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Production of *Arthrospira platensis* BEA 005B: Biomass characterisation and use as a colouring additive in macarons

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ABSTRACT

The present study aimed at (i) producing the microalga *Arthrospira platensis* BEA 005B using 80 m^2 raceway reactors and (ii) to assess the potential use of the produced biomass as a food colour in macarons. The biomass production was carried out during winter and the productivity of the system ranged between 8 and 10 g m^{-2}·day^{-1} depending on the depth of the culture (0.10–0.20 m). Illumina sequencing revealed that *A. platensis* represented over 85% of the prokaryotic populations; other alkaliphile strains were also identified. The produced biomass was mainly composed of protein (57.0 g·100 g^{-1}) and contained natural and valuable pigments including chlorophylls (6.7 mg·100 g^{-1}), carotenoids (1.8 mg·100 g^{-1}), phycocyanins (115.4 mg·100 g^{-1}), and allophycocyanins (36.9 mg·100 g^{-1}). The effect of pH variations and thermal processing (121 °C, 15 min) on the colour of the biomass and on the stability of the main pigments was studied. The produced biomass was used to mimic the colour of two synthetic pigments used in green macarons. The lowest colour difference between the commercial and the microalgae-containing macarons was around 5 at a concentration of 3.5 mg g^{-1}. The pigmentation capacity of the biomass was so high that the small quantities added did not affect the nutritional value of the macarons.

1. Introduction

Microalgae have been suggested by many researchers as one of the key sustainable foods of the future; the main reasons are that they are highly productive, they can grow almost anywhere, they have a low carbon footprint, and their varied composition could fulfil many nutritional gaps of a growing population. The biochemical composition of microalgae is highly variable; however, a hierarchical Bayesian analysis of data compiled from the literature concluded that the average protein, lipid, carbohydrate, and ash content of microalgae is 32.2, 17.3, 15.0, and 17.3 g·100 g^{-1}, respectively (Finkel et al., 2016). Microalgae are also rich in other valuable biomolecules such as carotenoids, phycobiliproteins (cyanobacteria), and chlorophylls that are interesting food ingredients not only because of their nutritional value or bioactive properties but also for their colouring potential.

Spirulina, which is the commercial name given to the strains *Arthrospira platensis* and *Arthrospira maxima* is the most widely produced and consumed microalga. Microalgae are produced in industrial facilities using photobioreactors. The most widely used photobioreactors are raceways, which despite being less productive than other more sophisticated designs such as closed tubular systems, they are easy to operate and build and they are less expensive (Barceló-Villalobos, Guzmán Sánchez, Martín Cara, Sánchez Molina, & Acién Fernández, 2018). The control of raceways is also more challenging than in closed systems; for this reason, they are generally built inside greenhouses to aid in the control of the culture conditions. Spirulina is the most commonly used microalga in the EU for different reasons but mainly because it is seen as a safe and sustainable ingredient by European consumers (Lafarga, Rodríguez-Bermúdez, et al., 2021) and because due to its long history of use it can be consumed without needing to comply with Regulation 2015/2283 known as the Novel Foods Regulation. Moreover, another important characteristic that makes Spirulina stand out is it high protein content, which is higher than that of other protein-rich foods such as legumes and meat.

Spirulina has been evaluated as a novel ingredient in different foods including baked products, beverages and snacks. If the goal of

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incorporating microalgae into foods is to increase their protein content, the intense colour of Spirulina is generally a drawback; for this reason, masking the colour of Spirulina using chocolate (Batista de Oliveira et al., 2021; Santos, Freitas, Moreira, Zanfonato, & Costa, 2016) or other naturally green ingredients such as broccoli (Lafarga, Acién-Fernández, et al., 2019) is a common strategy. The intense colour of Spirulina is not a problem when it is used as a marketing strategy and/or as a colouring additive to either change a foods' natural colour or to colour foods that would otherwise be colourless. The use of natural colourants has the advantage that these compounds generally show antioxidant or other health promoting properties. Consumers are now more aware about the relationship between food and health (and between food and the environment) and the utilisation of Spirulina as a natural colourant is gaining increased attention. Spirulina is one of the most relevant sources of phycocyanin, a photosynthetic pigment with biotechnological potential due to its intense blue colour and high antioxidant, anti-inflammatory, and anticarcinogenic capacities among others (Jaeschke, Teixeira, Ferreira Marczak, & Domeneghini Mercali, 2021). Spirulina is also rich in chlorophylls, which are green pigments with high antioxidant capacity and that can promote the rebalance of the gut microbiota (Queiroz Zepka, Jacob-Lopes, & Roca, 2019).

At the commercial scale, there are several examples of green products coloured using vegetable co-products rich in chlorophylls; for example, spinach. At the scientific level, the use of the whole biomass of Spirulina as a natural colour in beverages to avoid the utilisation of synthetic colourants (e.g. E−104, E−141, E−142, E−150d) is not common. In addition, it is not clear if the amounts required to mimic commercial colours alters the quality of foods beyond their colour. For this reason, the present work aimed at evaluating the potential of the microalga *Arthrospira platensis* BEA 005B as a novel colouring agent in macarons, which are generally pigmented using synthetic food colours. The microalga was produced at a pilot scale using 80 m^2 raceway reactors to ensure the traceability of the biomass. The produced biomass was characterised in terms of biochemical composition and the relative abundance of prokaryotic and eukaryotic populations was determined using illumina sequencing. The pigmentation capacity of the whole biomass was validated using macarons and compared against a commercial product pigmented using E−150d and E−142; the stability of the products was assessed during a 60-day storage period.

