

High-Pressure Homogenization for the Recovery of Value-Added Compounds from Vegetable Matrices

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High-pressure homogenization (HPH) has been recently reported to be an effective mechanical cell disruption technology to unlock the intracellular compounds, tightly entrapped in vegetable tissues, using only water as an extraction medium. In this work, HPH was used to promote the recovery of the bioactive compounds contained in white and black sesame seeds (*Sesamum indicum*). Aqueous suspensions (10% w/w) of the seeds, obtained by high-shear mixing (HSM) for 5 min at 20000 rpm, were treated by HPH at 100 MPa or 140 MPa for up to 10 passes and different temperatures (25 and 50 °C). The HPH treatment caused a considerable cell deagglomeration and fragmentation effect, as shown by the decrease in the size distribution of the suspended particles. At the same time, the HPH treatment also significantly increased, more than two-fold, the polyphenolic content and antioxidant activity of the aqueous extracts, in comparison to HSH. Remarkably, a significant decrease (-20%) in antioxidant activity was observed during HPH processing at a higher temperature, likely due to the degradation of thermolabile compounds. Higher operating pressures increased the antioxidant activity of the aqueous extracts but caused also the increased release of polyphenol oxidases, which induced a higher degradation of the antioxidant activity of the extracts over time in comparison with samples processed at lower pressure. However, spray drying of the HPH-treated suspensions, without any further treatment or additive, resulted in the efficient stabilization of the extracts.

1. Introduction

Sesame seeds are characterized by remarkable techno-functional (Zakidou and Paraskevopoulou, 2021) and health-beneficial properties, associated with the high content of bioactive molecules (Shahidi et al., 2006), including oleic acid, phytic acid, and lignans (Fukuda et al., 1985). However, being the bioactive compounds located in the intracellular space of the plant cells, their efficient extraction requires either increasing the driving force (the concentration gradient in the extraction solvent) or reducing the mass transfer resistances. The driving force can be increased by selecting solvents and operating conditions that maximize the solubilization of the analytes. However, this approach, when applied to the non-polar phenolic compounds or fatty acids of interest for sesame seeds, demands the use of organic solvents and high-temperatures, which are both undesirable not only in terms of sustainability of the process but also of the increasing cost of production of the bioactives (Chan et al., 2014), because of the need for extract purification from the traces of the solvents, as well as for the risk of degradation of thermolabile compounds (Wang and Weller, 2006).

An alternative approach, based on the reduction of mass transfer resistances, requires the permeabilization or disintegration of the cell membranes, through the use of enzymes, thermochemical lytic processes, or the use of physical methods. High-pressure homogenization (HPH) has recently emerged as a natural and sustainable approach for the recovery of intracellular compounds from plant tissue through a purely mechanical process of cell deagglomeration and disruption of cell walls and membranes, using minimal heating (Innings et al., 2020) and only water as an extraction solvent (Jurić et al., 2019). Previous studies on the HPH treatment of aqueous suspensions of different plant materials, such as corn bran (Wang et al., 2014), wheat bran (Wang et al., 2013), mustard bran (Donsi and Velikov, 2020), broccoli seeds (Xing et al., 2019), soybean okara (Fayaz et al., 2019), tomato peels and spent coffee grounds (Ferrari et al., 2017; Jurić et al., 2019; Mustafa et al., 2018), and aromatic plants (Gali et al., 2020), demonstrated the important contribution of HPH in achieving the

efficient extraction in aqueous phase also of lipophilic intracellular compounds (e.g. lycopene (Jurić et al., 2019), rutin and quercetin (Gali et al., 2020)), with yields which are often higher than with organic solvents. This work aims at investigating how the HPH process, through the purely physical-mechanical disruption of the cell structures, can contribute to the recovery of bioactive compounds from white and black sesame seeds, using only water as a process medium, hence avoiding the use of any environmentally harmful reagent. In particular, the main operating parameters of HPH processing, including operating pressure, temperature, and number of passes are investigated in terms of the effect on size distribution, and release of antioxidant compounds. In addition, the stabilization of the extracts through spray drying is also assessed.

