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Stabilization of *Arthrospira platensis* with high-pressure processing and thermal treatments: Effect on physico-chemical and microbiological quality

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1 **Stabilisation of *Arthrospira platensis* with high-pressure processing and thermal treatments: effect on physico-chemical**  
2 **and microbiological quality**

3

4 **Running title:** Thermal, non-thermal treatment on Spirulina

5

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10

11

12 **Abstract**

13 *Arthrospira platensis* (*Spirulina*) is a cyanobacterium that has been recently studied for food applications due to its high  
14 biological and nutritional value. When *A. platensis* is used as ingredient in food applications, proper treatments have to be  
15 applied in order to reduce microbial contamination. This work compared the effect of thermal treatments (sterilization at  
16 121°C and pasteurization at 90°C) and high-pressure processing (400, 600 MPa) on the chemical, physico-chemical and  
17 microbial quality of 5% (w/v) *A. platensis* aqueous suspensions. Total antioxidant capacity, total polyphenols content, colour  
18 and pigments content were not strongly lowered/modified by the HPP treatments. HPP at 400 MPa even improved the release  
19 of C-phycoerythrin from the biomass because of the breakage of cell walls. HPP treatments were comparable to pasteurization  
20 in reducing Yeasts, Coliforms, Staphylococci and total bacterial count. Conversely, sterilization was the only treatment that  
21 guaranteed the inactivation of spore-forming species but affecting the final quality.

22

23

24 **Keywords:** non-thermal processing; emerging technologies; high pressure processing; *Spirulina*; C-phycoerythrin

25

26 **Novelty Impact Statement** HPP treatments were found to be a good strategy to preserve or even improve some physical and  
27 chemical properties of *Arthrospira platensis* (*Spirulina*), in particular antioxidant capacity, polyphenols, colour and pigments  
28 content. Furthermore, HPP treatments were comparable to pasteurization in reducing microbial cell count, while sterilization  
29 was the only treatment able to ensure the inhibition of spore-forming species.

30

31

32 **1. Introduction**

33 *Arthrospira platensis*, a cyanobacterium commonly known as *Spirulina*, is widely cultivated and studied because of its  
34 richness in bioactive compounds with high nutritional value, that can be used in several food formulations (Niccolai, Chini  
35 Zittelli, et al., 2019; Golmakani et al., 2019; Batista et al., 2017; Barkallah et al., 2017).

36 It can be commercialized dehydrated as a food integrator, or as an ingredient for food formulations or, as recently reported,  
37 as a booster in the fermentation process or as natural antimicrobial (Martelli, Alinovi, Bernini et al., 2020; Zheng et al., 2020,

38 [Bancalari et al., 2020](#)). Moreover, several bioactive or functional compounds can be extracted from *A. platensis* biomass and  
39 used as ingredients ([Martelli, Cirlini, Lazzi et al., 2020](#); [Bancalari et al., 2021](#))  
40 *A. platensis* is commonly cultivated in raceway ponds that is cheaper than cultivations in photobioreactors ([Richardson et al.,](#)  
41 [2012](#)) but one of its major drawbacks is the high level of microbial contamination. In fact, alterative and spoilage bacteria are  
42 reported as contaminants in these cultivations and their presence could lead to safety issues for consumers ([Wang et al., 2013](#)).  
43 For this reason, microalgal biomass cannot be marketed fresh but needs to be dehydrated in order to reduce its water activity  
44 ( $a_w$ ) and avoid the potential bacterial proliferation. Otherwise, thermal or non-thermal treatments can be applied to inactivate  
45 alterative and foodborne pathogens, but, on the other hand they may cause an unwanted loss of nutritional and functional  
46 compounds ([Martelli et al., 2014](#)). One of the most promising emerging alternatives to thermal treatment is high pressure  
47 processing (HPP), that can reduce the microbial load and stabilize food products, avoiding losses of nutritional and functional  
48 compounds ([Paciulli et al., 2019](#)). Furthermore, high pressure processing could also improve the extraction of bioactive  
49 compounds by disrupting tissues, cell walls, membranes and organelles ([Cox et al., 2014](#); [Jun, 2013](#); [Martelli, Favari, Mena](#)  
50 [et al., 2020](#)). Reducing bacterial contamination to an acceptable level maintaining unchanged or even enhance the composition  
51 of *A. platensis* is a goal that must be reached to use it as a safe and functional ingredient in food formulations. Moreover, HPP  
52 treatment showed to have a positive effect in stabilizing the C-phycoyanin mainly in presence of proteins or hydrocolloids  
53 due to encapsulation induced by pressure ([Zhang et al., 2021](#)). In the last years, the extraction and stabilization of C-  
54 phycoyanin, an accessory photosynthetic blue-protein that is present in relatively high concentration in *A. platensis*, has been  
55 widely investigated for potential food applications (Zhang et al., 2021; Martelli et al., 2014; [Martinez et al., 2017](#)). The  
56 interest for this protein pigment has grown because the confectionary and drinks industry has a high demand in blue colorants,  
57 that are however uncommon in nature and have to be synthesized (Martelli et al., 2014) and because of the high protein yield  
58 and the relatively low cost of production of this pigment. Thus, the aims of the present study were to: i) investigate the effect  
59 of the above-mentioned treatments on: phenolic content, antioxidant capacity, chlorophyll, carotenoids and C-phycoyanin  
60 content, protein fraction and colour and ii) evaluate the efficacy of two thermal treatments (pasteurization and sterilization)  
61 and two high pressure treatments (HPP at 400 and 600 MPa for 3 min) in reducing the microbial contamination of *A. platensis*  
62 aqueous solution.

63

## 64 2. Materials and Methods

**Commentato [MA1]:** Martínez, J. M., Luengo, E., Saldaña, G., Álvarez, I., & Raso, J. (2017). C-phycoyanin extraction assisted by pulsed electric field from *Arthrospira platensis*. *Food Research International*, 99, 1042-1047.

65 **2.1 Materials**

66 Gallic acid, sodium carbonate, Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), DPPH (2,2-diphenyl-  
67 1-picrylhydrazyl free radical) and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA); Folin-Ciocalteu's  
68 phenol reagent solution was obtained from VWR (Milan, Italy). Bi-distilled water was produced by a Millipore Alpha Q  
69 purification system (Waters, Billerica, MA, USA).

70 Acrylamide, N,N'-methylenebisacrylamide, N,N,N,N, tetramethyl ethylene-diamine (TEMED), glycine, Sodium dodecyl  
71 sulphate (SDS), Tris aminomethane (TRIZMA® BASE), ammonium persulfate, 1,4-dithioeritrol (DTE), Bromophenol blue,  
72 glycerol, sigmamarker™ wide range protein ladder (200-6.5 kDa), Coomassie brilliant blue G250, orto-phosphoric acid  
73 (85%), aluminium sulphate, ethanol, and acetone were purchased from-Merck (Milan, Italy).

74

75 **2.2 Experimental design and processing treatments**

76 Dried *A. platensis* was kindly provided by S.A.Ba.R (Novellara, RE, Italy). One hundred grams of dried product were  
77 rehydrated in 2 L of distilled water (5% w/v) under gentle stirring at 4°C overnight. After rehydration, the suspension was  
78 divided into ten aliquots of 200 mL that were packed in sterilized bottles with an internal volume of 250 mL. The aliquots  
79 were grouped in 5 different treatment (two 200-mL aliquots for each treatment): i) raw, non-treated sample (control); ii) 400  
80 MPa for 3 min (HPP400), iii) 600 MPa for 3 min (HPP600), iv) pasteurized at 90°C for 10 min (PAST) and v) sterilized at  
81 121°C for 20 min (STER).

82 Both HPP treatments were performed with a QUINTUS FOOD PRESS QFP 35 L (Avure Technologies Inc., Middletown,  
83 OH, USA). Cold water (4 °C) was used as pressure medium; temperature during processing was not controlled but the  
84 temperature increase due to compression was not higher than 2-3 °C/100 MPa.

85 Sterilization was performed by means of Tuttnauer autoclave (Cavallo s.r.l., Milan, Italy), at 121°C for 20 min. Pasteurization  
86 treatment was performed by placing the samples in a temperature-controlled laboratory bath (type M418-BM, MPM  
87 instruments, Bernareggio, Italy) at 90°C for 10 min; the treatment durations of sterilization and pasteurization were  
88 respectively calculated when the inner temperature of the autoclave reached 121 ± 1°C and when the water inside the bath  
89 reached 90± 1°C after the immersion of the bottles. After both pasteurization and sterilization, the bottles were immediately  
90 cooled in a water bath and then, immediately analysed or stored at refrigerated temperature (4 ± 1°C).

ha formattato: Inglese (Stati Uniti)

91 [Five out of the ten bottles subjected to](#) different treatments were analysed immediately (0 d of storage) and [the other five](#)  
92 after a short period of refrigerated storage at 4°C (6 d of storage). [Two technical replicates for each analysis were performed](#)  
93 [for each sample.](#)

**Commentato [eb2]:** Forse ci diamo la zappa sui piedi ma è quello che abbiamo fatto, cosa ne pensate? Penso che il revisore volesse sapere questo

### 95 2.3 Total phenolic content determination

96 Total phenolic content was determined using the Folin-Ciocalteu's method, following the protocol reported by [Wu et al.](#)  
97 [\(2005\)](#) with some modifications. Briefly, 0.25 mL of water diluted sample (1/5, v/v) were transferred in the test tubes and  
98 added with 1 mL of Folin-Ciocalteu's phenol reagent solution (1/10, v/v in bi-distilled water) and with 2 mL of aqueous  
99 sodium carbonate (20 %, w/v). All the samples were incubated for 30 min, in the dark at room temperature. Absorbance was  
100 measured at 760 nm using a JASCO V-530 spectrophotometer (Easton, MD, USA), and water was used to adjust zero. A  
101 calibration curve was built analysing 5 different gallic acid standard solutions (10 – 100 mg GAE/L). Results were expressed  
102 as gallic acid equivalent (mg GAE/L).