2. Materials and methods

2.1. Biomass production

In the present work, the strain used was *Arthrospira platensis* BEA 005B, which was provided by the Spanish Bank of Algae (Spain). The inocula were prepared using 1–10 L bubble columns that were located in a lab and then using 1000 L raceway reactors located outdoors inside a greenhouse. The biomass production was carried out in winter using 80 m^2 raceway reactors described elsewhere (Sánchez-Zurano, Morillas-España, et al., 2021). The biomass production was carried out at the SABANA Demonstration Plant. The reactors were operated at a culture depth of 0.05, 0.10, or 0.15 m and a fixed dilution rate of 0.15 day^{-1}, which was calculated based on the batch phase of production. The semi-continuous production was maintained for 30 days. The culture medium contained 0.9 g L^{-1} $NaNO_3$, 0.14 g L^{-1} KH_2PO_4, 0.18 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.015 g L^{-1} $CaCl_2{\cdot}2H_2O$, 16.8 g L^{-1} $NaHCO_3$, and 0.03 g L^{-1} Karentol (Konegard, Barcelona, Spain); the latter is a commercial mixture of micronutrients (Gómez, Guzmán-Carrasco, Lafarga, & Acién-Fernández, 2021). The algal paste obtained after filtration was washed with water twice to remove the excess of salts. For this, the algal biomass was collected using a 100 μm membrane and water was passed through at a biomass:water ratio of 1:1 (w:w). This process was repeated twice.

The biomass concentration was calculated by dry weight, filtrating 50 mL of culture using 1 μm filters and then drying them in an oven at 80 °C until a constant weight. The chlorophyll fluorescence ratio (F_V/F_m) was assessed using an AquaPen AP 100 fluorometer (Photo System Instruments, The Czech Republic) after 15 min of dark adaptation. The biomass was harvested from the reactors by centrifugation using a SSD 6-06-007 centrifuge (GEA Westfalia Separator, Oelde, Germany) operating continuously at 12,000 rpm. The microalgal paste was frozen, freeze-dried and stored at −20 °C until further use.

2.2. Biomass characterisation

The crude protein, lipid, and ash content of the freeze-dried biomass was measured as described elsewhere (Ciardi et al., 2022). The total carbohydrate content was determined by difference. Phycobiliproteins, namely phycocyanin (C_P), allophycocyanin (C_A), and phycoerythrin (C_E) were estimated using a Genesys 10S UV spectrophotometer (Thermo Fisher Scientific, Spain) as described elsewhere (Rodrigues, de Lima, Santiago-Aguiar, & Rocha, 2019) using the equations:

$$C_P\ \left(g\cdot L^{-1}\right)=\frac{A_{615}-0.474{\cdot}A_{652}}{5.34}$$

$$C_A\ \left(g\cdot L^{-1}\right)=\frac{A_{652}-0.208{\cdot}A_{615}}{5.09}$$

$$C_E\ \left(g\cdot L^{-1}\right)=\frac{A_{562}-2.41{\cdot}C_P{\cdot}A_{615}-0.849{\cdot}C_A}{9.62}$$

where A_{615}, A_{652}, and A_{562} are the optical densities measured at 615, 652, or 562 nm, respectively. In addition, the total carotenoid content (*TCC*) and the concentration of chlorophylls (Chl_a and Chl_b) was calculated as described elsewhere (Ciardi et al., 2022) using a Genesys 10S UV spectrophotometer (Thermo Fisher Scientific, Spain) and the equations:

$$Chl_a\left(mg\cdot L^{-1}\right)=16.72\cdot A_{665}-9.16{\cdot}A_{652}$$

$$Chl_b\left(mg\cdot L^{-1}\right)=34.09\cdot A_{652}-15.28{\cdot}A_{665}$$

$$TCC\left(mg\cdot L^{-1}\right)=\frac{(1000{\cdot}A_{470}-1.63{\cdot}Chl_a-104.9{\cdot}Chl_b)}{221}$$

where A_{665}, A_{652}, and A_{470} are the optical densities at 665, 652, or 470 nm, respectively.

Finally, the DNA of 100 mg of the freeze-dried biomass was extracted using the DNeasy Plant® kit (Qiagen, Hilden, Germany) and illumina sequencing was performed for prokaryotic and eukaryotic communities at STAB VIDA (Lisbon, Portugal) as described elsewhere (Morillas-España et al., 2021). The purity and yield of the DNA was assessed using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Milan, Italy) as well as the eventual fragmentation, which was determined by gel electrophoresis using 1% (w/v) TAE agarose gels (Bio-Rad Laboratories, Madrid, Spain).

2.3. Effect of temperature and pH on the colour of the biomass

To assess the effect of pH and temperature on the colour of the biomass, the freeze-dried biomass was suspended in 250 mL of distilled water at a concentration of 10 g L^{-1}. The suspension was then homogenised using a UP400S ultrasonic processor (Hielscher Ultrasonics GmbH, Germany) operating at 400 W and 24 kHz for 30 s. The pH of the solutions was adjusted to 1–10 using either 1M NaOH or HCl and was kept constant during the measurements. The solutions were then pasteurised at 121 °C during 15 min using a 4004371 JP Selecta autoclave (JP Selecta, Barcelona, Spain). Colour recordings were taken before and after the thermal treatment using a Minolta CR-400 chroma meter (Minolta Inc., Tokyo, Japan) and the D65 illuminant, which approximates to daylight. Colour determinations were taken in triplicate per natural replicate.

2.4. Formulation of macarons

Test macarons were prepared following a traditional French recipe as described in a previous work (Albuquerque, Pinela, Barros, Oliveira, & Ferreira, 2020). The macarons contained 28% almond flour, 28% powdered sugar, 23% egg whites, and 23% sugar. The doughs were baked for 13 min at 130 °C using a Rational SCC WE-101 convection oven (Rational AG, Landsberg am Lech, Germany) and left to chill at room temperature (20 °C) for 1 h. After this period, the macarons were stored in plastic bags at 4 or 25 °C and protected from the light.

Commercial green macarons were purchased from a local retailer. According to the manufacturer, the colour of the macarons was achieved using a combination of E−150d and E−142, both commonly used in the food industry. To mimic the colour of the commercial additives E−150d and E−142, small amounts of microalgal biomass were added to the macaron recipe before mixing. The colour difference between two samples (ΔE^*) was calculated using the equation:

$$\Delta E^* = \sqrt{\left(L_A^* - L_B^*\right)^2 + \left(a_A^* - a_B^*\right)^2 + \left(b_A^* - b_B^*\right)^2}$$

where L_A^*, a_A^*, and b_A^* are the colour parameters of one of the samples (i.e. the microalgae-containing macarons) and L_B^*, a_B^*, and b_B^* are the colour parameters of the other one (i.e. commercial green macarons).

