Characterization of dried horseradish leaves pomace: phenolic compounds profile and antioxidant capacity, content of organic acids, pigments and volatile compounds

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Abstract

Horseradish (*Armoracia rusticana*) leaves pomace, which contains high-value bioactive compounds, is the product resulting from pressing horseradish leaves for juice production. The aim of the current research was to investigate the effect of convective, microwave-vacuum and freeze-drying on the content of bioactive compounds in horseradish leaves pomace. Convective hot air-drying was performed at 40, 60 and 80 °C. The total phenolic content (TPC), total flavonoid content (TFC), total flavan-3-ol content, total phenolic acid content, total flavonol content, chlorophylls and total carotenoids, and antioxidant activity were determined by spectrophotometric methods. Individual profiles of phenols and organic acids are estimated by high-performance liquid chromatography (HPLC), but volatile compounds are estimated by gas chromatography (GC). Totally, 14 individual phenolic compounds, 8 organic acids, and 49 volatile compounds were analysed in the studied samples. The main phenolic compound identified in horseradish leaves pomace was rutin (3231 mg/100 g DW), among organic acids—quinic and malic acids, and volatile compounds—allyl isothiocyanate, 3-butenenitrile and benzyl alcohol. In the drying process, the content of some (total flavan-3-ols, total carotenoids content) compounds increased, but others (TPC, total organic acids content) decreased, and it was drying method-dependent. Freeze-drying caused the reduction of TPC by 29%, whereas convective drying by 53–59%. Fresh pomace contains such isothiocyanates as allyl isothiocyanate and butyl isothiocyanate, which were completely lost in the drying process. Freeze-drying allowed the best retention of various phenolic and volatile compounds in horseradish leaves pomace.

Keywords Rutin · Chlorophylls · Carotenoids · Allyl isothiocyanate · Drying method

Introduction

Horseradish (*Armoracia rusticana*) leaves pomace is the product resulting from pressing horseradish leaves for juice production. Generally, pomace is considered to be a production waste, which gains a strong interest due to the environmental aspects related to the waste disposal. Additionally, it is well documented that production waste, such as peels, seeds, and pomace, contains high-value bioactive

compounds [1, 2]. Thus, pomace can be used as a source of phenolics and other bioactives possessing antioxidant power. However, the use of pomace is limited by its high moisture content, which reduces the shelf life. Therefore, processing of waste is required to extend its possible use. But, processing may result in unwanted changes of bioactive compounds [3]. Drying, among other processing methods, is widely used to inhibit enzyme and microbial activity [4]. In food processing, variety of drying technologies with various processing parameters are used [3].

Convective drying is one of the most popular drying methods, which is based on the moisture removal by hot air. As a result of thermal effect, volatile compounds may be lost during air-drying [5]. However, due to cell wall damage, some compounds may be released and become more easily extracted. Additionally, as a result of oxidative reactions or glycosidic hydrolysis of new compounds, which are not present in fresh samples, may be formed [6].

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To increase the process efficiency and reduce costs, as well as to preserve product quality, alternative dehydration methods, such as microwave-vacuum (MW) and freeze-drying (FD), are used.

In the MW drying, compared to the traditional drying technologies, lower temperatures are used and vacuum along with volumetric heating increases the drying rate [7]. It results in shorter drying time, which reduces the possible damage to food compounds due to oxidation and thermal degradation [8]. It results in a better preservation of thermally unstable bioactive compounds present in the product being dried.

Freeze-drying, which unlike other drying methods takes place at low temperatures, is suitable for elimination of heat-induced degradation of bioactives [8]. However, relatively high expense is the major limitation of this method [9].

In the drying process, fruit and vegetable phenolics are exposed to various temperatures and oxygen, resulting in chemical and biochemical degradation reactions, which may induce formation of new compounds of different bioactivity compared to the initial material. Scientific literature reveals contradictory data on drying effect on phenolic content in plant materials because bioactive compounds may have different response to temperature, oxygen, light, and other physical factors due to the biological diversity and chemical structure [10], as well as drying method and process parameters—temperature, duration, microwave power, pressure, etc. [3, 10].

Similarly, initial volatile composition, food matrix, variety, developmental stage, and drying method and its parameters affect volatile profile of dried products [6, 12]. Drying may cause interaction among volatile compounds as well as induce other reactions, resulting in loss of initially present volatiles responsible for aroma, flavour and taste of the product [13]. Earlier studies proved that the most effective method for preservation of volatiles in aerial parts of coriander is freeze-drying [14]. In hot air-drying, an increased loss of volatiles is observed at higher drying temperature. According to Calín-Sánchez et al. [11], the most suitable drying method for volatile preservation in sweet basil plants (*O. basilicum* L.) was combined method—convective pre-drying and microwave finish-drying (40 °C and 360 W) because of short drying time (approx. 250 min). In the final product, they reported high concentration of volatiles and sensory profile typical for high-quality dried products. Despite FD is not a very popular method for plant drying, it effectively retains volatile compounds in grape skins [15] and jabuticaba peel [16].

To the best of our knowledge, this is the first study that aimed to investigate the effect of convective, microwave-vacuum and freeze-drying on the content of total phenolics, flavonoids, flavan-3-ols, phenolic acids, individual phenols, antioxidative capacities, organic acids, pigments,

and volatile compound profiles in horseradish leaves pomace (HRLP).

Materials and methods

Plant materials and chemicals

Fresh horseradish (*Armoracia rusticana* L.) leaves were collected in Latvia (latitude: 56° 40' N, longitude: 23° 30' E) in July 2018. Leaves were washed, placed on the moisture-absorbing surface until fully dried (approximately 2 h), and cut into 5.0 ± 0.5 cm long pieces. The samples of 0.25 kg each were packaged in the hermetically sealed polypropylene (PP) pouches and then frozen at -20 °C and stored until processing (Fig. 1). Frozen horseradish leaves were ground for juice extraction in a basket press.

