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Analysis and sensory evaluation of volatile constituents of blackcurrant (*Ribes nigrum* L.) and redcurrant (*Ribes rubrum* L.) fruits

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TABLE OF CONTENTS

COMMON NAME

acetophenone	1-phenyl-1-ethanone
alloocimene	2,6-dimethyl-2,4,6-octatriene
allylphenol	(2-propen-1-yl)phenol
bicyclogermacrene	(1*S*,2*E*,6*E*,10*R*)-3,7,11,11-tetramethylbicyclo[8.1.0]undeca-2,6-diene
borneol	*rel*-(1*R*,2*S*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol
bornyl acetate	*rel*-(1*R*,2*S*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol acetate
γ-butyrolactone	5-hexyldihydro-2(3*H*)-furanone
δ-cadinene	(1*S*,8a*R*)-1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)naphthalene
camphene	2,2-dimethyl-3-methylenebicyclo[2.2.1]heptane
camphor	1,7,7-trimethylbicyclo[2.2.1]heptan-2-one
2-carene	3,7,7-trimethylbicyclo[4.1.0]hept-2-ene
Δ-3-carene	3,7,7-trimethylbicyclo[4.1.0]hept-3-ene
caryophyllene	(1*R*,4*E*,9*S*)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene
caryophyllene oxide	(1*R*,4*R*,6*R*,10*S*)-4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0.0^{4,6}]dodecane
1,8-cineole	1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane
citraconic anhydride	3-methyl-2,5-furandione
citronellol	3,7-dimethyl-6-octen-1-ol
citronellyl acetate	3,7-dimethyl-6-octen-1-yl acetate
m-cymene	1-methyl-3-(1-methylethyl)benzene
o-cymene	1-methyl-2-(1-methylethyl)benzene
p-cymen-8-ol	1-methyl-4-(1-hydroxy-1-methylethyl)benzene
p-cymene	1-methyl-4-(1-methylethyl)benzene
(*E*)-β-damascenone	(2*E*)-1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one
γ-decalactone	dihydro-5-octyl-2(*3H*)-furanone
3,4-dehydro-β-ionone	4-(2,6,6-trimethyl-1,3-cyclohexadienyl)-but-3-en-2-one
dill ether	(3*S*,3a*S*,7a*R*)-2,3,3a,4,5,7a-hexahydro-3,6-dimethylbenzofuran
p-α-dimethylstyrene	1-methyl-4-(1-methylethenyl)benzene
ebselen	2-phenyl-1,2-benzisoselenazol-3(2*H*)-one
eugenol	2-methoxy-4-(2-propen-1-yl)phenol
fenchene	7,7-dimethyl-2-methylenebicyclo[2.2.1]heptane
fenchol	1,3,3-trimethylbicyclo[2.2.1]heptan-2-ol
furaneol	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone

COMMON NAME	
furfural	2-furancarboxaldehyde
furfuryl alcohol	2-furanmethanol
geranial	(2*E*)-3,7-dimethyl-2,6-octadienal
geraniol	(2*E*)-3,7-dimethyl-2,6-octadien-1-ol
geranyl acetate	(*E*)-3,7-dimethyl-2,6-octadien-1-ol acetate
germacrene D	(1*E*,6*E*,8*S*)-1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene
glutaconic anhydride	2*H*-pyran-2,6(3*H*)-dione
γ-hexalactone	5-ethyldihydro-2(3*H*)-furanone
δ-hexalactone	tetrahydro-6-methyl-2*H*-pyran-2-one
HMF	5-(hydroxymethyl)-2-furancarboxaldehyde
hotrienol	(3*R*,5*E*)-3,7-dimethyl-1,5,7-octatrien-3-ol
humulene	(1*E*,4*E*,8*E*)-2,6,6,9-tetramethyl-1,4,8-cycloundecatriene
2-hydroxy 1,8-cineole	1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-ol
levulinic acid	4-oxopentanoic acid
limetol	2-ethenyltetrahydro-2,6,6-trimethyl-2*H*-pyran
limonene	1-methyl-4-(1-methylethenyl)cyclohexene
limonene-4-ol	4-methyl-1-(1-methylethenyl)-3-cyclohexen-1-ol
linalool	3,7-dimethyl-1,6-octadien-3-ol
(*E*)-linalool oxide	*rel*-(2*R*,5*R*)-5-ethenyltetrahydro-α,α,5-trimethyl-2-furanmethanol
(*Z*)-linalool oxide	*rel*-(2*R*,5*S*)-5-ethenyltetrahydro-α,α,5-trimethyl-2-furanmethanol
linalyl acetate	3,7-dimethylocta-1,6-dien-3-yl acetate
p-menth-1-en-9-al	dimethyl-3-cyclohexene-1-acetaldehyde
menthol	5-methyl-2-(1-methylethyl)cyclohexanol
mesifurane	4-methoxy-2,5-dimethyl-3(2*H*)-furanone
methional	3-methylsulfanylpropanal
methyl indole-3-pyruvate	methyl α-oxo-1*H*-indole-3-propanoate
4-methyl itaconate	methyl 1-carboxy-1-(methylene)-3-propanoate
methyl salicylate	methyl 2-hydroxybenzoate
p-methylacetophenone	1-(4-methylphenyl)ethanone
myrcene	7-methyl-3-methylene-1,6-octadiene
neral	(2*Z*)-3,7-dimethyl-2,6-octadienal
nerol oxide	3,6-dihydro-4-methyl-2-(2-methyl-1-propen-1-yl)-2*H*-pyran
neryl acetate	*Z*-3,7-dimethyl-2,6-octadien-1-ol acetate
(*E*)-β-ocimene	(3*E*)-3,7-dimethyl-1,3,6-octatriene
(*Z*)-β-ocimene	(3*Z*)-3,7-dimethyl-1,3,6-octatriene
(*E*)-ocimenol	(5*E*)-2,6-dimethyl-1,5,7-octatrien-3-ol

COMMON NAME	
(*Z*)-ocimenol	(5*Z*)-2,6-dimethyl-1,5,7-octatrien-3-ol
pantolactone	(3*R*)-dihydro-3-hydroxy-4,4-dimethyl-2(3*H*)-furanone
perillyl alcohol	4-(1-methylethenyl)-1-cyclohexene-1-methanol
α-phellandren-8-ol	α,α,4-trimethyl-2,4-cyclohexadiene-1-methanol
β-phellandren-8-ol	α,α-dimethyl-4-methylene-2-cyclohexene-1-methanol
α-phellandrene	2-methyl-5-(1-methylethyl)-1,3-cyclohexadiene
β-phellandrene	3-methylene-6-(1-methylethyl)cyclohexene
α-pinene	2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
β-pinene	6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane
α-pinene oxide	2,7,7-trimethyl-3-oxatricyclo[4.1.1.$0^{2,4}$]octane
(*E*)-piperitol	*rel*-(1*S*,6*S*)-3-methyl-6-(1-methylethyl)-2-cyclohexen-1-ol
(*Z*)-piperitol	*rel*-(1*R*,6*R*)-3-methyl-6-(1-methylethyl)-2-cyclohexen-1-ol
(*E*)-rose oxide	*rel*-(2*R*,4*R*)-tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-2*H*-pyran
(*Z*)-rose oxide	*rel*-(2*R*,4*S*)-tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-2*H*-pyran
sabinene	4-methylene-1-(1-methylethyl)bicyclo[3.1.0]hexane
sabinene hydrate	2-methyl-5-(1-methylethyl)bicyclo[3.1.0]hexan-2-ol
sotolon	3-hydroxy-4,5-dimethylfuran-2(*5H*)-one
spathulenol	(1a*R*,4a*R*,7*S*,7a*R*,7b*R*)-decahydro-1,1,7-trimethyl-4-methylene-1*H*-cycloprop[e]azulen-7-ol
sylvestrene	1-methyl-5-(1-methylethenyl)cyclohexene
terpin	4-hydroxy-α,α,4-trimethylcyclohexanemethanol
terpinen-4-ol	4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol
α-terpinene	1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene
β-terpinene	4-methylene-1-(1-methylethyl)cyclohexene
γ-terpinene	1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene
α-terpineol	α,α,4-trimethyl-3-cyclohexene-1-methanol
β-terpineol	1-methyl-4-(1-methylethenyl)cyclohexanol
γ-terpineol	1-methyl-4-(1-methylethylidene)cyclohexanol
terpinolene	1-methyl-4-(1-methylethylidene)cyclohexene
α-terpinyl acetate	2-(4-methyl-3-cyclohexen-1-yl)-2-propyl acetate
α-thujene	2-methyl-5-(1-methylethyl)bicyclo[3.1.0]hex-2-ene
vanillin	4-hydroxy-3-methoxybenzaldehyde

ABBREVIATIONS

AAT	alcohol acyltransferase
ADH	alcohol dehydrogenase
AEDA	aroma extract dilution analysis
ANOVA	analysis of variance
APCI-MS	atmospheric pressure chemical ionisation-mass spectrometry
ATP	adenosine-5´-triphosphate
cAMP	cyclic adenosine-3´,5´-monophosphate
CHARM	combined hedonic aroma response method
CoA	coenzyme A
DHS	dynamic headspace extraction
DMAPP	dimethylallyl diphosphate
DMF	dimethylformamide
EI	electron ionisation
FD	flavour dilution
FID	flame ionisation detector
FPD	flame photometric detector
FW	fresh weight
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GC/O	gas chromatography/olfactometry
HPL	hydroperoxide lyase
IF	isomerase factor
IPP	isopentenyl diphosphate
IR	infrared spectroscopy
LC-MS	liquid chromatography-mass spectrometry
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
LOX	lipoxygenase
MEP	methylerythritol phosphate
MRI	magnetic resonance imaging
MS	mass spectrometer
MVA	mevalonate
n.c.	not calculated
n.d.	not detectable
n.q.	not quantifiable

ABBREVIATIONS

NIF	nasal impact frequency
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance
OAV	odour activity value
PTR-MS	proton transfer reaction-mass spectrometry
RI	retention index
SAFE	solvent-assisted flavour evaporation
SDE	simultaneous distillation extraction
SDEV	simultaneous distillation extraction at reduced pressure
SIM	selected ion monitoring
SNIF	surface of nasal impact frequency
SPME	solid-phase microextraction
TIC	total ion chromatogram
VHS	vacuum-headspace extraction

1 INTRODUCTION AND OBJECTIVES

Blackcurrant (*Ribes nigrum* L.) and redcurrant (*Ribes rubrum* L.) belong to the family Grossulariacea, and the genus *Ribes* L.. Blackcurrant berries were originally collected from the wild for medical use and have been cultivated since the 16th century (Barney and Hummer 2005; Düll and Kutzelnigg 2016). Redcurrant berries have already been cultivated since the 14th century (Bundessortenamt 2002). During the last decade, the worldwide crop of currant fruits ranged between 650,000 t and 780,000 t per year (FAO 2017). They are used for the production of jam, juice, wine, liqueur, and spirit (Eisenbrand and Schreier 2006).

So far, investigations of blackcurrant fruits mainly focused on phenolic compounds and the antioxidant properties of these berries (Beattie *et al.* 2005, Heinonen 2007, Gopalan *et al.* 2012, Miller *et al.* 2013). First analyses of the volatile constituents of blackcurrant fruits date back to the 1960s, and a broad spectrum of terpenes, esters, and alcohols were identified. These investigations as well as following studies in the last decades, however, primarily focused on frozen blackcurrant berries (Andersson and von Sydow 1964, 1966a, b; Nursten and Williams 1969; von Sydow and Karlsson 1971a, b; Karlsson-Ekstrom and von Sydow 1973; Latrasse *et al*. 1982; Bricout *et al*. 1985; Marriott 1986, 1987; Mikkelsen and Poll 2002; Orav *et al*. 2002; Ruiz del Castillo and Dobson 2002a, b; Tiitinen *et al*. 2004; Christensen and Pedersen 2006; Kampuss *et al*. 2008; Hempfling *et al*. 2013a). To the best of the author's knowledge data on the sensory evaluation, for example, are still entirely limited to frozen fruits. Furthermore no odour activity values (OAVs) as well as no reconstitution experiments were carried out, even for frozen fruits (von Sydow and Karlsson 1971b; Latrasse *et al.* 1982; Mikkelsen and Poll 2002; Tiitinen *et al.* 2004). Therefore, the odour-active compounds of fresh fruits are still unknown. In one investigation in 1982, Latrasse *et al*. detected a 'catty urine odour' via gas chromatography/olfactometry (GC/O) in frozen blackcurrant berries, and in subsequent studies on blackcurrant buds the compound 4-methoxy-2-methyl-2-butanethiol was identified (Rigaud *et al.* 1986; Le Quéré and Latrasse 1990). However, until now only GC/O data indicated the presence of this sulphur-containing compound in frozen berries of blackcurrant (Latrasse *et al*. 1982; Mikkelsen and Poll 2002).

Due to these insufficient data on the volatile profiles of fresh blackcurrant fruits, the first objectives of this work were: (i) to unambiguously verify the presence of 4-methoxy-2-methyl-2-butanethiol in fresh blackcurrant berries; (ii) to identify and to quantify volatile compounds in fresh blackcurrant berries; (iii) to illustrate the effect of freezing and the state of ripeness on the contents of volatile constituents; (iv) to investigate the potential to classify blackcurrant berries based on the volatile profiles of fresh fruits, and (v) to assess the contributions of volatile constituents to the overall aroma by GC/O and aroma profile testing.

Recent analytical investigations of redcurrant berries have been concentrating on the phenolic compounds and the antioxidant properties of these fruits (Beattie *et al.* 2005, Mattila *et al.* 2016). Data on the volatile profiles of redcurrant berries are limited to three investigations. In 1956, free and bound acetic acid as well as formic acid have been identified in juice of redcurrant fruits (Mehlitz and Matzik 1956a, b). Later, Schreier *et al.* (1977) determined the qualitative and quantitative differences between the volatile profiles of fresh redcurrant fruits and juice. In addition, Boschetti *et al.* (1999) monitored six volatile compounds during the postharvest aging of redcurrant berries. However, the variability in the profiles of volatile compounds of fresh redcurrant berries as well as the impact of these compounds on the overall aroma was never investigated.

Therefore, the objectives of the second part of the present study were: (i) to identify and quantify volatile compounds in fresh redcurrant berries; (ii) to investigate the variability of the volatile profiles with respect to cultivar, location, season, and state of ripeness; and (iii) to assess the contributions of volatile constituents to the overall aroma by GC/O and aroma profile testing.

2 BACKGROUND

2.1 Currant

2.1.1 Botanical classification, history, and uses

The taxonomy of currants, such as blackcurrant (*Ribes nigrum* L.) and redcurrant (*Ribes rubrum* L.), was controversial in the beginning. Originally, in 1891, they were assigned into the family Saxifragaceae, but they were later relocated into the family Grossulariacea. The *Ribes* species were further classified into either subgenera, sections, or series, depending on the classification scheme applied. In the classification into subgenera, for example, blackcurrant was assigned to the subgenus *Coreosma,* and redcurrant was assigned to the subgenus *Ribesia* (Barney and Hummer 2005). In Table 1, the most recent taxonomy according to Erhardt *et al.* (2008) is listed.

Table 1: Taxonomy of blackcurrant and redcurrant according to Erhardt *et al.* (2008).

	blackcurrant	redcurrant
class:	Dicotyledoneae	
subclass:	Rosidae	
order:	Saxifragales	
family:	Grossulariacea	
genus:	*Ribes* L.	
species:	*nigrum*	*rubrum*
variety:		*domesticum* Wallr.

2.1.1.1 Blackcurrant

The blackcurrant wild species *Ribes nigrum* L. is native to Eurasia (Bundessortenamt 2002). Blackcurrant berries were originally not appreciated for their palatability, but they were collected from the wild for medical use and have been cultivated since the 16th century (Barney and Hummer 2005; Düll and Kutzelnigg 2016). In the late 17th century and the early 18th century, their cultivation became popular in Canada, but they were not common in the United States. In the middle of the 18th century, the cultivars changed markedly caused by breeding against resistance to spring frosts. In the beginning of the 20th century, the currant industry in North America was destroyed by a severe infestation of the currant population with the plant fungus white pine blister rust. Therefore, efforts were undertaken to breed rust-resistant blackcurrant cultivars, and the rust-resistant cultivar ‘Titania’ was introduced into North America from Europe (Barney and Hummer 2005).

In 2014, the worldwide production of currant fruits amounted to approximately 660,000 t (FAO 2017). Blackcurrant berries are, by far, the leading *Ribes* crop worldwide and are widely grown in Europe (Barney and Hummer 2005). In Germany, for example, the production volume amounted to 6,809 t in 2016 according to *Statistisches Bundesamt* (2017). Blackcurrant berries are mostly used for industrial exploitation and are commonly manufactured into jam, juice, wine, liqueur, and spirit. Additionally, they are recommended against cold and are used as a household remedy against gout, rheumatism, and quinsy (Keipert 1981; Eisenbrand and Schreier 2006).

2.1.1.2 Redcurrant

Cultivated redcurrants (i.e. *Ribes rubrum* L. var. *domesticum* Wall.) originated from a crossing of wild species (*Ribes petraeum* Wulf., *Ribes rubrum* L. var. *rubrum,* and *Ribes sativum* Syme), which are common in Europe and West Asia, with the wild species *Ribes multiflorum* Kitt., which is domestic in the Balkans and Central Italy. The resulting species hybrid and its offspring were first cultivated in the 14th century in monastery gardens (Bundessortenamt 2002; Pirc 2015).

Redcurrant berries are commonly used for the production of jam, juice, wine, liqueur, and spirit (Eisenbrand and Schreier 2006). In Germany, for example, the production volume of redcurrant and white currant amounted to 7,185 t in 2016 according to *Statistisches Bundesamt* (2017). White currant berries are a colour variant of the redcurrant species and are used in parts of Europe to produce baby food. In Finland, they are also used for the production of sparkling wine (Barney and Hummer 2005).

2.1.2 Compositional data

2.1.2.1 Blackcurrant

Blackcurrant berries consist of 81.3 % water, 6.78 % dietary fibres, 6.11 % carbohydrates, 2.63 % organic acids, 1.28 % proteins, 0.80 % minerals, and 0.22 % fat (Souci *et al.* 2015). For the main vitamin ascorbic acid, concentrations between 148 mg/100 g fresh weight (FW) and 541 mg/100 g FW were reported (Rodriguez *et al.* 1992; Bordonaba and Terry 2008; Nour *et al.* 2011; Vagiri *et al.* 2013; Souci *et al.* 2015). Other vitamins present are, e.g. pantothenic acid (389 - 403 µg/100 g FW), nicotinic acid (233 - 293 µg/100 g FW), and biotin (2.0 - 2.7 µg/100 g FW) (James 1952).

The mineral compositions of blackcurrant berries were analysed by Lefèvre *et al.* (2011) and Nour *et al.* (2011). Nour *et al.* (2011) showed that the berries contain several minerals, especially potassium (251 - 305 mg/100 g FW), magnesium (46 - 66 mg/100 g FW), calcium

(31 - 64 mg/100 g FW), and iron (1.1 - 1.7 mg/100 g FW). Lefèvre *et al.* (2011) confirmed the high amounts of the aforementioned minerals. However, in this study the concentrations were calculated in relation to the dry weights of the berries. A detailed overview of additional vitamin and mineral compositions is given by Nour *et al.* (2011), Herrmann (2001), and Souci *et al.* (2015).

As early as 1958, citric acid was shown to be the main organic acid in blackcurrant berries (Whiting 1958). In several studies, a large variation in the concentration, ranging between 28 mg/100 g FW and 5,979 mg/100 g FW, was demonstrated. Additional organic acids detected are oxalic acid (23 - 47 g/100 g FW), malic acid (2 - 731 mg/100 g FW), quinic acid (0 - 148 mg/100 g FW), tartaric acid (31 - 144 mg/ 100 g FW), shikimic acid (2.7 mg/100 g FW), and fumaric acid (950 µg/100 g FW) (Haila *et al.* 1992; Rodriguez *et al.* 1992; Bordonaba and Terry 2008; Milivojević *et al.* 2009; Nour *et al.* 2011; Mikulic-Petkovsek *et al.* 2012b).

In various studies, the concentrations of the monosaccharides fructose (1.9 - 12.7 g/100 g FW) and glucose (1.4 - 8.5 g/100 g FW), as well as the disaccharide sucrose (0.4 - 3.7 g/100 g FW) were determined at the ripe state. In each investigation, the amount of fructose was slightly higher than the amount of glucose (Haila *et al.* 1992; Bordonaba and Terry 2008; Milivojević *et al.* 2009; Mikulic-Petkovsek *et al.* 2012b). The concentrations of monosaccharides increased during ripening (Marriott 1986).

Recent analyses focused mainly on phenolic compounds and the antioxidant properties of blackcurrant berries (Beattie *et al.* 2005; Heinonen 2007; Gopalan *et al.* 2012; Miller *et al.* 2013). Phenolic compounds are a large group of secondary metabolites with antioxidant properties. They consist of one or more aromatic rings with variable degrees of hydroxylation, methoxylation, and glycosylation. Representatives found in berry fruits, such as blackcurrant berries, are flavonoids, tannins, phenolic acids, and stilbenes (Gopalan *et al.* 2012; Manganaris *et al.* 2014). The flavonoids can be further subdivided into anthocyanins, flavonols, flavanols, flavones, flavanones, and isoflavonoids (Manganaris *et al.* 2014). Anthocyanins are the main phenolic compounds (75 % of all phenolic compounds) in blackcurrant berries (Heinonen 2007). Delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-rutinoside were identified as the most common anthocyanins. In most cultivars, delphinidin-3-*O*-rutionoside was identified as the major anthocyanin followed by cyanidin-3-*O*-rutionoside (Mattila *et al.* 2016). In addition to these four major compounds in blackcurrant berries, twelve other anthocyanins (cyanidin-3-*O*-(6″-coumaroylglucoside), cyanidin-3-*O*-arabinoside, delphinidin-3-*O*-(6″-coumaroylglucoside), delphinidin-3-*O*-sophoroside, delphinidin-3-*O*-xyloside, malvidin-3-*O*-glucoside, malvidin-3-*O*-rutinoside, pelargonidin-3-*O*-glucoside, pelargonidin-3-*O*-rutinoside, peonidin-3-*O*-glucoside, peonidin-3-*O*-rutinoside, and petunidin-3-*O*-glucoside) were detected, but in much lower amounts (Frøytlog *et al.* 1998; Slimestad and Solheim 2002; Gavrilova *et al.* 2011; Veberic *et al.* 2015).

As the principal flavonols in blackcurrant berries, myricetin, quercetin, kaempferol, and their glycosides (with the main gylcosyl groups glucoside, rutinoside, and hexoside-malonate) were identified (Määttä *et al.* 2003; Mikulic-Petkovsek *et al.* 2012a; Mattila *et al.* 2016).

