Extraction Techniques of Brassica By-Products

Subjects: Food Science & Technology

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The Brassica genus (*Brassicaceae* family) is a large group of primarily herbaceous plants, one of the most important crops after soybean in world oilseed production, and as fresh vegetables, they are widely consumed throughout the year as part of salads or after cooking. This genus includes various types of well-known species such as cabbage, broccoli, brussels sprouts, kale, kohlrabi, pak choi, rape, turnip, mustard, and cress. Brassica plants are also distinguished from other vegetable plants by their high functional (phenolic and organosulfur compounds) and nutritional properties. Food losses and waste reduction are a worldwide challenge involving governments, researchers, and food industries. Therefore, by-product revalorization and the use of key extracted biocompounds to fortify innovative foods seems an interesting challenge to afford.

Keywords: Brassica By-Products ; Extraction Techniques ; Ultrasound-Assisted ; Microwave-Assisted ; Enzymatic-Assisted

1. Introduction

In the last few decades, sustainable and non-thermal techniques have been optimized to reduce costs due to conventional technologies' high energy consumption and the degradation of thermolabile nutritional compounds and the thermal instability of several bioactive compounds during the process. Therefore, it is essential to focus on innovative non-thermal 'Green Technologies' such as USAE, MWAE, and EAE, among others.

Most studies are focused on fruit by-products [1], finding a lack of clear evidence related to horticultural commodities, including Brassica by-products. Due to the interest in the effect of green and non-thermal treatments on Brassica by-products for phytochemical extraction, a compilation of the scientific evidence is needed to establish the optimum treatments and conditions (extraction, addition, processing, storage, and shelf-life). Additionally, the effect of processing, including blanching, drying, homogenization, and/or grinding into powder, should be studied as pretreatments of extraction techniques.

2. Ultrasound-Assisted Extraction from Brassica By-Products

USAE consists of the propagation of ultrasonic waves in a liquid medium, inducing a longitudinal displacement of particles that create cavities in the liquid, which is called acoustic cavitation [1]. This can occur with less solvent consumption, energy, and extraction time, making it an environmentally friendly and economical technique [2].

Table 1 shows the main conditions used for USAE of bioactive compounds from Brassica by-products. According to the research found, broccoli is the main Brassica studied, followed by cabbage, radish, cauliflower, and kale. The revalorization of Brassica by-products is mainly concentrated on leaves and stems, although there are articles focused on seeds. The frequency of USAE equipment ranged from 20 to 50 kHz. Power units depended on the equipment used, reporting values from 100 to 500 W, 50 W/L, or 0.228 W/cm^2. The best results were achieved with an aqueous solvent. Water was used as the extractant in ten of the studies found, and in seven of them it was combined with an organic solvent (ethanol, methanol, and acetonitrile), with ethanol being the main one [3][4][5][6]. In fact, Liu et al. [7] reported a better SFN extraction with a ratio of 1:10 for water compared to 1:50 for ethyl acetate. The solid:liquid ratio in most of the studies ranged between 1:2 and 1:50, and just one of the studies found that it worked with a more diluted extract (0.06:30) [8]. The extraction temperature used was determined by the target compound or the function to be achieved by the extraction. An extraction temperature below 30 °C was best for the GLS and SFN extractions [5][6][7][9][10][11]. However, MWAE pretreatment for a short time favored SFN extraction due to the inactivation of the myrosinase enzyme and GLS-SFN conversion. Temperatures above 45 °C were used for the extraction of phenolic compounds [2][6], and in the case of protein extraction, USAE was carried out in some studies [4][12][13].

Table 1. Ultrasound conditions (frequency, power parameters, solvent, time, and temperature) for the extraction of bioactive compounds from Brassica by-products.

