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Impact of high pressure homogenization on physical properties, extraction yield and biopolymer structure of soybean okara

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Title: Impact of high pressure homogenization on physical properties, extraction yield and biopolymer structure of soybean okara

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Abstract: The effect of high pressure homogenization (HPH) on soy okara was studied. To this purpose, okara dispersions (10 g/100 g) were subjected to 1 pass at 50, 100 and 150 MPa and to 5 passes at 150 MPa. Samples were analyzed for stability, particle size, microstructure, and viscosity. Results highlighted that the increase of HPH intensity was associated with the structural disruption of okara particles, leading to physically stable homogenates having increasing viscosity. This was mainly attributed to an increase in okara solubility, due to fibre and protein release. The latter resulted almost complete, reaching values up to 90% of the protein originally entrapped in okara matrix. Absorbance at 280 nm, SH groups and dimension of proteins revealed that HPH treatments favoured the extraction of the main protein fractions even if, at the higher intensity level, extracted proteins probably underwent conformational changes and reassembling phenomena.

Dear Editor,

We send to your attention the revised form of the research article entitled "Impact of high pressure homogenization on physical properties, extraction yield and biopolymer structure of soybean okara" by Goly Fayaz, Stella Plazzotta, Sonia Calligaris, Lara Manzocco, Maria Cristina Nicoli.

We carefully go through the text and further improved it by applying the minor corrections indicated by the reviewers.

Best regards,

Stella Plazzotta (on behalf of all the authors)

Answers to reviewers:

The authors made corrections but still used some units that are not SI units. All units should be SI units.

1. Viscosity unit is written as "cP" but it should be in "Pa.s" [see line 108 and may be other locations]

The viscosity unit was changed as suggested (line 108).

2. Line 128, 133: "12000 g" should be written as "12000 x g" to avoid confusion with mass gram.

The mistake was corrected (lines 128, 133).

3. Line 137 and table 4 (4th column heading): "M" for molar concentration should be written as "mol/L" to avoid confusion with mega

The molar concentration unit was changed as suggested (lines 137, 147 and Table 4).

4. Line 314: "Agriculture" should be "Agricultural"

The mistake was corrected (line 314).

*Highlights (for review)

- HPH is a promising technology for okara valorization
- HPH favours the release of okara proteins and soluble fibers
- Above 50 MPa HPH, physically stable okara dispersions are obtained
- HPH at 150 MPa for 5 passes leads to 90% protein extraction yield

23 **Keywords:** Soybean residue, Vegetable by-products, Waste valorization, Protein, Fiber.

24 **1. Introduction**

25 Okara is a general term defining the by-products obtained after milling and extraction of the
26 aqueous fraction of soybeans. Every kilogram of processed soybeans intended for the production
27 of soy milk and tofu generates about 1.1-1.2 kg of wet okara (containing about 80 g/100 g of
28 water) (O'Toole, 1999). Thus, large quantities of okara are today produced and treated as
29 industrial waste with high management costs and related issues (Rado & Dimi, 2010). However,
30 soy okara still contains various valuable components, mainly fibres (14.5-55.4 g/100 g on dry
31 matter basis), proteins (24.5-37.5 g/100 g) and lipids (9.3-22.3 g/100 g) (Jiménez-Escrig, Alaiz,
32 Vioque, & Rupérez, 2010). Okara can be considered an always-available and cheap source of
33 nutrients rather than waste and might be thus turned into a value-added ingredient by the
34 application of proper valorization strategies (Vong & Liu, 2016). On this regard, air-drying of
35 okara is one of the main solutions proposed. The resulting products are ambient stable flours that
36 can be exploited in the production of functional baked goods, cereal products and snacks
37 (Grizotto, Rufi, Yamada, & Vicente, 2010; O'Toole, 1999; Rado & Dimi, 2010). Nevertheless,
38 being water removal a costly process (Vong & Liu, 2016), other valorization strategies should be
39 developed.

40 Recently, the application of unconventional technologies has been proposed as an effective tool
41 to steer the functional properties of plant-based materials. In this context, high pressure
42 homogenization (HPH) has been shown as promising technology able to induce cell disruption,
43 particle size reduction and modification of macromolecule structure in vegetable matrices
44 (Lopez-Sanchez, Svelander, Bialek, Schumm, & Langton, 2011; Tan & Kerr, 2015). These
45 changes are associated with the intense mechanical stresses suffered by the product during the

46 process. In particular, in the homogenizer, a fluid is pumped through a narrow gap valve by
47 means of a pressure intensifier, undergoing intense mechanical forces and elongational stresses
48 at the valve entrance and in the valve gap. On the other hand, turbulence cavitation and impact
49 with the solid surface is expected to occur at the valve outlet (Floury, Bellettre, Legrand, &
50 Desrumaux, 2004). Cell disruption and modification of biopolymer physical properties are
51 reported to be highly dependent on matrix characteristics and HPH intensity in term of operating
52 pressure and number of passes through the homogenization valve (Augusto, Ibarz, & Cristianini,
53 2013).

