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Physical, chemical, and techno-functional properties of soy okara powders obtained by high pressure homogenization and alkaline-acid recovery

*Original*

*Availability:*

This version is available <http://hdl.handle.net/11390/1207247> since 2025-01-15T11:53:59Z

*Publisher:*

*Published*

DOI:10.1016/j.fbp.2021.04.017

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# Food and Bioproducts Processing

## Physical, chemical, and techno-functional properties of soy okara powders obtained by high pressure homogenization and alkaline-acid recovery

--Manuscript Draft--

<b>Manuscript Number:</b>	
<b>Article Type:</b>	Full Length Article
<b>Keywords:</b>	waste valorisation; food by-products; protein structure; foaming and emulsifying properties; protein food ingredients
<b>Corresponding Author:</b>	Sonia Calligaris  ITALY
<b>First Author:</b>	Stella Plazzotta, PhD
<b>Order of Authors:</b>	Stella Plazzotta, PhD  Martina Moretton  Sonia Calligaris, Prof.  Lara Manzocco, Prof.
<b>Abstract:</b>	High pressure homogenization (HPH) at increasing intensity (50, 100, 150 MPa and 150 MPa for 5 passes) was combined with an alkaline-acid recovery method to prepare protein-enriched okara powders intended as ingredients with improved techno-functional properties. The treatment allowed obtaining from 4 to 8 g powder per 100 g fresh okara. The intensity of HPH treatment significantly affected amount, composition, particle size, protein structure and techno-functional properties. Treatments at intermediate pressures in the range 50-100 MPa resulted the most feasible from an industrial perspective allowing to obtain a good balance between protein content and the techno-functionalities (e.g. solubility, foaming, gelling and emulsifying properties) of the okara ingredient. Thus, HPH combined with simple pH-driven precipitation can be considered an efficacious and industrially feasible pre-treatment for okara valorisation into innovative ingredients.
<b>Suggested Reviewers:</b>	Francesca Bot University College Cork francesca.bot@ucc.ie Expert in high pressure processing of vegetable products  Antonio Vicente University of Minho avicente@deb.uminho.pt Expert in food protein analysis  Francesco Donsi University of Salerno: Università degli Studi di Salerno fdonsi@unisa.it Expert in high pressure food processing  Nataša Poklar University of Ljubljana natasa.poklar@bf.uni-lj.si Expert in component recovery

Dear Editor,

we send to your attention the research article "**Physical, chemical, and techno-functional properties of soy okara powders obtained by high pressure homogenization and alkaline-acid recovery**" by Stella Plazzotta, Martina Moretton, Sonia Calligaris and Lara Manzocco. All the authors have read and approved the manuscript.

The research for waste valorisation into innovative ingredients has received increasing attention in recent years. In this context, soy okara, the waste material generated from soy-milk production, is a good candidate being still rich in proteins and other valuable components. Based on these considerations, in this study, we explored the use of high pressure homogenization (HPH) process followed by an alkaline-acid treatment to prepare protein-enriched powders from the soy okara with improved techno-functionalities. To this end, okara dispersions were subjected to HPH treatments at increasing intensity and an alkaline-acid method to obtain protein-enriched powders. The latter were fully characterised for physical, chemical and techno-functional properties (solubility, foaming, gelling and emulsifying ability).

The authors believe that the current research article may significantly contribute to shed light on the sustainable valorization of food wastes as sources of innovative food-grade ingredients for the food industry.

We hope that this article could satisfy the requirements of Food and Bioproduct Processing so that you might consider it for publication in this Journal.

We suggest the following reviewers:

- Francesca Bot  
francesca.bot@ucc.ie  
Expert in high pressure processing of vegetable products  
Food Ingredients Research Group, School of Food and Nutritional Sciences, University College Cork (UCC), Cork, Ireland
- Antonio Vicente  
avicente@deb.uminho.pt  
Expert in food protein analysis  
FIT Research Group, Industry and Processes Laboratory, Centre of Biological Engineering, University of Minho, Braga, Portugal
- Francesco Donsì

fdonsi@unisa.it

Expert in high pressure food processing

Dipartimento di Ingegneria Industriale/DIIN, Università of Salerno, Salerno, Italy