By-Product Characteristics	F (kHz)	Power Parameters	Solvent	S:L Ratio (w:v)	T (min)	T (°C)	Other Information	Main Findings	Ref.
Radish seeds cv. IPR 11 Particle size information NA	25	165 W	EtOH	1:12	20–60	30–60	USAE bath with indirect contact. After the extraction, seeds were separated by filtration, and the excess solvent was removed until reaching a constant weight.	The maximum yield (25%), a greater amount of phytosterols and tocopherols, and, consequently, greater oxidative stability.	[14]
Red radish cv. information NA Freeze dried 1–2 mm pieces	NA	138–358 W	H_2O	0.06:30	30–120	45	Before USAE by pulse cycles of 5 s on and 1 s off, extraction of anthocyanins was performed.	High-energy USAE treatment (120 min at 286–258 W) is adequate to enhance TAC but does not preserve anthocyanins.	[8]
Broccoli leaves, stems, and inflorescences cvs.: 'TSX 007', 'Monaco', 'BRO 2047', 'Parthenon', and 'Summer Purple' Dried (45 °C, 48 h) Particle size information NA	NA	NA	80% EtOH	10:60	60	45–50	Excess EtOH was removed by heating it at 37 °C in a rotary evaporator under vacuum. The resulting aqueous extracts were combined and lyophilized.	Extraction yield of 13.4–16.3% dw. High TAC and chlorophylls and phenolics (mainly kaempferol and quercetin glucosides) in leaf extracts ('Summer Purple') and high GLS content in inflorescence extract.	[15]
Broccoli leaves, stems, and inflorescences cv. Parthenon Dried (45 °C, 24–48 h) Particle size information NA	NA	220 V 360 W	H_2O	1:50	60	NA	Before USAE, the mixture was heated for 16 min at 121 °C. After US, four times its volume of ethanol was added, and after 12 h of incubation, it was dried at 45 °C in a forced-air oven.	USAE did not manage to modify the neutral sugar profile.	[16]
Broccoli by-products cv. information NA Dried (35 °C, 48 h) Particle size information NA	25	50 W/L	H_2O	1:10	60	15	The extract was dried at 30 °C in a vacuum oven. The residue was mixed with water and recovered by centrifugation (6000 rpm × 10 min).	USAE extracted more bioactive compounds than supercritical fluids but not as many as pressurized liquid.	[13]
Cauliflower by-products cv., drying, and particle size information NA	NA	175 W	H_2O (pH 11)	1:4	15	NA	The crude fiber and insoluble protein were removed from the extract first with 3 layer gauze and then by centrifugation (4000 rpm × 30 min).	Extraction yield of 53.1% and 12.066 g of soluble leaf protein kg^{-1}.	[9]