54 Based on these considerations, the use of HPH on okara dispersions might have different
55 advantages comprising: (i) extraction of proteinaceous and fibrous materials as a consequence of
56 cell disruption and (ii) increase of functionality resulting from biopolymer structure modification
57 and development of novel particle interactions and networking. Preece, Hooshyar, Krijgsman,
58 Fryer and Zuidam (2017) observed an improvement of the extraction yield of proteins from soy
59 okara dispersions after homogenization at 100 MPa for 1 pass. Besides these data, to our
60 knowledge, no information is available on the effect of HPH on okara biopolymer structure and
61 interactions.

62 The aim of the present study was to explore the potentialities of HPH in releasing protein and
63 fibre constituents from soy okara and turn this by-product into an added-value ingredient for the
64 food industry. To this aim, okara dispersions were subjected to HPH treatments and analyzed for
65 physical properties, protein and fibre extractability, and biopolymer structural changes.

66 **2. Materials and methods**

67 **2.1. Material**

68 A batch of soy okara (1 kg) was kindly provided by a local food processing industry engaged in
69 the production of soy derivatives based on the application of the “Japanese method” (O’Toole,
70 1999). Okara was frozen at -18 °C before using in the experiments.

71 **2.2. Preparation of okara dispersion**

72 Soy okara was dispersed in deionized water at 10 g/100 g concentration under magnetic stirring
73 for 30 min at 20 °C and subsequently pre-homogenized with a high-speed blender (Polytron, PT
74 3000, Littau, Swiss) at 8000 rpm for 1 min to increase the homogeneity of okara distribution in
75 aqueous phase as well as avoiding valve clogging during the subsequent high pressure
76 homogenization.

77 **2.3. High pressure homogenization (HPH)**

78 Okara dispersion was treated by a continuous lab-scale high-pressure homogenizer (Panda Plus
79 2000, GEA Niro Soavi, Parma, Italy) supplied with two Re+ type tungsten carbide
80 homogenization valves, with a flow rate of 10 L/h. Aliquots of 150 mL of okara dispersion at 20
81 °C were subjected to single-pass at a pressure of 50, 100 and 150 MPa and at 5 passes at 150
82 MPa. Sample temperature was measured immediately after HPH treatment (Ellab, Hillerød,
83 Denmark). After treatments, all the samples were cooled at room temperature (20 °C) by
84 immersion into an ice bath under gentle mixing. Untreated okara dispersion was used as control.

85 **2.4. Chemical composition**

86 Moisture, fat, protein and ash content of fresh okara were analyzed by the reference AOAC
87 (1997). Soluble (SDF) and insoluble dietary fibre (IDF) of fresh okara, untreated dispersion and
88 HPH-treated okara dispersions were also analyzed according to AOAC method using a total
89 dietary fibre (TDF) assay kit (TDF-100A, Sigma-Aldrich, St. Louis, Missouri, USA). The
90 SDF/TDF and IDF/TDF ratio were reported as g/100 g fibre. Total polyphenolic content (TPC)

91 was determined according to Singleton and Rossi (1965) method by using Folin-Ciocalteu
92 reagent. The absorbance was read at 750 nm using UV-Vis spectrophotometer (Shimadzu UV-
93 2501PC, UV-Vis recording spectrophotometer, Shimadzu Corporation, Kyoto, Japan). Results
94 were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample.

95 **2.5. Physical Stability**

96 To monitor the physical stability of okara dispersion treated by HPH, samples were transferred
97 into a 20 mL glass tube and stored up to 30 days at 4 °C. Images were acquired using an image
98 acquisition cabinet (Immagini & Computer, Bareggio, Italy) equipped with a digital camera
99 (EOS 550D, Canon, Milan, Italy). Images were saved in *jpeg* format resulting in 3456×2304
100 pixels.

101 **2.6. pH measurement**

102 The pH of samples was recorded at 20 °C by using a Basic 20 pH meter (Crison Instruments,
103 S.A., Barcelona, Spain).

104 **2.7. Particle size distribution**

105 The particle size distribution of samples was measured by using the dynamic light scattering
106 instrument Zetasizer Nano ZS (Malvern, Milan, Italy). Samples were diluted (1 mL/10 mL) in
107 deionized water prior to the analysis to avoid multiple scattering effects. Observation angle,
108 solution refractive index and viscosity were set at 173°, 1.333 and 0.00088 Pa·s, respectively,
109 corresponding to the values of pure water at 25 °C. Mean particle size diameter and peak area
110 corresponding to intensity distribution were measured.