2.4.1. Assessment of antioxidant capacity

The antioxidant capacity of the macarons was assessed using the ferric reducing antioxidant power (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) scavenging activity assays following previously described methods (Nicolau-Lapeña et al., 2019) with a minor modification. In the present study, the extract was obtained using 100 mg of freeze-dried macarons and 5 mL of a mixture of methanol:water (70:30, v/v). The extraction of the antioxidant compounds was carried out by stirring at 350 rpm for 30 min at 21 ± 1 °C. The preparation of the reagents and the determination of the antioxidant capacity was carried out as described in the above-mentioned work.

2.5. Statistical analysis

The normality and homoscedasticity of the data within each group were checked. Each photobioreactor was considered an experimental unit. The data were analysed using either a *t*-test (to assess differences between the means of two variables) or an ANOVA (to assess differences between the means of more than two variables). A Tukeys' HSD test was carried out to find where the sample differences occurred and the criterion for statistical significance was $p < 0.05$. The analyses were done with Statgraphics 18 (Statgraphics Technologies Inc., USA). Results are shown as mean values ± standard deviations (SD).

3. Results and discussion

3.1. Biomass production

Not only operational but also environmental conditions have an important effect on the production of microalgal biomass. Environmental data are shown in Fig. 1. Overall, the maximum solar radiation that reached the culture was on average 1517 ± 97 μmol $m^{-2}{\cdot}s^{-1}$. The daily average solar radiation that reached the culture, considering only the sunlight hours was 418 ± 58 μmol $m^{-2}{\cdot}s^{-1}$. It is important to highlight that the biomass production was carried out in winter, when the average, maximum and minimum daily temperature values were 13.1 ± 0.6, 17.4 ± 0.7, and 8.2 ± 0.8 °C. These values were measured using lightning detectors and temperature probes that are located at the SABANA Demonstration Plant and are available at http://www2.ual.es/sabana/data-center-2/. The biomass productivity achieved when operating at the different dilution rates is shown in Fig. 1. Overall, the depth of the culture had a significant effect on the biomass productivity

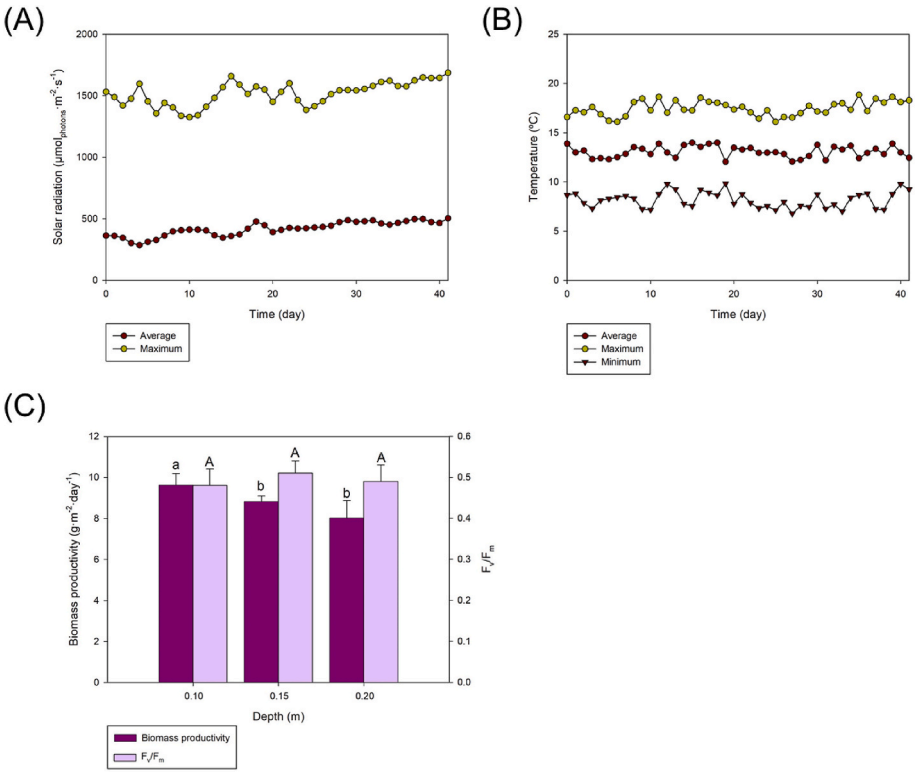

Fig. 1. (A) Solar radiation and (B) temperature during biomass production. The maximum and minimum values represent the maximum and minimum daily value. In the case of solar radiation, the average value represent the average during the sunlight hours. (C) Biomass productivity and (D) F_v/F_m values. Values represent means ± SD. Different letters indicate significant differences ($p < 0.05$).