- Nataša Poklar

natasa.poklar@bf.uni-lj.si

Expert in component recovery

Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Best regards,

Prof. Sonia Calligaris, PhD

Section of Food Chemistry and Technology

Department of Agricultural, Food, Environmental and Animal Sciences

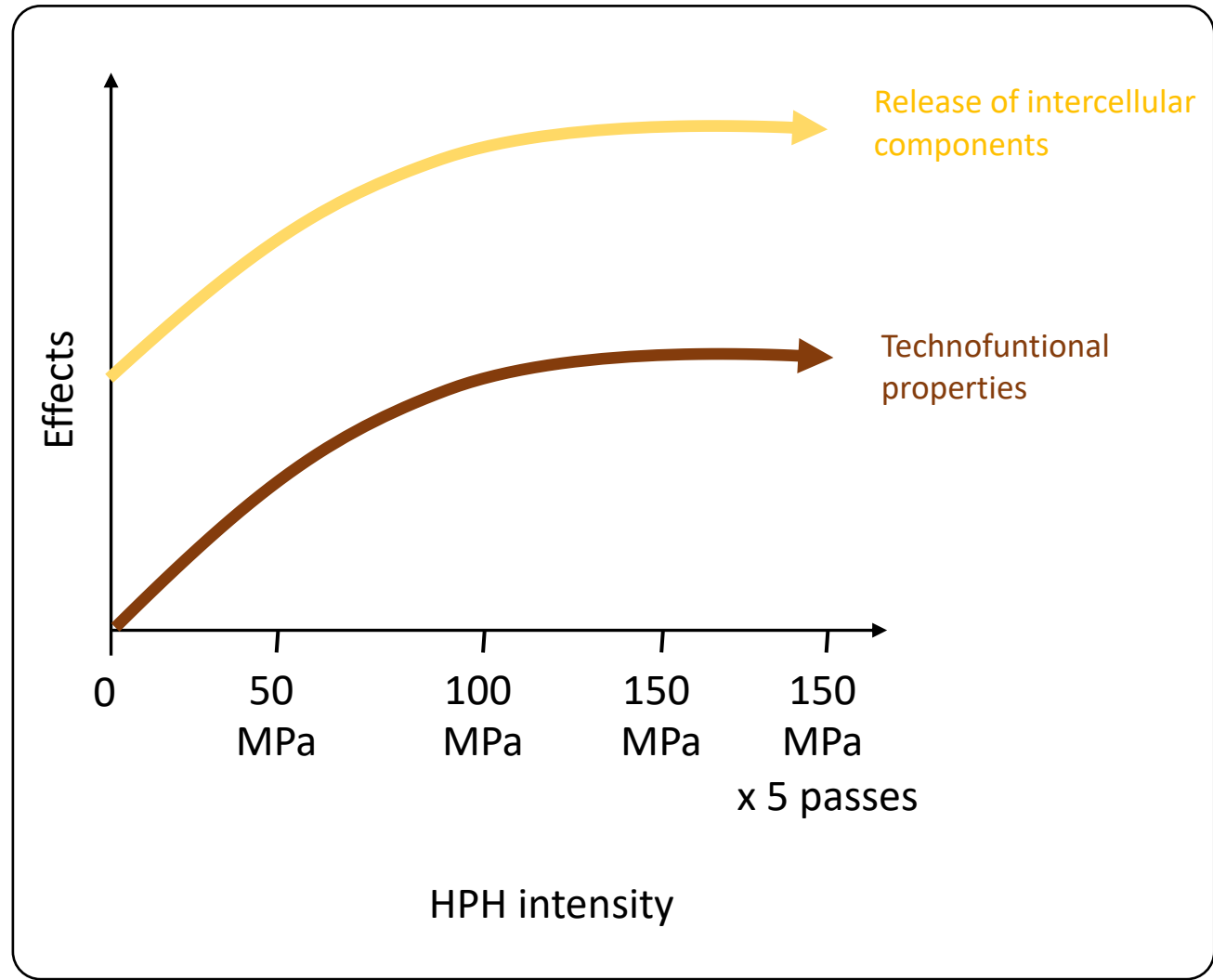
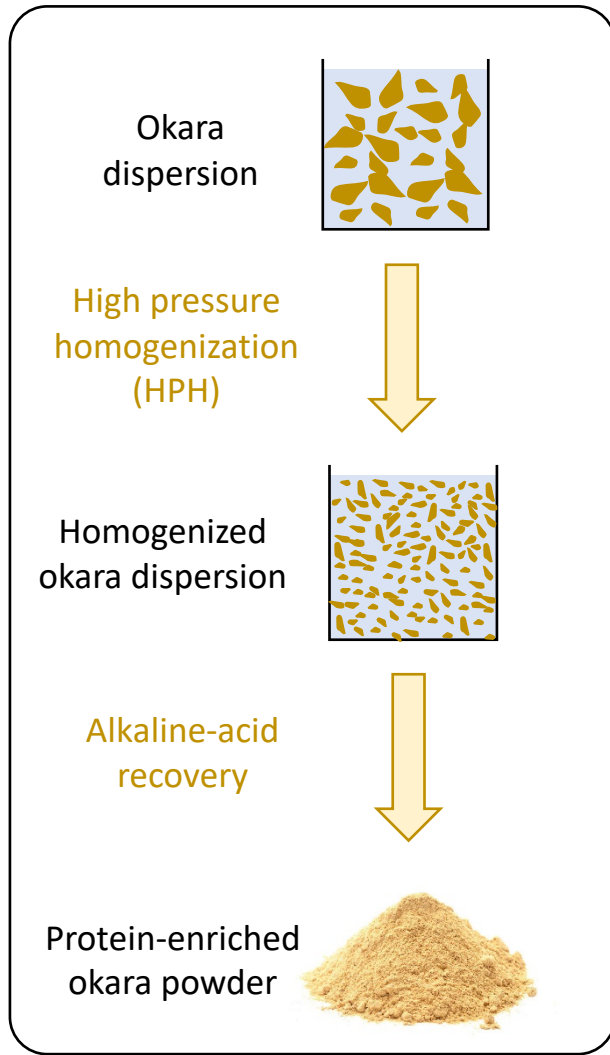
University of Udine

Via Sondrio 2/A

33100 Udine, Italy

## **Highlights**

- Okara powders can be obtained by HPH, alkaline-acid recovery and drying
- Okara powders obtained by 50-100 MPa HPH presented more than 67% protein content
- 50-100 MPa HPH enhances okara powder foaming, gelling and emulsifying properties
- HPH favours local interaction between okara proteins and carbohydrates



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4 **Physical, chemical, and techno-functional properties of soy okara powders obtained by high**  
5 **pressure homogenization and alkaline-acid recovery**  
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9 Stella Plazzotta <sup>a</sup>, Martina Moretton <sup>a</sup>, Sonia Calligaris <sup>a\*</sup>, Lara Manzocco <sup>a</sup>

10  
11 <sup>a</sup> Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via  
12 Sondrio 2/A, 33100 Udine, Italy  
13

14 \*corresponding author: Sonia Calligaris, Department of Agricultural, Food, Environmental and  
15 Animal Sciences, University of Udine, Via Sondrio 2/A, 33100 Udine, Italy; Tel: +39 0432-558571;  
16  
17 e-mail: sonia.calligaris@uniud.it;  
18

19 authors e-mails: stella.plazzotta@uniud.it; moretton.martina@spes.uniud.it; lara.manzocco@uniud.it  
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22  
23 **Abstract**

24 High pressure homogenization (HPH) at increasing intensity (50, 100, 150 MPa and 150 MPa for 5  
25 passes) was combined with an alkaline-acid recovery method to prepare protein-enriched okara  
26 powders intended as ingredients with improved techno-functional properties. The treatment allowed  
27 obtaining from 4 to 8 g powder per 100 g fresh okara. The intensity of HPH treatment significantly  
28 affected amount, composition, particle size, protein structure and techno-functional properties.  
29 Treatments at intermediate pressures in the range 50-100 MPa resulted the most feasible from an  
30 industrial perspective allowing to obtain a good balance between protein content and the techno-  
31 functionalities (e.g. solubility, foaming, gelling and emulsifying properties) of the okara ingredient.  
32 Thus, HPH combined with simple pH-driven precipitation can be considered an efficacious and  
33 industrially feasible pre-treatment for okara valorisation into innovative ingredients.  
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43 **Keywords:** waste valorisation; food by-products; protein structure; foaming and emulsifying  
44 properties; protein food ingredients.  
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48 **Abbreviations**<sup>1</sup>  
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54 <sup>1</sup> **Abbreviations:** HPH: high pressure homogenization; BCA: bichinchonic acid method; WR: working reagent; FTIR:  
55 Fourier transform infrared spectroscopy; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SH:  
56 sulfhydryl groups; A<sub>412</sub>: absorbance at 412 nm; C: sample concentration (mg extract/mL); D: dilution factor; W<sub>0</sub>: initial  
57 sample weight; W<sub>1</sub>: dried sample weight; FAI: foaming activity index; FSI: foaming stability index; V<sub>0</sub>: initial liquid phase  
58 volume; V<sub>1</sub>: volume of foam monitored every 10 min; G': storage modulus; G'': loss modulus.  
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## **Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.



