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Evaluation of Seasonal Variations in the Glucosinolate Content in Leaves and Roots of Four European Horseradish (*Armoracia rusticana*) Landraces

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In comparison with other cruciferous vegetables, horseradish has rarely been the object of scientific research, and the knowledge about the composition, content and distribution of glucosinolates (GLS) in different organs of horseradish plants is limited. Therefore, the aim of this study was to evaluate changes in the GLS content in leaves and roots of four horseradish landraces during the growing season.

The presence of 13 GLS was determined in the examined horseradish tissues, and glucoraphanin, glucoraphenin and napoleiferin were noted for the first time in the species. During the growing season, the content of individual GLS changed significantly. The rate and direction of these changes varied across the examined landraces and plant organs. In the leaves, between May and June, the content of sinigrin, the main GLS in all horseradish landraces, decreased in Bavarian (40%) and Hungarian (11%) horseradish, increased (22%) in Creamy horseradish, whereas in Danish horseradish, the difference was not significant. Despite the changes observed in the first two months, the highest content of sinigrin was noted in July in all horseradish landraces. During the growing season (August-October), the content of sinigrin fluctuated in the roots of Creamy and Danish landraces, reaching the highest level in October and September, respectively, whereas in the roots of Hungarian and Bavarian landraces, sinigrin concentrations continued to increase and peaked in October. Changes in the content of other, minor GLS during the growing season often differed from those noted in sinigrin levels.

INTRODUCTION

Horseradish (*Armoracia rusticana* Gaertn., C.A. Mey & Scherb) is a perennial and neglected plant cultivated mainly for its roots. Grated horseradish is characterised by a hot and spicy flavour, and is used as a condiment in many countries around the world [Wedelsbäck Bladh & Olsson, 2011]. The characteristic taste and aroma of horseradish are associated with the presence of sulphur glucosides known as glucosinolates (GLS) [Agneta *et al.,* 2013]. Glucosinolates are found in vacuoles of plant cells, and they are separated from myrosinase (EC 3.2.3.1), a membrane-bound enzyme, until cell damage [Redovnikovic *et al.,* 2008]. When horseradish tissue is damaged during grating or cutting, the enzyme is released and fused with GLS. The enzymatic hydrolysis of GLS results in the formation of various compounds, mostly isothiocyanates, as well as smaller amounts of nitriles, thiocyanates, epithionitriles, and oxazolidines [Agerbirk & Olsen, 2012]. The breakdown products are responsible for the taste and aroma of horseradish. For instance, sinigrin undergoes enzymatic degradation to form allyl isothiocyanate which has a bitter taste, a pungent odour and causes lachrymation [Horbowicz & Rogowska, 2006; Kosson & Horbowicz, 2009; Wedelsbäck Bladh *et al.,* 2013].

To date, nearly 130 GLS have been identified [Agerbirk & Olsen, 2012], including around only 20 in vegetables. The type and concentration of GLS in plants vary considerably subject to genetic traits [Fahey *et al*., 2001], climate [Ciska *et al.,* 2000] and agronomic factors such as sulphur and nitrogen fertilization [Alnsour 2013; De Maria *et al.,* 2016]. GLS can be divided into aliphatic, aralkyl and indole group. Horseradish is one of the richest sources of GLS [Fahey *et al.,* 2001; Li & Kushad, 2004; Agneta *et al.,* 2012, 2014; Alsnour, 2013]. Seventeen GLS have been identified in horseradish to date [Agneta *et al.,* 2014]. Sinigrin is an aliphatic GLS and is the main GLS in horseradish, followed by gluconasturtiin and glucobrassicin, as aralkyl and indole GLS, respectively, while the content of other GLS is marginal. The content of other GLS, both aliphatic and indole, is minimal. Differences in GLS profiles and content were observed between horseradish accessions and between plant organs. According to Li & Kushad [2004], the total GLS content in the roots

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of 27 horseradish accessions and the leaves of 9 accessions ranged from 2 to 296 μmol/g DW and from 34 to 201 μmol/g DW, respectively. The noted variations could also be attributed to the growth stage [Li & Kushad, 2004; Alnsour, 2013]. It is assumed that younger tissues are a richer source of indole GLS than senescent tissues [Mevy *et al.,* 1997].