111 **2.8. Optical and polarized light microscopy**

112 One drop of okara dispersion was placed on a glass slide, covered with a cover slide and
113 observed at 20 °C using a Leica DM 2000 optical microscope (Leica Microsystems, Heerbrugg,

114 Switzerland). The images were taken at 200× magnification using a Leica EC3 digital camera
115 and elaborated with the Leica Suite Las EZ software (Leica Microsystems, Heerbrugg,
116 Switzerland).

117 **2.9. Viscosity**

118 Viscosity determination was performed at 20 °C by a Haake Rheostress 6000 (Thermo
119 Scientific, Rheostress, Haake, Germany), connected to a thermostatic controller. The flow
120 behaviour of samples was measured using concentric cylinder geometry by recording apparent
121 viscosity against shear rate from 0.1 to 200 s⁻¹. The relationship between apparent viscosity and
122 the shear rate was described by Ostwald-de-Waele model, (eq. 1):

$$123 \eta_{app} = K \cdot \dot{\gamma}^{n-1} \quad \text{eq. 1}$$

124 where η_{app} is the apparent viscosity (Pa·s); $\dot{\gamma}$, the shear rate (s⁻¹); K , the consistency index (Pa·
125 sⁿ) and n , the flow behaviour index (dimensionless). Model fitting was performed using the
126 software Haake Rheowin v.4.60.0001 (Thermo Fisher Scientific).

127 **2.10. Protein extraction yield**

128 Okara dispersions were centrifuged at 12000 × g for 10 min at 4 °C (Beckman, Avanti TM J-25,
129 Palo Alto, CA, USA). The protein content of the supernatant was determined by the Kjeldahl
130 method (AOAC, 1997). Protein extraction yield (g/100 g protein) was calculated as the ratio
131 between the proteins in the supernatant and the total protein content.

132 **2.11. Absorbance at 280 nm**

133 Okara dispersions were centrifuged at 12000 × g for 10 min at 4 °C. The supernatant was
134 collected, diluted (1 mL/200 mL) and UV absorbance was measured at 280 nm using UV-2501
135 PC UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan).

136 **2.12. Determination of free sulfhydryl group content**

137 The concentration ($\mu\text{molL}^{-1}\text{g}^{-1}$) of free sulfhydryl groups (SH) of the okara dispersions was
138 determined using Ellman's reagent (5',5-dithiobis (2-nitrobenzoic acid), DTNB) (Sigma-
139 Aldrich, Milan, Italy) according to the method of Panozzo, Manzocco, Lippe and Nicoli (2016).

140 **2.13. HPLC-gel permeation analysis**

141 Okara dispersions were analyzed using an HPLC system Varian ProStar (model 230, Varian
142 Associates Ltd., Walnut Creek, CA, USA) equipped with a UV/VIS detector. Two columns were
143 used: BioSep-SEC-S 3000, 30 cm length, 7.80 mm internal diameter and BioSep-SEC-S 2000,
144 30 cm length, 7.80 mm internal diameter, 5 μm granulometry, 125 \AA porosity with separation
145 range among 5 and 670 kDa. Samples were filtered on 0.2 μm porosity filters (Econofilters,
146 Cenusco sul Naviglio, Italy). Injection volume was 20 μL and the mobile phase, delivered at a
147 flow rate of 0.6 mL min^{-1} , was 1 mol/L potassium phosphate buffer pH 7.0 in isocratic
148 conditions. The detection wavelength was 220 nm. Catalase (250 kDa), glucose oxidase (160
149 kDa), lipoxidase (108 kDa), lysozyme (14.3 kDa) and insulin (5.8 kDa) (Sigma-Aldrich, USA)
150 were used as calibration standards. Peaks integration was performed by CHROM-CARD
151 software (v. 1.19).

152 **2.14. Data analysis**

153 All determinations were expressed as the mean \pm standard deviation (SD) of at least three
154 repeated measurements from two experiment replicates ($n = 2$). Statistical analysis was
155 performed by using R v. 2.15.0 (The R Foundation for Statistical Computing). Bartlett's test was
156 used to check the homogeneity of variance, one-way ANOVA was carried out and the Tukey test
157 was used to determine statistically significant differences among means ($p < 0.05$).

