

INFLUENCE OF HARVEST TIME ON THE PHENOLIC CONTENT OF HORSERADISH LEAVES

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Abstract

A perennial herb horseradish (*Armoracia rusticana* L.) contains biologically active substances and cultivated in temperate regions of the world. The aim of the current research was to determine the content of phenolic compounds and antioxidant properties of horseradish leaves depending on the harvest time. For experiments horseradish leaves three years at six different times (during the period from May to October) were collected. Fresh plant material was extracted with ethanol using conventional extraction. For all extracts total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH[•], ABTS^{•+} scavenging activity, reducing power) were determined using a spectrophotometric methods. Results showed that content of phenolic compounds and antioxidant activity of horseradish leaves were significantly affected by harvest time. The highest TPC and ABTS^{•+} scavenging activity was observed in the horseradish leaves collected in May, but the highest TFC and antioxidant activity (DPPH radical scavenging activity and reducing power) was observed in June. The content of phenolics compounds and antioxidant activity significantly decreased during post flowering stage and continues till October. Horseradish leaves contained considerable amount of phenolics compounds and natural antioxidants. In further experiments, use of horseradish leaves as natural antioxidants in different food matrixes should be studied.

Keywords: horseradish, harvest time, phenolic, antioxidant.

Introduction

Plants are rich in natural antioxidants, which are very important for human health (Naczka, Shahidi, 2006). The antioxidant characteristics of plant raw materials can be attributed to their phenolic compounds. Chemically phenolic compounds are highly active substances and over the past twenty years their popularity as natural antioxidants has grown significantly (Kammerer, Carle, 2012; Naczka, Shahidi, 2003).

Biologically active substances, especially phenolic compounds, composition, content and function in plants is affected by different factors – harvest time, various external factors (cultivation, storage conditions, processing, climate), genetic background (variety, genotype) (Angela, Meireles, 2009; Marrelli et al., 2012). Plants phenolic compounds are synthesized in a normal stage of development, but they increasingly can be synthesized by biotic or abiotic stress conditions (ultra-violet (UV) radiation, mechanical injury or microbial infection) (Naczka, Shahidi, 2006). Different plant enzymatic systems, which are involved in the biosynthesis of phenols, vary the profiles significantly (Hilt et al., 2003), thereby the phenols have wide variations in function (Kammerer, Carle, 2012).

The plants development stage at harvest time is an important factor of product quality. Several authors have reported that antioxidant activity (AA) and chemical composition are influenced by the harvest time, as well as the content of compounds and properties vary from plant development stages (Imene et al., 2012; Tomsone et al., 2012; Marrelli et al., 2012). The content and composition of phenolic compounds and antioxidant potential of areal parts of *Calamintha nepeta* L. Savi (*Lamiaceae*) was significantly affected by ontogenic growth stages (Pacifico et al., 2015). Tunisian researchers have found

that for some plants (*O. ficus-indica* (L.) Mill. and *O. stricta* (Haw.) Haworth) significantly higher total phenolics content (TPC) and AA are directly during flowering (Imene et al., 2012), similar trend also was observed for phenolic compounds in horseradish roots (Tomsone et al., 2012). Whereas the lowest TPC of *Ficus carica* cv. 'Dottato' fruit was at the beginning of maturation and gradually increased with the pulp ripening stage (Marrelli et al., 2012). Such differences might be related to changes in the secondary metabolism (Imene et al., 2012).

Horseradish (*Armoracia rusticana* L.) belong to *Brassicaceae* family. There is few found data about phenolic quantitative content of horseradish in the literature. One of the studies showed that TPC of horseradish leaves ranged from 256 mg GAE 100 g⁻¹ DW to 385 mg GAE 100 g⁻¹ DW (Calabrone et al., 2015). That is significantly more than the kale (*Lathyrus* L.), spinach (*Spinacia* L.) and broccoli (*Bromelia* L.), but less than the potato (*Kochia scoparia* L.) and carrot (*Bromelia* L.) (Zhou et al., 2006). The plants of *Brassicaceae* contain a number of compounds that can act as natural antioxidants (Raghavan, 2000). Several authors reported that also the chemical composition of *Brassicaceae* plants varies depending on the harvest time, growing conditions (Kusznierewicz et al., 2008) and stage of development (Björkman et al., 2011).

