treatments were evaluated 6 times, except the control which was evaluated 12 times. The samples were presented in different orders for taster and session following a Williams' Latin square design (balanced for First-order Carry-over or Residual Effects) (Macfie et al., 1989).

All samples, coded with 3-digit random numbers, were analysed in a standardised tasting room with green light to mask colour differences between samples (ISO 8589, 2007). The tasters were provided with mineral water and apple slices to clean their palates between samples.

The trustworthiness of the panel was verified by means of the standard methodology (ISO 11132, 2021).

2.3.4. Nutritional labelling

The nutritional label of bars was elaborated using the compositional data reported on the commercial product labels and technical sheets provided by the suppliers (Table A1). The percentage with respect to the reference value (%RV) for vitamin B12 was calculated considering that the EU estimated average requirement (EAR) is 4 μg/day for adults (European Food Safety Authority, 2015).

2.3.5. Statistical analysis

To assess the effect of microalgal ingredients (*MI*), their level of addition (*LA*) and their combined effect on the physicochemical and sensory properties of the bars an analysis of variance (ANOVA) was performed. The initial model included both main effects (*MI* and *LA* and their interaction) as fixed effects. When the interaction was significant, a new one-way ANOVA was performed considering the combination of *MI* and *LA* as treatments (9 in total). Significant differences among the mean values were assessed using the Tukey post hoc test. F statistic value of the ANOVA test was expressed as the percentage that each independent factor represents with respect to the sum of the three F values obtained for each variable (F of Microalgae Ingredient (MI), F of Level of Addition (LA) and F of MIxLA). In this way the values of F for the different physicochemical and sensorial parameters considered were comparable. Additionally, a Principal Component Analysis (PCA) was performed based on the mean values for each treatment and variable. To do this data were autoscaled. The analysis was done on the correlation matrix, data were centered and reduced to be standardized. Confidence interval at 95% confidence level were calculated according to the bootstrap method (Lebart, 2007).

All experimental data were statistically analysed using XLSTAT® software version 2021.1.1.1110 (Addinsoft, Paris, France).

Table 3
Analysis of variance of the physicochemical and sensory characteristics of the samples reformulated with different microalgae ingredients and two levels of addition (ns: not significant; *: p ≤ 0.05; **: p ≤ 0.01; ***: p ≤ 0.001).

Parameters	Microalgae ingredient (MI)		Level of addition (LA)		(MI × LA)	
	*F%	Significance	*F%	Significance	*F%	Significance
M %	42.33	*	26.83	ns	30.84	ns
a_w	44.67	ns	3.89	ns	51.44	ns
L*	79.73	***	19.69	***	0.57	***
a*	96.82	***	1.40	***	1.78	***
b*	95.15	***	1.87	***	2.97	***
ΔE	71.42	***	27.76	***	0.82	***
Hardness (N)	34.93	***	11.81	ns	53.26	***
Adhesiveness (J)	68.51	***	18.34	ns	13.14	ns
Cohesiveness	47.05	***	36.00	*	16.94	ns
Springiness	45.63	**	0.16	ns	54.21	**
Chewiness (N)	52.38	**	1.18	ns	46.43	**
Resilience	8.02	ns	2.42	ns	89.55	***
Olfactory profile attributes						
Global Intensity	33.51	***	66.19	***	0.30	ns
Cereal	86.16	***	12.10	ns	1.74	ns
Glucose Syrup	94.48	***	4.04	ns	1.48	ns
"Wet Dog"	8.45	ns	85.52	*	6.02	ns
Amines	75.90	***	22.09	***	2.01	ns
Dehydrated Prawn	87.65	***	9.95	ns	2.40	ns
Dry Grass	13.84	ns	18.89	ns	67.27	ns
Taste profile attributes						
Global Intensity	77.58	***	16.08	ns	6.33	ns
Puffed Rice	67.41	***	31.51	*	1.07	ns
Sweet	98.35	***	0.07	ns	1.58	ns
Refreshing	85.86	***	0.40	ns	13.75	ns
Candies	96.27	***	0.99	ns	2.74	ns
Prawn	19.47	ns	78.22	**	2.30	ns
Amines	55.27	ns	33.40	ns	11.33	ns
Dry Grass	64.61	***	28.99	ns	6.39	ns
Green Grass	67.42	***	26.77	*	5.80	ns

* *F% = F statistic value of the ANOVA test calculated as the percentage that each independent factor represents with respect to the sum of the three F values obtained for each variable (F of Microalgae Ingredient (MI), F of Level of Addition (LA) and F of MIxLA).*

Table 4
Mean values of physicochemical properties of energy bars samples. Different letters (a–h) in the same column indicate significant (p < 0.05) differences between formulations for the given parameter.

