

## INFLUENCE OF FREEZING AND DRYING ON THE PHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF HORSERADISH AND LOVAGE

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### Abstract

A perennial herb lovage (*Levisticum officinale* L.) and horseradish (*Armoracia rusticana* L.) contains biologically active substances and are cultivated in temperate regions of the world. The aim of this work was to study the effect of the technological processes (freezing and drying) on the polyphenol content and antioxidant activity of horseradish leaves and lovage leaves and stems. The samples were processed using freezing (-20 °C) and drying (at temperature of +24 °C, in a dark, till moisture content 10±2%), and for a comparison fresh samples were analysed. Lovage leaves and stems and horseradish leaves were extracted with ethanol using conventional extraction. Total phenols, total flavonoids, antioxidant activity were determined spectrophotometrically, and individual polyphenolics were identified using HPLC. Analysis of the phenolic compounds and antioxidant activity of lovage and horseradish showed differences depending on the technological processes applied. The predominant phenolic acid in lovage samples was caffeic acid, but horseradish leaves – chlorogenic acid, and the major flavonoid was rutin. Only sinapic acid in lovage stems were found to be higher in the dried samples, compared to the fresh and frozen samples. From analysed methods better results for preserving phenolic compounds and antioxidant activity of lovage leaves and stems and horseradish leaves proved to be freezing.

**Keywords:** antioxidant, horseradish, lovage, phenolic, treatment

### Introduction

World scientists have been paid attention to preservation of biologically active substances in food. Plants provide abundant natural antioxidants, which are vitally important for human health (Naczki, Shahidi, 2006).

Horseradish (*Armoracia rusticana* L.) and lovage (*Levisticum officinale* L.) are perennial plants which belong to *Brassicaceae* and *Umbelliferae* families, respectively. Horseradish is with a particularly pungent flavour, rich in vitamin C (302 mg 100 g<sup>-1</sup>) (Raghavan, 2000) All parts of lovage are strongly aromatic with a characteristic earthy, celery-like flavour and smell (Szebeni-Galambsi et al., 1992; Raghavan, 2000). Both plants contain compounds that can act as natural antioxidants (Raghavan, 2000). 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 (Marrelli et al., 2012). Changes of the content of biologically active compounds in plants can cause climate, soil, vegetative stage of the plant and also technological processes applied – drying, freezing and other heat treatment methods, irradiation etc. (Angela, Meireles, 2009). Technological processes has important role in final product quality and especially to the antioxidant activity. Part of biologically active compounds are instable during technological processes applied, resulting in decrease of biological value of fruit and it is very important to find best methods for preserving their value.

Drying is widely used preservation method as the drying process inhibits enzymatic degradation and limits microbial growth (Ahrne et al., 2007) Drying method also influence the composition and biological activity of plant (Ahrne et al., 2007). Treatment at higher temperature decreases content of gallic acid

(Orkoula et al., 2004), whereas during drying of sweet potatoes total phenolic content and antioxidant activity increases (Yang et al., 2010).

Results of investigations about influence of freezing and storage in frozen state to the changes of phenolic composition differ, and tendencies depend on food matrix used. Freezing has not significant influence to the content of ellagic acid and total phenols content in raspberries (Ancos et al., 2000), but in frozen blueberries total phenols content decreases (Rizzolo et al., 2003).

Irish scientists reported that after processing of six Lamiaceae herbs (rosemary, oregano, marjoram, sage, basil, thyme) content of rosmarinic acid increased (Hossain et al., 2010), similar trend also was observed for polyphenolic compounds in horseradish roots (Tomsone et al., 2013).

The aim of this work was to study the effect of the technological processes (freezing and drying) on the polyphenol content and antioxidant activity of horseradish leaves and lovage leaves and stems.

### Materials and Methods

#### 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 (Na<sub>2</sub>CO<sub>3</sub>, ethanol) used in the research were obtained from Acros Organic (USA).

#### Sample preparation

Fresh horseradish leaves (*Armoracia rusticana* L.) and lovage leaves and stems (*Levisticum officinale* L.) were collected in Latvia in June 2013.

The samples were processed using the following methods: a) freezing (-20 °C), b) drying (in a dark, temperature of +24 °C, till moisture content 10±2%).

### Extraction procedure

The homogenized sample were extracted with ethanol in a conical flask with 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 extraction process was done in triplicate.