### 104 2.4 Radical-scavenging activity by DPPH method

105 The antioxidant capacity test was performed applying the DPPH radical scavenging assay on the basis of the protocol reported  
106 by [Dall'Asta et al. \(2013\)](#) with slight modifications: an aliquot of 0.1 mL of the 1/5 diluted sample was putted in a test tube  
107 and added with 2.9 mL of a methanolic DPPH solution (0.05 mM). The resulting mixture was kept in the dark at room  
108 temperature for 30 minutes, then the absorbance at 517 nm was registered on a JASCO V-530 spectrophotometer (Easton,  
109 MD, USA), and methanol was used for setting the autozero. At the same time, a blank sample was prepared and submitted to  
110 the same procedure. After that, the inhibition of DPPH radical was determined on the basis of the following formula:

$$111 I\% = \frac{Abs_0 - Abs_1}{Abs_0} \cdot 100 \quad (2)$$

112 where  $Abs_0$  was the absorbance of the blank and  $Abs_1$  was the absorbance of the sample. A calibration curve was constructed  
113 using Trolox as reference, preparing 5 different standard solutions in a concentration range of 0.1 – 1 mM. Then, the  
114 antioxidant capacity of samples was expressed as TEAC value (Trolox Equivalent Antioxidant Capacity; mmol Trolox eq/kg).

116 **2.5 Quantification of chlorophylls, carotenoids and phycocyanin content**

117 **2.5.1 Sample preparation for spectrophotometric assays**

118 To perform spectrophotometric analyses, the insoluble biomass of *A. platensis* was separated by centrifugation (6,500 g for  
119 20 min at 4°C) from the supernatant containing water-soluble C-phycocyanin. Supernatant was extracted by pipetting and an  
120 aliquot (100 µL) was diluted by adding 900 µL of ultrapure water for C-phycocyanin quantification. The pellet containing *A.*  
121 *platensis* biomass was weighed and resuspended in ~900 µL of pure acetone (1:20 dilution). The suspension was vortexed at  
122 2,000 rpm and sonicated for 15 min using a workbench bath (VWR ultrasonic Cleaner) (Hynstova et al. 2018). Extracted  
123 pigments (chlorophyll and carotenoids) were separated by centrifuging at 13,700 g for 10 min at 4°C. Supernatant was filtered  
124 by syringe filtration using 0.45 µm filters (VWR International, Milan, Italy) and 50 µL of permeate were diluted by adding  
125 950 µL of pure acetone. Samples preparation was performed in dim light to avoid pigment degradation. Each sample was  
126 prepared by means of two independent extractions.

127

128 **2.5.2 Spectrophotometric determination of the pigments**

129 For chlorophylls and carotenoids determination, methanol was used as the blank of analysis. Concentration of chlorophyll a  
130 (Ca), chlorophyll b (Cb), total chlorophylls (Ca+b), total carotenoids (Cx+c), total chlorophylls and carotenoids (Ccc) and  
131 pheophytins (Cph) were calculated according to Hynstova et al. (2018) and Lichtenthaler (1987) using equations 3-8:

132 
$$Ca (\mu g/mL) = 11.24 A_{662} - 2.04 A_{645} \quad (3)$$

133 
$$Cb (\mu g/mL) = 20.13 A_{645} - 4.19 A_{662} \quad (4)$$

134 
$$Ca + b (\mu g/mL) = 7.05 A_{662} + 18.09 A_{645} \quad (5)$$

135 
$$Cx + c (\mu g/mL) = \frac{(1,000 A_{470} - 1.90 Ca - 63.14 Cb)}{214} \quad (6)$$

136 
$$Ccc (\mu g/mL) = (Ca + b) + (Cx + c) \quad (7)$$

137 
$$Cph (\mu g/mL) = 321.3 A_{653} - 208.4 A_{654} \quad (8)$$

138 For C-phycoerythrin, milliQ water was used as the blank of analysis. Concentration of water-soluble C-phycoerythrin was  
139 determined according to the method reported by de Marco Castro et al. (2019). Quantification of C-phycoerythrin was  
140 performed according to equation (9):

$$141 \quad C - \text{phycoerythrin} \left( \frac{\text{mg}}{\text{mL}} \right) = \frac{A_{615} - 0.474 A_{652}}{5.34} \quad (9)$$

142 where  $A_{615}$  and  $A_{652}$  are absorbances of the sample measured at 615 nm and 652 nm.

143

## 144 2.6 Physical properties

145 Colorimetric parameters were evaluated with image analysis: samples were scanned by means of a desktop flatbed scanner  
146 (Hewlett Packard Scanjet 8200, Palo Alto, CA, USA) at 236 pixels/cm (600 dpi of resolution; true colour – 24 bit), equipped  
147 with a cold cathode lamp for reflective scanning. All images were scanned at the same conditions, during image acquisition,  
148 the scanner was held in a black box to exclude surrounding light and external reflections. Flatbed scanner colour (R, G and  
149 B) was corrected as previously reported by N'Dri et al. (2010) and converted to L\* (lightness), a\* (redness at positive values,  
150 greenness at negative values) and b\* (yellowness at positive values, blueness at negative values). Total colour differences  
151 ( $\Delta E$ ) between 0 and 6 days were also calculated.

152 Water holding capacity (WHC) of *A. platensis* suspensions was measured similarly to Barkallah et al. (2017). Samples aliquots  
153 (~1 g) were placed in 1.5 mL tubes and centrifuged at 6,000 g per 10 min at 4°C with a benchtop centrifuge (mod. 5810R,  
154 Eppendorf, Hamburg, Germany). WHC was calculated as follows (equation 1):

$$155 \quad WHC \left( \% \frac{w}{w} \right) = \left( \frac{W_1}{W_2} \right) \cdot 100 \quad (1)$$

156 where  $W_1$  is the weight of *A. platensis* centrifuged pellet and  $W_2$  is the weight of the original sample.

157

## 158 2.7 Optical microscopy observation

159 An aliquot of 10  $\mu\text{L}$  of each sample was observed by means of an optical light microscope Nikon 80i (Tokyo, Japan) equipped  
160 with a 10X objective. Pictures of each sample were acquired by the Nis Elements software (ver. 2.10 Nikon, Tokyo, Japan).

161



162 **2.8 Sodium Dodecyl Sulphate polyacrylamide gel electrophoresis (SDS PAGE)**

163 Sodium dodecyl sulphate (SDS) PAGE was performed according to the method of Laemmi (1970). Stacking and separating  
164 gels were casted by using a 4 and 15% acrylamide concentration, respectively. Diluted samples (proteins concentration of ~2  
165  $\mu\text{g}/\mu\text{L}$ ) each sample were loaded in the gel wells (5  $\mu\text{L}$  aliquots). Samples were run in non-reducing conditions at 150 V on a  
166 Mini-Protean II cube (Bio-Rad, Hercules, CA, USA).

167 SDS PAGE gels were stained with Coomassie Brilliant Blue G250 according to Kang et al. (2002), destained in several  
168 changes of distilled water and scanned with a flatbed scanner.

169

170 **2.9 Microbial counts**

171 Each sample was ten-fold (1:10 v/v) diluted, up to  $10^{-6}$  in Ringer's solution (Sigma Aldrich, Milan) and then inoculated in  
172 specific culture media using the spread-plate technique. Total microbial count (TBC) was obtained by plating onto Plate Count  
173 Agar (PCA, Oxoid, Basingstoke, Hampshire, UK) and incubating at  $37 \pm 1$  °C for 48 h. Chromocult® Coliform Agar (Merck,  
174 KGaA, Darmstadt, Germany) was used for the enumeration of Coliforms by incubating at  $37 \pm 1$  °C for 24 h. Staphylococci  
175 were counted by spreading the dilutions on Mannitol Salt Agar (Oxoid, UK) and incubated at  $37 \pm 1$  °C for 48 h. For the  
176 count of Listeria, Agar Listeria Ottaviani and Agosti medium (ALOA, Biolife Italiana, Milan) was used and plates incubated  
177 for 48 h at  $37 \pm 1$  °C. Yeast and molds were counted on Yeast Extract Glucose Chloramphenicol Agar (YGC, Merck KGaA,  
178 Darmstadt, Germany) that was incubated at  $25 \pm 1$  °C for 48–72 h.

179 For aerobic and anaerobic spore-forming bacteria, samples were heated to 80 °C for 10 min and then cooled before analysis.  
180 Then the samples were serially diluted in Ringer's solution, and plated on Tryptone Soy Agar (TSA, Merck KGaA, Darmstadt,  
181 Germany), followed by incubation for 24 h at  $30 \pm 1$  °C under aerobic condition for the determination of aerobic spore-forming  
182 bacteria (Abdelmassih et al., 2011). For the count of anaerobic spore-forming bacteria, 1 mL of each dilution was inoculated  
183 in the medium using the pour-plate technique and then covered with a thin layer of the same medium in order to obtain a  
184 double-layer. The plates were anaerobically incubated into the jars with the AnaeroGen sachet (Oxoid, UK), at  $30 \pm 1$  °C for  
185 24 h.

186

187 **2.10 Statistical analysis**

188 Statistical analyses were performed using SPSS Statistics v. 25 (IBM, Armonk, NY, USA): one-way ANOVA and Tukey  
189 HSD post hoc test were applied to test significant differences among the treatments and storage times ( $P<0.05$ ). Pearson  
190 correlation coefficients between total polyphenolic concentrations and antioxidant capacity values were also calculated  
191 considering a significant correlation for  $P<0.05$ .

192

### 193 3. Results and Discussion

#### 194 3.1 Total polyphenolic content (TPC)

195 Immediately after processing, HPP treatments did not affect the amount of polyphenols if compared to the control sample.  
196 Conversely, pasteurized and sterilized samples (0 d of storage) showed significantly ( $P<0.05$ ) lower concentrations of phenols  
197 in respect to the untreated suspension (Table 1). The loss of polyphenols in PAST and STER samples could be ascribed to the  
198 effect of the heating treatments.