Sample	L*	a*	b*	ΔE	M%	a_w
Control	72.94^{h}	0.574^{f}	16.85^{c}	0.00^{a}	13.9^{c}	0.692^{b}
WC25	68.65^{g}	-0.078^{e}	21.77^{d}	7.05^{b}	12.9^{ab}	0.673^{ab}
WC50	65.18^{f}	-0.030^{e}	24.49^{e}	11.31^{c}	12.2^{ab}	0.678^{ab}
HC25	65.41^{f}	3.518^{g}	36.69^{g}	21.55^{d}	12.3^{ab}	0.665^{a}
HC50	57.34^{e}	5.650^{h}	43.64^{h}	31.74^{f}	12.4^{ab}	0.677^{ab}
SC25	53.52^{d}	-5.415^{b}	26.92^{f}	22.83^{d}	12.6^{ab}	0.683^{ab}
SC50	45.87^{c}	-5.978^{a}	26.44^{f}	29.90^{e}	12.1^{a}	0.678^{ab}
SP25	40.02^{b}	-2.374^{c}	12.66^{b}	33.45^{f}	12.8^{ab}	0.682^{ab}
SP50	31.85^{a}	-1.955^{d}	10.03^{a}	41.86^{g}	13.0^{b}	0.675^{ab}

3. Results and discussion

The visual appearance of the energy bars samples is shown in Fig. A2.

The nutritional values of the bars are reported in Table 2, while the analysis of the effect of the microalgae ingredient and their level of addition on the physicochemical and sensory properties is analyzed in Table 3.

Talking about the nutritional labelling, as observable in Table 2, the addition of microalgae produced a very slight decrease in carbohydrates and a slight increase in fats amount. Regarding the protein content, of course, the microalgae ingredients increased it, with the highest contribution supplied by SP. However, in none of the cases was sufficient to reach the claim "source of proteins", according to regulation (EC) No 1924/2006, considering also the high content of carbohydrates in the product. To produce a relevant increase in the protein content of the bar, higher concentration of microalgae in the product are necessary, but this would imply a significant modification of the sensorial properties as already seen in the previous. On the contrary, the B12 supply of the ingredients is very interesting: all the bars can be labelled as "source of vitamin B12" and additionally bars WC25, WC50, H50, SP25 and SP50 can be labelled as "high in vitamin B12" according to regulation (EC) No 1169/2011. Moreover, the high value of the percentage amount with respect to the reference value (%RV) similar, and for some formulations even higher than the intake provided by the cooked meat (5–132%, Gille & Schmid 2015), also justified the proposed functionality of these bars as vitamin B12 supplements.

Regarding the physicochemical parameters, in Table 3 it is possible to observe that Microalgal Ingredients (*MI*) significantly influenced M%, colour and textural parameters, with the exception of resilience, as well as several sensory descriptors for both flavour (the only exceptions were wet dog and dry grass) and taste (prawn and amines were not significant).

On the other hand, *LA* significantly affected all the colour parameters ($p \leq 0.001$) but showed no significant influence on M%, a_w and textural parameters, except cohesiveness. The effect of *LA* on the sensory profile was also significant for few smell attributes (Global Intensity, "Wet Dog" and Amines) and tastes (Puffed Rice, Prawn, and Green grass).

Additionally, significant interactions between the two independent factors *MI* and *LA* were also evidenced for colour and most of the textural parameters (excluding adhesiveness and cohesiveness). Concerning colour parameters, their intensity was mainly controlled by MI (F%=70–97). On the other side, textural parameters were influenced by the effect of the two factors and their combined interaction. Regarding

Table 5
Mean values of textural properties of energy bars samples. Different letters (a–d) in the same column indicate significant ($p < 0.05$) differences between formulations for the given parameter.

