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Free and Bound Sulphur Containing and Other Volatile Compounds from Evergreen Candytuft (*Iberis sempervirens* L.)

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Volatile fractions of evergreen candytuft, *Iberis sempervirens* (Brassicaceae), were isolated by simultaneous hydrodistillation-extraction from fresh plant material prior and after autolysis and from dried plant material and were then analyzed using GC and GC-MS. Isothiocyanates, nitriles, aliphatic alcohols, carbonyls, fatty acids, hydrocarbons, terpene compounds, C_{13}-norisoprenoids and phenylpropane derivatives were identified – thirty-nine components in all. Major components of all samples were: 3-butenyl isothiocyanate (17.4–63.3 %), 5-methylthiopentanonitrile (2.2–9.5 %), 4-methylthiobutanonitrile (4.8–6.9 %), allyl isothiocyanate (4.0–6.6 %), 4-methylthiobutyl isothiocyanate (2.9–6.2 %) and 3-methylthiopropyl isothiocyanate (1.3–3.4 %). Oil isolated after autolysis is more complex than the other two oils. It is composed of a large number of compounds. Some of them are identified as *O*-aglycones such as eugenol, (*Z*)-3-hexene-1-ol and 1*H*-indole. *O*-glycosides of volatile compounds were isolated from fresh plant material and purified by selective extraction and column chromatography. After hydrolysis with β-glucosidase, the liberated volatile aglycones were also analyzed by GC and GC-MS. Nineteen aglycones were identified. The main aglycones identified were: eugenol (15.2 %), 2-phenylethanol (12.9 %), 2-hydroxy-β-ionone (10.6 %), 2-*tert*-butyl-5-methylphenol (9.2 %) and methyl-2,5-dihydroxybenzoate (4.5 %).

Keywords
Iberis sempervirens L.
evergreen candytuft
Brassicaceae
glucosinolates
O-glycosides
volatile aglycones
isothiocyanates
GC-MS

INTRODUCTION

Iberis sempervirens L. belongs to the Brassicaceae family. Plants of this family contain glucosinolates. Glucosinolates are a class of about 120 naturally occurring thioglucosides found in fifteen botanical families. The best-known family is Brassicaceae, which includes many plants that are important as human and animal food. These are cabbage, cauliflower, broccoli, horseradish, mustard, radish, turnip and oilseed rape. Glucosinolate breakdown products, which include nitriles, isothiocyanates, thiocyanates, epithionitriles and oxazolidine-2-thiones, are responsible for the biting taste and characteristic flavours of these vegetables. The presence of some glucosinolates in agricultural crops, such as oilseed rape and vegetables, is undesirable because of the toxic effects of their breakdown products. The glucosinolates from broccoli, cabbage and cauliflower have anticarcinogenic properties,[1–4] notably their aglucone sulphoraphane (*R*)-4-methylsulphinylbutyl isothiocyanate.[5,6] Unfortunately, some isothiocyanates show mutagenic potential in mammalian cells.[4] Glucosinolate breakdown products have been also proposed to act as alle-

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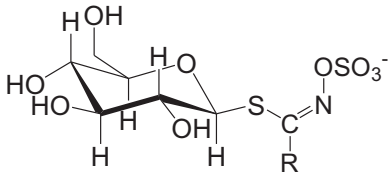
lochemicals and to play a role in plant defence against herbivores, pests, and pathogens and thus reduce the need for application of synthetic organic pesticides.[7]

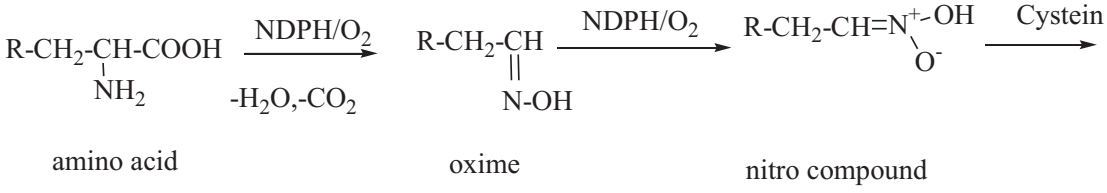
Glucosinolates are present in all vegetable organs, with the highest concentration in seed. Glucosinolates are anions, usually found as potassium salts, which are soluble in water. Their skeleton consists of a β-thioglucose moiety, a sulphonated oxime moiety (glucone) and a highly variable side chain (aglucone). The side chain and sulphate group have anti or *Z* configuration around the carbon nitrogen double bond.