2. Materials and Methods

2.1 Materials

Sesame seeds (*Sesamum indicum* L.) were tested in the white (origin Egypt, BMS Srl, Perugia, Italy, length, width, and thickness were 2.5, 1.7, and 0.9 mm respectively) and black variety (origin Lebanon, Chicchi e Baccelli, Naples, Italy, length, width, and thickness were 2.9, 1.6, and 0.8 mm respectively), without any further pre-treatment. All chemicals and solvents were purchased from Sigma Aldrich (Milan, Italy).

2.2 Mechanical disruption treatments

25 g of white or black sesame seeds (about 10^4 seeds), with an average water content of $8 \pm 1\%$ w/w, were suspended in water (225 g) at 10% w/w and processed by high-shear mixing (HSM) using an Ultra Turrax T25 (IKA Labortechnik), equipped with an S25 N18 G rotor at 20000 rpm for 5 min to cause the complete disruption of the sesame seeds.

The HSM suspensions were, subsequently, submitted to HPH processing, using an in-house developed unit, equipped with an interchangeable orifice valve (model WS1973, Maximator JET GmbH, Schweinfurt, Germany). Orifice diameters of 100 and 150 μm were used for operating pressures of 140 MPa and 100 MPa, respectively, for up to 10 passes, for a total processing time up to 10 min. Before processing and after each pass, the suspensions were cooled in a tube-in-tube heat exchanger at 25 °C or 50 °C to compensate for the inherent heating effects due to viscous dissipation of the pressure energy (about 0.17 °C/MPa). Treated suspensions were analyzed as collected for particle size distribution, or centrifuged ($5.289 \times g$ for 10 min at 4 °C) to measure the antioxidant activity and the total polyphenolic content of the aqueous supernatant.

Suspensions were also stabilized by direct spray drying in a Mini Spray Dryer B-290 Basic (Büchi Italia s.r.l., Milan, Italy) at a spray rate of 4 mL/min, with drying and outlet temperatures fixed at 140 and 70 °C, respectively. Dry airflow was set at 800 L/h, and the atomization pressure was set at 1.5 bar.

2.3 Particle size distribution

Particle size distributions of sesame seed suspensions were analyzed by laser diffraction at 25°C, using a Master Sizer 2000 particle size analyzer (Malvern Panalytical Ltd, Malvern, UK). The volume-weighted mean diameter $D[4,3]$ and surface weighted mean diameter $D[3,2]$, together with the diameters corresponding to the 10, 50, and 90 percentile of the cumulative distribution ($D_{0.1}$, $D_{0.5}$, and $D_{0.9}$, respectively), of the distribution, as previously defined (Mustafa et al., 2018), were recorded for each sample. The parameters used in the analysis were the properties of water at 25°C (refraction index = 1.33), which was used as a dispersant medium.

2.4 Antioxidant activity and total polyphenols

Analyses were carried out on the supernatant from sesame HSM- or HPH-treated suspensions stored under refrigerated conditions, as well as on spray-dried powders rehydrated to 10% w/w.

The antioxidant activity was evaluated using the ferric reducing antioxidant power (FRAP) (Guo and Jauregi, 2018), with some modifications. FRAP reagent was freshly prepared by mixing, at a ratio of 10:1:1, acetate buffer 300 mM (pH 3.6, made of 3.1 g of sodium acetate and 16 mL of glacial acetic acid dissolved in 1 L of distilled water), 20 mM ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), and 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM of hydrochloric acid (HCl). 2.5 mL of the reagent was mixed with 500 μL of the sample (supernatant from centrifugation of aqueous suspensions) and incubated at room temperature for 10 min before reading absorbance at 593 nm using a V-650 spectrophotometer (Jasco Inc., Easton, MD, USA). Results were expressed as mmol of ascorbic acid equivalent per kg of fresh seeds ($\text{mmol}_{\text{AAE}}/\text{kg}$).

