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ORIGINAL PAPER

Resveratrol and tryptophan in cherry, sweet cherry, sea buckthorn fruits and in japanese knotweed leaves

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Abstract

Research on the relationship between diet and human health has provided information on the prophylactic and auxiliary treatment of many diseases with the use of nutrients and non-food components of plant-origin products. However, the content of compounds with health-promoting effects in plants varies and depends on many factors, including a genotype, geographical area, agrotechnical practices, climatic and soil conditions. The subject of the study were fruits of cherry cv. Łutówka, cherry cv. Vanda, sea buckthorn cv. Sirola, and Japanese knotweed leaves as a potential source of resveratrol and tryptophan in human diet. The plants were grown at the Agricultural Experimental Station in Lipnik (53°20′35″N 14°58′10″E, altitude 7 m.a.s.l.), in northern Poland. The soil on which the experiment was conducted belongs to the typical rusty soil group, and is classified as a Haplic Cambisol. In the Ap level (arable-humus horizon), it has the grain size distribution of clay with slightly acidic pH. An analysis of the content soil minerals showed moderate levels of magnesium and potassium, and high levels of phosphorus. No fertilization or supplementary irrigation was used. The fruits were collected from representative trees (avoiding the edge trees). The leaves were collected from four plants, from four shoots of each sampled plant. An analysis of the tryptophan content in the study material was performed according to the methodology described by AOAC (2012). A UHPLC Thermo Ultimate 3000 RS system was used for an analysis of resveratrol in the plant material. In this study, no free resveratrol in the fruit of sea buckthorn and in the leaves of Japanese knotweed was detected. It was also undetected in the fruit of cherries and sweet cherries. However, Japanese knotweed leaves contained derivatives of resveratrol, piceids. Fruits of cherry and, above all, fruits of sweet cherry and sea buckthorn were characterized by a high content of tryptophan.

Keywords: fruits, glycosides, resveratrol, leaves, tryptophan

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INTRODUCTION

The progress in science and technology has stimulated the development of civilization, while simultaneously giving rise to many previously unknown threats. These are civilization diseases that prevail in highly urbanized countries and ones lacking proper prophylaxis. Unhealthy lifestyle (sedentary lifestyle, fast food and unhealthy nutrition, low physical activity) increases the risk of myocardial infarct, intracranial hemorrhage, diabetes, depression or cancer (Kitajewska et al. 2014). Research on the relationship between diet and human health provided information on prophylactic and auxiliary treatment of many diseases, nutrients and non-food components of plant-origin products. Compounds with beneficial effects on health were considered to include dietary fiber, oligosaccharides, amino acids, peptides, proteins, vitamins, minerals, and phytochemicals. Bioactive compounds have long-term health benefits owing to their wide range of biological properties, thereby acting as dietetic or nutraceutical substances. Secondary metabolites and natural antioxidants are the most bioactive plant substances (Sytar, Smetanska 2022).

An excellent source of plant origin phytochemicals are stone fruits and berries. Cherry fruits (*Prunus cerasus* L.) contain biologically active substances with anti-inflammatory and antioxidant properties (Pirhadi et al. 2019). Sweet cherries (*Prunus avium*) are characterized by high concentrations of anthocyanins, phenolic compounds, significant amounts of melatonin, serotonin and tryptophan (Gonçalves et al. 2017, Xia et al. 2020, Gonçalves et al. 2024). In sea buckthorn berries (*Hippophae rhamnoides* L.), there are over 190 various biologically active compounds that support the treatment of many diseases, e.g. cardiovascular diseases, urinary tract disorders, diabetes. They also contain 18 to 19 free amino acids, of which eight (threonine, valine, methionine, leucine, lysine, tryptophan, isoleucine and phenylalanine) are essential amino acids (Jastrząb, Skrzydlewska 2019).