The aim of the current research was to determine the content of phenolic compounds and antioxidant properties of horseradish leaves depending on harvest time.

Materials and Methods

Chemicals

Gallic acid, Folin-Ciocalteu phenol reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) were purchased from Sigma-Aldrich (Switzerland). All other chemicals

(Na₂CO₃, ethanol) used in the research were obtained from Acros Organic (USA).

Sample preparation

Fresh samples were collected from May to October in Jelgava Latvia during three-year period (2014–2016). Average temperature and precipitation during study in harvest place are shown in Figure 1.

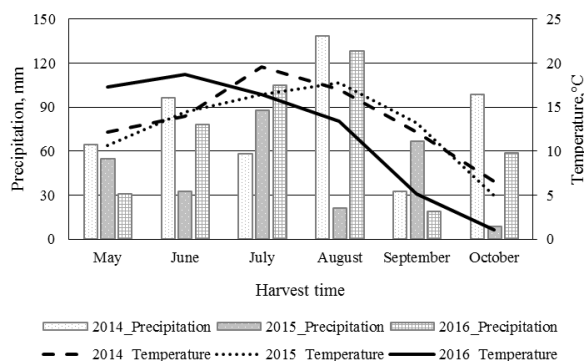


Figure 1. Average temperature and precipitation

Characterization of collected horseradish leaves are shown in Table 1.

Table 1

Height of collected horseradish plants, cm			
Harvest times	2014	2015	2016
May	10–15	10–14	9–14
June	14–20	12–21	13–20
July	15–28	17–30	13–26
August	17–33	17–38	18–33
September	20–45	18–44	22–40
October	24–50	27–53	27–52

Extraction procedure

Extraction procedure was applied as outlined by Tomson et al. (2012). The extraction process was done in triplicate.

Analytical methods

Determination of total phenolic content (TPC) and total flavonoid content (TFC). The TPC of the plant extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999). TPC was expressed as the gallic acid equivalents (GAE) 100 g⁻¹ dry weight (DW) of plant material. The TFC was measured by a colorimetric method (Kim et al., 2003). TFC was expressed as the catechin equivalents (CE) 100 g⁻¹ DW of plant material.

Determination of antioxidant activity (AA). AA of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) radical as outlined by Yu et al. (2003). The radical scavenging activity of extract was also measured by 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid (ABTS^{•+}) cation assay (Re et al., 1999). The radical scavenging activity was expressed as TE 100 g⁻¹ DW of plant material. The higher the Trolox equivalent antioxidant capacity (TEAC) of a sample, the stronger the antioxidant activity. The reducing power was determined by the method of Athukorala et al. (2006) and reducing power was expressed as the

ascorbic acid equivalents (AAE) 100 g⁻¹ DW of plant material. Additionally for all horseradish leaves 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 analysed 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, TFC, antioxidant activity such as DPPH[•], ABTS^{•+} and reducing power. Differences were considered as significant at p < 0.05.

Results and Discussion

Total phenolics and flavonoids content

The TPC determined in horseradish leaves depending on harvest time are shown in Table 2. ANOVA analysis of variance showed that TPC was significantly affected (p<0.05) by harvest time but not by harvest year.

Table 2

Total phenolic content of horseradish leaves at different harvest times, mg GAE 100g⁻¹ DW

Harvest times	2014	2015	2016
May	2634±57 ^{a*,A**}	2705±59 ^{a,A}	2590±55 ^{a,A}
June	2368±52 ^{b,A}	2351±53 ^{b,A}	2339±50 ^{b,A}
July	1853±56 ^{c,A}	1834±50 ^{c,A}	1814±54 ^{c,A}
August	1568±50 ^{d,A}	1616±52 ^{d,A}	1642±48 ^{d,A}
September	1339±59 ^{e,A}	1380±51 ^{e,A}	1368±58 ^{e,A}
October	1279±54 ^{e,A}	1235±51 ^{f,A}	1252±50 ^{e,A}

* Different small letters in the same column represents significant differences between values (Tukey's test, p<0.05).