Representatives of the flavanols, such as (+)-catechin (4.7 - 7.5 mg/100 g FW), epigallocatechin (5.9 - 6.0 mg/100 g FW), and (-)-epicatechin (4.7 mg/100 g FW), could be quantified (Gavrilova *et al.* 2011; Cyboran *et al.* 2014). Further, proanthocyanidins, better known as condensed tannins (148 mg/100 g FW), were detected by Gu *et al.* (2004).

Hydroxylated phenolic acids are the main fraction of phenolic acids (92 %) present in blackcurrant berries and most of the hydroxylated phenolic acids found are conjugated (96 %) (Russell *et al.* 2009). Gallic acid derivatives were identified by Russell *et al.* (2009) as the major hydroxylated phenolic acid compounds, followed by *p*-coumaric acid derivatives, protocatechuic acid derivatives, and *p*-hydroxybenzoic acid derivatives. Määttä *et al.* (2003) and Gavrilova *et al.* (2011) determined high concentrations for *p*-coumaric acid derivatives (1.8 - 4.8 mg/100 g FW), caffeic acid derivatives (2.1 - 2.6 mg/100 g FW), and ferulic acid derivatives (1 - 1.3 mg/100 g FW). Additionally, the phenolic acids chlorogenic acid, *p*-coumaroyl quinic acid, gentisic acid, neochlorogenic acid, salicylic acid, sinapic acid, syringic acid, and vanillic acid were found in free and/or bound form (Russell *et al.* 2009; Gavrilova *et al.* 2011; Cyboran *et al.* 2014).

2.1.2.2 Redcurrant

Redcurrant berries consist of 84.7 % water, 4.78 % carbohydrates, 3.50 % dietary fibres, 2.37 % organic acids, 1.13 % proteins, 0.63 % minerals, and 0.20 % fat. Ascorbic acid was identified as the main vitamin in redcurrant berries, but in lower amounts compared to blackcurrant berries (Souci *et al.* 2015). The amount of ascorbic acid ranges between 23 g/100 g FW and 110 mg/100 g FW (Rodriguez *et al.* 1992; Djordjević *et al.* 2010; Nour *et al.* 2011; Kevers *et al.* 2014; Souci *et al.* 2015). The vitamins nicotinic acid (130 µg/100 g FW), pantothenic acid (64 µg/100 g FW), and biotin (2.6 µg/100 g FW) could be quantified by James (1952). Additionally, a total concentration of 360 µg/100 g FW was determined for carotenoids in ripe redcurrant berries, with lutein and β-carotene as the main carotenoid compounds. It could be also shown that the concentrations of chlorophyll a and b as well as the total carotenoid concentration decrease from unripe to fully ripe berries (Gross 1982). The mineral compositions of redcurrant berries were analysed by Nour *et al.* (2011). In the investigated *Ribes* species a diverse set of minerals, especially potassium (240 - 256 mg/100 g FW), magnesium (27 - 38 mg/100 g FW), calcium (18 - 34 mg/100 g

FW), and iron (1.2 - 1.4 mg/100 g FW) was found. A detailed overview of the vitamin and mineral composition of redcurrant berries has been given by Nour *et al.* (2011), Herrmann (2001), and Souci *et al.* (2015).

Citric acid was identified as the major organic acid (17 - 3,084 mg/100 g FW), followed by malic acid (2 - 740 mg/100 g FW), tartaric acid (25 - 40 mg/100 g FW), shikimic acid (35 mg/100 g FW), and fumaric acid (720 µg/100 g FW) (Haila *et al.* 1992; Rodriguez *et al.* 1992; Milivojević *et al.* 2009; Nour *et al.* 2011; Mikulic-Petkovsek *et al.* 2012b).

The dominating sugars in redcurrant berries are fructose (2.1 - 12.1 g/100 g FW) and glucose (1.2 - 9.7 g/100 g FW). The concentration of the disaccharide sucrose was reported to range between 0.01 g/100 g FW and 2.0 g/100 g FW (Haila *et al.* 1992; Milivojević *et al.* 2009; Mikulic-Petkovsek *et al.* 2012b).

As for blackcurrant berries, recent analyses of redcurrant berries mainly focused on the phenolic compounds and the antioxidant properties of these fruits (Beattie *et al.* 2005; Mattila *et al.* 2016). Anthocyanins also represent the major group of phenolic compounds in redcurrant berries, albeit the concentration is 8.5 to 10 times lower than in blackcurrant berries (Määttä *et al.* 2003; Gavrilova *et al.* 2011). In two studies by Yang *et al.* (2013) and Veberic *et al.* (2015), cyanidin-3-*O*-xylosylrutinoside (6.9 - 9.4 mg/100 g FW), cyanidin-3-*O*-sambubioside (1.8 - 8 mg/100 g FW), cyanidin-3-*O*-glucosylrutinoside (5.3 mg/100 g FW), cyanidin-3-*O*-rutionoside (2.4 - 3.1 mg/100 g FW), and cyanidin-3-*O*-sophoroside (1.1 - 1.9 mg/100 g FW) were quantified as the major cyanidin-based anthocyanins. In addition to these five compounds, the anthocyanin cyanidin-3-*O*-glucoside was identified in redcurrant berries by Määttä *et al.* (2003). Mattila *et al.* (2016) analysed twelve different cultivars of redcurrant berries and showed high variations of the anthocyanin profiles depending on the cultivar.

First investigations on flavonols in redcurrant berries in the 1980s quantified quercetin, kaempferol, and myricetin after hydrolysis of the flavonol glycosides (Wildanger and Herrmann 1973; Starke and Herrmann 1976). Starke and Herrmann (1976) investigated two cultivars of redcurrant berries at the ripe and the unripe state and reported concentrations between 0.2 mg/100 g FW and 1.1 mg/100 g FW for quercetin and between 0.01 mg/100 g FW and 0.2 mg/100 g FW for kaempferol at the ripe state. The concentrations of flavonols generally decreased during ripeness, myricetin was only quantified in one cultivar at the unripe state. According to Määttä *et al.* (2003) and Yang *et al.* (2013), the quercetin glycosides represent the major flavonol glucosides in redcurrant berries. Though, Mikulic-Petkovsek *et al.* (2012a) showed that kaempferol-3-*O*-rutinoside represents 48.5 % of the analysed flavonols.

Representatives of the flavanols, such as (+)-catechin, (-)-epicatechin, (+)-gallocatechin, and (-)-epigallocatechin, were also detected in redcurrant berries (Stöhr and Herrmann 1975;

Gavrilova *et al.* 2011). In addition to flavanols, the phenolic acids *p*-coumaric acid, caffeic acid, ferulic acid, gallic acid, *p*-hydroxybenzoic acid, and protocatechuic acid were identified in berries of different redcurrant cultivars by Stöhr and Herrmann (1975). Määttä *et al.* (2003) and Gavrilova *et al.* (2011) quantified lower concentrations of the phenolic acid derivatives *p*-coumaric acid (n.d. - 1.2 mg/100 g FW), caffeic acid (0.3 - 0.9 mg/100 g FW), and ferulic acid (n.d. - 0.3 mg/100 g FW) compared to blackcurrant berries.

2.1.3 Aroma compounds in currant berries

2.1.3.1 Blackcurrant

First analyses of the volatile constituents in frozen blackcurrant fruits were published in 1964 and 1966 by Andersson and von Sydow. A broad spectrum of terpenes, esters, and alcohols were identified in these studies and in several following investigations on frozen berries in the 1960s and 1970s (Andersson and von Sydow 1964, 1966a, b; Nursten and Williams 1969a; von Sydow and Karlsson 1971a, b; Karlsson-Ekstrom and von Sydow 1973).

Further studies on the volatile profiles of blackcurrant fruits carried out in recent decades primarily focused on frozen fruits. A detailed overview is given in Table 2. Up until now, more than 200 volatile constituents were identified in blackcurrant fruits, including acids, alcohols, aldehydes, esters, ketones, lactones, terpenes, and others (Andersson and von Sydow 1964, 1966a, b; Nursten and Williams 1969a; von Sydow and Karlsson 1971a, b; Karlsson-Ekstrom and von Sydow 1973; Latrasse *et al.* 1982; Bricout *et al.* 1985; Marriott 1986; 1987; Mikkelsen and Poll 2002; Orav *et al.* 2002; Ruiz del Castillo and Dobson 2002a, b; Tiitinen *et al.* 2004; Christensen and Pedersen 2006; Kampuss *et al.* 2008; Hempfling *et al.* 2013a).

Kampuss *et al.* (2008) showed that esters and terpenes are the main compound classes. After analysing frozen berries of twelve blackcurrant cultivars via dynamic headspace extraction (DHS), Kampuss *et al.* demonstrated that esters comprise on average 55 % (2 - 83 %) and terpenes 35 % (13 - 81 %) of the total volatile compounds. Two other investigations confirmed this trend (Mikkelsen and Poll 2002; Christensen and Pedersen 2006). Large variations in the concentrations of the main volatile constituents, e.g. the esters ethyl butanoate (8 - 26,200 µg/kg), methyl butanoate (4 - 22,400 µg/kg), methyl hexanoate (n.d. - 1,200 µg/kg), ethyl hexanoate (n.d. - 710 µg/kg), and ethyl acetate (150 - 390 µg/kg) as well as the terpenes sabinene (12 - 9,900 µg/kg), Δ-3-carene (4 - 5,040 µg/kg), terpinolene (<1 - 2,330 µg/kg), γ-terpinene (7 - 1,520 µg/kg), (*Z*)-β-ocimene (11 - 1,120 µg/kg), 1,8-cineole (2 - 960 µg/kg), myrcene (14 - 780 µg/kg), and α-pinene (35 - 540 µg/kg) were observed (Marriott 1986; Christensen and Pedersen 2006; Kampuss *et al.* 2008).

Table 2: An overview of investigations of volatile compounds in blackcurrant fruits.

reference	sample material	sample preparation	analysis
Andersson and von Sydow (1964)	frozen berries (-40 °C; ten months)	extraction + steam distillation	GC, IR, GC-MS
Andersson and von Sydow (1966a)	frozen berries (-40 °C)	vacuum distillation + extraction or extraction + steam distillation	GC, IR, GC-MS
Andersson and von Sydow (1966b)	frozen berries (-40 °C; nine to ten months), six cultivars	extraction + steam distillation or headspace	GC
Nursten and Williams (1969a)	frozen berries (-20 °C)	extraction + distillation	GC, IR
von Sydow and Karlsson (1971a)	frozen berries (-40 °C)	extraction or headspace	GC, GC-MS
von Sydow and Karlsson (1971b)	frozen berries (-40 °C)	headspace	GC, GC/O
Karlsson-Ekstrom and von Sydow (1973)	frozen berries (-40 °C)	extraction or headspace	GC, GC-MS
Latrasse *et al.* (1982)	frozen berries, ten cultivars	vacuum distillation + extraction	GC-MS, GC/O
Bricout *et al.* (1985)	frozen berries (-20 °C), eleven cultivars	DHS	GC-MS
Marriott (1986)	fresh berries, three cultivars	extraction	GC-MS
Marriott (1987)	fresh berries	extraction	GC-MS
Ruiz del Castillo and Dobson (2002a)	fresh and frozen berries (-20 °C; 40 days), three cultivars	SPME	GC
Ruiz del Castillo and Dobson (2002b)	frozen berries (-20 °C), ten cultivars	SPME	GC
Mikkelsen and Poll (2002)	frozen berries (-15 °C; six months)	DHS	GC-MS, GC/O
Orav *et al.* (2002)	fresh berries	SDE	GC, GC-MS
Tiitinen *et al.* (2004)	frozen berries (two months)	SPME	GC-MS, GC/O
Christensen and Pedersen (2006)	frozen berries, 13 cultivars	DHS	GC, GC-MS
Kampuss *et al.* (2008)	frozen berries, twelve cultivars	DHS	GC, GC-MS
Hempfling *et al.* (2013a)	fresh berries	VHS	GC, GC-MS

GC: gas chromatography, IR: infrared spectroscopy, GC-MS: gas chromatography-mass spectrometry, GC/O: gas chromatography/olfactometry, DHS: dynamic headspace extraction; SPME: solid-phase microextraction, SDE: simultaneous distillation extraction, VHS: vacuum-headspace extraction.

However, data on sensory evaluations are scarce and entirely limited to frozen fruits. In 1971, von Sydow and Karlsson already evaluated the odour properties via GC/O of each discernible peak after headspace isolation of unheated and heated frozen blackcurrant berries (von Sydow and Karlsson 1971b). Latrasse *et al.* demonstrated in 1982, that ethyl butanoate, methyl butanoate, 1,8-cineole, diacetyl, and a 'catty urine odour' are important for the typical blackcurrant aroma (Latrasse *et al.* 1982). In a further study by Mikkelsen and Poll (2002), a 'catty urine odour' was also detected by GC/O together with another 21 (14 identified) volatile compounds. In Table 3, the odour-active volatile constituents identified in studies by Latrasse *et al.* (1982) and Mikkelsen and Poll (2002), as well as in a third study by Tiitinen *et al.* (2004), are listed. In particular, esters and terpenes were identified as odour-active compounds in these studies. It is important to emphasise, that the sensory contribution of the detected odour-active volatile compounds was not evaluated by OAVs or reconstitution experiments (Latrasse *et al.* 1982; Mikkelsen and Poll 2002; Tiitinen *et al.* 2004).

Table 3: Odour-active volatile compounds in frozen blackcurrant berries identified via GC/O, according to Latrasse *et al.* (1982), Mikkelsen and Poll (2002), and Tiitinen *et al.* (2004).

compound	reference	compound	reference
'catty urine odour'	*a, b*	methyl butanoate	*b, c*
Δ-3-carene	*c*	methyl hexanoate	*c*
1,8-cineole	*a, b*	β-myrcene	*c*
neo-alloocimene	*b*	(*E*)-non-2-enal	*b*
citronellol	*a*	oct-1-en-3-one	*b, c*
β-damascenone	*a, b*	(*E*)-ocimene	*c*
diacetyl	*a*	(*Z*)-ocimene	*c*
ethyl butanoate	*a, b, c*	octanol	*b*
ethyl hexanoate	*b*	α-pinene	*b, c*
ethyl 3-methylbutanoate	*c*	β-pinene	*c*
eugenol	*b*	α-phellandrene	*c*
geraniol	*a*	rose oxide	*b*
hexanal	*c*	sabinene	*c*
limonene	*c*	γ-terpinene	*c*
limonen-4-ol	*a*	α-terpineol	*a*
linalool	*a*	terpinen-4-ol	*a, b*
methoxyisopropylpyrazine	*a, b*	terpinolene	*c*
methylacetophenone	*a*	toluene	*c*

[a] Latrasse *et al.* (1982). [b] Mikkelsen and Poll (2002). [c] Tiitinen *et al.* (2004).

After Latrasse *et al.* (1982) detected a 'catty urine odour' in frozen fruits of blackcurrant, subsequent studies on blackcurrant buds were carried out and the sulphur-containing compound 4-methoxy-2-methyl-2-butanethiol was identified in essential oils obtained by steam distillation (Rigaud *et al.* 1986; Le Quéré and Latrasse 1990). According to an abstract from an oral presentation, the presence of this compound was confirmed in blackcurrant berries and leaf extracts (Joulain and Laurent 1989). However, no experimental data substantiating this statement were provided. Later, Mikkelsen and Poll (2002) detected a 'catty', blackcurrant-like odour by GC/O in an extract from frozen blackcurrant fruits and the authors assigned the compound as 4-methoxy-2-methyl-2-butanethiol. Though, this was solely based on a comparison of the odour and the chromatographic retention behaviour to those reported by Latrasse *et al.* (1982); no authentic reference compound was available and no confirmation via GC-MS data was provided.

As mentioned before, several studies were carried out to investigate the volatile profiles of blackcurrant fruits. Nevertheless, as shown in Table 2, most investigations on volatile compounds in blackcurrant berries were performed on frozen berries. Data on the overall volatile profiles of fresh blackcurrant berries are limited and restricted to investigations of terpenes (Marriott 1986; 1987; Ruiz del Castillo and Dobson 2002a) and a study with simultaneous distillation extraction (SDE), which is a method known to trigger the formation of heat-induced artefacts (Orav *et al.* 2002). Analyses based on a gentle isolation method, capable of isolating a broad complete spectrum of volatiles of fresh blackcurrant berries, in combination with investigations on the influence of cultivar, season, or ripeness on the volatile profile and the determination of impact aroma compounds of fresh blackcurrant berries are not available.

In addition to blackcurrant fruits, volatile constituents were investigated in buds and leaves of blackcurrant as well as in products of blackcurrant fruits, i.e. juice, nectar, concentrate, and wine.

In blackcurrant buds, various volatile constituents were identified besides the impact volatile compound 4-methoxy-2-methyl-2-butanethiol. The main constituents are terpenes, such as bicyclogermacrene, Δ-3-carene, β-caryophyllene, limonene, α-pinene, β-pinene, β-phellandrene, and terpinolene (Le Quéré and Latrasse 1990; Píry *et al.* 1995; Orav *et al.* 2002; Dvaranauskaite *et al.* 2008).

Terpenes, especially Δ-3-carene, β-caryophyllene, (*Z*)-β-ocimene, sabinene, and terpinolene as well as caryophyllene oxide were also identified in different studies of blackcurrant leaves as the major volatile compounds (Andersson *et al.* 1963; Marriott 1988; Orav *et al.* 2002).

In a first study on blackcurrant juice in 1956, free acetic acid and formic acid were detected (Mehlitz and Matzik 1956a, b). In subsequent investigations on juice, nectar, concentrate, and wine of blackcurrant berries, alcohols, aldehydes, esters, furans, ketones, terpenes, and other compounds were identified. It was shown that alcohols, esters, and terpenes are the qualitatively dominating compound classes (Leino and Kallio 1993; Kollmansberger and Berger 1994; Iversen *et al.* 1998; Varming and Poll 2003; Varming *et al.* 2004).

2.1.3.2 Redcurrant

Unlike for blackcurrant berries, data on the volatile profiles of redcurrant berries are limited, except for three studies reporting 45 volatile compounds in redcurrant fruits and juice, which are listed in Table 4 (Mehlitz and Matzik 1956a, b; Schreier *et al.* 1977; Boschetti *et al.* 1999).

In 1956, Mehlitz and Matzik identified free and bound acetic acid and formic acid in juice of redcurrant fruits. They calculated the amount of volatile acids as acetic acid between 56 mg/L and 285 mg/L and the amount of esters up to 32 mL 0.1 *n*-NaOH/L (determined by back titration with sulfuric acid after transesterfication with NaOH) (Mehlitz and Matzik 1956a, b).

Schreier *et al.* analysed the volatile profiles of fresh redcurrant berries in 1977. The qualitative and quantitative differences between the volatile compositions of fresh fruits and the corresponding juices were investigated by liquid-liquid extraction (LLE). A total of 38 compounds were identified, among them alcohols, aldehydes, C_6-compounds, and terpenes. C_6-compounds are a subgroup of alcohols and aldehydes and are generated by enzymatic pathways (described in more detail in section 2.2.2). Butan-1-ol, (*E*)-pent-2-enal, and *p*-α-dimethylstyrene were identified as the three main compounds in fresh redcurrant fruits with concentrations of 70 µg/L, 61 µg/L, and 40 µg/L, respectively. The substances were determined in methanolic press juice (4.71 L methanolic press juice resulted from an extraction of 3 kg redcurrant berries). Relatively low concentrations of terpenes (e.g. myrcene: 23 µg/L and β-pinene: 12 µg/L) were quantified compared to blackcurrant berries, and no representatives of esters could be identified in fresh redcurrant fruits. Hydrocarbon compounds were only determined as traces in juice of redcurrant berries, while the concentrations of C_6-compounds were higher in juice than in fresh berries, especially in juices analysed without prior inhibition of enzymes (Schreier *et al.* 1977).

In a recent study, Boschetti *et al.* characterised the postharvest aging of different berries (blackberry, blueberry, redcurrant, raspberry, strawberry, and white currant) by monitoring the concentrations of volatile organic compounds (e.g. acetaldehyde, acetic acid, ethyl acetate, methyl acetate, ethanol, and methanol) via proton transfer reaction-mass spectrometry (PTR-MS). They observed a berry-specific trend in emission and relative concentrations of these compounds as well as their rates of evolution during aging (Boschetti *et al.* 1999).

Table 4: Volatile compounds identified in redcurrant fruits and juice, according to Mehlitz and Matzik (1956a, b), Schreier *et al.* (1977), and Boschetti *et al.* (1999).

compound	reference	compound	reference
C_6-compounds[a]		*alcohols*	
(E)-hex-2-en-1-ol	*b*	methanol[e]	*d*
(Z)-hex-3-en-1-ol	*b*	oct-1-en-3-ol	*b*
(E)-hex-2-enal	*b*	pent-1-en-3-ol	*b*
(Z)-hex-3-enal	*b*	pentan-1-ol	*b*
hexan-1-ol	*b*	pentan-2-ol	*b*
hexanal	*b*	pentan-3-ol	*b*
esters		*terpenes*	
ethyl acetate[e]	*d*	δ-cadinene	*b*
methyl acetate[e]	*d*	β-caryophyllene	*b*
		α-humulene	*b*
aldehydes		limonene	*b*
3-methylbutanal	*b*	myrcene	*b*
acetaldehyde[e]	*d*	β-phellandrene	*b*
benzaldehyde	*b*	β-pinene	*b*
(E)-dec-2-enal	*b*	γ-terpinene	*b*
decanal	*b*	terpinen-4-ol	*b*
(E)-non-2-enal	*b*		
nonanal	*b*	*acids*	
octanal	*b*	acetic acid	*c, d*
(E)-pent-2-enal	*b*	formic acid[f]	*c*
pentanal	*b*		
		others	
alcohols		*n*-butylbenzene	*b*
2-methylpropan-1-ol	*b*	*p*-α-dimethylstyrene	*b*
3-methylbutan-1-ol	*b*	2-pentylfuran	*b*
butan-1-ol	*b*	toluol	*b*
butan-2-ol	*b*	*p*-xylol	*b*
ethanol[e]	*d*		

[a] C_6-compounds are a subgroup of alcohols and aldehydes and are generated by enzymatic pathways described in more detail in section 2.2.2. [b] Schreier *et al.* (1977). [c] Mehlitz and Matzik (1956a, b). [d] Boschetti *et al.* (1999). [e] Only identified in redcurrant berries. [f] Only identified in redcurrant juice.

2.2 Aroma compounds – a brief introduction

The consumption of foods depends on its quality, which can be characterised by different aspects, such as appearance, colour, texture, flavour, and nutritional value. In particular, flavour is one of the most important quality properties (Jiang and Song 2010). The flavour perception of food consists of taste, trigeminal stimulation, and olfactory stimulation, each triggered by different compounds in the food. Taste is perceived in the mouth. It is a sensation, which is induced by non-volatile compounds and mediated by specialised receptor cells, primarily located on the tongue. It can be divided into the five basic taste qualities bitter, salty, sour, sweet, and umami. Other perceptions such as spicy-hot, cooling, and tingle also originate in the mouth through trigeminal stimulation. On the contrary, odour is perceived in the olfactory system through an interaction of volatile compounds, in this case also called aroma compounds, with the olfactory receptors (Reineccius 2006; Paravisini and Guichard 2016). This stimulation is described in more detail in section 2.2.1. Depending on their concentrations, many volatile compounds induce not only an olfactory stimulation but also a trigeminal stimulation (Gingras-Lessard and Frasnelli 2016). A classic example is (-)-menthol in peppermint, which has a cooling effect in addition to the typical peppermint odour (Lawless and Lee 1993).