The remaining horseradish leaves pomace (HRLP) was then dried till dry matter content 92–97% using the following methods:

- freeze-drying in a vacuum freeze-dryer FT333 (Armfield Ltd, UK) at -40 °C and 6.4 Pa pressure, drying time 72 h;
- microwave-vacuum drying in a dryer Musson-1 (Ingredient, Russia) for at a drum rotation speed of 6 rpm, pressure of 12.00–14.63 kPa, programmed at gradually decreasing microwave power level, starting with 4 operating magnetrons, then 3, 2, 1 magnetrons (each 640 W), drying time for 2 kg of fresh product 45 min;
- convective hot air-drying in a Universal Oven UF160 (Mettmert GmbH + Co.KG, Germany) at 40, 60 and 80 °C until the moisture content was below 8%, which requires drying time 6, 5 and 4 h, respectively.

Fresh HRLP was used as a control sample.

The moisture content was determined in triplicate according to the standard ISO 6496:1999 (LVS 272:2000) for expressing results on dry basis.

All the following chemicals were of analytical or high-performance liquid chromatography (HPLC) grade. Gallic acid, Folin–Ciocalteu phenol reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) were purchased from Sigma-Aldrich (Switzerland). All other chemicals used in the research were obtained from Acros Organic (USA).

Fig. 1 Horseradish leaves processing flowchart

Determination of total phenolic content (TPC), total flavonoid content (TFC), total flavan-3-ol content, total phenolic acid content, total flavonol content and antioxidant activity

Extraction procedure

HRLP for spectrophotometric analysis was ground to fine particles and homogenized to a particle size of 0.5 μm . Weight of samples for multiple extractions was selected to obtain equal dry matter mass, namely, 1.00 ± 0.01 g of fresh pomace (Fr) and 0.25 ± 0.01 g of dried pomace. First, samples were twice extracted with 10 mL acetone in an ultrasonic bath YJ5120-1 (Oubo Dental, USA) at 35 kHz for 30 min at 20 ± 1 °C. The extracts were then separated in a

centrifuge CM-6MT (Elmi Ltd., Latvia) at 3,500 rpm for 10 min. The supernatants were combined into 25 mL graduated flask and filled up to the mark with the solvent. The residues were re-extracted with ethanol:water 80:20 (v/v) using the same procedure. Quantification was done using a JENWAY 6300 spectrophotometer (Baroworld Scientific Ltd., UK). Results were calculated as a sum of acetone and ethanol extracts using respective calibration curves. The extraction process was done in triplicate.

Spectrophotometric analysis

TPC was determined according to the Folin–Ciocalteu spectrophotometric method described by Singleton et al. [17]

and is expressed as GAE/100 g dry weight (DW) of plant material.

TFC was determined according to the colorimetric method described by Kim et al. [18] with modifications given by Blasco et al. [19] and is expressed as CE/100 g DW of plant material.

The flavan-3-ol (proanthocyanidins) content was determined by Zam et al. [20] method and is expressed as CE/100 g DW of plant material.

The phenolic acid content was determined by Gawlik-Dziki [21] method and is expressed as CAE/100 g DW of plant material.

The total flavonol content was determined by Ložiene et al. [22] method and is expressed as RE/100 g DW of plant material.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) radical-scavenging activity was determined by Yu et al. [23] method. The radical-scavenging activity of extract was also measured by 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid (ABTS^{•+}) radical cation assay by Re et al. [24] method. Both antioxidant activities are expressed as TE/100 g DW of plant material.

The reducing power was determined according to Athukorala et al. [25]. This parameter is expressed as AAE per 100 g DW of plant material.

Analysis of individual phenols

Weight of HRLP samples for extraction was selected to obtain equal dry matter mass, namely, 5.00 ± 0.01 g of fresh pomace (Fr) and 1.25 ± 0.01 g of dried pomace, and twice extracted with 10 mL of 1 N HCl/ET/H₂O (1/80/19 v/v/v) in an ultrasonic bath YJ5120-1 (Oubo Dental, USA) at 35 kHz for 10 min at 20 ± 1 °C. The extracts were then centrifuged in a centrifuge CM-6MT (Elmi Ltd., Latvia) at 3,500 rpm for 5 min. The supernatants were combined into 25 mL graduated flask and filled up to the mark with the solvent. The quantification was carried out using a Shimadzu liquid chromatograph LC-20AD with the analytical column C18, photodiode array detector SPD-M20A, according to the procedure described by Priečina et al. [26].

Determination of organic acids

Weight of HRLP samples for extraction was selected to obtain equal dry matter mass, namely, 5.00 ± 0.01 g for fresh pomace (Fr) and 1.25 ± 0.01 g for dried pomace, and extracted with freshly prepared m-phosphoric acid in distilled water ($\text{pH} = 3.00 \pm 0.20$). Extraction was performed using a magnetic stirrer (magnet 4.0×0.5 cm) at 700 rpm for 1 h at room temperature (20 ± 1 °C). The extracts were then filtered (paper No. 89). The supernatants were placed into 25 mL graduated flask and filled up to the mark with the solvent.

Determination of oxalic, tartaric, quinic, malic, ascorbic, citric, fumaric, and succinic acids was carried out using high-performance liquid chromatography (HPLC) (Shimadzu LC-20 Prominence, Shimadzu USA Manufacturing Inc., USA), detector DAD SPD-M20A, solvent delivery unit LC-20AD, column oven CTO-20A, auto sampler SIL-20A, system controller CBM-20A and data system LC solution software. Chromatographic parameters, such as the analytical column Perkin Elmer C18, $4.6 \text{ mm} \times 250 \text{ mm}$, $5 \mu\text{m}$ and temperature of column 35 °C,—were used for the separation of organic acids at wavelength 210 nm. Injection volume of sample was $10 \mu\text{L}$. Mobile phase: A (acetonitrile) and B (0.05 M KH₂PO₄) ratio 1:99 in the gradient conditions was used. Initial flow rate was 1.25 mL/min . Each measuring was carried out three times and the averages are reported. The calibration chromatogram is presented in Fig. 2.

Determination of chlorophylls and carotenoids

Chlorophyll *a*, chlorophyll *b* and total carotenoid content were determined by spectrophotometric method [27] with modifications described by Tomsone and Kruma [28]. Weight of HRLP samples for extraction was selected to obtain equal dry matter mass, namely, 0.50 ± 0.01 g of fresh pomace (Fr) and 0.10 ± 0.01 g of dried pomace, and extracted with 10 mL acetone using a magnetic stirrer (magnet size 4.0×0.5 cm) at 700 rpm for 15 min at room temperature (20 ± 1 °C). Extractable chlorophylls *a* and *b* as well as total carotenoids were detected at various wavelengths (470, 645, and 662 nm) using a spectrophotometer Jenway 6300

Fig. 2 HPLC calibration chromatogram of individual organic acids (g/L) recorded at 210 nm

(Baroworld Scientific Ltd., UK) and calculated according to equations described by Tomsone and Kruma [28]. Additionally, total extractable chlorophyll content was expressed as a sum of chlorophylls *a* and *b*, and the ratio between chlorophylls *R* (*a/b*) was calculated.