Total polyphenol content (TPC) was determined by the Folin-Ciocalteu reagent (Lowry et al., 1951). A volume of 200 μL of the sample was mixed with 2.6 mL of water, 1 mL of sodium carbonate (7%), and 200 μL of the Folin-Ciocalteu reagent. The mixture was incubated in the dark for 90 min and the absorbance was then read at 745 nm. Results were expressed as mg of gallic acid equivalent per kg of fresh seeds ($\text{mg}_{\text{GAE}}/\text{kg}$).

2.5 Statistical analysis

Treatments and analyses were performed in triplicate unless differently specified and the results were reported as means \pm standard deviations. Differences among mean values were analyzed by one-way variance (ANOVA), performed with SPSS 20 (SPSS IBM., Chicago, USA) statistical package, and Tukey test was performed to determine statistically significant differences ($p < 0.05$).

3. Results and Discussion

3.1 Effect of HPH processing on the particle size distribution of sesame seed suspensions

The effect of HPH treatments on white sesame suspensions is shown in Figure 1 for a pressure of 100 MPa and a temperature of 25 °C and a variable number of passes. The results show that the distribution obtained through HSM is clearly bimodal (around 2 μm and 200 μm), and did not significantly change upon prolonging the time of HSM processing beyond 5 min (results not shown). HPH processing caused the efficient disruption of the larger fraction of sesame seed suspension: already for $N=4$ passes, only a small residual population around 200 μm was visible, which disappeared for $N = 6$ passes, without any significant further change occurring for $N \geq 6$. Therefore, $N = 6$ passes was selected for the subsequent experiments.

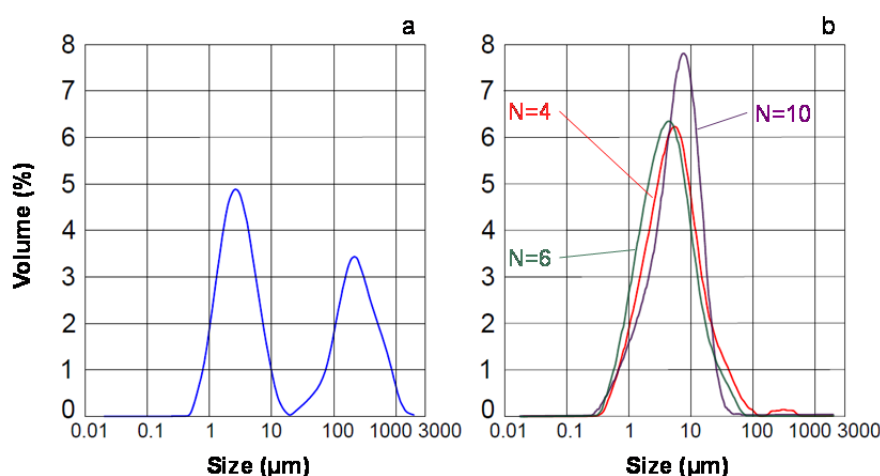


Figure 1: Particle size volume distribution of white sesame suspensions (10% w/w) treated by HSM (a) and HPH at 100 MPa and 25 °C for $N = 4, 6,$ and 10 passes (b).

The increase in HPH process intensity, by either increasing the operating pressure to 140 MPa or increasing the operating temperature to 50 °C, did not significantly change the size distribution of the white and black sesame suspensions, as shown in Table 1.

Table 1: Characteristic diameters of the particle size distribution of white and black sesame suspensions (10% w/w) treated by HSM (pressure of 0.1 MPa) and by HPH at different pressures and temperatures, for $N=6$ passes.

