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

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Corresponding Author: Dr. Stella Plazzotta, Ph.D.

Corresponding Author's Institution: University of Udine

First Author: Goly Fayaz, Ph.D.

Order of Authors: Goly Fayaz, Ph.D.; Stella Plazzotta, Ph.D.; Sonia Calligaris, Professor; Lara Manzocco, Professor; Maria Cristina Nicoli, Professor

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.

Cover Letter

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).

- 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

1   **Impact of high pressure homogenization on physical properties, extraction yield and biopolymer**

2   **structure of soybean okara**

3

4   Goly FAYAZ, Stella PLAZZOTTA*, Sonia CALLIGARIS, Lara MANZOCCO, Maria Cristina

5   NICOLI

6

7   *Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine,*

8   *Italy*

9

10   *e-mail: stella.plazzotta@uniud.it; Tel: +39 0432-558137

11   **Abstract**

12   The effect of high pressure homogenization (HPH) on soy okara was studied. To this purpose,

13   okara dispersions (10 g/100 g) were subjected to 1 pass at 50, 100 and 150 MPa and to 5 passes

14   at 150 MPa. Samples were analyzed for stability, particle size, microstructure, and viscosity.

15   Results highlighted that the increase of HPH intensity was associated with the structural

16   disruption of okara particles, leading to physically stable homogenates having increasing

17   viscosity. This was mainly attributed to an increase in okara solubility, due to fibre and protein

18   release. The latter resulted almost complete, reaching values up to 90% of the protein originally

19   entrapped in okara matrix. Absorbance at 280 nm, SH groups and dimension of proteins revealed

20   that HPH treatments favoured the extraction of the main protein fractions even if, at the higher

21   intensity level, extracted proteins probably underwent conformational changes and reassembling

22   phenomena.

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

2

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**

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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 ºC 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 ºC) 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-Ciocalteau

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 ºC 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 173º, 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    $\eta_{app} = K \cdot \dot{\gamma}^{n-1}$ 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   s^n) 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 $12000 \times g$ 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 $12000 \times g$ for 10 min at 4 ºC. 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 (μmolL^{-1}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 Å 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 ± 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

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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,

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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.

274 **References**

275 AOAC. (1997). *Official Methods of Analysis. Washington, DC: Association of Official*

276   *Analytical Chemists.*

277 Augusto, P. E. D., Ibarz, A., & Cristianini, M. (2012). Effect of high pressure homogenization

278   (HPH) on the rheological properties of tomato juice: Time-dependent and steady-state

279   shear. *Journal of Food Engineering*, *111*, 570–579.

280 Augusto, P. E. D., Ibarz, A., & Cristianini, M. (2013). Effect of high pressure homogenization

281   (HPH) on the rheological properties of tomato juice: Viscoelastic properties and the Cox-

282   Merz rule. *Journal of Food Engineering*, *114*, 57–63.

283 * Chau, C. F., Wang, Y. T., & Wen, Y. L. (2007). Different micronization methods significantly

284   improve the functionality of carrot insoluble fibre. *Food Chemistry*, *100*, 1402–1408. (This

285   paper elucidates the effects of HPH on the properties of vegetable fibres)

286 Chen, L., Liu, D., Wu, G., & Ma, Y. (2015). Soy 11S globulin acid subunits as the novel food

287   polymer carrier. *International Journal of Polymer Science*, *2015*, 1–9.

288 Cole, K. D., & Cousin, S. L. (1994). Size exclusion chromatography. *Analytical Chemistry*, *66*,

289   595R–620R.

290 * Floury, J., Bellettre, J., Legrand, J., & Desrumaux, A. (2004). Analysis of a new type of high

291   pressure homogeniser. A study of the flow pattern. *Chemical Engineering Science*, *59*, 843–

292   853. (This paper gives valuable information about the main mechanisms of HPH)

293 Grizotto, R. K., Rufi, C. R. G., Yamada, E. A., & Vicente, E. (2010). Evaluation of the quality of

294   a molded sweet biscuit enriched with okara flour. *Food Science and Technology*

295   *(Campinas)*, *30*, 270–275.

296 Hayes, M. G., & Kelly, A. L. (2003). High pressure homogenisation of raw whole bovine milk

13

297   (a) effects on fat globule size and other properties. *Journal of Dairy Research*, *70*, 297-305.

298   Hu, R., Zhang, M., Adhikari, B., & Liu, Y. (2015). Effect of homogenization and ultrasonication

299   on the physical properties of insoluble wheat bran fibres. *International Agrophysics*, *29*,

300   423–432.

