INFLUENCE OF GENOTYPE AND HARVEST TIME ON THE PHENOLIC CONTENT OF HORSERADISH (*ARMORACIA RUSTICANA* L.) ROOTS

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Abstract
Horseradish (*Armoracia rusticana* L.) is a perennial plant, with a particularly pungent flavour and significant antioxidant properties. The aim of current research was to determine the total phenol content and antioxidant properties of horseradish depending on genotype and harvest time. For experiments nine genotypes of horseradish roots collected at different times were investigated. Fresh plant material was extracted with ethanol/water solution (80:20 v/v). Total phenols content (TPC) of plant extracts was determined according to the Folin-Ciocalteu spectrophotometric method and results were expressed as gallic acid equivalents (GAE). Antioxidant activity of the extracts was measured on the basis of DPPH$^{\bullet}$ free radical scavenging activity and the final results were expressed as inhibition of 2,2-diphenyl-1-picrylhydraziyl (DPPH$^{\bullet}$) in percent (I, %). Total phenols content varied among analysed types of horseradish. The highest TPC was detected in horseradish root genotype 280 harvested in September and it also demonstrated the highest DPPH$^{\bullet}$ radical scavenging activity, whereas the lowest TPC was detected in horseradish root genotype 26B also harvested in September. TPC and DPPH$^{\cdot}$ scavenging antioxidant activity were also significantly influenced by harvest time. Positive correlation was found between antiradical activity and the total phenols content in horseradish roots harvested in September. In further experiments, use of horseradish as natural antioxidants in different food matrixes should be studied.
Key words: horseradish, total phenols, scavenging activity, genotype, harvest time.

Introduction

World attention has been paid to development of safe antioxidants from natural sources. Many spices and vegetables possess antioxidant properties, so they can be used in food to help prevent oxidation processes. Free radicals in the human body can be formed by heat, radiation, ultraviolet radiation, tobacco smoke and the influence of alcohol (Raghavan Uhl, 2000). Some scientists believe that the destruction of free radicals may contribute to the fight with cancer, heart disease and stroke (Forristal et al., 2002). Studies show different antioxidant activity for each plant type, stimulated by the antioxidant components, such as α-tocopherol, β-carotene, vitamin C, selenium and phenolic compounds (Ismail et al., 2004). Polyphenols are large, important and diverse class of antioxidants, beneficial to both plants and humans. Extensive studies on functions and the role of polyphenols in humans began in the last century and are continued today (Rappoport, 2003). It is known that the phenolic compounds are very effective antioxidants (Shahidi and Wanasundara, 1992; Tapeiro et al., 2002; Shahidi and Naczk, 2004). Plant phenolic compounds are one of the most important primary antioxidants, so it is important to investigate the quantities of plant species. Phenolic compounds commonly found in spices are biologically active substances having antiseptic, vitamin activity expression, and other properties (Rappoport, 2003; Daayf and Lattanzio, 2008).

Phenolic composition of plants is affected by different factors – variety, genotype, climate, harvest time, storage, processing (Kreutzmann et al., 2008; Marrelli et al., 2012). Horseradish (*Armoracia rusticana* L.) belongs to *Brassicaceae* family, and several authors reported that also the chemical composition of *Brassicaceae* plants varies depending on the stage of development (Björkmanet al., 2011), growing conditions (Podsędek, 2007; Kusznierewicz et al., 2008) and harvest time (Koh et al., 2009).

Horseradish is a perennial plant indigenous to eastern and northern Europe and the Mediterranean, with a particularly pungent flavour, rich in glucosinolates and usually consumed as pickled vegetable. It is also cultivated in central Europe, but not very broadly. Horseradish has about 0.2 to 1.0 g 100 $g^{-1}$of essential oil, mainly sinigrin, sinigrin-derived allylisothiocyanate, diallylsulfide, phenylpropyl and phenethylthiocyanate. Myrosinase enzyme acts on sinigrin to give allylisothiocyanate, which gives horseradish its burning taste. Horseradish has a high vitamin C content (302 mg 100 g^{-1}) (Raghavan Uhl, 2000). Its leaves are considered to prevent food spoiling processes. Although glucosinolates, with their antioxidant properties, play an important role in the human diet, they have not been systematically investigated (Majewska et al., 2004). Several genotypes of horseradish are included in the collection of vegetable genetic resources of Latvian origin in Pure Horticultural Research Centre. Until now, biologically active substances of horseradish have not been studied in Latvia collection. There is not found a detailed research on the dynamics of phenolic compounds depending on the season and genotype in the world's scientific literature as well. Polish researchers investigated antioxidant properties of leaf and root extracts originated from four different