199 The phenolic concentration of the aqueous suspension of *A. platensis* at 0 d (Table 1) was  $149.49 \pm 0.31$  mg GAE/L,  
200 significantly higher ( $P<0.05$ ) than after the storage period ( $216.48 \pm 2.07$  mg GAE/L).

201 After 6 d of refrigerated storage, control and HPP400 samples showed a significant increase ( $P<0.05$ ) of TPC. This trend  
202 could be probably due to a release of phenolic compounds from cells; polyphenols are contained in cells and for their  
203 completely extraction the lysis of cell walls results necessary (Parniakov et al., 2015). Conversely, HPP600 samples showed  
204 a significantly lower value than HPP400 and control samples, but still a significantly higher ( $P<0.05$ ) value than PAST and  
205 STER (Table 1). Differences observed between the two HPP treatments after the storage could be ascribed to a degradation  
206 of the compounds that react with Folin-Ciocalteu's reagent, such as C-phycoerythrin (İlter et al., 2018). By increasing the  
207 pressure applied in HPP processing, the degradation of C-phycoerythrin may be observed, as reported in a previous work which  
208 investigated the decomposition of this molecule at different pressure levels (50 – 600 MPa) (Li et al., 2020).

209

#### 210 3.2 Antioxidant activity

211 *A. platensis* aqueous sample at 0 d presented an antioxidant activity of  $0.472 \pm 0.036$  mmol Trolox eq/kg (Table 1). As already  
212 observed for TPC at 6 d, the antioxidant capacity of the control samples increased to  $0.578 \pm 0.027$  mmol Trolox eq/kg  
213 ( $P<0.05$ ). On the other hand, the other samples presented values comparable with those found in the control at 0 d, while after  
214 6 d of storage, all the samples presented a lower antioxidant activity in respect to the control. In particular, the pasteurized

215 sample showed the statistically lowest radical scavenging capacity ( $P < 0.05$ ) (Table 1). These results reflected those observed  
216 for total polyphenolic content: a strongly positive relationship ( $r = 0.81$ ) between the radical scavenging activity and the  
217 phenolic concentration values was found.

218 Along with polyphenolic compounds, several other molecules naturally present in *A. platensis* showed antioxidant properties,  
219 such as carotenoids, chlorophylls, phycocyanin and ascorbic acid (Chopra & Bishnoi, 2008). So, even if heating treatments  
220 caused a reduction of TPC as in the case of sterilized samples, the antioxidant capacity remained almost comparable in respect  
221 to the control, probably due to the presence of other compounds not belonging to the polyphenols class. The control sample,  
222 prepared at a concentration of 5 % of *A. platensis* (50  $\mu\text{g/mL}$ ), showed a concentration of antioxidant compounds of  $0.472 \pm$   
223  $0.036$  mmol Trolox eq/kg, corresponding to an inhibition of  $12.65 \pm 0.97$  %, resulting indeed higher as compared to  
224 data already reported in literature. Also ethanolic suspension that contained 25  $\mu\text{g/mL}$  of *A. platensis*, showed an inhibition  
225 of  $10.30 \pm 0.80$  % (Anbarasan et al., 2011). *A. platensis* is a well-known antioxidant source: some studies reported the  
226 antioxidant properties of *A. platensis* water extracts in reducing cell death due to apoptosis caused by the presence of free  
227 radicals (as DPPH), so the authors hypothesized that these products could be applied in food as natural preservatives (Chu et  
228 al., 2010).

229

### 230 3.3 Physical properties of *A. platensis* aqueous dispersions

231 In general, all the treatments showed a significant effect on the colour of the samples, as depicted in Fig. 1. Regarding  $L^*$   
232 values, all treatments caused a significant increase ( $P < 0.05$ ) (Table 2). Increase of  $L^*$  values could be due to rapid oxidation  
233 and/or pigment decomposition at high temperature, in accordance with Nouri et al. (2018).

234 Redness (positive  $a^*$  scores) was significantly reduced ( $P < 0.05$ ) by all treatments apart from HPP400, that showed  
235 an increase if compared to the control; the extent of reduction was in the following order: PAST>HPP600>STER.  
236 Pasteurization (PAST) caused the highest decrease of  $a^*$ ; in particular, PAST was the only sample that showed a negative  $a^*$   
237 value, indicating a green shade of the sample, that was also visually observable (Fig. 1). Interestingly, HPP400 showed a  
238 significantly higher ( $P < 0.05$ )  $a^*$  value than HPP600,

239

240 Yellowness (positive b\* scores) significantly increased with the thermal treatments (P<0.05), while HPP samples did not  
241 show significant differences (P>0.05) compared to the control. The thermal degradation of C-phycoerythrin in PAST and STER  
242 probably caused a loss of blue hue towards a greener and browner colour (Fig.1).

243 Changes in b\* and a\* values were due to the effect of different processes that can cause pigments' degradation: e.g. for  
244 chlorophylls with the formation of various coloured compounds (Koca et al., 2007), and for C-phycoerythrin which results in  
245 precipitation and fading of the blue pigment (increase of b\*) (Chaiklahan et al. 2012). On the contrary, the  
246 HPP treatments compared to the control did not show significantly different values in terms of the b\* parameter, despite the  
247 highest C-phycoerythrin content was observed in HPP400 (Fig. 5) and apparently led to a bluer shade (Fig. 1), with this latter  
248 characteristic probably caused by the higher, positive a\* score that led to a bluer hue.

249  
250  
251 . The highest total colour difference ( $\Delta E$ ) was higher for the thermally treated samples if compared to the HPP  
252 ones (Table 2). Surprisingly, the highest total colour difference ( $\Delta E$ ), with an evident variation of colour compared to the  
253 control, was observed for PAST and not for STER, that reported the second highest total colour difference with  
254 the control sample. On the contrary, the lowest  $\Delta E$  value was recorded for HPP600 and HPP400. These results  
255 confirmed the strongest impact of the thermal treatment on colour characteristics, that still represent an important limitation  
256 of *A. platensis* in food applications (Chaiklahan et al. 2012).

257 During refrigerated storage, the thermally-treated samples showed a considerable stability, conversely, the control and the  
258 HPP samples showed significant variations (P<0.05), especially a decrease in L\* and b\* and an increase in a\* values was  
259 observed, probably due to microbial activity and/or residual activity of enzymes.

260 As reported in Fig. 2, water holding capacity (WHC) of the control samples resulted  $13.3 \pm 0.1$  %, confirming the characteristic  
261 of *A. platensis* to retain water thanks to the high protein (Benelhadj et al., 2016) and extracellular polysaccharides (EPS)  
262 content (Trabelsi et al., 2009). All the treatments caused a significant increase (P<0.05) in WHC values except for HPP600.  
263 The increase in WHC was probably related to gelation of *A. platensis* proteins (Chronakis, 2001) due to dissociation into  
264 subunits and unfolding of the protein molecules. As temperature increases, reactive groups are exposed favouring protein-  
265 protein or other electrostatic and hydrogen bonding interactions. Thermally treated samples (PAST and STER) presented

266 significant higher values ( $P<0.05$ ) compared to high pressure treated ones. After 6 d of storage, WHC of the treatments  
267 remained the same with no significant ( $P>0.05$ ) variations, confirming the stability of the formed network.

268

### 269 3.4 Pigments concentration

270 Concentrations of chlorophyll a (Ca), carotenoids (Cx+c) and pheophytins (Cph) are reported in Table 3. The control sample  
271 showed a relatively high Ca content ( $135 \pm 8 \mu\text{g/mL}$  at 0 d, corresponding to  $2.71 \pm 0.15 \text{ mg/g}$  dry weight), comparable to  
272 values already reported in other studies (Hynstova et al., 2018; Park et al., 2018). Conversely, Cb content of raw *A. platensis*  
273 was low ( $6 \pm 4 \mu\text{g/mL}$ ) (Table 3).

274 Cph, the primary degradation product of chlorophylls due to thermal treatments, was found in a concentration of  $779 \pm 45$   
275  $\mu\text{g/mL}$  in the control. In particular, Cph content was found to be higher than Ca (Table 3) and this can be due to the initial  
276 degradation of chlorophylls during the *A. platensis* industrial drying step (Hynstova et al., 2018).

277 The concentration of C-phycoyanin (Fig. 3), the main phycobiliprotein present in *A. platensis*, was  $760 \pm 20 \mu\text{g/mL}$  in the  
278 control, corresponding to  $15.1 \pm 0.4 \text{ mg/g}$  dry weight, in accordance with de Marco Castro et al. (2019).

279 Afterwards, the different treatments showed significant differences ( $P<0.05$ ) in terms of pigments content (Table 3, Fig. 3).  
280 As expected, thermal treatments promoted a significant reduction of chlorophylls, pheophytin and C-phycoyanin. On the  
281 contrary, the reduction of carotenoids was only significant for STER ( $P<0.05$ ). Interestingly, both HPP treatments did not  
282 cause a significant variation of total chlorophylls, carotenoids and pheophytin content, and this result was in line with the  
283 colorimetric analyses. This observation highlights the benefits of applying HPP treatments, compared to the thermal ones,  
284 which lead to significant denaturation of these thermolabile compounds that are known to have an important positive bioactive  
285 activity (Ariede et al., 2017; Kumar et al., 2015).

286 C-phycoyanin concentration was slightly, but significantly higher ( $P<0.05$ ) for the HPP400 (Fig. 3) also if compared to the  
287 control, probably due to both an increase in extraction of this molecule from *A. platensis* cells and a limited denaturation.