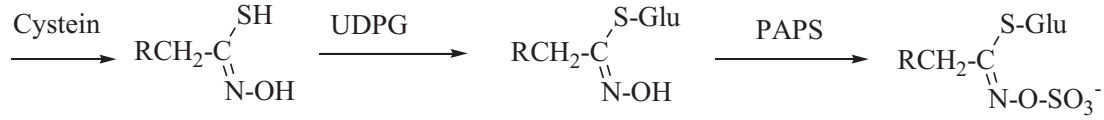
The side chains (R) are derived from amino acids. They can be alkyl, alkenyl, benzyl or heterocyclic groups.[8] Only eight of the glucosinolates correspond to amino acids (alanine, methionine, valine, leucine, isoleucine, phenylalanine, tyrosine and tryptophane) and are almost always derived from chain elongation protein amino acids. The side chains often have complex structures. Biosynthesis of the glucosinolates begins with oxidation, dehydratation and decarboxylation of amino acids to oximes and nitro compounds. Nitro compounds with cystein (sulphur donor) give thiohydroximates. Glucosylation of thiohydroximate gives desulphoglucosinolate, which in the last stage by sulphatation (3'-phosphoadenosine-5'-phosphosulphate) gives glucosinolate:[1]

Many methods have been reported for the determination of total glucosinolate, such as direct determination methods using degradation products (glucose, sulphate), HPLC methods of intact glucosinolates or desulphoglucosinolates, methods based on determination of trimethylsilylated desulphoglucosinolates with gas chromatography, GC, gas chromatography-mass spectrometry, GC–MS. On the other hand, volatile aglucones can be isolated by the Likens-Nickerson method and analyzed using the GC-MS technique.[11] Total isothiocyanates as the most important aglucones can be determined as derivatives of 1,2-benzenedithiol (1,3-benzodithiole-2-thiones) by GC-MS with the selected ion monitoring, SIM, technique.[12] Unfortunately, undesirable artefacts are easily generated in all cases of analytical determination.

O-Glycosides of volatile aglycones are present in many plants as water-soluble and nonvolatile compounds. They were previously investigated in aromatic plants, grape, vine and also identified in non aromatic plants.[13–15] They were not investigated in plants of the Brassicaceae family. They have β-configurations and hydrolyze with β-glycosidases (or acid/base) into aglycones and glycones. Pyrolytic degradation (by elimination) of these glycosides generates also volatile compounds. The most common glycones are glucose, arabinose or some disaccharides. The most common volatile aglycones are aliphatic and terpenic alcohols, phenols and carbonyl compounds. Further, the plants contain free volatile compounds, which belong to essential oils, plant aromas and epicuticular waxes.



amino acid oxime nitro compound



thiohydroximate desulphoglucosinolate glucosinolate

The mechanism of glucosinolate enzymatic hydrolysis has been studied using the myrosinase isolated from white mustard, *Synapis alba* seeds and various types of glucosinolates.[9] Myrosinases (β-thioglucosidases, glucohydrolases) are specific towards glucosinolates, with little or no activity towards any other *O*- or *S*-glycosides.[10] They do not hydrolyze the sulphate group from glucosinolate.

The aim of this study was to determine the chemical composition of the free and bonded volatile compounds of evergreen candytuft. The chemical composition of these compounds from evergreen candytuft has not been reported to date.

EXPERIMENTAL

Reagents

Solvents and β-glucosidase were purchased from Fluka Chemie, Buchs, Switzerland. Octyl-β-D-glucopyranoside, silica gel for column chromatography (Kieselgel 60, 0.040–0.063 mm), pre-coated silica plates (Kieselgel 60, thickness 0.2 mm) for thin layer chromatography, ammonia, and sodium sulphate were obtained from Merck, Darmstadt, Germany.

Plant Material

Plant material of evergreen candytuft, *Iberis sempervirens*, was collected in November 2004. The plants were cultivated near Sinj, in southern Croatia. Fresh plant material was used (stems *ca.* 15 cm, with leaves). The remaining part of plant material was dried in a shaded place at room temperature for fifteen days. The voucher specimens (No. 0011010–20) are deposited at the Department of Organic Chemistry and Natural Products, Faculty of Chemical Technology, Split.