The interest in GLS and their breakdown products has been spurred by their biological activity. These compounds play a very important role in plants by protecting them from herbivores and pathogens [Appel & Cocroft, 2014]. Glucosinolate breakdown products deliver health benefits also for humans. Numerous studies have demonstrated that isothiocyanates [Higdon *et al.,* 2007] and selected nitriles have anticarcinogenic effects [Tanii *et al.,* 2008; Hanschen *et al.,* 2015].

Horseradish is considered as the richest source of sinigrin in a human diet [Agneta *et al.,* 2013]. Allyl isothiocyanate, the main breakdown product of sinigrin, has been shown to have several biological activities. Antimicrobial [Shin *et al.,* 2004] and anticancer activity related to multimode mechanism of action [Xiao *et al.,* 2003; Zhang *et al.,* 2010], including the stimulation of cytoprotective protein and anti-inflammatory activity, have been reported in the previous studies [Wagner *et al.,* 2012]. The biological activity of horseradish has been discovered many centuries ago, and it was very often utilized for treatment purposes in the traditional medicine [Agneta *et al.,* 2013].

The use of horseradish as food is most wide spread in the Eastern and Mediterranean part of Europe [Wedelsback Bladh & Olsson, 2011]. The roots are used to flavour and preserve the plant food, or to support the vegetable fermentation [Sampliner & Miller, 2009]. Grated root is very popular as a condiment to cooked meat. The horseradish leaves can be used for salad and placed in the oven under baking bread to flavour and to prevent it from sticking.

The knowledge about the composition, content and distribution of GLS in different organs of horseradish plants is limited [Li & Kushad, 2004; Agneta *et al.,* 2014]. Various authors have recognized the need to examine GLS concentrations in intermediate periods of horseradish development [Agneta *et al.,* 2014]. Therefore, the aim of this study was to evaluate changes in the GLS content in leaves and roots in different horseradish accessions during the growing season.

MATERIALS AND METHODS

Plant material

The experimental material consisted of 4 horseradish landraces: Bavarian, Danish, Hungarian, and Creamy. White horseradish (Bavarian, Danish and Hungarian) has white, cylindrically-shaped roots and dark green, strongly toothed leaves. Creamy horseradish has light yellow, slightly conical roots and green, somewhat toothed leaves. All landraces originated from the south-western part of the Łódź Region, the centre of horseradish production in Poland.

Horseradish cultivation

Horseradish seedlings were obtained by vegetative multiplication of original accessions in 2004. They were planted in 2005 between 10 and 20 of April in the experimental fields of the Research Institute of Horticulture in Skierniewice, Poland (geographical coordinates: N51°57'50.1139" E20°9'49.6166"). The cultivation experiment was conducted on sandy loam soil with pH 5.7–6.0 and 1.7% organic matter content. Before planting, soil was fertilised with urea (80 kg N/ha), superphosphate (125 kg P_2O_5/ha) and potassium (250 kg K_2O/ha); potassium nitrate (60 kg N/ha) was applied during rapid growth. Weeds were removed manually, and the crops were watered according to need. Each type of horseradish was grown on 4 plots (15 m^2 each, 75 plants per plot) with a randomised block design. The distance between rows was 67.5 cm and within rows was 5 cm.

Sample preparation

Horseradish leaves were sampled for analysis from May to August (in the middle of each month; between 9 and 10 a.m.). Leaf sampling stopped in August due to leaf aging and yellowing. Root samples were collected from August to October. For analyses of the GLS content in leaves, one leaf was collected from 12 plants in one replication (around 50–100 g). A quarter of the central part of roots (around 100 g) was collected for GLS analysis. Plant samples were immediately frozen at -25°C and lyophilised using the Christ Alpha 1–2LD plus laboratory freeze-dryer (Osterode am Harz, Germany).

