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(Article begins on next page)

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 (ΔE) 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 μL 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 Laemmli (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 μL 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 ± 1 °C for 48 h. Chromocult® Coliform Agar (Merck,
174 KGaA, Darmstadt, Germany) was used for the enumeration of Coliforms by incubating at 37 ± 1 °C for 24 h. Staphylococci
175 were counted by spreading the dilutions on Mannitol Salt Agar (Oxoid, UK) and incubated at 37 ± 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 ± 1 °C. Yeast and molds were counted on Yeast Extract Glucose Chloramphenicol Agar (YGC, Merck KGaA,
178 Darmstadt, Germany) that was incubated at 25 ± 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 ± 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 ± 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 ± 0.31 mg GAE/L,
200 significantly higher ($P<0.05$) than after the storage period (216.48 ± 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 ± 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 ± 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 $0.036 \text{ mmol Trolox eq/kg}$, 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 (ΔE) was higher for the thermally treated samples if compared to the HPP
252 ones (Table 2). Surprisingly, the highest total colour difference (ΔE), 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 ΔE 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 ± 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 ± 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-phycoerythrin concentration, in
293 accordance with [Li et al. \(2020\)](#), that observed a heavier C-phycoerythrin denaturation at higher pressure treatments. According
294 to its low thermal stability ([Martelli et al., 2014](#)), C-phycoerythrin 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 phycoerythrin 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-phycoerythrin 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-phycoerythrin ([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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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 α and β subunits (Benelhadj et al., 2016). Chaiklahan et al. (2011) reported that C-phycoyanin α and β
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 α and β 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 ± 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**

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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 ^{ab} ± 0.31	0.472 ^{ab} ± 0.036	12.65 ^{ab} ± 0.97
	HPP400	144.02 ^{ab} ± 5.53	0.424 ^{ab} ± 0.026	11.36 ^{ab} ± 0.70
	HPP600	146.58 ^{ab} ± 2.72	0.437 ^{ab} ± 0.015	11.71 ^{ab} ± 0.39
	PAST	95.80 ^{ba} ± 6.45	0.372 ^{ab} ± 0.028	9.97 ^{ab} ± 0.74
	STER	112.62 ^{ba} ± 6.42	0.450 ^{ab} ± 0.021	12.05 ^{ab} ± 0.56
6	Control	216.48 ^{ab} ± 2.07	0.578 ^{ab} ± 0.027	15.50 ^{ab} ± 0.72
	HPP400	205.94 ^{ab} ± 5.67	0.461 ^{ba} ± 0.011	12.35 ^{ba} ± 0.29
	HPP600	142.68 ^{ba} ± 0.82	0.427 ^{ba} ± 0.004	11.45 ^{ba} ± 0.12
	PAST	113.89 ^{ca} ± 6.31	0.348 ^{ca} ± 0.008	9.33 ^{ba} ± 0.22
	STER	110.31 ^{ca} ± 1.97	0.413 ^{ba} ± 0.016	11.06 ^{ba} ± 0.44

561 ^{a-c} lower case superscripts highlight differences and/or analogies among different treatments within a column.

562 ^{A-B} 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 ^{ba} ± 0.92	6.03 ^{bb} ± 1.00	1.25 ^{ba} ± 0.28	-
	HPP400	9.04 ^{aa} ± 0.77	8.67 ^{ab} ± 2.07	1.34 ^{ba} ± 0.36	5.85 ^{ca} ± 1.36
	HPP600	10.31 ^{aA} ± 1.09	0.61 ^{dB} ± 0.99	1.73 ^{ba} ± 0.29	8.37 ^{ca} ± 0.63
	PAST	11.85 ^{aA} ± 2.32	-11.25 ^{ca} ± 1.58	13.11 ^{aA} ± 3.11	22.45 ^{ab} ± 3.54
	STER	11.66 ^{aA} ± 2.04	3.39 ^{ca} ± 0.35	12.19 ^{aA} ± 1.97	13.60 ^{bA} ± 2.73
6	Control	6.23 ^{bcA} ± 1.35	12.75 ^{bA} ± 1.47	0.32 ^{bb} ± 0.50	-
	HPP400	5.68 ^{cb} ± 0.84	17.08 ^{aA} ± 0.88	-1.46 ^{bb} ± 0.54	4.81 ^{ca} ± 0.78
	HPP600	6.30 ^{bcB} ± 1.17	6.80 ^{ca} ± 0.87	0.97 ^{bb} ± 0.15	6.08 ^{cb} ± 0.87
	PAST	11.36 ^{aA} ± 2.83	-11.50 ^{ca} ± 1.94	12.23 ^{aA} ± 3.35	27.66 ^{aA} ± 3.55
	STER	9.67 ^{abA} ± 1.60	1.14 ^{dA} ± 0.47	10.67 ^{aA} ± 1.29	15.86 ^{bA} ± 1.20

573 ^{a-c} lower case superscripts highlight differences and/or analogies among different treatments within a column.