Analysis of volatile compounds

Volatiles from the horseradish leaves pomace were extracted using SPME. 2.00 ± 0.01 g sample of fresh pomace (Fr) and 1.00 ± 0.01 g of dried pomace were weighed into a 20 mL headspace vial. For SPME extraction, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (Supelco Inc., Bellefonte, PA, USA) was used. The headspace SPME is based on the absorption of the analytes on a fiber coating placed in the sample's headspace volume and on the partition of the target analytes between the sampling matrix and the fiber. SPME parameters were: incubation time 10 min; extraction temperature 35 ± 1 °C; and extraction duration 10 min. After the extraction, the volatile compounds were thermally desorbed and transferred onto the chromatographic column where they were separated. Desorption parameters were set for 15 min at 250 °C. For the analysis of the SPME extracts, a Perkin Elmer Clarus 500 GC/MS and an Elite-Wax ETR columns (60 m $\times$ 0.25 mm i.d.; DF: 0.25 μ m) were used. Working conditions were as described by Tomsone et al. [29]. All analyses were performed in triplicate.

Statistical analysis

Experimental results are means of three parallel measurements and were analysed by Microsoft Excel 2010 and SPSS 17.00. Analysis of variance (ANOVA) and Tukey's test were used to determine the differences among samples. A linear correlation analysis was performed to determine the relationship between TPC, TFC, total flavan-3-ol, total flavonols, total phenolic acid and individual phenol contents, and antioxidant activity, such as DPPH[•], ABTS^{•+} and reducing power. Differences were considered as significant at $p \leq 0.05$. The principal component analysis (PCA) was used for the evaluation of data on the composition of phenolic compounds and antioxidative activity, as well as the composition of volatiles in HRLP depending on drying conditions.

Results and discussion

Phenolic compounds

TPC in fresh horseradish leaves pomace (HRLP) was 4539 mg GAE/100 g DW (Table 1), being higher than TPC in fresh horseradish leaves determined in our previous study

[30], which ranged from 19 mg GAE/100 g DW (using conventional extraction with n-hexane) to 3193 mg GAE/100 g DW (using Soxhlet extraction with 95% ethanol). Another study of TPC in horseradish leaves depending on clones demonstrated the variation between 339 and 2369 mg GAE/100 g DW [31]. According to Marelli et al. [32], the differences in TPC could be explained both by climatic factors, growing conditions, developmental stages and processing conditions.

Drying of HRLP resulted in the significant decrease ($p \leq 0.05$) in the phenolic content (Table 1) and it was drying method-dependent. Compared to all drying methods studied in the current research, FD better preserved phenolic compounds, namely, FD caused the reduction by 29%, whereas convective drying by 53–59%. Also, drying of guava (*Psidium guajava* L.) by different methods caused a decrease of TPC [33]. TFC in HRLP, similarly to TPC, was also decreased by 62% on average after drying compared to fresh pomace. It was in agreement with ginger drying, which showed a decrease of TFC in samples dried at 60 °C temperature and using microwave power [34]. Conversely, Xiong et al. [35] observed TFC increase in sorghum (*Sorghum bicolor* L. Moench) grain after treatment at 100 °C and 150 °C.

Influence of convective drying on HRLP was temperature-dependent and better preservation of TPC was achieved using higher temperature and shorter time. Similar trends for pumpkin by-products were observed by Kława et al. [36] who revealed that convective drying at 80 °C resulted in 21–39% higher TPC compared to product dried at 40 °C. It is recognized that higher temperatures improve the solubility of phenolic compounds as the hot air facilitates cell structure breakdown, which leads to the release of phenolics from macromolecules [36, 37]. Additionally, TPC reduction could occur due to oxidation enzyme activity (polyphenoloxidase, glycosidase and peroxidase) during lower-temperature (40 °C) drying [39].

At the same time, the total phenolic acid content was by 92% higher, but total flavan-3-ol content was by 56% higher in HRLP dried in a convective drier at 80 °C when compared to the convective drying at 40 °C. In contrast, Wojdyło et al. [9] reported a decrease of 38–44% in total flavan-3-ol content in jujube fruits after convective drying at increased temperatures (50, 60 and 70 °C).

In HRLP, TFC decreased, which could be explained by thermal degradation during high-temperature treatment [40]. Higher TFC content of guava powder was reported in FD sample compared to oven-dried powder [33]. At increased drying temperature and reduced duration, TFC of HRLP was higher, but opposite trend was observed by Wolfe and Liu [1] in apple peels.

Microwave-vacuum (MW) drying caused a decrease in TPC by 28%, but according to Lim and Murtijaya [40], fast

Table 1 Phenolic compounds, antioxidant activity, and pigment content in horseradish leaves pomace (HRLP) dried by different methods