### Determination of total phenolic and total flavonoid compounds

The total phenolic content (TPC) of the plant extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. The absorbance was measured at 765 nm and total phenols were expressed as the gallic acid equivalents (GAE) 100 g<sup>-1</sup> dry weight (DW) of plant material. The total flavonoid content (TFC) was measured by a colorimetric method (Kim et al., 2003) with minor modification. The absorbance was measured at 415 nm and total flavonoids were expressed as the catechin equivalents (CE) 100 g<sup>-1</sup> DW of plant material.

### Determination of antioxidant activity

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. (2003). The absorbance was measured at 517 nm. The radical scavenging activity (RSA) of extract was also measured by 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid (ABTS<sup>•+</sup>) radical cation assay (Re et al., 1999). For the assessment of extracts, the ABTS<sup>•+</sup> solution was diluted with a phosphate buffer solution to obtain the absorbance of 0.800±0.030 at 734 nm. The RSA was expressed as TE 100 g<sup>-1</sup> DW of plant material. 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 et al. (2006). 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 plant material.

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

### 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 mL min<sup>-1</sup>. 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.

### 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.

## Results and Discussion

### Total phenolics and flavonoids content

The TPC and TFC determined in horseradish leaves and lovage leaves and stems extracts depending on treatment are shown in Table 1. Results of multivariate dispersion analyses showed that both type of treatment and plant material are significant factors affecting TPC and TFC (p<0.05). The highest content of phenolic compounds was determined in horseradish leaves.

Table 1

**Total phenolic and flavonoid content in plants depending on treatment**

Plant material	Type of treatment	TPC, mg GAE 100 g <sup>-1</sup> DW	TFC, mg CE 100 g <sup>-1</sup> DW
Horseradish leaves	Fresh	2368.48±2.03 <sup>b*</sup>	5889.85±6.02 <sup>b</sup>
	Frozen	2722.13±2.03 <sup>a</sup>	6178.03±6.27 <sup>a</sup>
	Dried	123.60±0.17 <sup>h</sup>	287.79±0.28 <sup>h</sup>
Lovage leaves	Fresh	1593.61±1.09 <sup>d</sup>	2965.40±2.69 <sup>d</sup>
	Frozen	1601.87±1.47 <sup>c</sup>	3548.33±3.08 <sup>c</sup>
	Dried	359.75±0.37 <sup>g</sup>	551.01±0.55 <sup>g</sup>
Lovage stems	Fresh	381.15±0.39 <sup>f</sup>	1208.18±1.83 <sup>f</sup>
	Frozen	458.03±0.40 <sup>e</sup>	1259.94±1.51 <sup>e</sup>
	Dried	44.83±0.07 <sup>i</sup>	184.04±0.14 <sup>i</sup>

Processing technologies has similar influence to the content of bioactive compounds for all types of plant materials investigated. It is possible to observe that for all plant materials after freezing content of phenolic compounds increased and the highest increase in lovage stems was detected – 20%. Whereas in lovage leaves TFC in frozen samples are for 20% higher. The same tendency was also observed in investigations about fresh and frozen horseradish roots (Tomsone et al., 2013). Similar results that at lower temperature increase TPC also were reported in *Etlingera elatior* and *Morus alba* L. (Chan et al., 2013). Obtained results could be explained by fact that ice crystals formed within the plant matrix can rupture cell structure. This allows the exit of cellular components and access of solvent (Asam et al., 2003). Whereas in maringold after freezing TPC and TFC slightly decreased

(Siriamornpun et al., 2012), that are opposite tendency comparing to our results.

Drying is one of the oldest methods of food preservation that is used for extending plants availability throughout the year. Drying results in significant losses in phenolic compounds in all studied materials. For example in lovage leaves losses of TPC after drying is 23%, but TFC – 19% comparing to fresh leaves. The possible reason for this could be that the preliminary process resulted in severe damage and deterioration of the integrity of leaf tissue. The release of active enzyme could cause enzymatic degradation and lose extractable phenolics. The enzyme inactivation required additional energy and during the periods of drying, the enzymes were inactivated due to decreased water activity (Lin et al., 2012). According to the study of Hossain et al. (2010), drying makes the plant tissue more brittle, which leads to rapid cell wall breakdown during the extraction procedure. Several scientists reported that drying reduced TPC of plant materials, but changes in treatment in low temperatures are dependent on plant variety and genotype (Chan et al., 2009; Ahmad-Qasem et al., 2013). Oposite to our results in the studies about sweet potatoes (Yang et al.,