Sample	Hardness [N]	Adhesiveness [J]	Cohesiveness	Springiness	Chewiness [N]	Resilience
Control	662.2^{bc}	−90.42^{c}	0.231^{a}	0.464^{ab}	72.48^{ab}	0.054^{ab}
WC25	703.1^{b}	−45.26^{a}	0.205^{c}	0.290^{c}	42.13^{bc}	0.051^{bcd}
WC50	698.7^{b}	−51.95^{a}	0.208^{bc}	0.302^{bc}	40.35^{c}	0.050^{cd}
HC25	657.8^{bc}	−62.86^{abc}	0.210^{bc}	0.436^{abc}	62.03^{abc}	0.048^{d}
HC50	822.9^{a}	−49.34^{a}	0.206^{c}	0.357^{abc}	61.32^{abc}	0.056^{a}
SC25	673.7^{bc}	−83.49^{bc}	0.223^{ab}	0.361^{abc}	56.49^{abc}	0.052^{abc}
SC50	610.6^{c}	−56.19^{ab}	0.208^{bc}	0.464^{ab}	73.17^{ab}	0.051^{bcd}
SP25	691.1^{bc}	−87.71^{c}	0.222^{ab}	0.486^{a}	78.40^{a}	0.052^{abc}
SP50	686.5^{bc}	−86.58^{bc}	0.217^{abc}	0.437^{abc}	70.74^{abc}	0.048^{cd}

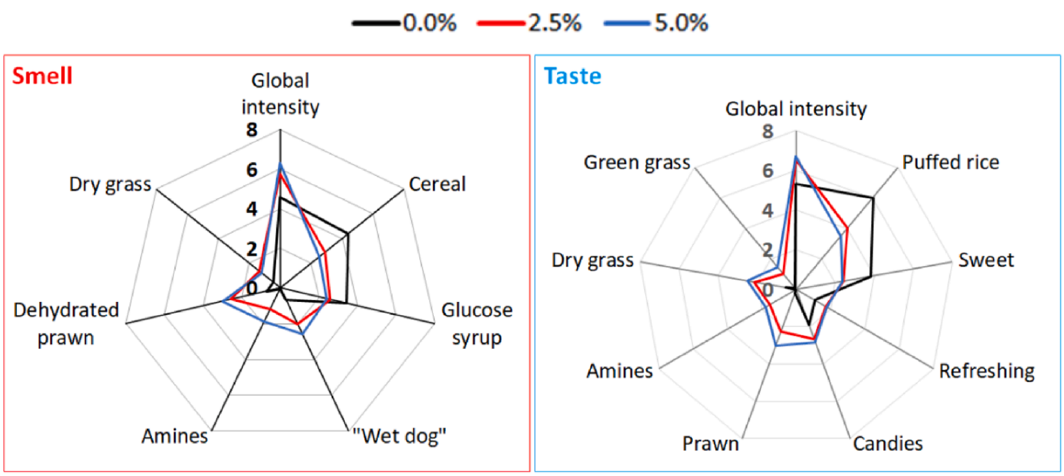

Fig. 1. Influence of microalgae ingredient inclusion level (black line 0%, red line 2.5% and blue line 5%) on the sensory profiles of the energy bars samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