($p < 0.05$), being higher at a depth of 0.10 m, which was the lowest studied. These results are in line with previous works where a negative correlation was observed between the depth of the culture and the biomass productivity (Sánchez-Zurano, Morillas-España, et al., 2021). The main reason for this is that lower depths allow to maximise light availability inside the culture because of the self-shading effect of microalgae. Indeed, thin-layer cascade photobioreactors that operate at culture depths lower than 0.05 m are highly productive because they allow maximising light availability (Apel et al., 2017). A second advantage of operating at a lower depth is that the working volume of the system was lower and therefore, the amount of culture that was daily harvested was smaller. This facilitates harvesting and processing of the biomass by reducing the size (and costs) of the equipment needed. The biomass productivity of the system was low when compared to that of other microalgal strains such as *Scenedesmus almeriensis* that allowed a biomass productivity in winter of 10–15 g m^{-2}·day^{-1} when working at dilution rates of 0.2 or 0.3 day^{-1} (Morillas-España, Lafarga, Gómez-Serrano, Acién-Fernández, & González-López, 2020). The main reason for the observed lower productivity is that the microalga produced in the present work is *A. platensis*, which is not naturally adapted to winter conditions. Indeed, a previous work reported that the metabolic activity of different *Arthrospira* strains decreased significantly at temperatures below 17 °C (De Oliveira, Monteiro, Robbs, & Leite, 1999). In a more recent work, carried out in different regions of Brazil, the authors demonstrated that the productivity of *Arthrospira* sp. LEB-18 in the south of the country, where the temperature was lower, was much lower when compared to the north, where the climatic conditions were more suitable for the production of spirulina (de Jesus et al., 2018). Finally, the Fv/Fm value at the different culture depths is shown in Fig. 1. No differences were observed between the different experimental runs and the value was in all cases around 0.5, which is the optimal for this strain. This suggests that the cells were not subjected to a stress condition and that all the studied conditions were suitable for biomass production.

3.2. Biomass characterisation

The composition of the produced biomass is shown in Table 1. The biomass was mainly composed of protein (57.0 g·100 g^{-1}) and carbohydrates (26.5 g·100 g^{-1}), which is comparable to that of commercial spirulina powders. The high protein content was expected as spirulina is mainly known and used for its high protein content (Lafarga, Rodríguez-Bermúdez et al., 2021). The present work compared the composition of the biomass produced in this work and a commercial spirulina powder produced in China (unknown conditions, photobioreactor design, and processing methods). Overall, despite the slightly lower protein content of the commercial product ($p < 0.05$), the composition of both powders was comparable and in line with that of commercial spirulina powders available at the USDA FoodData Central database available at https://fdc.nal.usda.gov/index.html. The lipid and ash content were in both cases around 5 and 10%, respectively.

Spirulina can be used not only as a source of protein but as a source of pigments. It is known that spirulina is a rich source of phycobiliproteins (Martínez, Luengo, Saldaña, Álvarez, & Raso, 2017). A recent study demonstrated the potential of using phycobiliproteins extracted from *A. platensis* to colour isotonic drinks and tonic waters (García, Longo, & Bermejo, 2021). In the present study, the produced biomass was rich in different pigments including chlorophylls, carotenoids, and phycobiliproteins. Phycobiliproteins are proteins with linear tetrapyrrole prosthetic groups that absorb light and are involved in photosynthesis. These molecules are especially interesting not only because the limited number of natural blue colour dyes but also because of their antioxidant, anti-inflammatory, or anti-carcinogenic properties (Jaeschke, Rocha Teixeira, Damasceno Ferreira Marczak, & Domeneghini Mercali, 2021). The phycobiliprotein content of the biomass was higher when compared to that of the commercial spirulina, probably because the commercial one was spray-dried and the one used in this work was dried by lyophilisation.

Table 1
Composition of the produced biomass.

Compound	*A. platensis* BEA 005B	Commercial spirulina
Proteins (g·100 g^{-1})	57.0 ± 2.3 [a]	53.3 ± 1.3 [b]
Lipids (g·100 g^{-1})	6.0 ± 0.7 [a]	4.4 ± 0.6 [a]
Ashes (g·100 g^{-1})	11.6 ± 0.5 [a]	10.5 ± 0.7 [a]
Carbohydrates (g·100 g^{-1})	26.5 ± 2.3 [b]	30.7 ± 2.4 [a]
Total carotenoids (mg·100 g^{-1})	1.78 ± 0.02 [a]	0.42 ± 0.01 [b]
Total chlorophylls (mg·100 g^{-1})	6.67 ± 0.01 [b]	9.63 ± 0.00 [a]
Phycocyanin (mg·100 g^{-1})	115.37 ± 0.35 [a]	90.59 ± 0.47 [b]
Allophycocyanin (mg·100 g^{-1})	36.95 ± 0.29 [a]	28.00 ± 0.34 [b]

Different letters in the same line indicate significant differences between *A. platensis* BEA 005B and a commercial powdered spirulina ($p < 0.05$).

The microbial composition of the biomass is shown in Fig. 2. Overall, despite being produced in an open system, *A. platensis* managed to be the predominant strain in the culture. The relative abundance of *Arthrospira* strains was higher than 85%, which compares favourably with previous works that identified a large variety of prokaryotes in microalgal cultures. For example, in *Scenedesmus* cultures the most abundant prokaryotic phyla were Proteobacteria followed by Bacteroidetes independently of the operational conditions studied (Sánchez-Zurano, Lafarga, et al., 2021). Moreover, in a different study, the relative abundance of the phylum cyanobacteria ranged between 55 and 60% in cultures of *Anabaena* sp. And *Dolichospermum* sp. (Morillas-España et al., 2021). The higher diversity of microorganisms in these two works when compared to the present study can be attributed to two main factors. In the first place, that the above-mentioned studies were carried out using waste streams as the nutrient sources and their microbial load was very high before microalgae inoculation. In the second place, because *A. platensis* is an alkaliphile (extremophile) strain, which means that it grows at alkaline conditions that avoid or minimise the growth of non-alkaliphile microorganisms (Lafarga, Rodríguez-Bermúdez, et al., 2021). Indeed, the second most abundant prokaryote was a halophilic bacteria belonging to the genus *CK06-06-Mud-MAS4B-21* (2.5 ± 0.8%), previously identified in fracture waters outflowing from Canadian boreholes (Wilpiszeski, Lollar, Warr, & House, 2020), and the third most abundant prokaryotic microorganism was the extremophile *Alkalimonas* sp. (1.6 ± 0.4), also identified in saline-alkali soil lands (Yadav et al., 2021). Not only prokaryotes but also eukaryotes were identified in the biomass. The two most abundant eukaryotes were an unclassified metazoan (87.4 ± 1.5) followed by *Brachionus calyciflorus* (2.9 ± 0.5%). The latter is a freshwater planktonic rotifer previously reported to ingest the microalgae *Scenedesmus obliquus* (Cheng et al., 2021) and *Chlorella kesslery* (Pradeep et al., 2015). This is, to the best of the authors' knowledge, the first time that this algal predator was identified in a culture of *A. platensis*. Other microalgae including *Tetradesmus obliquus* (0.5 ± 0.0%) and *Euplotes antarcticus* (0.6 ± 0.1%) were also identified in the culture. Previous studied revealed that microalgae and other microorganisms can be transported in aerosols (Wiśniewska, Lewandowska, & Śliwińska-Wilczewska, 2019). This could explain the presence of *T. obliquus* in the culture as a thin-layer cascade photobioreactor located inside the same greenhouse was being used to produce *Tetradesmus* sp. During the production of *A. platensis*. This phenomenon was also observed in previous works, where *Chlorella* strains passed from one reactor to another (Morillas-España et al., 2021).