288 Contrarily, Li et al. (2020) observed a significant reduction in the C-phycoyanin content in HPP-treated *A. platensis* at 400  
289 MPa for 3.5 min and hypothesized that its decrease was related to the denaturation promoted by the HPP treatments. The  
290 microscopic observations allowed to observe that HPP treatment induced cell breakage, although not complete (Fig. 4). This  
291 can explain the increase in the C-phycoyanin concentration in sample treated at 400 MPa. On the contrary, stronger HPP

292 conditions (600 MPa x 3 min) caused a marked denaturation and a consequent reduction of C-phycoyanin concentration, in  
293 accordance with [Li et al. \(2020\)](#), that observed a heavier C-phycoyanin denaturation at higher pressure treatments. According  
294 to its low thermal stability ([Martelli et al., 2014](#)), C-phycoyanin was also heavily denatured by PAST and STER thermal  
295 treatments that showed about 87 and 97 % of reduction, respectively. It is quite well known that solutions containing C-  
296 phycoyanin are sensitive to heat treatment already at temperature above 47°C ([Chaiklahan et al., 2012](#)) with at least 50% of  
297 reduction after 30 min at 60°C. By considering first-order rate constants reported by ([Chaiklahan et al., 2012](#)) calculated  
298 residual activities agree with experimental data.

299 After 6 d of storage, a general increase of pigments, although not always significant, was observed; in particular, HPP400 and  
300 pasteurized samples showed a significant increase ( $P<0.05$ ) in the total chlorophyll and carotenoids concentration, while the  
301 control, HPP400, HPP600 samples showed a significant increase ( $P<0.05$ ) in the C-phycoyanin concentration. This result  
302 was partly in accordance with the increase of antioxidant capacity of the control and of the polyphenols content of the control  
303 and HPP400 (section 3.4). As stated before, an increase in the pigments' concentrations could be explained by the presence  
304 of residual enzymatic activities that may cause the release of these compounds from algae cells. Previous studies demonstrated  
305 that enzymes produced by bacteria can improve the extraction of C-phycoyanin ([de Marco Castro et al., 2019](#); [Zhu et al.,](#)  
306 [2007](#)).

307

### 308 3.5 Optical microscopy observation

309 To further investigate whether each treatment affected the microstructure of *A. platensis*, the samples were observed using  
310 light microscopy (Fig. 4). *A. platensis* is characterized by a particular morphological feature, such as the arrangement in an  
311 open left-hand helix along the entire length ([Ali & Saleh, 2012](#)). The untreated *A. platensis* (Fig. 4a) already showed some  
312 breakdown of the cell walls and the presence of cell's fragments, probably mainly related to the drying process of the biomass  
313 ([CIT](#)). In the thermally treated samples, and particularly in STER (Fig. 4e), the appearance of some aggregates can be  
314 observed. These are assumed to be caused by the sterilization process that may lead to aggregation of denatured proteins, that  
315 are the major constituent of the total biomass ([Bernaerts et al., 2017](#)). These aggregates are probably the main responsible for  
316 the higher WHC (Fig. 2). Both HPP treatments (Fig. 4b, c), affected the structure of *A. platensis* to a lesser extent, by causing  
317 a lower but observable breakdown of the cell walls, if compared to the more intact number of cells observed in the control (Li  
318 et al., 2020).

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319

### 320 3.6 Protein determination by SDS PAGE

321 Results of SDS PAGE are reported in Fig. 5. Several proteins bands were visible and the majority were present in the  
322 medium molecular weight range (30-60 kDa) (Aiello et al., 2019). Biliproteins were the most intense bands present in the low  
323 molecular weight range of the electrophoretogram (Li et al., 2020; de Marco Castro et al., 2019), which are mainly represented  
324 by C-phycoyanin  $\alpha$  and  $\beta$  subunits (Benelhadj et al., 2016). Chaiklahan et al. (2011) reported that C-phycoyanin  $\alpha$  and  $\beta$   
325 subunits were characterized by a molecular weight of about 18.4 and 21.3 kDa, respectively; Rajakumar & Muthukumar,  
326 (2018) reported a molecular weight range of 19-22 kDa. Our results were consistent with these observations, as C-phycoyanin  
327  $\alpha$  and  $\beta$  subunits were found to have a molecular weight of about 18.5 and 20.5 kDa, respectively.

328 Other well-defined bands were observable at higher molecular weights (~44, 60, 102 kDa, corresponding to peaks 3, 4, 5  
329 reported in Fig. 5). In particular, peak 3 (~44 kDa) is probably indicative of the presence of chlorophyll complexes (Kalaji et  
330 al., 2017) and peak 5 (~102 kDa) is probably related to a core protein linker of the phycobilisomes to thylakoid membrane  
331 (Rajakumar & Muthukumar, 2018).

332 In general, protein bands showed a reduction because of both thermal and non-thermal treatments. The decrease of intensity  
333 was more evident for thermally-treated samples than HPP ones; this was expected as C-phycoyanin is denatured at  
334 temperatures above 40°C (Liu et al., 2016), and it was in accordance with pigments quantification (section 3.4). No observable  
335 protein bands were present in the sterilized sample.

336 Concerning HPP treatments, HPP400 showed a smaller reduction in protein bands than HPP600, if compared to the control  
337 sample.

338

### 339 3.7 Microbial counts

340 The result of total microbial count (TBC) on raw samples, showed a value of  $5.96 \pm 0.05$  Log CFU/mL (Table 4), confirming  
341 the high values of microbial contamination previously observed (Morais et al., 2009; Yu et al., 2019; Martelli, Cirlini, Lazzi  
342 et al, 2021; Martelli, Marrella, Lazzi et al 2021). Regarding the treated samples, a different microbial reduction was observed  
343 depending on the treatment: pasteurization, together with HPP400 and HPP600 allowed a reduction of respectively 4.30, 4.32

344 and 4.96 Log CFU/mL. Sterilization achieved a significantly higher reduction of microbial load, for all the species considered  
345 (Table 4).

346 If compared to both HPP treatments, pasteurization was more effective on Salmonella, allowing a reduction of 4 Log CFU/mL.  
347 Despite no significant differences ( $P>0.05$ ) were found between HPP600 and HPP400 on Salmonella, a greater reduction of  
348 2.68 Log CFU/mL was observed in samples treated with HPP600 (Table 1). This could be due to the higher pressure, that  
349 could induce a greater denaturation of enzymes and proteins, the breakdown of the cell membrane with the consequent loss  
350 of internal substances, and a higher bacterial death (Abe, 2007).

351 Results on spores confirmed that neither pasteurization nor HPP treatments can completely inactivate endospore-forming  
352 strains: their effect on those bacteria count was not consistent, allowing a small reduction of only about 1 Log CFU/mL. Our  
353 results agreed with Reddy et al. (2006), who reported that bacterial spores cannot be inactivated by high pressure alone and  
354 that they can survive at pressure treatments above 1000 MPa, probably due to the thickness structure of bacterial spore coat.  
355 Under such conditions, the pressure tolerance of the endospore substantially exceeds that of the microorganism's original  
356 vegetative state (Leggett et al., 2012). From these results, HPP effect on *A. platensis* microbial count, was comparable to the  
357 effect of a high temperature pasteurization. Sterilization was the only treatment that can ensure the inhibition of spore-forming  
358 species. Some authors reported that to achieve sterilization levels, HPP treatments can be used combined with an appropriate  
359 temperature treatment or, in combination with other pressure cycle, to effectively inhibit endospore activity (Huang et al.,  
360 2014).

361 Regarding the analysis after 6 d of refrigerated storage, the overall microbial count (TBC) increased for all the treated samples;  
362 probably due to the presence of heat-resistant, psychrotrophic gram-positive bacteria (Fromm & Boor, 2004). Significant  
363 ( $P<0.05$ ) increments were also found in the yeast and Staphylococci enumeration.

364 For *Salmonella*, a significant reduction ( $P<0.05$ ) of concentration was found after 6 d of storage for both HPP treatments  
365 while spore forming bacteria, showed a small reduction in number for all the treatments applied.

366 Concerning the sterilized samples, no significative differences ( $P>0.05$ ) were found during the storage.

367

#### 368 4. Conclusions



369 In this study different thermal and HPP treatments were applied on 5% (w/v) *A. platensis* aqueous suspensions, to assess  
370 which treatments was the best compromise between the achievement of microbial safety and the preservation bioactive  
371 compounds. HPP treatments were found to be the best process in preserving the biological value and the physical properties  
372 of *A. platensis*. In particular, HPP400, even improved the release of C-phycoerythrin from *A. platensis* probably because of the  
373 breakage of cell walls promoted by the mechanical effect of high pressures.

374 From the microbiological analysis it was observed that HPP treatments were comparable to pasteurization in reducing  
375 microbial cell count, while sterilization was the only treatment able to ensure the inhibition of spore-forming species.

376 These results suggest that HPP, despite being the best treatments in preserving the biological values of *A. platensis*, were not  
377 sufficient to achieve microbial stabilization. In conclusion, a combination of thermal and HPP treatments or a combination of  
378 HPP cycles, could be further investigated as a strategy to obtain a good compromise between microbiological and physico-  
379 chemical, nutritional quality. These results may be useful to design strategies to stabilize and transform food products  
380 containing *A. platensis* as ingredient.

381

#### 382 **Declarations of Interest**

383 None

384

#### 385 **References**

386 Abdelmassih, M., Planchon, V., Anceau, C. & Mahillon, J. (2011). Development and validation of stable reference materials  
387 for food microbiology using *Bacillus cereus* and *Clostridium perfringens* spores. *Journal of Applied Microbiology*, 110, 1524-  
388 1530. <https://doi.org/10.1111/j.1365-2672.2011.05007.x>

389 Abe, F. (2007). Exploration of the effects of high hydrostatic pressure on microbial growth, physiology, and survival:  
390 perspectives from piezophysiology. *Bioscience, Biotechnology, and Biochemistry*, 71, 2347-2357.  
391 <https://doi.org/10.1271/bbb.70015>

392 Aiello, G., Li, Y., Boschin, G., Bollati, C., Arnoldi, A., & Lammi, C. (2019). Chemical and biological characterization of  
393 spirulina protein hydrolysates: Focus on ACE and DPP-IV activities modulation. *Journal of Functional Foods*, 63, 103592.  
394 <https://doi.org/10.1016/j.jff.2019.103592>

395 Ali, S. K., & Saleh, A. M. (2012). Spirulina - an overview. *International Journal of Pharmacy and Pharmaceutical Sciences*,  
396 4(3), 9-15.