158 **3. Results and discussion**

159 Table 1 shows the chemical composition of okara obtained from the waste stream of soy milk
160 processing. Okara presented a high moisture content and was particularly rich in insoluble fibre,
161 proteins and lipids. Interestingly, it also contained significant amounts of polyphenols. Obtained
162 compositional data are in the range of proximal composition analysis reported in the literature
163 (Vong & Liu, 2016). The compositional variability of okara can be associated with the soybean
164 starting material characteristics used in the production of soy derivatives as well as to the process
165 applied during the soy milk production. In any case, significant quantities of valuable
166 compounds still remain in this by-product, mainly entrapped in the fibrous cellular material.

167 A 10 g/100 g okara aqueous dispersion was subjected to HPH by applying pressures up to 150
168 MPa and number of passes up to 5. Sample temperature increased with the treatment intensity up
169 to 63 °C (Supplementary Table S1), due to the mechanical stresses suffered by the sample during
170 the passage through the homogenization valve (Hayes & Kelly, 2003).

171 The visual observation of the samples revealed that the physical stability of HPH-treated
172 dispersions was higher than that of the untreated one, which immediately separated after
173 preparation. By contrast, after 1 day-storage, HPH-treated dispersions showed no evident phase
174 separation, with the only exception of the samples treated at 50 MPa, which showed a beginning
175 of phase separation (Supplementary Figure S1). However, all samples gradually revealed phase
176 separation within 30 days, except for the okara sample subjected to 5 passes at 150 MPa. Thus,
177 the stability of okara dispersion increased with the HPH intensity. These results can be attributed
178 to HPH-induced modifications of okara constituent structure.

179 To study HPH-induced modifications, the particle size distribution of samples was determined
180 (Figure 1). Dispersions treated at 50 MPa showed a trimodal distribution, with about 71%, 26%
181 and 3% of the particles presenting a mean diameter around 200 nm, 750 nm and 5000 nm,

182 respectively (Supplementary Table S2). The application of increasing pressure led to a
183 progressive particle downsizing with the disappearance of the intermediate peak, a reduction of
184 the largest particles and a concomitant increase of particles with 350 nm mean diameter. These
185 results agree with literature data (Augusto, Ibarz, & Cristianini, 2012; Song, Zhou, Fu, Chen, &
186 Wu, 2013) and can be attributed to the intense mechanical stresses delivered by HPH, able to
187 disrupt soy components. The disruptive ability of HPH can be well noted observing the
188 microscopy images of samples (Figure 2). Untreated okara dispersion showed a dense
189 microstructure with colloidal material dispersed throughout the aqueous environment. A portion
190 of this material is represented by partially denatured proteins. Okara is actually produced by
191 heating soybeans at 80 °C, which is a temperature higher than that required for thermal
192 denaturation of the main soy storage protein β -conglycinin (74-77 °C) (Wang, Qin, Sun, & Zhao,
193 2014). Untreated okara also showed clearly visible aggregates of fragmented fibrous cell
194 material. As reported by Preece et al. (2015), okara is composed of intact cotyledon cells, walls
195 of disrupted cells and other protein-polysaccharide agglomerated materials. In agreement with
196 literature data, these materials partially retained the original crystalline structures, as well
197 highlighted by polarized light microscopy images (Liu, Chien, & Kuo, 2013).

198 HPH treatment at 50 MPa induced the breakage of these large aggregates into smaller ones,
199 resulting in a more homogeneous particle dispersion (Figure 2). The further increase of
200 homogenization pressure caused a progressive reduction of dimension, number and crystallinity
201 of particles, possibly due to an increase of their solubility upon HPH (Figure 2).

202 To study the macroscopic effect of these microstructural changes, flow curves of okara
203 dispersions were determined. Data were elaborated with the Ostwald-de-Waele model ($R>0.94$)
204 and the estimated parameters are reported in Table 2. Except from sample treated at 50 MPa, all

205 samples exhibited a shear thinning flow behaviour ($n < 1$) and the application of more intense
206 treatments increased both consistency index (K) and apparent viscosity (η_{100}). Samples treated at
207 150 MPa for 5 passes revealed an apparent viscosity about 3 times higher than that of the sample
208 treated at 100 MPa. This result can be due to different phenomena. From one side, sample
209 viscosity can rise as a consequence of the increased system crowding, associated with the
210 progressively higher number of small particles; from the other side HPH-induced cell breakage is
211 expected to promote extraction of okara components, leading to a higher content of soluble
212 materials in the dispersions (Preece et al., 2017). To confirm this hypothesis and better
213 understand the nature of the extracted material, samples were analyzed for total (TDF), insoluble
214 (IDF) and soluble (SDF) dietary fibre (Table 3). TDF content decreased with the increase of
215 HPH intensity. A concomitant increase in the ratio between SDF and TDF was also observed.
216 The redistribution of fibres in favour of the soluble fraction has been reported for different
217 vegetable matrices subjected to homogenization treatments. To this regard, Chau et al. (2007)
218 and Hu, Zhang, Adhikari and Liu (2015) reported an increase in soluble/insoluble fibre ratio of
219 carrot pomace and wheat bran upon the application of microfluidization and high pressure
220 homogenization at 80 and 100 MPa, respectively. The observed changes in fibre content and
221 solubility (Table 3) can be attributed to the progressive rupture of the fibrous aggregates upon
222 HPH, favouring the solubilization of okara polysaccharides. This structure breakage was also
223 associated with a progressive pH decrease, which can be attributed to the release of organic acids
224 and polyphenols originally held in cotyledon cells (Table 3).