574 ^{A-B} 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 ^{aA} ± 8	5.9 ^{aA} ± 4.1	186 ^{aA} ± 6	46 ^{aA} ± 4	233 ^{aA} ± 10	779 ^{aA} ± 45
	HPP400	148 ^{aB} ± 5	3.5 ^{aBA} ± 0.4	201 ^{aB} ± 6	47 ^{aA} ± 1	249 ^{aB} ± 7	820 ^{aB} ± 23
	HPP600	142 ^{aA} ± 13	2.8 ^{aBA} ± 0.3	192 ^{aA} ± 18	45 ^{aB} ± 2	237 ^{aA} ± 20	784 ^{aA} ± 73
	PAST	92 ^{bB} ± 8	2.1 ^{aBA} ± 0.6	125 ^{bB} ± 11	43 ^{aB} ± 5	168 ^{bB} ± 16	476 ^{bB} ± 43
	STER	43 ^{cA} ± 3	0.6 ^{bA} ± 0.2	57 ^{cA} ± 4	26 ^{bA} ± 1	83 ^{cA} ± 5	184 ^{cA} ± 11
6	Control	192 ^{aA} ± 21	5.0 ^{aA} ± 2.3	260 ^{aA} ± 30	60 ^{aA} ± 6	320 ^{aA} ± 36	1062 ^{aA} ± 123
	HPP400	189 ^{aA} ± 10	5.0 ^{aA} ± 2.1	257 ^{aA} ± 11	58 ^{aA} ± 4	315 ^{aA} ± 14	1017 ^{aA} ± 49
	HPP600	185 ^{aA} ± 10	4.6 ^{aBA} ± 1.8	251 ^{aA} ± 11	59 ^{aA} ± 3	309 ^{aBA} ± 15	1034 ^{aA} ± 43
	PAST	138 ^{bA} ± 7	1.2 ^{aBA} ± 0.6	185 ^{bA} ± 9	60 ^{aA} ± 3	245 ^{bCA} ± 12	702 ^{aBA} ± 44
	STER	105 ^{bA} ± 21	0.7 ^{bA} ± 0.5	141 ^{bA} ± 29	53 ^{aA} ± 12	194 ^{cA} ± 41	484 ^{bA} ± 113

584 ^{a-c} lower case superscripts highlight differences and/or analogies among different treatments within a column.

585 ^{A-B} 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 ^{aA}	2.66 ± 0.19 ^{aA}	5.96 ± 0.05 ^{aA}	4.44 ± 0.41 ^{aA}	4.53 ± 0.15 ^{aA}	5.06 ± 0.08 ^{aA}	4.94 ± 0.19 ^{aA}
	HPP400	1.88 ± 0.29 ^{bA}	1.83 ± 0.27 ^{bA}	1.66 ± 0.08 ^{bA}	<1 ^{bA}	2.70 ± 0.17 ^{bA}	nd ^{bA}	1.70 ± 0.20 ^{bA}
	HPP600	1.81 ± 0.31 ^{bA}	1.70 ± 0.20 ^{bA}	1.00 ± 0.17 ^{bA}	<1 ^{bA}	1.85 ± 0.08 ^{bA}	nd ^{bA}	1.70 ± 0.10 ^{bA}
	PAST	1.86 ± 0.32 ^{bA}	1.66 ± 0.25 ^{bA}	1.66 ± 0.19 ^{bA}	nd ^{bA}	nd ^{cA}	nd ^{bA}	<1 ^{bA}
	STER	nd ^{cA}	nd ^{cA}	nd ^{cA}	nd ^{bA}	nd ^{cA}	nd ^{bA}	nd ^{bA}
6	Control	1.44 ± 0.29 ^{aB}	2.65 ± 0.17 ^{aA}	6.90 ± 0.07 ^{aB}	5.14 ± 0.12 ^{aB}	2.10 ± 0.10 ^{aB}	6.83 ± 0.15 ^{aB}	6.76 ± 0.21 ^{aB}
	HPP400	1.86 ± 0.26 ^{bA}	1.85 ± 0.19 ^{bA}	3.40 ± 0.09 ^{bB}	1.18 ± 0.17 ^{bA}	nd ^{bB}	nd ^{bA}	1.83 ± 0.08 ^{bB}
	HPP600	1.71 ± 0.21 ^{bB}	1.56 ± 0.17 ^{bB}	2.35 ± 0.12 ^{bB}	1.30 ± 0.08 ^{bB}	nd ^{bB}	nd ^{bA}	1.81 ± 0.13 ^{bB}
	PAST	1.48 ± 0.26 ^{bB}	1.50 ± 0.31 ^{bB}	2.00 ± 0.08 ^{bB}	nd ^{cA}	nd ^{bA}	nd ^{bA}	1.78 ± 0.31 ^{bB}
	STER	nd ^{cA}	nd ^{cA}	nd ^{cA}	nd ^{cA}	nd ^{bA}	nd ^{bA}	nd ^{cA}

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

600 ^{a-c} Different lower-case superscript letters highlight significant differences (P<0.05) among treatments within each column

601 ^{A-B} 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