** Different capital letters in the same row represents significant differences between values (Tukey's test, p<0.05).

Comparing harvest years similar tendency in all analysed samples was observed - the highest TPC was in May and by October it gradually decreased by 52% on average. No significant differences between TPC of horseradish leaves collected in May 2014, 2015 and 2016 were determined while comparing weather conditions – in May 2015 there was the lowest average air temperature (+10.6 °C), compared with 2014 (+12.2 °C) and 2016 (+17.3 °C). Environmental stress conditions (heat, cold, ozone, drought, intense light before the harvest) have a positive impact on the TPC in fruit and vegetables – lettuce (*Leucojum vernum* L.), for sweet potatoes (*Salix* L.), strawberries (*Zantedeschia aethiopica* L.), tomatoes and maize (*Kniphofia uvaria* L.) (Capanoglu, 2010).

A group of researchers found that content of phenolic acids (for example caffeic acid) of areal parts of *Calamintha nepeta* L. Savi was significantly higher at the flowering stage than at the post flowering stage (Pacífico et al., 2015). There is no general tendency for differences of TPC in horseradish roots depending on genotype and harvest time (Tomson et al., 2012).

The highest TFC (Table 3) was determined in June 2016, when the average air temperature was higher (+18.7 °C) compared to 2014 (+14.0 °C) and 2015 (+14.4 °C). The TFC in July decreased on average by 37%. This may be due to the intensity of UV radiation, influenced by the daylight hours and ontogenic growth stages of plants. There was a tendency, that after June to October (during post flowering stage) TFC in horseradish leaves gradually decreased by an average of 76% in three analysed years.

Flavones and flavonols are highly UV absorbers, which accumulates mainly in the cells of the epidermis. Consequently, in different parts of one plant is significantly different flavonoid content and leaves contain more flavonoids than roots (Gould, Lister, 2006; Naczka, Shahidi, 2003).

The obtained results showed that the largest content of phenolic compounds of horseradish leaves was in the period from May to June, which coincides with the horseradish development period until the flowering and the flowering period (Raghavan, 2000). A similar trend was also observed in *W. somnifera* leaves (Fernando et al., 2013). The phenolic compounds synthetic reinforced surface plant parts until flowering period.

Perennial plants post flowering period, virtually all biological processes are allocated to the root development and nutrient accumulation in roots. The same tendency can be observed in certain genotypes of horseradish roots (Tomson et al., 2012), where a higher content of phenolic compounds were directly in the post flowering stage. Pacifico et al. (2015) reported that quercetin derivative of areal parts of *Calamintha nepeta* L. Savi was significantly higher at the flowering stage than at the post flowering stage.

Antioxidant activity (AA)

Phenolic compounds have been reported to have strong AA (Li et al., 2006). The antioxidant potential of these biological active compounds is dependent on the structural conformation (Elzaawely et al., 2007). Results of multivariate dispersion analyses showed that harvest time is significant ($p < 0.05$) factor affecting AA.

Scavenging activity of DPPH radicals for all samples has similar tendencies that the higher activity was in June, and during post flowering stage by October it gradually decreased by an average of 45%. The highest DPPH[•] scavenging activity was determined in horseradish leaves in June 2016 (Table 4) similar to TFC. It allows thinking that exactly flavonoids of horseradish leaves have a high DPPH[•] scavenging activity.