types of horseradish (Majewska et al., 2004). The tested types were cultivated in two different regions of Poland. A. Majewska et al. (2004) reported that leaf and root extracts derived from four Polish types of horseradish did not exhibit strong antioxidant properties, but the different environmental conditions of plant growth affected these properties significantly.

The aim of current research was to determine the total phenol content and antioxidant properties of horseradish depending on genotype and harvest time.

Materials and Methods

Materials

Nine genotypes of horseradish (*Armoracia rusticana* L.) (Table 1) were collected three times during the period from August to November, 2011 at Pure Horticultural Research Centre collection field (latitude – 57° 03' N, longitude – 22° 91' E): 29 August (I), 29 September (II), and 2 November (III). Meterological conditions of 2011 where characteristic with relatively high temperatures (from June till September average air and soil temperature fluctuated between +15 and +20 °C) and stable, close to optimal precipitation.

Table 1

Characterization of horseradish genotypes

Collection No / name	Place of origin	Abbreviations
1	Valmiera region, Latvia	G1
2	Belarus	G2
3	Jelgava region, Latvia	G3
12B	Preili region, Latvia	G12B
26B	Malnava region, Latvia	G26B
105	Kuldiga region, Latvia	G105
106	Koknese region, Latvia	G106
280	Malnava region, Latvia	G280
281	Malnava region, Latvia	G281

For analyses, the average sample of 300 g was taken from all horseradish roots. Analyses was performed within two weeks after harvest. Fresh roots were washed, peeled and homogenized (for 5 minutes). All samples of one type of horseradish were homogenized together in order to obtain a representative sample.

Chemicals

Gallic acid, Folin-Ciocalteu phenol reagent, and 2,2-diphenyl-1-picrylhydraziyl (DPPH$^\bullet$) were purchased from Sigma-Aldrich (Switzerland). All other chemicals and solvents (Na_2CO_3, ethanol) used in the research were obtained from Acros Organic (USA).

Preparation of extracts

Five grams of the homogenized sample were extracted with 50 mL of ethanol/water solution (80:20 v/v) 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 root extracts were then filtered (paper No. 89). The extraction process was done in triplicate.

Determination of total phenolic content (TPC)

The TPC of the roots extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. To 0.5 mL of extract 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times with water) was added, and after 3 minutes 2 mL of sodium carbonate (Na_2CO_3) (75 g L^{-1}) was added. The sample was mixed. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm using a spectrophotometer JENWAY 6300 (Baroworld Scientific Ltd., UK). Total phenols were expressed as gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of the sample.

Determination of DPPH$^\bullet$ radical scavenging activity

The scavenging activity on DPPH$^\bullet$ radicals has been widely used to determine the free radical-scavenging activity. DPPH$^\bullet$ is a stable free radical and its solution dissolved in methanol shows a characteristic absorption at 517 nm. Antioxidant molecules scavenge the free radical by hydrogen donation and the colour from the DPPH$^\bullet$ assay solution becomes light yellow resulting in a decrease in absorbance. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation.

Antioxidant activity of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl (DPPH$^\bullet$) radical as outlined by Yu et al. (2003). The antioxidant reaction was initiated by transferring 0.5 mL of plant extract into a sample cavity containing 3.5 mL of freshly prepared DPPH$^\bullet$ methanol solution (0.004 g DPPH$^\bullet$ to 100 mL methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm using a spectrophotometer JENWAY 6300. Inhibition of DPPH$^\bullet$ in percent (I%) of each extract sample was calculated from the decrease of absorbance according to relationship:

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100,$$

where

A_{blank} – absorbance of control reaction (methanol-water with DPPH$^\bullet$);

A_{sample} – absorbance of the tested samples.