301   Huang, C. C., Chen, Y. F., & Wang, C. C. R. (2010). Effects of micronization on the physico-

302   chemical properties of peels of three root and tuber crops. *Journal of the Science of Food*

303   *and Agriculture*, *90*, 759–763.

304   Jiménez-Escrig, A., Alaiz, M., Vioque, J., & Rupérez, P. (2010). Health-promoting activities of

305   ultra-filtered okara protein hydrolysates released by in vitro gastrointestinal digestion:

306   Identification of active peptide from soybean lipoxygenase. *European Food Research and*

307   *Technology*, *230*, 655–663.

308   Liu, H. H., Chien, J. T., & Kuo, M. I. (2013). Ultra high pressure homogenized soy flour for tofu

309   making. *Food Hydrocolloids*, *32*, 278–285.

310   Lopez-Sanchez, P., Svelander, C., Bialek, L., Schumm, S., & Langton, M. (2011). Rheology and

311   microstructure of carrot and tomato emulsions as a result of high-pressure homogenization

312   conditions. *Journal of Food Science*, *76*, E130- E140.

313   * O'Toole, D. K. (1999). Characteristics and use of okara, the soybean residue from soymilk

314   production − a review. *Journal of Agricultural and Food Chemistry*, *47*, 363–371. (This

315   paper reviews traditional okara valorisation strategies)

316   Panozzo, A., Manzocco, L., Lippe, G., & Nicoli, M. C. (2016). Effect of pulsed light on structure

317   and immunoreactivity of gluten. *Food Chemistry*, *194*, 366-372.

318   Preece, K. E., Drost, E., Hooshyar, N., Krijgsman, A., Cox, P. W., & Zuidam, N. J. (2015).

319   Confocal imaging to reveal the microstructure of soybean processing materials. *Journal of*

320   *Food Engineering*, *147*, 8–13.

321   * Preece, K. E., Hooshyar, N., Krijgsman, A. J., Fryer, P. J., & Zuidam, N. J. (2017).

322   Intensification of protein extraction from soybean processing materials using hydrodynamic

323   cavitation. *Innovative Food Science and Emerging Technologies*, *41*, 47–55. (This paper

324   gives valuable information about the effect of HPH on protein extraction from soybeans)

325   Rado, O., & Dimi, E. (2010). Valorization of wet okara, a value-added functional ingredient, in a

326   coconut-based baked snack. *Cereal Chemistry*, *30*, 256–262.

327   Singleton, V., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-

328   phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

329   * Song, X., Zhou, C., Fu, F., Chen, Z., & Wu, Q. (2013). Effect of high-pressure homogenization

330   on particle size and film properties of soy protein isolate. *Industrial Crops and Products*,

331   *43*, 538–544. (This paper presents the effects of HPH on selected physical and functional

332   properties of soy protein isolate)

333   Stanojevic, S. P., Barac, M. B., Pesic, M. B., & Vucelic-Radovic, B. V. (2012). Composition of

334   proteins in okara as a byproduct in hydrothermal processing of soy milk. *Journal of

335   Agricultural and Food Chemistry*, *60*, 9221–9228.

336   Tan, J., & Kerr, W. L. (2015). Rheological properties and microstructure of tomato puree subject

337   to continuous high pressure homogenization. *Journal of Food Engineering*, *166*, 45–54.

338   Vong, W. C., & Liu, S. Q. (2016). Biovalorisation of okara (soybean residue) for food and

339   nutrition. *Trends in Food Science and Technology*, *52*, 139–147.

340   Wang, T., Qin, G. X., Sun, Z. W., & Zhao, Y. (2014). Advances of research on glycinin and β-

341   conglycinin: a review of two major soybean allergenic proteins. *Critical Reviews in Food

342   Science and Nutrition*, *54*, 850–862.

343   Yu, C. (2018). Effects of high-pressure homogenisation on structural and functional properties of

344   mussel (Mytilus edulis) protein isolate. *International Journal of Food Science and*

345   *Technology*, 53, 1157–1165.

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.