Parameters	Drying methods					
	Fr	FD	MW	C-40	C-60	C-80
<i>Phenolic compounds</i>						
TPC, mg GAE/100 g DW	4539 ± 57 ^a	3232 ± 44 ^b	1975 ± 29 ^d	1870 ± 28 ^e	2092 ± 28 ^c	2119 ± 23 ^c
Total phenolic acids, mg CAE/100 g DW	3214 ± 39 ^a	2904 ± 39 ^b	1624 ± 26 ^e	1065 ± 11 ^f	1978 ± 24 ^d	2046 ± 26 ^c
TFC, mg RE/100 g DW	3365 ± 28 ^a	1589 ± 21 ^b	1443 ± 16 ^c	1049 ± 16 ^f	1229 ± 29 ^e	1275 ± 13 ^d
Total flavan-3-ol, mg CE/100 g DW	10,713 ± 98 ^c	13,408 ± 124 ^b	10,626 ± 104 ^{c,d}	9332 ± 92 ^e	13,393 ± 124 ^b	14,598 ± 129 ^a
Total flavonols, mg RE/100 g DW	1172 ± 14 ^a	1050 ± 19 ^b	659 ± 17 ^c	479 ± 15 ^f	546 ± 17 ^e	619 ± 18 ^d
<i>Antioxidant activity</i>						
DPPH [•] , mmol TE/100 g DW	36 ± 1 ^d	46 ± 1 ^c	53 ± 1 ^a	47 ± 1 ^{b,c}	48 ± 1 ^b	53 ± 1 ^a
ABTS ^{•+} , mmol TE/100 g DW	359 ± 9 ^a	291 ± 3 ^{b,c}	299 ± 8 ^b	252 ± 6 ^e	272 ± 6 ^d	288 ± 6 ^{b,c}
Reducing power, mg AAE/100 g DW	782 ± 18 ^{d,e}	1116 ± 25 ^b	1272 ± 24 ^a	805 ± 20 ^d	891 ± 18 ^c	905 ± 22 ^c
<i>Pigments</i>						
Chlorophyll <i>a</i> , mg/g DW	2.44 ± 0.02 ^a	1.45 ± 0.02 ^b	1.04 ± 0.03 ^e	1.11 ± 0.04 ^d	1.29 ± 0.06 ^c	1.33 ± 0.01 ^c
Chlorophyll <i>b</i> , mg/g DW	0.91 ± 0.05 ^a	0.78 ± 0.04 ^b	0.23 ± 0.04 ^{e,f}	0.69 ± 0.01 ^c	0.35 ± 0.07 ^d	0.29 ± 0.05 ^{d,e}
Chlorophyll <i>total</i> , mg/g DW	3.35 ± 0.06 ^a	2.23 ± 0.02 ^b	1.27 ± 0.07 ^e	1.80 ± 0.05 ^c	1.64 ± 0.11 ^{c,d}	1.62 ± 0.04 ^d
Ratio <i>a b</i> ⁻¹	2.68 ± 0.13 ^d	1.86 ± 0.10 ^e	4.42 ± 0.17 ^{a,b}	1.62 ± 0.11 ^f	3.70 ± 0.03 ^c	4.68 ± 0.19 ^a
Total carotenoids, mg/g	0.10 ± 0.01 ^e	0.15 ± 0.01 ^d	0.33 ± 0.01 ^b	0.28 ± 0.01 ^{b,c}	0.36 ± 0.08 ^b	0.53 ± 0.01 ^a

All data are means ± standard deviation ($n = 3$, dry basis)

Fr fresh, FD freeze-drying, MW microwave-vacuum drying, C-40 convective hot air-drying at 40 °C, C-60 convective hot air-drying at 60 °C, C-80 convective hot air-drying at 80 °C, TPC total phenolic content, TFC total flavonoid content, DPPH 2,2-diphenyl-1-picrylhydrazyl radical activity, ABTS 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid radical activity, GAE gallic acid equivalent, CAE caffeic acid equivalent, RE rutin equivalent, CE catechin equivalent, TE Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent, AAE ascorbic acid equivalent, DW dry weight

^{a,b,c...} values with different superscripts in the same row are significantly different ($p \leq 0.05$)

and intensive microwave power accelerated thermal degradation of phenolic compounds. Similar trends were observed in the investigations about ginger where An et al. [34] established 29% reduction. Whereas about ginger contrasting data are also reported with increase of TPC during microwave drying [41]. MW drying resulted in a decrease of total phenolic acids in HRLP by 49%. In turn, it did not have a significant effect on total flavan-3-ol content. MW drying indicated better retention of TFC and total flavonols compared to convective drying C-40 and C-60 possibly due to a shorter drying time and, thus, reduced time for enzymatic reactions and vacuum conditions which eliminate oxygen availability.

FD was the most effective method for preserving flavonoids. Similar to other groups of phenolic compounds, flavonols were better preserved in FD samples. Low temperature and vacuum during FD reduced the activity of enzymes, resulting in the lower degradation of biologically active compounds. FD of ginger even caused increase of TPC [34]. Also for drying of apple peels, FD was reported as the most effective [1] for preserving phenolic compounds. Similar results were observed by Nunes et al. [33] in guava powder, where FD preserved 60% of TPC from fresh guava. But also contrary data are found, showing lower effectiveness of FD, for TPC preserving, namely, Kłava et al. [36]

reported twice higher TPC in hot air-dried (80 °C) pumpkin by-products compared to freeze-dried. Compared to fresh samples in FD apple peels, no significant changes of TFC were observed by Wolfe and Liu [1].

Thus, FD was found to be the most effective drying method for preservation of TPC, TFC, total flavonol content, and total phenolic acid content.

Profile of individual phenolic compounds

Totally, 14 individual phenolic compounds were determined in the studied HRLP samples (Table 2). Specifically, 12 compounds in fresh pomace, 11 in each of FD, MW and C-80 samples, and 10 in each of C-40 and C-60 were noted. Gallic acid and 3-hydroxycinnamic acid were detected only in the fresh HRLP 0.17 ± 0.01 mg/100 g DW and 0.09 ± 0.00 mg/100 g DW, respectively. But, 5-hydroxybenzoic acid was detected only in the FD sample of HRLP (1.17 ± 0.06 mg/100 g DW). Significant quantities of rutin were present in all samples. The variation of rutin content was drying type-dependent. Its content decreased in MW and FD by 31% and 19%, respectively. Conversely, convective drying at 80 °C demonstrated rutin content increase by 12%. Possibly covalent bonds could be broken down and

Table 2 Individual phenolic compounds and organic acid profile (mg/100 g DW) in horseradish leaves pomace (HRLP) dried by different methods