225 Moreover, protein extraction yield dramatically increased from 11 to about 90 g/100 g protein
226 with the increase of HPH pressure and number of passes (Table 4). Okara proteins are
227 represented by proteins that were not extracted during the soy milk process, due to their

228 entrapment in soybean cells or engagement in protein-fibre complexes. It is thus likely that HPH
229 allowed the release of proteins, due to the physical rupture of both cells and polymeric
230 complexes. Proteins were analyzed for conformational changes by determining the absorbance at
231 280 nm and free sulfhydryl (SH) group content (Table 4). Absorbance at 280 nm and free SH
232 groups of proteins in okara dispersions significantly increased with HPH pressure. This increase
233 is consistent with the change in protein content and conformation, resulting in increased
234 exposure of aromatic and SH groups of amino acids on the protein surface and in the rupture of
235 S-S bonds within protein molecules. However, the application of the most intense treatment (5
236 passes at 150 MPa) was associated with a decrease in both these indexes. This is generally
237 associated with reassembling phenomena of extracted proteins, probably by both inter- and intra-
238 molecular interactions (Yu, 2018). To confirm this hypothesis, okara dispersions were analyzed
239 by HPLC-gel permeation analysis (Table 4). The chromatogram relevant to untreated okara
240 dispersion showed 4 main protein fractions (19, 70, 110, 290 kDa). The most abundant protein
241 fraction (70 kDa) can be attributed to α and α' subunits of β -conglycinin (Cole & Cousin, 1994;
242 Stanojevic, Barac, Pesic, & Vucelic-Radovic, 2012). The fraction corresponding to 19 kDa can
243 be associated with the basic polypeptide of glycinin. The largest protein fraction (290 kDa) was
244 represented by soy 11S globulin which is made up of acid and alkaline sub-units (Chen, Liu, Wu,
245 & Ma, 2015). Finally, lipoxygenase was also present (110 kDa) (Cole & Cousin, 1994;
246 Stanojevic et al., 2012). HPH treatments resulted in a progressive area increase of peaks
247 corresponding to β -conglycinin, lipoxygenase and globulin, supporting the hypothesis of protein
248 release from the fibrous matrix upon HPH. However, in the samples subjected to 150 MPa for 1
249 and 5 passes, the polypeptide band of glycinin was no more present, suggesting its embedding
250 into multimeric aggregates. This result might be consistent with the occurrence of a new peak,

251 not observed in the untreated sample (166 kDa), probably resulting from protein reassembling, as
252 also suggested by the decrease in SH groups and absorbance at 280 nm.

253 **4. Conclusions**

254 Results obtained in this study highlighted that HPH can be used as an efficient tool to induce a
255 progressive disruption of okara native structure, leading to the release of entrapped proteins and
256 soluble fibres. HPH might thus be applied as a pretreatment to favour extraction of proteins and
257 fibres, allowing okara by-product to be turned into added-value ingredients for the food industry.
258 Moreover, the possibility to directly exploit HPH-treated okara dispersions to develop physically
259 stable soy-based beverages cannot be underestimated. The valorization of okara by its complete
260 re-use in novel functional products could actually represent an interesting market opportunity.
261 Although the case here presented was relevant to soy okara, obtained results could be easily
262 extended to by-products deriving from vegetable sources other than soybeans, largely broaden
263 their applicability and impact. This effort is worth making considering that HPH is being
264 increasingly introduced as processing operation in different industrial contexts, showing good
265 feasibility and cost-effectiveness.

266 **Conflict of interest**

267 The authors have declared no conflicts of interest.

268 **Acknowledgements**

269 S. Calligaris and L. Manzocco conceived the study in conjunction with M.C. Nicoli; G. Fayaz
270 carried out the experiments and, in conjunction with S. Plazzotta, wrote the first paper draft. All
271 authors participated in manuscript revision and discussion, coordinated and critiqued by S.
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346

347 **Figure Captions**

348 Figure 1. Particle size distribution of 10 g/100 g okara aqueous dispersions subjected to HPH
349 treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes.