## 1 Introduction

Processing of soybeans into soymilk, tofu, protein isolates and oil generates a by-product called “okara” (Ma et al., 1997). Similar to other plant waste, okara contains about 50% fibre, 30% proteins and 9% lipids (Mateos-Aparicio et al., 2010), but it is currently used for animal feeding, composted into fertilizers or landfilled (O’Toole, 1999). In these ways, its valuable protein fraction is underutilized or lost (Papargyropoulou et al., 2014). Okara protein fraction actually presents interesting nutritional quality, due to the high essential aminoacid content (Chan and Ma, 1999), which makes this waste material a potential exploitable source of low-cost protein for human consumption (Ma et al., 1997). Based on these considerations, research efforts have been recently concentrated on maximizing the extraction of the protein fraction that remains entrapped in the non-starch matrix of the plant cell walls and into the intracellular cotyledons (Preece et al., 2017b, 2015). This approach is still a challenge since it requires not only the application of proper techniques to disrupt the compact okara matrix but also of downstream processing to recovery and purify the protein fraction (Cai et al., 2020; Tao et al., 2019). As regards disintegration of plant cellular integrity, different techniques, including sub- and supercritical fluid extraction, ultrasounds, microwave, pulsed electric fields and high pressure homogenization (HPH), have been proposed (Barba et al., 2015; Pojić et al., 2018). Among them, HPH has been indicated as particularly promising to release proteins from okara (Fayaz et al., 2019; Preece et al., 2017a). During the process, the treated fluid is forced through a narrow homogenizing valve, suffering pressures from 50 to 150 MPa and intense mechanical stresses that cause the downsizing of suspended particles (Paquin, 1999).

The application of HPH to soy-okara has been demonstrated to be non-selective, causing the release not only of the desired protein fraction but also of other compounds, apparently less interesting, such as polysaccharides (Preece et al., 2017a). In addition, beside favouring extraction of okara constituents, HPH might also modify their structure (Fayaz et al., 2019; Tao et al., 2019). For instance, in the case of proteins, treatments up to 100 MPa favoured protein unfolding, leading to the exposure of hydrophobic groups, while, beyond this pressure value, unfolded proteins reassemble by interparticle interactions (Fayaz et al., 2019). As regards polysaccharides, HPH seems to promote conformational changes and favour chain-to-chain association of pectins (Shpigelman et al., 2015). In the case of starch, HPH improved rheological properties by favouring particle size reduction and partial gelatinization (Peressini et al., 2020). Similarly, oil holding, swelling capacities, and emulsifying capacity of dietary fibers results improved by a HPH treatment at 200 MPa (Xie et al., 2017). These data suggest that application of HPH might positively affect the techno-functional

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4 properties of okara, due to effects exerted not only on the protein fraction but also on the fibrous  
5 components.  
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8 To reduce the presence of other non-protein components, a further purification step has been proposed  
9 after okara cell disruption process (Tao et al., 2019). An alkaline extraction, at pH from 9 to 12, was  
10 proven to reduce cell wall rigidity as well as modify globular protein structure with a consequent  
11 modification of their functional properties (Jiang et al., 2009; Ma et al., 1997; Tao et al., 2019).  
12 Subsequently, precipitation at isoelectric point (pH 4.2 – 4.5) is performed followed by a drying phase  
13 to obtain okara powders enriched in proteins (about 73% of protein on dry basis) (Tao et al., 2019).  
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15 Despite these interesting results, literature information is available only considering a defined HPH  
16 process as pretreatment (50 MPa). To claim the industrial applicability of an HPH-alkaline recovery  
17 combined process to generate high-protein ingredients from okara, the understanding of the effect of  
18 increasing pressures and number of passes appears necessary.  
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21 Based on these considerations, in the present work we investigate the effect of homogenization pre-  
22 treatments at increasing intensities before an alkaline treatment on the final compositional, physical  
23 and techno-functional properties of the obtained protein-enriched okara powders. The final aim was  
24 to identify processing conditions to easily obtain okara derivatives to be used as food-grade  
25 ingredients with improved techno-functional properties. The resulting okara powders were  
26 characterised for physical (FTIR, particle size), chemical (protein content and molecular fractions, SH  
27 groups, absorbance at 280nm) and techno-functional properties (solubility, foaming, emulsifying and  
28 gelling ability).  
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## 31 32 33 34 35 36 37 38 39 40 41 **2 Materials and methods**

### 42 43 44 *2.1 Fresh okara*

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46 Fresh soy okara (1 kg) was kindly provided by Unigrà (Conselice, Italy). Okara was frozen at -30 °C  
47 and, before use, thawed at 4 °C for 8 h and equilibrated at 20 °C for 2 h. The composition of the okara  
48 used in the experiments was previously characterized by Fayaz et al. (2019): moisture  $76.22 \pm 0.40$   
49 g/100 g, lipid  $6.53 \pm 0.01$  g/100 g, fibre  $12.50 \pm 0.05$  g/100 g and ash  $0.59 \pm 0.05$  g/100 g. By contrast,  
50 the protein content was determined by using the BCA assay (paragraph 2.3) and resulted  $8.41 \pm 0.40$   
51 g/100 g okara.  
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### 57 58 *2.2 Okara powder*

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4 Aqueous fresh okara dispersion (20 g/100 g) was prepared and homogenized at increasing pressure  
5 (0, 50, 100 and 150 MPa) by using a lab-scale high-pressure homogenizer supplied with two Re +  
6 type tungsten carbide homogenization valves (Panda Plus 2000; GEA Niro Soavi, Parma, Italy)  
7 following the method described by (Fayaz et al., 2019). An additional sample was prepared by  
8 subjecting the okara dispersion to HPH to 5 successive passes at 150 MPa, to assess the effect of an  
9 extreme treatment intensity. HPH treated okara dispersions were submitted to alkaline-acid recovery  
10 (Tao et al., 2019). In particular, homogenized okara dispersions were adjusted to pH 12.0 (Hanna  
11 Instruments pH 301, Padova, Italy) with 1 M NaOH (Carlo Erba Reagent, Milan, Italy), stirred for 1  
12 h at 50 °C and centrifuged at 9,462 x g for 20 min at 4 °C (AVANTI TM J-25, Beckman Coulter s.r.l.,  
13 Cassina De' Pecchi, Milan, Italy). The supernatant was collected, acidified to pH 4.2 with 1 M HCl  
14 (J. T. Baker, Deventer, Holland) and centrifuged at 9,462 x g for 20 min at 4 °C. The precipitate was  
15 then washed with deionized water, dispersed in deionized water in a 1:5 (w/w) ratio, and adjusted to  
16 a pH of 7.0 with 1 M NaOH. Samples were then frozen at -30 °C and freeze-dried for 72 h at 4053 Pa  
17 (Mini Fast 1700, Edwards Alto Vuoto, Milan, Italy) to obtain soy-okara powder. The latter was sealed  
18 in plastic trays and stored into desiccators (P<sub>2</sub>O<sub>5</sub>, 5% RH) at 25 °C until use.  
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### 32 *2.3 Protein content*

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34 Protein content was determined using the bicinchoninic acid method (BCA) adapted to a 96-well  
35 microplate spectrophotometer procedure. The working reagent (WR) was obtained by mixing the  
36 bicinchoninic acid solution (reagent A) (Sigma-Aldrich, Milan, Italy) with the cupric sulphate solution  
37 (4% w/v) (reagent B) (Sigma-Aldrich, Milan, Italy) (50:1 v/v). The samples were dispersed in  
38 deionized water (8.0 mg/mL), added with 100 µL NaOH 0.1 M (Carlo Erba Reagent, Milan, Italy)  
39 and diluted with deionized water (1:5 v/v). The sample (25 µL) was mixed with 200 µL of WR and  
40 incubated at 37 °C for 30 min in the dark. Absorbance was measured at 562 nm using a microplate  
41 reader (Sunrise, Tecan, Männedorf, Switzerland). Water blanks were run in each assay. Protein  
42 content was determined by comparison with a calibration curve (0-2000 µg/mL bovine serum  
43 albumin, R<sup>2</sup> = 0.994) (Sigma-Aldrich, Milan, Italy).  
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### 53 *2.4 Particle size distribution*