2010) and onion (Arslan, Musa Özcan, 2010) fresh samples contained less TPC, but dried samples – the highest TPC. Our findings are similar to the data from a previous study by Miean and Mohamed (2001) and Erbay and Icier (2009) who reported decreased after thermal processing in holy basil and olive leaves, respectively. TFC proportion in the content of total phenols in analysed samples differed. In literature it was found that for fruit the proportion between TFC/TPC ranged from 0.15 to 0.56, but for vegetables 0.07–0.78 (Marinova et al., 2005). The highest raio between TFC/TPC was observed for frozen horseradish leaves and lovage leaves. In horseradish and lovage leaves ratio of flavonoids after freezing increases, and similar trend for frozen horseradish roots also were observed (Tomsone et al., 2013). Results showed that freezing results in release of flavonoids, that in fresh samples are linked with other compounds. The results showed that both methods of treatment could lead to significantly different outcomes.

*Individual phenolic compounds*

The distribution of individual phenolic compounds (presenting more than 5 mg 100 g<sup>-1</sup> DW) as affected by treatment and plant material is presented in Table 2.

Table 2

**Total phenolic and flavonoid content depending on treatment**

Plant material	Type of treatment	Rutine	Catechin	Caffeic acid	Chlorogenic acid	Coumaric acid	Sinapic acid	Ferulic acid
Horseradish leaves	Fresh	2376.16±2.09 <sup>h*</sup>	3.19±0.72 <sup>c</sup>	1.22±0.11 <sup>a</sup>	10.16±0.91 <sup>d</sup>	1.14±0.09 <sup>b</sup>	3.41±0.27 <sup>d</sup>	0.24±0.05 <sup>a,b</sup>
	Frozen	954.08±1.49 <sup>g</sup>	1.91±0.32 <sup>b</sup>	0.93±0.02 <sup>a</sup>	2.15±0.12 <sup>a,b</sup>	0.17±0.02 <sup>a</sup>	1.20±0.33 <sup>a,b,c</sup>	0.84±0.02 <sup>a,b,c</sup>
	Dried	191.07±0.99 <sup>f</sup>	0.85±0.58 <sup>a</sup>	0.1±0.00 <sup>a</sup>	0.95±0.28 <sup>a</sup>	0.01±0.00 <sup>a</sup>	1.08±0.58 <sup>a,b,c</sup>	ND
Lovage leaves	Fresh	65.06±0.50 <sup>e</sup>	0.25±0.02 <sup>a</sup>	39.29±0.72 <sup>c</sup>	3.90±0.70 <sup>c</sup>	23.92±0.72 <sup>g</sup>	8.57±0.62 <sup>f</sup>	5.71±0.22 <sup>e</sup>
	Frozen	55.46±0.50 <sup>d</sup>	0.09±0.01 <sup>a</sup>	31.08±0.29 <sup>b</sup>	3.37±0.31 <sup>b,c</sup>	20.47±0.19 <sup>f</sup>	5.85±0.42 <sup>e</sup>	2.65±0.23 <sup>d</sup>
	Dried	15.24±0.21 <sup>c</sup>	0.03±0.00 <sup>a</sup>	0.71±0.58 <sup>a</sup>	1.19±0.09 <sup>a</sup>	0.02±0.00 <sup>a</sup>	0.26±0.01 <sup>a</sup>	0.09±0.01 <sup>a</sup>
Lovage stems	Fresh	67.72±0.72 <sup>e</sup>	12.19±0.36 <sup>d</sup>	95.03±0.78 <sup>d</sup>	85.91±0.95 <sup>e</sup>	8.5±0.06 <sup>e</sup>	1.54±0.07 <sup>b,c</sup>	5.91±0.39 <sup>e</sup>
	Frozen	8.42±0.90 <sup>b</sup>	ND	0.72±0.52 <sup>a</sup>	3.24±0.26 <sup>b,c</sup>	7.54±0.59 <sup>d</sup>	0.99±0.08 <sup>a,b</sup>	1.34±0.84 <sup>c</sup>
	Dried	3.08±0.32 <sup>a</sup>	0.47±0.04 <sup>a</sup>	1.01±0.08 <sup>a</sup>	10.46±0.09 <sup>d</sup>	2.87±0.23 <sup>c</sup>	2.01±0.18 <sup>c</sup>	1.09±0.09 <sup>b,c</sup>

\* Each value is the mean (mg 100 g<sup>-1</sup> DW) of three replications ± standard deviation; \*\* Mean values within the same column followed by different letters significantly differ according to the LSD test (p<0.05). ND – not detected.