So far, over 10,000 volatile compounds have been identified in foods. Depending on their odour thresholds and their concentrations in the evaluated food, each of them could potentially contribute to the aroma of foods (Jelen and Gracka 2016). To identify the key aroma compounds in foods Dunkel *et al.* (2014) performed a meta-analysis on the basis of a comprehensive literature survey. In total 226 key aroma compounds were identified in 227 food samples. This result indicates that less than 3 % of the 10,000 volatile compounds that occur in foods contribute to the specific aroma. Moreover, 16 volatile compounds were detected as key odour-active volatile compounds in more than 25 % of the 227 investigated food samples (Dunkel *et al.* 2014).

Odour-active volatile compounds belong to different classes of chemical organic compounds, including alcohols, acids, esters, phenolic compounds, carbonyl compounds, heterocyclic compounds, sulphur- and nitrogen-containing aliphatic compounds, terpenes, and norisoprenoids (Jelen and Gracka 2016). Their biosynthesis proceeds via different biochemical pathways, which are described in more detail in section 2.2.2.

2.2.1 Odour perception

The odour perception occurs at the olfactory mucosa (*Regio olfactoria*), which is part of the nasal cavity and contains between 10 and 25 million sensory cells with olfactory receptors. The olfactory process is initiated at the surface of the olfactory epithelium, when a volatile compound binds to an olfactory receptor. This induces a transduction cascade provoking the communication of a sensory response into higher brain regions (Mücke and Lemmen 2010; Tromelin 2016).

On the molecular level, a binding event of a volatile compound causes a conformational change of the corresponding olfactory receptor protein. This conformational change further induces a G protein-controlled signal transduction cascade, leading to the activation of adenylate cyclase, which converts adenosine-5´-triphosphate (ATP) to cyclic adenosine-3´,5´-monophosphate (cAMP), a second messenger in the signal cascade. The binding of cAMP to cation-selective ion channels causes an opening of the ion channels, allowing positively charged ions (Na^+ and Ca^{2+}) to influx into the cell interior. This reduces the membrane potential. The changes in membrane potential generate an electronical signal into the olfactory bulb (*Bulbus olfactorius*), where the single signals of the axons are bundled into the glomeruli and further sent to higher brain regions, such as the limbic system, for decoding. Two structures of the limbic system, the amygdala, which is responsible for emotional evaluations, and the hippocampus, which contains the memory centre, are critical for the reception of olfactory information (Herman 2002; Mücke and Lemmen 2010; Tromelin 2016).

For trained humans it is possible to differentiate between 10,000 distinct odours. However, until now, only approximately 400 different active olfactory receptors are known. Therefore, a simple one-to-one correspondence between receptor and aroma compound is not realistic. Generally, a single volatile molecule can bind to a multiplicity of olfactory receptor types, and vice versa, a single olfactory receptor can recognise multiple different volatile molecules but with variable sensitivity. Furthermore, it was proven that different aroma compounds are recognised by different combinations of olfactory receptors (Herman 2002; Gottfried 2010; Mücke and Lemmen 2010; Dunkel *et al.* 2014).

Aroma compounds can reach the olfactory epithelium by two pathways. If volatile compounds enter the olfactory region directly during sniffing via the nostrils, it is termed orthonasal perception. The term retronasal perception is used, if volatile molecules reach the olfactory epithelium via the mouth after eating or drinking (Hummel and Seo 2016). The in-mouth release of aroma compounds during consumption of food may be influenced by various factors and can be monitored by different *in vivo* methods, such as mass spectrometric methods (Burdach and Doty 1987; Romano 2016). The mass spectrometric *in vivo* methods most often applied are based on atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS) or PTR-MS (Romano 2016). Moreover, the transfer of aroma compounds from the oral to the nasal cavity during food consumption was studied by Büttner *et al.* by means of videofluoroscopy and real-time magnetic resonance imaging (MRI). It was shown that no continuous release of volatile compounds into the nasal cavity is possible due to the velum-tongue border. Oropharyngeal actions such as swallowing are necessary to allow or increase aroma perception. An influence of food texture and the amount of food material in the oral cavity on the aroma perception was also demonstrated (Büttner *et al.* 2001; Büttner *et al.* 2002). Additionally, an adsorption of volatile compounds and food matrix to the oral mucosa was shown (Büttner *et al.* 2002). In several other studies, the effect of salvia on volatile compounds and their precursors was analysed. No enzymatic degradation via salvia was observed for alcohols, methoxyphenols, methoxypyrazines, and sotolon. In contrast, the concentrations of aldehydes, esters, and thiols decreased in the presence of salvia (Büttner 2002a, b). Additionally, a transformation of cysteine-*S*-conjugates into free volatile thiols by bacterial enzymes could be shown (Starkenmann *et al.* 2008).

2.2.2 Biosynthesis of aroma compounds

Volatile compounds can be classified in primary and secondary compounds. Primary compounds are already present in the intact tissue and are formed through the anabolic and/or catabolic pathways of the plant. In contrast, secondary compounds are generated upon damage of intact cells by enzymatic, oxidative, or thermal processes from non-volatile compounds. In general, volatile compounds are biosynthesised from the three main precursors (i) amino acids, (ii) fatty acids, and (iii) carbohydrates via different biochemical pathways (Matheis 1991; Siegmund 2015).

Amino acids, such as alanine, aspartic acid, leucine, isoleucine, methionine, phenylalanine, and valine, are important direct precursors of the biosynthesis of different primary volatile compounds, i.e. aliphatic, branched and aromatic alcohols, acids, carbonyls, and esters (Sanz and Pérez 2010; El Hadi *et al.* 2013). The biosynthesis of these volatile compounds was proven in different isotopic and radioactive labelling studies, for example, in banana (Tressl and Drawert 1973) and apple (Rowan *et al.* 1996), as well as in studies with unlabelled precursors and is summarised in different articles (Sanz and Pérez 2010; El Hadi *et al.* 2013). In addition to representing important direct precursors of primary volatile compounds, amino acids are also indirect precursors of secondary volatile compounds via the enzymatic transformation of non-volatile glucosinolates and alk(en)yl cysteine sulfoxides upon cell distribution (Sanz and Pérez 2010).

In the fatty acid metabolism, volatile compounds are formed by two main pathways, the β-oxidation and the lipoxygenase (LOX) pathway (Sanz and Pérez 2010). Two other oxidation routes are the α-oxidation and the autoxidation (Jiang and Song 2010). The β-oxidation occurs in intact tissues, whereas volatile compounds are formed via the LOX pathway in disrupted tissues. However, there are some suggestions that with increasing ripeness the LOX pathway is also relevant in intact tissues. Reasons for this may be some shift in the LOX pathway activity or increasing membrane permeability followed by a higher availability of free fatty acids (Siegmund 2015). The importance of enzymes from the LOX pathway for the aroma development was demonstrated in different studies (Hatanaka and Harada 1973; Phillips *et al.* 1979; Salas *et al.* 1999), and the generation of volatile compounds, i.e. saturated and unsaturated C_6-, C_8-, and C_9-compounds, was verified in various fruits and vegetables (Tressl *et al.* 1981; Hsieh 1994). These compounds, which belong to the chemical groups of alcohols and aldehydes, are formed from linoleic acid and linolenic acid. As an example, the biosynthesis of C_6-compounds from linolenic acid is shown in Figure 1.

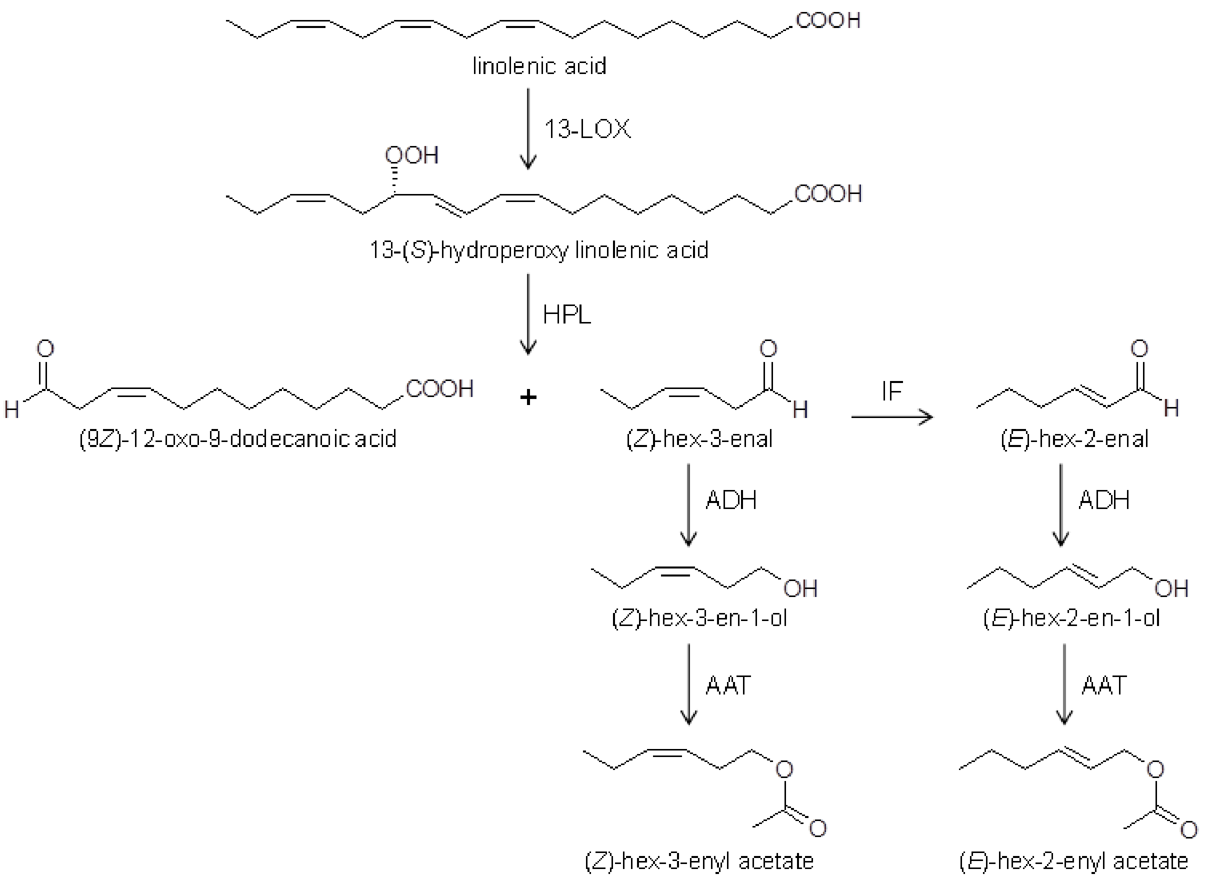

Figure 1: The 13-lipoxygenase (13-LOX) pathway based on linolenic acid as initial substrate; adapted with permission from Sanz and Pérez (2010) supplemented by Matsui (2006). HPL: hydroperoxide lyase; ADH: alcohol dehydrogenase; IF: isomerase factor; AAT: alcohol acyltransferase.

In the LOX pathway, unsaturated fatty acids are stereospecifically oxygenated by 9- or 13-LOX to either 9- (precursors of C_9-compounds) or 13- (precursors of C_6-compounds) hydroperoxy intermediates in an initial step, depending on the plant source and the isoenzymes present (Sanz and Pérez 2010). In tomato (Galliard and Matthew 1977), for example, a preferential generation of 9-hydroperoxy intermediates was proven and in grape (Cayrel *et al.* 1983) and apple (Grosch *et al.* 1977), a biosynthesis of 13-hydroperoxy intermediates was shown. In contrast, no preference was determined for gooseberries (Kim and Grosch 1978). In mushrooms, a generation of the C_8-compound oct-1-en-3-ol via the

10-hydroperoxy intermediate was demonstrated (Grosch and Wurzenberger 1985). In the case of 13-hydroperoxy intermediates, C_6-aldehydes and C_{12}-ω-keto fatty acids are formed in the next step by hydroperoxide lyase (HPL). The C_6-aldehyde (*Z*)-hex-3-enal can then be further isomerised to aldehydes such as (*E*)-hex-2-enal. Furthermore, C_6-aldehydes and their isomers can be metabolised by alcohol dehydrogenase (ADH) to the corresponding alcohols. Subsequently, the alcohol acyltransferase (AAT) can catalyse the formation of esters such as (*Z*)-hex-3-en-1-yl acetate from the reactants acetyl coenzyme A (CoA) and (*Z*)-hex-3-en-1-ol (Matsui 2006; Osorio *et al.* 2010).

The generation of volatile compounds via β-oxidation is based on a successive removal of two carbon atoms per cycle from the acetyl-CoA derivates of fatty acids. A detailed scheme is postulated by Sanz *et al.* (1997) and Baker *et al.* (2006). Depending on many different factors, this breakdown may be stopped at any point and the metabolites can further react in several enzymatically catalysed reactions to saturated and unsaturated esters, lactones, methylketones, alcohols, and acids (Siegmund 2015). Regarding lactones however, it is assumed that the LOX fatty acid pathway and the β-oxidation fatty acid pathway are both concertedly involved in the biosynthesis (Sanz *et al.* 1997). Two classical examples for fruits with β-oxidation as the main biosynthetic pathway for aroma compound generation are apple and pear (Paillard 1990).

Carbohydrates are direct precursors of two groups of volatile compounds, the terpenoids and the furanones, which are generated in different biosynthetic pathways. Furanones, for example, can be biosynthesised by several plants, such as pineapple and strawberry plants. Additionally, furanones can also be formed during Maillard reactions or fermentation processes (Siegmund 2015).

Terpenoids are the largest class of secondary metabolites and more than 40,000 different terpenoid-like molecules are known (Osorio *et al.* 2010). They are derivatives from the two universal C_5 precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). These substances are formed via two independent pathways from either acetyl-CoA (mevalonate (MVA) pathway) or pyruvate and glyceraldehyde-3-phosphate (methylerythritol phosphate (MEP) pathway). The MVA pathway provides precursors to sesquiterpenes (C_{15}) and triterpenes (C_{30}), while hemiterpenes (C_5), monoterpenes (C_{10}), and diterpenes (C_{20}) are generated via the MEP pathway (Dudareva *et al.* 2013; El Hadi *et al.* 2013). The generation of these substances involves a large family of enzymes, the terpene synthases, and is described in more detail by several articles, for example, by Sanz and Pérez (2010) and Dudareva *et al.* (2013). Many volatile terpenes are directly formed by these synthases, whereas others are further transformed by oxidation, dehydrogenation, acylation, and other reaction types, which is reviewed by Dudareva *et al.* (2004).

2.3 Analysis of aroma compounds

The analytical approach of aroma analysis involves as a first step an isolation of volatile compounds from other non-volatile constituents, which is described in more detail in section 2.3.1. The separation as well as the identification and quantification of the volatile compounds follow in a second step. The most important and efficient separation technique is gas chromatography (GC). An important detector for the identification of volatile compounds is the mass spectrometer (MS), while for the determination of odour-active compounds the coupling of olfactometric detection to gas chromatography (GC/O) is used in combination with different application techniques (see section 2.3.2) (Mehinagic and Le Quéré 2010).

2.3.1 Sample preparation methods – a brief overview

Volatile compounds are a group of substances, which are only present in low concentrations (below 1 part in 10^{12}) in foods. The chemical structures of volatile compounds are very diverse (Siegmund 2015). Their only common attribute is the volatility, which can vary considerably. Therefore, a multiplicity of isolation methods for volatile compounds from other food constituents, such as water, carbohydrates, lipids, proteins, minerals, or vitamins, were developed. These isolation methods are based on the principles of volatility and/or solubility (Reineccius 2010) and are summarised in numerous publications (Sides *et al.* 2000; Werkhoff *et al.* 2002; Augusto *et al.* 2003; Reineccius 2010). On the basis of model systems, the performance of different methods was investigated (Jennings and Filsoof 1977; Schultz *et al.* 1977; Leahy and Reineccius 1984; Prost *et al.* 1993). Additionally, several studies compared the results of the isolation of volatile compounds with different state-of-the-art methods from various matrices, for example, apple (Mehinagic *et al.* 2003), paprika oleoresin (Guadayol *et al.* 1997), parmesan cheese (Careri *et al.* 1994), tomato (Zhang *et al.* 2008), and whisky (Caldeira *et al.* 2007). In an investigation by Werkhoff *et al.* (1998), the comparison of four isolation methods, i.e. DHS, SDE, simultaneous distillation extraction at reduced pressure (SDEV), and vacuum-headspace extraction (VHS), showed that the most representative and typical extract of passion fruit was obtained by means of VHS. In a further study, this result was confirmed by analysing various fruits, such as pear and strawberries (Güntert *et al.* 1998). Another common gentle method for isolation of volatile compounds from complex matrices is the solvent-assisted flavour evaporation (SAFE) method developed by Engel *et al.* (1999). In general, the results of the studies mentioned above showed qualitative and quantitative differences in dependence of the isolation method and demonstrated that no universal method exists. Therefore, it is important to consider the objectives of the analysis, when selecting an isolation method (Reineccius 2010).

Sulphur-containing compounds are for example important compounds with respect to aroma isolation due to their low odour thresholds. They often occur only in low concentrations in different food materials but contribute significantly to the characteristic aroma of many foods. Thus, several different methods for separation and concentration of sulphur-containing compounds were developed (Blank 2002; Rouseff 2002). In 1994, Full and Schreier described, for the first time, an isolation method, which enabled a separation of thiols from other compounds based on an enrichment step of thiols on mercurated agarose gel (Full and Schreier 1994). Another efficient method is based on the trapping of thiols with *p*-hydroxymercuribenzoic acid (Blank 2002). This method was, for example, applied to coffee in order to isolate six sulphur-containing compounds, among others 4-methoxy-2-methyl-2-butanethiol (Poisson *et al.* 2004; Kerler *et al.* 2005). This substance, which was identified in blackcurrant buds (see section 2.1.3.1), was also isolated with the method by Full and Schreier (1994) from olive oil via enrichment on mercurated agarose gel (Reiners and Grosch 1998; 1999).

2.3.2 Methods for sensory evaluation

The aroma of different foods consists of a complex mixture of several hundred volatile compounds. Nevertheless, in many cases ten to 30 volatile compounds most dominantly contribute to the sensory perception (Reineccius 2010). In mango, for example, more than 300 different volatile compounds were identified, but only 15 were necessary to imitate the authentic aroma profile (Pino *et al.* 2005; Munafo *et al.* 2016).

A valuable method for identification of aroma-active compounds is GC/O, proposed by Fuller *et al.* (1964). It is a combination of instrumental-analytical and sensorial methods. Volatile compounds are sensorially evaluated via a sniffing port after capillary gas chromatographic separation (Brattoli *et al.* 2013). For the evaluation of the relative importance of aroma compounds, different GC/O techniques were developed and are summarised in various publications (van Ruth 2001; Delahunty *et al.* 2006; d'Acampora Zellner *et al.* 2008; Brattoli *et al.* 2013). Van Ruth (2001) and d'Acampora Zellner *et al.* (2008) classified these techniques into four main categories: (i) dilution analysis methods, (ii) detection frequency methods, (iii) posterior intensity methods, and (iv) time-intensity methods.

The dilution analysis methods are the techniques most often applied in order to evaluate the relative odour potency of volatile compounds in an extract. They are based on a successive dilution of an extract until no odour is recognisable anymore. Two separate dilution analysis methods were developed by two different research groups. The aroma extract dilution analysis (AEDA) was first proposed by Ullrich and Grosch (1987) and the combined hedonic aroma response method (CHARM) by Acree *et al.* (1984). In the AEDA, the diluted extracts are investigated with an increasing dilution order until no odour is detectable. The results are reported in the flavour dilution (FD) factors. The FD factors are calculated on the basis of the dilution levels at which an odour is still detectable. With CHARM, on the contrary, the extracts are analysed in a random order and the duration of odour perception is measured. Each odour is then reported using the CHARM values (van Ruth 2001; d'Acampora Zellner *et al.* 2008; Brattoli *et al.* 2013).

The detection frequency method was first proposed by Linssen *et al.* (1993) and further developed and formalised by Pollien *et al.* (1997). In this method, a panel of six to twelve participants analyse an extract under the same GC/O conditions. The intensity of an odour is assessed by the proportion of the panel that is able to detect the odour and is described using nasal impact frequency (NIF) values or surface of nasal impact frequency (SNIF) values (Delahunty *et al.* 2006; Brattoli *et al.* 2013).

In the posterior intensity method, participants assess the odour intensity based on a scale after the compounds eluted. On the contrary, in the method termed Osme, a time-intensity method, the odour intensity and the duration are directly recorded while a compound is eluting. In this method, the intensity of the odour is assessed by a trained panel based on a 16 point scale (van Ruth 2001).

The screening of odour-active compounds in foods can also be evaluated with the OAV concept, a method that was developed by Rothe and Thomas (1963) and is often used in combination with GC/O. The OAV is defined as the ratio of the concentration of a volatile compound to its odour threshold. A volatile compound with an OAV ≥ 1 contributes to the aroma of the analysed food, whereas a compound with a concentration below the odour threshold (OAV < 1) is expected to be not odour-active (d'Acampora Zellner *et al.* 2008; Reineccius 2010).

The odour threshold is defined as the lowest concentration that is recognisable by the panellists and can be influenced by several factors (Ohloff 1990; d'Acampora Zellner *et al.* 2008). For example, an interaction of different classes of non-volatile compounds such as alcohols, carbohydrates, lipids, proteins, polyphenols, and salts with aroma compounds was observed (Paravisini and Guichard 2016).

However, with the OAV concept and all the different GC/O-based methods, only single compounds can be identified and no information about their behaviour in a mixture is obtained. Therefore, reconstitution experiments in a food matrix constitute an important final step of sensory evaluation and can be used to estimate the correct selection of odour-active compounds by combining potential odour-active compound candidates in a simplified aroma model. After a good correlation of the aroma model to the original food, further experiments, the so-called omission experiments, can be performed. In this type of experiments, one or more compounds are omitted from the original aroma model and the omission model is then compared to the overall odour of the aroma model without omission. In this way, the compounds, which contribute to the aroma profile, can be identified among other potentially potent aroma compounds (Grosch 2001; van Ruth 2001).

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant materials

Blackcurrant, redcurrant, and white currant fruits were purchased from local grocery stores or directly obtained from the grower. The origin of every single batch is listed in Table 5, Table 6, and Table 7 together with the date of harvest or the date of purchase, respectively. The berries were stored at 4 °C and brought to room temperature before analysis. Some of the blackcurrant and redcurrant fruit batches were additionally stored for three, six, nine, and twelve months at -20 °C and thawed overnight before analysis.