Overall, the produced biomass did not contain any known human pathogen and the cyanobacterium *A. platensis*, the inoculated strain, managed to be the predominant strain in the culture after 30 days of semi-continuous production in an open system. The biochemical composition of the produced biomass suggested that it was suitable for being used as a protein-rich food ingredient or as a blue/green food colourant (because of the presence of chlorophylls and phycocyanins).

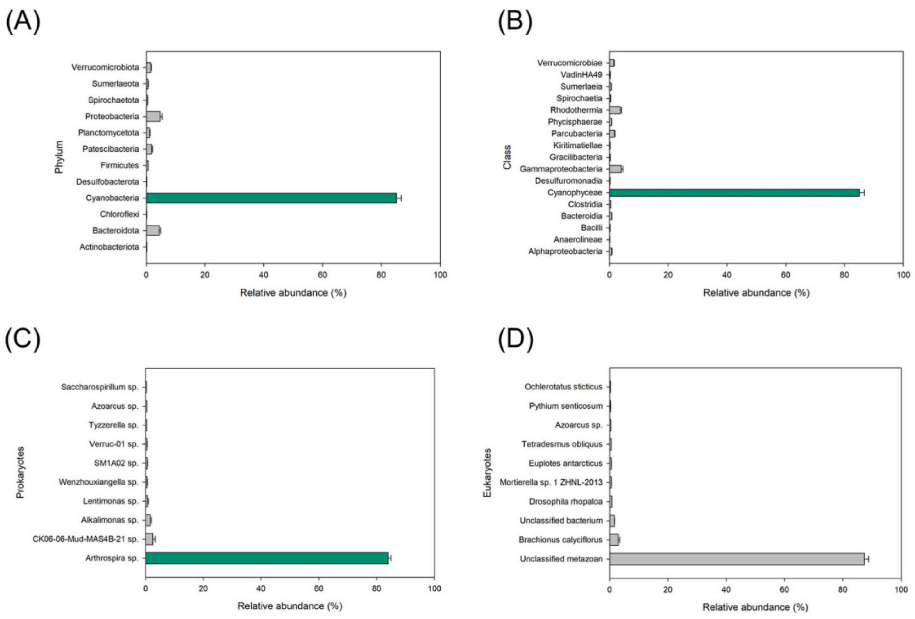

Fig. 2. Prokaryotic diversity at (A) phylum and (B) class level. Only those phyla and classes with a relative abundance higher than 0.1% are shown. Top 10 most abundant (C) prokaryotes and (D) eukaryotes. Values represent the average of three independent determinations ± SD.

3.3. Stability of microalgal pigments

The produced biomass was used to colour macarons with the goal of mimicking the green colour of a commercial product. The use of microalgae-derived extracts as colouring agents in beverages is not new; for example, phycocyanins derived from *A. platensis* have been recently used to colour isotonic drinks, tonic waters, and wines (García, Longo, & Bermejo, 2021) and phycoerythrins derived from *Porphyridium cruentum* were used to simulate the colour of synthetic additives in different dairy products (García, Longo, Murillo, & Bermejo, 2021). One of the main advantages of using the whole biomass is that the need for expensive protein purification and separation steps used in the above-mentioned works are avoided, maximising the economic viability of the process. Moreover, a second main advantage is avoiding the use of the additives E−150d and E−142. The former is known as "caramel dye" and contains 4-methylimidazole, a cytotoxic molecule that is obtained via chemical synthesis, although it can form naturally during the caramelisation of carbohydrates (Maillard reaction). A recent study concluded that E−150d reduced the survival and induced alterations of muscle tissue in a zebrafish model and induced teratogenic effects and reduced their swimming performance with a dose dependent manner (Capriello, Visone, Motta, & Ferrandino, 2021). The latter, E−142, is known as "green S" and has been associated with the presence of carcinogenic residues and to negative health effects when consumed in very high doses (Silva, Reboredo, & Lidon, 2022). It is important to highlight that these additives, at the concentrations used, were approved by the FDA and the European Food Safety Authority and are therefore safe. However, consumers prefer natural additives, and the current trend is to avoid the utilisation of non-renewable or synthetic colourants if a natural replacement is available (Carocho, Morales, & Ferreira, 2015).

Initially, the colour of the produced biomass was assessed at different pH values; results are shown in Table 2. Overall, the colour of the biomass was highly influenced by the pH of the solution ($p < 0.05$). Results suggested that the stability of the pigments was affected by the pH variations. The L* value, which represents the lightness of the sample was the highest at pH 3.0 and decreased significantly at lower and especially higher pH values. For example, the L^* value at pH 14.0 was 5.6. In turn, a^* and b^* express the four colours of human vision green/red and yellow/blue, respectively. The lowest a^* value, which expresses a greener hue, was observed at pH 3.0. The green colour of microalgae is due to the presence of chlorophylls (Table 1), which are highly sensitive to pH variations. Indeed, the conversion of chlorophylls to pheophytin and pheophorbide by the influence of pH is one of the major causes of the green decolouration of vegetables during processing (Andrés-Bello, Barreto-Palacios, García-Segovia, Mir-Bel, & Martínez-Monzó, 2013). This reaction turns the green colour of vegetables or algae from a bright green to a dull olive green or brown. No major changes in the blue hue of

Table 2
Effect of temperature and pH on the colour attributes of *A. platensis*.