By-Product Characteristics	F (kHz)	Power Parameters	Solvent	S:L Ratio (w:v)	T (min)	T (°C)	Other Information	Main Findings	Ref.
Cauliflower by-products Blanching cv. information NA Dried (50–55 °C overnight) Particle size 0.5 mm	24	400 W	H_2O 70% MeOH 80% Ac	50:100	0–10	NA	Amplitude USAE from 20–100%. After US, centrifugation at 1500× *g* for 15 min, and the pellet was centrifuged with 100 mL of solvent. Both supernatants were collected, combined, and filtered under vacuum conditions.	The amplitude affected the extraction of isothyocyanates (80% amplitude for 3 min) and phenolics (100% amplitude for 3 min).	[12]
Rapeseed meal cv., drying, and particle size information NA	28	0.228 W/cm^2	H_2O	1:30	41.48	NA	Other extraction conditions were pH 11.71 and USAE power 40%.	High protein yield of 43.3% and nitrogen solubility of 18.1%.	[3]
Broccoli cv., drying, and particle size information NA	40	500 W	Ch 80% EtOH Ac	100:500	60	40	Extracts were combined to metal-organic framework nanocubes. They were dispersed by an ultrasonic probe in 100 mL, then triethylamine as a capping agent was added, and the mixture was agitated and heated for 12 h at 130 °C.	Broccoli extract combined with MOF-5-NCs showed synergistic activity against *P. aeruginosa* bacteria in standard and clinical strains.	[2]
Kale cv. information NA Convective dryer (39 °C) Particle size information NA	20	100 W	80% EtOH	2:40	60	60	USAE in two cycles of 30 min Extracts were filtered, combined, and evaporated. The residues were dissolved in methanol and filtered.	High isolation of phenolic acids and high yield of biocompounds in short time and reduced solvent volume with easy handling.	[4]
Broccoli seeds cv., drying, and particle size information NA	NA	200–500 W	H_2O EA	1:10–1:50	5–40 s	25–35	Before USAE, broccoli seeds were treated in a MWAE oven for 1–4 min at low power.	The highest SFN formation was under a MWAE pretreatment of 3 min and a US treatment of 20 s, 500 W, and 1:10 for water or 1:50 ethyl acetate.	[7]
Broccoli stems and leaves cv. information NA. Dried (30–35 °C, 48 h). Particle size information NA	25	50 W/L	H_2O	1:10	60	NA	After homogenization, the extract was dried at 30 °C in a vacuum oven. The residue was mixed with water (25 mL) and recovered by centrifuging at 6000 rpm for 10 min.	High-quality extract in terms of antimicrobial efficacy against *Pseudomonas* spp. and *Candida krusei*.	[17]
White cabbage cv. information NA Oven-dried (60 °C, 72 h) Particle size information NA	40	132 W	60% EtOH	2:10	120	30–70	Ultrasonic intensity of 0.46 W/cm^2. The obtained extracts were hydrolyzed before analyzing.	Richer extract at 30 °C. Antimicrobial activities only of the hydrolyzed extracts.	[5]

By-Product Characteristics	F (kHz)	Power Parameters	Solvent	S:L Ratio (w:v)	T (min)	T (°C)	Other Information	Main Findings	Ref.
Broccoli heads cv., drying, and particle size information NA	23	NA	H_2O	1:20	1–12	25–60	Amplitude was set at 135 µm.	Higher myrosinase inactivation and SFN content at 60 °C for 4 min. Activation energy was 3.6-fold lower regarding traditional blanching.	[10]
Camelina sativa oil cv., drying, and particle size information NA	35	60–120 W	40–80 EtOH	1:5–1:15	10–20	30	USAE in 2–4 cycles of 5 min each. A solid-phase extraction procedure to obtain an extract rich in GLS and to perform cellular assays.	High-GLS extraction with 65% EtOH, 1:15, and 10 min. The purified extract (800 mg from 10 g) showed chemopreventive action against colorectal cancer cells.	[6]
Thirty-six *Brassica oleracea* var. *acephala* accessions Dried in an oven (105 °C) or freeze-dried Particle size information NA.	40	300 W	80% MetOH	0.03:1.5	30	20	After USAE, extracts were centrifuged at 15,000× *g* for 5 min.	Higher GLS content, TAC, TPC, and sugars with freeze-dried samples and USAE compared with hot extraction.	[11]
Cabbage leaves, fresh and steamed (100 °C, 2 min) cv., and drying info NA Particle size 1.7–2.55 mm.	37	320 W	H_2O	5:50	40	NA	Absorbed US power of 0.03 W/g extraction + MWAE or vaccum.	Higher glucoraphanin content with USAE + vacuum or MWAE More effective (87%) when leaves were steamed, presenting higher myrosinase inactivation.	[18]

NA: Data not available; cv.: cultivar; Ac: acetone; EA: ethyl acetate; Ch: chloroform; TPC: total phenolic content; TFC: total flavonoid content; TAC: total antioxidant capacity; GLS. Glucosinolates; SFN: sulforaphane; S:L: solid:liquid.

