

VOLATILE CONSTITUENTS OF *VALERIANELLA LOCUSTA*

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Key Word Index—*Valerianella locusta*; Valerianaceae; blended lamb's lettuce; aroma substances.

Abstract—Concentrates of blended lamb's lettuce obtained by combined high-vacuum distillation/solvent extraction were analysed by HRGC, HRGC-MS and, in part, HRGC-FTIR. In total, 103 aroma substances were identified for the first time. Qualitatively and quantitatively a number of 3-methylbutanoates and 2-phenethyl esters together with various volatile lipid cleavage products were predominant.

INTRODUCTION

Lamb's lettuce (*Valerianella locusta*) is one of the important European winter salads [1]. Our knowledge about its constituents is rather scarce. Studies on a few non-volatile compounds such as ascorbic and chlorogenic acid have been carried out [2], but the volatile substances formed after disruption of the tissue have not been previously investigated.

RESULTS AND DISCUSSION

The volatile substances identified by HRGC, HRGC-MS and, in part, HRGC-FTIR, are represented in Table 1.

In total, 103 components were identified for the first time as lamb's lettuce constituents.

The quantitative distribution of the major volatiles of lamb's lettuce is given in Table 2. The total quantity of aroma substances was determined to be 23 mg/kg (fr. wt) of which 85% was found in *ca* equal amounts in silica gel fractions II and III. The compounds listed in Table 1 and not represented in Table 2 were found in amounts < 30 µg/kg. Hydrocarbons do not feature amongst the volatiles identified. They were mostly found in traces and be contaminants of solvents used.

The ester fraction, in which 3-methylbutanoates and 2-phenethyl esters predominated, was important among the volatiles. Qualitatively and quantitatively, with 21 sub-

Table 1. Volatile compounds identified in blended lamb's lettuce by HRGC, HRGC-MS and, in part, HRGC-FTIR

Compound	Identified in fraction no.*	Mass spectral data (m/z)†	Compound	Identified in fraction no.*	Mass spectral data (m/z)†
Hydrocarbons			Hexyl acetate	II	43-56-41-55
Undecane	I	43-41-57-42	(Z)-Hex-3-enyl acetate	II	67-43-82-41
Dodecane	I	57-43-71-41	(E)-Hex-2-enyl acetate	II	43-67-41-82
Tridecane	I	57-43-71-41	2-Phenethyl acetate	II	43-104-91-65
Tetradecane	I	57-41-43-85	2-Phenethyl butanoate	II	104-43-71-91
Pentadecane	I	57-71-43-41	Methyl 3-methylbutan- oate‡	II	74-41-43-59
Hexadecane	I	57-43-71-41	Methylpropyl 3-methyl- butanoate‡	II	57-41-85-56
Heptadecane	I	57-43-41-71	Butyl 3-methylbutan- oate‡	II	41-57-56-85
Octadecane	I	57-43-41-71	3-Methylbutyl 3-methyl- butanoate‡	II	70-43-41-57
Nonadecane	I	57-43-41-71	(E)-Pent-2-enyl 3-meth- ylbutanoate‡	II	57-85-41-69
Toluene	I	91-92-65-51	Hexyl 3-methylbutan- oate‡	II	43-41-56-84
Ethylbenzene	I	91-106-51-65	(Z)-Hex-3-enyl 3-meth- ylbutanoate‡	II	67-82-57-41
p-Xylene	I	91-106-51-105	(E)-Hex-2-enyl 3-methyl- butanoate‡	II	57-41-67-82
m-Xylene	I	91-106-51-105	Octyl 3-methylbutan- oate‡	II	57-103-43-85
o-Xylene	I	91-106-105-51	Benzyl 3-methylbutan- oate‡	II	91-57-41-65
2-Ethyltoluene	I	91-106-105-51			
p-Cymene	I	119-91-134-65			
Naphthalene	I	128-127-51-129			
1-Methylnaphthalene	I	142-141-115-139			
2-Methylnaphthalene	I	142-141-115-139			
Phenanthrene	I	178-71-76-89			
Limonene	I	68-67-93-41			
Esters					
Ethyl acetate‡	II/III	43-61-45-70			