Table 5: List of analysed blackcurrant berries.

origin/cultivar	date of harvest	date of purchase	harvested at/ purchased from
Freising, Germany			
'Andega'	01/06/2014	-[a]	Allotment Garden of the University of Applied Sciences Weihenstephan-Triesdorf
'Andega'	13/07/2015	-	Allotment Garden of the University of Applied Sciences Weihenstephan-Triesdorf
'Andega'	16/07/2015	-	Allotment Garden of the University of Applied Sciences Weihenstephan-Triesdorf
Deutenkofen, Germany			
'8 Bona'	20/06/2014	-	Lehr- und Beispielsbetrieb für Obstbau
'8 Bona'	29/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau
'Ben Sarek'	20/06/2014	-	Lehr- und Beispielsbetrieb für Obstbau
'Ben Sarek'	03/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
'Rosenthals'	20/06/2014	-	Lehr- und Beispielsbetrieb für Obstbau
'Rosenthals' (unripe)	17/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau
'Rosenthals'	03/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
'Silvergieters'	20/06/2014	-	Lehr- und Beispielsbetrieb für Obstbau
'Silvergieters'	29/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau
'Supernova'	20/06/2014	-	Lehr- und Beispielsbetrieb für Obstbau
'Supernova'	29/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau
'Supernova'	03/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
'Titania'	20/06/2014	-	Lehr- und Beispielsbetrieb für Obstbau
'Titania'	03/07/2014	-	Lehr- und Beispielsbetrieb für Obstbau
'Titania' (unripe)	11/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau
'Titania' (unripe)	17/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau
'Titania' (unripe)	23/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau
'Titania'	29/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau

Table 5: Continued.

origin/cultivar	date of harvest	date of purchase	harvested at/ purchased from
‘Titania’	03/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Titania’	14/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Tsema’	20/06/2014	-	Lehr- und Beispielsbetrieb für Obstbau
‘Tsema’	29/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Ometa’	03/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Ometa’	20/06/2014	-	Lehr- und Beispielsbetrieb für Obstbau
Bühl, Germany			
‘Supernova’[b]	-	22/07/2014	Früchtehandel Roth
‘Supernova’	-	20/07/2015	Früchtehandel Roth
‘Tsema’	-	04/08/2015	Früchtehandel Roth
Oberkirch, Germany			
‘Supernova’	-	04/07/2016	Obstgroßmarkt Mittelbaden eG
‘Tenah’	-	28/07/2015	Obstgroßmarkt Mittelbaden eG
Tettnang, Germany			
unknown cultivar	-	10/07/2014	BayWa AG
unknown cultivar	-	12/07/2014	BayWa AG
Gleinstätten, Austria			
‘Tenah’[b]	-	24/07/2014	Fritz Oswald GmbH
‘Tenah’	-	29/07/2014	Fritz Oswald GmbH

[a] Not applicable. [b] After sample preparation and weighing, parts (500 g each) of this batch were additionally stored at -20 °C.

Table 6: List of analysed redcurrant berries.

origin/cultivar	date of harvest	date of purchase	harvested at/ purchased from
Freising, Germany			
‘Jonkheer van Tets’	16/06/2014	-[a]	Allotment Garden of the University of Applied Sciences Weihenstephan-Triesdorf
‘Red Poll’	25/07/2014	-	Allotment Garden of the University of Applied Sciences Weihenstephan-Triesdorf
‘Red Poll’	16/07/2015	-	Allotment Garden of the University of Applied Sciences Weihenstephan-Triesdorf
‘Roodneus’	18/07/2014	-	Allotment Garden of the University of Applied Sciences Weihenstephan-Triesdorf
‘Roodneus’	16/07/2015	-	Allotment Garden of the University of Applied Sciences Weihenstephan-Triesdorf
‘Rovada’	18/07/2014	-	Allotment Garden of the University of Applied Sciences Weihenstephan-Triesdorf

Table 6: Continued.

origin/cultivar	date of harvest	date of purchase	harvested at/ purchased from
‘Rovada’	13/07/2015	-	Allotment Garden of the University of Applied Sciences Weihenstephan-Triesdorf
Deutenkofen, Germany			
‘Jonkheer van Tets’	20/06/2014	-	Lehr- und Beispielsbetrieb für Obstbau
‘Jonkheer van Tets’	29/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Junifer’	29/06/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Red Lake’	10/07/2014	-	Lehr- und Beispielsbetrieb für Obstbau
‘Red Lake’	08/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Rosetta’	03/07/2014	-	Lehr- und Beispielsbetrieb für Obstbau
‘Rosetta’	10/07/2014	-	Lehr- und Beispielsbetrieb für Obstbau
‘Rosetta’ (unripe)	03/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Rosetta’	14/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Rosetta’	20/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Rovada’	10/07/2014	-	Lehr- und Beispielsbetrieb für Obstbau
‘Rovada’ (unripe)	03/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
‘Rovada’	14/07/2015	-	Lehr- und Beispielsbetrieb für Obstbau
Fahrenzhausen, Germany			
‘Rovada’	01/07/2014	-	Hofladen Obsthof Bauer
‘Telak’	01/07/2014	-	Hofladen Obsthof Bauer
Weinsberg, Germany			
‘Maraton’	02/07/2014	-	Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg
‘Red Poll’	31/07/2013	-	Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg
‘Red Poll’	19/07/2014	-	Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg
‘Roodneus’	29/07/2013	-	Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg
‘Roodneus’	19/07/2014	-	Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg
‘Rovada’	30/06/2014	-	Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg
‘Rovada’	15/07/2014	-	Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg
‘Rovada’	21/07/2014	-	Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg
‘Tatran’	15/07/2014	-	Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg
Ladenburg, Germany			
‘Rovada’	-	28/06/2014	D. & B. Hege GbR

Table 6: Continued.

origin/cultivar	date of harvest	date of purchase	harvested at/ purchased from
Oberkirch, Germany			
‘Rovada’	-	01/08/2016	Obstgroßmarkt Mittelbaden eG
Gleinstätten, Austria			
‘Rovada’	-	29/07/2013	Fritz Oswald GmbH
‘Rovada’	-	05/08/2013	Fritz Oswald GmbH
‘Rovada’[b]	-	12/08/2013	Fritz Oswald GmbH
‘Rovada’	-	04/08/2014	Fritz Oswald GmbH
‘Rovada’	-	05/08/2014	Fritz Oswald GmbH
‘Rovada’	-	06/08/2014	Fritz Oswald GmbH
Marbach-Rieslingshausen, Germany			
unknown cultivar	-	27/07/2015	Obstbau Stirm GbR
Tettnang, Germany			
‘Rovada’[b]	-	12/08/2013	BayWa AG
unknown location			
unknown cultivar	-	03/08/2015	Schweiger’s Früchte
Weisenheim/Sand, Germany			
‘Rovada’	-	10/08/2015	BayWa AG

[a] Not applicable. [b] After sample preparation and weighing, parts (500 g each) of this batch were additionally stored at -20 °C.

Table 7: List of analysed white currant berries.

origin/cultivar	date of harvest	harvested at
Deutenkofen, Germany		
‘Weißer Versailler’	08/07/2015	Lehr- und Beispielsbetrieb für Obstbau
‘Zitavia’	03/07/2015	Lehr- und Beispielsbetrieb für Obstbau

3.1.2 Chemicals

Authentic reference chemicals were purchased from Alfa Aesar (Karlsruhe, Germany), Fluka (Steinheim, Germany), Merck (Darmstadt, Germany), SAFC (Steinheim, Germany), and Sigma-Aldrich (Steinheim, Germany) or provided by Frey+Lau GmbH (Henstedt-Ulzburg, Germany) and Silesia (Neuss, Germany). The internal standards heptan-1-ol and 2-methyl-5,6-diethylpyrazine were provided by Frey+Lau GmbH (Henstedt-Ulzburg, Germany), heptan-2-ol was purchased from Fluka (Steinheim, Germany), and 4-methyl-4-sulfanylpentan-2-one from Alfa Aesar (Karlsruhe, Germany). Additional chemicals used are listed in Table 8.

Table 8: List of used chemicals.

substance	supplier
Affi-Gel® 10	Bio-Rad, Munich, Germany
2-aminoethanol (≥ 98 %)	Merck-Schuchard, Hohenbrunn, Germany
ascorbic acid (AnalaR Normapur)	VWR, Leuven, Belgium
calcium chloride dihydrate (p.a.)	Sigma-Aldrich, Steinheim, Germany
citric acid (p.a.)	Bernd Kraft, Duisburg, Germany
chloroform-d ($CDCl_3$) (99.96 atom % D)	Sigma-Aldrich, Steinheim, Germany
dichloromethane (p.a.)	Merck, Darmstadt, Germany
diethyl ether (p.a.)	Merck, Darmstadt, Germany
dimethylformamide (DMF) (pure)	Amresco, Solon, USA
DL-dithiothreitol (≥ 98 %)	Sigma, Steinheim, Germany
ethanol (p.a.)	Sigma-Aldrich, Steinheim, Germany
fructose (≥ 99 %)	Sigma-Aldrich, Steinheim, Germany
glucose (≥ 99.5 %)	Sigma-Aldrich, Steinheim, Germany
indole-3-acetic acid (98 %)	Sigma-Aldrich, Steinheim, Germany
malic acid (puriss.)	Fluka, Steinheim, Germany
methanol (HiPerSolv Chromanorm)	VWR, Fontenay-sous-Bois, France
n-pentane (p.a.)	VWR, Fontenay-sous-Bois, France
p-aminophenylmercuri acetate (≥ 90 %)	Sigma, Steinheim, Germany
propan-2-ol (p.a.)	Merck, Darmstadt, Germany
sodium sulphate, anhydrous (p.a.)	VWR, Leuven, Belgium
sucrose (≥ 99 %)	Sigma-Aldrich, Steinheim, Germany
sulfuric acid (98 %)	Merck, Darmstadt, Germany
tartaric acid (AnalaR Normapur)	VWR, Leuven, Belgium

The solvents diethyl ether and *n*-pentane were distilled via a packed column prior to use.

Synthesis and NMR spectroscopy of methyl indole-3-acetate

In order to synthesise methyl indole-3-acetate, 1 mL methanol, acidified with sulfuric acid (pH 2), was added to 175 mg indole-3-acetic acid and the mixture was stirred for 24 h at 63 °C under reflux. Subsequently, the mixture was cooled to room temperature and 10 mL of diethyl ether were added. The organic phase was washed with water (2 x 1 mL), dried over sodium sulphate, and the solvent was evaporated. Mass spectrometric and NMR data were in accordance with those from the National Institute of Standards and Technology (NIST) database and Tobisu *et al.* (2010), respectively. For the NMR spectroscopy analysis, $CDCl_3$ was used as solvent. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz and 126 MHz, respectively, with Avance500 spectrometers. ^{1}H-detected experiments were done with an inverse ^{1}H/^{13}C probehead. Direct ^{13}C-measurements were performed with a QNP ^{13}C/^{31}P/^{29}Si/^{19}F/^{1}H cryoprobe. The experiments were done in full automation using standard parameter sets of the TOPSPIN 3.0 software package (Bruker, Bremen, Germany). ^{13}C NMR spectra were recorded in proton-decoupled mode. The spectra were recorded at 27 °C. Data processing was done with the MestreNova software.

3.2 Methods

3.2.1 Sample preparation

The isolation of volatile constituents of currant berries was performed after removal of the peduncles of fresh berries that had been brought to room temperature. Frozen berries, that had been stored at -20 °C, were thawed overnight before the isolation of volatile constituents was conducted.

Volatile constituents were isolated via VHS from blackcurrant berries and via LLE from redcurrant and white currant berries.

3.2.1.1 Vacuum-headspace extraction (VHS)

Five hundred grams of blackcurrant fruits were homogenised (30 s) in a laboratory blender (Moulinex Turbo blender) with 400 mL of water and heptan-2-ol as internal standard (150 µg). The homogenate was transferred into a 2 L round bottom flask, and the laboratory blender was rinsed with 150 mL of water, which was additionally transferred into the 2 L round bottom flask. The flask was placed into a water bath at approximately 35 °C, and after connecting to a vacuum pump (type D4A, Leybold-Heraeus, Cologne, Germany), the isolation was performed at 1-10 mbar for 2 h according to Werkhoff *et al.* (1998). The aqueous distillate was condensed in three cooling traps, which were cooled by a water-ice-mixture (I and II) and liquid nitrogen (III). The aqueous, frozen condensates were thawed, pooled, and extracted three times with 50 mL of a mixture of diethyl ether and *n*-pentane

(1:1; v/v). After drying over anhydrous sodium sulphate, the extract was concentrated at 40 °C to 1 mL using a Vigreux column. All extracts were further concentrated to a final volume of 0.5 mL under a gentle nitrogen flow and analysed within ten days after extraction by capillary gas chromatography and by capillary gas chromatography-mass spectrometry. The storage of concentrated extracts occurred at -18 °C. This isolation of volatiles via VHS was performed in triplicate for each batch of blackcurrant berries.

For isolations involving the inhibition of enzymes, 400 mL of saturated calcium chloride solution was added 30 s, 60 s, 90 s, and 180 s after the homogenisation had started, respectively, instead of water.

For identification and quantification of the sulphur-containing target compound 4-methoxy-2-methyl-2-butanethiol, 4-methyl-4-sulfanylpentan-2-one was used in addition to heptan-2-ol as internal standard (1,900 ng for ‘Andega’ and 50 ng for all other cultivars) and the isolation via VHS was followed by an enrichment step on mercurated agarose gel as described below.

3.2.1.2 Liquid-liquid extraction (LLE)

LLE was carried out with 500 g of redcurrant or white currant berries in a Kutscher-Steudel extractor (Wieland and Gattermann 1982). The fruits were homogenised (30 s) in a laboratory blender (Moulinex Turbo blender) with 75 µg of heptan-1-ol as internal standard. 400 mL of saturated calcium chloride solution were added after additional 150 s and the suspension was homogenised for additional 10 s. Subsequently, the blender was rinsed with 100 mL of water, which was collected and combined with the homogenate. The homogenate was centrifuged (5 min, 2500 rpm) and the supernatant was transferred into a liquid-liquid extractor. A suspension of the residue and 150 mL of water was further centrifuged; the supernatant was additionally transferred into the extractor and filled up with water to a final volume of 1 L. Volatiles were extracted for 24 h using 150 mL of a mixture of *n*-pentane and diethyl ether (1:1; v/v) as solvent. After drying over anhydrous sodium sulphate, the extract was concentrated at 40 °C to 1 mL using a Vigreux column. The extract was further concentrated to a final volume of 0.5 mL under a gentle nitrogen flow and analysed within eleven days after extraction by capillary gas chromatography and capillary gas chromatography-mass spectrometry. The storage of concentrated extracts occurred at -18 °C.

To follow the impact of enzymatic reactions on the formation of C_6-compounds over time, an enzyme inhibition was carried out 30 s, 60 s, 90 s, and 180 s after the homogenisation had started, respectively, by adding 400 mL of saturated calcium chloride solution.

3.2.1.3 Enrichment on mercurated agarose gel

For the preparation of the mercurated agarose gel, 100 mL of Affi-Gel® 10 were washed with 300 mL of anhydrous 2-propanol. *p*-Aminophenylmercuric acetate (1.5 g dissolved in 30 mL DMF) was added to the washed Affi-Gel® 10 and stirred at ambient temperature for 4 h. After addition of 1 mL 2-aminoethanol, the mixture was again stirred for 1 h and washed with DMF (250 mL) and propan-2-ol (700 mL). After the final washing step, the mercurated agarose gel was kept under propan-2-ol (300 mL) at 4 °C.

For the enrichment step, approximately 2 mL of mercurated agarose gel were transferred into a Pasteur pipet and conditioned with propan-2-ol (5 mL) and a mixture of *n*-pentane and dichloromethane (2:1; v/v; 5 mL). Three to six VHS-extracts were combined, filled up to 10 mL with a diethyl ether/*n*-pentane mixture (1:1; v/v) and passed through the column. After washing with a mixture of *n*-pentane/dichloromethane (2:1; v/v; 25 mL), the thiols were eluted with 4 mL DL-dithiothreitol-solution (10 mM in a diethyl ether/*n*-pentane mixture (1:1; v/v)). This extract was washed with 1 mL of water, dried over anhydrous sodium sulphate, concentrated to a final volume of 0.1 mL under a gentle nitrogen flow, and analysed by capillary gas chromatography-mass spectrometry in selected ion monitoring (SIM) mode.

3.2.2 Identification

Volatile constituents were analysed by capillary gas chromatography (see section 3.2.3.1) and capillary gas chromatography-mass spectrometry (see section 3.2.2.2). The identifications were carried out by comparison of mass spectrometric and capillary gas chromatographic data (retention indices) to those of authentic reference compounds.

3.2.2.1 Determination of retention indices (RIs)

For determination of retention indices (RIs), alkane series (*n*-alkanes) were used as standard compounds. RIs were calculated based on the retention times of *n*-alkanes and unknown or reference substances, respectively, as described in equation 1 (Kováts 1958).

$$\mathrm{RI} = 100 \times \left[\mathrm{N} + \frac{\mathrm{RT} - \mathrm{RT_n}}{\mathrm{RT_{n+1}} - \mathrm{RT_n}}\right] \qquad (1)$$

RI	retention indices
RT	retention time of unknown or reference substance
$\mathrm{RT_n}$	retention time of *n*-alkane
$\mathrm{RT_{n+1}}$	retention time of *n*+1-alkane
N	number of carbon atoms of *n*-alkane

The determinations of RIs were performed on the Carlo Erba HRGC Mega II 8575 system (see section 3.2.3.1) and for 4-methoxy-2-methyl-2-butanethiol on the GC 8000^{TOP} system (see section 3.2.2.2).

3.2.2.2 Gas chromatography-mass spectrometry (GC-MS)

<u>GC-MS conditions:</u>

Instrument:	GC 8000^{TOP} interfaced with a Voyager GC-MS (Thermo Fisher Scientific, Dreieich, Germany)
Capillary columns:	DB-WaxEtr (30 m x 0.25 mm i.d., 0.5 µm film thickness; J&W Scientific) DB1 (30 m x 0.25 mm i.d., 1 µm film thickness; J&W Scientific) RTX-200MS (30 m x 0.32 mm i.d., 1 µm film thickness; Restek)
Temperature programs:	blackcurrant: 40 °C/ 5 min// 4 °C/min/ 240 °C/ 35 min redcurrant and white currant: 40 °C/ 5 min// 4 °C/min/ 240 °C/ 65 min
Carrier gas:	helium (5.0); constant inlet pressure (75 kPa)
Injector:	split/splitless injector (220 °C); split ratio 1:50; injection volume 1 µL
Detector:	quadrupole mass filters in EI-mode; ionisation energy: 70 eV; source temperature: 200 °C; interface temperature: 240 °C

Data acquisition was done with the software Xcalibure, version 1.4 (Thermo Fisher Scientific).

3.2.3 Quantification

Quantification of volatile constituents in blackcurrant berries was based on heptan-2-ol as internal standard; 1 mL of a 1:5 diluted stock solution (0.150 g/200 mL of water) was added to blackcurrant berries before homogenisation and extraction of volatile constituents occurred. For redcurrant and white currant berries heptan-1-ol was used as internal standard; 0.5 mL of a 1:5 diluted stock solution (0.150 g/200 mL of water) was added to redcurrant and white currant berries before homogenisation and extraction of volatile constituents. Preceding screenings had shown that heptan-2-ol is not present in blackcurrant fruits as well as heptan-1-ol in redcurrant fruits. Quantifications were carried out on the Carlo Erba HRGC Mega II 8575 system (see section 3.2.3.1) taking into account response factors and recovery rates. Substances with a recovery rate less than 25 % were not quantified. For isolation via VHS acids were generally not quantified, because of their low recoveries.

$$c_X = \frac{A_X \times RF_X \times M_S}{A_S \times M \times R} \qquad (2)$$

c_x	concentration of substance x
A_X	peak area of substance x
RF_X	response factor of substance x
M_S	mass of added internal standard
A_S	peak area of internal standard
M	mass of analysed berries
R	recovery rate of substance x

The quantification of odour-active compounds in blackcurrant and redcurrant berries, which were present below the limits of quantification using GC/FID, was performed via GC-MS (see section 3.2.3.1) in the SIM mode. Ten extracts obtained via VHS (‘Supernova’, 4 July, 2016, Oberkirch) of blackcurrant fruits were pooled, concentrated to a volume of 0.25 mL, and analysed using 740 ng heptan-2-ol and 51 ng 2-methyl-5,6-diethylpyrazine (for the pyrazines), respectively, as internal standards. For the analysis of redcurrant berries, twelve extracts obtained by LLE (‘Rovada’, 1 August, 2016, Oberkirch) were pooled, concentrated to a volume of 0.25 mL, and analysed using 760 ng of heptan-1-ol and 50 ng of 2-methyl-5,6-diethylpyrazine, respectively, as internal standards. A preceding screening had shown that 2-methyl-5,6-diethylpyrazine is not present in blackcurrant and redcurrant fruits.

Quantification of 4-methoxy-2-methyl-2-butanethiol in blackcurrant berries was carried out on the GC 8000^{TOP} system (see section 3.2.3.1) based on 4-methyl-4-sulfanylpentan-2-one as internal standard in SIM mode. A preceding screening had shown that this compound is not present in blackcurrant fruits. The response factors and the relative recovery rates via VHS and the enrichment step on mercurated agarose gel were considered as described in the equation 3.

$$c_X = \frac{A_X \times RF_X \times M_S}{A_S \times M \times R_V \times R_M} \quad (3)$$

c_X	concentration of 4-methoxy-2-methyl-2-butanethiol
A_X	peak area of 4-methoxy-2-methyl-2-butanethiol
RF_X	response factor of 4-methoxy-2-methyl-2-butanethiol
M_S	mass of added internal standard (4-methyl-4-sulfanylpentan-2-one)
A_S	peak area of internal standard (4-methyl-4-sulfanylpentan-2-one)
M	mass of analysed berries
R_V	recovery rate in VHS
R_M	recovery rate in the enrichment step on mercurated agarose gel

For the quantification in the SIM mode, characteristic fragment ions were used (Table 9).

Table 9: Characteristic fragment ions for the quantification in the SIM mode.

compound	quantifier ion [*m*/*z*]	qualifier ions [*m*/*z*]
(*E*)-non-2-enal	55	111, 122
(*E*)-β-damascenone	69	121, 190
(*Z*)-rose oxide	139	140, 154
2-isobutyl-3-methoxypyrazine	124	94, 151
2-isopropyl-3-methoxypyrazine	137	124, 152
2-methoxy-3-methylpyrazine	124	106, 109
2-methyl-5,6-diethylpyrazine	150	135, 149
2-*sec*-butyl-3-methoxypyrazine	138	124, 151
4-methoxy-2-methyl-2-butanethiol	85	100, 134
geraniol	69	123, 154
heptan-1-ol	56	69, 83
heptan-2-ol	45	83, 98
methyl pentanoate	74	85, 87
oct-1-en-3-one	55[a] 70[b]	70, 97[a] 56, 111[b]
pent-1-en-3-one	55	29, 57
γ-decalactone	85	100, 128

[a] Used for the quantification in blackcurrant berries. [b] Used for the quantification in redcurrant berries.