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351 Figure 2. Optical and polarized light microscopy of 10 g/100 g untreated okara aqueous
352 dispersion and samples subjected to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa
353 with 5 passes.

Table 1. Chemical composition of soy okara

| Parameter | Amount |
|--|--------------|
| Moisture (g/100 g) | 76.22 ± 0.40 |
| Protein (g/100 g) | 6.53 ± 0.01 |
| Lipid (g/100 g) | 1.57 ± 0.06 |
| Total dietary fiber (g/100 g) | 12.50 ± 0.05 |
| Insoluble fiber (g/100 g) | 12.19 ± 0.04 |
| Soluble fiber (g/100 g) | 0.31 ± 0.01 |
| Total phenolic content (mg GAE/g dry matter) | 1.92 ± 0.04 |
| Ash (g/100 g) | 0.59 ± 0.05 |

Table 2. Flow behavior index (n), apparent viscosity at 100 s^{-1} (η_{100}), consistency index (K) of 10 g/100 g okara aqueous dispersions subjected to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes.

| HPH treatment | n | η_{100} (Pa's) | K (Pa's ⁿ) |
|------------------|---------------------|---------------------|--------------------------|
| Untreated | n.a. | | |
| 50 MPa | 1.054 ± 0.031^a | 0.001 ± 0.00^c | 0.001 ± 0.0^c |
| 100 MPa | 0.512 ± 0.022^b | 0.010 ± 0.001^b | 0.111 ± 0.020^b |
| 150 MPa | 0.494 ± 0.001^b | 0.012 ± 0.000^b | 0.140 ± 0.003^b |
| 150 MPa-5 passes | 0.309 ± 0.025^c | 0.029 ± 0.000^a | 0.803 ± 0.104^a |

Data points Means \pm SD ($n = 2$); n.a. not analyzed since immediately separating; ^{a, b, c} In the same column, means indicated by different letters are significantly different ($p < 0.05$).

Table 3. pH, total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) /TDF ratio and total phenolic compounds (TDC) of 10 g/100 g untreated okara aqueous dispersion and samples subjected to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes.

| HPH treatment | pH | TDF (g/100 g dm) | IDF/TDF (g/100 g fiber) | SDF/TDF (g/100 g fiber) | TPC (mg GAE/ g dm) |
|------------------|--------------------------|----------------------------|----------------------------|----------------------------|--------------------------|
| Untreated | 8.29 ± 0.06 ^a | 52.57 ± 0.18 ^a | 97.51 ± 0.061 ^a | 2.49 ± 0.06 ^c | 2.58 ± 0.03 ^c |
| 50 MPa | 8.22 ± 0.02 ^a | 51.49 ± 2.45 ^a | 96.37 ± 0.30 ^{ab} | 3.63 ± 0.30 ^{bc} | 5.08 ± 0.06 ^b |
| 100 MPa | 8.11 ± 0.04 ^a | 45.82 ± 1.06 ^{ab} | 94.77 ± 0.99 ^b | 5.23 ± 0.99 ^b | 5.11 ± 0.05 ^b |
| 150 MPa | 7.90 ± 0.05 ^b | 47.56 ± 3.14 ^{ab} | 94.93 ± 0.42 ^b | 5.07 ± 0.42 ^b | 5.31 ± 0.05 ^b |
| 150 MPa-5 passes | 7.67 ± 0.02 ^c | 41.82 ± 2.52 ^b | 89.28 ± 0.32 ^a | 10.72 ± 0.32 ^a | 8.08 ± 0.35 ^a |

Data points Means ± SD (n = 2); ^{a, b, c, d} In the same column, means indicated by different letters are significantly different (p<0.05).

Table 4. Protein extraction yield, absorbance at 280 nm, free sulfhydryl groups and peak areas relevant to proteins with a molecular weight of 19, 70, 110, 166 and 290 kDa of 10 g/100 g untreated okara aqueous dispersion and samples subjected to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes.