Parameters	Drying methods					
	Fr	FD	MW	C-40	C-60	C-80
<i>Individual phenolic compounds</i>						
(+)-Catechin	0.19 ± 0.01 ^f	2.85 ± 0.14 ^d	2.40 ± 0.12 ^e	5.66 ± 0.28 ^c	8.31 ± 0.42 ^b	12.02 ± 0.60 ^a
4-Hydroxybenzoic acid	0.26 ± 0.01 ^{b,c}	0.46 ± 0.02 ^a	0.23 ± 0.01 ^d	0.24 ± 0.01 ^{c,d}	0.26 ± 0.01 ^{b,c}	0.28 ± 0.01 ^b
Chlorogenic acid	0.67 ± 0.03 ^c	1.00 ± 0.05 ^a	0.54 ± 0.03 ^e	0.56 ± 0.02 ^{d,e}	0.61 ± 0.03 ^{c,d}	0.81 ± 0.04 ^b
(-)-Epicatechin	16.14 ± 0.81 ^b	18.12 ± 0.91 ^a	10.02 ± 0.50 ^d	7.93 ± 0.40 ^f	8.81 ± 0.44 ^e	13.89 ± 0.69 ^c
Syringic acid	ND	2.09 ± 0.10 ^a	1.00 ± 0.05 ^c	0.34 ± 0.02 ^e	0.60 ± 0.03 ^d	1.41 ± 0.07 ^b
p-Coumaric acid	0.58 ± 0.03 ^a	0.47 ± 0.02 ^b	0.23 ± 0.01 ^c	0.01 ± 0.00 ^f	0.03 ± 0.00 ^e	0.04 ± 0.00 ^d
Sinapic acid	0.12 ± 0.01 ^e	ND	1.48 ± 0.07 ^{b,c}	1.28 ± 0.06 ^d	1.52 ± 0.08 ^b	2.80 ± 0.14 ^a
t-Ferulic acid	0.23 ± 0.01 ^b	0.32 ± 0.02 ^a	0.05 ± 0.00 ^c	ND	ND	0.02 ± 0.00 ^d
2-Hydroxycinnamic acid	0.28 ± 0.01 ^e	1.83 ± 0.09 ^b	1.77 ± 0.09 ^{b,c}	1.43 ± 0.07 ^d	1.81 ± 0.09 ^b	2.85 ± 0.14 ^a
Rutin	3231.07 ± 161.55 ^b	2598.89 ± 129.94 ^d	2212.90 ± 110.64 ^e	2909.07 ± 145.45 ^c	3294.70 ± 164.74 ^b	3649.39 ± 182.47 ^a
Luteolin	1.28 ± 0.06 ^f	3.62 ± 0.18 ^a	1.96 ± 0.10 ^c	1.55 ± 0.08 ^c	1.76 ± 0.09 ^d	2.72 ± 0.14 ^b
Other*	0.26 ± 0.01 ^b	1.17 ± 0.06 ^a	ND	ND	ND	ND
<i>Organic acids</i>						
Oxalic acid	44.4 ± 2.2 ^c	178.1 ± 8.9 ^a	83.2 ± 4.2 ^b	86.1 ± 4.3 ^b	86.9 ± 4.4 ^b	87.8 ± 4.4 ^b
Quinic acid	197.7 ± 10.0 ^a	26.4 ± 1.3 ^d	149.6 ± 7.6 ^b	132.8 ± 6.8 ^c	133.6 ± 6.8 ^c	134.7 ± 6.8 ^c
Malic acid	135.0 ± 6.7 ^c	16.7 ± 0.8 ^d	202.3 ± 10.1 ^a	164.9 ± 8.2 ^b	166.2 ± 8.3 ^b	167.2 ± 8.3 ^b
Ascorbic acid	7.8 ± 0.4 ^a	3.6 ± 0.2 ^b	0.6 ± 0.0 ^c	ND	ND	ND
Citric acid	175.0 ± 8.2 ^a	150.4 ± 7.0 ^{b,c}	154.7 ± 7.2 ^b	153.4 ± 7.2 ^b	153.7 ± 7.2 ^b	154.0 ± 7.2 ^b
Fumaric acid	4.9 ± 0.2 ^b	9.6 ± 0.4 ^a	1.6 ± 0.1 ^e	3.1 ± 0.1 ^d	3.4 ± 0.2 ^{c,d}	3.6 ± 0.2 ^c
Succinic acid	135.1 ± 6.8 ^b	50.1 ± 2.5 ^d	181.3 ± 9.2 ^a	66.9 ± 3.4 ^c	67.8 ± 3.4 ^c	68.5 ± 3.5 ^c
Other**	45.7 ± 2.4 ^a	ND	ND	ND	ND	ND

All data are means ± standard deviation ($n=3$, dry basis)

Fr fresh, FD freeze-drying, MW microwave-vacuum drying, C-40 convective hot air-drying at 40 °C, C-60 convective hot air-drying at 60 °C, C-80 convective hot air-drying at 80 °C, DW dry weight, ND not detected.

*Gallic acid, 3,5-dihydroxybenzoic acid, 3-hydroxycinnamic acid

**Tartaric acid, lactic acid, acetic acid

a, b, c... = values with different superscripts in the same row are significantly different ($p \leq 0.05$)

phytochemicals are released from chromoplasts by microwave energy, resulting in an increment in the concentration in the final product [42]. Inconsistencies between spectrophotometrically determined total content of phenolic acids and individual phenolic acids determined by HPLC have been noticed. This possibly is because of the methodology used. At this stage, results are inconclusive and further research is needed by including in HPLC analysis of other phenolic acids and/or improving extraction procedure for spectrophotometric analysis.

(-)-Epicatechin content after FD increased by 12%, but all other drying methods caused its decrease by 14–50% compared to fresh HRLP.

Aguilera et al. [43] described that the increase in phenolics during FD may be related to the changes in plant cell structure due to ice crystal formation, which can cause degradation of cells. In the plant cell, bioactive compounds

are protected from the surrounding environment; therefore, for compound extraction are required specific physical and chemical conditions to release them from the matrix. For example, ferulic acid is esterified and bound to specific polysaccharides, and then, it is possible to extract only 0.5–2%. In the case of free compounds, extraction rate can reach higher level depending on the used method [44].

HPLC analysis of individual phenolic compounds revealed that total amount of phenolic compounds increased by 13% after convective drying at 80 °C with shorter drying time, but it decreased by 31% in MW drying. For the effective preservation of 4-hydroxybenzoic, syringic, ferulic and chlorogenic acids, epicatechin and luteolin in HRLP FD may be recommended, but for the preservation of sinapic acid, 2-hydroxycinnamic acid, (+)-catechin and rutin, convective air-drying at 80 °C temperature may be recommended.