GLS analysis

The isolation, desulphatation and HPLC separation of GLS were carried out according to the guidelines of the Commission of European Communities [1990], as previously described by Ciska *et al.* [2008]. The separation was performed using the HPLC system with an autosampler (LC-10, Shimadzu, Japan) and a UV-VIS detector (SPD-10A, Shimadzu, Japan) using the LiChrospher® 100 RP-18 (250 × 4 mm; 5 μm) column (Merck). Desulpho-GLS was separated with a gradient of water (A) and 20% acetonitrile (B) as previously described [Ciska *et al.,* 2008]. Glucosinolates were identified by comparing their retention times with those of known reference compounds or on the basis of available literature data. The presence of aliphatic GLS not having a standard was also additionally confirmed with the GC-MS analysis of respective degradation products using a gas chromatograph Agilent 7890A equipped with a mass detector 5975C VL, as described earlier [Ciska & Pathak, 2004]. Glucotropaeolin (Merck, Darmstadt, Germany) was used as the internal standard for quantification. All experiments and analytical measurements were performed in triplicate. The sample content of GLS was quantified based on the internal standard and relevant relative response factors [Commission of European Communities, 1990]. Data were processed with the use of standard statistical procedures, and the least significant difference between means was calculated using the Newman-Keuls test at $p<0.05$.

RESULTS AND DISCUSSION

Thirteen GLS, including nine aliphatic, three indole and one aryl GLS, were identified in the analysed horseradish. For all landraces, the concentrations of these compounds during the growing season (from May to August in leaves, and from August to October in roots) are presented in Tables 1–4. The presence of nine GLS in horserad-

TABLE 1. The GLS content (μmol/g DW) in leaves and roots of Bavarian landrace.

Month	Aliphatic GLS									Aryl GLS	Indole GLS		
	Sinigrin	Gluconapin	Glucobrassicanapin	Progoitrin	Napoliferin	Glucoiberverin	Glucoiberin	Glucoraphanin	Glucoraphenin	Gluconasturtiin	Glucobrassicin	4-Hydroxy-glucobrassicin	4-Methoxy-glucobrassicin
Leaves													
May	85.14±1.07^{b*}	0.07±0.00^{a}	0.15±0.01^{b}	0.43±0.01^{a}	tr**	tr	0.80±0.01^{a}	0.30±0.03^{a}	0.34±0.05^{b}	0.15±0.01	tr	tr	tr
June	50.82±1.21^{c}	tr	0.13±0.02^{b}	0.36±0.01^{b}	tr	tr	0.67±0.00^{b}	0.08±0.01^{c}	0.54±0.04^{a}	tr	0.05±0.00^{a}	tr	0.10±0.00^{b}
July	106.22±5.25^{a}	0.09±0.01^{a}	0.25±0.02^{a}	0.17±0.01^{c}	tr	tr	0.63±0.04^{b}	0.21±0.02^{b}	0.16±0.01^{c}	tr	0.07±0.01^{a}	tr	0.05±0.00^{c}
August	76.23±4.12^{b}	0.07±0.01^{a}	0.17±0.01^{b}	0.14±0.01^{d}	tr	tr	0.36±0.00^{c}	0.22±0.01^{b}	0.13±0.01^{c}	tr	0.06±0.01^{a}	tr	0.12±0.00^{a}
Roots													
August	72.06±2.56^{b}	0.12±0.02^{a}	0.42±0.01^{b}	0.13±0.01^{a}	0.06±0.01^{a}	0.10±0.01^{a}	0.21±0.02^{c}	0.17±0.01^{b}	0.10±0.01^{a}	6.79±0.21^{a}	0.77±0.09^{b}	0.07±0.01^{b}	tr
September	73.49±2.86^{b}	0.12±0.00^{a}	0.38±0.03^{b}	0.11±0.01^{a}	tr	0.10±0.00^{a}	0.30±0.01^{b}	0.15±0.01^{b}	0.12±0.01^{a}	4.95±0.08^{c}	0.86±0.04^{b}	0.05±0.00^{b}	0.06±0.01^{a}
October	100.31±2.96^{a}	0.15±0.00^{a}	0.83±0.05^{a}	0.13±0.01^{a}	0.08±0.01^{a}	0.08±0.00^{a}	1.23±0.03^{a}	0.45±0.05^{a}	0.10±0.00^{a}	5.6±0.166^{b}	1.25±0.06^{a}	0.13±0.02^{a}	0.05±0.00^{a}

(*) Mean values (n = 3) with different letters in the same column for groups are significantly different ($p<0.05$) (Newman-Keuls Test, ANOVA). (**)Trace < 0.05 μmol/g DW.