397 Anbarasan, V., Kumar, V. K., Kumar, P. S., & Venkatachalam, T. (2011). In vitro evaluation of antioxidant activity of blue  
398 green algae *Spirulina platensis*. *Journal of Pharmaceutical Science and Research*, 2, 2616-2618.

399 Antelo, F. S., Costa, J. A. V., & Kalil, S. J. (2008). Thermal degradation kinetics of the phycocyanin from *Spirulina platensis*.  
400 *Biochemical Engineering Journal*, 41, 43–47. <https://doi.org/10.1016/j.bej.2008.03.012>

401 Ariede, M. B., Candido, T. M., Jacome, A. L. M., Velasco, M. V. R., de Carvalho, J. C. M., & Baby, A. R. (2017). Cosmetic  
402 attributes of algae—A review. *Algal Research*, 25, 483–487. <https://doi.org/10.1016/j.algal.2017.05.019>

403 Bancalari, E., Martelli, F., Berinini, V., Neviani, E., & Gatti, M. (2020). Bacteriostatic or bactericidal? Impedometric  
404 measurements to test the antimicrobial activity of *Arthrospira platensis* extract. *Food Control*, 118, 107380.  
405 <https://doi.org/10.1016/j.foodcont.2020.107380>

406 Bancalari, E., Martelli, F., Bottari, B., V., Neviani, E., & Gatti, M (2021). *Arthrospira platensis* Extract: A Non-Invasive  
407 Strategy to Obtain Adjunct Attenuated Cultures. *Foods*, 10, 588. <https://doi.org/10.3390/foods10030588>

408 Barkallah, M., Dammak, M., Louati, I., Hentati, F., Hadrich, B., Mechichi, T., Ayadi, M. A., Fendri, I., Attia, H., & Abdelkafi,  
409 S. (2017). Effect of *Spirulina platensis* fortification on physicochemical, textural, antioxidant and sensory properties of yogurt  
410 during fermentation and storage. *LWT – Food Science and Technology*, 84, 323–330.  
411 <https://doi.org/10.1016/j.lwt.2017.05.071>

412 Bartlett, D. H. (2002). Pressure effects on in vivo microbial processes. *Biochimica et Biophysica Acta (BBA)-Protein Structure  
413 and Molecular Enzymology*, 1595, 367-381. [https://doi.org/10.1016/S0167-4838\(01\)00357-0](https://doi.org/10.1016/S0167-4838(01)00357-0)

414 Batista, A. P., Nicolai, A., Fradinho, P., Fragoso, S., Bursic, I., Rodolfi, L., Biondi, N., Tredici, M. R., Sousa, I., &  
415 Raymundo, A. (2017). Microalgae biomass as an alternative ingredient in cookies: Sensory, physical and chemical properties,  
416 antioxidant activity and in vitro digestibility. *Algal Research*, 26, 161–171. <https://doi.org/10.1016/j.algal.2017.07.017>

417 Benelhadj, S., Gharsallaoui, A., Degraeve, P., Attia, H., & Ghorbel, D. (2016). Effect of pH on the functional properties of  
418 *Arthrospira* (*Spirulina*) *platensis* protein isolate. *Food Chemistry*, 194, 1056-1063.  
419 <https://doi.org/10.1016/j.foodchem.2015.08.133>

420 Bernaerts, T. M. M., Panozzo, A., Doumen, V., Foubert, I., Gheysen, L., Goiris, K., Moldenaers, P., Hendrickxa, M.E., &  
421 Van Loey, A. M. (2017). Microalgal biomass as a (multi)functional ingredient in food products: Rheological properties of  
422 microalgal suspensions as affected by mechanical and thermal processing. *Algal Research*; 25, 452–463.  
423 <https://doi.org/10.1016/j.algal.2017.05.014>

424 Chaiklahan, R., Chirasuwan, N., & Bunnag, B. (2012). Stability of phycocyanin extracted from *Spirulina* sp.: influence of  
425 temperature, pH and preservatives. *Process Biochemistry*, 47, 659-664. <https://doi.org/10.1016/j.procbio.2012.01.010>

426 Chaiklahan, R., Chirasuwan, N., Loha, V., Tia, S., & Bunnag, B. (2011). Separation and purification of phycocyanin from  
427 *Spirulina* sp. using a membrane process. *Bioresource Technology*, 102(14), 7159-7164.  
428 <https://doi.org/10.1016/j.biortech.2011.04.067>

429 Chopra, K., & Bishnoi, M. (2008). *Spirulina in Human Nutrition and Health*. In M. E. Gershwin, A. Belay (Eds) Antioxidant  
430 profile of Spirulina: a blue-green microalga. (pp. 101- 116) CRC Press, Boca Raton, FL, USA,

431 Chronakis, I. S. (2001). Gelation of edible blue-green algae protein isolate (*Spirulina platensis* strain pacifica): thermal  
432 transitions, rheological properties, and molecular forces involved. *Journal of Agricultural and Food Chemistry*, 49, 888-898.  
433 <https://doi.org/10.1021/jf0005059>

434 Chu, W-L., Lim, Y-W., Radhakrishnan, A.K., & Lim, P-E. (2010). Protective effect of aqueous extract from *Spirulina*  
435 *platensis* against cell death induced by free radicals. *BMC Complementary and Alternative Medicine*, 10, 53.  
436 <https://doi.org/10.1186/1472-6882-10-53>

437 Cox, S., Hamilton Turley, G., Rajauria, G., Abu-Ghannam, N., & Jaiswal, A. K. (2014). Antioxidant potential and  
438 antimicrobial efficacy of seaweed (*Himanthalia elongata*) extract in model food systems. *Journal of Applied Phycology*, 26,  
439 1823–1831. <https://doi.org/10.1007/s10811-013-0215-0>

440 da Silva, M. F., Casazza, A. A., Ferrari, P. F., Aliakbarian, B., Converti, A., Bezerra, R. P., Porto, A. F. L., & Perego, P.  
441 (2017). Recovery of phenolic compounds of food concern from *Arthrospira platensis* by green extraction techniques. *Algal*  
442 *Research*, 25, 391–401. <https://doi.org/10.1016/j.algal.2017.05.027>

443 Dall'Asta, C., Cirlini, M., Morini, E., Rinaldi, M., Ganino, T., & Chiavaro, E. (2013). Effect of chestnut flour supplementation  
444 on physico-chemical properties and volatiles in bread making. *LWT - Food Science and Technology*, 53, 233-239.  
445 <https://doi.org/10.1016/j.lwt.2013.02.025>

446 de Marco Castro, E., Shannon, E., & Abu-Ghannam, N. (2019). Effect of Fermentation on Enhancing the Nutraceutical  
447 Properties of *Arthrospira platensis* (Spirulina). *Fermentation*, 5, 28. <https://doi.org/10.3390/fermentation5010028>

448 Fromm, H. I., & Boor, K. J. (2004). Characterization of pasteurized fluid milk shelf-life attributes. *Journal of Food Science*,  
449 69, 207-214. <https://doi.org/10.1111/j.1365-2621.2004.tb09889.x>

450 Fromm, H. I., & Boor, K. J. (2004). Characterization of pasteurized fluid milk shelf-life attributes. *Journal of Food Science*,  
451 69, 207-214. <https://doi.org/10.1111/j.1365-2621.2004.tb09889.x>

452 Golmakani, M.-T., Soleimani-Zad, S., Alavi, N., Nazari, E., & Eskandari, M. H. (2019). Effect of Spirulina (*Arthrospira*  
453 *platensis*) powder on probiotic bacteriologically acidified feta-type cheese. *Journal of Applied Phycology*, 31, 1085–1094.  
454 <https://doi.org/10.1007/s10811-018-1611-2>

455 Huang, H-W., Lung, H-M., Yang, B.B., & Wang, C-Y. (2014). Responses of microorganisms to high hydrostatic pressure  
456 processing, a Review. *Food Control*, 40, 250-259. <https://doi.org/10.1016/j.foodcont.2013.12.007>

457 Hynstova, V., Sterbova, D., Klejdus, B., Hedbavny, J., Huska, D., & Adam, V. (2018). Separation, identification and  
458 quantification of carotenoids and chlorophylls in dietary supplements containing *Chlorella vulgaris* and *Spirulina platensis*  
459 using High Performance Thin Layer Chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, 148, 108–118.  
460 <https://doi.org/10.1016/j.jpba.2017.09.018>

461 İtter, I., Akyıl, S., Demirel, Z., Koç, M., Conk-Dalay, M., & Kaymak-Ertekin, F. (2018). Optimization of phycocyanin  
462 extraction from *Spirulina platensis* using different techniques. *Journal of Food Composition and Analysis*, 70, 78–88.  
463 <https://doi.org/10.1016/j.jfca.2018.04.007>

464 Jun, X. (2013). High-Pressure Processing as Emergent Technology for the Extraction of Bioactive Ingredients From Plant  
465 Materials. *Critical Reviews in Food Science and Nutrition*, 53, 837–852. <https://doi.org/10.1080/10408398.2011.561380>

466 Kalaji, M. H., Goltsev, V. N., Żuk-Golaszewska, K., Zivcak, M., & Brestic, M. (2017). *Chlorophyll fluorescence:  
467 understanding crop performance—basics and applications*. CRC Press, Boca Raton, FL, USA.