Isolation of Volatiles

The volatiles were isolated from fresh and dried plant material (200 or 100 g) by simultaneous hydrodistillation-extraction in a Likens–Nickerson apparatus for 3 hours using pentane-ether, ψ = 1:1 for trapping. After distillation, the pentane-ether extract was separated and dried over anhydrous sodium sulphate. The extract was made to a small volume (*ca*. 3 mL) and 1 μL of this solution was used for each GC and GC-MS analysis.

Autolysis and Volatiles Isolation

Fresh plant material (200 g) was ground (in a coffee grinder), stirred with 200 mL of water, and allowed to autolyze at 27 °C for 24 hours. Volatile compounds were isolated under the same distillation conditions in a Likens–Nickerson apparatus and analyzed by GC and GC-MS.

O-*Glycosides Isolation*

A hundred grams of fresh plant material was submitted to boiling water (400 mL) for 20 min to inactivate enzymes and simultaneously extract water soluble compounds and partially remove volatile compounds by evaporation. Thereafter, the aqueous extract was separated, and the residual plant material was ground and extracted once more with boiling water (300 mL). The pooled aqueous extracts were concentrated to 50 mL in a rotating evaporator under reduced pressure. Ballast components were removed by precipitation with ethanol (300 mL). Final precipitation of acidic ballast compounds was performed in an ethanol solution (almost non-aqueous) with several drops of conc. ammonia. Finally, purification was carried out by flash chromatography on a silica gel column applying ethyl acetate : ethanol : ammonia, ψ = 6:3:1. TLC analyses showed the absence of free carbohydrates in the glycosidic fraction. The obtained glycosidic fraction was concentrated to dryness, dissolved in a citrate buffer (pH = 5.5; 5 mL), octyl-β-D-glucoside (500 μg; internal standard for glycosides) was added, and the remaining volatile compounds in traces were removed with pentane-dichloromethane extraction as described in a previous paper.[16]

O-*glycosides Hydrolysis*

β-Glucosidase from almonds (20 mg, 5–8 units/mg) was added to the glycosidic solution along with 3 mL pentane to trap the liberated aglycones. Hydrolysis was carried out for 72 h at 30 °C with the mixture being shaken occasionally. After hydrolysis, the pentane layer was separated and the remaining aglycones were extracted from the aqueous layer with pentane (10 × 2 mL). The combined pentane extract was dried (Na_2SO_4) and concentrated to the final volume of 0.5 mL, and 1 μL was used for GC and GC-MS analyses.

Gas Chromatography (GC-FID)

Gas chromatography analysis was performed on a Hewlett-Packard Model 5890 Series II gas chromatograph equipped with a flame ionization detector and capillary column HP-101 (Methyl silicone fluid), 25 m × 0.2 mm i.d., coating thickness 0.2 mm. Chromatographic conditions were as follows: helium as carrier gas at 1.0 mL/min; injector and detector temperatures, 250 °C and 300 °C. Oven temperature was isothermal at 70 °C for 2 min, then increased to 200 °C at a rate of 3 °C min^{-1} and held isothermal for 15 min. Volume injected was 1 mL. Split ratio was 1:50.

Gas Chromatography-Mass Spectrometry (GC-MS)

Isolated volatiles were also analyzed by a Hewlett Packard GC-MS (model 5890 series II) with a mass selective detector (model 5971A). Two columns of different polarity were used: a HP-101 column (dimethyl-polysiloxane fluid), Hewlett Packard; 25 m × 0.2 mm i.d., film thickness 0.2 mm and a HP-20M column (polyethyleneglycol) Hewlett Packard; 50 m × 0.2 mm i.d., film thickness 0.2 mm. Oven temperature was programmed as follows: isothermal at 70 °C for 4 min, then increased to 180 °C, at a rate of 4 °C min^{-1} and subsequently held isothermal for 15 min (for HP-20M column); isothermal at 70 °C for 2 min, then increased to 200 °C at a rate of 3 °C min^{-1} and held isothermal for 15 min (for HP-101 column). Carrier gas was helium, flow rate: 1 mL min^{-1}; injector temperature: 250 °C; volume injected: 1 μL; split ratio: 1/50. MS conditions: ionization voltage: 70 eV; ion source temperature: 280 °C; mass range: 30–300 mass units.

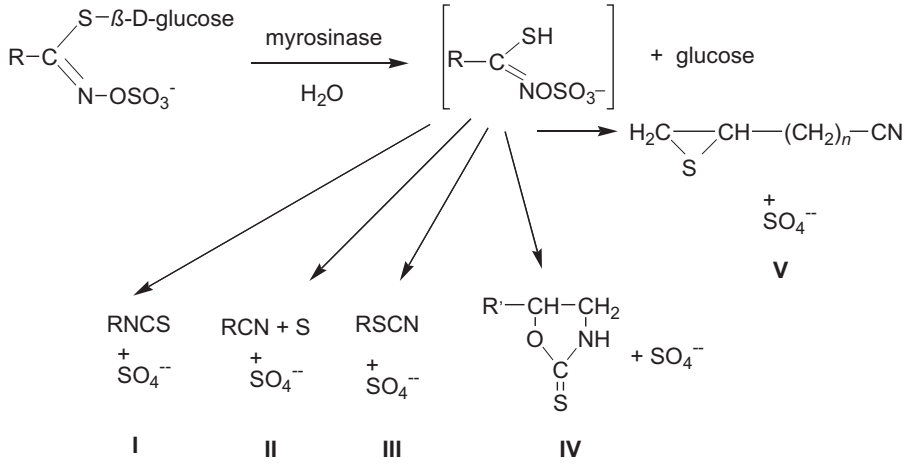
Qualitative and Quantitative Determination

Individual peaks were identified by comparison of their retention indices to those of authentic samples, as well as by comparing their mass spectra with the Wiley 6.0 library (Wiley, New York) and NIST98 (National Institute of Standards and Technology, Gaithersburg) mass spectral database and literature.[17,18] The percentage composition of the

samples was computed from the GC peak areas using the normalization method (without correction factors). The gravimetric method was used for quantification of total volatiles (after removing solvents by careful fractional distillation). The content of *O*-aglycones was estimated from the GC-peak areas related to the GC-peak area of 1-octanol (liberated from octyl-β-D-glucoside as internal standard for *O*-glycosides). Preliminary GC-MS analysis showed the absence of 1-octanol as potential aglycone. Yields of volatiles were expressed as mg/kg with respect to the masses of starting fresh or air-dried plant material. Quantitative results are means of the data derived from duplicate GC analyses.

RESULTS AND DISCUSSION

Volatile compounds isolated by hydrodistillation from evergreen candytuft as well as *O*-aglycones obtained by hydrolysis with β-glucosidase from *O*-glycosides were subjected to detailed GC and GC-MS analyses. Table I shows the yields and composition of the volatiles. Yields of volatiles obtained from fresh plant material by the Likens-Nickerson method were 828 mg/kg (without autolysis) and 426 mg/kg (with autolysis), and the yield from dried plant material was 1200 mg/kg of dried plant material. Lower yield of oil obtained by hydrodistillation after autolysis is probably due to the fact that ground plant material has higher sorption power compared to not ground plant material. In this case, hydrodistillation is slower and incomplete. Simultaneous hydrodistillation-solvent extraction (Likens-Nickerson apparatus) ensures isolation and concentration of free volatile compounds and volatile aglucones obtained by chemical degradation of glucosinolates during isolation. Thirty-seven compounds were identified in oil isolated from ground plant material after autolysis, twenty were identified in oil isolated from fresh plant material, and only seventeen in oil isolated from dried plant material. The compounds are listed in order of their elution on the HP-101 column. 90.6–94.1 % of total oil could be identified. Degradation of glucosinolates begins with hydrolysis by myrosinase, which generates unstable aglucones that are rearranged depending on the structure of the side chains and degradation conditions. The main breakdown products are isothiocyanates (**I**) at pH > 7 and nitriles (**II**) at pH < 4. In the presence of Fe^{++} ions, there is an increase of nitriles at all pH values. Allyl-, benzyl- and 4-methylthiobuthyl glucosinolates generate thiocyanates (**III**). The presence of OH-group in β-position of side chains causes spontaneous cyclization to produce the corresponding oxazolidine-2-thiones (**IV**), and the terminal double bond in the side chain results in generation of epithionitriles (**V**) in the presence of Fe^{++} and epithiospecifier protein. Thus, hydrolysis of sinigrin, allyl glucosinolate (main glucosinolate in cruciferous plants) gives allyl isothiocyanate, allyl cyanide, allyl thiocyanate and 1-cyano-2,3-epithiopropane.