TABLE 2. The GLS content (μmol/g DW) in leaves and roots of Danish landrace.

Month	Aliphatic GLS									Aryl GLS	Indole GLS		
	Sinigrin	Gluconapin	Glucobrassicanapin	Progoitrin	Napoliferin	Glucoiberverin	Glucoiberin	Glucoraphanin	Glucoraphenin	Gluconasturtiin	Glucobrassicin	4-Hydroxy-glucobrassicin	4-Methoxy-glucobrassicin
Leaves													
May	57.36±0.81^{bc*}	0.06±0.01^{a}	0.07±0.00^{b}	2.46±0.19^{b}	tr**	tr	0.80±0.01^{a}	0.23±0.04^{a}	0.36±0.04^{b}	0.11±0.02	tr	tr	tr
June	51.29±1.26^{c}	tr	0.09±0.00^{b}	2.82±0.10^{a}	tr	tr	0.79±0.00^{a}	0.11±0.00^{b}	0.53±0.05^{a}	tr	tr	tr	0.10±0.01^{a}
July	84.48±2.73^{a}	0.07±0.01^{a}	0.18±0.03^{a}	0.63±0.04^{c}	tr	tr	0.52±0.01^{b}	0.13±0.01^{ab}	0.11±0.04^{c}	tr	0.06±0.01	tr	tr
August	63.73±3.81^{b}	0.06±0.00^{a}	0.11±0.02^{b}	0.54±0.05^{c}	tr	tr	0.26±0.03^{c}	0.19±0.01^{a}	0.18±0.02^{c}	tr	tr	tr	0.12±0.01^{a}
Roots													
August	98.33±3.46^{a}	0.25±0.02^{a}	0.65±0.01^{a}	0.13±0.01^{ab}	0.12±0.01^{a}	0.27±0.01^{a}	0.26±0.01^{c}	0.17±0.02^{b}	0.09±0.01^{a}	7.74±0.01^{a}	0.71±0.05^{b}	0.21±0.02^{a}	0.06±0.01^{a}
September	71.74±3.30^{b}	0.09±0.01^{c}	0.41±0.04^{b}	0.11±0.01^{b}	tr	0.06±0.01^{b}	0.34±0.01^{b}	0.16±0.02^{b}	0.09±0.00^{a}	3.78±0.17^{c}	0.45±0.05^{c}	0.11±0.00^{b}	tr
October	90.23±3.26^{a}	0.17±0.03^{b}	0.64±0.04^{a}	0.14±0.01^{a}	0.08±0.01^{a}	0.08±0.01^{b}	1.12±0.01^{a}	0.28±0.00^{a}	0.06±0.01^{b}	5.47±0.36^{b}	1.40±0.03^{a}	0.25±0.04^{a}	0.05±0.00^{a}

(*) Mean values (n = 3) with different letters in the same column for groups are significantly different ($p<0.05$) (Newman-Keuls Test, ANOVA). (**)Trace < 0.05 μmol/g DW.

TABLE 3. The GLS content (μmol/g DW) in leaves and roots of Hungarian landrace.