Higher amounts of all phenolic compounds were found in fresh samples. The predominant phenolic acid in lovage samples was caffeic acid, but in horseradish leaves – chlorogenic acid. The highest content of chlorogenic acid was in the lovage stems. The major flavonoid was rutin, and highest content in fresh horseradish leaves were identified. Comparing results of TPC, TFC and individual phenolic compounds (Table 1 and Table 2) it is possible to conclude that horseradish and lovage leaves contain other flavonoids, and rutin forms only part of TFC. Also in horseradish roots rutin, caffeic acid and chlorogenic acid in significant amounts were identified (Tomsone et al., 2013). Only sinapic acid in lovage stems were found to

be higher in the dried samples, compared to the fresh and frozen samples. In research about horseradish roots higher content of rutin and caffeic acid in frozen samples were detected (Tomsone et al., 2013). Also literature studies showed that each phenolic compound behave different depending on treatment method. Content of rosmarinic acid after freezing and drying decreases, but carnosic acid content in the same conditions increased (Mulinaccia et al., 2011). Caffeic acid and ferulic acid both are hydroxycinnamic acids, and depending on treatment they behave different. In hara leaves content of caffeic acid after freezing increased, but ferulic acid has opposite effect (Lin et al., 2012). In literature it is found that phenolic

compounds can fast degradates in high temperatures in oxygen presence (Iguar et al., 2012). Spanish scientists reported that in red onions frozen with CO<sub>2</sub> and stored at temperature individual phenolic content decreases for 24% (Pérez-Gregorio et al., 2011). Also flavonoids content in rosmary leaves during freezing and drying decreased (Mulinaccia et al., 2011). Whereas in hara leaves better results were obtained for samples dried in room temperatures (Lin et al., 2012). Phenolic compounds may differ from one another with respect to their binding status, depending on specific aspects of their chemical structures. Thus, processes may differ in their effectiveness in liberating phenolic acids from plant tissues. Phenolic acids occur in plants as metabolic intermediates, and they also accumulate in the vacuoles (Chism, Haard, 1996). Thermal processing may release more bound phenolic acids due to the breakdown of cellular constituents. Although disruption of cell walls also releases the oxidative and hydrolytic enzymes that can destroy the antioxidants in

fruits and vegetables (Chism, Haard, 1996), thermal processing deactivates these enzymes to avoid the loss of phenolic acids (Dewanto et al., 2002).

#### *Antioxidant activity*

Phenolic compounds are commonly found in plants and they have been reported to have strong antioxidant activities (Bors et al., 2001; Li et al., 2006). The antioxidant potential of phenolic compounds is dependent on the number and arrangement of the hydroxyl groups as well as the presence of the electron donating substitute in the ring structure (Elzaawely et al., 2007). It is considered that in plant material poor in vitamin C, main antioxidants are phenolic acids and flavonoids (Iguar et al., 2012).

Results of multivariate dispersion analyses showed that both type of treatment and plant material are significant factors affecting ( $p < 0.05$ ) for antioxidant activity (Table 3). Secondary antioxidant activity (DPPH<sup>·</sup> and ABTS<sup>·+</sup>) for all samples are similar tendencies.

Table 3

**Analysis of antioxidant capacity depending on treatment**

Plant material	Type of treatment	DPPH <sup>·</sup> , mM TE 100 g <sup>-1</sup> DW	ABTS <sup>·+</sup> , mM TE 100 g <sup>-1</sup> DW	Reducing power, mg AAE 100 g <sup>-1</sup> DW
Horseradish leaves	Fresh	22.84±0.07 <sup>b</sup>	154.36±0.12 <sup>a</sup>	9573.38±9.07 <sup>d</sup>
	Frozen	13.21±0.04 <sup>d</sup>	88.62±0.09 <sup>d</sup>	15994.88±13.02 <sup>c</sup>
	Dried	8.87±0.02 <sup>h</sup>	12.80±0.03 <sup>b</sup>	2254.65±2.71 <sup>i</sup>
Lovage leaves	Fresh	10.90±0.02 <sup>e</sup>	98.66±0.07 <sup>c</sup>	21225.22±20.04 <sup>b</sup>
	Frozen	10.12±0.02 <sup>f</sup>	99.61±0.07 <sup>b</sup>	22857.82±22.06 <sup>a</sup>
	Dried	8.72±0.01 <sup>i</sup>	30.13±0.05 <sup>g</sup>	4196.27±4.08 <sup>g</sup>
Lovage stems	Fresh	21.25±0.06 <sup>c</sup>	32.50±0.05 <sup>f</sup>	4330.21±4.04 <sup>f</sup>
	Frozen	35.06±0.07 <sup>a</sup>	42.46±0.06 <sup>e</sup>	6684.49±5.94 <sup>e</sup>
	Dried	9.82±0.03 <sup>g</sup>	3.98±0.01 <sup>i</sup>	2389.65±2.43 <sup>h</sup>

\* Mean values within the same column followed by different letters significantly differ according to the LSD test ( $p < 0.05$ ).