Effect of drying method on antioxidant activity of HRLP

The highest DPPH[•] antioxidant activity among studied products was in the samples dried in the convective dryer at 80 °C (Table 1), while the microwave-vacuum-dried samples exhibited the highest ABTS^{•+} antioxidant activity. Both microwave energy and high temperature may cause cell structure damage resulting in the release of the compounds, which may be hydrogen atom or electron donors, resulting in free radical-scavenging activity. HRLP dried at 80 °C contained the highest amount of rutin and high content of chlorogenic acid, which according to Braham et al. [45] may be associated with significant scavenging properties of plant extracts. Strong correlation between ABTS^{•+} and total flavonoid content (TFC), $r=0.935$ in HRLP was observed. Overall, ABTS^{•+} values closely correlated with TPC ($r=0.856$) and total flavonol content ($r=0.818$).

For evaluation of antioxidant activity, it is advised to use several approaches to get a broader overview of activity. In the current investigation, three assays were tested as DPPH[•], ABTS^{•+}, and reducing power. Their reactivity differs due to the type of free radicals used for the evaluation of antioxidant properties of extracts. ABTS method is based on ABTS cation radical formation and inhibition. It uses intensely coloured ABTS^{•+} cation radicals, which accept hydrogen electrons or atoms from antioxidants [46]. This method is suitable for the detection of both hydrophilic and lipophilic antioxidants. The DPPH[•] method is based on the

measurement of the ability of antioxidants to donate hydrogen atom, thus, reducing free radicals in the solution. In this test, the reaction of product with DPPH[•] reacts with sample in aqueous and nonpolar organic solvents. Each test uses different free radicals and reactions, which depend on the compounds present in the product; therefore, there can be observed the differences between antioxidant capacities determined by different methods. Similar to our study, for the drying of pumpkin by-products, as the most effective method for preserving the antioxidant activity (both with DPPH[•] and ABTS^{•+} assays), Kļāva et al. [36] suggested drying at 80 °C. Contrary to the current results, for the drying of quince fruits [4] and jujube fruits [9], the highest antioxidant activity (using ABTS^{•+} assay) was achieved in FD samples. For obtaining guava powder with the highest antioxidant activity, a forced air circulation oven at 55 °C for 22 h was proposed [33].

Dried horseradish leaves pomace samples exhibited higher reducing power compared to the fresh pomace. Reducing power for MW-dried pomace was by 62%, but for FD by 42% higher than in fresh pomace dry matter.

Principal component analysis (PCA) showed that PC1 explained 49.53% of total variances (Fig. 3). Cumulative input from the first and second principal component reached 72.36%.

PCA allowed allocating the studied samples in separate groups. Fresh HRLP (Fr) is characterised by higher content of TPC, total phenolic acids, TFC, total flavonols, *p*-Coumaric acid and ABTS^{•+}. Separate group is formed by

Fig. 3 Principal component analysis (PCA) plot of data from phenolic compounds and antioxidant activity of horseradish leaves pomace (HRLP) processed using different drying methods (TPC total phenolic content, TFC total flavonoid content, DPPH 2,2-diphenyl-1-picrylhydrazyl radical activity, ABTS 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid radical activity)

six individual phenolic compounds, such as 4-hydroxybenzoic acid, chlorogenic acid, (–)-epicatechin, syringic acid, t-ferulic acid, and luteolin, which were found to have higher content in FD samples compared to other studied products. MW drying can be suggested for better preservation of antioxidant activities in HRLP. Hot air-dried samples form another group, which is characterised by higher content of total flavan-3-ol, (+) catechin, sinapic acid, 2-hydroxycinnamic acid and rutin. Also, DPPH[•] activity is well preserved in the convective dried HRLP.

Differences in antioxidant activities between MW, C-80 and C-40 dried samples could be due to polyphenol oxidases. These enzymes catalyse the oxidation of phenols to quinones with subsequent nonenzymatic rapid polymerization [36]. Thus, possibly, there were better conditions for enzymatic reactions in C-40 and C-60 samples, resulting in lower phenolic content and lower antioxidant activity.

Organic acid profile

Among organic acids in fresh HRLP, the highest content was for quinic, citric, succinic and malic acids (Table 2). Depending on the organic acid type and the dehydration method, acid content varied significantly after drying. The increase of oxalic acid content was affected by drying method. Thus, its content in FD sample was fourfold higher in all other dried samples and it was doubled compared to the fresh sample dry matter. Priecina et al. [26] observed contrary trend—oxalic acid content decreased when drying celery roots in the convective or MW driers.

Drying significantly reduced quinic acid content in HRLP by 87% (FD), 32% (C-80), and 24% (MW), respectively. Priecina et al. [26] revealed that quinic acid was found only in few dried celery root samples after blanching. Change in quinic acid content possibly resulted from redistribution of plant resources towards other shikimic acid pathway products [47].

Despite the small ascorbic acid content in fresh HRLP, it demonstrated similar trend as described by Priecina et al. [26] in dried celery root. In the drying process of pomace, ascorbic acid significantly degraded and its content after FD treatment decreased by 54%, but MW reduced it by 92%. The reason for ascorbic acid content decrease and oxalate increase may be oxidative degradation pathway [48].

Citric acid content also decreased by 12–14% as a result of HRLP drying, which possibly is caused by thermal decomposition as described by Wyrzykowski et al. [49].

Fumaric acid content in HRLP increased by 95% after FD, whereas in the case of increased treatment temperature, such as MW and C-80, fumaric acid content decreased by 68% and 28%, respectively.

Succinic acid content in HRLP increased by 34% after MW drying, whereas in C-80 and FD samples, succinic acid

content decreased by 63% and 49%, respectively, which had the same trend as Priecina et al. [26] confirmed in celery root.

Fresh HRLP contained 45.7 ± 2.4 mg/100 g DW tartaric acid, which was completely degraded and it was not found in any of the dried HRLP samples.

The most effective preservation of malic acid was observed in MW-dried HRLP, while oxalic and fumaric acids were the most retained in FD samples.

Overall, the best method for organic acid preservation in HRLP was MW drying. Total content of organic acids decreased in C-80 by 17%, but in FD even by 42%.

Chlorophylls and total carotenoids

The content of chlorophyll *a* observed in HRLP (0.80 ± 0.01 mg/g FW) was higher than its content in peppermint leaves of different varieties (0.321 till 0.849 mg/g) [27]. But, it was smaller than the values reported for the leaves of peanut (*Arachis hypogaea* L.) (1.606 mg/g) [50] or the leaves of Indian mustard (*Brassica juncea* L.) (11.30 mg/g) [51].