3.2.3.1 Gas chromatography (GC)

GC conditions:

Instrument:	Carlo Erba HRGC Mega II 8575 (Thermo Fisher Scientific, Dreieich, Germany)
Capillary column:	DB-Wax (60 m x 0.32 mm i.d., 0.25 µm film thickness; J&W Scientific)
Temperature program:	40 °C/ 5 min// 4 °C/min/ 240 °C/ 25 min
Carrier gas:	hydrogen (5.0); constant inlet pressure (110 kPa)
Injector:	split/splitless injector (215 °C); split ratio 1:10; injection volume 1 µL
Detectors:	FID (235 °C) and FPD (235 °C); eluentsplit via press-fit t-piece 1:1

Data acquisition was done with Chromcard software, version 2.5 (Thermo Fisher Scientific).

3.2.3.2 Determination of response factors

The response factors were determined with solutions of authentic reference compounds relative to the internal standards heptan-1-ol, heptan-2-ol, 2-methyl-5,6-diethylpyrazine, and 4-methyl-4-sulfanylpentan-2-one (0.1 µg/µL in diethyl ether), respectively.

$$\mathrm{RF_x} = \frac{\mathrm{A_S}}{\mathrm{A_X}} \qquad (4)$$

RF_x	response factor of substance x
A_S	peak area of internal standard
A_X	peak area of substance x

3.2.3.3 Determination of recovery rates

Recovery rates of volatile constituents occurring in blackcurrant berries

Recovery rates were determined in triplicate from aqueous solutions or assumed from previous studies (Schrade 2014); 100 µL of stock solution (3 mg/mL of reference and heptan-2-ol in ethanol) was added to 1 L of water and isolated via VHS. In addition, one aliquot of the stock solution was diluted (1:9) with diethyl ether and directly analysed via capillary gas chromatography. The relative recovery rate of a specific substance was calculated based on the comparison of the peak areas of the substance and the internal standard before and after isolation, as described in equation 5.

$$R = \frac{A_{XI} \times A_{SO}}{A_{XO} \times A_{SI}} \qquad (5)$$

R	recovery rate of substance x
A_{XI}	peak area of substance x after isolation
A_{XO}	peak area of substance x before isolation
A_{SI}	peak area of internal standard after isolation
A_{SO}	peak area of internal standard before isolation

Recovery rates of 4-methoxy-2-methyl-2-butanethiol

The recovery rates of the VHS isolation and the enrichment step on mercurated agarose gel were determined in triplicate for 0.3 mg/mL, 0.6 mg/mL, and 3 mg/mL in ethanol (100 µL of a stock solution, a 1:5- or a 1:10-diluted stock solution were spiked to 1 L of water for VHS or to 10 mL of a diethyl ether/*n*-pentane mixture (1:1; v/v) for the enrichment on mercurated agarose gel). Calculations were performed according to equation 5.

Recovery rates of volatile constituents occurring in redcurrant and white currant berries

Recovery rates were determined in triplicate from aqueous solutions; 100 µL of stock solution (3 mg/mL of reference and heptan-1-ol in ethanol) was added to 600 mL of water and 400 mL of saturated calcium chloride solution. Before isolation via LLE, 30 min were allowed to elapse after adding the stock solution to imitate the centrifugation step. Recovery rates of C_6-compounds were determined using separate stock solutions of the individual substances. For some substances, recovery rates were determined in 500 mL of a redcurrant-type matrix (the composition of the redcurrant-type matrix is described below) instead of water. Calculations were performed according to equation 5.

Composition of the redcurrant-type matrix

The redcurrant-type matrix was prepared according to the concentrations of sugars and organic acids described by Haila *et al.* (1992), Mikulic-Petkovsek *et al.* (2012b), and Nour *et al.* (2011): 22 g/L fructose, 17.5 g/L glucose, 600 mg/L sucrose, 20 g/L citric acid, 2.9 g/L malic acid, 350 mg/L tartaric acid, and 330 mg/L ascorbic acid.

3.2.3.4 Determination of limits of detection (LOD) and limits of quantification (LOQ)

The limits of detection (LOD) and the limits of quantification (LOQ) were determined using the method of Vogelgesang and Hädrich (1998). Five different concentrations of octanal, (*E*)-oct-2-enal, ethyl hexanoate, methyl 3-hydroxybutanoate, and pent-1-en-3-ol in diethyl ether ranging between 780 ng/mL and 12,500 ng/mL were analysed in triplicate (Carlo Erba

HRGC Mega II 8575 system), and by determining a calibration curve, the LOD and LOQ were calculated (assumption: recovery rate and response factors = 1) as described in the equations 6, 7, and 8.

The residual standard deviation s_y is calculated by setting up a calibration line y = a + bx and using equation 6.

$$s_y = \sqrt{\frac{\sum_{i=1}^{n}(y_i-(a+bx_i))^2}{n-2}} \qquad (6)$$

a	y intercept
b	slope
n	number of measured values
y_i	peak area of sample i
x_i	fortification concentration of sample i
i	index of calibration analyses

This results in the LOD:

$$\mathrm{LOD} = \frac{s_y}{b} \times t_{f;a} \times \sqrt{1 + \frac{1}{n} + \frac{\overline{x}^2}{\sum_{i=1}^{n}(x_i-\overline{x})^2}} \qquad (7)$$

and the LOQ:

$$\mathrm{LOQ} = \frac{\left(\left(\overline{y} + b\,(\mathrm{ID}-\overline{x}) + s_y \times t_{f;a} \times \sqrt{1 + \frac{1}{n} + \frac{(\mathrm{ID}-\overline{x})^2}{\sum_{i=1}^{n}(x_i-\overline{x})^2}}\right) - a\right)}{b} \qquad (8)$$

$t_{f;a}$	1.771: quantil of t-distribution for f = n - 2 degrees of freedom and a probability of 95 % (error probability α=0.05)
$\overline{y}$	mean value of peak area of all calibration analyses
$\overline{x}$	mean value of all concentrations
ID	identification limit (ID = 2 x LOD)

The resulting LOD and LOQ showed slight differences for the five substances. Therefore, the LOD (0.5 µg/kg) and the LOQ (1.5 µg/kg) with the highest determined value were used.

For 4-methoxy-2-methyl-2-butanethiol the LOD and LOQ were determined on the GC 8000^{TOP} system in triplicate with five concentrations in total ion chromatogram (TIC) mode

(656 ng/mL - 10.5 µg/mL) and SIM mode (20.5 - 328 ng/mL). The resulting LODs and LOQs were 0.4 µg/mL and 1.3 µg/mL in TIC mode as well as 34.2 ng/mL and 101.5 ng/mL in SIM mode.

3.2.4 Statistical analysis

Each experiment was performed in triplicate and results were presented as mean ± standard deviation, if the data were normally distributed or as median (minimum-maximum), if the data were not normally distributed. Normal distribution was tested with Shapiro-Wilk test and equality of variances with Fisher´s F-Test. If normal distribution and equality of variances were shown, unpaired Student's t-test was used to test for equality of means. Welch's t-test was performed, if the data were normally distributed but equality of variances was not shown. Non-parametric Wilcoxon-Mann-Whitney U-test was used to compare medians, if data were not normally distributed. All tests were two-tailed and differences were considered as statistically significant at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). Statistical analyses were performed using XLSTAT 2017 (Addinsoft, Paris, France).

3.2.5 Sensory evaluation of volatile constituents

For the sensory evaluation of volatile constituents in blackcurrant and redcurrant fruits, the AEDA was applied according to Ullrich and Grosch (1987). Extracts obtained by VHS for blackcurrant berries (cultivar: 'Titania', 3 kg) and by LLE for redcurrant berries (cultivar: 'Rovada', 3 kg) were pooled, concentrated to 1.5 µL under a gentle nitrogen flow, and subsequently used for AEDA. The concentrated extracts were gradually diluted with a mixture of diethyl ether and *n*-pentane (1:1; v/v) and analysed by two panellists via GC/O until no odour was detectable. The odour quality and the FD factors were determined as described in equation 9.

$$\mathrm{FD} = 2^{\mathrm{n}} \qquad (9)$$

FD	flavour dilution factor
n	dilution factor of the last perception

3.2.5.1 Gas chromatography/olfactometry (GC/O)

GC/O conditions:

Instrument:	Trace GC Ultra (Thermo Fisher Scientific, Dreieich, Germany)

Capillary column:	DB-Wax (60 m x 0.32 mm i.d., 0.25 µm film thickness; J&W Scientific).
Temperature programs:	35 °C/ 1 min// 30 °C/min/ 40 °C/ 4 min// 4 °C/min/ 240 °C/ 25 min purity check: 35 °C/ 1 min// 10 °C/min/ 240 °C/ 15 min
Carrier gas:	hydrogen (5.0); constant inlet pressure (75 kPa)
Injector:	cold-on-column injector; injection volume 0.5 µL
Detectors:	FID (250 °C) and a sniffing port (200 °C); eluent split via press-fit t-piece 1:1

3.2.5.2 Determination of odour thresholds

Odour thresholds of 4-methoxy-2-methyl-2-butanethiol were determined in water and a blackcurrant-type matrix (for the composition of the blackcurrant-type matrix see below) by an untrained panel of 18 participants (13 females and five males, age from 20 to 75 years, median: 29 years). A triangle-test in combination with the ‘forced choice’ technique, a method, which forces all panel members to answer even in case of non-perception of any odour difference between the samples, was applied. In order to rule out hits by chance, only the sample, from which each further dilution stage (more precisely the higher concentration) was correctly recognised, was evaluated as correctly.

The calculation of odour thresholds of individual participants was done according to the procedure described by Mehinagic *et al.* (2006).

$$\mathrm{OT_i} = \sqrt{\mathrm{C_x} \times \mathrm{C_{x-1}}} \qquad (10)$$

OT_i	individual odour threshold value
C_x	concentration of the first recognised sample
C_{x-1}	concentration of the previous sample

The geometric mean was used for the determination of the group odour threshold.

$$\overline{\mathrm{OT}} = \sqrt[n]{\prod_{i=1}^{n} \mathrm{OT_i}} \qquad (11)$$

$\overline{\mathrm{OT}}$	group odour threshold value
n	number of persons
$\mathrm{OT_i}$	individual odour threshold value
Π	product of individual thresholds

The purity of 4-methoxy-2-methyl-2-butanethiol was checked by GC/O.

Composition of the blackcurrant-type matrix

In order to imitate a blackcurrant-type matrix, the natural amounts of sugars and organic acids, occurring in blackcurrant berries, were dissolved in water according to Bordonaba and Terry (2008), Haila *et al.* (1992); Milivojević *et al.* (2009); Mikulic-Petkovsek *et al.* (2012b) Nour *et al.* (2011), and Vagiri *et al.* (2013): 49 g/L fructose, 37 g/L glucose, 10 g/L sucrose, 2.5 g/L ascorbic acid, 22 g/L citric acid, 3 g/L malic acid, and 0.9 g/L tartaric acid.

3.2.5.3 Calculation of odour activity values (OAVs)

In order to evaluate the aroma-relevance of volatile constituents, the concept of OAV according to Rothe and Thomas (1963) was used as described in equation 12.

$$\mathrm{OAV} = \frac{\mathrm{C}}{\mathrm{OT}} \qquad (12)$$

OAV	odour activity value
C	concentration of individual compound in food
OT	odour threshold of individual compound

3.2.5.4 Aroma profile tests

The aroma profile tests were performed by a panel of twelve to 15 participants (ten females and five males, age from 21 to 76 years, median: 29 years). They had been trained in pre-sessions to assess the odorants used as descriptors and had given informed consent to participate. Samples (15 mL) were placed into glasses with lids and were orthonasally evaluated by the panel. The odorants (descriptors) ethyl butanoate (pineapple-like), methyl butanoate (cheesy-fruity), 1,8-cineole (eucalyptus-like), (*Z*)-hex-3-enal (grassy), (*E*)-hex-2-enal (apple-marzipan-like), 4-methoxy-2-methyl-2-butanethiol (catty), 2-isopropyl-3-methoxypyrazine (musty), and pent-1-en-3-one (pungent-solvent-like) were used for the aroma profile test of blackcurrant berries. (*Z*)-Hex-3-enal (grassy), (*E*)-hex-2-enal (apple-marzipan-like), (*E*)-β-damascenone (boiled apple), acetic acid (acid), 2-isopropyl-3-methoxypyrazine (musty), and pent-1-en-3-one (pungent-solvent-like) were used for the aroma profile test of redcurrant berries. The odorants were dissolved in water and the panellists assessed each descriptor on a seven-point discontinuous scale from 0 (not detectable) to 3 (strong). The sensory evaluations of the blackcurrant and redcurrant berries were performed with 15 g of freshly mashed berries.

3.2.5.5 Reconstitution experiments

The reconstitution model of blackcurrant berries was prepared based on the concentrations of odour-active compounds determined in the cultivar 'Tsema' (4 August, 2015) and in the cultivar 'Supernova' (4 July, 2016) listed in Table 10. In a second reconstitution experiment, a lower concentration of (*E*)-hex-2-enal (22 µg/L) was used to access the influence of this volatile compound on the aroma profile. For the reconstitution model of redcurrant berries the concentrations of odorants with an OAV ≥ 1 in the cultivar 'Rovada' from Freising (13 July, 2015), Deutenkofen (14 July, 2015), and Oberkirch (1 August, 2016) were used (see Table 10). All reference substances were checked for purity by GC/O. Appropriate amounts of stock solutions of the odorants were dissolved in a berry-type matrix (see section 3.2.3.3 and section 3.2.5.2).

Table 10: Concentrations of aroma compounds used for the reconstitution experiments of blackcurrant and redcurrant berries.

compound	blackcurrant	redcurrant
	µg/L	
(*E*)-hex-2-enal	632	1,236
(*Z*)-hex-3-enal	285	52
(*Z*)-hex-3-en-1-ol	90	31
pent-1-en-3-one	4	1.27
2-isobutyl-3-methoxypyrazine	0.06	0.14
oct-1-en-3-one	0.24	0.10
2-isopropyl-3-methoxypyrazine	0.05	0.04
methyl butanoate	795	
ethyl butanoate	240	
α-pinene	85	
hexanal	70	
citronellol	52	
1,8-cineole	44	
decanal	6	
linalool	5	
methional	0.29	

Table 10: Continued.

compound	blackcurrant	redcurrant
	µg/L	
4-methoxy-2-methyl-2-butanethiol	0.35	
(*Z*)-rose oxide	1.59	
(*E*)-non-2-enal	0.57	
2-*sec*-butyl-3-methoxypyrazine	0.04	
acetic acid		1,597
vanillin		20
(*E*)-β-damascenone		0.79

4 RESULTS AND DISCUSSION

4.1 Investigation of volatile compounds in blackcurrant berries

4.1.1 Identification and quantification of 4-methoxy-2-methyl-2-butanethiol in blackcurrant berries

The volatile profiles of blackcurrant berries have been investigated for several decades (Andersson and von Sydow 1964, 1966a, b; Nursten and Williams 1969a; von Sydow and Karlsson 1971a, b; Karlsson-Ekstrom and von Sydow 1973; Latrasse *et al.* 1982; Bricout *et al.* 1985; Marriott 1986; 1987; Mikkelsen and Poll 2002; Orav *et al.* 2002; Ruiz del Castillo and Dobson 2002a, b; Tiitinen *et al.* 2004; Christensen and Pedersen 2006; Kampuss *et al.* 2008; Hempfling *et al.* 2013a). In 1982, Latrasse *et al.* reported a ‘catty urine odour’ note in extracts of frozen blackcurrant fruits (Latrasse *et al.* 1982). In subsequent studies, the sulphur-containing compound 4-methoxy-2-methyl-2-butanethiol was identified in essential oils obtained by steam distillation of blackcurrant buds (Rigaud *et al.* 1986; Le Quéré and Latrasse 1990). According to an abstract from an oral presentation, the presence of this compound was confirmed in blackcurrant berry and leaf extracts (Joulain and Laurent 1989). However, no experimental data substantiating this statement were provided. Later, a ‘catty’, blackcurrant-like odour was detected by GC/O in an extract from frozen blackcurrant fruits, and the authors assigned the compound as 4-methoxy-2-methyl-2-butanethiol (Mikkelsen and Poll 2002). Though, this was solely based on a comparison of the odour and the chromatographic retention behaviour to those reported by Latrasse *et al.* (1982); no authentic reference compound was available and no confirmation via GC-MS data was provided.

In the meantime, 4-methoxy-2-methyl-2-butanethiol was identified in various foods, such as coffee (Poisson *et al.* 2004; Kerler *et al.* 2005), green tea (Kumazawa and Masuda 1999), and olive oil (Guth and Grosch 1991; 1993; Reiners and Grosch 1998; 1999; Vichi *et al.* 2014), in the twigs of a wild growing *Ruta* species (*Ruta chalepensis* L.) (van de Waal *et al.* 2002; Escher *et al.* 2006), and in the flowers of wintercurrant (*Ribes sanguineum*) (Brunke *et al.* 1997). Quantifications were performed with the use of isotope-labelled 4-methoxy-2-methyl-2-butanethiol or 3-sulfanylpentan-2-one as internal standard via GC-MS (Guth and Grosch 1993; Reiners and Grosch 1998, 1999). In one approach, derivatisation with ebselen and subsequent quantification with derivatised 4-methoxy-α-toluenethiol as internal standard via liquid chromatography-mass spectrometry (LC-MS) was described (Vichi *et al.* 2014). In addition, an enrichment of thiols on mercurated agarose gel or *p*-hydroxymercuribenzoic acid was applied (Reiners and Grosch 1998; 1999; Poisson *et al.* 2004; Kerler *et al.* 2005).

The objective of this part of the work was to provide unambiguous evidence for the presence of 4-methoxy-2-methyl-2-butanethiol in blackcurrant fruits. The compound should be quantified and the potential impact of the blackcurrant cultivar on the content should be investigated.

Volatile constituents of fresh blackcurrant berries were isolated via VHS, a procedure previously applied to various foods, such as passion fruits, rhubarb, gooseberries, and jostaberries (Güntert *et al.* 1998; Werkhoff *et al.* 1998; Dregus and Engel 2003; Hempfling *et al.* 2013a; Hempfling *et al.* 2013b). In order to increase the selectivity regarding the detection of the sulphur-containing target compound 4-methoxy-2-methyl-2-butanethiol, the isolation via VHS was followed by an enrichment step on mercurated agarose gel. The enrichment of volatile thiols on mercurated agarose gel has been described for the first time by Full and Schreier (1994) on Affi-Gel 501. In the present work, the mercurated agarose gel was prepared from Affi-Gel® 10 according to Steinhaus *et al.* (2007) and the enrichment was performed according to Schneider *et al.* (2003). The quantification of 4-methoxy-2-methyl-2-butanethiol was based on the use of 4-methyl-4-sulfanylpentan-2-one as internal standard; a preceding screening had shown that this compound is not present in blackcurrant fruits. For the final calculations, the relative recovery rates of 4-methoxy-2-methyl-2-butanethiol (see section 8.1, Table 24) compared to the internal standard during VHS isolation (98 ± 9 %) and enrichment on mercurated agarose gel (87 ± 9 %) were taken into account. In addition, a response factor of 0.69, reflecting the higher intensity observed for the quantifier ion (*m*/*z* 85) of 4-methoxy-2-methyl-2-butanethiol compared to the quantifier ion (*m*/*z* 132) of 4-methyl-4-sulfanylpentan-2-one in the SIM mode, was used.

In an experimental allotment garden located in Freising, access to the blackcurrant cultivar ‘Andega’, a cross between ‘Noir de Bourgogne’ and ‘Öjebyn’ (Le Quéré and Latrasse 1990; Bundessortenamt 2002) was enabled. Latrasse *et al.* (1982) described the blackcurrant cultivars ‘Noir de Bourgogne’ and ‘Royal de Naples’ as the most aromatic cultivars. Investigations of 23 cultivars also revealed the highest contents of 4-methoxy-2-methyl-2-butanethiol in the buds of ‘Noir de Bourgogne’, ‘Royal de Naples’, and ‘Burga’ (Le Quéré and Latrasse 1990). Therefore, the search for the presence of 4-methoxy-2-methyl-2-butanethiol in blackcurrant berries was started with the cultivar ‘Andega’.

As shown in Figure 2, 4-methoxy-2-methyl-2-butanethiol could indeed be directly identified by GC-MS in the TIC mode of an extract obtained by VHS. The identification was confirmed by comparison of the retention indices to those of the authentic reference compound on three stationary phases (DB-1: 901, DB-WaxEtr: 1224, RTX-200MS: 1029). The concentrations of 4-methoxy-2-methyl-2-butanethiol determined in ‘Andega’ at two points of harvest via quantification in the SIM mode based on the added internal standard 4-methyl-4-sulfanylpentan-2-one are shown in Table 11.

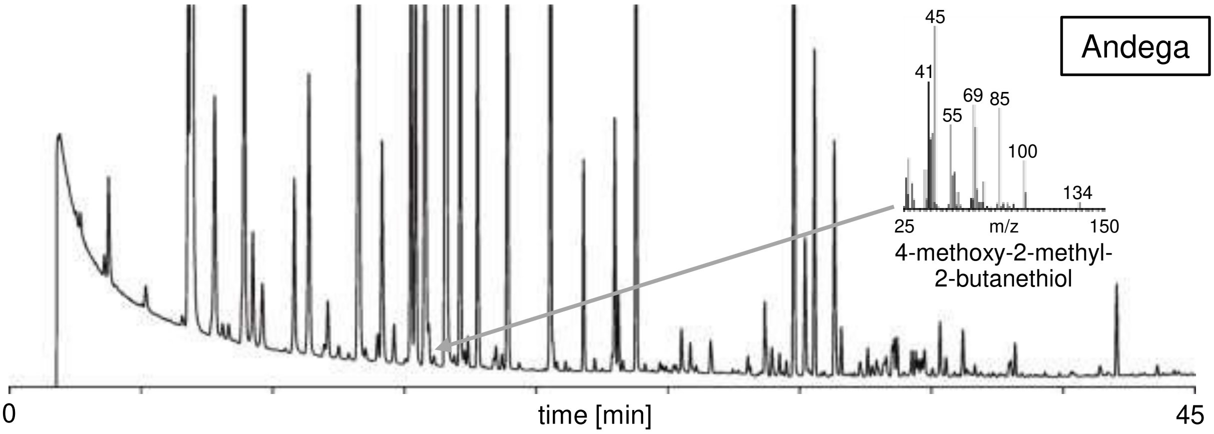

Figure 2: Capillary gas chromatographic separation (DB-WaxEtr; total ion chromatogram (TIC)) of volatiles isolated from blackcurrant berries (‘Andega’, 13 July, 2015) by vacuum-headspace extraction (VHS) (extract from 500 g berries) and the mass spectrum of the 4-methoxy-2-methyl-2-butanethiol peak in the chromatogram of the VHS-extract. For gas chromatography-mass spectrometry (GC-MS) conditions see section 3.2.2.2.