Table 3

Total flavonoid content of horseradish leaves at different harvest times, mg CE 100 g⁻¹ DW

Harvest times	2014	2015	2016
May	8515±223 ^{b*,A**}	9192±237 ^{b,B}	9087±229 ^{b,A,B}
June	11136±233 ^{a,A}	11099±261 ^{a,A}	11697±257 ^{a,A}
July	7036±183 ^{c,A,B}	7409±245 ^{c,B}	6873±199 ^{c,A}
August	5486±211 ^{d,A}	5038±253 ^{d,A}	5070±226 ^{d,A}
September	3921±230 ^{e,A}	3518±245 ^{e,A}	776±219 ^{e,A}
October	2486±220 ^{f,A}	2970±252 ^{e,A}	2784±231 ^{f,A}

Table 4

DPPH[•] scavenging activity of horseradish leaves at different harvest times, mM TE 100 g⁻¹ DW

Harvest times	2014	2015	2016
May	52.84±2.18 ^{b*,A,B**}	50.92±2.02 ^{b,A}	57.41±2.31 ^{b,B}
June	69.33±1.92 ^{a,A}	71.09±2.11 ^{a,A}	73.38±2.07 ^{a,A}
July	51.69±2.27 ^{c,A}	57.30±2.40 ^{c,A,B}	61.50±2.35 ^{b,c,B}
August	48.03±2.09 ^{c,A}	49.66±1.89 ^{c,A}	53.04±2.12 ^{c,A}
September	43.74±1.95 ^{d,A}	40.97±2.30 ^{d,A}	45.53±2.28 ^{d,A}
October	39.18±1.83 ^{d,A}	39.51±1.75 ^{d,A}	37.13±1.99 ^{e,A}

Table 5

ABTS^{•+} scavenging activity of horseradish leaves at different harvest times, mM TE 100 g⁻¹ DW

Harvest times	2014	2015	2016
May	148.92±5.74 ^{a*,A**}	150.83±5.44 ^{a,A}	146.71±6.3 ^{a,A}
June	140.08±4.99 ^{a,A}	143.13±5.16 ^{a,A}	142.52±3.69 ^{a,A}
July	125.50±3.93 ^{b,A}	127.70±3.65 ^{b,A}	126.24±4.03 ^{b,A}
August	121.44±4.34 ^{b,c,A}	122.20±4.47 ^{b,c,A}	124.24±5.25 ^{b,A}
September	112.04±3.62 ^{c,A}	111.63±3.03 ^{c,A}	111.48±3.23 ^{c,A}
October	91.76±2.98 ^{e,B}	88.07±2.48 ^{d,A,B}	81.42±2.68 ^{d,A}

* Different small letters in the same column represents significant differences between values (Tukey's test, $p < 0.05$).

** Different capital letters in the same row represents significant differences between values (Tukey's test, $p < 0.05$).

Reducing power of horseradish leaves at different harvest times, mg AAE 100 g⁻¹ DW

Harvest times	2014	2015	2016
May	8548±210 ^{b*,A**}	7834±223 ^{b,B}	7639±198 ^{b,B}
June	9000±207 ^{a,A}	9135±190 ^{a,A,B}	9573±192 ^{a,B}
July	7990±187 ^{c,B}	7437±216 ^{b,A}	7576±205 ^{b,A,B}
August	5708±150 ^{d,A}	5532±178 ^{c,A}	5457±161 ^{c,A}
September	4621±197 ^{e,B}	3691±209 ^{d,A}	3981±176 ^{d,A}
October	2873±165 ^{f,A}	2638±176 ^{e,A}	2998±190 ^{e,A}

* Different small letters in the same column represents significant differences between values (Tukey's test, p<0.05).

** Different capital letters in the same row represents significant differences between values (Tukey's test, p<0.05).

The same tendency was observed with *Calamintha nepeta* L. Savi where an antioxidant potential (DPPH') decreased during the post flowering stage (Pacifico et al., 2015).

The highest ABTS^{•+} scavenging activity was determined in horseradish leaves in May 2015 (Table 5), similar to TPC. Apparently other phenolic compounds fractions (not flavonoids) have a way of better scavenging activity of ABTS^{•+}. The highest ABTS^{•+} scavenging activity was in May for all analysed samples and by October it gradually decreased on average by 41%.