350

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 $\pm$ 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 ($\mu molL^{-1}g^{-1}$)	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 ± 0.19^d	0.224 ± 0.014^d	15.77 ± 0.47^c	51.7 ± 16.5^b	189.7 ± 31.0^b	53.1 ± 0.3^c	n.d.	54.9 ± 0.2^d
50 MPa	37.11 ± 1.09^c	0.428 ± 0.002^c	51.42 ± 2.93^a	886.8 ± 96.81^a	168.1 ± 42.7^b	200.4 ± 42.7^{bc}	n.d.	321.1 ± 8.3^{bc}
100 MPa	61.14 ± 1.16^b	0.653 ± 0.004^a	51.67 ± 3.74^a	672.6 ± 54.9^a	63.9 ± 29.9^b	125.4 ± 33.4^c	n.d.	182.9 ± 76.5^{cd}
150 MPa	65.94 ± 2.70^b	0.646 ± 0.006^a	47.49 ± 0.87^a	n.d.	290.4 ± 82.4^b	374.7 ± 82.4^b	111.3 ± 15.5^a	433.8 ± 64.4^b
150 MPa-5 passes	89.69 ± 2.24^a	0.577 ± 0.002^b	20.70 ± 1.13^b	n.d.	830.9 ± 3.3^a	620.3 ± 3.3^a	907.7 ± 11.9^a	831.0 ± 63.0^a