Plants widely recognized as weeds are abundant in nutrients, and they have bioactive, pro-health and therapeutic effects. Japanese knotweed (*Polygonum cuspidatum* Siebold & Zucc.) is thought to be a harmful weed in Europe and North America. In native Asia, its root is dried and used to produce Itadori tea, a traditional herbal medicine that prevents many diseases, including heart disease and stroke (Cucu et al. 2021). Japanese knotweed contains resveratrol, polysaccharides, flavonoids and large amounts of condensed tannins (Nosalova et al. 2013, Peng et al. 2013, Słowiński 2024).

Resveratrol and tryptophan are food ingredients characterized by high biological activity that have a wide range of effects on the human body.

Resveratrol (3,4’,5-trihydroxystilbene) is a chemical compound, a polyphenol derivative of stilbene. Together with its 3-O-β-d glucoside, piceid, they form two of many secondary metabolites produced by plants that have gained much attention as functional food components. In nature, both compounds exist as both trans and cis isomers. Until now, the biological activity of trans-resveratrol has been more widely studied than other isomers (cis-resveratrol, trans-piceid and cis-piceid), but all of them probably have a positive impact on an organism (Liu et al. 2013, Nawaz et al. 2017). Since its anticancer activity was reported in 1997, its health benefits have been intensively investigated. Resveratrol has antioxidant, anti-inflammatory, immunomodulatory, glucose and lipid regulatory, neuroprotective, and cardiovascular properties, and therefore it can protect against diverse chronic diseases, such as cardiovascular diseases (CVDs), cancer, liver diseases, obesity, diabetes, Alzheimer's disease, and Parkinson's disease (Meng et al. 2020, Marko, Kursvietiene et al. 2023, Pawliczak et al. 2023, Koc et al. 2024). Resveratrol could also be an effective and safe compound for the prevention and treatment of aging and age-related diseases (Zhou et al. 2020). Resveratrol occurs in nature in many plants, with its highest concentration found in roots of Japanese knotweed (*Polygonum cuspidatum*) (Mikuła-Pietrasik et al. 2015). In large quantities, it can also be found in fruits, which are part of human diet, such as grapes (*Vitaceae*), blueberries (*Vaccinium* spp.), blackberries (*Morus* spp.) and peanuts (*Arachis hypogaea*) (Gambini et al. 2015).

Amino acids play an important role in maintaining the health of every organism. Although they are intensively researched, knowledge about them is still limited. Data on the content of free amino acids in plants come from 20 years ago, and current findings are rare. Therefore, efforts are made to build a new database of free amino acids found in various plant foods, including vegetables, mushrooms and fruits (Ito et al. 2017).

Tryptophan, 2-amino-3-indolopropanoic acid, is an essential exogenous amino acid, thus it is necessary to provide it with food because it cannot be synthesized in the human body (Stępień et al. 2014). It plays an important role in the biosynthesis of proteins and as a precursor of various biologically active compounds, including serotonin, melatonin, kynurenine and quinoline acid. Tryptophan is also a precursor of nicotinamide adenine dinucleotide coenzymes (NAD) and NAD phosphate (NADP); it can replace niacin as an essential nutrient. It is widely used as a natural remedy for depression, pain, insomnia, hyperactivity and eating disorders (Garrido et al. 2012). Dietary tryptophan and its metabolites seem to have the potential to contribute to the therapy of autism, cardiovascular disease, cognitive function, chronic kidney disease, depression, inflammatory bowel disease, multiple sclerosis, sleep, social function, and microbial infections. Tryptophan can also facilitate the diagnosis of certain conditions such as human cataracts, colon neoplasms, renal cell carcinoma, and the prognosis of diabetic nephropathy (Mendel 2018). Good natural sources of tryptophan are protein-abundant products.

The content of compounds with health-promoting effects in plants varies and depends on many factors, such as a genotype, geographical area, agrotechnical practices, climatic and soil conditions, physiological status of plants and degree of their ripeness. For example, in the vine, it is the weather and the presence of fungi (Gambini et al. 2015, Viljevac et al. 2017, He et al. 2023).

The aim of this research was to verify the following hypothesis: fruits of *Prunus cerasus* L., Prunus avium, *Hippophae rhamnoides* L., and leaves of *Polygonum cuspidatum* are a good source of resveratrol and tryptophan.

Currently, there is little information on the content of resveratrol and tryptophan in fruits of *Prunus cerasus* L., Prunus avium, *Hippophae rhamnoides* L., and in leaves of *Polygonum cuspidatum*, cultivated in Central Europe (northern Poland). Therefore, the aim of the present study was to assess whether selected species cultivated in the conditions of Central Europe (northern Poland) can be a good source of resveratrol and tryptophan in the context of their application in health promoting diet.

MATERIALS AND METHODS

Plant material

The subject of the study were fruits of cherry cv. Łutówka, cherry cv. Vanda, sea buckthorn cv. Sirola, and Japanese knotweed leaves. The plants was grew at the Agricultural Experimental Station in Lipnik (53°20′35″N 14°58′10″E, altitude 7 m.a.s.l.), in north Poland. The aim of the experiment was to determine the effect of a geographical area on the content of resveratrol and tryptophan in the fruits of *Prunus cerasus* L., *Prunus avium*, *Hippophae rhamnoides* L., and in leaves of *Polygonum cuspidatum*.

The soil on which the experiment was conducted belongs to the typical rusty soil group and is classified as a Haplic Cambisol. In the Ap level (arable-humus horizon), it has the grain size distribution of clay with slightly acidic pH (5.6). The content analysis of soil minerals showed moderate levels of magnesium (33 mg kg^{-1} Mg) and potassium (94,5 mg kg^{-1} K) and high levels of phosphorus (71 mg kg^{-1} P).

The plants grew in natural conditions, and no agrotechnical treatments, e.g. fertilization or supplementary irrigation were used. There was lawn between the experimental trees, but selective herbicide fallow was kept in the rows. The trees were planted at a spacing of 3.5 x 3 m. Japanese knotweed grew within concrete rings, with a single pot covering an area of 0.8 m^2. The sea buckthorn shrubs were planted at a spacing of 4 x 3 m. The area of a single plot was 12 m^2.

During the vegetation season, the air temperature and sum of rainfalls were measured at the Weather Station in Lipnik, located near the experimental plots. The mean monthly temperature and total rainfall were slightly varied in the experimental year. During the experiment (April – August), the mean monthly temperature was 15.6°C and total rainfall reached 190.1 mm.

Cherry and sweet cherry fruits were harvested in early July, while sea buckthorn berries were picked in late August. Leaves of Japanese knotweed (without any signs of mechanical damage) were collected for analysis before the flowering of plants (third week of July). Only fruits and leaves without any signs of aging or mechanical damage were sampled. Fruits were collected from representative trees (avoiding the edge trees). Fresh fruits were harvested by hand and kept in a refrigerator at 4°C (24 h) before pre-treatment and drying. The leaves were collected from four plants, from four shoots of each plant. After harvest, the leaves were cleaned and dried in a convection chamber laboratory oven. Until the examination could take place, the leaves had been stored in a dry, shaded, and airy place.

Samples of plant material (100 mg in triplicate from each plant) were mixed with diatomaceous earth and extracted with 70% (v/v) methanol using an automatic pressure extractor (Dionex ASE 200). The extractions were carried out in three cycles at a pressure of 10 MPa at 100°C. Extracts evaporated under reduced pressure were dissolved in 1 ml of methanol and stored at -20°C until LC-MS analyses. In order to verify the potential decomposition of resveratrol or its derivatives during high temperature pressure extraction, the plant material was also extracted by applying 70% methanol at room temperature and using an ultrasonic bath.