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55 Samples were dispersed in deionized water (0.01 g/mL). Particle size distribution of the samples  
56 relevant to volume distribution was assessed by using the Zetasizer Nano ZS instrument (Malvern,  
57 Milan, Italy) by setting the observation angle at 173°. The solution refractive index and viscosity were  
58 set at 1.333 and 0.00088 Pa·s, respectively, corresponding to the values of pure water at 25 °C.  
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## 2.5 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method of Laemmli (1970), optimized on okara samples (Tao et al., 2019). Three mg of sample was mixed with 500  $\mu\text{L}$  of sample buffer 2 $\times$  (Bio-Rad Laboratories, Inc., Hercules, California) and 10  $\mu\text{L}$  of  $\beta$ -mercaptoethanol (Bio-Rad Laboratories, Inc., Hercules, California), incubated for 1 h at room temperature, heated at 90  $^{\circ}\text{C}$  for 5 min and centrifuged at 9,500  $\times g$  for 10 min at 20  $^{\circ}\text{C}$  (D3024, DLAB Scientific Europe S.A.S, Schiltigheim, France). An aliquot of 10  $\mu\text{L}$  of the sample was loaded into SDS-PAGE pre-stained gels (Mini-PROTEAN TGX Stain-Free Gels, Bio-Rad Laboratories, Inc., Hercules, California) and the electrophoresis was performed at 220 V for 45 min. A bioanalytical imaging system (G:Box, SYNGENE UK, Cambridge, UK) was used to visualize protein bands and acquire gel images. Protein identification was based on the comparison with protein standards in the molecular weight range 10-170 kDa (Precision Plus Protein Standards, Kaleidoscope, Bio-Rad Laboratories, Inc., Hercules, California).

## 2.6 Absorbance at 280 nm

The samples were dispersed in deionized water (0.01 g/mL), stirred for 20 min at room temperature and centrifugated at 13,680  $\times g$  for 5 min (D3024, DLAB Scientific Europe S.A.S, Schiltigheim, France). Supernatant absorbance was measured at 280 nm using a UV-2501 PC UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan).

## 2.7 Free SH groups

Total free SH groups content was determined according to the method described by Panozzo et al., (2014) using Ellman's reagent (5',5-dithiobis (2-nitrobenzoic acid), DTNB) (Sigma-Aldrich, Milan, Italy) (Ellman, 1959). Free SH groups were calculated as follows (Eq. 1):

$$\mu\text{M SH/g} = \frac{73.53 \times A_{412} \times D}{C} \quad (\text{Eq. 1})$$

where  $A_{412}$  is the absorbance at 412 nm,  $C$  is the sample concentration (mg extract/mL),  $D$  is the dilution factor (10) and 73.53 is derived from  $10^6/(1.36 \cdot 10^{-4})$ , where  $1.36 \cdot 10^{-4}$  is the molar absorptivity of Ellman's reagent.

## 2.8 Fourier transform infrared spectroscopy (FTIR) measurement

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4 FTIR spectra were recorded at 25 °C by using an FTIR spectrometer equipped with an ART accessory  
5 and a Zn-Se crystal that allowed the collection of FTIR spectra directly on the sample, without sample  
6 preparation (Alpha-P, Bruker Optics, Milan, Italy). The pressure arm of the instrument was used to  
7 apply constant pressure to the samples positioned on the top of the Zn-Se crystal, to ensure good  
8 contact between the sample and the incident IR beam. Background scan of the clean Zn-Se crystal  
9 was acquired prior to sample scanning. FTIR spectra were obtained in the wavenumber range from  
10 400 to 4000 cm<sup>-1</sup> at a spectral resolution of 4 cm<sup>-1</sup> and with 32 co-added scans by using the OPUS  
11 software (version 7.0 for Microsoft Windows, Bruker Optics).  
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### 19 *2.9 Water solubility*

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21 Samples ( $W_0$ , 10 mg) were suspended in 1 mL deionized water, stirred at room temperature for 1 h  
22 and centrifuged at 21,380 x  $g$  rpm for 5 min at 4 °C (D3024, DLAB Scientific Europe S.A.S,  
23 Schiltigheim, France). The insoluble precipitates were dried in a vacuum oven at 40 °C overnight  
24 (Vuotomatic 50, Bicasa, Milan, Italy) and weighted ( $W_I$ , mg). Protein solubility was calculated as  
25 follows (Eq. 2):  
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$$32 \text{ Powder solubility (\%)} = \frac{W_0 - W_I}{W_0} \times 100 \quad (\text{Eq. 2})$$

### 34 *2.10 Foaming properties*

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36 Samples were dispersed in deionized water (0.01 g/mL) and stirred at room temperature for 1 h.  
37 Aliquots of 25 mL ( $V_0$ , mL) of sample was placed into a 50 mL graduated cylinder and mixed with a  
38 high-speed blender (Ika-Werke, DI 25 basic, Staufen, Germany) at 800 x  $g$  for 3 min. The volume of  
39 the developed foam ( $V_I$ , mL) was measured and visually monitored every 10 min for 1 h. The foaming  
40 activity index (FAI) and the foaming stability index (FSI) were calculated as follows (Eq. 3, Eq. 4):  
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$$48 \text{ FAI (\%)} = (V_1/V_2) \times 100 \quad (\text{Eq. 3})$$

$$49 \text{ FSI (\%)} = (V_{60}/V_0) \times 100 \quad (\text{Eq. 4})$$

### 50 *2.11 Emulsifying properties*

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52 Samples were dispersed in deionized water (0.025 g/mL) and stirred for 1 h at room temperature,  
53 mixed with sunflower oil (9:1 w/v sample:oil ratio) and pre-homogenized with a high-speed blender  
54 (Polytron, PT 3000, Littau, Swiss) at 800 x  $g$  for 1 min. The pre-emulsion was homogenized using a  
55 continuous lab-scale high-pressure homogenizer (Panda Plus 2000; GEA Niro Soavi, Parma, Italy),  
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4 with the first valve at 50 MPa and the second at 5 MPa. The obtained emulsions were placed into  
5 transparent 10 mL-glass vials and stored for 25 days at 4 °C. During this time, emulsion stability was  
6 monitored by sampling 0.1 mL of sample from the central layer and measuring particle size  
7 distribution as described in paragraph 2.4. The Z-average mean particle size diameter was calculated  
8 through the Cumulant Analysis.  
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### 10 11 12 13 14 *2.12 Gelling properties*