	L*		a*		b*	
pH	Fresh	Sterilised[a]	Fresh	Sterilised[a]	Fresh	Sterilised[a]
1.0	14.1 ± 0.2 ^{dA}	9.1 ± 0.1 ^{gB}	−6.0 ± 0.1 ^{gB}	−3.2 ± 0.1 ^{fA}	1.4 ± 0.0 ^{gA}	0.5 ± 0.2 ^{jB}
2.0	14.1 ± 0.1 ^{dA}	12.1 ± 0.3 ^{eB}	−7.0 ± 0.1 ^{hB}	−2.7 ± 0.0 ^{eA}	2.6 ± 0.1 ^{dA}	2.2 ± 0.3 ^{fgA}
3.0	20.7 ± 0.1 ^{aB}	23.3 ± 0.2 ^{cA}	−13.3 ± 0.1 ^{kB}	−3.6 ± 0.1 ^{gA}	2.4 ± 0.1 ^{aB}	9.1 ± 0.1 ^{cA}
4.0	19.8 ± 0.2 ^{bB}	25.0 ± 0.6 ^{aA}	−16.7 ± 0.2 ^{lB}	−3.9 ± 0.1 ^{hA}	2.6 ± 0.1 ^{cdB}	10.7 ± 0.1 ^{aA}
5.0	17.5 ± 0.2 ^{cB}	24.5 ± 0.2 ^{bA}	−13.3 ± 0.1 ^{jB}	−4.6 ± 0.1 ^{iA}	2.4 ± 0.2 ^{dB}	9.3 ± 0.1 ^{bA}
6.0	12.9 ± 0.1 ^{eB}	20.3 ± 0.2 ^{dA}	−7.3 ± 0.1 ^{iB}	−4.6 ± 0.1 ^{iA}	3.4 ± 0.0 ^{bB}	7.5 ± 0.1 ^{dA}
7.0	10.9 ± 0.1 ^{fA}	10.3 ± 0.1 ^{fB}	−3.6 ± 0.1 ^{bB}	−3.1 ± 0.0 ^{fA}	4.1 ± 0.3 ^{aA}	3.5 ± 0.1 ^{eB}
8.0	10.7 ± 0.1 ^{fgA}	8.5 ± 0.2 ^{hB}	−3.6 ± 0.1 ^{bB}	−2.4 ± 0.1 ^{dA}	4.1 ± 0.1 ^{aA}	2.4 ± 0.1 ^{fB}
9.0	10.4 ± 0.3 ^{gA}	8.0 ± 0.1 ^{iB}	−3.9 ± 0.1 ^{cB}	−2.0 ± 0.1 ^{cA}	2.9 ± 0.2 ^{cA}	2.1 ± 0.1 ^{gB}
10.0	7.9 ± 0.1 ^{hA}	6.4 ± 0.2 ^{kB}	−5.6 ± 0.1 ^{fB}	−2.0 ± 0.1 ^{cA}	1.5 ± 0.2 ^{fgA}	1.7 ± 0.1 ^{hA}
11.0	6.4 ± 0.1 ^{iA}	5.2 ± 0.2 ^{lB}	−5.0 ± 0.0 ^{eB}	−2.3 ± 0.1 ^{dA}	1.7 ± 0.1 ^{efA}	1.3 ± 0.3 ^{iA}
12.0	5.9 ± 0.2 ^{jB}	6.6 ± 0.2 ^{kA}	−4.5 ± 0.2 ^{dB}	−1.0 ± 0.2 ^{bA}	1.8 ± 0.2 ^{eA}	0.1 ± 0.1 ^{kB}
13.0	5.0 ± 0.2 ^{lB}	8.0 ± 0.1 ^{iA}	−3.5 ± 0.0 ^{bB}	2.2 ± 0.1 ^{aA}	1.7 ± 0.1 ^{efA}	−1.4 ± 0.1 ^{lB}
14.0	5.6 ± 0.5 ^{kB}	7.4 ± 0.2^{jA}	−3.0 ± 0.1 ^{aB}	2.2 ± 0.1 ^{aA}	1.3 ± 0.3 ^{gA}	−1.4 ± 0.1 ^{lB}

Different lower case letters indicate significant differences between pH values ($p < 0.05$). Different capital letters indicate significant differences between fresh and pasteurised samples ($p < 0.05$).

[a] Thermally treated at 121 °C for 15 min.

the suspensions were observed as an effect of pH, with values ranging between 1 and 5 for all the pH values. The blue hue of spirulina is caused by allophycocyanin and (mainly) phycocyanin. The stability of these pigments against pH variations is shown in Fig. 3. Overall, the concentration of phycobiliproteins and chlorophylls was highly influenced by the pH variations ($p < 0.05$). Phycocyanins were more stable at pH values within 6.0 and 8.0 while allophycocyanins showed an overall higher stability to pH variations, especially at pH values above 6.0. These values correlate well with those shown in Table 2, as the highest a^* values, which indicate a more intense blue hue, were obtained at pH values of 7.0 and 8.0. The degradation of chlorophylls as an effect of the pH variations was not as intense as in the case of phycocyanins; however, a lower concentration of chlorophylls was observed especially at lower pH values. The degradation of the green colour in spirulina due to pH variations was comparable to that reported in previous works using green vegetables. For example, the stability of the chlorophylls found in green peas was found to be higher at a pH value of 7.5 and the green colour loss was suggested to be higher at lower pH conditions (Koca, Karadeniz, & Burdurlu, 2007).