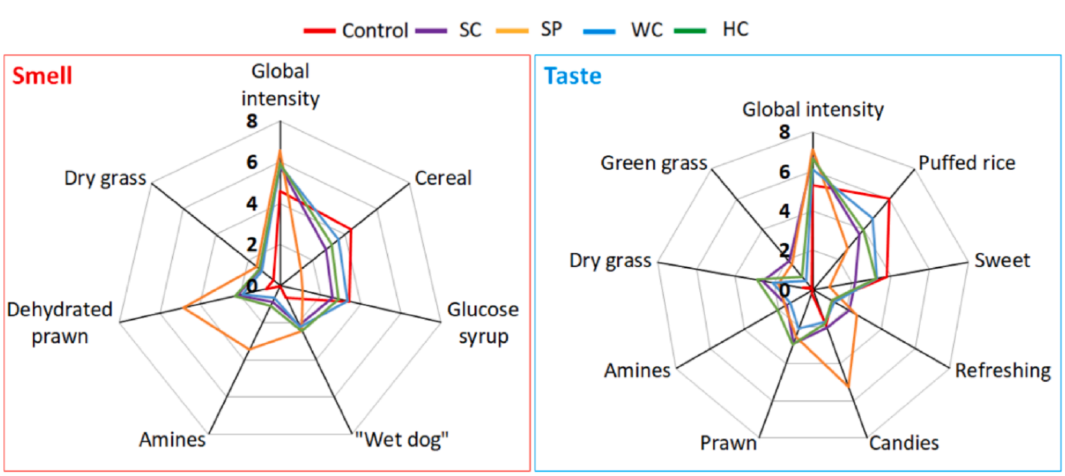

Fig. 2. Sensory profile of the energy bars samples evaluated as a function of the microalgae ingredient added (red line – Control, purple line – SC, orange line – SP, blue line – WC and green line – HC). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

M%, a_w, and all the sensory attributes, the interaction between *MI* and *LA* was not significant. There is no combined effect of the two factors. For this reason, in the case of sensory evaluation, the influences of factors *MI* and *AL* were analysed without taking into account their interaction effect and considering the two effects independently.

Table 4 shows the physicochemical parameters for the nine energy bar formulations.

Looking at Table 4 it can be observed that the addition of microalgae single-cell ingredients decreased the lightness (L^*) in all the samples evaluated. A significant decrease of a^* (corresponding to an increase in greenness) was also observed. This effect was especially pronounced in SP and SC treatments and limited for WC as observable in Fig. A2.

These colour changes can be related to the high chlorophyll content in blue–green algae (like SP and SC), and have been observed in other food products (e.g. cookies and bread) reformulated with microalgal ingredients (Batista et al., 2017; García-Segovia et al., 2017). On the other hand, the inclusion of yellowish microalgal ingredients (HC, WC) caused the opposite effect, with a significant increase of a^* in comparison with the control sample.