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16 Gelling properties were determined according to the method of Zhao et al., (2017) by heating the  
17 sample suspensions (0.12 g/mL) at 90 °C for 30 min, cooling at 4 °C for 12 h. Samples viscoelastic  
18 properties (moduli G' and G'') were tested at 20 °C using an RS6000 Rheometer (Thermo Scientific  
19 RheoStress, Haake, Germany), equipped with a Peltier system for temperature control and by using a  
20 parallel plate geometry (25 mm diameter) with a gap of 1.0 mm. Oscillatory sweep tests to identify  
21 the linear viscoelastic region (LVR) were performed increasing stress from 0.1 to 100 Pa at 1 Hz.  
22 Frequency sweep tests were then performed increasing frequency from 0.1 to 10 Hz at stress values  
23 selected in the LVR.  
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### 31 32 *2.13 Data analysis*

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34 All determinations were expressed as the mean  $\pm$  standard deviation (SD) of at least three repeated  
35 measurements from two experiment replicates (n = 2). Statistical analysis was performed by using R  
36 v. 2.15.0 (The R Foundation for Statistical Computing, Wien, Austria). Bartlett's test was used to  
37 check the homogeneity of variance, one-way ANOVA was carried out and Tukey test was used to  
38 determine statistically significant differences among means (p<0.05).  
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## 44 **3 Results and Discussion**

### 45 46 *3.1 Effect on protein content and physical properties*

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48 Soy okara was submitted to high pressure homogenisation at different intensity levels. Samples were  
49 then enriched in protein content by pH-driven precipitation and dried to obtain soy-okara powder. The  
50 amount of powder obtained after HPH treatments resulted almost twice as compared to that obtained  
51 in the absence of the treatment (Tab. 1). This result is in agreement with the well-known capacity of  
52 HPH of producing intense mechanical forces, which disrupt okara fibrous matrix and favour an  
53 efficacious release of tightly packed cell components. The latter include not only proteins with high  
54 added-value but also non protein fractions, such as lipids and fibers (Fayaz et al., 2019; Preece et al.,  
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4 2017a). The presence of non-protein components was confirmed by FTIR analysis (Fig. 1). Peaks  
5 associated with the presence of lipids ( $2700\text{-}3000\text{ cm}^{-1}$  and  $1740\text{ cm}^{-1}$ ) (Gurdeniz et al., 2007) and  
6 polysaccharides ( $3000\text{-}3700\text{ cm}^{-1}$ ,  $2900\text{ cm}^{-1}$ , and  $600\text{ to }1400\text{ cm}^{-1}$ ) (Coimbra et al., 1998; Mateos-  
7 Aparicio et al., 2010; Yang et al., 2019) were clearly observed. The peaks relevant to lipids and  
8 especially to polysaccharides resulted higher in the spectrum of the samples obtained by the most  
9 intense HPH treatment. All samples also showed the typical FTIR spectral profile of proteins,  
10 characterised by the IR bands of amides I and II at around  $1650$  and  $1550\text{ cm}^{-1}$ , respectively (Barth,  
11 2007). The protein fraction actually resulted the most important from a quantitative point of view,  
12 representing *circa* 70% of the obtained powder (Tab. 1), which was more than twice that of the  
13 untreated okara (30 %) (Mateos-Aparicio et al., 2010). However, the increase in HPH treatment  
14 intensity was associated with a progressive decrease in powder protein content (Tab. 1). This result  
15 confirms that, while increasing the overall amount of powder obtained from okara, HPH  
16 concomitantly favours the release of intracellular components other than proteins. To study the  
17 disruption efficacy of HPH on okara matrix, particle size distribution of the powders suspended in  
18 water was studied by dynamic light scattering (Fig. 2). The suspension of okara non subjected to HPH  
19 (0 MPa) showed a broad distribution in a diameter range from 100 nm to 1  $\mu\text{m}$ , and large aggregates  
20 at about 10  $\mu\text{m}$ . A downsizing accompanied with a homogeneity increase was observed in the  
21 dispersions treated at 50 and 100 MPa, both showing a bimodal distribution, with a major particle  
22 family at about 700 nm and a minor one at 100 nm. These results are consistent with the increase in  
23 the amount of powder obtained from HPH treated okara (Tab. 1) and can be attributed to the intense  
24 mechanical stresses delivered by HPH (Augusto et al., 2012; Fayaz et al., 2019; Song et al., 2013).  
25 Interestingly, the sample obtained upon the application of 150 MPa showed most of the particles  
26 organized into large aggregates with dimensions around 1200 nm and minor particle fractions at about  
27 50 and 200 nm. Finally, the application of multiple passes at 150 MPa allowed obtaining samples  
28 which presented a particle distribution characterised by two balanced peaks around 300 and 80 nm,  
29 respectively. These results suggest that HPH would promote structural rearrangements of okara  
30 components, leading to the formation of smaller particles able to reassemble into new aggregates. It  
31 can be inferred that these novel aggregates could result from the interaction among proteins and  
32 polysaccharides released upon HPH treatment of okara matrix. As known, when polyelectrolytes  
33 characterised by different energy landscape are forced to coexist in the same environment, complex  
34 coacervates might be formed (Cooper et al., 2005), depending on the capacity of proteins to  
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4 efficaciously interact with polysaccharides. This interaction is generally favoured by the presence of  
5 relatively small and unfolded proteins (Klemmer et al., 2012).  
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### 8 *3.2 Effect on chemical properties*

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10 To better understand the effect of HPH on protein conformation, okara powders were analysed for  
11 their content in free SH groups, absorbance at 280 nm (Tab. 1). The free SH content increased ( $p<0.05$ )  
12 in samples treated at pressure up to 100 MPa, indicating that HPH would promote exposure of surface  
13 SH-groups, supporting the hypothesis of protein unfolding. On further increase in HPH treatment  
14 intensity, a decrease in free SH content was detected, indicating a rearrangement of proteins by SH-  
15 disulphide exchange, as also previously reported (Fayaz et al., 2019). The absorbance at 280 nm  
16 significantly decreased ( $p<0.05$ ) by increasing HPH pressure and number of passes. This is likely to  
17 be associated to progressive burying of protein residues of cysteine, tyrosine and tryptophan inside  
18 the protein structure (Layne, 1957). Changes in SH and absorbance at 280 nm were thus consistent  
19 with a mechanism of protein unfolding. SDS-PAGE analysis was carried out to enlighten HPH effects  
20 on okara protein configuration (Fig. 3). Patterns largely agree with those reported in the literature  
21 (Stanojevic et al., 2012; Tao et al., 2019). In particular, the  $\alpha'$ ,  $\alpha$ ,  $\beta$ , Bg subunits of  $\beta$ -conglycinin (7S)  
22 were detected at 85, 75, 50, 27 and 16 kDa respectively, and the AS and BS subunits of glycinin (11S)  
23 were detected around 34, 18 and 15 kDa. Independently on treatment intensity, HPH caused the  
24 disappearance of the low molecular weight bands (10 and 15 kDa), along with the formation of protein  
25 aggregates (at about 100 kDa), not detected in the control sample. These result confirm that HPH  
26 mechanical forces led to unfolding and fragmentation of globular proteins, exposing residues which  
27 governed the interaction with non-protein compounds.  
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### 44 *3.3 Effect on techno-functional properties*