Data points Means ± 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).
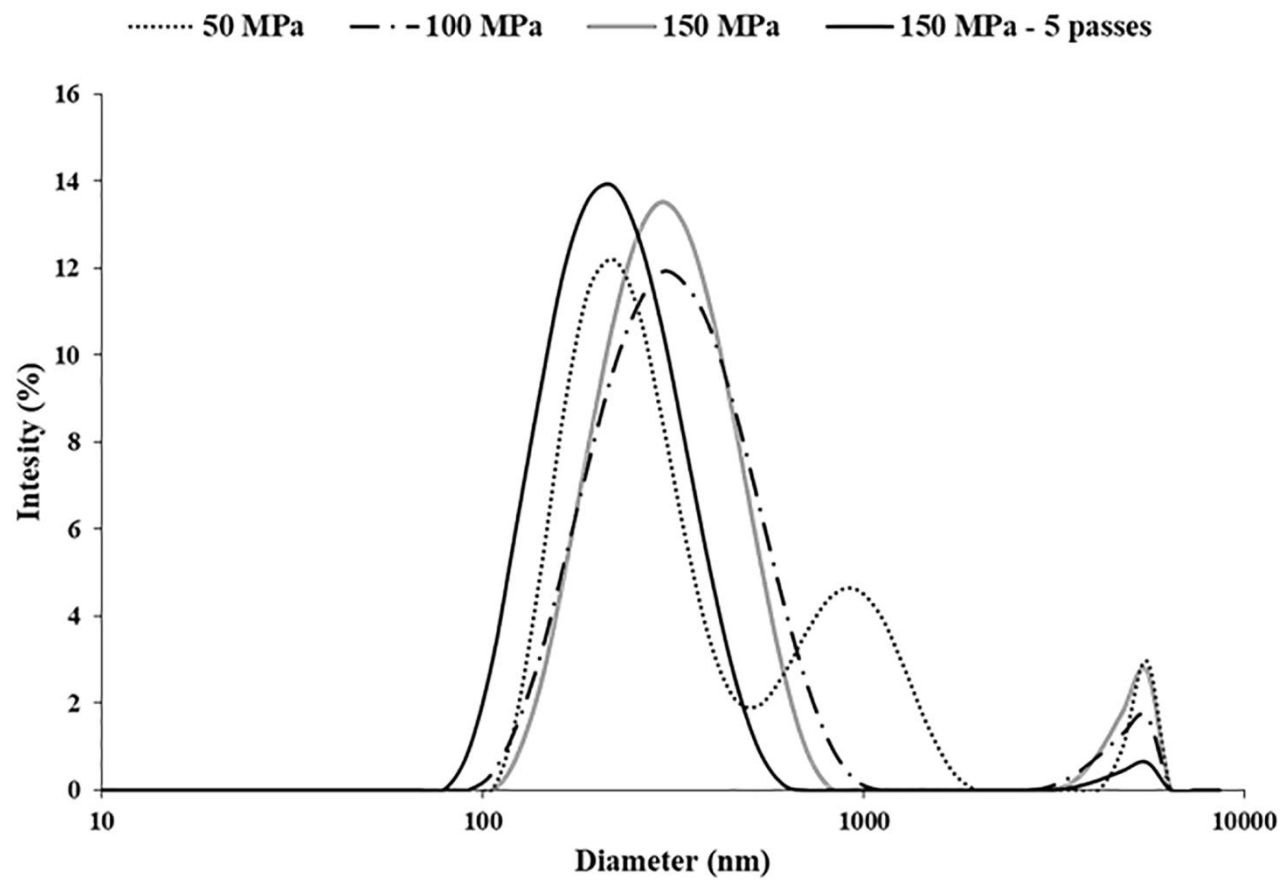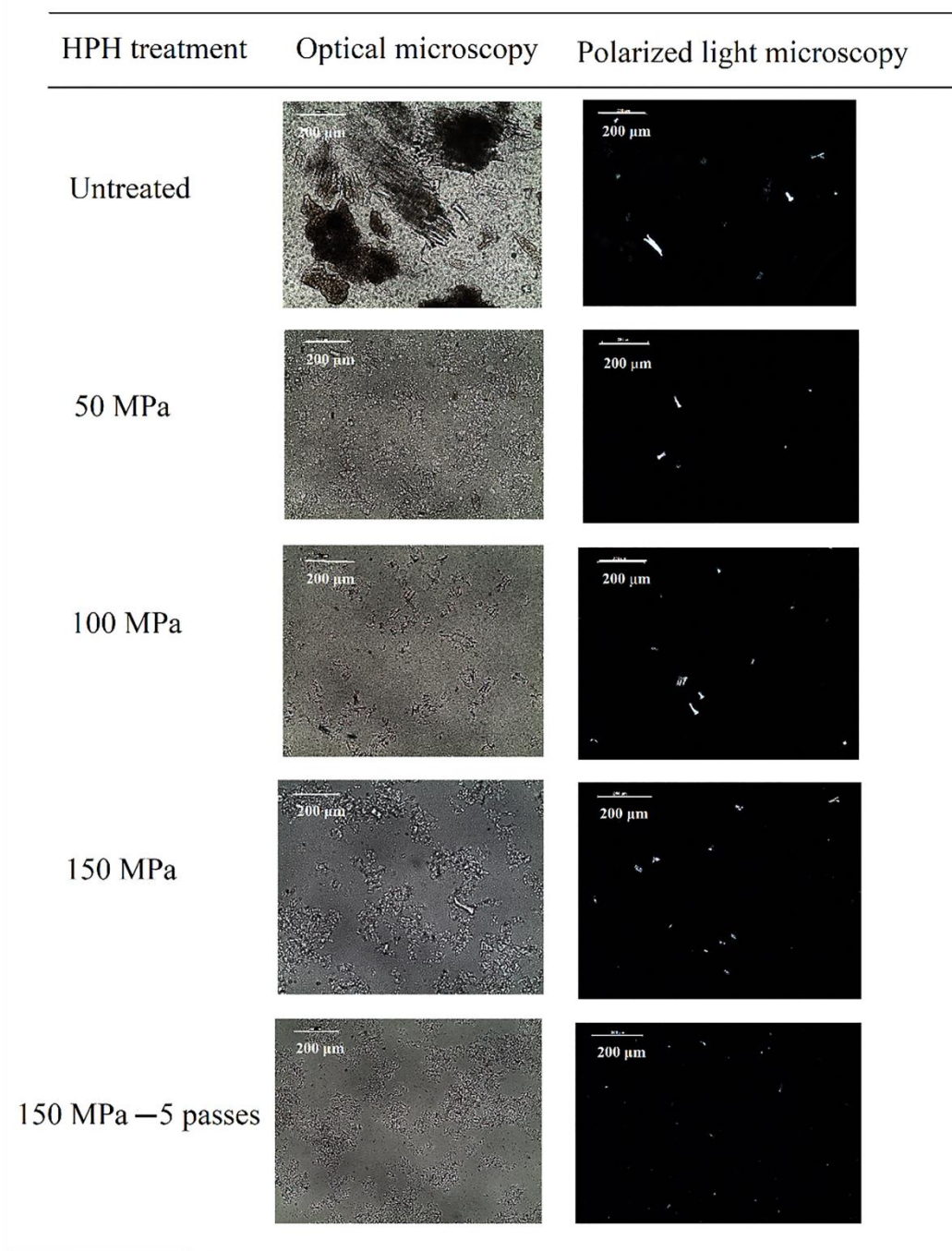
Figure

Figure 1

Figure

Figure 2

HPH treatment	Optical microscopy	Polarized light microscopy
Untreated		
50 MPa		
100 MPa		
150 MPa		
150 MPa —5 passes		

Supplementary Material

Supplementary Material

Conflict of Interest and Authorship Conformation Form

The Authors declare that:

- ✓   All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

- ✓   This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

- ✓   The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript