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Antioxidant properties of leaf and root extract and oil from different types of horseradish (*Armoracia rusticana* Gaertn.)

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ABSTRACT

Antioxidant properties of leaf and root extracts and mustard oil originated from four different types of horseradish ('Lipnik', 'Mazaniec', 'Osjaków', 'Tądle') were investigated. The tested types were cultivated in two different regions of Poland: near the town Wieluń (sand-originated yellow soil) and in the area near Warsaw (Wilanów – dust-originated middle mud). Antioxidant properties in roots and leaves were determined by two methods: reduction of Fe^{3+} ions and inhibition of deoxiribose oxidation process. Additionally, the root-derived oil was subjected to

Total Antioxidant Status (TAS) determination. In addition, experiments with mice (line C57BL/6J) were also performed. The animals were divided into two groups. The first group was fed a standard diet while the second group was fed the same diet enriched with 1% horseradish. Although leaf and root extracts derived from 4 Polish types of horseradish did not exhibit strong antioxidant properties, the different environmental conditions of plant growth affected these properties significantly. Volatile oil obtained from horseradish roots revealed stronger antioxidant properties than pure allyl isothiocyanate and was significantly affected by types as well as environmental factors. Diet containing horseradish had no effect on antioxidant plasma and heart activity in mice. The biological attributes of horseradish probably do not come from antioxidant properties of compounds contained in the useful parts of this vegetable.

INTRODUCTION

The increasing interest in plant-derived chemicals has been observed during the last years, especially because of their medical attributes. Special attention is paid to vitamins: E (tocopherol), A (retinol), C (ascorbic acid), and carotenoids (β-carotene) as well as to flavonoids and glucosinolates. These antioxidants, when present in the diet, prevent damage to various tissues caused by free radicals.

Plants from *Brassicaceae* family are the main source of glucosinolates in the human diet. Glucosinolates are at present considered as important anti-cancer factors (Shapiro et al. 1998, Murillo and Mehta 2001). The epidemiological experiments lead to the conclusion that cruciferous vegetables especially prevent lung cancer and food-conduit cancer. Cytotoxic activities of glucosinolates as well as their degradation products (isothiocyanates) were demonstrated in experiments with animals and cell cultures. However, the concentration of those compounds used in those tests is relatively higher than their real concentration in food products.

Glucosinolates in plant cells are exposed to myrosinase activity while preparing the meal (cutting, rubbing). This enzyme catalyses decomposition of glucosinolates to volatile compounds such as isothiocyanates, thiocyanates, and nitrils (Yu et al. 2001). A substantial part of them is lost during boiling.

Horseradish is a perennial plant, with a particularly pungent flavour, rich in glucosinolates and usually consumed as a fresh vegetable. Its leaves are considered to prevent food spoiling processes.

Although glucosinolates, with their antioxidant properties, play an important role in the human diet and possess a very common appearance in many plant species, they have not been systematically investigated.

In the present study the authors evaluate some antioxidant properties of leaf and root extracts and mustard oil from four different types of horseradish (‘Lipnik’,

'Mazaniec', 'Osjaków', 'Tądle') growing in different environmental conditions (Wieluń, Wilanów). Additionally, the dietary effect of horseradish on oxidant status in mice was investigated.

MATERIAL AND METHODS

Studies were conducted on four Polish types of horseradish ('Lipnik', 'Mazaniec', 'Osjaków', 'Tądle') originated from one-year-old plantation, in the years 2001 and 2002. They were grown in two different regions: near the town of Wieluń (sand-originated yellow soil) and near Warsaw (dust-originated middle mud). The field experiments were performed in a randomized block design in 4 replications. Samples of raw material (20 developed healthy leaves and central parts of 12 roots) were isolated from 12 randomly selected plants of each type of horseradish. The samples for the chemical analyses were taken at the same time from two localizations of the cultivation.

Antioxidant properties of water extracts (20 mg cm^{-3}) of lyophilized leaves and roots were determined using two methods: reduction of Fe^{3+} ions and inhibition of deoxiribose oxidation process. Additionally, volatile oil obtained from roots by the steam distillation method (Farmakopea Polska VI 2002), was subjected to Total Antioxidant Status (TAS) determination. Antioxidant properties were estimated as an ability to reduce Fe^{3+} ions (Oyaizu 1986) and inhibition of deoxiribose oxidation (Halliwell et al. 1987). Fe^{3+} reduction level was evaluated using light absorbance at a wavelength of 700 nm. In this method the extracts with high absorbance ratio were considered as strongly reducing. Inhibition of deoxiribose oxidation was defined as a reciprocal of concentrations of substances reacting with thiobarbituric acid (TBARS). In this method the increasing inhibition of oxidation was in accordance with strong antioxidant abilities of tested substances. Results obtained with TBARS tests were presented as a concentration of malondialdehyde, using 1,1,3,3-tetraoxypropane as a standard. Total Antioxidant Status was determined using reagents from Randox (cat. nr NX2332). All analyses were performed in triplicates.

The experiment with mice (line C57BL/6J) was conducted as follows: the animals were divided into two groups with 10 mice in each group. The first group (control) was fed a standard diet while the second group received the same diet enriched with 1% horseradish. After 3 weeks of treatment the animals were killed. Their blood was placed into heparinized tubes and centrifuged. Plasma TAS was then determined. Hearts were immediately washed with physiological salt solution and frozen in liquid nitrogen, then homogenized, and used to evaluate the level of substances reacting with thiobarbituric acid (TBARS). The results were evaluated statistically using the Statgraphics Program.

RESULTS AND DISCUSSION

According to Hur JongMoon et al. (1998), horseradish leaves exert antioxidant activity: methanol extract from horseradish leaves reduced by 64% lipid oxidation in rat's liver whereas kaempferol glycosides isolated from leaves decreased the formation of lipid peroxide in the range 16 and 39%. However there have been no reports on the effect of horseradish types and localisation of its cultivation on antioxidant activity of horseradish leaves. In the present study the place of horseradish cultivation appeared to be an important factor which had impact on the reducing power and antioxidant activity of leaf extract (Table 1), whereas differences between types were very rare. The 'Tądle' type was distinguished positively from other types.

Velioglu et al. (1998) indicated 57.4% activity of root extracts (when compared with α-tocopherol activity 97.3%) and very strong activity of oil (99.1%). However, up to now the effect of horseradish types and localisation of its cultivation on antioxidant activity of horseradish roots has not been investigated. In the present study the authors observed that every root extract from plants grown in the Wieluń region revealed stronger antioxidant properties than from Wilanów (Table 1). The four tested types differed from each other in term of their antioxidant activity and also reducing power. The strongest antioxidant activity had the type 'Mazaniec'. The most important factor which had impact on the reducing power and antioxidant activity of leaf extract (Table 1) was the place of horseradish cultivation, whereas differences between types were very rare. The 'Tądle' type was distinguished positively from other types.

Table 1. Antioxidant properties of extracts derived from lyophilised horseradish leaves and roots

Type (t) / localisation (l)	Reducing power (absorbance, λ = 700 nm)		Antioxidant activity (nM^{-1} TBAR)	
	leaves	roots	leaves	roots
'Lipnik' / Wieluń	0.20	0.27	1.45	1.97
'Lipnik' / Wilanów	0.15	0.21	1.23	1.27
Mean	0.16	0.24	1.34	1.62
'Mazaniec' / Wieluń	0.17	0.24	1.28	2.38
'Mazaniec' / Wilanów	0.15	0.21	1.41	1.71
Mean	0.175	0.23	1.34	2.04
'Osjaków' / Wieluń	0.19	0.26	1.47	1.92
'Osjaków' / Wilanów	0.17	0.24	1.49	1.16
Mean	0.18	0.25	1.48	1.54
'Tądle' / Wieluń	0.22	0.26	1.52	1.97
'Tądle' / Wilanów	0.15	0.24	1.51	1.14
Mean	0.19	0.25	1.51	1.56
$LSD_{0.05}$ (t)	n.s.	0.01	0.13	0.09
$LSD_{0.05}$ (l)	0.02	0.01	n.s.	0.05
$LSD_{0.05}$ (lxt)	0.03	0.01	0.22	n.s.

All the methods used in this study for evaluating antioxidant abilities of oil derived from horseradish roots (Table 2) allowed the conclusion that they were in correlation with the place of cultivation and with types. Only while using the method with TBARS did it turned out that the type did not affect antioxidant activity. On the basis of the reducing power and TAS analyses it was concluded that oil derived from the roots cultivated in Wilanów in most cases possessed stronger antioxidant activity than oil obtained from the same types but cultivated in Wieluń region. However, the method with TBARS revealed stronger antioxidant activity of oil derived from roots of types cultivated in Wieluń than those cultivated in Wilanów. Among the tested types, 'Tądle' exhibited strongest antioxidant activity while using the TAS method. Allyl isothiocyanate was found to be a less powerful antioxidant than mustard oil with every analytical method applied. Significant differences in antioxidant activity of horseradish raw material originating from different regions indicate that accumulation of biological active compounds is probably affected by climatic and soil factors.

Table 2. Antioxidant properties of mustard oil derived from horseradish roots

Type (t) / localisation (l)	Reducing power (absorbance, λ = 700 nm)	Antioxidant activity (nM^{-1} TBAR)	TAS (mM)
'Lipnik' / Wieluń	0.35	0.092	308.15
'Lipnik' / Wilanów	0.49	0.075	359.40
Mean	0.42	0.083	333.76
'Mazaniec' / Wieluń	0.48	0.084	312.00
'Mazaniec' / Wilanów	0.57	0.077	287.17
Mean	0.52	0.081	299.59
'Osjaków' / Wieluń	0.55	0.075	290.35
'Osjaków' / Wilanów	0.49	0.073	347.35
Mean	0.52	0.074	318.85
'Tądle' / Wieluń	0.44	0.083	407.60
'Tądle' / Wilanów	0.55	0.077	415.68
Mean	0.50	0.081	411.64
Allyl isothiocyanate	0.43	0.066	218.45
$LSD_{0.05}$ (t)	0.07	n.s.	25.24
$LSD_{0.05}$ (l)	0.03	0.01	12.86
$LSD_{0.05}$ (lxt)	0.12	n.s.	44.19

Plumb et al. (1996) investigated antioxidant properties of extracts derived from vegetables belonging to *Cruciferae* as well as pure glucosinolates, by using oxidizing mixtures very similar to the present authors' to define reducing power or inhibition of the oxidation process. They found that pure glucosinolates have rather weak antioxidant properties. Also most of the extracts from vegetables they

submitted to analyses seemed to possess rather weak antioxidant properties, whereas extract from boiled brussels sprouts increased lipids oxidation in liver microsomes.

Manesh and Kuttan (2003) recovered abolishing activity of allyl isothiocyanate, which was able to remove free radicals OH. In the authours' experiments they did not find such an effect, but during the tests with deoxiribose (abolition of free radicals) they noticed that pure allyl isothiocyanate significantly increased the substrate oxidation level.

In animal model (Table 3) the authors focused their attention on the activity of plasma and hearts isolated from mice consuming a diet enriched in 1% horseradish. The authours found that dietary horseradish administered to mice for 3 weeks did not significantly affect these indices, although Khan et al. (1997) proved that white mustard seeds could inhibit oxidation of lipids in rats subjected to a high-fat diet. Also Manesh and Kuttan (2003) in their studies concerning a red-ox state of organisms treated with allyl isothiocyanate and phenyloethyl isothiocyanate found that both substances inhibited oxidation in mouse liver homogenates.

Table 3. Antioxidant ability of plasma and heart of mice treated with diet containing 1% horseradish

Diet	TAS – plasma (μM)	TBARS – heart (nmol g^{-1})
Standard	339 ± 41	1656 ± 43
Standard + horseradish	368 ± 51	1568 ± 43

CONCLUSIONS

1. Although leaf and root extracts derived from 4 Polish types of horseradish did not exhibit strong antioxidant properties, the different environmental conditions of plant growth affected these properties significantly.
2. Volatile oil obtained from horseradish roots revealed stronger antioxidant properties than pure allyl isothiocyanate. They were significantly affected by types as well as environmental conditions.
3. Diet containing horseradish addition had no effect on antioxidant ability of plasma and heart in mice.
4. Biological attributes of horseradish probably do not originate from antioxidant properties of compounds found in roots and leaves of this vegetable. This phenomenon, very interesting for scientists as well as for practical utilities, needs special, interdisciplinary research.

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WŁAŚCIWOŚCI PRZECIWUTLENIAJĄCE EKSTRAKTÓW Z LIŚCI I KORZENI ORAZ OLEJKU RÓŻNYCH TYPÓW CHRZANU (*ARMORACIA RUSTICANA* GAERTN.)

Streszczenie: Do badań użyto czterech typów chrzanu: 'Lipnik' 'Mazaniec', 'Osjaków', 'Tądle' uprawianych w dwóch miejscach: w okolicy Wielunia (gleba płowa wytworzona z piasków) oraz w Wilanowie (mada średnia wytworzona z utworu pyłowego ilastego). Określono właściwości przeciwutleniające w korzeniach i liściach stosując dwie metody, a mianowicie: redukcji żelaza oraz hamowania utleniania deoksyrybozy. W olejku uzyskanym z korzeni chrzanu oprócz w/w metod oznaczono całkowity potencjał przeciwutleniający (TAS – Total Antioxidant Status). Przeprowadzono również badania na myszach rasy C57BL/6J. Zwierzęta były podzielone na dwie grupy: grupa pierwsza otrzymywała dietę standardową i grupa druga karmiona była dietą standardową z 1% dodatkiem chrzanu. Chociaż ekstrakty z liści i korzeni czterech polskich typów chrzanu nie wykazywały silnych właściwości przeciwutleniających, to jednak istotny wpływ na nie miały warunki klimatyczno-glebowe. Olejek gorczyczny uzyskany z korzeni chrzanu charakteryzował się silniejszymi właściwościami przeciwutleniającymi niż czysty izotiocyjanian allilu, a jednocześnie również tutaj stwierdzono istotny wpływ warunków klimatyczno-glebowych. Natomiast dieta zawierająca dodatek chrzanu nie wpłynęła na potencjał przeciwutleniający osocza i serca myszy. Działanie biologiczne chrzanu prawdopodobnie nie wynika z właściwości przeciwutleniających związków zawartych w częściach użytkowych tego warzywa.

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