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46 Collected results demonstrate that HPH strongly affected physical and chemical properties of okara  
47 powder, possibly altering its techno-functional properties. To have an insight into these aspects, the  
48 solubility of the powders was evaluated (Tab. 1). HPH treatment strongly decreased powder solubility  
49 ( $p<0.05$ ), reasonably due to the occurrence of complex aggregates (Fig. 3). A similar decrease in  
50 solubility was also observed upon HPH treatment of soy proteins at pressures in the range 200-600  
51 MPa (Wang et al., 2008). The foaming properties of the powders were also assessed (Tab. 1). HPH  
52 treatment did not affect the foaming ability (FAI) ( $p\geq 0.05$ ) but increased foam stability (FSI) ( $p<0.05$ )  
53 (Tab. 1). This is consistent with data obtained by Tao et al., (2019) and suggests that the  
54 conformational and configurational changes induced by HPH on okara proteins, as well as the  
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4 formation of novel aggregates (Tab. 1, Fig. 3), allowed a better stabilization of the water-air interface  
5 (Nakai, 1983). The gelling properties of the powders were also evaluated. In all cases, weak gels were  
6 achieved, as indicated by the  $G'$  and  $G''$  patterns, which resulted reciprocally parallel and dependent  
7 on the frequency (Fig. 4). These self-standing gels are based on the self-assembling of globulins,  
8 driven by hydrophobic interactions and S-S bonds (Kohyama et al., 1995). The increase in HPH  
9 treatment up to 100 MPa allowed a progressive improvement of the gelling ability of the powders, as  
10 shown by the higher ( $p < 0.05$ ) elastic modulus ( $G'$ ) (Fig. 4). The increasing elasticity could be  
11 attributed to the formation of a mixed gel with a stronger network, since locally reinforced by the  
12 interactions among unfolded proteins and polysaccharides (Doublier et al., 2000; Wijaya et al., 2017).  
13 This hypothesis is also supported by a tendency of the gel with 150 MPa x 5 passes-treated powder to  
14 create syneresis. Finally, the effects of HPH treatment on the emulsifying properties of the powders  
15 were evaluated (Fig. 5). Immediately after preparation, all the emulsions showed a mean diameter in  
16 the range 500-600 nm. However, during storage, the droplet diameter of the powder not subjected to  
17 HPH (0 MPa) rapidly increased and the emulsion separated within 7 days. By contrast, the emulsions  
18 obtained from the HPH-treated powders showed a slight progressive increase of oil droplet size but  
19 no phase separation during the considered storage period (21 days). Partial protein unfolding and  
20 aggregate formation induced by HPH would allow a more efficient adaptation of particles to oil  
21 droplet surface as well as potential Pickering effects (Yang et al., 2019). Noticeably, the powder  
22 obtained by 5 passes at 150 MPa showed lower stability as compared to the others (Fig. 5). This result  
23 can be probably explained in the light of both the conformational changes induced by this intense  
24 treatment on okara proteins and the lower concentration of proteins in the obtained extract, in favour  
25 of a higher presence of non-surface-active compounds (Tab. 1).  
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#### 45 **4 Conclusions**