Month	Aliphatic GLS									Aryl GLS	Indole GLS		
	Sinigrin	Gluconapin	Glucobrassicanapin	Progoitrin	Napoliferin	Glucoiberverin	Glucoiberin	Glucoraphanin	Glucoraphenin	Gluconasturtiin	Glucobrassicin	4-Hydroxy-glucobrassicin	4-Methoxy-glucobrassicin
Leaves													
May	52.89±1.13^{b*}	0.08±0.00^{a}	0.20±0.01^{b}	0.26±0.03^{b}	tr**	tr	0.61±0.02^{a}	0.30±0.03^{ab}	0.54±0.04^{a}	0.10±0.01	tr	tr	tr
June	48.47±3.76^{b}	0.08±0.02^{a}	0.23±0.03^{b}	0.31±0.03^{b}	tr	tr	0.52±0.06^{a}	0.19±0.01^{b}	0.47±0.07^{a}	tr	tr	tr	0.11±0.03^{b}
July	70.78±4.06^{a}	0.09±0.02^{a}	0.46±0.01^{a}	0.27±0.01^{b}	tr	tr	0.47±0.06^{a}	0.23±0.00^{b}	0.16±0.00^{b}	tr	tr	tr	0.05±0.00^{c}
August	50.09±2.86^{b}	0.06±0.01^{a}	0.49±0.04^{a}	0.56±0.01^{a}	tr	tr	0.25±0.01^{b}	0.39±0.06^{a}	0.15±0.01^{b}	tr	0.06±0.01	tr	0.18±0.02^{a}
Roots													
August	70.21±5.20^{b}	0.13±0.02^{a}	0.40±0.04^{ab}	0.28±0.01^{a}	0.06±0.01^{b}	0.14±0.01^{a}	0.13±0.01^{c}	0.13±0.01^{b}	0.11±0.01^{a}	2.52±0.38^{a}	0.23±0.06^{a}	0.14±0.01^{b}	0.06±0.00^{a}
September	85.80±5.81^{ab}	0.14±0.02^{a}	0.30±0.01^{b}	0.31±0.03^{a}	0.07±0.01^{b}	0.18±0.01^{a}	0.17±0.01^{b}	0.17±0.02^{b}	0.11±0.01^{a}	2.11±0.02^{a}	0.18±0.01^{a}	0.12±0.01^{b}	tr
October	96.30±6.05^{a}	0.17±0.01^{a}	0.50±0.04^{a}	0.23±0.02^{a}	0.16±0.00^{a}	0.19±0.01^{a}	0.32±0.01^{a}	0.25±0.03^{a}	tr	1.66±0.10^{a}	0.25±0.01^{a}	0.27±0.02^{a}	0.07±0.00^{a}

(*) Mean values (n = 3) with different letters in the same column for groups are significantly different ($p<0.05$) (Newman-Keuls Test, ANOVA). (**)Trace < 0.05 μmol/g DW.

TABLE 4. The GLS content (μmol/g DW) in leaves and roots of Creamy landrace.

