

## Influence of Technological Processes on the Phenol Content and Antioxidant Properties of Horseradish Roots (*Armoracia rusticana* L.)

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**Abstract.** Horseradish (*Armoracia rusticana* L.) is a perennial herb belonging to the *Brassicaceae* family and containing biologically active substances. The aim of this work was to study the effect of the technological processes (freezing, microwave-vacuum drying, and freeze-drying) on the polyphenol content and antioxidant activity of the horseradish roots. The samples were processed using the following methods: a) freezing, b) microwave-vacuum drying, c) freeze-drying, and for a comparison fresh samples were analyzed. Extracts of the horseradish samples were prepared using Soxhlet extraction and total phenols, total flavonoids, antioxidant activity were determined spectrophotometrically, individual polyphenols were determined using HPLC. Analysis of the phenolic compounds and antioxidant activity of horseradish roots showed differences depending on the technological processes applied. The best method for preserving phenolic compounds and antioxidant activity of horseradish roots proved to be freezing.

**Keywords:** Horseradish roots, Phenolics, Antioxidants, Technological processes.

### 1. Introduction

Extraction technologies and applications of natural antioxidants recently have become very important topic for many studies. Plants provide abundant natural antioxidants, which are vitally important for human health [1]. Horseradish (*Armoracia rusticana* L.) is a perennial plant which belongs to *Brassicaceae* family with a particularly pungent flavour, rich in vitamin C (302 mg 100 g<sup>-1</sup>) and other compounds that can act as antioxidants [2]. The antioxidant characteristics of plant derived materials can be attributed to their polyphenols. Phenolic composition of plants is affected by different factors – variety, genotype, climate, harvest time, storage, processing, and treatment [3].

A viable new dehydration technology should not only exhibit higher efficiency and lower costs, but also should have little or no loss in the quality of dried products. Drying is an important process for raw materials in order to prolong shelf life, as the drying process inhibits enzymatic degradation and limits microbial growth [4]. In recent years, microwave-vacuum drying (MVD) has been investigated as a potential method for the obtaining high quality dried foodstuffs. Various fruits and grains have been successfully dried by the MVD [5] and [6]. Freeze drying (FD) is an existing technology that is able to retain product quality, yet providing all the benefits of dried foods in terms of the shelf life, transport, and storage costs. However, the major disadvantage of FD is its relatively high cost [7]. Drying method also influence the composition and biological activity of plant. Radical scavenging activity and levels of polyphenolic compounds in mulberry leaves air-dried at 60 °C or below were not different from those of FD leaves, whereas both values in mulberry leaves air-dried at 70 °C and over decreased significantly [8]. Some researchers [9] highlighted that the air-dried and FD apple peel retained much better their phenols, flavonoids, and anthocyanins (with similar contents to those of the fresh apple peel) than the oven-dried samples at 40, 60 or 80 °C.

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Freezing (F) is also popular method for extending shelf-life of a product and it influences composition and activity of plants. Scientists reported that after freezing at  $-20\text{ }^{\circ}\text{C}$  of grape pomace the yield of polyphenol increases [10], but polyphenol content and antioxidant activity in black currant decreases [11]. Technological processes play an important role in the quality of the final product, especially, in terms of its antioxidant activity. There have been no published reports on the effects of F, MVD, and FD on the phenol content and antioxidant properties of horseradish.

The aim of this work was to study the effect of the technological processes (freezing, microwave-vacuum drying, and freeze-drying) on the polyphenol content and antioxidant activity of horseradish roots.

## 2. Material and Methods

### 2.1. Chemicals

Gallic acid, Folin-Ciocalteu phenol reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) were purchased from Sigma-Aldrich (Switzerland). All other chemicals and solvents ( $\text{Na}_2\text{CO}_3$ , ethanol) used in the research were obtained from Acros Organic (USA).

### 2.2. Sample Preparation

Fresh horseradish roots (*Armoracia rusticana* L.) were collected from Pure Horticultural Research Centre collection field (latitude –  $57^{\circ} 03' \text{ N}$ , longitude –  $22^{\circ} 91' \text{ E}$ ), Latvia in October 2012. For analyses the average sample of 300 grams was taken. Fresh roots were washed and peeled.

The samples were processed using the following methods: a) freezing ( $-20\text{ }^{\circ}\text{C}$ ), b) microwave-vacuum drying (10 min at drum rotation speed of 6 rpm and pressure of 56-70 mm Hg), c) freeze-drying in a freeze-dryer (72 hours at temperature of  $-40\text{ }^{\circ}\text{C}$  and pressure of 0.064 mbar).

### 2.3. Sample Analysis

*Extraction procedure.* Three grams of a homogenized sample were placed in the filter cartridge (paper No. 89) in a classical Soxhlet apparatus and extracted with 170 ml of an ethanol for 2 h. Extracts were cooled to room temperature.