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47 Our results demonstrated the effectiveness of combining moderate HPH treatments with a pH-driven  
48 purification, to prepare okara powders intended as novel ingredients. The latter presented improved  
49 techno-functionality, probably due to the synergistic effects of proteins and polysaccharides. The most  
50 effective processes resulted those performed at intermediate intensities (50-100 MPa). Within this  
51 range it could be possible to obtain a good balance between protein content and techno-functionalities  
52 of the okara ingredient. The final choice of the homogenization pressure could be then based on the  
53 target functional property (e.g. solubility, foaming, emulsifying, gelling) to be enhanced. Interestingly,  
54 the most effective homogenization pressures (<100 MPa) appear compatible with the commercially  
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4 available HPH plants intended for large scale productions, making feasible its application in an  
5 industrial environment. Although the case here presented was relevant to soy okara, this approach  
6 could be extended to by-products derived from other plant sources. The combination of HPH and  
7 simple recovery techniques can be considered a key strategy in bio-refinery processes, aiming at  
8 exploiting all the components embedded into complex waste matrices, without limitation to the protein  
9 fraction solely, in the optic of optimizing the use of natural resources.  
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4 **Figure captions**  
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8 **Fig. 1** FTIR spectra of powder obtained from soy-okara submitted to HPH treatment at 0, 50, 100,  
9 150 MPa and 5 passes at 150 MPa.  
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13 **Fig. 2** Particle size distribution of powder obtained from soy-okara submitted to HPH treatment at 0,  
14 50, 100, 150 MPa and 5 passes at 150 MPa.  
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18 **Fig. 3** SDS-PAGE patterns of powder obtained from soy-okara submitted to HPH treatment at 0, 50,  
19 100, 150 MPa and 5 passes at 150 MPa. MW: molecular weight.  
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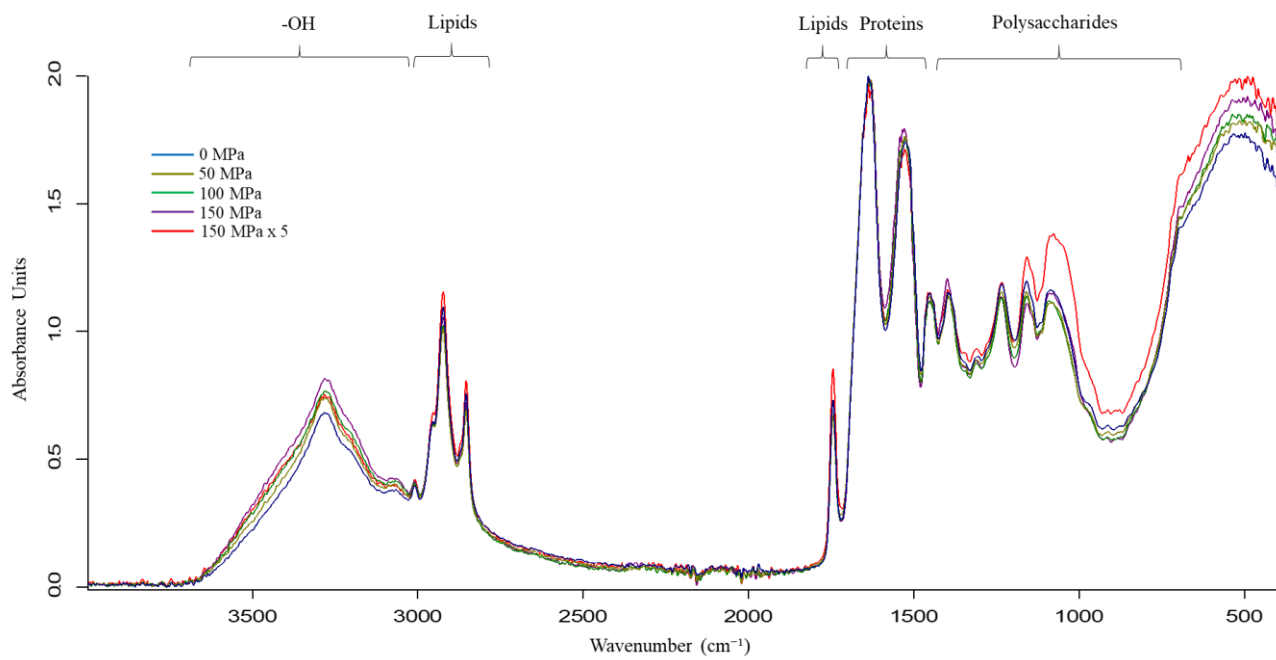
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23 **Fig. 4** Mechanical spectra of the storage ( $G'$ ) and the loss ( $G''$ ) moduli of soy-okara powder gel  
24 submitted to HPH treatment at 0, 50, 100, 150 MPa and 5 passes at 150 MPa.  
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28 **Fig. 5** Z-average mean diameter of emulsions stabilized by powder obtained from soy-okara submitted  
29 to HPH treatment at 0, 50, 100, 150 MPa and 5 passes at 150 MPa. Data points Means  $\pm$  SD (n = 2).  
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35 **Table captions**  
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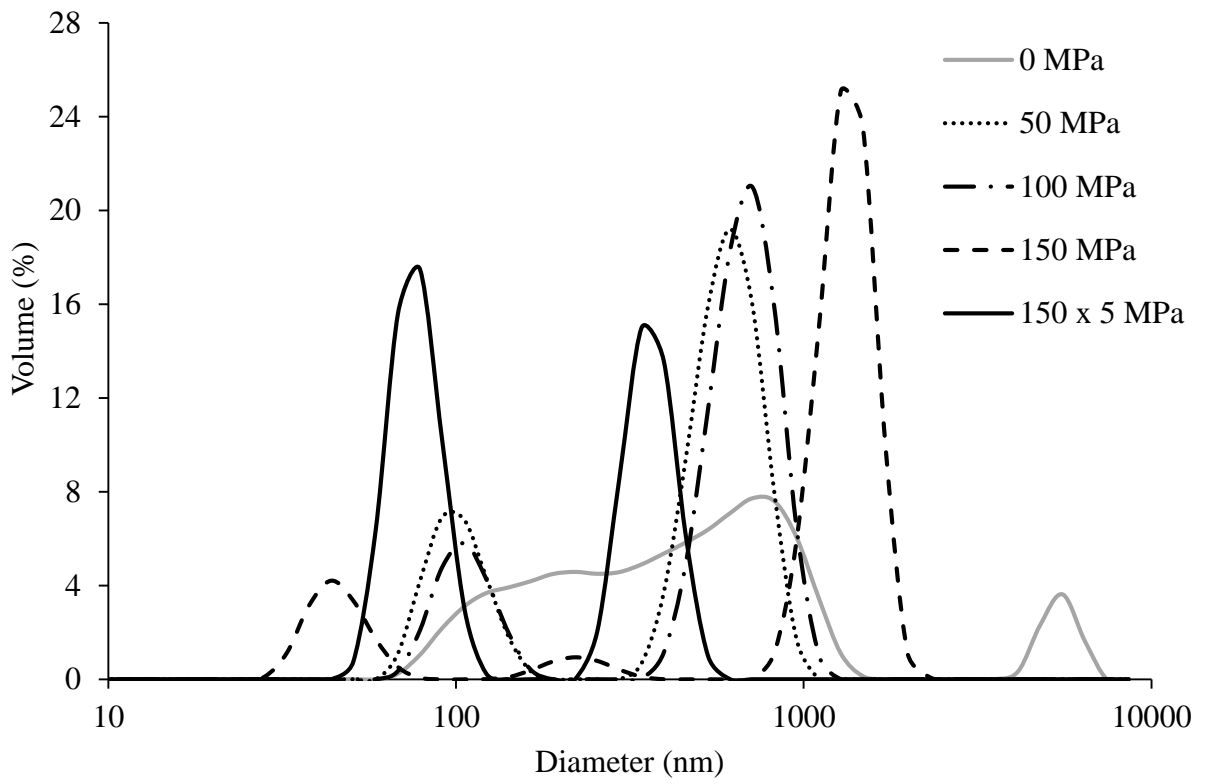
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38 **Tab. 1** Amount, protein content, free SH groups, absorbance at 280 nm, water solubility and foaming  
39 properties of powder obtained from soy-okara submitted to HPH treatment at 0, 50, 100, 150 MPa  
40 and 5 passes at 150 MPa.  
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Figure 1



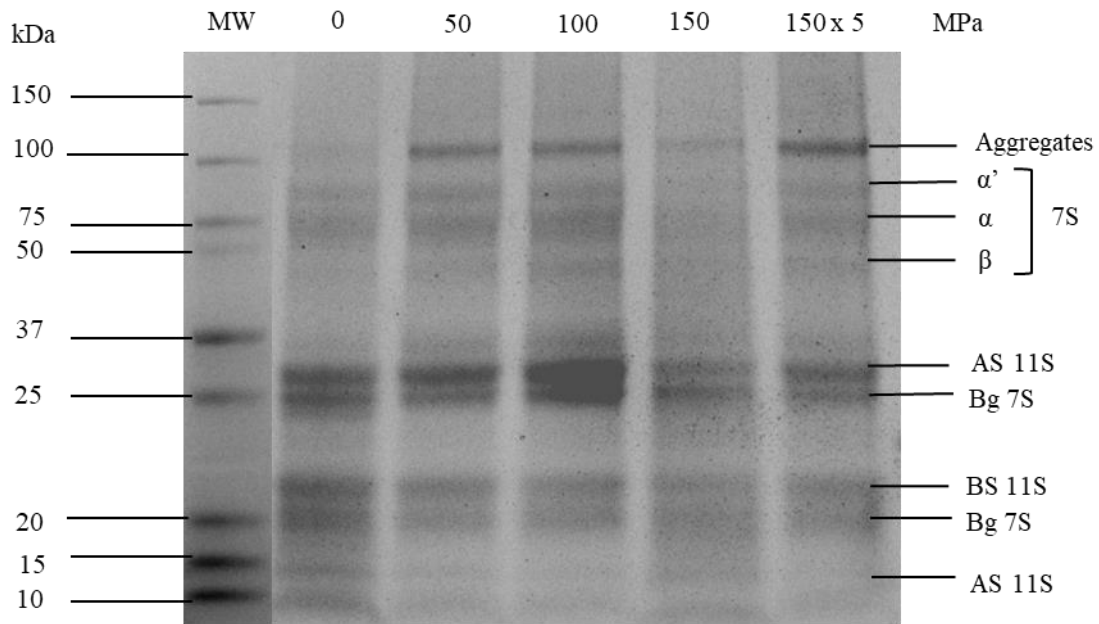
**Fig. 1** FTIR spectra of powder obtained from soy-okara submitted to HPH treatment at 0, 50, 100, 150 MPa and 5 passes at 150 MPa.