Month	Aliphatic GLS									Aryl GLS	Indole GLS		
	Sinigrin	Gluconapin	Glucobrassicanapin	Progoitrin	Napoliferin	Glucoiberverin	Glucoiberin	Glucoraphanin	Glucoraphenin	Gluconasturtiin	Glucobrassicin	4-Hydroxy-glucobrassicin	4-Methoxy-glucobrassicin
Leaves													
May	64.42±0.90^{d*}	0.30±0.01^{a}	0.20±0.02^{a}	0.42±0.06^{a}	0.16±0.04^{a}	0.24±0.05^{a}	1.01±0.00^{a}	0.61±0.02^{a}	0.37±0.01^{a}	0.09±0.01^{a}	0.07±0.01^{b}	0.05±0.01	0.06±0.01^{c}
June	78.46±1.51^{c}	0.15±0.01^{b}	0.12±0.03^{a}	0.32±0.06^{ab}	0.05±0.00^{b}	0.09±0.00^{b}	0.92±0.02^{a}	0.40±0.01^{c}	0.27±0.06^{ab}	0.09±0.03^{a}	0.11±0.00^{a}	tr**	0.12±0.01^{b}
July	121.36±0.94^{a}	0.31±0.03^{a}	0.18±0.01^{a}	0.23±0.01^{b}	tr	0.11±0.00^{b}	0.72±0.06^{b}	0.30±0.01^{d}	0.21±0.02^{bc}	0.07±0.01^{a}	tr	tr	0.08±0.01^{bc}
August	106.28±1.41^{b}	0.28±0.01^{a}	0.17±0.01^{a}	0.19±0.04^{b}	tr	0.12±0.00^{b}	0.35±0.00^{c}	0.46±0.03^{b}	0.12±0.01^{c}	tr	0.08±0.01^{ab}	tr	0.24±0.02^{a}
Roots													
August	88.27±2.50^{b}	0.40±0.01^{b}	0.45±0.04^{b}	0.12±0.01^{a}	0.36±0.04^{b}	0.45±0.01^{b}	0.23±0.01^{a}	0.17±0.01^{b}	0.06±0.01^{a}	7.18±0.38^{a}	0.14±0.00^{b}	0.33±0.01^{b}	0.07±0.01^{a}
September	74.89±1.37^{c}	0.28±0.00^{c}	0.35±0.02^{c}	0.11±0.01^{a}	0.29±0.01^{b}	0.32±0.01^{c}	0.28±0.01^{a}	0.19±0.00^{b}	0.07±0.00^{a}	5.54±0.02^{b}	0.11±0.00^{b}	0.30±0.01^{b}	0.11±0.01^{a}
October	116.92±2.91^{a}	0.50±0.01^{a}	0.61±0.01^{a}	0.19±0.04^{a}	0.57±0.00^{a}	0.58±0.01^{a}	0.37±0.06^{a}	0.35±0.03^{a}	tr	8.13±0.10^{a}	0.72±0.00^{a}	0.67±0.06^{a}	tr

(*) Mean values (n = 3) with different letters in the same column for groups are significantly different ($p<0.05$) (Newman-Keuls Test, ANOVA). (**)Trace < 0.05 μmol/g DW.

ish: sinigrin, glucoiberin, gluconapin, glucobrassicanapin, progoitrin, gluconasturtiin, glucobrassicin, 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin, was confirmed by other researchers [Li & Kushad, 2004; Agneta *et al.,* 2012; Wedelsbäck Bladh *et al.,* 2013]. Glucoiberverin (identified only based on the corresponding mustard oils) was also identified by Grob & Matile [1980], whereas the presence of glucoraphanin, glucoraphenin and napoleiferin was noted in horseradish for the first time.

In all analysed landraces, sinigrin was the predominant GLS in both leaves and roots in all growth stages. In the leaves, the percentage of sinigrin in total GLS content varied from 92% in Danish horseradish in June to more than 98% in Creamy and Bavarian horseradish in July and August, and in the Danish accession in July. In the roots, the highest content of sinigrin (94–96%) was observed in the Hungarian landrace, and the lowest content (89%) was noted in Creamy and Bavarian horseradish in August. Other researchers also reported high sinigrin levels in the leaves and roots of horseradish [Li & Kushad, 2004; Redovniković *et al.,* 2008; Alnsour, 2013].

Sinigrin concentrations fluctuated across growth stages. At the first stage of leaf growth (May to June), sinigrin content decreased by 40% and 11% in Bavarian and Hungarian horseradish, respectively, but increased by 22% in the Creamy accession (Table 1). In Danish horseradish, the observed difference in the sinigrin content in leaves was not statistically significant, but a decreasing trend was noted. At successive stages of development (July-August), sinigrin concentrations increased significantly in July and decreased in August in all horseradish landraces. In July, the highest 2-fold increase in sinigrin levels was observed in Bavarian horseradish, whereas the smallest 46% increase was noted in the Hungarian type. In August, sinigrin concentrations decreased by 20–29% in Bavarian, Danish and Hungarian landraces, and by only 12% in Creamy horseradish.