Before analysis, samples were diluted at a 1:1 ratio (v/v) with distilled water containing the internal standard, digoxin (final concentration 50 pmol^{-1} µl). An UHPLC Thermo Ultimate 3000 RS system was used for analysis, equipped with a pump, an automatic sample feeder and a corona discharge detector in a spray (CAD). Chromatographic separations were carried out on a Waters BEH C18 column maintained at 30°C (2.1 × 100 mm, grain 1.7 µl) by a gradient of acetonitrile containing 0.1% (v/v) formic acid (mobile phase B) in distilled water with 0.1% formic acid (mobile phase A).

The outflow from the column was separated in a 3:1 ratio between the corona discharge detector (Thermo Corona Veo RS) and the ion source of the QTOF mass spectrometer (Bruker Impact II HD), which worked in the anion scanning mode within the *m/z* range from 120 to 2000. Data were collected at a frequency of 5 Hz. In each scan cycle, for two ions with intensities above 2500 counts, MS/MS decays were performed with automatically selected collision energy depending on the m/z in the range from 15 to 60 eV. The mass calibration was carried out using sodium formate provided to the ion source with 20 µl loop at the beginning and end of each separation. From the obtained data, ion chromatograms for ions with formulas $C_{14}H_{11}O_3$ were extracted and integrated ($[M\text{-}H]^-$, m/z 227.0714±0.01), $C_{20}H_{21}O_8$ ($[M\text{-}H]^-$, m/z 389.1242±0.01) and $C_{41}H_{64}O_{14}$ ($[M\text{+}FA\text{-}H]^-$, m/z 825.4278±0.01), which represented ionized forms of resveratrol, piceid and digoxin, respectively.

Sample preparation and analysis of tryptophan

Dried and ground plant material was weighed on an analytical balance to an amount of 2 g (no more than 400 mg of protein) and placed into conical flasks.

An analysis of the tryptophan content in the study material was performed according to the methodology described by AOAC (2012). The tryptophan indole ring in the acidic environment gives a colored condensation product with *p*-dimethylaminobenzaldehyde (DMAB), the intensity of which was measured on a spectrophotometer at a wavelength λ = 590 nm. The tryptophan content was calculated from the formula:

$$\frac{\dfrac{(E_{pr} - E_{śl}) \times 2}{(E_{tryp} - E_{śl})} - \dfrac{(E_{enz} - E_{śl}) \times 2}{(E_{tryp} - E_{śl})}}{\text{sample weight}} \ (\text{g kg}^{-1} \text{ sample})$$

E_{pr} – extinction of the test sample,
$E_{śl}$ – extinction of the blank,
E_{tryp} – extinction of tryptophan,
E_{enz} – extinction of the enzyme.

Statistical analysis

All calculations were made using an ANOVA version 13.3 software (StatSoft, Poland), after assessing the normality and homogeneity of variance. The content of *trans*- and *cis*-piceid in Japanese knotweed leaves was calculated with descriptive statistics. The results were presented as means ± SD (standard deviation). The content of tryptophan in the material under study was submitted to analysis of variance (ANOVA) at a significance level of P<0.05. The significance of differences between means was compared by the Tukey multiple range tests.

RESULT AND DISCUSSION

Results of previous studies confirm that both vegetables and fruits are an excellent source of resveratrol. Among the fruit and vegetables used in daily diet, this promising compound with a strong anti-inflammatory, antioxidant and anti-cancer effect has been detected in dates, strawberries and tomatoes (Sebastia et al. 2017), sea buckthorn (Chen et al. 2023) or in less popular fruit such as jamun (*Syzygium cumini* L.), jackfruit (*Artocarpus heterophyllus*) and mulberry (*Morus rubra*), and in other fruit and vegetables (Shrikanta et al. 2015, Shreelakshmi et al. 2021). However, different parts of plants may have a different chemical composition and thus be used for different therapeutic purposes.

The plant growth habitats, which are diverse in terms of environmental conditions, may affect the content of active components in plants. Even the time of harvest determines the composition of plants (Naik et al. 2017). In the present experiment, no free resveratrol was detected in any of the fruit samples tested, regardless of the method applied to extract the plant material. Resveratrol was undetected in samples of cherry fruits from Spain by Sebastia et al. (2017). Japanese knotweed, especially its root, is commonly considered to be the best source of resveratrol. The analyzed Japanese knotweed leaf samples originating from northern Poland did not contain free resveratrol. The content of resveratrol in Japanese knotweed was determined by Chen et al. (2013) and Cucu et al. (2021). According to the cited researchers, the root contained a much higher level of resveratrol than the stem and leaves. The content of resveratrol in Japanese knotweed from China and Canada was similar, although there was a huge difference in climatic and environmental factors between these two locations.

One sample appeared to contain less resveratrol, indicating the effect of environmental factors. The analyzed Japanese knotweed leaves, apart from 3-O-glucosides of resveratrol, also contained its malonyl glucoside (Table 1, Figure 1).

Table 1

Resveratrol derivatives found in a knotweed leaf sample

#	RT (min)	Measured m/z	Formula ion *a*(M-H)-	Calculated m/z	Error (ppm)	mσ	rdb	Identification	Comments
1.	12.1	389.124189	$C_{20}H_{21}O_8$	389.124191	0	2.0	10.5	*trans*-piceid	ChEBI: 8198, identification on basis of authentic pattern
2.	14.2	475.124588	$C_{23}H_{23}O_{11}$	475.124585	0	39.1	12.5	3-O malonyl glucoside *trans*-resveratrol	CAS: 1816954-96-7, Ref. 1, identification on basis of disintegration in source
3.	15.0	389.124444	$C_{20}H_{21}O_8$	389.124191	-0.7	1.8	10.5	*cis*-piceid	ChEBI: 76155, identification on basis of authentic pattern
4.	16.0	475.125029	$C_{23}H_{23}O_{11}$	475.124585	-0.9	91.5	12.5	3-O malonyl glucoside *cis*-resveratrol	none in databases, identification based on the dissolution of MS and compared retention time from *trans*- and *cis*

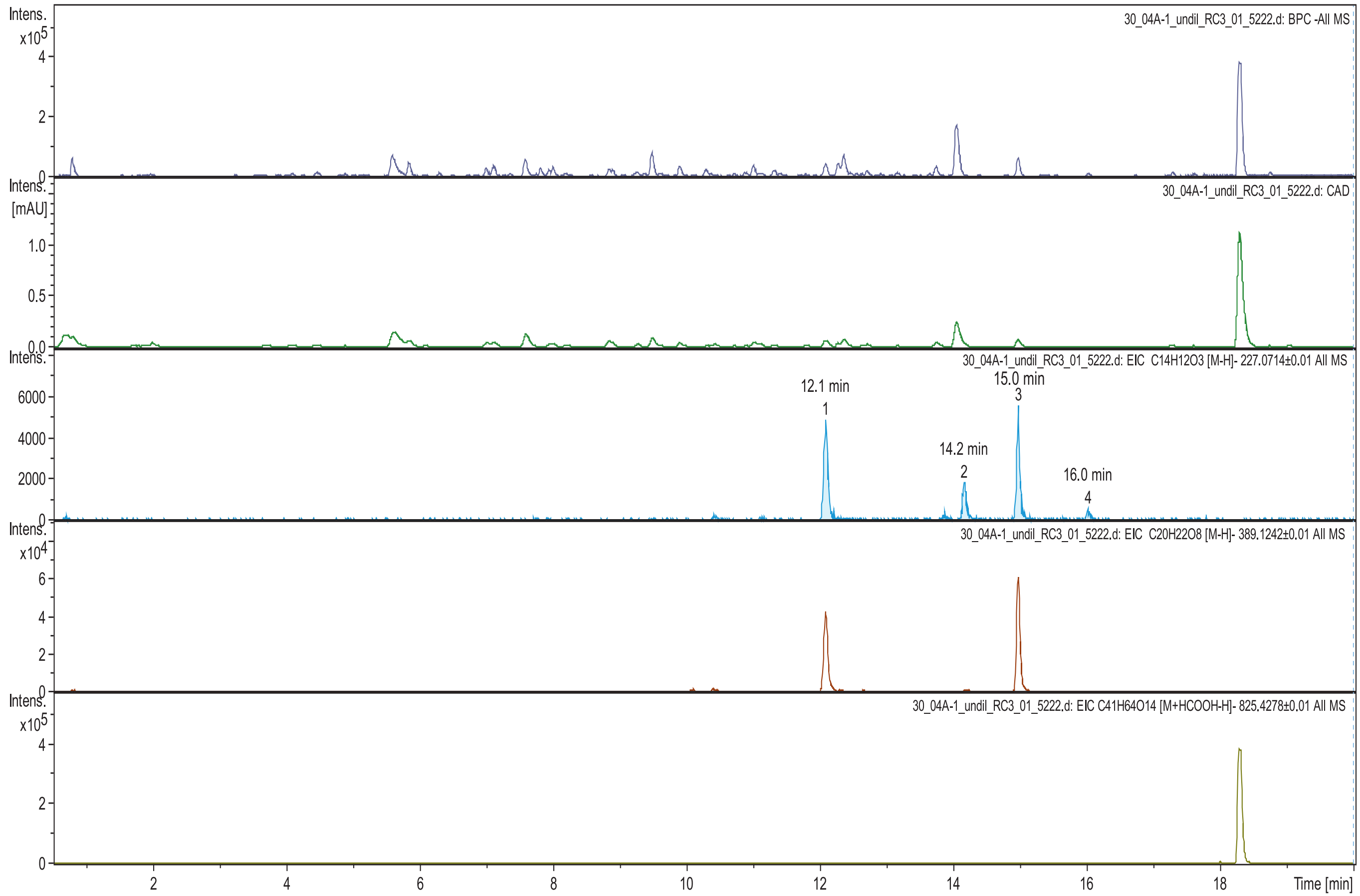

Fig. 1. Chromatograms of resveratrol in samples of Japanese knotweed leaves

Cherry and sweet cherry fruits did not contain resveratrol glucoside (piceid); in the sea buckthorn fruit, piceid isomers were detected at concentrations below the detection limit. Japanese knotweed leaf and sea buckthorn fruit samples contained resveratrol glucosides, although in fruit, they were present below the detection limit (signal-to-noise ratio lower than 3) – Table 2. *Trans*-piceid (T-Pc) is abundant in *Polygonum cuspidatum* and in grapes and grape products such as wine. Piceid, also known as polydatin, has anti-proliferative activity in Caco-2 intestinal epithelial cells, which is not related to the release of resveratrol (Storniolo et al. 2014). The analyzed leaves of *Polygonum cuspidatum* grown in northern Poland contained from 2 to 7 times more piceid compared to those originating from Canada and China (from 0.06 mg g^{-1} to 0.17 mg g^{-1}, on average) (Chen et al. 2013), which can indicate the impact of habitat factors.

Table 2

Content of *trans*- and *cis*-piceid in fruits of cherry, sweet cherry, sea buckthorn and in Japanese knotweed leaves (mg g^{-1} d.m. ± SD)

Sample	*Trans*-piceid	*Cis*-piceid	Sum
Fruits of cherry	n.f.	n.f.	-
Fruits of sweet cherry	n.f.	n.f.	-
Fruits of sea buckthorn	b.d.	b.d.	-
Leaves of Japanese knotweed	0.44±0.12	0.53±0.12	0.97±0.16

d.m. – dry matter, n.f. – not found, b.d – below the detection limit

Like other bioactive compounds, free amino acids should be able to provide beneficial health effects through fruit consumption if they contain a remarkable amount of free amino acids. The content of tryptophan in the analyzed plant material is shown in Figure 2. The tested fruits contained from 1.54 to 5.70 g kg^{-1} d.m. of tryptophan. Its significantly largest amount was found in sea buckthorn fruits (5.7 g kg^{-1}), while the least of tryptophan