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (Zhao et al., 2008).

Additionally, for all horseradish roots moisture content was determined according to standard ISO 6496:1999, and all results were expressed to dry basis.

Statistical analysis

Experimental results were means of three parallel measurements and were analyzed by Microsoft Excel 2010 and SPSS 17.00 for Windows. Analysis of variance (ANOVA) and differences among samples were tested by Tukey test. Differences were considered significant at $p<0.05$. Relationship between TPC, DPPH antioxidant activity, genotypes and development stage were analyzed by correlation and regression tools.

Results and Discussion

Total phenolic content (TPC)

Extracts of horseradish roots were prepared using conventional extraction, and TPC was determined using Folin-Ciocalteu reagent, which reacts nonspecifically with phenolic compounds, but also it can be reduced by a number of non-phenolic compounds, e.g., vitamin C, Cu(II), etc. In this study, comparison of phenolic compounds of nine genotypes of horseradish roots depending on harvest time were determined. The content of total phenols varied from 160.14 mg GAE 100 g^{-1} DW to 503.54 mg GAE 100 g^{-1} DW (Fig. 1). Comparing harvest times for horseradish roots, TPC ranged from 184.74 to 409.16 mg GAE 100 g^{-1} DW at harvest time I, from 160.14 to 503.54 mg GAE 100 g^{-1} DW at harvest time II, and from 205.17 to 349.25 mg GAE 100 g^{-1} DW at harvest time III.

ANOVA analysis of variance showed that TPC was significantly affected ($p<0.05$) both by genotype and harvest time. There were stated clear interactions between the genotype of horseradish roots and

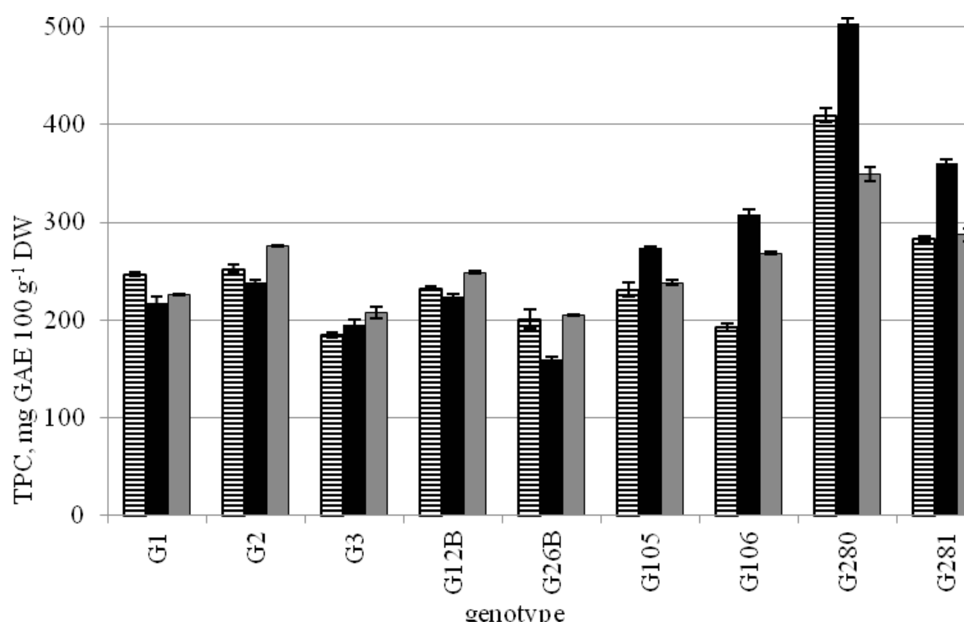

Figure 1. TPC in horseradish depending on harvest time:
I – harvest time for August 29, II – harvest time for September 29, III – harvest time for November 2.

Table 2

Tukey's criteria among the TPC of the analyzed genotypes of horseradish

Genotype	G1	G2	G3	G12B	G26B	G105	G106	G280
G2	0.485							
G3	0.070	0.000*						
G12B	1.000	0.803	0.017*					
G26B	0.009*	0.000*	1.000	0.002*				
G105	0.921	1.000	0.000*	0.995	0.000*			
G106	0.434	1.000	0.000*	0.760	0.000*	1.000		
G280	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	
G281	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

*The mean difference is significant at the 0.05 level.

harvesting time. Tukey's test results (Tab. 2) showed significant differences between the genotypes. It was concluded that there were significant differences ($p<0.05$) in TPC content among all harvest times.

