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

Original

Availability:
This version is available http://hdl.handle.net/11390/1152882 since 2020-03-10T15:50:38Z

Publisher:

Published
DOI:10.1016/j.lwt.2019.108324

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(Article begins on next page)

12 October 2024

Elsevier Editorial System(tm) for LWT - Food Science and Technology

Manuscript Draft

Manuscript Number: LWT-D-19-01686R2

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

Article Type: Research paper

Keywords: Soybean residue; Vegetable by-products; Waste valorization; Protein; Fiber

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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.

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).

***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

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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.

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Material

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

2.2. Preparation of okara dispersion

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

2.3. High pressure homogenization (HPH)

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

2.4. Chemical composition

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

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

2.5. Physical Stability

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

2.6. pH measurement

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

2.7. Particle size distribution

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

2.8. Optical and polarized light microscopy

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

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

2.9. Viscosity

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

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

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

2.10. Protein extraction yield

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

2.11. Absorbance at 280 nm

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

2.12. Determination of free sulfhydryl group content

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

2.13. HPLC-gel permeation analysis

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

2.14. Data analysis

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

3. Results and discussion

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

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

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

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

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

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

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

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

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

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

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

4. Conclusions

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

Conflict of interest

The authors have declared no conflicts of interest.

Acknowledgements

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

Authors are grateful to Deborah Lizzi and Anna De Monte for contributing to analyses.

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Figure Captions

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

Figure 2. Optical and polarized light microscopy 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.

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 ± 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 ($p<0.05$).

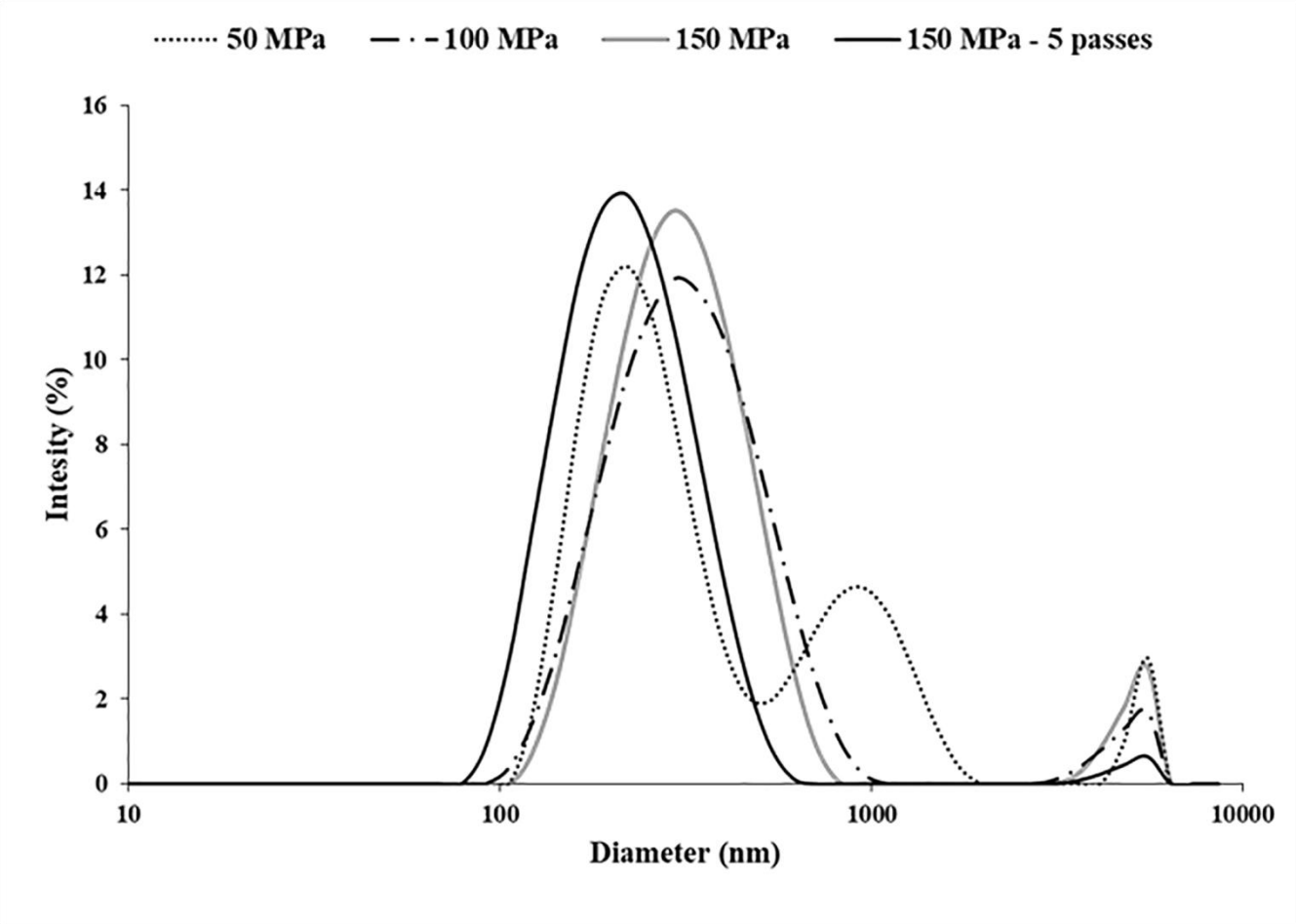
Table 4. Protein extraction yield, absorbance at 280 nm, free sulfhydryl groups and peak areas relevant to proteins with a molecular weight of 19, 70, 110, 166 and 290 kDa of 10 g/100 g untreated okara aqueous dispersion and samples subjected to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa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