Chlorophyll *b* content in HRLP was 0.26 ± 0.01 mg/g FW, being higher than in peppermint leaves (0.066 till 0.179 mg/g) reported by Straumite et al. [27] and much lower than in the leaves of peanut (*Arachis hypogaea* L.) (0.474 mg/g) [51] or the Indian mustard (*Brassica juncea* L.) leaves (5.79 mg/g FW) [51].

The drying method had a significant effect ($p \leq 0.05$) on all studied pigment types (Table 1). Drying reduced chlorophyll content in HRLP. Chlorophyll *a* content was reduced by 40–57%, but chlorophyll *b* by 14–74% of DW depending on the drying method. According to Di Cesare et al. [52], chlorophyll *b* is more stable in pheophytinization reactions compared to chlorophyll *a* [53]. Other researchers also reported the effect of processing temperature, presence of oxygen, enzyme activity and other factors on chlorophyll stability [54].

Chlorophyll degradation may occur via chemical, biochemical or enzymatic reactions. The most effective method for both chlorophyll *a* and chlorophyll *b* preservation proved to be FD, where products are not exposed to high temperatures and the process takes place under vacuum, which reduces the oxygen availability and the rate of enzymatic reactions. In all other studied drying methods, there was increased temperature, which may induce chemical reactions resulting in the replacement of manganese ion in porphyrin ring and the formation of pheophytin. During thermal treatment may occur decarbomethoxylation, which induces other reactions until the formation of pyropheophytin [55].

Unfortunately, even the application of low temperature during drying did not guarantee high chlorophyll content [51, 56]. It is related to the longer drying time and heat

transfer through the surface, which were confirmed in the current study, where chlorophyll *a* content at 40 °C decreased by 54%, but at 80 °C, it decreased only by 45% compared to fresh HRLP. Longer drying time and lower temperature allow oxidative enzyme activity (lipoxygenase, chlorophyll oxidase, and peroxidase), which contributes to chlorophyll loss and formation of oxidized chlorophyll catabolites [57].

The total carotenoid in HRLP (0.10 ± 0.01 mg/g DW) was similar to the value in the leaves of *Mentha spicata* varieties (0.04–0.10 mg/100 g) [27] or pak choi (*Brassica rapa* ssp. *chinensis*) sprouts (70–500 ng/mg DW) [58].

In the current study, total carotenoid content significantly increased irrespective of applied drying method and the highest content was found in C-80 samples. When drying at 80 °C, total carotenoid content in pomace was by 86% bigger compared to the pomace dried at 40 °C. Data from the study of Multari et al. [38] show similar trend that moderate heat improved the extraction efficiency from quinoa seeds, peaking at 60 °C. According to Kotíková et al. [59], moderate heat might have caused the disruption of the carotenoid protein complexes and the inactivation of carotenoid-oxidizing enzymes prevented the degradation of pigments. Many studies provided contradictory results, indicating thermal degradation of carotenoids [60–62]; however, their data revealed that the difference in the type of food matrix, carotenoid structure, and the type of processing had a significant effect.

Effect of drying on volatile compounds in HRLP

In HRLP totally, 49 volatiles were identified. Table 3 shows the major volatile compounds, while compounds whose content did not exceed 2% are combined under others. The main volatile in fresh HRLP was allyl isothiocyanate (23.39%), which is confirmed also by Petrović et al. [62] and Kroener and Buettner [63]. Allyl isothiocyanate gives pungent, mustard-like, horseradish-like, onion-like odour notes [63]. The following major volatile compounds, such as 5-methylthiopentyl isothiocyanate (berteroin) and 6-methylthiohexyl isothiocyanate (lesquerellin) that ranged between 55.0–59.0% and 34.1–36.4%, respectively, were detected by Petrović et al. [62] in a wild plant *Armoracia macrocarpa*. The major volatile compounds in dried HRLP were 3-butenenitrile, acetic acid, and benzyl alcohol. Qualitative and quantitative profile of volatiles is similar to phenolic compounds affected by genotype, growing conditions, and weather conditions [32], which are confirmed also by other researchers [33].

The drying methods had various effects on the volatile compositions in HRLP. In convective drying, at increased temperature and reduced drying time, the content of pentanal decreased, simultaneously, with the increased content of acetic acid and benzaldehyde. Some studies

demonstrated that the formation of new volatile compounds has positive correlation with drying time as well as temperature [5]. Thus, air-drying of oregano significantly decreased the content of volatile compounds [13].

Bigger volatile compound losses from HRLP were observed in convective (hot air) drying compared to FD or MW drying. This fact may be related to the increased volatile compound evaporation due to longer drying time. Szumny et al. [64] observed the biggest loss of volatiles from rosemary in the MW-dried samples.

Several compounds (such as carbonyl disulphide, alpha-phellandrene, and others) were detected exceptionally in fresh HRLP, which may indicate their volatility.

Overall, better preservation of volatile compounds in HRLP FD can be recommended.

PCA of volatile compounds (Fig. 4) indicated that PC1 explained 43.61% of total variances. Cumulative contribution of PC1 and PC2 reached 66.13%.

According to PCA of volatile compounds, FR sample can be allocated in a separate group similar to PCA of phenolics and antioxidant activities. Fr pomace contains such isothiocyanates as allyl isothiocyanate and butyl isothiocyanate, which were completely lost in the drying process along with some other volatiles. Loss of volatiles occurs at various rates and to various extent because they degrade due to the activity of enzymes (mirosynase and other), presence of air oxygen or metal ions. Thus, only fresh HRLP is characterised by the presence of the following volatile compounds, which give specific aroma to the products: isothiocyanates (strong, pungent, mustard, green), allantoic acid (garlic like), carbonyl sulphide (sulfide like), carbonyl disulphide (aromatic), α -phelanderene (citrus, herbal, terpene, green, woody, peppery) and β -phelanderene (mint, terpentine).

Despite large losses of volatile compounds, among all the dried HRLP, only FD sample retained some amounts of such compounds as 2-nitro ethanol (woody), 2-methyl-2-propenenitrile (bitter almond), and (Z)-2-hexen-1-ol (green, cortex, leafy, green, bean, nasturtium, herbal, soapy, aldehydic, narcissus, phenolic). Compared to other samples, FD pomace contained a higher amount of 3-butenenitrile (onion odour), but benzyl alcohol and benzaldehyde were in the lowest concentration.