In addition, five different cultivars, hand-picked at Deutenkofen, another location in Southern Germany, were investigated. In none of these cultivars 4-methoxy-2-methyl-2-butanethiol could be detected directly after isolation via VHS. Therefore, combined VHS-extracts corresponding to totals of 1.5 kg and 3 kg fruits, respectively, were subjected to enrichment with the mercurated agarose gel for the cultivars ‘Rosenthals’, ‘Silvergieters’, ‘Supernova’, ‘Titania’, and ‘Tsema’. As shown in Figure 3 for the cultivar ‘Supernova’, this step enabled the detection of the compound via GC-MS in the TIC mode. The quantification was performed in the SIM mode.

The concentrations of 4-methoxy-2-methyl-2-butanethiol in blackcurrant berries from the location Deutenkofen ranged from 0.16 µg/kg to 0.54 µg/kg (see Table 11).

In addition to the hand-picked berries, three batches of commercially available blackcurrant berries were purchased at a local market. As shown in Table 11, the concentrations of 4-methoxy-2-methyl-2-butanethiol in these three cultivars ranged from 0.35 µg/kg to 0.72 µg/kg.

Among the investigated cultivars, ‘Andega’ is outstanding regarding its high content of 4-methoxy-2-methyl-2-butanethiol. The average concentration determined in fruits from two points of harvest (4.5 µg/kg) is approximately 13 times higher than the average concentration (0.34 µg/kg) determined in the other cultivars. Except for the cultivar ‘Silvergieters’, the concentrations of 4-methoxy-2-methyl-2-butanethiol in all other cultivars hand-picked at the location Deutenkofen varied only in a rather small range (0.16 - 0.23 µg/kg). For the cultivar ‘Tsema’ the concentration was independent from the degree of ripeness. In the commercially obtained cultivars, the concentrations of 4-methoxy-2-methyl-2-butanethiol were slightly higher than those in the berries from Deutenkofen. A rough extrapolation of the quantitative

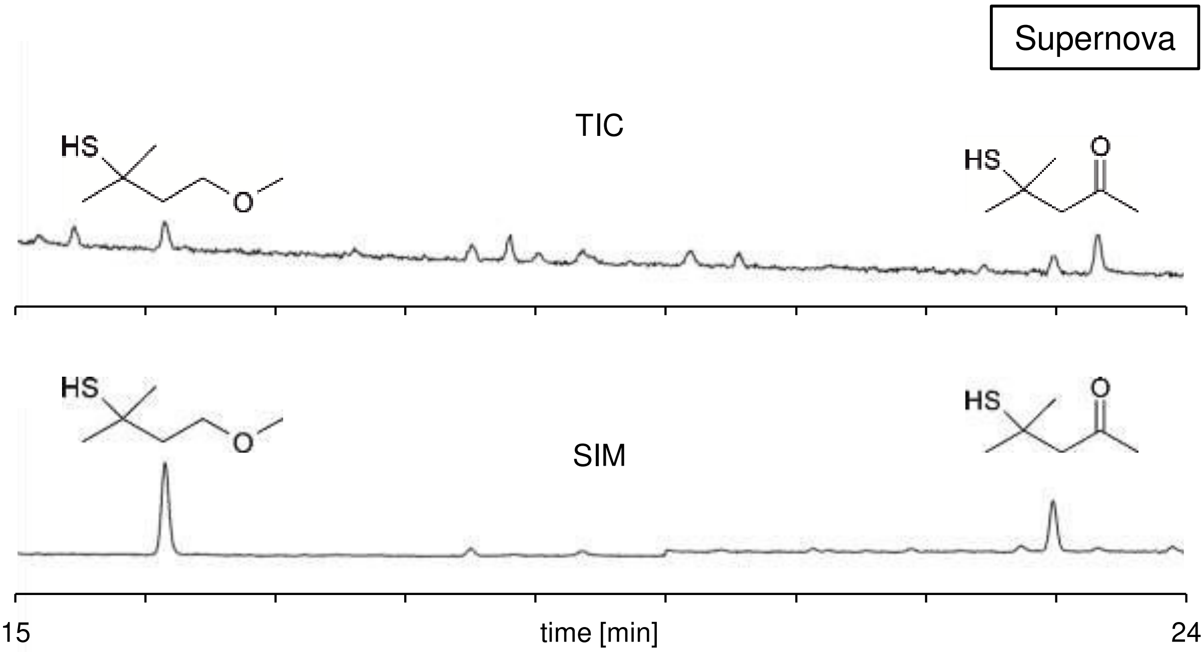

Figure 3: Capillary gas chromatographic separation (DB-WaxEtr) of volatiles isolated from blackcurrant berries ('Supernova', 29 June and 3 July, 2015) by vacuum-headspace extraction (VHS) and enrichment on mercurated agarose gel (pooled extracts from a total of 3 kg berries) in total ion chromatogram (TIC) and selected ion monitoring (SIM) mode. The SIM chromatogram was obtained by screening for *m/z* 85, 100, 134 (4-methoxy-2-methyl-2-butanethiol) from 0 min to 20 min and for *m/z* 75, 99, 132 (4-methyl-4-sulfanylpentan-2-one) from 20 min to 40 min. For gas chromatography-mass spectrometry (GC-MS) conditions see section 3.2.2.2.

information provided by Le Quéré and Latrasse, i.e. 2 - 5 mL of essential oil/kg blackcurrant buds and contents of 4-methoxy-2-methyl-2-butanethiol in the oil of up to 0.04 %, reveals that the concentrations of this sulphur-containing compound in the blackcurrant buds (approximately 800 - 2,000 µg/kg) are several orders of magnitude higher than those in the berries (Le Quéré and Latrasse 1990). On the other hand, the concentrations determined in blackcurrant berries are significantly higher than those reported in coffee (14 - 22 ng/kg) (Poisson *et al.* 2004; Kerler *et al.* 2005). In olive oil, concentrations of this sulphur-containing compound between 0.1 ng/kg and 1.8 µg/kg were reported (Guth and Grosch 1993; Vichi *et al.* 2014). In two studies, the concentration of 4-methoxy-2-methyl-2-butanethiol detected in olive oil amounted to 4.3 µg/kg and was thus in a similar range as in the blackcurrant cultivar 'Andega' (Reiners and Grosch 1998; 1999). For 4-methoxy-2-methyl-2-butanethiol, an odour threshold in water of 0.001 µg/kg was described (Rigaud *et al.* 1986; Blank 2002). Further odour thresholds were reported in coffee (0.085 µg/kg) (Kerler *et al.* 2005) and sunflower oil (0.02 - 0.06 µg/kg) (Guth and Grosch 1991; Reiners and Grosch 1998). Sensory evaluations by an own panel confirmed the odour threshold of 0.001 µg/kg in water as well as in a blackcurrant-type matrix containing sugars and organic acids. Therefore, this value was used for calculation of the OAVs listed in Table 11. The high OAVs obtained for 'Andega' indicated that 4-methoxy-2-methyl-2-butanethiol is a prominent contributor to the aroma of berries from this blackcurrant cultivar. The odour thresholds suggested that this sulphur-containing compound also contributes to the aroma of berries from the other investigated cultivars that contained the compound in much lower amounts.

Table 11: Concentrations of 4-methoxy-2-methyl-2-butanethiol in blackcurrant berries of different cultivars originating from locations in Southern Germany.

origin/cultivar	date of harvest/ purchase	concentration [µg/kg]	OAV[a]
Freising[b]			
'Andega'	13 July, 2015	3.62 ± 0.03[c]	3,620
'Andega'	16 July, 2015	5.37 ± 1.64[c]	5,370
Deutenkofen[b]			
'Rosenthals'	3 July, 2015	0.22[d]	220
'Silvergieters'	29 June, 2015	0.54[d]	540
'Supernova'	29 June and 3 July, 2015	0.20[e]	200
'Titania' (unripe)	23 June, 2015	0.23[e]	230
'Tsema' (unripe)	23 June, 2015	0.16[d]	160
'Tsema'	29 June, 2015	0.16[d]	160
Bühl[f]			
'Supernova'	20 July, 2015	0.72[e]	720
'Tsema'	4 August, 2015	0.35[e]	350
Oberkirch[f]			
'Tenah'	28 July, 2015	0.52[e]	520

[a] Odour activity value (OAV) calculated on the basis of an odour threshold of 0.001 µg/kg determined in water and in a blackcurrant-type matrix, respectively. [b] Fruits were hand-picked, dates refer to the time of harvest. [c] Data of three batches (500 g each); triplicated analysis: mean ± standard deviation. [d] Analysis of pooled extracts from three batches (500 g each). [e] Analysis of pooled extracts from six batches (500 g each). [f] Fruits were purchased at a local grocery store in Freising; dates refer to the time of purchase.

4.1.2 Identification and quantification of volatile compounds in blackcurrant berries via VHS

Earliest investigations of the volatile constituents of blackcurrant fruits date back to the 1960s. In these studies, a broad spectrum of terpenes, esters, and alcohols were identified (Andersson and von Sydow 1964, 1966a, b; Nursten and Williams 1969a; von Sydow and Karlsson 1971a, b; Karlsson-Ekstrom and von Sydow 1973). In the following decades, several additional studies on the volatile profile of blackcurrant berries were carried out (Latrasse *et al.* 1982; Bricout *et al.* 1985; Marriott 1986; 1987; Mikkelsen and Poll 2002; Orav *et al.* 2002; Ruiz del Castillo and Dobson 2002a, b; Tiitinen *et al.* 2004; Christensen and Pedersen 2006; Kampuss *et al.* 2008; Hempfling *et al.* 2013a). Despite all of this research activity on the volatile profiles of blackcurrant berries, most of these previous studies focused on the analysis of frozen berries (see section 2.1.3.1, Table 2) (Andersson and von Sydow 1964, 1966a, b; Nursten and Williams 1969a; von Sydow and Karlsson 1971a, b; Karlsson-Ekstrom and von Sydow 1973; Latrasse *et al.* 1982; Bricout *et al.* 1985; Mikkelsen and Poll 2002; Ruiz del Castillo and Dobson 2002b; Tiitinen *et al.* 2004; Christensen and Pedersen 2006; Kampuss *et al.* 2008). Previous investigations on fresh blackcurrant berries either concentrated only on terpenes (Marriott 1986; 1987; Ruiz del Castillo and Dobson 2002a) or only on the analysis of volatile compounds isolated with a non-gentle isolation method, i.e. SDE (Orav *et al.* 2002).

Therefore, one of the objectives of the present work was to identify and quantify volatile compounds in fresh blackcurrant berries comprehensively, applying a gentle isolation method, such as VHS, and to illustrate the effect of freezing on the volatile profile. The influence of enzyme inhibition and the state of ripeness should be also analysed. Additionally, the potential to classify blackcurrant berries based on the volatile profiles of fresh fruits should be investigated.

4.1.2.1 Volatile profiles of fresh blackcurrant berries

Volatile constituents of fresh blackcurrant berries were isolated via VHS, which is a gentle method preventing the formations of thermally induced reaction products. Commercially available blackcurrant berries as well as berries hand-picked at two locations in Southern Germany were investigated. The obtained VHS-extracts were analysed via GC/FID and GC-MS. Quantification was based on the use of heptan-2-ol as internal standard; a preceding screening had shown that this compound is not present in blackcurrant fruits. For the final calculations, the relative recovery rates and response factors were taken into account.

The recovery rates, determined in this work (see section 8.1, Table 23) as well as in earlier studies by Schrade (2014) and Dregus and Engel (2003), showed a discrimination of non-volatile and very polar substances. For instance, organic acids, such as citric acid, malic acid, or oxalic acid, are not isolated by means of VHS (Dregus and Engel 2003). The study by Schrade (2014) also demonstrated that a berry-type matrix containing sugars and organic acids has no effect on recovery rates.

In total, 155 compounds (29 tentatively) were identified in 34 batches of blackcurrant berries after isolation via VHS. Ten C_6-compounds, 39 esters, 25 alcohols, eleven aldehydes, eight ketones, 46 terpenes, eight acids, and eight other substances were determined. Fifty-six (22 tentatively) of these 155 compounds were reported for the first time in blackcurrant berries. The distributions of volatile compounds determined in the cultivars ‘8 Bona’, ‘Ben Sarek’, and ‘Ometa’ (location: Deutenkofen, season: 2014) are shown as example in Table 12. Compounds identified in blackcurrant berries in addition to those listed in Table 12 are summarised in Table 13, and a typical example chromatogram of a VHS-extract is shown in Figure 4.

In agreement with previous studies, the major classes of volatile compounds isolated from fresh blackcurrant fruits are esters and terpenes. In addition, the alcohol 2-methylbut-3-en-2-ol also occurred in high concentrations. However, the rather high concentrations of C_6-compounds, a typical class of so-called ‘secondary’ aroma compounds, constituted a striking difference compared to data from previous studies (Andersson and von Sydow 1964, 1966a, b; Nursten and Williams 1969a; von Sydow and Karlsson 1971a, b; Karlsson-Ekstrom and von Sydow 1973; Latrasse *et al.* 1982; Bricout *et al.* 1985; Marriott 1986; 1987; Mikkelsen and Poll 2002; Orav *et al.* 2002; Ruiz del Castillo and Dobson 2002a, b; Tiitinen *et al.* 2004; Christensen and Pedersen 2006; Kampuss *et al.* 2008). The fact that in most of these investigations frozen blackcurrant berries were analysed, prompted to specifically address the impact of freezing on the spectrum of blackcurrant volatile compounds.

Table 12: Volatile compounds isolated via vacuum-headspace extraction (VHS) from berries of three hand-picked batches of the blackcurrant cultivars ‘8 Bona’, ‘Ben Sarek’, and ‘Ometa’ (location: Deutenkofen, season: 2014).

no.[a]	compound	RI[b]	‘8 Bona’	‘Ben Sarek’	‘Ometa’	remark
				[µg/kg][c]		
	C_6-compounds					
43	(*E*)-hex-2-enal	1199	5,977 ± 601	403 ± 84	3,074 ± 881	*d, f*
26	(*Z*)-hex-3-enal	1128	591 ± 496	n.d.[k]	786 ± 124	*d, f*
82	(*E*)-hex-2-en-1-ol	1394	478 ± 155	169 ± 29	749 ± 57	*d, f*
14	hexanal	1067	335 ± 59	15 ± 4	111 ± 38	*d, f*
77	(*Z*)-hex-3-en-1-ol	1373	135 ± 14	26 ± 1	508 ± 30	*d, f*
25	(*E*)-hex-3-enal	1123	83 ± 4	n.d.	55 ± 2	*d, h, j*
69	hexan-1-ol	1346	73 ± 18	16 ± 2	127 ± 24	*d, f*
72	(*E*)-hex-3-en-1-ol	1353	5 ± 1	n.q.[l]	14 ± 3	*e, i, j*

Table 12: Continued.

no.[a]	compound	RI[b]	'8 Bona'	'Ben Sarek'	'Ometa'	remark
				[µg/kg][c]		
83	(*Z*)-hex-2-en-1-ol	1404	2 ± 1	n.d.	8 ± 1	*d, g*
40	(*Z*)-hex-2-enal	1185	n.d.	3 ± 1	n.d.	*e, i, j*
	esters					
6	ethyl butanoate	1028	2,827 ± 1,357	1,066 ± 705	295 ± 145	*d, f*
1	methyl butanoate	973	445 ± 199	26,389 ± 3,491	990 ± 234	*d, f*
106	bornyl acetate	1563	47 ± 4	5 ± 1	3 ± 0	*d, f*
87	ethyl octanoate	1425	23 ± 5	13 ± 10	6 ± 5	*d, g*
13	butyl acetate	1060	21 ± 16	5 ± 2	n.d.	*d, f*
36	methyl hexanoate	1175	20 ± 9	546 ± 51	50 ± 8	*d, f*
81	ethyl 2-hydroxybutanoate	1389	12 ± 5	2 ± 1	n.d.	*e, i*
67	(*E*)-hex-2-enyl acetate	1322	7 ± 3	4 ± 2	2 ± 0	*d, f*
123	citronellyl acetate	1649	5 ± 1	14 ± 1	21 ± 3	*d, g*
56	hexyl acetate	1262	4 ± 4	n.d.	n.q.	*d, f*
115	methyl benzoate	1597	4 ± 2	66 ± 18	22 ± 7	*d, f*
98	ethyl 3-hydroxybutanoate	1499	4 ± 1	n.d.	n.d.	*d, g, j*
63	(*Z*)-hex-3-enyl acetate	1302	3 ± 1	8 ±1	10 ± 8	*d, f*
141	methyl salicylate	1746	2 ± 1	8 ± 2	9 ± 3	*d, f*
79	methyl octanoate	1378	2 ± 2	59 ± 9	6 ± 2	*d, f*
104	linalyl acetate	1544	2 ± 0	n.d.	6 ± 1	*d, g*
122	ethyl benzoate	1642	n.q.	6 ± 4	5 ± 3	*d, f*
118	ethyl decanoate	1626	n.q.	n.d.	n.q.	*d, f*
107	methyl 2-hydroxybutanoate	1362	n.q.	48 ± 4	3 ± 1	*e, i, j*
113	methyl decanoate	1584	n.q.	8 ± 3	2 ± 0	*d, g*
22	propyl butanoate	1111	n.q.	n.d.	n.d.	*e, i, j*
140	geranyl acetate	1742	n.d.	n.d.	5 ± 2	*d, g*
121	methyl 3-hydroxybutanoate	1463	n.d.	7 ± 1	n.d.	*d, g, j*
94	methyl 3-hydroxyoctanoate	1634	n.d.	4 ± 1	n.d.	*e, i, j*
9 10	methyl (*Z*)-but-2-enoate methyl but-3-enoate	1040	n.d.	4 ± 1	n.d.	*e, i, j*
15	methyl pentanoate	1072	n.d.	4 ± 1	n.d.	*d, f, j*
133 134	neryl acetate benzyl acetate	1706	n.d.	9 ± 0	5 ± 1	*d, g*
128	α-terpinyl acetate	1680	n.d.	n.d.	9 ± 1	*d, g*
	alcohols					
8	2-methylbut-3-en-2-ol	1034	541 ± 153	1,530 ± 294	899 ± 166	*d, f*
65	(*Z*)-pent-2-en-1-ol	1311	67 ± 7	28 ± 10	59 ± 2	*d, f, j*
28	butan-1-ol	1136	33 ± 40	18 ± 9	n.d.	*d, f*

Table 12: Continued.

no.[a]	compound	RI[b]	'8 Bona' [µg/kg][c]	'Ben Sarek' [µg/kg][c]	'Ometa' [µg/kg][c]	remark
53	pentan-1-ol	1242	2 ± 1	2 ± 1	4 ± 0	*d, g*
92	heptan-1-ol	1448	2 ± 1	2 ± 0	3 ± 1	*d, f*
110	propan-1,2-diol	1572	n.c.[m]	n.c.	n.c.	*d, j*
95	2-ethylhexan-1-ol	1482	n.q.	n.q.	n.q.	*d, g*
62	(*E*)-pent-2-en-1-ol	1301	n.q.	n.d.	2 ± 0	*e, i, j*
147	benzyl alcohol	1852	n.q.	3 ± 0	2 ± 0	*d, g*
124	nonan-1-ol	1651	n.q.	2 ± 1	2 ± 1	*d, g*
91	oct-1-en-3-ol	1442	n.q.	n.q.	3 ± 0	*d, f*
105	octan-1-ol	1549	n.q.	n.d.	n.q.	*d, f*
16	2-methylpropan-1-ol	1083	n.d.	4 ± 1	3 ± 2	*d, f*
99	hept-2-en-1-ol	1500	n.d.	n.q.	n.q.	*e, i, j*
153	allylphenol	2478	n.d.	10 ± 1	n.q.	*e, i*
	aldehydes					
21	(*E*)-pent-2-enal	1109	35 ± 9	7 ± 1	46 ± 2	*d, f, j*
64	(*E*)-hept-2-enal	1304	8 ± 3	2 ± 0	5 ± 1	*d, f*
80	nonanal	1379	6 ± 0	6 ± 0	7 ± 3	*d, g*
84	(*E*)-oct-2-enal	1411	2 ± 0	n.d.	2 ± 0	*d, g*
139	(*E*)-undec-2-enal	1736	n.d.	n.q.	2 ± 1	*d, g, j*
97	decanal	1485	n.d.	4 ± 1	4 ± 0	*d, f*
59	octanal	1274	n.d.	n.d.	2 ± 0	*d, g*
	ketones					
2	pent-1-en-3-one	1008	47 ± 9	4 ± 1	19 ± 4	*d, f, j*
34	heptan-2-one	1165	2 ± 0	n.q.	2 ± 0	*d, g*
89	3-methyl-3-cyclohexen-1-one	1427	n.d.	n.q.	n.q.	*e, i, j*
	terpenes					
41	β-phellandrene	1189	166 ± 46	74 ± 5	283 ± 57	*d, f*
48	(Z)-β-ocimene	1224	147 ± 18	202 ± 198	340 ± 192	*d, f*
30	α-phellandrene	1148	111 ± 57	15 ± 3	30 ± 3	*d, f*
18	β-pinene	1090	92 ± 15	98 ± 7	54 ± 14	*d, f*
32	myrcene	1151	86 ± 35	15 ± 2	30 ± 3	*d, f*
42	1,8-cineole	1194	68 ± 36	357 ± 153	222 ± 63	*d, f*
27	Δ-3-carene	1133	66 ± 16	231 ± 16	1,140 ± 258	*d, f*
38	limonene	1181	65 ± 15	40 ± 1	147 ± 35	*d, f*
57	terpinolene	1266	48 ± 14	212 ± 11	797 ± 231	*d, f*
20	sabinene	1106	44 ± 13	285 ± 84	2,436 ± 308	*d, f*

Table 12: Continued.