Figure 2



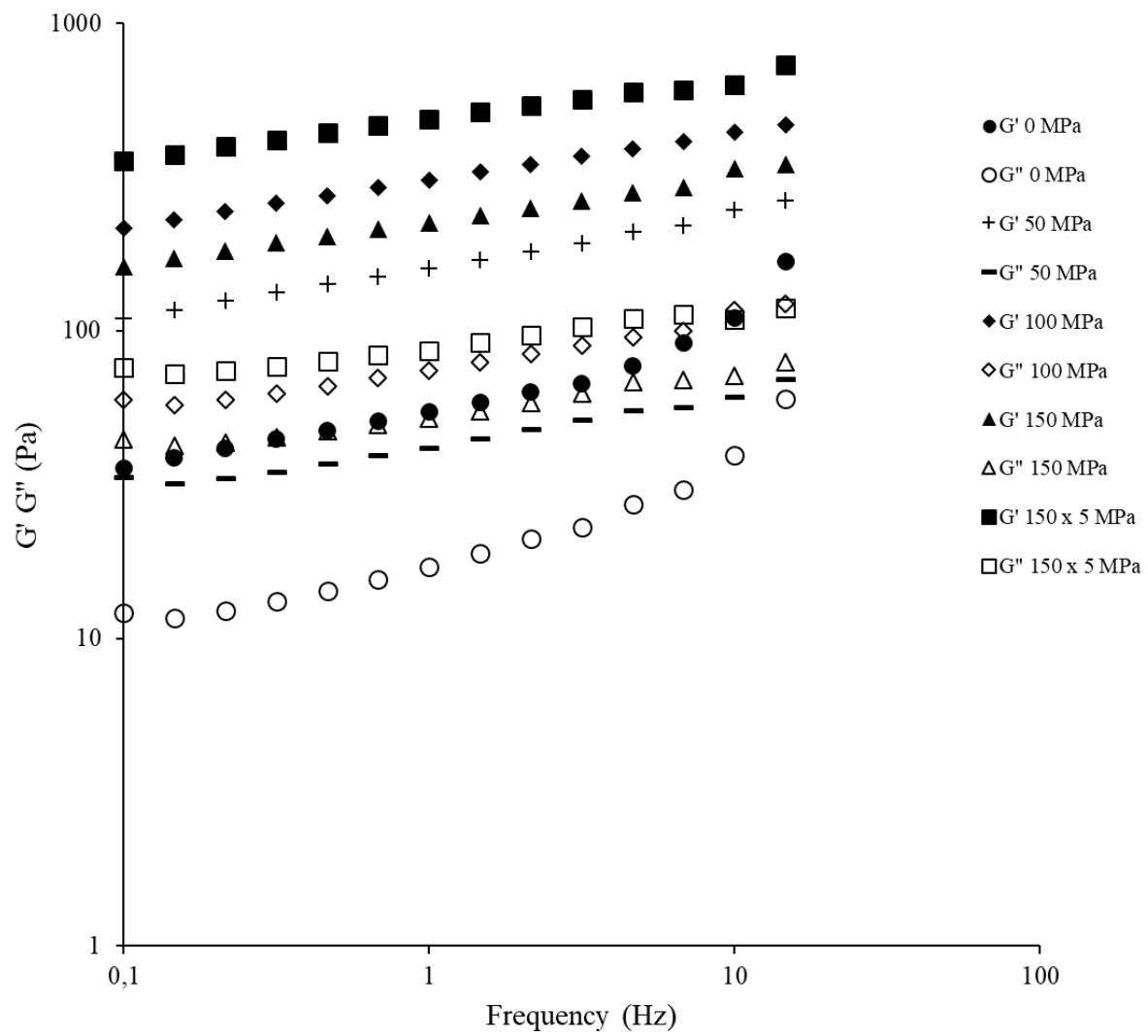
**Fig. 2** Particle size distribution of powder obtained from soy-okara submitted to HPH treatment at 0, 50, 100, 150 MPa and 5 passes at 150 MPa.





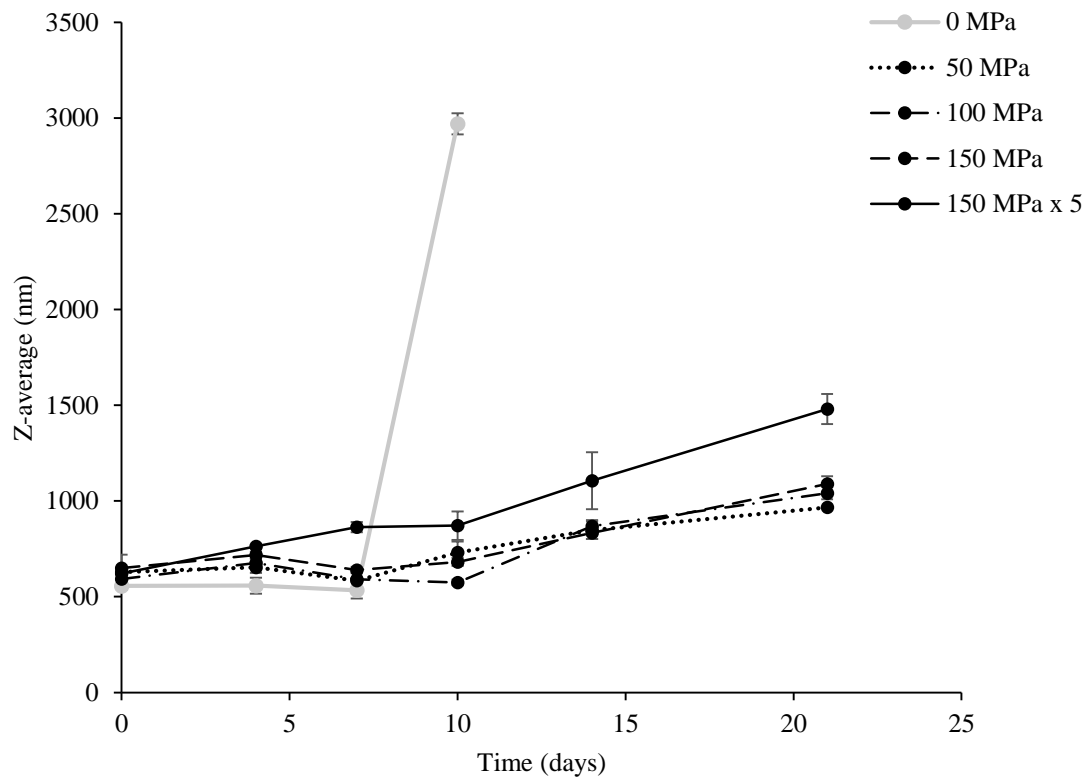
**Fig. 3** SDS-PAGE patterns of powder obtained from soy-okara submitted to HPH treatment at 0, 50, 100, 150 MPa and 5 passes at 150 MPa. MW: molecular weight.

Figure 4



**Fig. 4** Mechanical spectra of the storage ( $G'$ ) and the loss ( $G''$ ) moduli of soy-okara powder gel submitted to HPH treatment at 0, 50, 100, 150 MPa and 5 passes at 150 MPa.

Figure 5



**Fig. 5** Z-average mean diameter of emulsions stabilized by powder obtained from soy-okara submitted to HPH treatment at 0, 50, 100, 150 MPa and 5 passes at 150 MPa. Data points Means  $\pm$  SD (n = 2).



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**Table**

Tab 1.pdf

## **Conflict of Interest**

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.