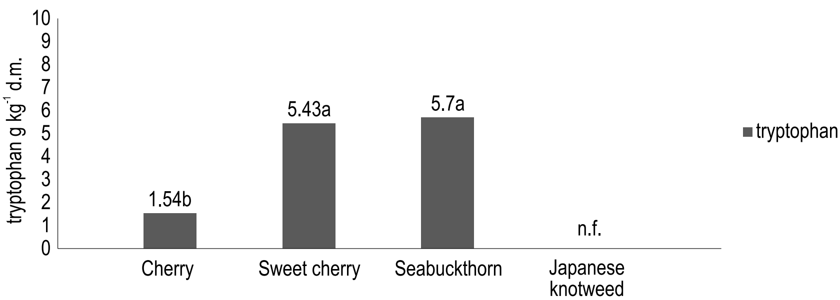

Fig. 2. Tryptophan in fruits of cherry, sweet cherry, sea buckthorn and in Japanese knotweed leaves: n.f. – not found, mean values with the same letter in each line are not significantly different at $P \leq 0.05$ according to the Tukey test

was detected in cherry fruits. According to the literature (Chen et al. 2023), sea buckthorn contains on average 5 mg kg^{-1} i.e. 0.005 g kg^{-1} tryptophan (in pulp). Sea buckthorn berries contained almost three times more (by 4.16 g kg^{-1} d.m.) tryptophan than cherry fruits (1.54 g kg^{-1}). Sweet cherry fruit was also abundant in tryptophan. The concentration of the examined amino acid in sweet cherries was on a similar level and did not differ significantly from the content of tryptophan in sea buckthorn (0.27 g kg^{-1} less compared to sea buckthorn). In the fruit of cherry trees grown in the Spanish region of Extremadura, the concentration of tryptophan varied from 36.53 to 82.65 mg kg^{-1} d.m., i.e. from 0.037 to 0.083 g kg^{-1} d.m., depending on a variety (Cubero et al. 2010). In the literaturę, there is little information about the content of tryptophan in Japanese knotweed. In the present experiment, no tryptophan was detected in Japanese knotweed leaves.

CONCLUSIONS

In conclusion, the results of the present study provide complementary data to the information available in the literature concerning the content of resveratrol and tryptophan in fruits of *Prunus cerasus* L., *Prunus avium*, *Hippophae rhamnoides* L., and in *Polygonum cuspidatum* leaves.

In this study, the content of free resveratrol in the fruit of sea buckthorn and Japanese knotweed leaves has not been demonstrated. It was not found in the fruit of cherries and sweet cherries, either. Leaves of Japanese knotweed contained derivatives of resveratrol, piceids, at an average level of 0.97 mg kg^{-1}.

Fruits of cherry (1.54 g kg^{-1}) and, above all, sweet cherry (5.43 g kg^{-1}) and sea buckthorn (5.7 g kg^{-1}), were characterized by a high content of tryptophan, which suggests that they may be a good source of this amino acid. Consumption of these fruits can provide significant amounts of this amino acid, thus fulfilling the health-promoting functions. No tryptophan was detected in Japanese knotweed leaves.

The results of this research seem to confirm that the chemical composition and nutritional value of plants can be shaped by genetic (plant species, parts of a plant) and environmental (soil and climatic conditions) factors. Therefore, it is necessary to continue the research in order to constantly update the database of nutrients and non-nutritive substances with health effects occurring in various plant foods.

Author contributions

WB, AJ – conceptualization, WB, AJ, MK – methodology, WB, AJ, MK – formal analysis, WB, AJ writing – original draft preparation,, AJ – editing. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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