Table 1. *Continued*

Compound	Identified in fraction no.*	Mass spectral data (<i>m/z</i>)†	Compound	Identified in fraction no.*	Mass spectral data (<i>m/z</i>)†
2-Phenethyl 3-methyl- butanoate‡	II	104-57-105-85	Pentan-1-ol	III	42-41-55-70
Methyl hexanoate	II	74-87-59-55	Pentan-3-ol	III	59-41-57-58
2-Phenethyl hexanoate	II	104-43-105-71	(<i>Z</i>)-Pent-3-en-1-ol‡	III	55-56-41-68
Methyl heptanoate	II	74-43-87-55	(<i>Z</i>)-Pent-2-en-1-ol‡	III	57-41-67-68
Methyl octanoate	II	74-43-87-41	Pent-1-en-3-ol‡	III	57-67-58-68
Carbonyls			3-Methylpentan-1-ol‡	III	56-41-43-55
But-2-enal	III	41-70-69-44	4-Methylpentan-1-ol‡	III	56-41-43-42
Pentanal	II	44-41-58-43	Hexan-1-ol‡	III	56-43-41-55
(<i>E</i>)-Pent-2-enal‡	II/III	55-83-41-84	(<i>Z</i>)-Hex-3-en-1-ol‡	III	41-67-55-69
(<i>Z</i>)-Pent-2-enal	II/III	55-41-83-84	(<i>E</i>)-Hex-2-en-1-ol‡	III	57-41-67-82
2-Methylpent-2-enal	II	41-98-69-55	2-Ethylhexan-1-ol	III	57-43-41-55
Hexanal	II	44-43-41-56	Heptan-1-ol‡	III	56-43-70-41
(<i>Z</i>)-Hex-3-enal‡	II/III	41-69-55-42	Octan-1-ol	III	56-41-55-43
(<i>E</i>)-Hex-2-enal‡	II/III	41-55-69-42	Oct-1-en-3-ol‡	III	57-43-41-72
Heptanal	II	44-43-42-41	Benzyl alcohol	III	79-77-108-107
Octanal	II	43-44-42-41	2-Phenethyl alcohol‡	III	91-92-65-122
Nonanal	II	41-43-44-57	Linalool	III	71-41-43-55
(<i>E</i>)-Non-2-enal	II/III	44-41-43-55	Nerol	III	69-41-93-68
(<i>E</i> , <i>E</i> , <i>E</i>)-Nona-2,4,6- trienal	II/III	79-77-41-136	Geraniol	III	69-41-93-68
Decanal	II	41-43-57-44	Citronellol‡	III	41-69-55-67
Myrtenal‡	II/III	79-41-107-77	Myrtenol‡	III	79-91-41-43
Citronellal‡	II	69-41-55-95	Carveol	III	119-91-41-134
Furfural	III	95-96-67-51	Perilla alcohol	III	68-67-79-41
5-Methylfurfural	III	109-110-53-51	Farnesol	III	69-41-81-93
2-Formylpyrrol	III	95-66-94-96	Phenols		
Pentan-2-one	II/III	43-86-41-58	Phenol	III	94-66-65-63
2-Methylhexan-3-one	II	43-71-41-114	<i>o</i> -Cresol	III	108-107-77-79
2,6,6-Trimethyl- cyclohex-2-en- 1,4-dione	III	68-96-41-152	<i>m</i> -Cresol	III	108-107-79-77
Acetophenone	II	77-105-51-120	<i>p</i> -Cresol	III	107-108-77-79
Camphor	II	41-95-81-55	2,3-Dimethylphenol	III	107-122-77-121
Alcohols			2,6-Dimethylphenol	III	107-122-77-91
2-Methylpropan-1-ol‡	III	43-42-41-74	3-Ethylphenol	III	107-77-122-51
Butan-1-ol‡	III	43-42-41-74	Eugenol‡	III	55-77-164-51
2-Methylbut-3-en-2-ol	III	71-43-59-58	Miscellaneous		
			2,3-Epoxy-pinane‡	II/III	67-41-43-55
			2-Phenethyl isothio- cyanate	II	91-163-90-65

*I-III: silica gel fractions.

†The four most abundant peaks are represented.

‡Compounds additionally identified by HRGC-FTIR.

stances and, in total, an amount of 5 mg/kg, they made an important contribution to the complex aroma composition. The occurrence of the 3-methylbutyl structure as acyl and alkyl moiety in esters suggested an intensive leucine metabolism, which has been thoroughly studied in banana [3]. In a similar way, other amino acids can be metabolized, for example from isoleucine, valine and phenylalanine the alcohols 2-methylbutan-1-ol, methylpropanol and 2-phenethyl alcohol as well as the corresponding acyl compounds can be formed [4]. Recently, an alcohol-acetyltransferase has been isolated from banana fruit catalysing the formation of 3-methylbutyl acetate [5]. In lamb's lettuce, the relation of acyl and alkyl components formed varied depending on the amino acid

precursor. Obviously, from leucine both 3-methylbutyl esters and 3-methylbutanoates, but not 3-methylbutan-1-ol, were formed, whereas phenylalanine was preferably transformed to 2-phenethyl alcohol leading only to 2-phenethyl esters.

Carbonyls comprised only a few ketones, but a number of aldehydes, from which most can be considered as lipid peroxidation products formed due to cell disruption, for example, by lipoxygenase and hydroperoxide lyase activities [6]. With an amount of 4 mg/kg fresh weight, they can be assumed to contribute with a 'green-grassy' note to the overall flavour of lamb's lettuce.