| HPH treatment | Protein extraction yield (g/100 g protein) | Absorbance at 280 nm | Free sulfhydryl groups ($\mu\text{molL}^{-1}\text{g}^{-1}$) | Peak area of proteins with different MW (arbitrary absorbance unit $\times 10^4$) | | | | |
|------------------|--|--------------------------------|---|--|-------------------------------|--------------------------------|-------------------------------|--------------------------------|
| | | | | 19 kDa | 70 kDa | 110 kDa | 166 kDa | 290 kDa |
| Untreated | 11.49 \pm 0.19 ^d | 0.224 \pm 0.014 ^d | 15.77 \pm 0.47 ^c | 51.7 \pm 16.5 ^b | 189.7 \pm 31.0 ^b | 53.1 \pm 0.3 ^c | n.d. | 54.9 \pm 0.2 ^d |
| 50 MPa | 37.11 \pm 1.09 ^c | 0.428 \pm 0.002 ^c | 51.42 \pm 2.93 ^a | 886.8 \pm 96.81 ^a | 168.1 \pm 42.7 ^b | 200.4 \pm 42.7 ^{bc} | n.d. | 321.1 \pm 8.3 ^{bc} |
| 100 MPa | 61.14 \pm 1.16 ^b | 0.653 \pm 0.004 ^a | 51.67 \pm 3.74 ^a | 672.6 \pm 54.9 ^a | 63.9 \pm 29.9 ^b | 125.4 \pm 33.4 ^c | n.d. | 182.9 \pm 76.5 ^{cd} |
| 150 MPa | 65.94 \pm 2.70 ^b | 0.646 \pm 0.006 ^a | 47.49 \pm 0.87 ^a | n.d. | 290.4 \pm 82.4 ^b | 374.7 \pm 82.4 ^b | 111.3 \pm 15.5 ^a | 433.8 \pm 64.4 ^b |
| 150 MPa-5 passes | 89.69 \pm 2.24 ^a | 0.577 \pm 0.002 ^b | 20.70 \pm 1.13 ^b | n.d. | 830.9 \pm 3.3 ^a | 620.3 \pm 3.3 ^a | 907.7 \pm 11.9 ^a | 831.0 \pm 63.0 ^a |

Data points Means \pm SD (n = 2); MW molecular weight; n.d. not detected; ^{a, b, c, d} In the same column, means indicated by different letters are significantly different (p<0.05).