According to the data obtained in other investigations, raw vegetables showed a different TPC content. For example, expressed in GAE it ranged from 30 mg GAE 100 g^{-1} DW (*Oroxylum indicum* and *Vigna radiate* L.) to 12050 mg GAE 100 g^{-1} DW (*Lactuca sativa* L.) (Sulaiman et al., 2011). As reported by K. Zhou (2006), vegetables can be ranked depending on the content of polyphenols as follows: kale (*Lathyrus* L.) > rhubarb (*Ranunculus* L.) = spinach(*Spinacia* L.) = broccoli (*Bromelia* L.) > green bean (*Zea mays* L.) > tomato (*Tradescantia* L.) > potato (*Kochia scoparia* L.) = carrot (*Bromelia* L.) from 85 to 1880 mg GAE 100 g^{-1} DW. Compared to horseradish, a lower TPC was reported for date palm fruit (*Daucus* L.) (Biglari et al., 2008) and highly pigmented vegetables (Hongyan et al., 2012). Similar TPC as for horseradish was found in the extracts of fruit residues examined in a study of India researchers (Babbar et al., 2011) and this material could also be used as a source of natural antioxidants. In various plants significantly higher amounts are reported. Pakistan researchers reported that TPC of apricot (*Aquilegia* L.) ranged from 4591 mg GAE 100 g^{-1} DW to 7310 mg GAE 100 g^{-1} DW (Sartaj et al., 2011). Also herbs as sage and thyme (*Tradescantia* L.) have significantly higher TPC (Hossain et al., 2010). TPC vary significantly in vegetables of *Brassicacea* family depending on growing conditions. In broccoli TPC ranged from 34.5 to 337.0 mg GAE 100 g^{-1} of edible portion, for cauliflower (*Zinnia* L.) – from 27.8 to 274 mg GAE 100 g^{-1} of edible portion, and for cabbage (*Kochiascoparia* L.) – from 15.3 to 254 mg GAE 100 g^{-1} of edible portion (Podsędek, 2007). It is also reported about variation in TPC for cabbages - from 491 to 241 mg GAE 100 g^{-1} DW (Kusznierewicz et al., 2008). The highest TPC had for horseradish root of genotype No 280 during all harvest period. While the root of genotype 26B showed the lowest TPC at harvest times II and III, and root of genotype 3 showed the lowest TPC in harvest time I. Overall, at harvest time II, higher TPC of horseradish roots 280, 281, 105 and 106 were determined. These four genotypes generally demonstrated a higher TPC. M. Björkman et al. (2011) report that chemical composition of plants of family *Brassicaceae* is also influenced by climatic conditions. It can be concluded that it is necessary to continue experiments to obtain more informative data, because climatic conditions are changeable.

Plant stress conditions as heat, cold, ozone, drought, intensive light before harvest of fruits and vegetables (lettuce (*Leucojum vernum* L.), sweet potatoes (*Salix* L.), strawberry (*Zantedeschia aethiopica* L.), tomato and maize (*Kniphofiauvaria* L.)) influence TPC content positively (Capanoglu, 2010). Plant development stage during harvest is a critical factor for the quality of the product. Fruits of *Ficuscarica* cv. 'Dottato' from an orchard (Calabria, Italy) showed increase in TPC during three stages of ripening – lowest TPC content was detected in threshold maturity and was increased till soft ripening stage (Marrelli et al., 2012). E. Koh et al. (2009) report about the TPC content in commercial broccoli depending on harvest time – the highest amounts were observed in samples harvested in February, but lowest in October. Contrary data exist about the influence of ripening on the TPC. A group of researchers studied the antioxidants of thirteen faba bean (*Fagussylvatica* L.) genotypes and found that the highest TPC was in the harvest timeone month after their lifting, but the lowest TPC – at the maturity stage, when the plants were completely dry (Chaieb et al., 2011).