468 Kang, D., Gho, Y. S., Suh, M., & Kang, C. (2002). Highly sensitive and fast protein detection with Coomassie brilliant blue  
469 in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Bulletin of the Korean Chemical Society*, 23, 1511–1512.  
470 <https://doi.org/10.5012/bkcs.2002.23.11.1511>

471 Koca, N., Karadeniz, F., & Burdurlu, H. S. (2007). Effect of pH on chlorophyll degradation and colour loss in blanched green  
472 peas. *Food Chemistry*, 100, 609–615. <https://doi.org/10.1016/j.foodchem.2005.09.079>

473 Kumar, K., Mishra, S. K., Shrivastav, A., Park, M. S., & Yang, J.-W. (2015). Recent trends in the mass cultivation of algae  
474 in raceway ponds. *Renewable and Sustainable Energy Reviews*, 51, 875–885. <https://doi.org/10.1016/j.rser.2015.06.033>

475 Laemmi, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227,  
476 680–685. <https://doi.org/10.1038/227680a0>

477 Leggett, M. J., McDonnell, G., Denyer, S. P., Setlow, P., & Maillard, J.-Y. (2012). Bacterial spore structures and their  
478 protective role in biocide resistance. *Journal of Applied Microbiology*, 113, 485–498. [https://doi.org/10.1111/j.1365-  
479 2672.2012.05336.x](https://doi.org/10.1111/j.1365-2672.2012.05336.x)

480 Li, Y., Zhang, Z., Paciulli, M., & Abbaspourrad, A. (2020). Extraction of phycocyanin—A natural blue colorant from dried  
481 spirulina biomass: Influence of processing parameters and extraction techniques. *Journal of Food Science*, 85, 727 – 735.  
482 <https://doi.org/10.1111/1750-3841.14842>

483 Lichtenthaler, H. K. (1987). Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Methods in  
484 Enzymology*, 148, 350–382. [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)

485 Liu, Q., Huang, Y., Zhang, R., Cai, T., & Cai, Y. (2016). Medical application of *Spirulina platensis* derived C-phycocyanin.  
486 *Evidence-Based Complementary and Alternative Medicine*, 2016, 7803846. <https://doi.org/10.1155/2016/7803846>

487 Machu, L., Misurcova, L., Vavra Ambrozova, J., Orsavova, J., Mlecek, J., Sochor, J., & Jurikova, T. (2015). Phenolic Content  
488 and Antioxidant Capacity in Algal Food Products. *Molecules*, 20, 1118–1133. <https://doi.org/10.3390/molecules20011118>

489 Martelli, F., Alinovi, M., Bernini, V., Gatti, M., & Bancalari, E. (2020). *Arthrospira platensis* as Natural Fermentation Booster  
490 for Milk and Soy Fermented Beverages. *Foods*, 9, 350. <https://doi.org/doi:10.3390/foods9030350>

491 Martelli, F., Cirlini, M., Lazzi, C., Neviani, E., & Bernini, V. (2020). Edible Seaweeds and Spirulina Extracts for Food  
492 Application: In Vitro and In Situ Evaluation of Antimicrobial Activity towards Foodborne Pathogenic Bacteria. *Foods*, *9*,  
493 1442. <https://doi.org/10.3390/foods9101442>

494 Martelli, F., Favari, C., Mena, P., Guazzetti, S., Ricci, A., Del Rio, D., Lazzi, C., et al. (2020). Antimicrobial and Fermentation  
495 Potential of *Himantalia elongata* in Food Applications. *Microorganisms*, *8*, 248.  
496 <http://doi.org/10.3390/microorganisms8020248>

497 Martelli, F., Cirlini, M., Lazzi, C., Neviani, E., & Bernini, V. (2021). Solid-State Fermentation of *Arthrospira platensis* to  
498 Implement New Food Products: Evaluation of Stabilization Treatments and Bacterial Growth on the Volatile Fraction. *Foods*,  
499 *10*, 67. <https://doi.org/10.3390/foods10010067>

500 Martelli, F., Marrella, M., Lazzi, C., Neviani, E., & Bernini, V. (2021). Microbiological contamination of ready to eat algae  
501 and evaluation of *Bacillus cereus* behavior by microbiological challenge test. *Journal of food protection*, *10*, 4315.

502 Martelli, G., Folli, C., Visai, L., Daglia, M., & Ferrari, D. (2014). Thermal stability improvement of blue colorant C-  
503 Phycocyanin from *Spirulina platensis* for food industry applications. *Process Biochemistry*, *49*, 154–159.  
504 <https://doi.org/10.1016/j.procbio.2013.10.008>

505 Morais, M. G., Radmann, E. M., Andrade, M. R., Teixeira G. G., Bruschi, L. R. F., & Costa J. A. V. (2009). Pilot scale  
506 semicontinuous production of Spirulina biomass in southern Brazil. *Aquaculture* *294*, 60–64.  
507 <https://doi.org/10.1016/j.aquaculture.2009.05.009>

508 N'Dri, D., Calani, L., Mazzeo, T., Scazzina, F., Rinaldi, M., Del Rio, D., Pellegrini, N., & Brighenti, F. (2010). Effects of  
509 different maturity stages on antioxidant content of Ivorian Gnagnan (*Solanum indicum* L.) berries. *Molecules*, *15*, 7125–7138.  
510 <https://doi.org/10.3390/molecules15107125>

511 Niccolai, A., Chini Zittelli, G., Rodolfi, L., Biondi, N., & Tredici, M. R. (2019). Microalgae of interest as food source:  
512 Biochemical composition and digestibility. *Algal Research*, *42*, 101617. <https://doi.org/10.1016/j.algal.2019.101617>

513 Nouri, E., Abbasi, H., & Rahimi, E. (2018). Effects of processing on stability of water-and fat-soluble vitamins, pigments (C-  
514 phycocyanin, carotenoids, chlorophylls) and colour characteristics of *Spirulina platensis*. *Quality Assurance and Safety of*  
515 *Crops & Foods*, *10*, 335–349. <https://doi.org/10.3920/QAS2018.1304>

516 Paciulli, M., Palermo, M., Chiavaro, E., & Pellegrini, N. (2017). Chlorophylls and colour changes in cooked vegetables. In  
517 E. M. Yahia (Ed.) *Fruit and Vegetable Phytochemicals: Chemistry and Human Health: Second Edition*, 703–719.  
518 <https://doi.org/10.1002/9781119158042.ch31>

519 Paciulli, M., Rinaldi, M., Rodolfi, M., Ganino, T., Morbarigazzi, M., & Chiavaro, E. (2019). Effects of high hydrostatic  
520 pressure on physico-chemical and structural properties of two pumpkin species. *Food Chemistry*, *274*, 281–290.  
521 <https://doi.org/10.1016/j.foodchem.2018.09.021>

522 Park, W. S., Kim, H. J., Li, M., Lim, D. H., Kim, J., Kwak, S. S., Kang, C.-M., Ferruzzi, M. G., & Ahn, M. J. (2018). Two  
523 classes of pigments, carotenoids and C-phycoyanin, in Spirulina powder and their antioxidant activities. *Molecules*, *23*, 2065.  
524 <https://doi.org/10.3390/molecules23082065>

525 Parniakov, O., Apicella, E., Koubaa, M., Barba, F. J., Grimi, N., Lebovka, N., Pataro, G., Ferrari, G., & Vorobiev, E. (2015).  
526 Ultrasound-assisted green solvent extraction of high-added value compounds from microalgae *Nannochloropsis* spp.  
527 *Bioresource Technology*, *198*, 262–267, <https://doi.org/10.1016/j.biortech.2015.09.020>

528 Rajakumar, M. S., & Muthukumar, K. (2018). Influence of pre-soaking conditions on ultrasonic extraction of *Spirulina*  
529 *platensis* proteins and its recovery using aqueous biphasic system. *Separation Science and Technology*, *53*, 2034-2043.  
530 <https://doi.org/10.1080/01496395.2018.1442860>

531 Reddy, N. R., Tetzloff, R. C., Solomon, H. M., & Larkin, J. W. (2006). Inactivation of *Clostridium botulinum* non proteolytic  
532 type B spores by high pressure processing at moderate to elevated high temperatures. *Innovative Food Science & Emerging*  
533 *Technologies*, *7*, 169–175. <https://doi.org/10.1016/j.ifset.2006.03.002>

534 Richardson, J. W., Johnson, M. D., & Outlaw, J. L. (2012). Economic comparison of open pond raceways to photo bio-  
535 reactors for profitable production of algae for transportation fuels in the Southwest. *Algal Research*, *1*, 93–100.  
536 <https://doi.org/10.1016/j.algal.2012.04.001>

537 Trabelsi, L., M'sakni, N. H., Ouada, H. B., Bacha, H., & Roudesli, S. (2009). Partial characterization of extracellular  
538 polysaccharides produced by cyanobacterium *Arthrospira platensis*. *Biotechnology and Bioprocess Engineering*, *14*, 27-31.  
539 <https://doi.org/10.1007/s12257-008-0102-8>

540 Wang, H., Zhang, W., Chen, L., Wang, J., & Liu, T. (2013). The contamination and control of biological pollutants in mass  
541 cultivation of microalgae. *Bioresource Technology*, *128*, 745–750. <https://doi.org/10.1016/j.biortech.2012.10.158>

542 Wu, L-C., Ho, J-A. A., Shien, M-C., & Lu, I-W. (2005). Antioxidant and Antiproliferative Activities of Spirulina and  
543 Chlorella Water Extracts. *Journal of Agricultural and Food Chemistry*, *53*, 4207-4212. <https://doi.org/10.1021/jf0479517>

544 Yu, J., Hu, H., Wu, X., Wang, C., Zhou, T., Liu, Y., Ruan, R., & Zheng H. (2019). Continuous cultivation of *Arthrospira*  
545 *platensis* for phycocyanin production in large-scale outdoor raceway ponds using microfiltered culture medium. *Bioresource*  
546 *Technology*, *287*, 121420. <https://doi.org/10.1016/j.biortech.2019.121420>