*Reversed phase high performance liquid chromatography (HPLC) analysis of the extracts.* The analyses were carried out using a Shimadzu liquid chromatograph LC - 20AD with the analytical column C18, photo diode array detector SPD M20A. As eluting solvents were used methanol (A, 20%), water (B, 78.4%), and acetic acid (C, 1.6%) using a gradient mode: 17,50 minutes – 40.3% A concentration, 58.5% B concentration, C concentration of 1.2%, 35<sup>th</sup> minute till end. The sample injection into the chromatograph was performed using an automatic sample injection system SIL - 20AC. Eluent flow rate was  $1.0\text{ ml min}^{-1}$ . Several wavelengths were used to define polyphenols. Using wavelength 253 nm 4-hydroxybenzoic acid and rutin were determined; 263 nm – gallic acid; 278 nm – catechin, caffeic acid, syringic acid; 298 nm – chlorogenic acid, epicatechine, coumaric acid, sinapic acid, and ferulic acid.

*Determination of total phenolic compounds.* The total phenolic content (TPC) of the roots extract was determined according to the Folin-Ciocalteu spectrophotometric method [12] with some modifications. The absorbance was measured at 765 nm and total phenols were expressed as the gallic acid equivalents (GAE)  $100\text{ g}^{-1}$  dry weight (DW) of horseradish. The total flavonoid content (TFC) was measured by a colorimetric method [13] with minor modification. The absorbance was measured at 415 nm and total flavonoids were expressed as the catechin equivalents (CE)  $100\text{ g}^{-1}$  DW of the horseradish.

*Determination of antioxidant capacity.* Antioxidant activity of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) radical as outlined by Yu et al. [14]. The absorbance was measured at 517 nm and the radical scavenging capacity (RSC) was expressed as Trolox mM equivalents (TE)  $100\text{ g}^{-1}$  DW of the horseradish. The RSC of extract was also measured by 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid (ABTS<sup>•+</sup>) radical cation assay [15]. For the assessment of extracts, the ABTS<sup>•+</sup> solution was diluted with a phosphate buffer solution to obtain the absorbance of  $0.800 \pm 0.030$  at 734 nm. The RSC was expressed as TE  $100\text{ g}^{-1}$  DW of the horseradish. The higher the Trolox equivalent antioxidant capacity (TEAC) of a sample, the stronger the antioxidant activity. The reducing power can be determined by the method of Athukorala [16]. The absorbance was measured at

700 nm and reducing power was expressed as the ascorbic acid equivalents (AAE) 100 g<sup>-1</sup> DW of the horseradish.

Additionally for all horseradish root samples the moisture content was determined according to the standard ISO 6496:1999 and all results were expressed on dry basis.

*Statistical analysis:* Experimental results are means of three parallel measurements and were analyzed by Microsoft Excel 2010 and SPSS 17.00. Analysis of variance (ANOVA) and Tukey's test were used to determine differences among samples. A linear correlation analysis was performed in order to determine relationship between TPC, TF, antioxidant activity such as DPPH<sup>•</sup>, ABTS<sup>•+</sup> and reducing power. Differences were considered as significant at P<0.05.

### 3. Results and Discussion

#### 3.1. Polyphenol Content

The polyphenols detected in the analyzed horseradish roots samples are presented in Table I Results of Tukey's test showed that technological process has significant ( $p < 0.05$ ) influence on the content of phenolic compounds. In general, the highest content of individual polyphenols was determined in the fresh samples (DW), except caffeic acid and rutin, which were found at higher concentrations in the frozen samples (DW). Also results of the study about marigold (*Tagetes erecta* L.) flower [17] showed that in the fresh samples concentration of polyphenols was higher, but the content of rosmarinic acid in fresh *Lamiaceae* herbs was lower compared to the freeze-dried samples [18]. For each technological process the major phenolic compound (from the tested compounds) differed, for example, in the fresh sample – chlorogenic acid, in frozen – rutin, in microwave-vacuum dried – catechin, and in freeze dried - caffeic acid.

Table I: Content of individual polyphenols, total flavonoids, and total phenols in the horseradish roots depending on treatment