3. Microwave-Assisted Extraction from Brassica By-Products

The application of MWAE to enhance extraction consists of the ability to extract bioactive compounds from structural changes in cells due to the electric and magnetic fields generated by this technology. The conditions reported in previous studies to be considered in MWAE are summarized in **Table 2**. The main studied by-products came from broccoli, cabbage, and radish. Although the cv. is an important parameter to know since the phytochemical content may vary, it was not detailed in the reported manuscripts. The power intensity ranged from 130 to 400 W under atmospheric conditions, except in one study in which vacuum was applied together with MWAE to improve the extractability [18]. The solvents used for MWAE were different in each study, including water, water + ethanol, dichloromethane, nitric acid, or methanol. The most concentrated solid:liquid ratio used was 1:4 [19], and the most diluted was 0.5:31.5 [20]. Both obtained good results, because the extraction conditions (time, solvents, and temperature) were different. The temperature ranged from 20 to 90 °C, always below 100 °C to avoid bioactive compound degradation. The extraction time varied from 1 to 25 min, obtaining the best results with times of less than 20 min.

Table 2. Microwave conditions (power parameters, solvent, time, and temperature) for the extraction of bioactive compounds from Brassica by-products.

By-Product Characteristics	Power (W)	P	Solvent	S:L Ratio (w:v)	T (min)	T (°C)	Other Information	Main Findings	Ref.
Purple-heart radish cv. information NA. Dried in the oven (60 °C) Particle size 117-µm.	NA	Atm	H_2O andEtOH	0.5:31.5	20	70	Twenty grams of broccoli powder were pre-extracted with petroleum ether II at 80 °C for 6 h.	Polysaccharide yield (29%) was higher than hot (~24%) and USAE (27%) extraction.	[20]
White cabbage leaves are chopped. cv. information NA. Fresh or dried with a hot air dryer (60 °C) Particle size information NA.	130–390	Atm	DCh H_2O	5:50	1–5	22–38(DCh)22–98(H_2O)	After extraction with a domestic MW oven, the extract was filtered and dehydrated using the rotary evaporator at 30 °C (for DCh) or 45 °C (for H_2O).	Higher SFN yield in less time. Higher MW powers resulted in a shorter extraction time.No differences between fresh and semi-dried samples, nor between the solvents used.	[21]
Broccoli florets, stems, and leaves. cv., drying, and particle size information NA.	NA	Atm	40–80% MetOH	1:20	10–20	55–75	After extraction, the mixture was centrifuged for 20 min at 10,350 rpm and 4 °C. The supernatant was filtered and stored at −20 °C.	The optimum conditions were 74.5, 80, 80% MetOH, 15.9, 10, 18.9 min, and 74.5, 73.3, 75 °C for stalks, leaves, and florets, respectively. Increased the phenolic yield up to 65.3, 45.70, 133.6% for stalks, leaves, and florets, respectively, in less time.	[22]
Purple and white cabbages cv. information NA. Sun-dried. Particle size 80–100 µm.	200–400	Atm	NAc	1:4–1:7	10–25	60–90	After extraction, the extract was completed with 10 mL.	Optimum conditions: 201 W at 60 °C for 10 min at a 1:4 ratio. A polynomial regression was the best-fitting model.	[19]
Cabbage leaves (1.7–2.55 mm) cv. information NA. Fresh and steamed. (100 °C for 2 min). Particle size information NA.	180	Atm 70 kPa	H_2O	5:50	10	NA	Combined with USAE	Higher glucoraphanin content using vacuum MWAE with USAE than atmospheric MWAE. More effective (87%) when leaves were previously steamed, and a higher inactivation of the myrosinase enzyme.	[18]

NA: Data not available; cv.: cultivar; SFN: sulforaphane; NAc: Nitric acid; DCh: Dichloromethane; Atm: Atmospheric; P: pressure; S:L: solid:liquid.