Pressure (MPa)	Temperature (°C)	D[4,3] (μm)	D[3,2] (μm)	D _{0.1} (μm)	D _{0.5} (μm)	D _{0.9} (μm)
<i>White sesame</i>						
0.1	25	202 \pm 21.3 ^a	5.7 \pm 1.0 ^a	1.9 \pm 0.1 ^a	139.8 \pm 26.0 ^a	545.8 \pm 28.2 ^a
100	25	8.6 \pm 1.4 ^b	3.4 \pm 0.5 ^b	1.6 \pm 0.3 ^{a,b}	5.1 \pm 0.9 ^b	20.1 \pm 3.9 ^b
100	50	9.1 \pm 0.2 ^b	3.5 \pm 0.2 ^b	1.5 \pm 0.1 ^b	5.4 \pm 0.3 ^b	22.0 \pm 0.9 ^b
140	25	12.3 \pm 4.0 ^b	4.0 \pm 0.6 ^b	1.8 \pm 0.2 ^{a,b}	7.7 \pm 2.5 ^b	23.4 \pm 6.8 ^b
<i>Black sesame</i>						
0.1	25	205.4 \pm 5.3 ^a	7.0 \pm 0.2 ^a	2.1 \pm 0.1 ^a	131.8 \pm 1.9 ^a	535.6 \pm 30.6 ^a
100	25	16 \pm 0.6 ^b	3.9 \pm 0.1 ^b	1.6 \pm 0.1 ^b	6.6 \pm 0.2 ^b	29.2 \pm 1.0 ^b

The results are expressed as mean \pm standard deviation ($n = 3$) of independently prepared samples. Different lowercase letters indicate significant differences within the same type of sesame and characteristic diameter for different treatments ($p < 0.05$).

The volume-weighted and surface-weighted diameters were significantly reduced by HPH processing in comparison with HSM but did not show any statistically significant difference ($p < 0.05$) among different HPH

operating conditions. It must be highlighted that the reduction induced by HPH in D[4,3] (~95% reduction) is higher than in D[3,2] (~35% reduction) because D[4,3] is greatly influenced by the presence of large particles, whereas the D[3,2] is more influenced by smaller ones (Bengtsson and Tornberg, 2011), which are more difficult to disrupt, as shown by the distributions in Figure 1. This is also confirmed by the values of $D_{0.1}$, which, differently from $D_{0.5}$ and $D_{0.9}$, was not significantly affected by HPH processing. Moreover, it must be remarked that the reduction of the value of $D_{0.9}$ well below the typical size of plant cells (~100 μm) suggests that most of the sesame seed cells were completely disrupted, as previously observed for tomato skin (Jurić et al., 2019), mustard bran (Donsi and Velikov, 2020) and *Ruta chalepensis* plant material (Gali et al., 2020).

3.2 Effect of HPH processing on the extraction of polyphenols and antioxidant compounds

The total polyphenolic content and antioxidant activity (expressed as ferric reducing antioxidant power) are reported in Table 2 for the aqueous supernatant recovered from white and black seed suspensions, treated by HSM and HPH. HPH processing caused an increase of total polyphenolic content of more than 30% for an operating temperature of 25 °C, for both white and black sesame. Increased pressure levels led to a slightly but statistically significantly higher value in total polyphenolic content at 25 °C. A higher processing temperature of 50 °C caused the degradation of the extracted polyphenolic compounds, as can be observed both at 100 MPa and 140 MPa (-35% and -20%, respectively, in comparison with respective samples treated at 25 °C). The antioxidant compounds extracted in the aqueous supernatant exhibited a similar trend to the polyphenolic compounds with respect to temperature, with statistically significant degradation of the activity of 20% and 15%, respectively for 100 and 140 MPa, when increasing the operating temperature to 50 °C. Black sesame suspensions exhibited a significantly higher total polyphenolic content and antioxidant activity than white sesame suspensions, for both HSM and HPH treatment. Remarkably, in addition to the observed thermal degradation processes occurring during processing, other degradative processes caused the reduction in the antioxidant activity of the extracts during storage. Both white and black suspensions, stored for 2 and 3 d under refrigerated conditions, exhibited a significant decrease in antioxidant activity, which can be attributed to the release of polyphenol oxidases following cell damage (Taranto et al., 2017). The antioxidant activities in the supernatant of the HPH-treated samples rapidly decreased already after two or three days of storage, reaching the values recorded in the HSM-treated samples. Interestingly, in the case of black sesame, the initial higher content in polyphenols and antioxidant compounds is likely counterbalanced by a higher release of polyphenol oxidases, with a resulting proportionally greater decrease than what was observed for white sesame after 2 and 3 d. Further studies are, however, needed to better clarify this issue.