Figure 1

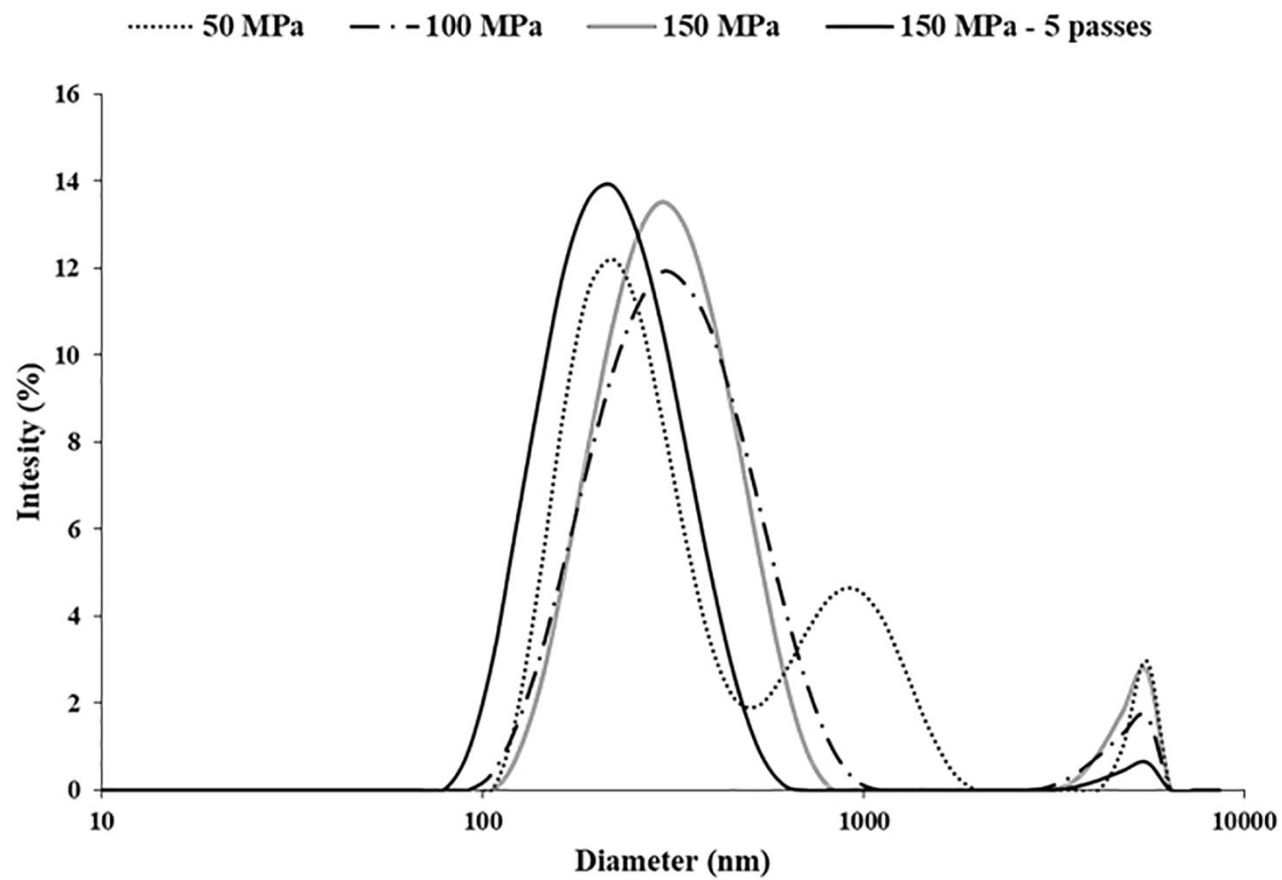
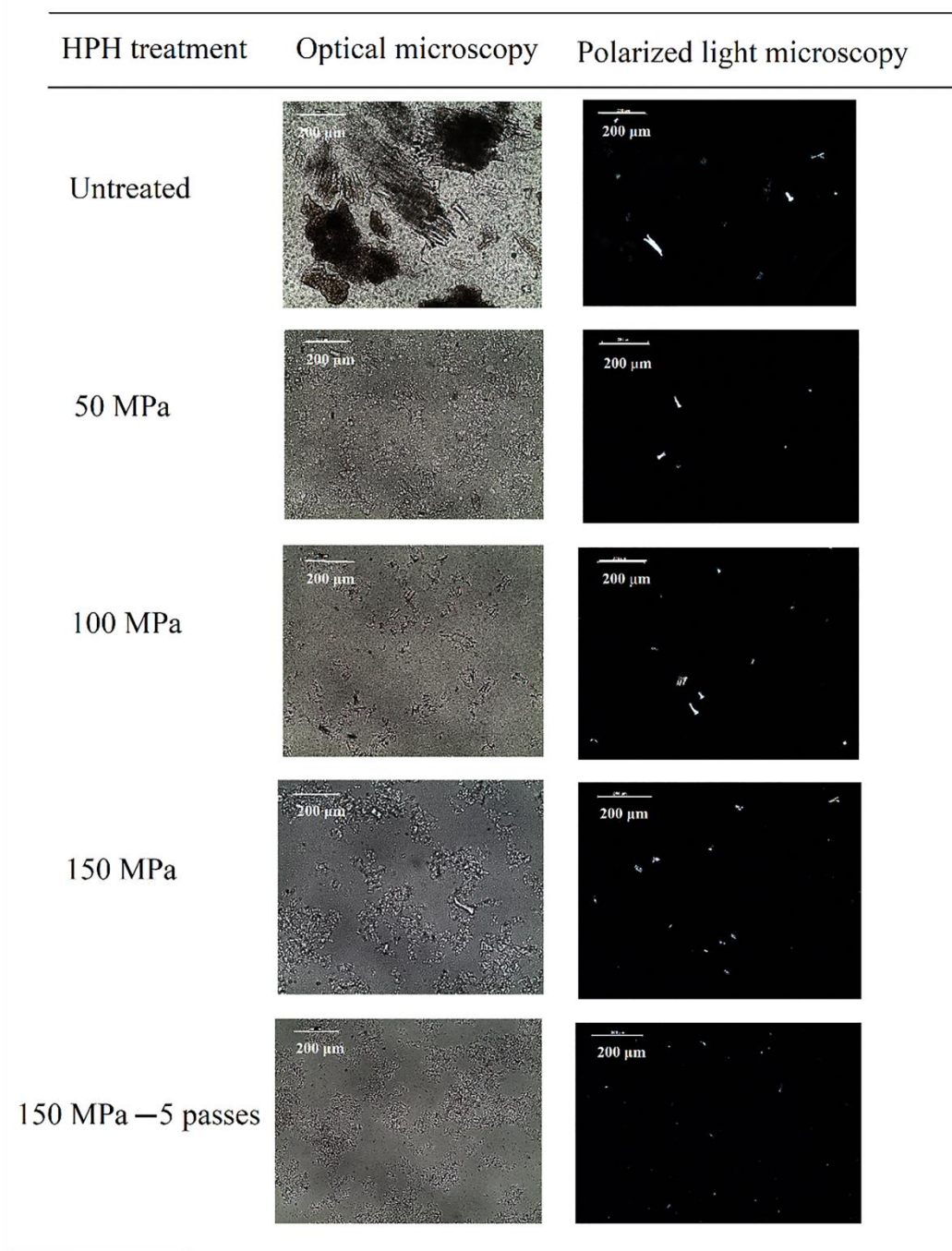


Figure 2



Supplementary Material

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Supplementary Material

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