The major components in all samples were sulphur and nitrogen containing compounds, such as 3-butenyl isothiocyanate (17.4–63.3 %), 5-methyl thiopentanonitrile (2.2–9.5 %), 4-methyl thiobutanonitrile (4.8–6.9 %), allyl isothiocyanate (4.0–6.6 %), 4-methyl thiobutyl isothiocyanate (2.9–6.2 %), 3-methyl thiopropyl isothiocyanate (1.3–3.4 %), phenyl acetonitrile (1.0–2.3 %), phenyl propanonitrile (0.5–1.7 %), 1-cyano-4,5-epithiopentane (0.7–1.4 %) and 4-pentenyl isothiocyanate (0.1–1.0 %). Thiocyanates and oxazolidine-2-thiones were not identified in these oils. The oil obtained after autolysis and isolation was qualitatively and quantitatively different from the other two oils. This oil, except for the above mentioned compounds, contains compounds without nitrogen and sulphur: (*Z*)-3-hexene-1-ol (17.2 %), palmitic acid (12.2 %), (*E*)-2-hexene-1-ol (3.1 %) and (*E*)-2-hexenal (2.2 %) as major components and methyl ester of linoleic and linolenic acids, eugenol, *p*-vinyl guaiacol, β-ionone, *trans*-β-damascenone and *trans*-anethole as

TABLE I. Volatile compounds from evergreen candytuft (*Iberis sempervirens* L.)

	Identified compounds	$1^{(a)}$ / %	$1^{(b)}$ / %	$1^{(c)}$ / %	Mode of identification
1.	(*E*)-2-hexenal	–	2.2	–	–, I_2,MS
2.	2-ethyl thiophene	–	0.7	–	I_1, –,MS
3.	pyridine	–	tr	–	I_1, –,MS
4.	(*Z*)-3-hexene-1-ol	–	17.2	–	I_1, I_2, MS
5.	(*E*)-2-hexene-1-ol	–	3.1	–	I_1, –,MS
6.	allyl isothiocyanate	4.0	6.6	5.8	I_1, I_2, MS
7.	butyl isothiocyanate	0.2	0.4	tr	I_1, I_2, MS
8.	3-butenyl isothiocyanate	63.3	17.4	48.2	I_1, I_2, MS
9.	phenyl acetaldehyde	tr	tr	1.0	I_1, I_2, MS
10.	4-methyl thiobutanonitrile	4.8	6.9	6.6	I_1, I_2, MS
11.	2-butenyl isothiocyanate*	–	6.2	–	– , –, MS
12.	phenyl acetonitrile	1.0	1.6	2.3	I_1, I_2, MS
13.	4-pentenyl isothiocyanate	0.8	1.0	0.1	I_1, I_2, MS
14.	5-methyl thiopentanonitrile	4.9	2.2	9.5	I_1, I_2, MS
15.	1-cyano-4,5-epithiopentane	0.7	1.4	0.9	I_1, I_2, MS
16.	β-cyclocitral	0.1	0.3	–	I_1, I_2, MS
17.	phenyl propanonitrile	0.6	0.5	1.7	I_1, I_2, MS
18.	*trans*-anethole	–	0.1	–	I_1, I_2, MS
19.	3-methyl thiopropyl isothiocyanate	3.4	1.3	1.9	I_1, I_2, MS
20.	benzyl isothiocyanate	0.2	0.1	–	I_1, I_2, MS
21.	4-vinyl guaiacol	–	0.5	–	I_1, I_2, MS
22.	*trans*-β-damascenone	–	0.2	–	I_1, I_2, MS
23.	eugenol	–	0.8	–	I_1, I_2, MS
24.	4-methyl thiobutyl isothiocyanate	6.2	–	2.9	I_1, I_2, MS
25.	1*H*-indole	–	0.1	–	I_1, I_2, MS
26.	2-phenyl ethyl isothiocyanate	0.3	0.2	0.7	I_1, I_2, MS
27.	β-ionone	–	0.3	0.7	I_1, I_2, MS
28.	2,6-dimethylbenzaldehyde	–	0.1	–	I_1, I_2, MS
29.	lauric acid	–	0.8	0.8	I_1, I_2, MS
30.	myristic acid	–	0.3	–	I_1, I_2, MS
31.	2,6,10-trimethylneophytadiene	–	0.1	–	I_1, I_2, MS
32.	α-gurjunene	0.1	0.2	–	I_1, I_2, MS
33.	ethyl palmitate	0.1	0.9	–	I_1, I_2, MS
34.	palmitic acid	0.7	12.2	–	I_1, I_2, MS
35.	methyl linoleate	–	2.3	–	I_1, I_2, MS
36.	methyl linolenate	–	2.8	–	I_1, I_2, MS
37.	phytol	0.8	3.0	1.0	I_1, I_2, MS
38.	heneicosane	0.5	–	6.6	I_1, I_2, MS
39.	linoleic acid	–	0.5	–	I_1, I_2, MS
Total identified		92.7	94.1	90.7	
Yield / mg kg^{-1}		828	426	1200	