Table 2: Total polyphenolic content (TPC) and ferric reducing antioxidant power (FRAP) of the supernatant from white and black sesame suspensions (10% w/w) treated by HSM (pressure of 0.1 MPa) and by HPH at different pressures and temperatures, for N=6 passes. The analyses have been carried out on supernatants obtained immediately after processing (day 0) and after 2 and 3 days from processing.

Pressure (MPa)	Temperature (°C)	TPC (mg _{GAE} /kg) day 0	FRAP (mmol _{AAE} /kg)		
			day 0	day 2	day 3
<i>White sesame</i>					
0.1	25	20.6±0.6 ^c	0.16±0.01 ^{dA}	0.18±0.01 ^{aA}	0.17±0.01 ^{abA}
100	25	28.4±3.1 ^b	0.36±0.02 ^{aA}	0.19±0.03 ^{ab}	0.20±0.01 ^{ab}
100	50	18.1±2.0 ^d	0.29±0.01 ^{cA}	0.16±0.01 ^{bB}	0.14±0.01 ^{bcB}
140	25	33.5±0.2 ^a	0.33±0.03 ^{abA}	0.17±0.01 ^{abB}	0.16±0.01 ^{abB}
140	50	26.6±0.3 ^c	0.28±0.03 ^{cA}	N.A.	N.A.
<i>Black sesame</i>					
0.1	25	29.4±0.6 ^b	0.44±0.03 ^{bA}	0.22±0.01 ^{bB}	0.21±0.01 ^{ab}
100	25	37.7±3.1 ^a	0.60±0.06 ^{aA}	0.26±0.02 ^{ab}	0.23±0.02 ^{ab}

The results are expressed as mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences within the same type of sesame and same time of analysis for different treatments (p < 0.05). Different uppercase letters indicate significant differences for different times of analysis for the same treatment (p < 0.05). N.A.: data not available.

3.3 Stabilization of HPH-treated sesame suspensions by spray drying

The results of section 3.2 suggest that the white and black sesame suspensions need to be stabilized to maintain the desired content of antioxidant compounds over time. This is particularly crucial in the potential use of sesame seeds as functional ingredients in food formulation. In this work, the stabilization of the suspensions was carried out through direct spray drying of the HPH-treated suspensions, without any further

processing or additives, to improve the stability of the antioxidant activity of the supernatant obtained from rehydrated powders (Figure 2).