The samples dried at lower temperatures (C-40 and C-60), which required a longer time to reach final moisture, are allocated in a separate group according to PCA. These samples contained such volatile compounds as carbondioxide (odourless), pentanal (fermented, bread, fruity, nutty, berry), 2-ethyl-1-hexanal (citrus, fresh, floral, oily, sweet), and benzyl alcohol (floral, rose, phenolic, balsamic), which may be formed in the degradation reactions of other compounds.

Table 3 Volatile fractions in horseradish leaves pomace (HRLP)

Nr.p.k	R.T (min)	Compounds	Flavor/odor description	Fraction, %					
				Fr	FD	MW	C-40	C-60	C-80
1	4.02	Carboondioxide	/odorless ^a	ND	2.32	4.43	5.70	6.28	3.73
2	4.03	Allantoic acid	Garlic ^b	2.42	ND	ND	ND	ND	ND
3	4.04	Carbonyl sulfide	/sulfide like ^a	2.02	ND	ND	ND	ND	ND
4	4.96	Carbonyl disulfide	-sweet and aromatic ^c	11.11	ND	ND	ND	ND	ND
5	6.01	Acetone	/solvent ethereal apple pear ^a	13.15	ND	5.44	21.39	16.13	13.16
6	6.05	Manganese (II) acetate	n.i	ND	2.28	ND	ND	ND	4.14
7	9.01	Ethylalcohol	/strong alcoholic ethereal medical ^a	8.47	7.40	18.28	11.08	9.83	7.00
8	9.03	Methane, nitroso	-mild fruity	ND	ND	2.46	ND	ND	ND
9	9.06	Ethanal, 2-nitro	/woody ^a	ND	2.16	ND	ND	ND	ND
10	10.44	Pentanal	winey, fermented, bready, cocoa chocolate notes/fermented bready fruity nutty berry ^a	0.51	1.36	ND	2.94	1.76	0.92
11	13.86	Hexanal	green, woody, vegetative, apple, grassy, citrus and orange with a fresh, lingering aftertaste/fresh green fatty aldehydic grass leafy fruity sweaty ^a	2.38	2.39	1.65	ND	ND	0.89
12	14.51	2-Propenenitrile, 2-methyl-	-bitter almond	ND	2.45	ND	ND	ND	ND
13	14.53	2-Butenenitrile	n.i	ND	3.41	4.00	ND	ND	ND
14	16.27	α -Phellandrene	terpenic, citrus lime with a fresh green note/citrus herbal terpene green woody peppery ^a	8.31	ND	ND	ND	ND	ND
15	16.47	2-Penten-1-ol, (Z)-	ethereal green nasturtium spicy mustard horseradish/green phenolic nasturtium ethereal medicinal aldehydic cherry narcissus metallic fruity ^a	ND	4.86	ND	ND	ND	3.44
16	16.49	2-Hexen-1-ol, (Z)	green nasturtium vegetable herbal tomato leaf potato fusel weedy/green cortex leafy green bean nasturtium herbal soapy aldehydic narcissus phenolic ^a	ND	5.73	ND	ND	ND	ND
17	17.25	3-Butenenitrile	-onion	ND	39.54	3.75	ND	ND	3.46
18	17.64	β -phellandrene	/mint terpine ^a	4.47	ND	ND	ND	ND	ND
19	19.66	Butyl isothiocyanate	/sulfury pungent green ^a	3.74	ND	ND	ND	ND	ND
20	22.17	Allyl isothiocyanate	mustard horseradish wasabi/strong pungent mustard ^a	23.39	9.42	ND	ND	ND	5.78
21	24.41	Acetic acid	pungent sour overripe fruit/sharp pungent sour vinegar ^a	ND	5.60	22.92	17.11	18.08	16.23
22	24.87	1-Hexanal, 2-ethyl	sweet fatty fruity/citrus fresh floral oily sweet ^a	1.59	ND	1.32	2.04	1.92	ND
23	25.76	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	herbal dill ^a	4.71	ND	ND	ND	ND	ND
24	26.11	Benzaldehyde	sweet, oily, almond, cherry, nutty and woody/strong sharp sweet bitter almond cherry ^a	4.54	2.61	10.79	10.10	10.36	13.00
25	32.16	Benzyl alcohol	chemical fruity cherry almond balsamic bitter/floral rose phenolic balsamic ^a	3.48	2.28	11.05	27.8	35.64	13.55
		Other		5.72	6.20	13.89	1.81	ND	14.70

ND not detected, fresh (Fr), freeze-drying (FD), microwave-vacuum drying (MW), convective hot air-drying at 40 °C (C-40), convective hot air-drying at 60 °C (C-60), convective hot air-drying at 80 °C (C-80)

^aFrom The Good Scents Company Information System

^bOxford Dictionary of Biochemistry and Molecular Biology (2006)

^cAir quality guidelines WHO (2000) Ch.5. Carbon disulfide

Conclusion

Fresh horseradish leaves pomace contained significantly higher amounts of TPC, TFC, total flavonol content and total

phenolic acid content compared to dried pomace. Only the content of flavan-3-ols increased after drying. In the drying process, gallic acid and 3-hydroxycinnamic acid were totally degraded. Rutin content increased in horseradish

Fig. 4 Principal component analysis (PCA) plot of data from volatile compounds of horseradish leaves pomace (HRLP) from different drying methods (*Fr* fresh, *FD* freeze-dried, *MW* microwave-vacuum dried, *C-40* convective dried at 40 °C, *C-60* convective dried at 60 °C, *C-80* convective dried at 80 °C)

leaves pomace after convective hot air-drying at 80 °C. The highest DPPH[•] antioxidant activity was in the samples dried in the convective dryer at 80 °C, but ABTS^{•+} antioxidant activity was in the microwave-vacuum-dried pomace. The major volatile compound in fresh pomace was allyl isothiocyanate; however, it was not found in any of the dried samples. Dried horseradish leaves pomace has the potential as the food ingredient containing valuable bioactive compounds and possessing antioxidant activity.

Drying can be used to extend the shelf life of horseradish leaves pomace. Overall, the most effective drying technology for retaining phenolic compounds and volatile compounds in horseradish leaves pomace is freeze-drying. In the case of convective drying, the majority of the studied bioactives are better preserved when drying at higher temperatures and shorter time.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study does not contain any studies with human or animal subjects.

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