547 Zarbà, C., Chinnici, G., & D'Amico, M. (2020). Novel Food: The Impact of Innovation on the Paths of the Traditional Food  
548 Chain. *Sustainability*, *12*, 555. <https://doi.org/10.3390/su12020555>

549 Zhang, Z., Cho, S., Dadmohammadi, Y., Li, Y., & Abbaspourrad, A. (2021). Improvement of the storage stability of C-  
550 phycoyanin in beverages by high-pressure processing. *Food Hydrocolloids*, *110*, 106055.  
551 <https://doi.org/10.1016/j.foodhyd.2020.106055>

552 Zheng, J. X., Yin, H., Shen, C. C., Zhang, L., Ren, D. F., & Lu, J. (2020). Functional and structural properties of spirulina  
553 phycoyanin modified by ultra-high-pressure composite glycation. *Food Chemistry*, *306*, 125615.  
554 <https://doi.org/10.1016/j.foodchem.2019.125615>

555 Zhu, Y., Chen, X. B., Wang, K. B., Li, Y. X., Bai, K. Z., Kuang, T. Y., & Ji, H. B. (2007). A simple method for extracting C-  
556 phycocyanin from *Spirulina platensis* using *Klebsiella pneumoniae*. *Applied Microbiology and Biotechnology*, 74, 244-248.  
557 <https://doi.org/10.1007/s00253-006-0636-7>

558

559 **Table 1.** Total phenolic content and antioxidant capacity of aqueous suspension of *A. platensis* analysed after different  
 560 treatment at time zero and after 6 days of storage

| Storage time (d) | Treatment | Total phenolic content (mg/L GAE) | Antioxidant capacity (mmol Trolox eq/kg) | Antioxidant capacity (%)   |
|------------------|-----------|-----------------------------------|--|----------------------------|
| 0                | Control   | 149.49 <sup>ab</sup> ± 0.31       | 0.472 <sup>ab</sup> ± 0.036              | 12.65 <sup>ab</sup> ± 0.97 |
|                  | HPP400    | 144.02 <sup>ab</sup> ± 5.53       | 0.424 <sup>ab</sup> ± 0.026              | 11.36 <sup>ab</sup> ± 0.70 |
|                  | HPP600    | 146.58 <sup>ab</sup> ± 2.72       | 0.437 <sup>ab</sup> ± 0.015              | 11.71 <sup>ab</sup> ± 0.39 |
|                  | PAST      | 95.80 <sup>ba</sup> ± 6.45        | 0.372 <sup>ab</sup> ± 0.028              | 9.97 <sup>ab</sup> ± 0.74  |
|                  | STER      | 112.62 <sup>ba</sup> ± 6.42       | 0.450 <sup>ab</sup> ± 0.021              | 12.05 <sup>ab</sup> ± 0.56 |
| 6                | Control   | 216.48 <sup>ab</sup> ± 2.07       | 0.578 <sup>ab</sup> ± 0.027              | 15.50 <sup>ab</sup> ± 0.72 |
|                  | HPP400    | 205.94 <sup>ab</sup> ± 5.67       | 0.461 <sup>ba</sup> ± 0.011              | 12.35 <sup>ba</sup> ± 0.29 |
|                  | HPP600    | 142.68 <sup>ba</sup> ± 0.82       | 0.427 <sup>ba</sup> ± 0.004              | 11.45 <sup>ba</sup> ± 0.12 |
|                  | PAST      | 113.89 <sup>ca</sup> ± 6.31       | 0.348 <sup>ca</sup> ± 0.008              | 9.33 <sup>ba</sup> ± 0.22  |
|                  | STER      | 110.31 <sup>ca</sup> ± 1.97       | 0.413 <sup>ba</sup> ± 0.016              | 11.06 <sup>ba</sup> ± 0.44 |

561 <sup>a-c</sup> lower case superscripts highlight differences and/or analogies among different treatments within a column.

562 <sup>A-B</sup> upper case superscripts indicate a significant difference (within a column) between the same treatment analysed at 0 d and 6 d of storage  
 563 time.

564 [Abbreviations: raw, non-treated sample \(control\); 400 MPa for 3 min \(HPP400\), 600 MPa for 3 min \(HPP600\), pasteurized](#)  
 565 [\(PAST\) and sterilized \(STER\).](#)

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Tabella formattata



569 **Table 2.** Colorimetric parameters (L\*, a\*, b\*, ΔE) of *A. platensis* aqueous suspensions (5% w/v). Treatments of *A. platensis*  
 570 aqueous dispersion were: fresh, non-treated *A. platensis* (control); high pressure processed (HPP) sample at 400MPa  
 571 (HPP400); HPP sample at 600MPa (HPP600); 90°C x 10 min pasteurized sample (PAST); 121°C x 20 min sterilized sample  
 572 (STER). Treatments were analysed at time zero (0 d) and after 6 days of refrigerated storage.

| Storage time (d) | Treatment | L*                         | a*                          | b*                         | ΔE                         |
|------------------|-----------|----------------------------|-----------------------------|----------------------------|----------------------------|
| 0                | Control   | 4.06 <sup>ba</sup> ± 0.92  | 6.03 <sup>bb</sup> ± 1.00   | 1.25 <sup>ba</sup> ± 0.28  | -                          |
|                  | HPP400    | 9.04 <sup>aa</sup> ± 0.77  | 8.67 <sup>ab</sup> ± 2.07   | 1.34 <sup>ba</sup> ± 0.36  | 5.85 <sup>ca</sup> ± 1.36  |
|                  | HPP600    | 10.31 <sup>aA</sup> ± 1.09 | 0.61 <sup>dB</sup> ± 0.99   | 1.73 <sup>ba</sup> ± 0.29  | 8.37 <sup>ca</sup> ± 0.63  |
|                  | PAST      | 11.85 <sup>aA</sup> ± 2.32 | -11.25 <sup>ca</sup> ± 1.58 | 13.11 <sup>aA</sup> ± 3.11 | 22.45 <sup>ab</sup> ± 3.54 |
|                  | STER      | 11.66 <sup>aA</sup> ± 2.04 | 3.39 <sup>ca</sup> ± 0.35   | 12.19 <sup>aA</sup> ± 1.97 | 13.60 <sup>bA</sup> ± 2.73 |
| 6                | Control   | 6.23 <sup>bcA</sup> ± 1.35 | 12.75 <sup>bA</sup> ± 1.47  | 0.32 <sup>bb</sup> ± 0.50  | -                          |
|                  | HPP400    | 5.68 <sup>cb</sup> ± 0.84  | 17.08 <sup>aA</sup> ± 0.88  | -1.46 <sup>bb</sup> ± 0.54 | 4.81 <sup>ca</sup> ± 0.78  |
|                  | HPP600    | 6.30 <sup>bcB</sup> ± 1.17 | 6.80 <sup>ca</sup> ± 0.87   | 0.97 <sup>bb</sup> ± 0.15  | 6.08 <sup>cb</sup> ± 0.87  |
|                  | PAST      | 11.36 <sup>aA</sup> ± 2.83 | -11.50 <sup>ca</sup> ± 1.94 | 12.23 <sup>aA</sup> ± 3.35 | 27.66 <sup>aA</sup> ± 3.55 |
|                  | STER      | 9.67 <sup>abA</sup> ± 1.60 | 1.14 <sup>dA</sup> ± 0.47   | 10.67 <sup>aA</sup> ± 1.29 | 15.86 <sup>bA</sup> ± 1.20 |

573 <sup>a-c</sup> lower case superscripts highlight differences and/or analogies among different treatments within a column.

574 <sup>A-B</sup> upper case superscripts indicate a significant difference (within a column) between the same treatment analysed at 0 d and 6 d of storage  
 575 time.

576 [Abbreviations: lightness \(L\\*\), Redness \(a\\*\), Yellowness \(b\\*\) and total colour differences \(ΔE\) between 0 and 6 days.](#)

577  **ha formattato: Inglese (Stati Uniti)**

578

579 **Table 3.** Total content of chlorophyll a (Ca), chlorophyll b (Cb), total chlorophylls (Ca + Cb), total carotenoids (Cx+c), total  
580 chlorophylls and carotenoids (Ccc), pheophytins (Cph) of different treatments of *A. platensis* in aqueous suspension (5% w/v).  
581 Treatments of *A. platensis* aqueous dispersion were: fresh, non-treated *A. platensis* (control); high pressure processed (HPP)  
582 sample at 400MPa (HPP400); HPP sample at 600MPa (HPP600); 90°C x 10 min pasteurized sample (PAST); 121°C x 20  
583 min sterilized sample (STER). Treatments were analysed at time zero (0 d) and after 6 days of shelf life.