With 27 substances and an amount of 9 mg/kg, the alcohols formed the major fraction among the volatiles

Table 2. Quantitative distribution of major aroma components of blended lamb's lettuce*

	Weight range (mg/kg)		
0.03–0.2	0.2–0.5	0.5–1	> 1
Esters			
Ethyl acetate (Z)-Hex-3-enyl acetate	Methyl 3-methyl- butanoate	(E)-Hex-2-enyl-3- methylbutanoate Benzyl 3-methyl- butanoate	2-Phenethyl-3- methylbutanoate
2-Phenethyl butanoate			
Methylpropyl 3-methylbutanoate			
Butyl 3-methylbutanoate			
3-Methylbutyl 3-methylbutanoate			
(E)-Pent-2-enyl 3-methylbutanoate			
2-Phenethyl hexanoate			
Carbonyls			
Pentanal	(Z)-Hex-3-enal		(E)-Hex-2-enal
(E)-Pent-2-enal	Myrtenal		
(Z)-Pent-2-enal			
Hexanal			
Decanal			
Alcohols			
2-Methylpropan-1-ol	(Z)-Pent-2-en-1-ol	2-Phenethyl alcohol	(E)-Hex-2-en-1-ol
Butan-1-ol	Pent-1-en-3-ol	Myrtenol	
4-Methylhexan-1-ol	Hexan-1-ol		
2-Ethylhexan-1-ol	(Z)-Hex-3-en-1-ol		
Oct-1-en-3-ol			
Benzyl alcohol			
Citronellol			
Miscellaneous			
2,3-Epoxy-pinane			
2-Phenethyl isothiocyanate			

*Standard controlled HRGC determination without consideration of calibration factors.

identified. Similar to the carbonyls, two principal biogenetic pathways can be considered for the alcohols, amino acid metabolism leading to volatiles such as 2-methylpropan-1-ol or 2-phenethyl alcohol and the lipid peroxidation pathways giving rise to a number of C₅ and C₆ alcohols. Additionally, several terpene alcohols were found.

The identified phenols were typically trace components. In the group of substances with miscellaneous structures, 2,3-epoxy-pinane, a well-known constituent of pepper [7], and 2-phenethyl isothiocyanate, known as enzymatically formed cleavage product from the corresponding glucosinolate [8], were identified.

EXPERIMENTAL

Sample preparation. Fresh lamb's lettuce (1.4 kg) obtained from the local market was washed with dist. H₂O and blended (30 sec) at room temp in a Waring blender with 3 l of dist. H₂O. After addition of int stds (0.32 mg butylbenzene; 0.35 mg ethyl heptanoate; 0.68 mg 2-methylhexan-3-ol) the homogenate was subjected to combined high-vacuum dist./solvent extraction

(pentane-CH₂Cl₂, 2:1) as previously described [9]. After a preliminary fractionation of the carefully conc [9] exts by LC on silica gel in three fractions using a pentane-Et₂O gradient (fraction I, eluted with pentane; fraction II, Et₂O-pentane 1:9; fraction III, Et₂O) [9] samples were concd to 0.1 ml before HRGC, HRGC-MS and HRGC-FTIR analysis.

HRGC. A 30 m × 0.259 mm id J & W fused silica DB-Wax capillary column (d.f. = 0.25 μm) with a 2 m uncoated fused silica capillary precolumn ('retention gap') [10] was used. On-column inj with an air-cooled inj. system was employed. Temp. prog. 50°, 3 min isothermal, then 50–250° at 4°/min. Carrier gas, 2.5 ml/min He; make-up gas 30 ml/min N₂; detector gases, 30 ml/min H₂ and 300 ml/min air. Detector temp. 220°. Results of qualitative analyses were confirmed by comparison of HRGC R_t, MS and, in part, FTIR data with those of authentic ref. substances. Quantitative determinations were carried out by standard controlled calculations without consideration of distillation and extraction yields, i.e. response factor = 1 for all compounds.

HRGC-MS. A GC equipped with a H₂O-cooled on-column inj. coupled by an open-split interface to a quadrupole MS was used. The same type of column as used for HRGC analysis was employed. Temp. program, 50°, 5 min isothermal, then 50–240°

5°/min. Carrier gas, 2.5 ml/min He; ion source and connection parts, temp. 200°. Electron energy, 70 eV; cathodic current, 0.8 mA. Scanning, m/z 41–250.

HRGC-FTIR. A Nicolet 20SXB system interfaced by a GC equipped with FID was used for the analysis of silica gel CC fractions II and III. The same type of column as described above was employed. Total sample inj. mode using prog. temp. vaporization (PTV) (40–240°, 0.1 min) was performed. The temp. prog. was 3 min isothermal at 50° and then 50–250° at 4°/min. Light pipe and transfer lines were held at 250°; carrier gas, 2.5 ml/min He. Vapour phase FTIR spectra were recorded from 400 to 4000 cm^{-1} with a resolution of 8 cm^{-1} .

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