no.[a]	compound	RI[b]	'8 Bona'	'Ben Sarek'	'Ometa'	remark
				[µg/kg][c]		
52	(*E*)-β-ocimene	1239	37 ± 19	52 ± 3	206 ± 110	*d, f*
3	α-pinene	1010	33 ± 9	17 ± 2	74 ± 11	*d, f*
113	terpinen-4-ol	1588	26 ± 12	459 ± 116	878 ± 138	*d, f*
129	α-terpineol	1683	14 ± 9	77 ± 29	42 ± 10	*d, f*
111	caryophyllene	1577	13 ± 7	14 ± 1	66 ± 18	*d, f*
50	γ-terpinene	1229	11 ± 3	161 ± 5	249 ± 78	*d, f*
152	spathulenol	2105	10 ± 2	n.d.	n.d.	*e, i, j*
33	α-terpinene	1162	9 ± 2	113 ± 4	186 ± 58	*d, f*
93	sabinene hydrate	1456	9 ± 2	56 ± 14	207 ± 57	*d, g*
12	camphene	1047	9 ± 2	n.q.	n.q.	*d, g*
142	citronellol	1755	9 ± 1	5 ± 1	23 ± 0	*d, f*
103	linalool	1538	7 ± 0	27 ± 6	86 ± 18	*d, f*
130	borneol	1685	3 ± 1	3 ± 1	4 ± 2	*d, g*
144	*p*-cymen-8-ol	1830	2 ± 0	3 ± 0	5 ± 1	*d, g*
136	β-phellandren-8-ol	1711	2 ± 0	4 ± 0	5 ± 1	*e, i, j*
55	*p*-cymene	1251	2 ± 0	6 ± 0	8 ± 2	*d, f*
126	neral	1658	2 ± 2	n.d.	7 ± 3	*d, f, j*
132	α-phellandren-8-ol	1703	2 ± 1	2 ± 0	5 ± 1	*e, i, j*
137	2-hydroxy 1,8-cineole	1712	n.q.	n.d.	n.q.	*e, i, j*
146	2-hydroxy 1,8-cineole	1844	n.q.	6 ± 1	3 ± 1	*e, i, j*
54	*o*/*m*-cymene	1250	n.q.	n.q.	3 ± 1	*e, i*
4	α-thujene	1015	n.q.	3 ± 1	11 ± 2	*e, i*
24	2-carene	1116	n.d.	3 ± 0	5 ± 1	*d, g, j*
74	alloocimene	1358	n.d.	n.d.	n.q.	*d, g*
11	fenchene	1040	n.d.	n.d.	3 ± 1	*e, i*
145	geraniol	1834	n.d.	2 ± 1	2 ± 1	*d, f*
131	germacrene D	1689	n.d.	4 ± 0	12 ± 3	*d, f*
127	(*Z*)-piperitol	1665	n.d.	2 ± 0	3 ± 1	*e, i, j*
138	(*E*)-piperitol	1732	n.d.	3 ± 1	4 ± 1	*e, i*
49	β-terpinene	1226	n.d.	n.q.	7 ± 2	*e, i, j*
37	sylvestrene	1179	n.d.	n.d.	3 ± 1	*e, i, j*
	acids					
90	acetic acid	1430	n.c.	n.c.	n.c.	*d, j*
101	propanoic acid	1515	n.d.	n.c.	n.d.	*d, j*
117	butanoic acid	1607	n.c.	n.c.	n.c.	*d, j*
143	hexanoic acid	1825	n.c.	n.c.	n.c.	*d, j*
150	(*E*)-hex-2-enoic acid	1943	n.c.	n.c.	n.c.	*d, j*

Table 12: Continued.

no.[a]	compound	RI[b]	'8 Bona'	'Ben Sarek'	'Ometa'	remark
				[µg/kg][c]		
149	(*E*)-hex-3-enoic acid	1914	n.d.	n.c.	n.d.	*d, j*
154	hexadecanoic acid	2855	n.c.	n.c.	n.c.	*d*
155	octadecanoic acid	3097	n.c.	n.d.	n.c.	*d, j*
	others					
151	caryophyllene oxide	1961	3 ± 0	7 ± 1	23 ± 2	*d, g*
85	*p*-α-dimethyl styrene	1416	n.d.	n.q.	2 ± 1	*d, g*

[a] Numbers correspond to the chromatogram shown in Figure 4. [b] Linear retention index on a DB-Wax-column (see section 3.2.2.1 and section 3.2.3.1). [c] Triplicate analysis of blackcurrant berries: mean ± standard deviation. [d] Identification based on comparison of GC and mass spectral data with those of authentic reference compounds. [e] Tentatively identified by comparison of mass spectral data with those from database (National Institute of Standards and Technology (NIST)). [f] Quantitation on the basis of recovery rate and response factor. [g] Quantitation on the basis of response factor, no recovery rate considered. [h] Quantitation on the basis of recovery rate, no response factor considered. [i] No recovery rate and response factor were considered. [j] Identified for the first time in blackcurrant berries. [k] Below limit of detection (0.5 µg/kg). [l] Below limit of quantification (1.5 µg/kg). [l] Detected but concentration not calculated because of too low recovery via VHS.

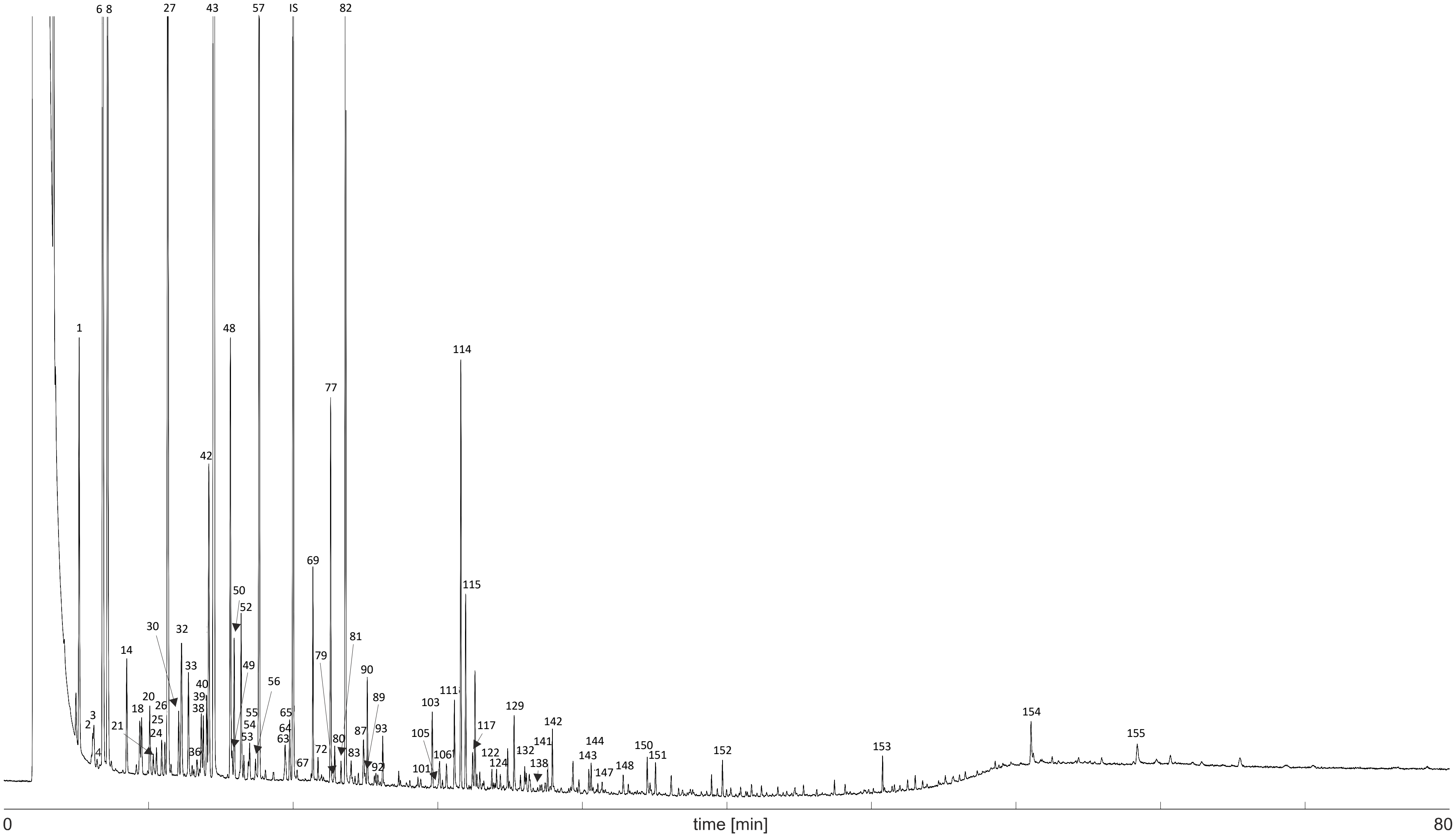

Figure 4: Typical gas chromatographic separation of volatile compounds isolated from fresh blackcurrant berries (‘Supernova’, Deutenkofen, 20 June, 2014) by vacuum-headspace extraction (VHS). Peak numbers correspond to Table 13; for gas chromatography (GC) conditions see section 3.2.3.1; IS: internal standard heptan-2-ol.

Table 13: Compounds identified in fresh blackcurrant berries in addition to those listed in Table 12.

no.[a]	compound	RI[b]	n[c]	remark
	esters			
17	methyl (*E*)-but-2-enoate	1090	22	*d, h, k*
29	ethyl (*E*)-but-2-enaote	1148	12	*d, h, k*
29	2-methylpropyl 3-methylbutanoate	1182	2	*d, i, k*
47	ethyl hexanoate	1224	13	*d, h*
51	3-methylbut-2-enyl acetate	1239	10	*d, i, k*
58	methyl hex-2-enoate	1272	5	*f, j, k*
60	3-methylbutyl 3-methylbutanoate	1286	5	*d, i, k*
75	3-methylbut-3-enyl 3-methylbutanoate	1360	5	*f, j, k*
76	methyl 2-hydroxyhexanoate	1563	4	*f, j, k*
	alcohols			
5	butan-2-ol	1020	1	*d, i*
7	propan-1-ol	1031	4	*d, i*
19	pentan-3-ol	1104	3	*d, i, k*
23	pentan-2-ol	1116	1	*d, i*
31	pent-1-en-3-ol	1151	31	*d, i*
45	2-methylbutan-1-ol	1200	23	*d, j*
46	3-methylbutan-1-ol	1200	28	*d, j*
66	3-methylbut-2-en-1-ol	1311	24	*d, i*
116	oct-2-en-1-ol	1600	12	*f, j*
148	2-phenylethanol	1886	2	*d, i*
	aldehydes			
35	heptanal	1170	17	*d, h, k*
102	(*E*)-non-2-enal	1517	7	*d, h*
119	(*E*)-dec-2-enal	1626	25	*d, i*
135	geranial	1708	7	*d, h*
	ketones			
61	oct-1-en-3-one	1290	1	*d, h*
68	6-methylhept-5-en-2-one	1322	18	*d, i, k*
73	(*Z*)-octa-1,5-dien-3-one	1354	1	*g, k*
78	nonan-2-one	1376	3	*d, i, k*
112	2-undecanone	1582	11	*d, i, k*
	terpenes			
70	(*Z*)-rose oxide	1341	7	*d, h*
71	(*E*)-rose oxide	1351	10	*d, h*

Table 13: Continued.

no.[a]	compound	RI[b]	n[c]	remark
108	fenchol	1563	2	*d, i*
120	menthol	1628	7	*d, h, k*
125	humulene	1665	14	*f, j*
	others			
44	4-methoxy-2-methyl-2-butanethiol	1199	12[l]	*d, h, k*
86	2-isopropyl-3-methoxypyrazine	1416	- [e]	*h, k*
88	methional	1426	- [e]	*h, k*
96	2-*sec*-butyl-3-methoxypyrazine	1482	- [e]	*h, k*
100	2-isobutyl-3-methoxypyrazine	1512	- [e]	*h, k*
109	mesifurane	1568	4	*d, k*

[a] Numbers correspond to the chromatogram shown in Figure 4. [b] Linear retention index on a DB-Wax-column (see section 3.2.2.1 and section 3.2.3.1). [c] Identified in *n* of 34 batches. [d] Identification based on comparison of mass spectral and GC data with those of authentic reference compounds. [e] Identified in a pooled extract resulting from a total of 5 kg blackcurrant berries, based on comparison of mass spectral and GC data with those of authentic reference compounds in SIM mode. [f] Tentatively identified by comparison of mass spectral data with those from database (National Institute of Standards and Technology (NIST)). [g] Tentatively identified by comparison of retention time and odour with literature data (Rychlik *et al.* 1998; Baba and Kumazawa 2014). [h] Quantitation on the basis of recovery rate and response factor. [i] Quantitation on the basis of response factor, no recovery rate considered. [j] No recovery rate and no response factor were considered. [k] Identified for the first time in blackcurrant berries. [l] 4-methoxy-2-methyl-2-butanethiol was identified in three batches directly after VHS and in nine batches after enrichment on mercurated agarose gel of 3 - 6 pooled VHS-extracts (corresponding to 1.5 - 3 kg blackcurrant berries).

4.1.2.2 Impact of freezing on the volatile compositions of blackcurrant berries

Many factors can influence the volatile constituents of fruits. One of these factors are the storage conditions, including temperature, irradiation, storage atmosphere, and chemical applications (El Hadi *et al.* 2013). The influence of storage temperature has been analysed in various investigations. In addition to an influence of elevated temperature on the volatile constituents of juice (Siegmund *et al.* 2001; Perez-Cacho and Rouseff 2008), an effect of freezing was determined in different fruits, such as kiwi (Talens *et al.* 2003), pineapple (Kaewtathip and Charoenrein 2012), and strawberries (Douillard and Guichard 1990). As these studies revealed the impact of freezing on the volatile compositions of a variety of different fruits and as most of the studies on blackcurrant berries were realised with frozen fruits (Andersson and von Sydow 1964, 1966a, b; Nursten and Williams 1969a; von Sydow and Karlsson 1971a, b; Karlsson-Ekstrom and von Sydow 1973; Latrasse *et al.* 1982; Mikkelsen and Poll 2002; Ruiz del Castillo and Dobson 2002b; Tiitinen *et al.* 2004; Christensen and Pedersen 2006; Kampuss *et al.* 2008), the influence of freezing on the volatile profile of blackcurrant berries was determined.

Blackcurrant berries of two cultivars (‘Supernova’ and ‘Tenah’) were investigated directly after purchase and the same batches were analysed after three, six, nine, and twelve months of storage at -20 °C. The frozen berries showed a less intensive odour and their odour was reminiscent of cooked berries. The concentrations of volatile main compound classes determined from batches of the aforementioned cultivars, which were analysed either directly after purchase or after storage at -20 °C for up to twelve months are depicted in Figure 5. In addition, the concentrations of individual major volatiles compounds determined in the batches, which were analysed directly after purchase or after nine months of storage at -20 °C, are listed in Table 14.

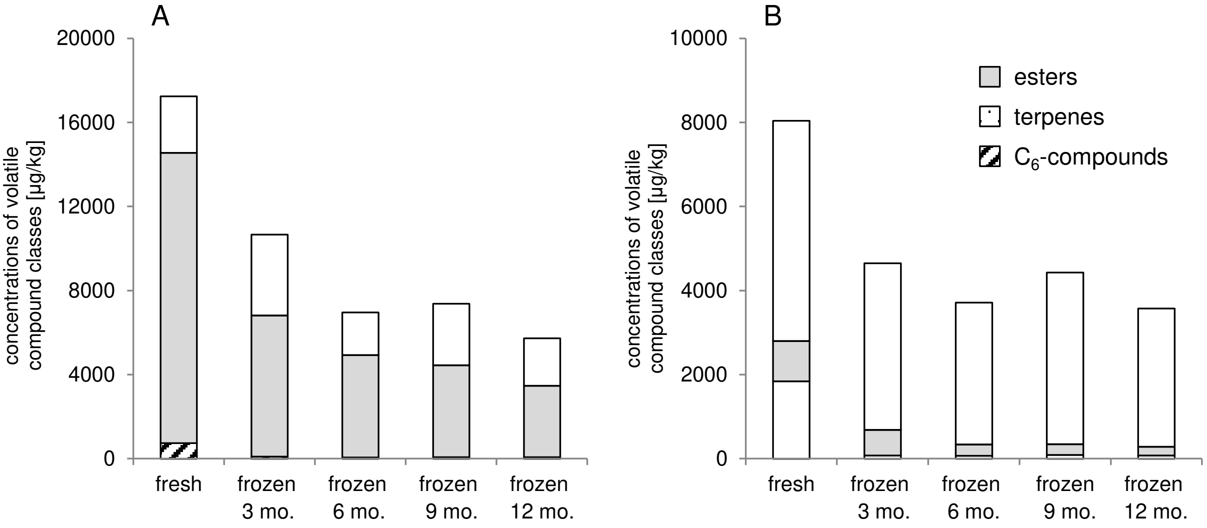

Figure 5: Concentrations of volatile main compound classes in fresh and frozen (-20°C) blackcurrant berries of the cultivars (A) 'Supernova' (Bühl, 22 July, 2014) and (B) 'Tenah' (Gleinstätten, 24 July, 2014). mo.: months.

The most pronounced influence of the freezing step was observed for the C_6-compounds. The concentrations of unsaturated C_6-aldehydes (e.g. (*Z*)-hex-3-enal and (*E*)-hex-2-enal) and their corresponding alcohols (e.g. (*Z*)-hex-3-en-1-ol, (*E*)-hex-2-en-1-ol), typical representatives of enzymatically formed degradation products of unsaturated fatty acids, decreased upon storage in frozen state; except for (*Z*)-3-hex-3-en-1-ol, they were all below their limits of detection (see Table 14). On the other hand, the concentrations of hexanal, heptanal, (*E*)-hept-2-enal, (*E*)-oct-2-enal, decanal, and nonanal, all of them known autoxidation products of either linoleic acid or oleic acid (Grosch 1987), were increased after freezing. These results are in agreement with the low concentrations or the absence of C_6-compounds in nearly all studies in which frozen blackcurrant fruits were investigated (Andersson and von Sydow 1964, 1966a; Nursten and Williams 1969a, b; von Sydow and Karlsson 1971a; Mikkelsen and Poll 2002). The increased concentration of hexanal upon storage in frozen state for nine months is in agreement with the fact that this C_6-compound was quantitated at concentrations between 192 µg/kg and 1.180 µg/kg in studies with frozen

Table 14: Concentrations of main volatile compounds in fresh and frozen blackcurrant berries.

	'Supernova'[a]		'Tenah'[b]	
compound	fresh	frozen[c]	fresh	frozen[c]
	[µg/kg][d]		[µg/kg][d]	
C_6-compounds				
(*E*)-hex-2-enal	423 ± 147	n.d.[e]	295 ± 67	n.d.
(*E*)-hex-2-en-1-ol	197 ± 55	n.d.	32 ± 8	n.d.
(*Z*)-hex-3-en-1-ol	46 ± 13	3 ± 0 (*)	147 (113 - 160)	3 (3 - 3)
(*Z*)-hex-3-enal	41 ± 58	n.d.	1,292 ± 329	n.d.
hexan-1-ol	35 ± 4	28 ± 5	21 ± 6	17 ± 2
hexanal	14 ± 11	62 ± 23 (*)	47 ± 9	80 ± 8 (**)
(*E*)-hex-3-en-1-ol	2 ± 0	n.d.	2 ± 0	n.d.
(*Z*)-hex-2-en-1-ol	2 ± 0	n.d.	n.d.	n.d.
(*E*)-hex-3-enal	n.d.	n.d.	18 ± 3	n.d.
esters				
ethyl butanoate	10,219 ± 3,959	2,761 ± 1,074 (*)	46 ± 45	60 ± 17
methyl butanoate	1,633 (710 - 6752)	1,395 (923 - 1,658)	770 ± 481	133 ± 50
methyl hexanoate	45 (20 - 133)	24 (21 - 24)	51 ± 18	10 ± 2
ethyl octanoate	22 ± 5	4 ± 0 (*)	n.q.[f]	n.d.
methyl octanoate	4 ± 3	n.q.	7 ± 1	3 ± 0 (**)
alcohols				
2-methylbut-3-en-2-ol	3,888 ± 141	2,448 ± 55 (*)	99 (62 - 231)	46 (38 - 77)
aldehydes				
nonanal	4 ± 1	7 ± 1 (*)	8 ± 1	8 ± 1
(*E*)-hept-2-enal	3 ± 0	6 ± 1 (**)	4 ± 1	9 ± 1 (**)
decanal	n.d.	8 ± 1	5 ± 0	8 ± 1 (**)
heptanal	n.d.	n.d.	2 ± 1	4 ± 1 (*)
(*E*)-oct-2-enal	n.d.	n.d.	2 ± 0	5 ± 0 (***)
terpenes				
Δ-3-carene	620 ± 32	669 ± 127	1,255 ± 274	978 ± 74
terpinolene	479 ± 36	464 ± 71	904 ± 55	706 ± 34 (**)
(*Z*)-β-ocimene	373 (212 - 395)	196 (180 - 205)	370 ± 41	238 ± 16 (**)
β-phellandrene	224 ± 177	525 ± 163	1,261 ± 200	1,058 ± 86
α-phellandrene	162 (161 - 186)	13 (13 - 19)	33 ± 5	25 ± 2
myrcene	114 ± 29	149 ± 17	257 ± 47	207 ± 14
terpinen-4-ol	98 ± 38	69 ± 6	49 ± 8	37 ± 6
1,8-cineole	92 ± 39	183 ± 11 (*)	32 ± 13	11 ± 4

Table 13: Continued.

compound	'Supernova'[a] fresh	'Supernova'[a] frozen[c]	'Tenah'[b] fresh	'Tenah'[b] frozen[c]
	[µg/kg][d]		[µg/kg][d]	
limonene	86 ± 51	163 ± 40	383 ± 61	311 ± 21
(*E*)-β-ocimene	85 ± 16	81 ± 9	247 ± 21	158 ±10 (**)
sabinene	74 ± 10	102 ± 13 (*)	42 ± 15	35 ± 5
α-pinene	61 ± 5	57 ± 12	98 ± 32	75 ± 11
β-pinene	39 ± 3	42 ± 8	27 ± 8	16 ± 2
α-terpinene	39 ± 4	32 ± 4	49 ± 5	38 ± 3 (*)
caryophyllene	33 ± 6	15 ± 5 (*)	67 ± 32	40 ± 11
γ-terpinene	25 ± 6	21 ± 3	22 ± 2	16 ± 1 (*)
sabinene hydrate	17 ± 5	21 ± 2	12 ± 1	15 ± 2 (*)
α-terpineol	16 ± 4	34 ± 7 (*)	9 ± 5	8 ± 0

[a] Bühl (July 22, 2014). [b] Gleinstätten (July 22, 2014). [c] Blackcurrant berries were stored for nine months at -20 °C. [d] Triplicate analysis of fresh and frozen fruits; if data were normally distributed, values are mean ± standard deviation; if data were not normally distributed, values are median (minimum-maximum). Unpaired Student's t-test was used to test for equality of means between fresh and frozen blackcurrant berries of the same variety, if the prerequisites of normal distribution (Shapiro-Wilk test) and equality of variances (Fisher´s F-test) were met; Welch's t-test was performed, if data were normally distributed but equality of variances was not shown. Non-parametric Wilcoxon-Mann-Whitney U-test was used to compare medians, if normal distribution was shown. All tests were two-tailed. (***), $p < 0.001$; (**), $p < 0.01$; (*), $p < 0.05$. [e] Below limit of detection (0.5 µg/kg). [f] Below limit of quantification (1.5 µg/kg).

blackcurrant berries (Christensen and Pedersen 2006; Kampuss *et al.* 2008). Only one study performed with frozen fruits also reported the presence of (*E*)-hex-2-enal at high concentrations (up to 201 µg/kg) (Kampuss *et al.* 2008). Otherwise, the C_6-compounds (*E*)-hex-2-enal, (*Z*)-hex-3-enal, (*E*)-hexen-2-en-1-ol, and (Z)-hex-3-en-1-ol were only reported as volatile constituents in one of the few studies dealing with fresh blackcurrant fruits (Hempfling *et al.* 2013a). A decrease in the concentrations of C_6-compounds upon freezing has been described earlier for other fruits. In 1980, a decrease of C_6-compounds was shown for strawberry fruits after six weeks of storage at -18 °C by Schreier (1980). Similar results were found for strawberries by Douillard and Guichard (1990), as well as for kiwis (Pfannhauser 1988; Talens *et al.* 2003). As C_6-compounds are formed during the LOX pathway from linoleic acid and linolenic acid (see section 2.2.2), possible reasons for the decrease of C_6-compounds upon freezing can be a decrease of linoleic acid and linolenic acid or their intermediates as well as a decrease of the enzymatic activities. In previous investigations, the autoxidation of linoleic acid (and/or its 9- and 13-hydroperoxy intermediates as well as the methylated form of the 9-hydroperoxy intermediate) and linolenic acid (only its 9- and 13-hydroperoxy intermediates) were investigated in model experiments (Grosch *et al.* 1981; Schieberle and Grosch 1981; Schieberle *et al.* 1985; 1986; Schieberle *et al.* 1988). Hexanal and propanal were identified as the main volatile compounds generated during the autoxidation of linoleic acid and linolenic acid, respectively (Grosch *et al.* 1981). However, it was also shown that non-volatile compounds are formed during the autoxidation (Schieberle *et al.* 1985; 1986; Schieberle *et al.* 1988). Furthermore, autoxidation of aldehydes, such as hexanal, leads to their corresponding fatty acids (Schieberle and Grosch 1981). In contrast to the volatile autoxidation products quantified in this study (i.e. hexanal, heptanal, (*E*)-hept-2-enal, (*E*)-oct-2-enal, decanal, and nonanal), fatty acids and non-volatile autoxidation products possibly generated during the freezing process could not be quantified by the techniques applied.