The degree of yellowness (b^*) was always higher than in the control

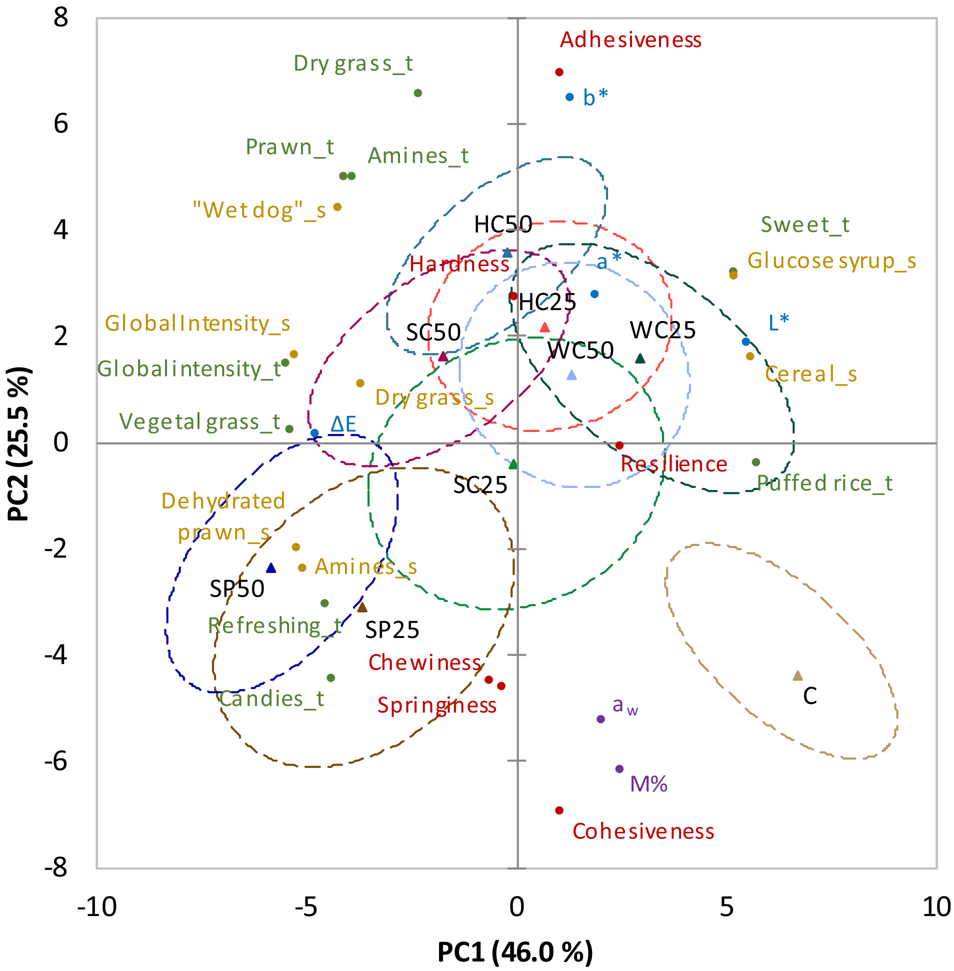

Fig. 3. Biplot of the first two components PC1 and PC2 derived from the PCA analysis of the physicochemical characteristics and sensory attributes of the samples. Black triangles represent the bar samples while the points indicative the variables (blue = colour parameters, purple = M % and a_w, red = textural parameters, dark yellow = smell attributes, green = taste attributes). Ellipses represent 95% confidence intervals calculated with the bootstrap method (Lebart, 2007). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. A1. Samples of the four microalgae powder ingredients used for the bars reformulation (from the left to the right: C. vulgaris White (WC), C. vulgaris Honey (HC), C. vulgaris Smooth (SC) and Spirulina (SP)).

in the treatments reformulated with *C. vulgaris* ingredients, while those containing Spirulina (SP25 and SP50) showed values significantly lower than the control for this parameter. This effect could be due to the yellow pigments, mainly carotenoids, which are produced in higher amounts by *C. vulgaris* spp. compared to Spirulina (Aruldass et al., 2018; Gouveia et al., 2006; Schüler et al., 2020; Uribe-Wandurraga et al., 2021).

The total colour variation (ΔE) was always higher than 7.0 (i.e. considered perceivable in most of the cases by human eye) in the samples containing microalgal ingredients (Table 4). Colour changes were less noticeable in WC and maximum in SP formulations. All the changes in the colour parameters were significantly more pronounced when increasing the *LA* as already observed in other studies with vegetable creams and pasta for example (Boukid et al., 2021; Şahin, 2020).

The addition of microalgae ingredients always reduced the M% of the energy bars in comparison to the control sample, probably because their inclusion in the form of powder ingredients allowed them to absorb more water (Table 4). This effect on M% was partially reflected in the values of a_w, which were generally lower than the control product in samples with microalgal ingredients, even if the difference were only significant for HC25. Similar results in terms of M% and a_w were observed also by other authors with savory products and cookies reformulated with microalgae single-cell ingredients (Batista et al., 2017,2019).

The crispiness and sensory acceptance of low/medium moisture foods are strongly influenced by their water content and/or a_w. When these values increase, the food materials become harder and increase