| Storage time (d) | Treatment | Ca (µg/mL)             | Cb (µg/mL)               | Ca+Cb (µg/mL)          | Cx+c (µg/mL)          | Ccc (µg/mL)             | Cph (µg/mL)              |
|------------------|-----------|------------------------|--------------------------|------------------------|-----------------------|-------------------------|--------------------------|
| 0                | Control   | 135 <sup>aA</sup> ± 8  | 5.9 <sup>aA</sup> ± 4.1  | 186 <sup>aA</sup> ± 6  | 46 <sup>aA</sup> ± 4  | 233 <sup>aA</sup> ± 10  | 779 <sup>aA</sup> ± 45   |
|                  | HPP400    | 148 <sup>aB</sup> ± 5  | 3.5 <sup>abA</sup> ± 0.4 | 201 <sup>aB</sup> ± 6  | 47 <sup>aA</sup> ± 1  | 249 <sup>aB</sup> ± 7   | 820 <sup>aB</sup> ± 23   |
|                  | HPP600    | 142 <sup>aA</sup> ± 13 | 2.8 <sup>abA</sup> ± 0.3 | 192 <sup>aA</sup> ± 18 | 45 <sup>aB</sup> ± 2  | 237 <sup>aA</sup> ± 20  | 784 <sup>aA</sup> ± 73   |
|                  | PAST      | 92 <sup>bb</sup> ± 8   | 2.1 <sup>abA</sup> ± 0.6 | 125 <sup>bb</sup> ± 11 | 43 <sup>aB</sup> ± 5  | 168 <sup>bb</sup> ± 16  | 476 <sup>bb</sup> ± 43   |
|                  | STER      | 43 <sup>ca</sup> ± 3   | 0.6 <sup>ba</sup> ± 0.2  | 57 <sup>ca</sup> ± 4   | 26 <sup>ba</sup> ± 1  | 83 <sup>ca</sup> ± 5    | 184 <sup>ca</sup> ± 11   |
| 6                | Control   | 192 <sup>aA</sup> ± 21 | 5.0 <sup>aA</sup> ± 2.3  | 260 <sup>aA</sup> ± 30 | 60 <sup>aA</sup> ± 6  | 320 <sup>aA</sup> ± 36  | 1062 <sup>aA</sup> ± 123 |
|                  | HPP400    | 189 <sup>aA</sup> ± 10 | 5.0 <sup>aA</sup> ± 2.1  | 257 <sup>aA</sup> ± 11 | 58 <sup>aA</sup> ± 4  | 315 <sup>aA</sup> ± 14  | 1017 <sup>aA</sup> ± 49  |
|                  | HPP600    | 185 <sup>aA</sup> ± 10 | 4.6 <sup>abA</sup> ± 1.8 | 251 <sup>aA</sup> ± 11 | 59 <sup>aA</sup> ± 3  | 309 <sup>abA</sup> ± 15 | 1034 <sup>aA</sup> ± 43  |
|                  | PAST      | 138 <sup>ba</sup> ± 7  | 1.2 <sup>abA</sup> ± 0.6 | 185 <sup>ba</sup> ± 9  | 60 <sup>aA</sup> ± 3  | 245 <sup>bca</sup> ± 12 | 702 <sup>abA</sup> ± 44  |
|                  | STER      | 105 <sup>ba</sup> ± 21 | 0.7 <sup>ba</sup> ± 0.5  | 141 <sup>ba</sup> ± 29 | 53 <sup>aA</sup> ± 12 | 194 <sup>ca</sup> ± 41  | 484 <sup>ba</sup> ± 113  |

584 <sup>a-c</sup> lower case superscripts highlight differences and/or analogies among different treatments within a column.

585 <sup>A-B</sup> upper case superscripts indicate a significant difference (within a column) between the same treatment analysed at 0 d and 6 d of storage  
586 time.

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597 **Table 4.** Microbial concentration at 0 d and after 6 d of storage at refrigerated temperatures. Microbial concentration was  
 598 expressed as Log CFU/ml.

| Storage time (d) | Treatment | Microbial species         |                           |                           |                           |                           |                           |                           |
|------------------|-----------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                  |           | Aerobic spor.             | Anaerobic spor.           | TBC                       | Yeast                     | Salmonella                | Coliform                  | Staphylococci             |
| 0                | Control   | 2.84 ± 0.12 <sup>aA</sup> | 2.66 ± 0.19 <sup>aA</sup> | 5.96 ± 0.05 <sup>aA</sup> | 4.44 ± 0.41 <sup>aA</sup> | 4.53 ± 0.15 <sup>aA</sup> | 5.06 ± 0.08 <sup>aA</sup> | 4.94 ± 0.19 <sup>aA</sup> |
|                  | HPP400    | 1.88 ± 0.29 <sup>bA</sup> | 1.83 ± 0.27 <sup>bA</sup> | 1.66 ± 0.08 <sup>bA</sup> | <1 <sup>bA</sup>          | 2.70 ± 0.17 <sup>bA</sup> | nd <sup>bA</sup>          | 1.70 ± 0.20 <sup>bA</sup> |
|                  | HPP600    | 1.81 ± 0.31 <sup>bA</sup> | 1.70 ± 0.20 <sup>bA</sup> | 1.00 ± 0.17 <sup>bA</sup> | <1 <sup>bA</sup>          | 1.85 ± 0.08 <sup>bA</sup> | nd <sup>bA</sup>          | 1.70 ± 0.10 <sup>bA</sup> |
|                  | PAST      | 1.86 ± 0.32 <sup>bA</sup> | 1.66 ± 0.25 <sup>bA</sup> | 1.66 ± 0.19 <sup>bA</sup> | nd <sup>bA</sup>          | nd <sup>cA</sup>          | nd <sup>bA</sup>          | <1 <sup>bA</sup>          |
|                  | STER      | nd <sup>cA</sup>          | nd <sup>cA</sup>          | nd <sup>cA</sup>          | nd <sup>bA</sup>          | nd <sup>cA</sup>          | nd <sup>bA</sup>          | nd <sup>bA</sup>          |
| 6                | Control   | 1.44 ± 0.29 <sup>aB</sup> | 2.65 ± 0.17 <sup>aA</sup> | 6.90 ± 0.07 <sup>aB</sup> | 5.14 ± 0.12 <sup>aB</sup> | 2.10 ± 0.10 <sup>aB</sup> | 6.83 ± 0.15 <sup>aB</sup> | 6.76 ± 0.21 <sup>aB</sup> |
|                  | HPP400    | 1.86 ± 0.26 <sup>bA</sup> | 1.85 ± 0.19 <sup>bA</sup> | 3.40 ± 0.09 <sup>bB</sup> | 1.18 ± 0.17 <sup>bA</sup> | nd <sup>bB</sup>          | nd <sup>bA</sup>          | 1.83 ± 0.08 <sup>bB</sup> |
|                  | HPP600    | 1.71 ± 0.21 <sup>bB</sup> | 1.56 ± 0.17 <sup>bB</sup> | 2.35 ± 0.12 <sup>bB</sup> | 1.30 ± 0.08 <sup>bB</sup> | nd <sup>bB</sup>          | nd <sup>bA</sup>          | 1.81 ± 0.13 <sup>bB</sup> |
|                  | PAST      | 1.48 ± 0.26 <sup>bB</sup> | 1.50 ± 0.31 <sup>bB</sup> | 2.00 ± 0.08 <sup>bB</sup> | nd <sup>cA</sup>          | nd <sup>bA</sup>          | nd <sup>bA</sup>          | 1.78 ± 0.31 <sup>bB</sup> |
|                  | STER      | nd <sup>cA</sup>          | nd <sup>cA</sup>          | nd <sup>cA</sup>          | nd <sup>cA</sup>          | nd <sup>bA</sup>          | nd <sup>bA</sup>          | nd <sup>cA</sup>          |

599 Results are shown as the mean ± standard deviation. nd, not detected

600 <sup>a-c</sup> Different lower-case superscript letters highlight significant differences (P<0.05) among treatments within each column

601 <sup>A-B</sup> Different upper-case superscript letters highlight significant differences (P<0.05) between the same treatments at 0 and 6  
 602 days of storage

603 [Abbreviations: aerobic spore-forming bacteria \(Aerobic spor\), anaerobic spore-forming bacteria \(Anaerobic spor.\), Total](#)  
 604 [Bacteria Count \(TBC\).](#)

605

606 **Figure captions**

607 **Fig.1** Visual appearance of *A. platensis* in aqueous suspensions (5% w/v) colour characteristics at 0 and 6 d of storage time.

608 Samples were: fresh, non-treated *A. platensis* (control); high pressure processed (HPP) sample at 400 MPa (HPP400); HPP

609 sample at 600 MPa (HPP600); 90°C x 10 min pasteurized sample (PAST); 121°C x 20 min sterilized sample (STER).

610 Treatments were analysed at time zero (0 d) and after 6 days of refrigerated storage.

611

612 **Fig.2** Water holding capacity (WHC) of *A. platensis* in aqueous suspensions (5% w/v) at 0 and 6 d of storage time. Samples

613 were: fresh, non-treated *A. platensis* (control); high pressure processed (HPP) sample at 400 MPa (HPP400); HPP sample at

614 600 MPa (HPP600); 90°C x 10 min pasteurized sample (PAST); 121°C x 20 min sterilized sample (STER). Treatments were

615 analysed at time zero (0 d) and after 6 days of refrigerated storage.

616

617 **Fig.3** Concentration of phycocyanin ( $\mu\text{g/mL}$ ) of different treatments of *A. platensis* in aqueous suspension (5% w/v).

618 Treatments of *A. platensis* aqueous dispersion were: fresh, non-treated *A. platensis* (control); high pressure processed (HPP)

619 sample at 400MPa (HPP400); HPP sample at 600MPa (HPP600); 90°C x 10 min pasteurized sample (PAST); 121°C x 20

620 min sterilized sample (STER). Treatments were analysed at time zero (0 d) and after 6 days of shelf life.

621

622 **Fig.4** Light microscopy observations (100x magnification) of *A. platensis* in aqueous suspensions (5% w/v) processed by

623 applying different treatments. (a) raw, control suspension; (b) HPP sample processed at 400 MPa x 3 min; (c) HPP sample

624 processed at 600 MPa x 3 min; (d) sample pasteurized at 90 °C x 10 min; (e) sample sterilized at 121 °C x 20 min. **CF: cell's**

625 **fragments; CB: cell's breakdown; AG: aggregates.**

626

627 **Fig.5** Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) results of *A. platensis* aqueous suspensions

628 (5 w/v) at 0 d of storage. Treatments of *A. platensis* aqueous dispersion were: fresh, non-treated *A. platensis* (control); high

629 pressure processed (HPP) sample at 400MPa (HPP400); HPP sample at 600MPa (HPP600); 90°C x 10 min pasteurized sample

630 (PAST); 121°C x 20 min sterilized sample (STER). Treatments were analysed at time zero (0 d) and after 6 days of shelf life.

631 Band 1: subunit of C-phycocyanin; band 2: subunit of C-phycocyanin; band 3: chlorophyll a complex; band 4: chlorophyll a

632 complex

ha formattato: Tipo di carattere: Grassetto