The decrease in the sinigrin content in all horseradish landraces, excluding Creamy, at the first stage of development (May-June) most probably resulted from dilution of sinigrin in growing leaves [Cleembut & Becker, 2012]. Changes in the GLS content of both leaves and roots during the growing season have not been investigated to date. For this reason, the distinct profile of changes in the sinigrin content in the Creamy landrace is difficult to explain without more extensive research. The unexpected increase in sinigrin concentrations observed in June only in Creamy landrace could be attributed to genetic factors which would also be responsible for differences in the growth rate and/or the rate of GLS accumulation. Therefore, it is possible that the drop in GLS concentrations, which was observed in Bavarian, Danish and Hungarian landraces in June, took place later or earlier in Creamy horseradish and remained unnoticed because the samples were analysed only once a month.

Agneta *et al.* [2014] and Mølmann *et al.* [2015] also reported high levels of aliphatic GLS in photosynthetic tissues, which indicates that the rate of photosynthesis is related to GLS content. Other authors also found that light can induce the expression of genes involved in GLS biosynthesis [Huseby *et al.* 2013].

Sinigrin content gradually increased in the roots of Bavarian and Hungarian landraces. In Danish and Creamy horseradish, sinigrin concentrations fluctuated throughout the growing season (August-October) and were lowest in September.

In our study, the decrease in sinigrin levels at the last stage of leaf development (August) and the increase in the GLS content in roots in October could be caused by the aging of above-ground tissues or the transfer of GLS from leaves to roots [Chen & Andreasson, 2001]. Similar results were reported by Alnsour [2013] who noted a rapid drop in GLS concentrations in fully matured horseradish leaves at the end of the growing season. The above authors attributed their findings to active translocation of GLS between leaves and roots, the sink organs of a plant. Previous studies suggested that the transport of GLS follows the allocation of assimilates and that intact GLS have properties that satisfy the permeability criterion for phloem mobility [Chen *et al.,* 2001]. The cited authors concluded that GLS are readily loaded into and transported by the phloem. Phloem-mediated transport of GLS may coordinate *de novo* biosynthesis and promote the use of GLS as defence compounds in various organs. Recent studies demonstrated the presence of GLS in the root xylem sap of *Arabidopsis,* which indicates that a transport pathway is involved in root-to-shoot GLS allocation [Madsen *et al.,* 2014; Jørgensen *et al.,* 2015]. In contrast, Li *et al*. [1999] did not report correlations between the GLS content in the leaves, stems and roots of *Brassica napus*, and they concluded that both synthesis and accumulation of GLS are controlled by plant tissues. A similar explanation for the lack of correlations between individual GLS in horseradish leaves and roots was proposed by Li & Kushad [2004].

In addition to the predominant sinigrin, significant quantities of gluconasturtiin were found in the roots of all horseradish landraces, and the leaves of Danish horseradish were abundant in progoitrin. During the growing season, the gluconasturtiin content in roots fluctuated in Creamy, Bavarian and Danish horseradish. Gluconasturtiin concentrations decreased in September and increased in October. In Creamy horseradish, the content of gluconasturtiin at harvest maturity was even higher than at the beginning of root development, reaching 8.13 μmol/g DW, *i.e.* the highest value noted in all accessions. In Hungarian landrace a decreasing tendency was observed, however the differences were not significant. The studied horseradish landraces contained only trace amounts of gluconasturtiin in leaves. Li & Kushad [2004] also reported higher gluconasturtiin levels in horseradish roots than leaves. Unlike the remaining horseradish landraces, the Danish landrace was characterised by a relatively high content of progoitrin in the leaves. The highest progoitrin levels were observed in May (2.46 μmol/g DW) and June (2.82 μmol/g DW) when they accounted for nearly 4% of total GLS content. The content of progoitrin decreased by 78% between June and July. In the leaves of other landraces, progoitrin concentrations did not exceed 0.4 μmol/g DW. The relatively high progoitrin content in Danish horseradish and low progoitrin levels in other landraces point to genetic differentiation of horseradish. In a study by Wedelsbäck Bladh *et al.*

[2013], measurable amounts of progoitrin were detected in only 2 of 168 accessions of Nordic horseradish.