4. Enzymatic-Assisted Extraction from Brassica By-Products

EAE is based on the use of enzymes to break down the cell walls of plant material and improve the extraction yield of its bioactive compounds. The main conditions to be considered are shown in **Table 3**. Most of the Brassica by-products used in the studies come from broccoli, radish, cauliflower, and cabbage. Before EAE, by-products are usually pretreated by

grounding and drying (oven at 45–60 °C or using a freeze-dryer), although particle size is rarely detailed. The enzymes used were determined by the compound to be extracted. The main enzymes found were cellulase, hemicellulase, protease, pectinase, and glucanase, among others. Papaioannou and Liakopoulou-Kyrikides [23] used a fungus to facilitate the β-carotene production from Brassica by-products. Other green technologies combined with EAE, such as MWAE [20] and USAE [24], have been used to increase the extraction yield prior to enzymatic rupture of the cell walls. Only half of the articles summarized in **Table 3** detail the enzyme inactivation conditions; two of them used heating for a few minutes and one used refrigeration. The solid:liquid ratio ranged from 10:40 to 5:500, like other extraction methods using green technologies. Extraction time was highly variable, ranging from 8.4 to 1200 min, but the temperature was limited between 26 [23] and 68 °C [20].

Table 3. Enzymatic conditions (enzyme, pressure, time, and temperature) for the extraction of bioactive compounds from Brassica by-products.

By-Product Characteristics	Combined with	Enzymes	Inactivation Enzymes	S:L Ratio (w:v)	T (min)	T (°C)	Main Findings	Ref.
Purple-heart radish cv. information NA. Oven-dryer (60 °C).	MW	Papain	NA	1:55–1:65	8.4	68	EAE combined with MWAE facilitated cell rupture and enzymolysis, improving the extraction yields and shortening the extraction time.	[20]
Broccoli by-products (leaves, stems, and inflorescences). cv. Parthenon Forced-air oven dryer (45 °C, 24–48 h)	NA	Cellulase	Cooled at room temperature	1:50.8	120	50	Decreased the sugar content and increased the uronic acid content. Non-extractable phenolics were found higher in inflorescences and increased with EAE and TAC.	[16]
Radish root ground with a mortar. cv. and drying information NA.	US	Cellulases Pectinases Amylases Glucanases Hemicellulases	Few minutes at 90 °C	10:40	66–84	46–64	Higher TAC with the highest extraction of TPC.	[24]
Canola (*Brassica napus*) oil pressing residues. Particle size: 0.5 mm cv. and drying information NA.	NA	Protamex® Alcalase® Viscozyme® Phyzyme®	NA	1:10	240–1200	45–50	The applied enzymes effectively enhanced the solubility of proteins, despite the lower yield of crude proteins compared to the alkaline extraction (40–82 vs. 91 g/100 g dw).	[25]
Cauliflower florets and leaves cv. information NA Pre-extraction with 96% ethanol (1:5) for 30 min at 100 °C. Residue was dried at 40 °C.	NA	Proteases Cellulases Endopolygalacturonase II Rhamnogalacturonan hydrolase Pectin methyl esterases Rapidase Liq+	10 min at 100 °C	5:500	240	50	Higher methoxy pectins of high molar mass were extracted with three enzyme mixtures. Health benefit pectic oligosaccharides were obtained after pectin extraction. Seventy percent of the by-products were consumed to extract two products of interest.	[26]

By-Product Characteristics	Combined with	Enzymes	Inactivation Enzymes	S:L Ratio (w:v)	T (min)	T (°C)	Main Findings	Ref.
Cabbage (91.5% humidity)	NA	*Blakeslea trispora* (mould)	NA	1:10	NA	26	Higher biomass accumulation and carotenoid production.	[23]

NA: Data not available; cv.: cultivar; TPC: total phenolic content; TAC: total antioxidant capacity; S:L: solid:liquid.

5. Other Extraction Methods from Brassica By-Products

Although the most commonly cited green technologies in the bibliography have already been described, a considerable number of works have studied other technologies to extract bioactive compounds from Brassica sources. Previous research has shown that extracting pectin from broccoli stalks with 0.1 M nitric acid under reflux for 30 minutes [27] is effective, and that by-products of broccoli florets are an excellent source of glucoraphanin and phenolics after extraction in a thermostatic bath mixed with ethanol (0, 40, and 80%) for 10, 40, or 70 minutes [28]. Nevertheless, despite the recent publication of these works, only the scientific studies that include novel and green technologies to enhance the extraction ability of Brassica by-products are shown in **Table 4**.