Plant material and Isolation Methods

[a]fresh plant material-Likens Nickerson method
[b]fresh plant material-autolysis-Likens Nickerson method
[c]dried plant material-Likens Nickerson method

Identification methods

I_1: retention indices on HP-20M; I_2: retention indices on HP-101; MS: mass spectra; tr: trace (< 0.1 %); –: not detected; *: correct isomer not identified

accessory components. Some of them were identified among the *O*-aglycones. (*E*)-2-Hexenal, (*Z*)-3-hexene-1-ol and (*E*)-2-hexene-1-ol, which are identified only in the volatile fraction obtained from fresh plant material after autolysis, are compounds formed after damage of plant tissue. Palmitic, lauric, linoleic, linolenic, myristic acids and their esters are mainly present in this fraction with the highest percentage. Aliphatic volatile compounds (alcohols, aldehydes, acids and their esters) can originate from fatty acid catabolism.[19–20] Eugenol, 4-vinyl guaiacol and *trans*-anethole are present only in the oil after autolysis. These aromatic compounds can originate from cinnamic acid catabolism.[20] Furthermore, phytol, β-cyclocitral, β-ionone originating by degradation of carotenoids present in evergreen candytuft.

O-*aglycones*

Isolated and purified *O*-glycosides were hydrolyzed by β-glucosidase and the liberated *O*-aglycones were analyzed by GC and GC-MS. Compounds with sulphur and nitrogen, which are characteristic of glucosinolate, were not identified among these compounds. The content of glycosidically bound volatile compounds in fresh plant material was 45.5 mg kg^{-1}. Nineteen aglycones were identified, representing 66.6 % of total volatile *O*-aglycones. Aliphatic alcohols, derivatives of phenyl propanes and C_{13}-norisoprenoides were identified. The results are shown in Table II. The main aglycones were: eugenol (15.2 %), 2-phenyl ethanol (12.9 %), 2-hydroxy-β-ionone (10.6 %), 2-*tert*-buthyl-5-methylphenol (9.2 %) and methyl-2,5-dihydroxybenzoate (4.5 %). 3-Oxo-α-ionol, benzyl alcohol, (*Z*)-3-hexen-1-ol, *o*- and *p*-methyl benzyl alcohol and other compounds in lower percentages were identified as well. Many of these compounds were identified as the most common aglycones in many plants. Comparing the chemical composition of *O*-aglycones (Table II) with the essential oil obtained after autolysis (Table I, 1[(a)]), they had only three identified compounds in common: eugenol, *cis*-3-hexene-1-ol and 1*H*-indole. These results show little correlation in the chemical composition of oil obtained after autolysis and volatile *O*-aglycones in this plant. The aglycones, such as eugenol, benzyl alcohol, 2-phenylethanol, aliphatic alcohols are ubiquitous in aglycone fractions of many plant families.[13] Eugenol and other *p*-hydroxyphenylpropanes identified in many plants as the main aglycones can be connected with lignin biosynthesis *via* the peroxidase-hydrogen peroxide system according to Siegel.[21] Aliphatic volatiles (alcohols, carbonyls, acids) can originate from fatty acid catabolism, and aromatic volatiles

TABLE II. Volatile O-aglycones from evergreen candytuft (*Iberis sempervirens* L.)