In addition to major GLS, the analysed horseradish landraces also contained small amounts of aliphatic GLS such as glucoiberin, glucoiberverin, glucoraphanin, glucoraphenin, napoleiferin, gluconapin and glucobrassicanapin in roots and leaves. During the growing season, the pattern of changes in the concentrations of aliphatic GLS often differed from that of the major GLS such as sinigrin and gluconasturtiin. Those differences can probably be attributed to the common biosynthesis pathways of individual compounds. Glucosinolates with three carbon atoms in a side chain, including sinigrin, glucoiberverin and glucoiberin, belong to the same biosynthesis pathway which is regulated by 2-oxoglutarate--dependent dioxygenases. Therefore, sinigrin levels are determined by the content of the precursor glucoiberin which, in turn, depends on glucoiberverin concentrations [Magrath *et al.,* 1994; Ishida *et al.,* 2014]. In our study, such relationships between individual GLS were observed between May and July when sinigrin content increased and the concentrations of glucoiberin and glucoiberverin decreased. The fluctuations in the levels of progoitrin, gluconapin and glucoraphanin resulting from S-oxygenation can be also explained by the fact that those four-carbon side-chain GLS belong to the same biosynthetic pathway [Magrath *et al.,* 1994; Ishida *et al.,* 2014].

Indole GLS, glucobrassicin, 4-hydroxy-glucobrassicin and 4-methoxy-glucobrassicin, were also found in both leaves and roots of the analysed horseradish plants. Leaves often contained trace amounts of these compounds, but in the roots of all horseradish landraces, the concentrations of indole GLS increased or tended to increase throughout the growing season. As a result, the glucobrassicin content in roots was relatively high at harvest maturity, and in Danish and Bavarian landraces exceeded even 1 μmol/g DW. Our results contradict most published findings which suggest that indole GLS levels are higher in younger tissues [Mevy *et al.,* 1997]. Some authors placed glucobrassicin in the group of predominant GLS [Li & Kushad, 2004], but in our study, the proportion of glucobrassicin in total GLS varied across landraces and was predominant only in Danish and Bavarian horseradish.

CONCLUSIONS

GLS in *Brassicaceae* plants are well described in the literature. However, as compared to other vegetables belonging to this family, the GLS pattern in horseradish has been relatively poorly characterised. Therefore, novel compounds that belong to the GLS group are still being discovered during studies of horseradish plants. A total of 13 GLS were found in the horseradish landraces examined in this study. Glucoraphanin, glucoraphenin and napoleiferin were noted in horseradish tissues for the first time.

In our study, the content of individual GLS in horseradish fluctuated significantly during the growing season, and the rate and direction of these changes varied across the analysed landraces. At the first stage of leaf growth (May to June), the content of sinigrin, the main GLS in horseradish, decreased by 40% and 11% in Bavarian and Hungarian horseradish, respectively and increased by 22% in the Creamy landrace. In Danish landrace, the difference in the sinigrin content in leaves was not statistically significant. Despite the differences noted in the first two months, sinigrin concentrations in all horseradish types peaked in July, probably due to a higher rate of photosynthesis. Further studies are needed to confirm the effect of temperature, sun exposure and photoperiod on GLS accumulation in horseradish in July.

Similarly to leaves, changes in the sinigrin content of roots were also observed in horseradish landraces during the growing season (August – October). Sinigrin content increased gradually in the roots of Hungarian and Bavarian landraces, and it fluctuated in Creamy and Danish types.

In our study, the fluctuations in the levels of other, minor GLS followed a different pattern than the changes in sinigrin levels. The differences in the direction and rate of changes in individual GLS during the growing season can probably be attributed to mutual relationships between GLS belonging to the same biosynthesis pathways. However, further research is required to confirm this hypothesis.

Among all the Brassica vegetables consumed by humans, horseradish is the richest source of the precursor of beneficially active isothiocyanates – sinigrin. Therefore, the intake of this plant, not only as a condiment made from root but also in the form of leaves containing even more sinigrin than the roots, should be propagated in the human diet. Horseradish leaves may be a valuable additive to salads, simultaneously enhancing the attractiveness and supplementing our diet with minerals, vitamins and fibre, as well as in compounds having a potential anticancer activity.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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