Table 4. Other green technologies used for the extraction of bioactive compounds from Brassica by-products.

By-Product Characteristics	Green Technology Used	S:L Ratio (w:v)	T (min)	T (°C)	Other Parameters to Be Monitored	Main Findings	Ref.
Broccoli leaves, stems, and inflorescences. cv. ParthenonDried in a forced-air oven (45 °C, 24–48 h).	Supercritical fluids using CO_2	NA	120	45–55	Dynamic extraction. Flow: 2 L/min. Three hundred bar at 55 °C or one-hundred and fifty bar at 45 °C.	The content of non-extractable phenolics and TAC increased and were higher in inflorescences.	[16]
Broccoli by-products. Dried (35 °C, 48 h).	Supercritical fluids using CO_2	NA	140	35	Two pumps: (i) Supercritical CO_2 (ii) Organic co-solvent (20% EtOH). 150 bar Flow: 2 L/min	Presented the worst results regarding the extraction of bioactive compounds.	[29]
Broccoli by-products. Dried (35 °C, 48 h)	Pressurized liquid	15:25	10	60	Steps: (i) Filling the cell with 70% EtOH, 2–3 min; (ii) Upto 1500 psi; (iii) Five minutes at 60 °C + 5 min extraction; (iv) Static and 30 s depressurization; (v) Washing the cell for 50 s; (vi) Purge the solvent with N_2 2 min. Drying in a vacuum oven (30 °C).	The highest content of bioactive compounds and TAC.	[13]

By-Product Characteristics	Green Technology Used	S:L Ratio (w:v)	T (min)	T (°C)	Other Parameters to Be Monitored	Main Findings	Ref.
Yellow mustard flour (30.7% oil, 30.9% protein, 4% ash, and 9% fiber).	**Ultrafiltration**	**NA**	**NA**	**25**	**Before ultrafiltration, defatting was carried out with hexane. Film composite membrane (150–300 Da, pH tolerance range 2–10 at 25 °C, max. T[a] of 80 °C, and pressure of 40 bar).**	**In acidic conditions, 77% of the phenolic compounds were recovered. Combination of diafiltration with nanofiltration was beneficial only when processing under acidic conditions.**	[30]
Broccoli stems and leaves Dried (30–35 °C, 48 h).	**Supercritical fluids using CO_2**	**NA**	**140**	**35**	**Two pumps:** (i) Deliver solvent; (ii) Organic co-solvent (100% EtOH). **50 bar Flow: 2 L/min Drying in a vacuum oven (30 °C)**	**High-quality extract in terms of antimicrobial efficiency against *Pseudomonas* spp. and *Candida krusei*.**	[17]
Broccoli stems and leaves cv. Parthenon and Naxos.	**Supercritical fluids using CO_2**	**NA**	**NA**	**NA**	**Two pumps:** (i) Supercritical CO_2; (ii) Co-solvent (20% EtOH).	**High yield of β-carotene, phenolic compounds, chlorophylls, and phytosterols. Great TAC. Reduced organic solvent consumption.**	[31]

NA: Data not available; cv: cultivar; TAC: total antioxidant capacity; S:L: solid:liquid.

As shown, four works used supercritical fluids, one used ultrafiltration, and another used pressurized liquids. All these techniques showed higher yields for recovering bioactive compounds from Brassica by-products. Nevertheless, such techniques are even more expensive than those previously described and take longer to extract the phytochemicals, although they use lower temperatures (35–60 °C) to avoid their degradation and do not require high amounts of solvents to complete the extraction. The solid:liquid ratio is not a relevant parameter in supercritical fluid technology. However, the solvent flow rate is detailed in almost all the works found as being 2 L/min. Superficial fluid technology facilitated the extraction of bioactive compounds and antioxidants, except in the work of Marinelli et al. [13], where this technology showed the worst results compared to pressurized liquid technology.

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