Imene et al. (2012) reported about scavenging activity depending on the harvest time – as chemical composition, amounts and nature of compounds vary within development stages and species; it can be influenced by changes in secondary metabolism. AA shows a marked variation with ontogenic growth stages and the maximum of AA is observed during post flowering stage for the two species – *Opuntia ficus-indica* (L.) Mill. and *O. stricta* (Haw.) Haworth (Imene et al., 2012). It is the opposite trend by horseradish leaves AA and areal parts of *Calamintha nepeta* L. Savi (Pacifico et al., 2015).

Reducing power (RP) associated with indirect antioxidants and it can serve as a significant reflection of activities (Oktay et al., 2003). Literature reports show that antioxidant activity of plant and herb is related to the reducing power that interrupts radical chain reactions (Singh, Rajini, 2004). The existence of reductants is key to reducing power, which shows the antioxidant activity because donate hydrogen atoms and interrupt free radical chain reactions (Xing et al., 2005).

The experiments showed that reducing power is dependent on the plant harvest time. The highest reducing power was determined in June 2016 (Table 6), similar to TFC. It is considered that in plant material poor in vitamin C, main antioxidants are flavonoids and phenolic acids (Igual et al., 2012). Apparently horseradish leaves flavonoids are able to donate hydrogen atoms by interrupting free radical chain reactions.

As mentioned above, higher reducing power was in June, and by October (during post flowering stage) it gradually decreased by an average of 69%. It can be explained by the modification of chemical composition of horseradish leaves in the post flowering stage. The

antioxidant potential (reducing power) of areal parts of *Calamintha nepeta* L. Savi was significantly higher at the flowering stage than at the post flowering stage (in October) (Pacifico et al., 2015).

The results show that pre-flowering and flowering stages corresponds to the maximum accumulation of phenolic compounds and antioxidant activities.

Correlation between phenolic content and antioxidant activity (AA)

The AA is influenced by the phenolic composition. A various correlation coefficients were obtained by analysing relationship between phenolics compounds and AA in. Overall, positive strong correlation between TPC, TFC, DPPH', ABTS^{•+} and reducing power was determined. This can be explained by the fact that phenolic compounds are the most important antioxidant of horseradish leaves. Further research is necessary to identify individual phenolic compounds and analyse their influence on the overall free radical scavenging activity.

Prasad et al. (2009) found, that statistical correlations between TPC and total antioxidant capacity of litchi seed were strong (r=0.98), that is similar to results of the current research. But Pacifico et al. (2015) found that correlation between TPC and ABTS^{•+} scavenging activity of areal parts of *Calamintha nepeta* L. Savi were moderate (r=0.64). Whereas stronger correlation between TFC – DPPH' and TFC – reducing power was observed in June (r=0.861 and r=0.853, respectively). Strong correlation between phenolic compounds and AA was also found in seabuckthorn (*Hippophae rhamnoides* L.) leaves (Kumar et al., 2011), canola meal (Hassas-Roudsari et al., 2009) and lychee (*L. chinensis* Sonn.) flowers (Liu et al., 2009). It coincides with the highest concentration and activity of these compounds. The correlation ranged from medium to weak at the others harvest times. Fresh *L. lucidus* Turcz. roots were harvested at three different times and there was significant correlation between phenolic content and different antioxidant assays (Lu et al., 2015).

Conclusions

This research is contribution to the determination of the content of phenolic compounds in horseradish leaves and its variability depending on the harvest time. Results showed that content of phenolic compounds and antioxidant activity was significantly affected both

by harvest time and year. The highest TPC and ABTS^{•+} was observed in the horseradish leaves collected in the May, but TFC, DPPH[•] and reducing power – in the plants collected in June. The content of phenolics compounds and antioxidant activity significantly decreased during post flowering stage and continued till October. Horseradish leaves contained considerable content of phenolics compounds and natural antioxidants. Also a strong correlation between the TPC and ABTS^{•+} as well as TFC and DPPH[•], reducing power at harvest time in May was detected. Further experiments are necessary to evaluate antioxidant activity of horseradish leaves extracts in food matrixes.

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