HPH treatment	Protein extraction yield (g/100 g protein)	Absorbance at 280 nm	Free sulfhydryl groups ($\mu mol L^{-1} g^{-1}$)	Peak area of proteins with different MW (arbitrary absorbance unit × 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 ($p<0.05$).

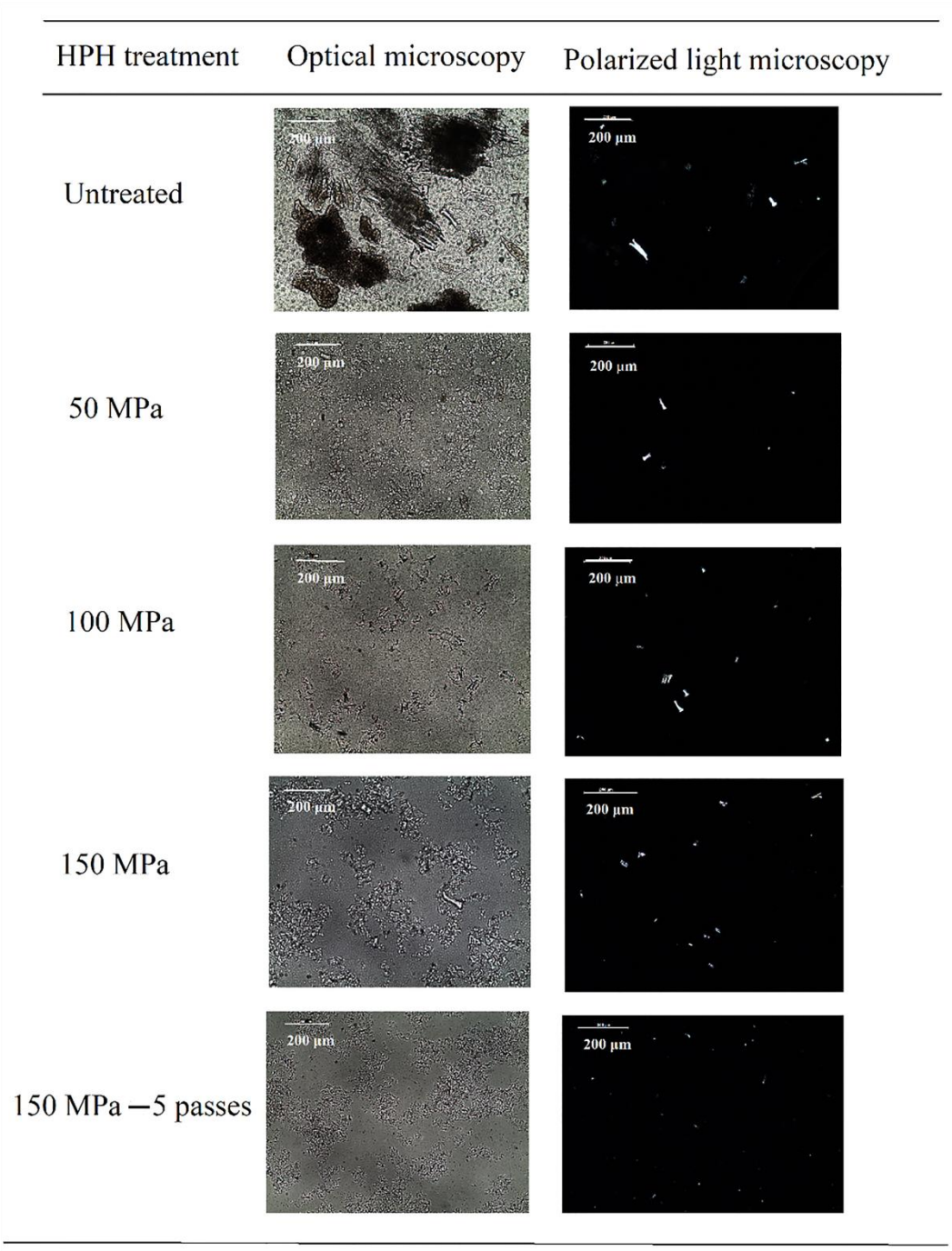
Figure

Figure 1



Figure

Figure 2



Supplementary Material

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***Conflict of Interest Form**

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.

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