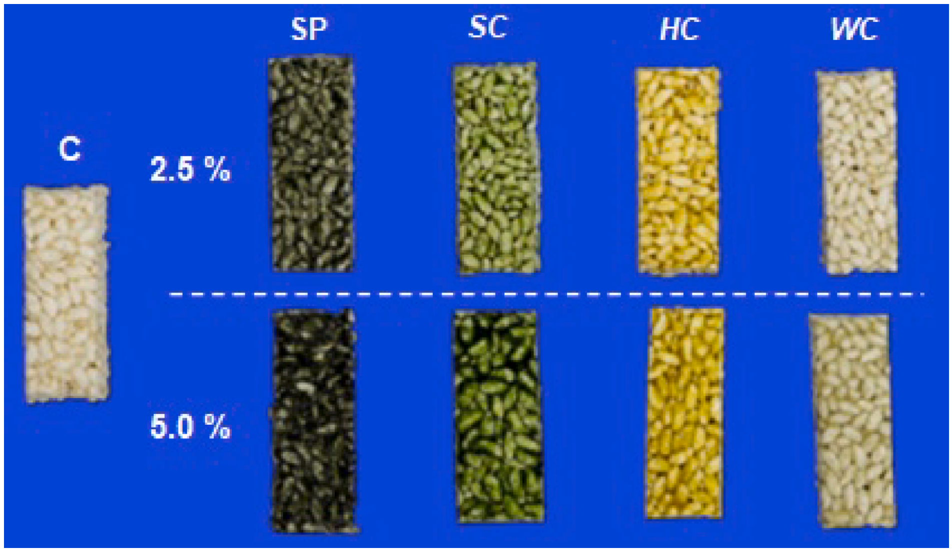

Fig. A2. Samples of the nine energetic bar formulations (C – Control sample; SP – Spirulina; SC – C. vulgaris Smooth; HC – C. vulgaris Honey; WC – C. vulgaris White) with two different levels of addition (2.5% and 5.0%).

Table A1
Nutritional values of the ingredients used in bars formulation (quantities are referred to 100 g of product).

Ingredient	Energy [kcal]	Carbohydrates [g]	Sugars [g]	Protein [g]	Fats [g]	Vitamin B12 [μg]
Puffed Rice	366.0	79.5	21.0	6.3	2.0	0.0
Glucose Syrup	332.0	83.0	25.0	0.0	0.0	0.0
C. vulgaris"White" (WC)	550.0	56.0	3.0	32.5	10.0	25.0
C. vulgaris "Honey" (HC)	357.5	30.0	10.0	32.5	7.5	15.0
C. vulgaris "Smooth" (SC)	338.0	25.0	2.0	30.0	8.0	55.0
Spirulina	354.5	6.0	2.0	65.0	6.5	190.0

Table A2
Smell sensory evaluation of energy bars samples for different microalgae species and addition levels. Different letters (a–c) in the same column indicate significant ($p < 0.05$) differences between samples for the given attribute.

Sample	Global intensity	Cereal	Glucose syrup	"Wet dog"	Amines	Dehydrated prawn	Dry grass
Control	4.58^{c}	4.39^{a}	3.44^{a}	0.66^{b}	0.04^{c}	0.72^{c}	0.44^{b}
WC	5.84^{b}	3.61^{b}	3.35^{a}	2.21^{a}	0.65^{b}	1.86^{b}	1.14^{ab}
HC	5.93^{b}	3.20^{bc}	2.90^{b}	2.44^{a}	1.08^{b}	2.27^{b}	1.29^{a}
SC	5.79^{b}	2.82^{c}	2.60^{b}	2.11^{a}	0.85^{b}	2.13^{b}	1.28^{a}
SP	6.59^{a}	1.29^{d}	1.12^{c}	2.42^{a}	3.43^{a}	4.81^{a}	1.47^{a}
Level of addition	**Global intensity**	**Cereal**	**Glucose syrup**	**"Wet dog"**	**Amines**	**Dehydrated prawn**	**Dry grass**
0	4.58^{c}	4.39^{a}	3.44^{a}	0.66^{c}	0.04^{c}	0.72^{c}	0.44^{b}
2.5	5.78^{b}	2.92^{b}	2.59^{b}	2.03^{b}	1.15^{b}	2.54^{b}	1.37^{a}
5.0	6.30^{a}	2.54^{c}	2.39^{b}	2.56^{a}	1.85^{a}	3.00^{a}	1.21^{a}

their crispiness. Anyway, considering the restricted variations, the impact on the product properties produced by the microalgae, in this study appears to be limited and not able to cause changes in the texture of the bars.