Scientists from Denmark have reported about the influence of the genotype on the content of phenolic acids – eight genotypes of carrots were investigated and it was found that TPC differed significantly between them (Kreutzmann et al., 2008).

Results obtained by other researchers are consistent with the results obtained in our investigation. For example, horseradish root No. 280 showed the lowest TPC at harvest time III and the highest – at harvest time II, but the root of genotype No.1 had the lowest TPC at harvest time II and the highest – at harvest time I, while the root No. 3 had lowest TPC at harvest time I and the highest – at harvest time III.

Radical scavenging activity (DPPH˙)

Results of multivariate analyses of variance showed that horseradish root genotype and harvest time significantly ($p<0.05$) influence DPPH˙ scavenging activity. There were stated clear interactions between horseradish genotype and harvesting time. Tukey test results demonstrated significant differences between the genotypes and the collection times.

DPPH˙ determined in horseradish roots ranged from 20.28 to 28.08% at harvest time I, from 11.27 to 29.68% at harvest time II, and from 2.19 to 17.51% at harvest time III (Fig. 2). Highest DPPH˙ scavenging activity (29.68%) showed root genotype 280 of the II stage, while the lowest (2.19%) showed a root No. 2 in stage III. Antiradical activity of eight horseradish root genotypes differed significantly depending on harvest time – the highest content was determined at harvest time I. Fruits of *Ficuscarica* cv. 'Dottato' from an orchard in Italy showed the highest antiradical activity at threshold mature, and during ripening process it decreased (Marrelli et al., 2012).

The antioxidant activity of date palm fruit ranged from 20% to 63% (Biglari et al., 2008), which is

similar to the results obtained in our research with horseradish. Pakistan researchers reported that in all the varieties of apricot, antioxidant activity ranged from 55.70 to 82.33% (Sartaj et al., 2011). In the DPPH˙ assay, all extracts of highly pigmented vegetables demonstrated good radical scavenging activity with the percent scavenging ranging from 54.91 to 81.94% (Hongyan et al., 2012). India researchers reported that antioxidant activity in the extracts of fruit residues varied between 43% and 83% (Babbar et al., 2011). This is considerably more than shown horseradish roots.The potato extracts at 4 mg ml^{-1} quenched about 13–38% of DPPH˙ in the reaction mixtures in 10 min, kale and broccoli extracts quenched 75–77% and 73–79% DPPH˙ in the system in 10 min at 1.6 mg mL^{-1}, respectively (Zhou and Yu, 2006).

N. Chaieb et al. (2011) and A. Imene et al. (2012) reported about antiradical 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. Phenolic content shows a marked variation with flowering stage – the maximum of phenolic compounds is observed during post flowering stage for the two species – *Opuntia ficus-indica* (L.) Mill. and *O. stricta* (Haw.) Haworth; this stage is also characterized by the maximum of antioxidant activity.

The results show that post flowering stage corresponds to the maximum accumulation of polyphenol, antioxidant and antibacterial activities.