Compounds	Type of treatment			
	fresh	frozen	microwave-vacuum dried	freeze dried
4-hydroxybenzoic acid *	0.50±0.02 <sup>a****</sup>	0.40±0.01 <sup>b</sup>	ND	ND
Rutin *	6.61±0.05 <sup>b</sup>	10.11±0.06 <sup>a</sup>	0.17±0.01 <sup>c</sup>	0.08±0.00 <sup>d</sup>
Gallic acid *	0.22±0.01 <sup>a</sup>	0.08±0.00 <sup>c</sup>	0.11±0.01 <sup>b</sup>	ND
Catechin *	4.34±0.03 <sup>a</sup>	2.67±0.02 <sup>b</sup>	0.53±0.02 <sup>c</sup>	0.26±0.01 <sup>d</sup>
Caffeic acid *	0.24±0.01 <sup>d</sup>	7.39±0.03 <sup>a</sup>	0.42±0.02 <sup>b</sup>	0.39±0.01 <sup>c</sup>
Syringic acid *	ND	0.16±0.01 <sup>a</sup>	0.11±0.01 <sup>b</sup>	0.06±0.00 <sup>c</sup>
Vaniline *	2.21±0.02 <sup>a</sup>	0.23±0.01 <sup>b</sup>	0.11±0.01 <sup>c</sup>	0.03±0.00 <sup>d</sup>
Chlorogenic acid *	8.77±0.04 <sup>a</sup>	0.17±0.01 <sup>c</sup>	0.25±0.01 <sup>b</sup>	0.18±0.01 <sup>c</sup>
Epicatechine *	0.31±0.02 <sup>a</sup>	0.11±0.01 <sup>b</sup>	0.01±0.00 <sup>d</sup>	0.02±0.00 <sup>c</sup>
Coumaric acid *	0.50±0.01 <sup>a</sup>	0.07±0.00 <sup>b</sup>	ND	ND
Sinapic acid *	0.40±0.01 <sup>a</sup>	0.12±0.01 <sup>b</sup>	0.01±0.00 <sup>c</sup>	0.01±0.00 <sup>c</sup>
Ferulic acid *	0.80±0.01 <sup>a</sup>	0.26±0.01 <sup>b</sup>	0.08±0.00 <sup>c</sup>	0.04±0.00 <sup>d</sup>
Sum of identified compounds *	24.97 <sup>a</sup>	21.75 <sup>b</sup>	1.81 <sup>c</sup>	1.08 <sup>d</sup>
TFC **	738.38±4.29 <sup>a</sup>	666.68±1.66 <sup>b</sup>	621.58±3.00 <sup>c</sup>	603.50±1.50 <sup>d</sup>
TPC ***	399.84±5.83 <sup>a</sup>	394.46±2.30 <sup>a</sup>	325.63±1.40 <sup>b</sup>	285.70±1.41 <sup>c</sup>
Flavonoids:Phenolics	0.54	0.59	0.52	0.47

\* Each value is the mean (mg 100 g<sup>-1</sup> DW) of three replications ± standard deviation; \*\* TFC - total flavonoid content (mg CE 100 g<sup>-1</sup> DW);

\*\*\*TPC - total phenolic content (mg GAE 100 g<sup>-1</sup> DW); ND - not detected. \*\*\*\* Mean values within the same row followed by the different letters significantly differ at the P<0.05.

TPC and TFC of the horseradish roots also differ significantly depending on the technological processes applied ( $P < 0.05$ ) and the highest content was determined in the fresh samples. These results are similar to those obtained in the study about mango peel [19]. Whereas in the studies about sweet potatoes [6] and onion [5] fresh samples contained less TPC, but dried samples – the highest TPC. The ratio of flavonoids to phenolics for all types of treatment was not significantly different ( $P < 0.05$ ), and flavonoids compose about a half of all phenolic compounds. Correlation between the content of individual polyphenols ranged from very strong (between vaniline and chlorogenic acid, 0.995) till very weak (between epicatechine and caffeic acid - 0.21), and correlation between TPC and TFC was strong (0.885).

### 3.2. Antioxidant Capacity

The variations were observed in antioxidant activity of horseradish roots depending on the type of treatment (Table II).

Table II: Antioxidant activity of the horseradish roots depending on the treatment

Methods	Type of treatment			
	fresh	frozen	microwave-vacuum dried	freeze dried
DPPH <sup>·</sup> *	53.57 ± 1.17 <sup>****</sup>	20.87 ± 0.17 <sup>b</sup>	7.44 ± 0.11 <sup>d</sup>	13.71 ± 0.07 <sup>c</sup>
ABTS <sup>·+</sup> *	9.50 ± 2.08 <sup>c</sup>	30.10 ± 0.42 <sup>a</sup>	13.05 ± 0.29 <sup>b</sup>	5.41 ± 0.90 <sup>d</sup>
Reducing power **	5272.92 ± 13.16 <sup>b</sup>	6825.37 ± 19.59 <sup>a</sup>	2534.03 ± 3.84 <sup>c</sup>	2261.58 ± 10.15 <sup>d</sup>

\* mM TE 100 g<sup>-1</sup> DW; \*\* mg AAE 100 g<sup>-1</sup> DW, \*\*\*\* Mean values within the same row followed by the different letters significantly differ at the  $P < 0.05$ .

For horseradish roots the results of analyses showed that the technological processes were significant factors affecting DPPH<sup>·</sup>, ABTS<sup>·+</sup> and reducing power ( $P < 0.05$ ). The highest DPPH<sup>·</sup> scavenging activity was detected for the fresh samples, whereas ABTS<sup>·+</sup> and reducing power assays showed higher results for the frozen samples. Similar tendency in the study about mango peel was detected [19], where sweet potatoes showed the highest activity after microwave vacuum drying, less active were frozen samples and fresh samples showed the lowest results [6]. Correlations between different antioxidant assays are medium or weak.

## 4. Conclusions

Analysis of the phenolic compounds and antioxidant activity of the horseradish roots showed differences depending on the technological processes applied. The best method for preserving phenolic compounds and antioxidant activity of the horseradish roots is freezing.

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