Moreover, looking at the textural parameters (Table 5) some significative differences were observed. In many cases the *C. vulgaris* ingredients provided adhesiveness and cohesiveness significantly lower than in the control sample, suggesting that these ingredients can in part make chewing more difficult. On the other hand, the bars added with spirulina showed always values not significantly different from the control in terms of texture (except for the difference in resilience presented by sample SP50). Even if specific studies with bars are not available, controversial results can be found in the literature about the influence of microalgae addition up to 5–10 % w/w on food texture. Spirulina and *C. vulgaris* influenced the texture of bakery products by increasing the hardness (from 1% to 6% level of inclusion) (Batista et al., 2017; Şahin, 2020), in other cases, on the contrary, reduced hardness and crispiness (1.5 % level of inclusion) (Uribe-Wandurraga et al., 2019). Other studies with bread reported that the textural parameters were not modified by the addition (inclusion at 1.5%) of several species of microalgae (García-Segovia et al., 2017). The controversial results about the influence of microalgae ingredients on texture probably reflect a limited impact considering the low inclusions level.

The results of the sensory evaluation are reported in Fig. 1, as a function of the level of addition of microalgae, and in Fig. 2, as a function of the microalgae ingredient (numerical values and significative differences are reported in Tables A2 and A3). The incorporation of microalgae produced an increase in global smell intensity, as well as of the "wet dog", amines, dehydrated prawn, and dry grass smells. On the contrary, cereal and glucose syrup smells were reduced. All these

Table A3
Taste sensory evaluation of energy bars samples for different microalgae species and addition levels. Different letters (a–d) in the same column indicate significant ($p < 0.05$) differences between samples for the given attribute.

Sample	Global intensity	Puffed rice	Sweet	Refreshing	Candies	Prawn	Amines	Dry grass	Green grass
Control	5.31^{d}	6.03^{a}	3.81^{a}	1.08^{b}	1.90^{b}	0.19^{c}	0.00^{b}	0.53^{c}	0.11^{c}
WC	6.12^{c}	4.73^{b}	3.31^{a}	1.23^{b}	1.75^{b}	2.11^{b}	1.34^{a}	2.07^{b}	0.55^{bc}
HC	6.64^{b}	3.96^{c}	3.23^{a}	1.11^{b}	1.83^{b}	2.97^{a}	2.08^{a}	2.90^{a}	0.87^{b}
SC	6.68^{b}	3.68^{c}	2.19^{b}	2.13^{a}	2.07^{b}	2.91^{a}	1.54^{a}	2.61^{a}	1.87^{a}
SP	7.12^{a}	2.72^{d}	0.83^{c}	2.58^{a}	5.27^{a}	2.54^{ab}	1.63^{a}	1.78^{b}	1.69^{a}
Level of addition	Global intensity	Puffed rice	Sweet	Refreshing	Candies	Prawn	Amines	Dry grass	Green grass
0	5.31^{b}	6.03^{a}	3.81^{a}	1.08^{b}	1.90^{b}	0.19^{c}	0.00^{b}	0.53^{c}	0.11^{c}
2.5	6.55^{a}	4.06^{b}	2.41^{b}	1.74^{a}	2.65^{a}	2.24^{b}	1.53^{a}	2.17^{b}	1.04^{b}
5.0	6.73^{a}	3.49^{c}	2.37^{b}	1.79^{a}	2.82^{a}	3.03^{a}	1.77^{a}	2.51^{a}	1.44^{a}

changes were positively correlated with the level of inclusion and of higher intensity when microalgae content went from 2.5% to 5% except for glucose syrup and dry grass smells. SP was the ingredient that showed the maximum intensity values of these parameters.

Concerning taste profile, the microalgae addition increased the global intensity, prawn, amines, dry grass, and green grass tastes and decreased puffed rice and sweet taste attributes. Moreover, the addition of SP increased the candy taste of bars significantly compared to all the other species (see Fig. 2). Finally, the effect of the inclusion level when the content went from 2.5% to 5% was significant only for puffed rice, prawn, dry grass, and green grass tastes (Fig. 1).

The introduction of microalgae ingredients in food products has been associated with umami, sweet and bitter flavours (Bruhn et al., 2019; Zamuz et al., 2019). In this work, the bitter attribute was not perceived, because it was probably masked by the intense sugar flavour provided by the glucose syrup. The tastes and odours detected could be associated with the aminoacidic profile of the microalgae ingredients, with alanine, for example, giving the sweet perception (Sahin et al., 2022). Kuatrakul et al. (2017) stated that fish, salty, seaweed, and green/grassy aromas, also detected in this study, are characteristic sensory attributes of dried microalgae ingredients. However, these attributes do not necessarily prejudice the overall acceptability of the product, because, as stated by Lucas et al. (2020) for snack bars, the inclusion of microalgae ingredients at low levels also caused an improvement in the products pleasantness. This could be related for example to the increase in candies/sweet attributes observed in some products in the present study.