Frozen lovage stem scavenging activity increases for 65%, and similar tendencies also were reported for horseradish roots (Tomson et al., 2013). In horseradish leaves opposite tendency were observed, and scavenging activity decreases for 43% comparing to fresh samples. Increase of antioxidant activity could be explained by fact that splitting of molecular structure covalent complex deliberates antioxidant compounds as flavonoids, carotenoids, lycopene etc. (Siriamornpun et al., 2012). Many antioxidant phenolic compounds in plants are most frequently present in a covalently bound form with insoluble polymers (Siriamornpun et al., 2012).

In dried lovage leaves remains 80% of antioxidant activity, comparing to fresh material. In hara leaves DPPH radical scavenging activity after freezing is lower, comparing to drying at room temperatures (Lin et al., 2012). Decrease of antioxidant activity due to thermal degradation of phenolic compounds could be explained by to loss of antioxidant enzyme activities and activity of degradative enzymes (Lim, Murtijaya, 2007).

Similar like phenolic content, also primary antioxidant activity (reducing power) for all frozen samples are higher, compared to fresh samples. The same tendency for horseradish roots is observed (Tomson et al., 2013). The highest increase in horseradish leaves and lovage stems were observed, 67% and 54%, respectively. Whereas after drying the higher primary antioxidant activity was maintained in lovage stem samples. Other authors reported similar tendencies in changes of antioxidant activity and TPC (Chan et al., 2009; Ahmad-Qasem et al., 2013).

#### *Correlation between phenolic content and antioxidant activity*

Phenolic compounds have radical scavenging activity. Correlation analysis was performed to determine relationship between these parameters. The antiradical capacity of an extract is often related to its polyphenolic constituents. In our study all correlations between analysed samples are positive and the strongest correlation was for lovage stems (Table 4), but for horseradish and lovage leaves medium or weak correlations (data not shown).



Table 4

**Pearson`s coefficients between total antioxidant capacity, total phenolic and flavonoid content for lovage stems**

	TPC	TFC	DPPH	ABTS
TPC	1			
TFC	0.943**	1		
DPPH	0.999**	0.940**	1	
ABTS	0.979**	0.991**	0.977**	1
Reducing power	0.997**	0.966**	0.996**	0.992**

\*\*Correlation is significant at the 0.01 level (2-tailed).

For horseradish roots correlation between TPC and TFC was strong ( $r=0.86$ ), but between different antioxidant assays are medium or weak (Tomsone et al., 2013), that is similar to results of current research. Kubola and Siriamornpun (2008) studied bitter melon (*Momordica charantia* L.) leaf, stem and fruit fraction and referred that correlation between TPC and antioxidant activity (DPPH) was moderate ( $r=0.7$ ), and between TPC and primary antioxidant activity (Reducing Power) very strong ( $r=0.95$ ), but antioxidant activity (DPPH) and primary antioxidant activity (Reducing Power) show moderate correlation ( $r=0.55$ ). Statistical correlations between TPC and total antioxidant capacity of litchi seed extract were strong ( $r=0.98$ ) (Prasad et al., 2009). Strong correlation between phenolic compounds and antiradical activity was also found in experiments about seabuckthorn (*Hippophae rhamnoides* L.) leaves (Kumar et al., 2011), lychee (*L. chinensis* Sonn.) flowers (Liu et al., 2009) and canola meal (Hassas-Roudsari et al., 2009).

### Conclusions

Analysis of the phenolic compounds and antioxidant activity of the horseradish leaves and lovage leaves and stems showed differences depending on the technological processes applied. In order to select the best treatment method criteria such as phenolic compounds and antioxidant activity should be considered. The predominant phenolic acid in lovage samples was caffeic acid, but horseradish leaves – chlorogenic acid, and the major flavonoid was rutin. Only sinapic acid in lovage stems were found to be higher in the dried samples, compared to the fresh and freeze samples. Based on the total phenolic and flavonoid content, one of the best traditional methods for preserving phenolic compounds and antioxidant activity of the horseradish leaves and lovage leaves and stems proved to be freezing.

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