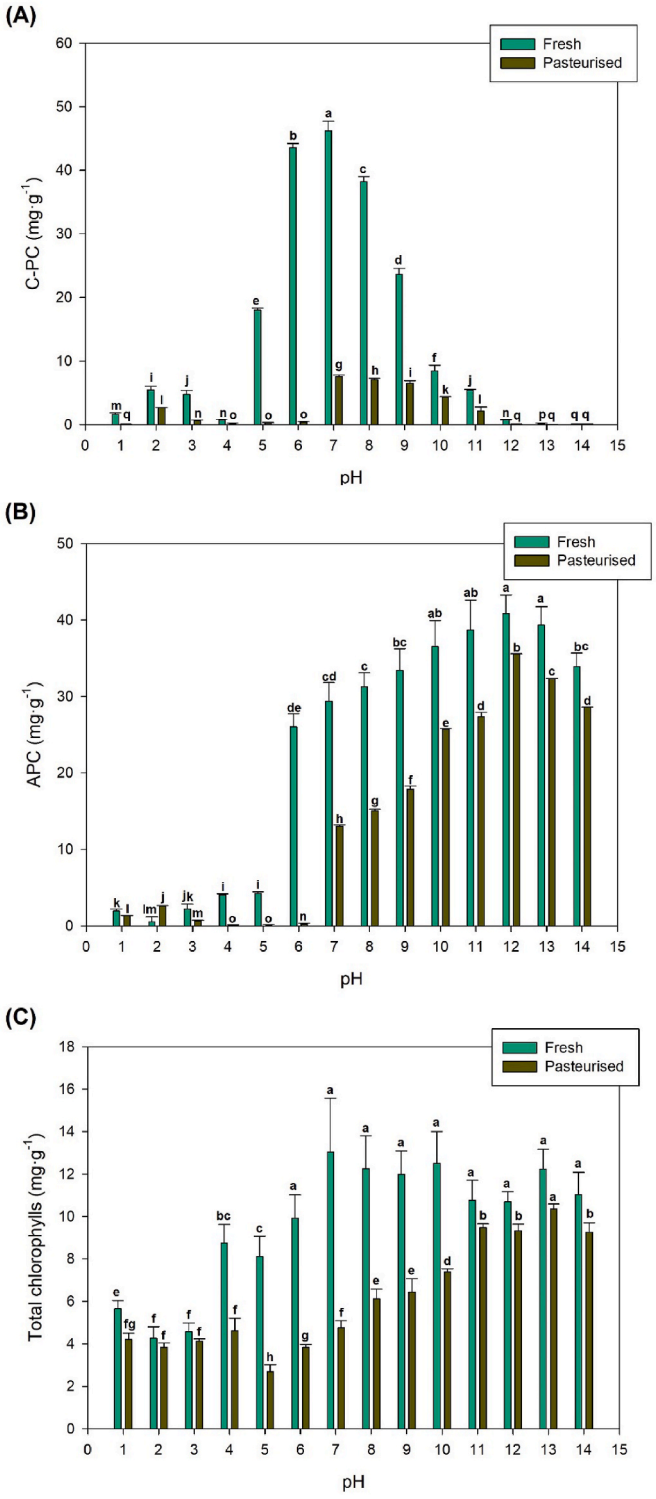

Fig. 3. Stability of (A) phycocyanin, (B) allophycocyanin, and (C) chlorophylls. Values represent the average of three independent determinations ± SD. Different letters indicate significant differences ($p < 0.05$).

The effect of thermal processing (121 °C, 15 min) on the colour of the biomass is shown in Table 2. Overall, the thermal process significantly affected the colour of the microalgal extracts ($p < 0.05$). These colour changes were attributed to the degradation of the main pigments present in the biomass (Fig. 3). The results showed that phycocyanins were almost completely degraded after the thermal processing with a decrease in the phycocyanin content higher than 80% at all the pH values studied. Similarly, the concentration of allophycocyanins and chlorophylls was also significantly reduced. The observed reduction in the concentration of allophycocyanin and chlorophylls was higher at pH values between 5.0 and 9.0. Heat treatments also generate the formation of pheophytin and the denaturation of phycobiliproteins. The stability of phycobiliproteins during the processing of foods has been revised recently (Nowruzi, Konur, & Anvar, 2022); overall, the studies reviewed in that work agree with the data reported in this study.

3.4. Pigmentation capacity of the algal biomass

The biomass of *A. platensis* BEA 005B was assessed as a potential food colourant aiming at mimicking the colour of a commercial macarons. The selection of the food matrix was made because of four main reasons. In the first place, because of the pH of the matrix, which is between 7 and 8 where the pigments identified were more stable. In addition, because the thermal process is not as intense as in other baked products such as bread and also because commercial green macarons are commonly pigmented using synthetic pigments. In this case, according to the products label, the pigments used were E−150d and E−142. Another reason for selecting macarons was their high sugar content, which could contribute to the stability of the colour. Previous works revealed that monosaccharides could be used to stabilise phycobiliproteins during thermal processes. Indeed, a recent study revealed that glucose enhanced the half-life of phycocyanin from 2.09 to 5.37 h when heated at 85 °C (Sharma et al., 2021). In that same study, and under the same conditions, the half-life of allophycocyanins was increased from 4.9 to 13.5 h by means of adding glucose. Similar protective effects of sugars were reported in a different work. In this case, the addition of sugars protected the stability of phycobiliproteins against high pressures (600 MPa, 300 s); the antioxidant capacity of these pigments was also improved in the sugar-containing samples (Faieta, Neri, di Michele, di Mattia, & Pittia, 2021).