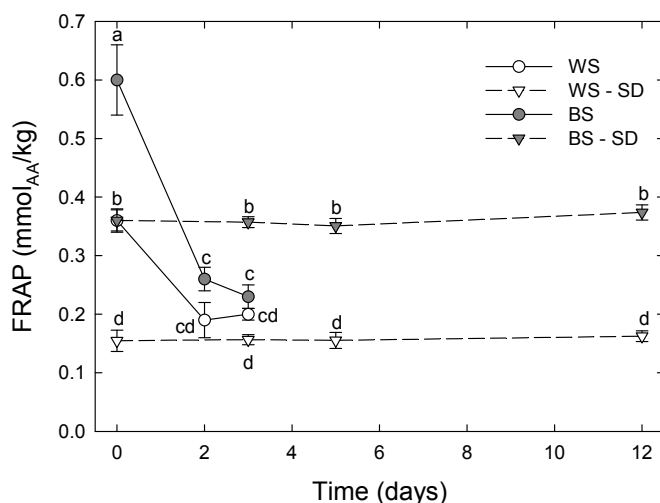


Figure 2: Ferric reducing antioxidant power (FRAP) of the supernatant from white (WS) and black sesame (BS) suspensions (10% w/w) treated by HPH at 100 MPa and 25 °C, for N=6 passes. The analyses have been carried out on supernatants obtained immediately after processing (day 0) and after 2 and 3 d from processing and for the supernatant obtained from spray-dried samples (WS – SD for white sesame, BS – SD for black sesame), upon rehydration and centrifugation at different storage times (0 - 12 d). Different lowercase letters indicate significant differences ($p < 0.05$).

The spray drying process, carried out to bring residual water content to $5 \pm 1\%$, caused a significant reduction in the FRAP values, which decreased from $0.36 \text{ mmol}_{\text{AAE}}/\text{kg}$ to $0.16 \text{ mmol}_{\text{AAE}}/\text{kg}$ for white sesame and from $0.44 \text{ mmol}_{\text{AAE}}/\text{kg}$ to $0.36 \text{ mmol}_{\text{AAE}}/\text{kg}$. The observed decrease for white sesame reached the FRAP value non-statistically different from those of the supernatant of the suspensions stored under refrigerated conditions ($0.20 \text{ mmol}_{\text{AAE}}/\text{kg}$), suggesting that oxidative and thermal degradative processing occurring during spray drying are relevant. However, the FRAP values of spray-dried samples did not change over 12 d of storage, suggesting that the enzymatic activity was efficiently inhibited by the reduced water activity. In the case of black sesame, the FRAP values measured for the spray-dried samples were significantly higher than the suspensions after 3-d storage ($0.36 \text{ mmol}_{\text{AAE}}/\text{kg}$ vs. $0.23 \text{ mmol}_{\text{AAE}}/\text{kg}$), especially because of the higher degradation observed for the antioxidant activity of black sesame suspensions. Similar to what was observed for white sesame, also the FRAP values of spray-dried black sesame did not change over 12 d of storage.

4. Conclusion

The high-pressure homogenization (HPH) process was studied in the mechanical disruption of sesame seed cells to promote the extraction and recovery of intracellular bioactive compounds, using only water as an extraction medium. Aqueous suspensions (10% w/w) of white and black sesame seeds were initially treated by high-shear mixing (HSM) for 5 min at 20000 rpm to break down the seeds, and then subsequently processed by HPH at 100 MPa or 140 MPa for up to 10 passes and different temperatures (25 and 50 °C).

The HPH treatment caused a considerable decrease in the size distribution of the suspended particles, well below the typical vegetable cell size, suggesting the occurrence of complete cell fragmentation. The associated disruption of cell walls and membranes caused a significant reduction of the mass transfer resistances for the release of intracellular compounds into the aqueous extraction solvent, which was highlighted by a more than two-fold increase in the polyphenolic content and antioxidant activity of the supernatant, in comparison to HSH treatment. Moreover, black sesame seeds exhibited a greater content of polyphenols and antioxidants. However, the results of this work highlighted also that two degradative phenomena of the extracted bioactive compounds take place. Heat-induced degradation of thermolabile bioactive compounds was observed (-20% in antioxidant activity) when HPH processing was carried out at higher temperatures (50 °C vs. 25 °C). Besides, it can be hypothesized that degradative enzymes, such as polyphenol oxidases, are also released by HPH processing, causing the progressive reduction in antioxidant activity of the extracts over time. Remarkably, the stabilization by spray drying of the HPH-treated

suspensions, without the need for further treatments or additives, demonstrated to be effective in realizing a facile and versatile integrated process for prolonging the shelf-life of the extracted bioactive compounds, which are well protected by a matrix of biopolymers naturally contained in the sesame seeds over a prolonged time.

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