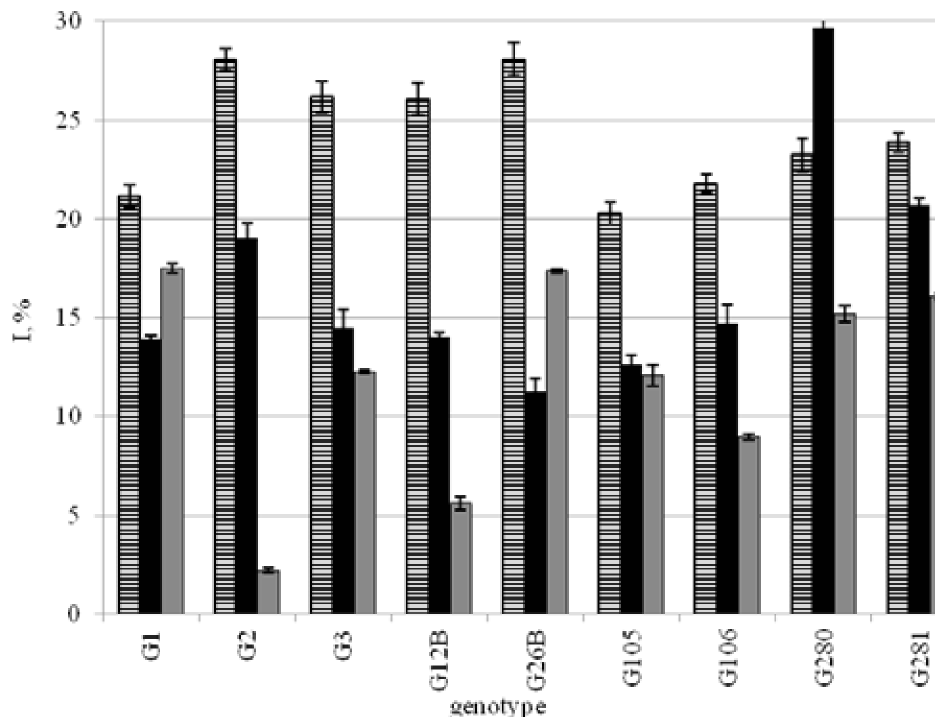

Figure 2. Scavening activity of DPPH˙ radicals of horseradish depending on harvest time: ▤ I – harvest time for August 29, ■ II – harvest time for September 29, ▩ III – harvest time for November 2.

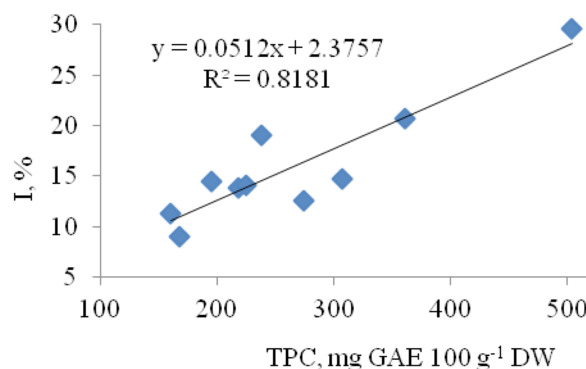

Figure 3. Correlation between TPC and DPPH˙ scavenging activity at harvest time II (n = 10) ($p<0.05$).

Correlation between total phenolic content and radical scavenging activity

The free radical scavenging activity is influenced by the phenolic composition. A various correlation coefficients were obtained by analysing relationship between TPC and DPPH˙ scavenging activity in different harvest times. Correlation between TPC and DPPH˙ antioxidant activity of the studied horseradish roots at harvest stages III and I was weak and very weak, respectively. By contrast, at the stage II, horseradish roots showed a high correlation between TPC and DPPH˙ antioxidant activity (Fig. 3). Overall, correlation between TPC and DPPH˙ antioxidant activity was weak. This can be explained by the fact that at harvest phenolic compounds are the most important radical scavengers of horseradish root. Further research is necessary to identify individual phenolic compounds and analyze their influence on the overall free radical scavenging activity. For example, N. Babbar et al. (2011) investigated extracts obtained from six fruit residues and found a weak correlation between total phenolic content and DPPH˙ antioxidant activity (r^2=0.36). Contrary, mustard greens (*Solanum dulcamra* L.) showed a high correlation between the TPC and DPPH˙ antioxidant activity, with the correlation coefficient ranging from 0.743 to 0.949 (Fang et al., 2008).

N. Chaieb et al. (2011) found a significant linear correlation between DPPH˙ antioxidant activity and TPC for thirteen faba bean genotypes, indicating the substantial contribution of phenolic compounds to related antioxidant activity. Green vegetables of Malaysia showed different correlation between TPC and DPPH˙ – from very weak (r=-0.0485) to very close (r=0.9408) (Sulaiman et al., 2011).

Conclusion

This research is contribution to the determination of the TPC in horseradish roots and its variability according to the genotype and the harvest time. Results showed that TPC and DPPH˙ scavenging activity were significantly affected both by horseradish genotype and harvest time. The highest TPC was observed in the roots collected at the end of September. Also a significant correlation between the TPC and DPPH˙antioxidant activity at harvest time II was detected. TPC could not be used as a major indicator of antioxidant activity. Further experiments are necessary to evaluate antioxidant activity of horseradish root extracts in food matrixes.

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