Fig. 4 shows the effect of adding small quantities of spirulina on the colour of the macarons; ΔE refers to the colour difference between the microalgae-containing macarons and the commercial macarons pigmented using E−150d and E−142. The ΔE value is minimal at a concentration of 3.5 mg g^{-1}, which means that adding 3.5 mg of biomass to 1.0 g of batter would allow getting as close as possible to the commercial colour. In this sense, with 1.0 kg of microalgae it would be possible to pigment approximately 286 kg of macarons using a natural ingredient that is trendy and gaining increased acceptance by consumers (Lafarga, Rodríguez-Bermúdez et al., 2021). The colour was not exactly the same although it was very similar; a ΔE value of around 5 suggests that the colour difference was visible to a human eye. Previous works evaluated the pigmentation capacity of microalgal extracts obtaining similar results, that is products that were not the same colour than the commercial

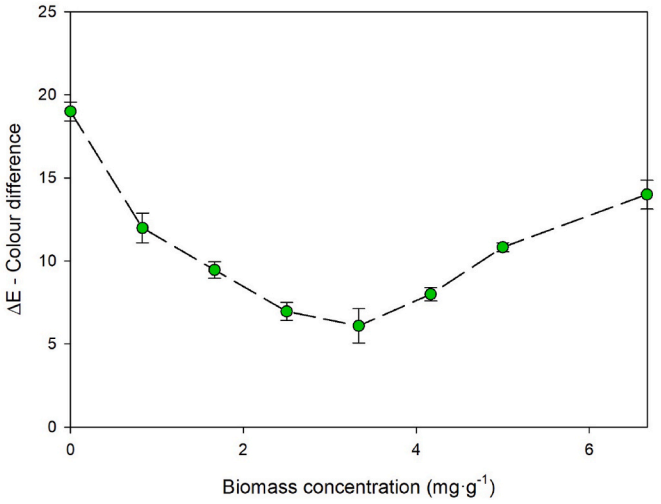

Fig. 4. Colouring curve for the biomass of *Arthrospira platensis*. The colour difference refers to the difference in the colour of the commercial and the microalgae-containing macarons. Values represent the average of three independent determinations ± SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

ones, but with colours that could be commercialised with a high acceptance (Carmona, Murillo, Lafarga, & Bermejo, 2022; García, Longo, & Bermejo, 2021). Pigments including chlorophylls and phycobiliproteins present in the biomass of *A. platensis* BEA005B are interesting for the food industry not only because of their natural nature but also because of their potential health-promoting properties. One of the most reported bioactivities of microalgal extracts, that contain the above-mentioned pigments and carotenoids and phenolic compounds, are their antioxidant capacities (Coulombier, Jauffrais, & Lebouvier, 2021). In the present work, the antioxidant capacity of the macarons containing a biomass concentration of 3.5 mg g^{-1} was compared against the control macarons. The antioxidant capacity of the macarons was 12.2 ± 0.4 and 7.8 ± 0.7 mg·100 g^{-1} when assessed using the FRAP and DPPH method, respectively. This value is very low when compared to, for example, fruits and vegetables (Lafarga, Ruiz-Aguirre, et al., 2019). Overall, no differences were observed in the antioxidant capacity of the macarons produced using microalgae and synthetic colourants, which suggests that the small quantity used to colour the product was not enough to improve its antioxidant activity.

The optimal biomass concentration to achieve a colour comparable to that of the commercial macarons was validated; the production of the macarons was repeated and their colour is shown in Table 3. The present study also evaluated the stability of the colour, as microalgal pigments (and other natural pigments) have a lower stability when compared to that of their synthetic counterparts. As highlighted previously, the stability of natural pigments can be improved by selecting a suitable food matrix (i.e. mild pH and temperature variations). However, the pigment degradation might continue during storage (Sharma et al., 2021) and, for this reason, the colour of the microalgae-containing macarons was evaluated during a 60-day storage period. Results, shown in Table 3, demonstrated that no colour changes were observed during the 60 days of analysis independently of the storage temperature (4 or 25 °C).

4. Conclusions

The microalga *A. platensis* BEA 005B shows potential for production and use in the food industry based on the biomass productivity achieved and in the nutritional value of the produced biomass. Illumina sequencing revealed that most of the produced biomass was spirulina. Algal predators were identified in the culture. Their presence does not represent a hazard to human health, although the biomass productivity could be affected during a longer production period. Further studies are needed to assess their evolution and their effect on the culture as well as to identify strategies for their control. The produced biomass was rich in proteins and pigments, which could be used to mimic the colour of synthetic colourants. The colour of products was stable after a 60-day storage period at room temperature. Finally, the present work demonstrated that the quantities used to mimic the colour of commercial products might not be enough to increase the antioxidant capacity of food products.

Table 3
Colour attributes of the microalgae-containing macarons.

	L^*	a^*	b^*	ΔE^*
Microalgae (day 0)	78.2 ± 0.5 ^{a}	−3.2 ± 0.8 ^{a}	11.2 ± 0.8 ^{a}	–
Microalgae (day 15; 4 °C)	79.6 ± 1.5 ^{a}	−3.2 ± 0.8 ^{a}	11.0 ± 1.0 ^{a}	1.7 ± 1.0
Microalgae (day 30; 4 °C)	79.1 ± 0.8 ^{a}	−3.3 ± 0.5 ^{a}	10.4 ± 0.6 ^{a}	0.7 ± 0.3
Microalgae (day 60; 4 °C)	78.2 ± 1.9 ^{a}	−2.8 ± 0.9 ^{a}	11.1 ± 0.6 ^{a}	2.1 ± 1.0
Microalgae (day 15; 20 °C)	78.9 ± 0.8 ^{a}	−3.4 ± 0.7 ^{a}	10.6 ± 0.7 ^{a}	1.4 ± 0.3
Microalgae (day 30; 20 °C)	78.2 ± 1.1 ^{a}	−3.4 ± 1.0 ^{a}	10.3 ± 0.5 ^{a}	1.5 ± 0.1
Microalgae (day 60; 20 °C)	77.7 ± 1.6 ^{a}	−3.8 ± 0.8 ^{a}	10.9 ± 0.3 ^{a}	1.9 ± 1.0

The colour difference refers to the difference between the microalgae-coloured macarons at day 0 and at days 15, 30, or 60.
Different letters in the same column indicate significant differences ($p < 0.05$).

CRediT authorship contribution statement

Silvia Villaró: Investigation, Formal analysis, Writing – original draft. **Gabriel Acién:** Supervision, Funding acquisition, Project administration. **Cynthia Victoria González-López:** Supervision, Funding acquisition. **Elisa Clagnan:** Formal analysis. **Tomás Lafarga:** Supervision, Funding acquisition, Project administration.

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.

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