	Identified compound	Peak area / %	Mode of identification
1.	(*Z*)-3-hexene-1-ol	1.5	I_1, I_2, MS
2.	benzaldehyde	0.4	–, I_2, MS
3.	α-methyl benzyl alcohol	0.4	I_1, –, MS
4.	benzyl alcohol	1.8	I_1, I_2, MS
5.	2-phenyl ethanol	12.9	I_1, I_2, MS
6.	*p*-methyl benzyl alcohol	1.3	–, I_2, MS
7.	*o*-methyl benzyl alcohol	0.5	–, I_2, MS
8.	2,4-dimethyl phenol	1.3	–, –, MS
9.	eugenol	15.2	I_1, I_2, MS
10.	4-hydroxy-3-methoxy-benzaldehyde	0.3	I_1, I_2, MS
11.	1*H*-indole	1.3	I_1, I_2, MS
12.	3-hydroxy-β-damascone	1.4	I_1, –, MS
13.	1-naphtalenemethanol	0.4	–, I_2, MS
14.	2-naphtalenemethanol	0.6	–, I_2, MS
15.	3-oxo-α-ionol	2.6	I_1, I_2, MS
16.	zingerone	0.4	–, I_2, MS
17.	2-hydroxy-β-ionone	10.6	I_1, I_2, MS
18.	methyl-2,5-dihydroxybenzoate	4.5	–, I_2, MS
19.	2-*tert*-butyl-5-methyl phenol	9.2	–, I_2, MS
Total identified		66.6	
Yield / mg kg^{-1}		45.5	

I_1: retention indices on HP-20M; I_2: retention indices on HP-101; MS: mass spectra; –: not detected

(alcohols, acids, carbonyls) from cinnamic acid catabolism.[20] This is the first investigation of volatile *O*-aglycones in cruciferous plants to date.

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SAŽETAK

Slobodni i vezani hlapljivi spojevi snješka (*Iberis sempervirens* L)

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Eterično ulje snješka, *Iberis sempervirens* (Brassicaceae) izolirano je simultanom vodenom destilacijom-ekstrakcijom i analizirano pomoću GC i GC-MS. Ulje je izolirano iz svježeg biljnog materijala prije i poslije autolize, te iz suhog biljnog materijala. Identificirani su izotiocijanati, nitrili alifatski alkoholi, karbonilni spojevi, masne kiseline, ugljikovodici, terpenski spojevi, C_{13}-norizoprenoidi i fenilpropanski derivati. Identificirano je tridesetdevet komponenata. Glavne komponente u svim uzorcima bile su: 3-butenil-izotiocijanat (17.4–63.3 %), 5-metiltiopentanonitril (2.2–9.5 %), 4-metiltiobutanonitril (4.8–6.9 %), alil-izotiocijanat (4.0–6.6 %), 4-metiltiobutil- izotiocijanat (2.9–6.2 %) i 3-metiltiopropil-izotiocijanat (1.3–3.4 %). Ulje izolirano poslije autolize je kompleksnijeg sastava od druga dva ulja, te sadrži veći broj spojeva. Neki od njih su identificirani u *O*-aglikonima kao eugenol, (*Z*)-3-heksene-1-ol, 1*H*-indol. *O*-glikozidi hlapljivih spojeva izolirani su iz svježeg biljnog materijala, a pročišćeni su selektivnom ekstrakcijom i kolonskom kromatografijom. Poslije hidrolize s β-glukozidazom oslobođeni hlapljivi aglikoni su također analizirani pomoću GC i GC-MS. Identificirano je devetnaest aglikona. Glavni aglikoni bili su: eugenol (15.2 %), 2-feniletanol (12.9 %), 2-hidroksi-β-jonon (10.6 %), 2-*tert*-butil-5-metilfenol (9.2 %) i metil-2,5-dihidroksibenzoat (4.5 %). Kemijski sastavi slobodnih i vezanih isparljivih spojeva snješka nisu do sada publicirani.