A PCA was carried out with all the sensory and physicochemical parameters to visualize the differences among formulations. Fig. 3 shows the biplot of the two principal components (PCs) of the PCA. The two principal components accounted for 71.5% of the total variance. Specifically, the first principal component, represented mainly by most of the sensory attributes and the colour parameters L* and ΔE, accounted for 46.0%. The second principal component, expressed as a function of M%, a_w and textural parameters, accounted for 25.5%. Observing the graphical representation, it is possible to see that the samples can be aggregated into three main groups, the spirulina one, the control bar and the *Chlorella* samples (highlighted with confidence intervals ellipses in the graph). Among samples with *Chlorella*, it can be seen that SCs had scores more similar to Spirulina compared to HC and WC that were significantly differently positioned in the chart. SP bars have opposite properties compared to the control bar and were the ones with the higher global intensity of smell and taste, characterized by refreshing, candies, grass taste and dehydrated prawns, amines and wet dog smell. The control bar, on the contrary, presented the highest scores for puffed rice and sweet taste and glucose syrup and cereal smells. Talking about the textural properties the bars with spirulina presented a little bit higher chewiness and springiness than the control ones. The control bar was higher in cohesiveness. *Chlorella* samples again were located in the middle, with higher hardness and resilience and, especially in the case of HC, also higher adhesiveness. These results are in line with the previously discussed results. However, PCA is just a graphical representation of the interactions so, taking into account this, to verify if the differences presented and described are actually significant, the reader should refer to what is reported in Tables 4, 5, A2 and A3.

4. Conclusions

Incorporation of single-cell ingredients from four microalgae species provided significant changes on the physicochemical and sensory characteristics of model energy bars prepared with puffed rice and glucose syrup, but also contribute to improve their nutritional values, enhancing the vitamin B12 and protein contents.

On the other hand, depending on the characteristics of the microalgal ingredient, significant changes in colour parameters were observed in the final products, especially with Spirulina wich gave the bar an intense dark green color, while the *Chlorella* species provide different colourways going from pale yellow, to intense yellow till light green.

In some cases (White and Honey *Chlorella*), the microalgal ingredients significantly influenced the texture of the bars, namely adhesiveness and cohesiveness, springiness, and resilience, even if the overall perceived impact during chewing could be considered limited.

From the organoleptic point of view, the incorporation of microalgae ingredients increased the global smell and taste intensity associated with sea/fishy sensory attributes but also enhanced other positive attributes, like the grass or the candy tastes in the case of Spirulina, which could be interesting for application in commercial sweet products.

One main limitation of the microalgae ingredients is their high cost compared to other sources of functional ingredients, their use in the formulation of energy bars targeted to a specific consumer group is still industrially feasible considering the low percentages of inclusions, as the products developed by the industrial partner in the project ProFuture demonstrated (www. pro-future.eu).

Even if our work was carried out in a simplified model matrix and can be considered preliminary, it clearly indicates that, considering the effects of microalgae inclusion on the sensorial profiles of the final product, an accurate selection of the microalgal ingredients is crucial during the formulation of commercial products since they could negatively affect the global acceptability of the energy bars. In this sense, a future consumer study could provide indications on the product acceptability and consumers' willingness to pay, giving more insights about the potential market for these products.

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CRediT authorship contribution statement

Fabio Fanari: Writing – review & editing, Writing – original draft, Software, Project administration, Data curation. **Josep Comaposada:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Conceptualization. **Fatma Boukid:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Elia Climent:** Writing – review & editing, Investigation. **Anna Claret Coma:** Writing – review & editing, Validation, Methodology, Data curation. **Luis Guerrero:** Writing – review & editing, Validation, Supervision, Software, Methodology, Data curation. **Massimo Castellari:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, 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.

Appendix A

Figs. A1, A2 and Tables A1–A3.

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