For all detected esters, except for ethyl butanoate in cultivar ‘Tenah’, the mean contents after storage at -20 °C for nine months were lower than those in the fresh fruits. However, owing to the lack of normal distribution and equality of variances, respectively, the differences in concentrations could only be shown to be statistically significant for ethyl butanoate in the cultivar ‘Supernova’ and for ethyl octanoate and methyl octanoate. A decrease in the concentrations of esters upon freezing has been described earlier for other fruits, such as strawberry fruits (Schreier 1980; Douillard and Guichard 1990), kiwis (Pfannhauser 1988; Talens *et al.* 2003), and pineapples (Kaewtathip and Charoenrein 2012).

For the third major group of volatile compounds, the terpenes, there were sporadic statistically significant differences, i.e. decreases of the concentrations of hydrocarbons and increases of the concentrations of 1,8-cineole and the terpene alcohols sabinene hydrate and α-terpineol, in frozen compared to fresh berries. However, these differences were only observed in one of the two investigated cultivars, and there was no consistent trend. This is in agreement with Ruiz del Castillo and Dobson (2002a), who analysed the influence of freezing on terpenes in blackcurrant berries. They demonstrated that the relative distribution of terpenes was not affected by freezing and that there were no significant changes in the enantiomeric distributions of chiral terpenes (Ruiz del Castillo and Dobson 2002b). A potential freezing effect on terpenes was also analysed in raspberry fruits, and no effect on the eight main terpenes was detected after three, six, nine, and twelve months of storage at -20 °C (de Ancos *et al.* 2000).

For the main alcohol 2-methylbut-3-en-2-ol a statistical significant increase upon freezing was observed for the cultivar ‘Supernova’. However, no statistical significant increase was observed in the cultivar ‘Tenah’, due to the lack of normal distribution.

The findings of this work, in combination with similar observations reported in the literature for other types of fruits, strongly indicate that the previous studies on frozen blackcurrant berries do not reflect the original distribution of volatile compounds. The data show that storage of blackcurrant fruits in frozen state results in drastically reduced concentrations of C_6-compounds. In combination with lowered concentrations of esters and 2-methylbut-3-en-2-ol, this leads to a shift of the distribution of volatile constituents in favour of the terpenes in the frozen material. Therefore, the volatile profiles of different cultivars of fresh blackcurrant berries should be investigated in order to unambiguously describe the original distribution of volatile compounds in fresh blackcurrant berries.

4.1.2.3 Impact of enzymatic reactions on profiles of C_6-compounds

C_6-compounds are formed from the unsaturated fatty acids linoleic acid and linolenic acid via the LOX pathway after disruption of plant tissues. However, the original profile of C_6-compounds can be rapidly changed by isomerisation or reduction reactions (Sanz and Pérez 2010). Therefore, in addition to the effect of freezing, the influence of enzymatic reactions on the formation of C_6-compounds was monitored over a specified time period.

Various enzyme inhibitors were used in a wide range of fruits and vegetables, such as ammonium sulphate in apricot (Guichard and Souty 1988), calcium chloride in cherries (Girard and Kopp 1998), sodium sulphate in breadfruit (Iwaoka *et al.* 1994), and stannous chloride in kiwi (Bartley and Schwede 1989). Buttery *et al.* (1987) analysed the effect of calcium chloride and sodium chloride on enzyme inhibition in tomatoes. The concentration of (*Z*)-hex-3-enal was unchanged 3 h after inhibition with calcium chloride. In contrast, sodium chloride showed a slow isomerisation of (*Z*)-hex-3-enal to (*E*)-hex-2-enal (Buttery *et al.* 1987). Therefore, enzymatic activities were inhibited in the present work by addition of saturated aqueous calcium chloride solution to crushed berries at different time points (30 s, 60 s, 90 s, and 180 s after the homogenisation had started, respectively). The results were compared with the profiles of C_6-compounds in uninhibited blackcurrant berries of the same batches.

As shown in Figure 6 for three batches, the total amounts of C_6-compounds after isolation via VHS increased in general over time. The decrease in total amounts of C_6-compounds observed for a few time points could be explained by the natural inhomogeneity of the blackcurrant fruits; as only a single determination was conducted due to time constraints. However, the spectrum of C_6-compounds was changing over time due to the activity of the isomerase and the alcohol dehydrogenase. The content of (*Z*)-hex-3-enal decreased in favour of the isomer (*E*)-hex-2-enal and the corresponding alcohols (*E*)-hex-2-en-1-ol and (*Z*)-hex-3-en-1-ol. The compounds generated from linoleic acid, hexanal and hexan-1-ol, also increased. Similar changes were observed in rhubarb (Dregus and Engel 2003). The extent of the isomerisation of (*Z*)-hex-3-enal to (*E*)-hex-2-enal was different in the three cultivars; different proportions were observed upon enzyme-inhibition after 180 s and these differences were even more pronounced after work-up of the berries without inhibition of enzymes.

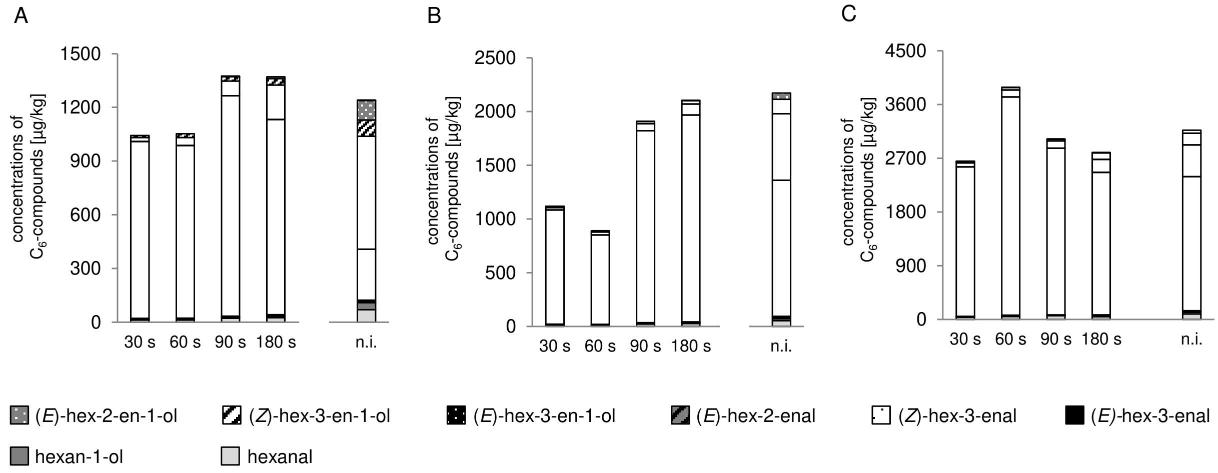

Figure 6: C_6-compounds isolated via vacuum-headspace extraction (VHS) from fresh blackcurrant berries of the cultivars (A) ‘Tsema’ (Bühl, 4 August, 2015), (B) ‘Tenah’ (Oberkirch, 28 July, 2015), and (C) an unknown cultivar (Tettnang, 10 July, 2014), after enzyme inactivation with calcium chloride at defined time points (30 s, 60 s, 90 s, and 180 s after homogenisation had started, respectively) and without inhibition (n.i.).

4.1.2.4 Variability in the compositions of volatile compounds isolated from fresh blackcurrant berries depending on cultivar and season

In addition to postharvest handling, such as freezing, genetic and preharvest factors, such as cultivar and harvest year, can affect the volatile profiles of fruits (El Hadi *et al.* 2013). In earlier investigations, the influence of cultivar on the volatile profiles of frozen blackcurrant berries (Bricout *et al.* 1985; Christensen and Pedersen 2006; Kampuss *et al.* 2008) has been analysed. Additionally, Marriott (1986) as well as Ruiz del Castillo and Dobson (2002b) determined the impact of cultivar on the profile of terpenes and Andersson and von Sydow (1966b) investigated the influence of cultivar on six volatile compounds. However, up until now no influence of the harvest year on the distribution of single volatile compounds was evaluated. The profiles of volatile compounds were analysed in this work in fruits of nine blackcurrant cultivars. All investigated cultivars met the following conditions: (i) In contrast to previous studies on varietal differences (Bricout *et al.* 1985; Christensen and Pedersen 2006; Kampuss *et al.* 2008), fresh rather than frozen berries were analysed due to the proven effects of freezing on the volatile profile of blackcurrant berries; (ii) the fruits were grown at the same location in Southern Germany (except for cultivar ‘Andega’); (iii) in order to minimize the effect of the stage of ripeness, the fruits were hand-picked at similar states of colour and firmness; (iv) to confirm the consistencies of the observed differences, fruits were harvested and analysed in two consecutive seasons (2014 and 2015).

In Figure 7 to Figure 9, the distributions of the concentrations of selected representatives of the three compound classes C_6-compounds, esters, and terpenes are depicted for eight cultivars harvested at the location Deutenkofen (‘8 Bona’, ‘Ben Sarek’, ‘Rosenthals’,

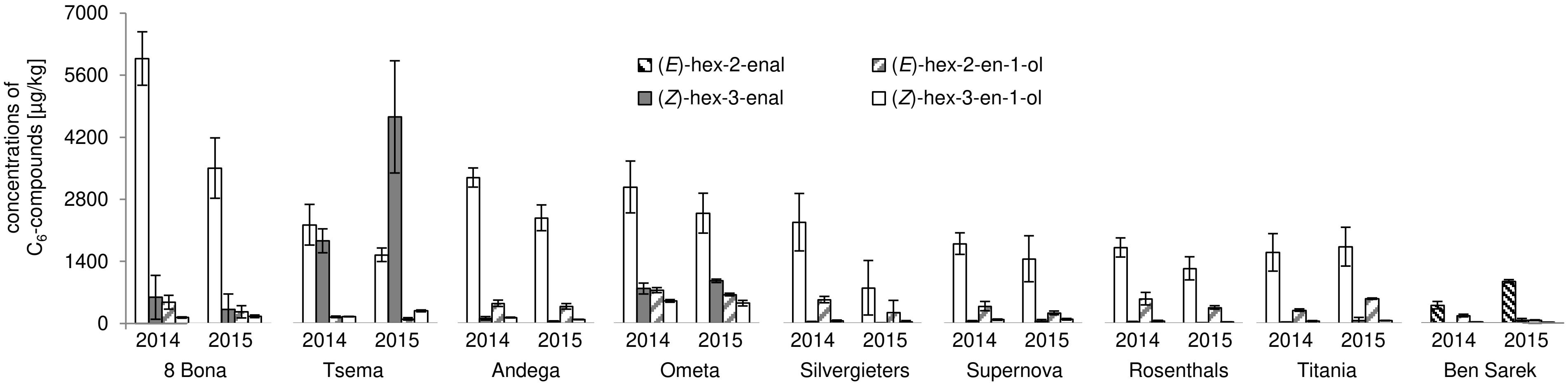

Figure 7: Concentrations of major C_6-compounds isolated via vacuum-headspace extraction (VHS) from ripe, fresh blackcurrant berries hand-picked at the locations Deutenkofen and Freising (‘Andega’) in 2014 and 2015.

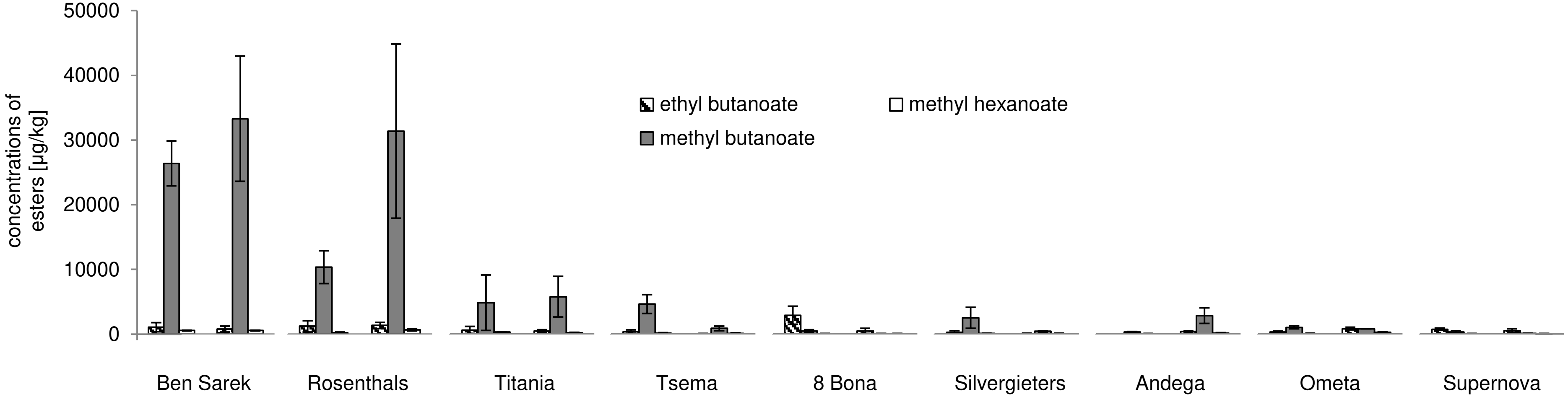

Figure 8: Concentrations of major esters isolated via vacuum-headspace extraction (VHS) from ripe, fresh blackcurrant berries hand-picked at the locations Deutenkofen and Freising (‘Andega’) in 2014 and 2015.

‘Silvergieters’, ‘Supernova’, ‘Titania’, ‘Tsema’, and ‘Ometa’) and for one cultivar harvested in Freising (‘Andega’).

C_6-compounds have previously been quantified in high concentrations in gooseberries (Hempfling *et al.* 2013b) and jostaberries (Hempfling *et al.* 2013a), two other *Ribes* species, as well as in other fruits, such as kiwi (Talens *et al.* 2003) and guava (Pino and Bent 2013). These aldehydes and alcohols, produced by plants after disruption of the plant structure, are important contributors to the characteristic aroma of fruits (El Hadi *et al.* 2013). Data on varietal differences in the spectra of C_6-compounds of blackcurrant berries are not available, owing to the nearly exclusive investigation of frozen berries (Andersson and von Sydow 1964, 1966a, b; Nursten and Williams 1969a, b; von Sydow and Karlsson 1971a, b; Karlsson-Ekstrom and von Sydow 1973; Latrasse *et al.* 1982; Bricout *et al.* 1985; Mikkelsen and Poll 2002; Ruiz del Castillo and Dobson 2002b; Tiitinen *et al.* 2004; Christensen and Pedersen 2006; Kampuss *et al.* 2008). In Figure 7, the main C_6-compounds (*E*)-hex-2-enal, (*Z*)-hex-3-enal, (*E*) hex-2-en-1-ol, and (*Z*)-hex-3-en-1-ol, generated from linolenic acid, are depicted. The aldehyde (*E*)-hex-2-enal was identified in all batches, except in one of the cultivar ‘Tsema’, as the quantitatively dominating C_6-compound varying in concentrations between 403 µg/kg and 5,977 µg/kg. It could also be found as the dominating C_6-compound in other fruits, such as jostaberries (Hempfling *et al.* 2013a), kiwi (Talens *et al.* 2003), and nectarine (Engel *et al.* 1988). In addition, the present work demonstrated that, with one exception (‘Tsema’), the profiles of the C_6-compounds were similar in two different harvest years (2014 and 2015).

Esters are one of the largest group of volatile constituents in fruits (Sanz and Pérez 2010), such as in Asian pear (Takeoka *et al.* 1992) and in pineapple (Takeoka *et al.* 1989; Kaewtathip and Charoenrein 2012). They are also a major group of volatile constituents in blackcurrant berries. The ester profiles of these berries are dominated by short-chain esters, especially saturated butanoic acid esters (see Figure 8). Large variations among the main esters methyl butanoate (37 - 33,300 µg/kg), ethyl butanoate (31 - 2,827 µg/kg), and methyl hexanoate (5 - 634 µg/kg) were observed in the nine analysed cultivars and are in agreement with data reported for frozen blackcurrant berries (Christensen and Pedersen 2006; Kampuss *et al.* 2008).

However, even if individual main esters strongly varied in dependence on the cultivar, for the first time, a stable distribution of the single main esters in the different cultivars could be observed over two consecutive harvest years in fresh blackcurrant berries. A quantitative preponderance of methyl butanoate compared to ethyl butanoate was observed in six of the nine investigated cultivars. In the other two cultivars, ‘8 Bona’ and ‘Supernova’, ethyl butanoate predominated in two consecutive harvest years. For the cultivar ‘Ometa’, no obvious trend could be observed. In all cultivars, the octanoic acid esters showed the same

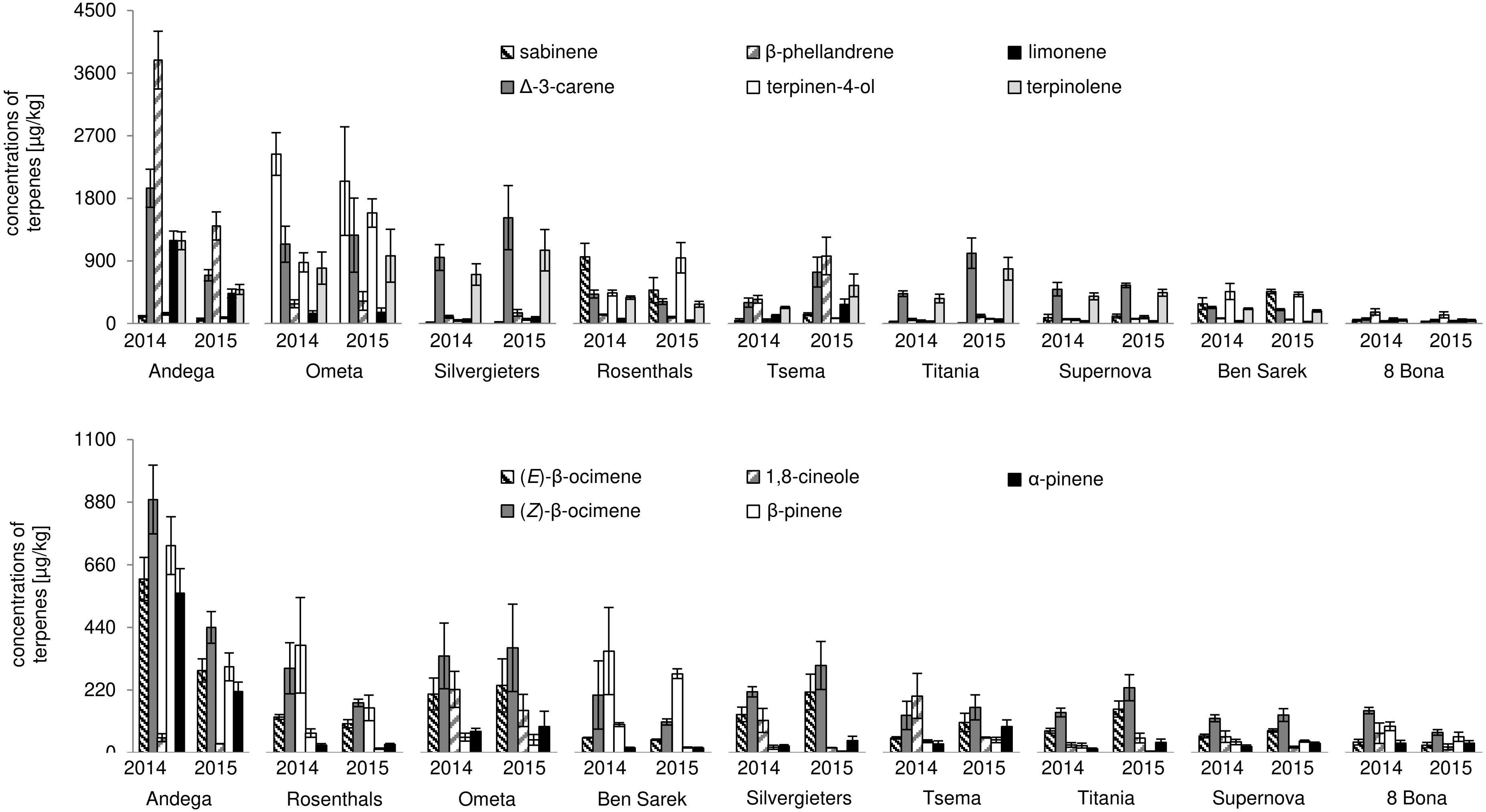

Figure 9: Concentrations of major terpenes isolated via vacuum-headspace extraction (VHS) from ripe, fresh blackcurrant berries hand-picked at the locations Deutenkofen and Freising (‘Andega’) in 2014 and 2015.

pattern of predominance between methyl and ethyl esters as observed for butanoic acid esters (data not shown). For 'Tsema' a predominance of methyl butanoate was also observed in a previous study on frozen blackcurrant berries (Christensen and Pedersen 2006), but in most cultivars investigated the ethyl ester showed higher concentrations than the methyl ester (Christensen and Pedersen 2006; Kampuss *et al.* 2008). In most other fruits ethyl and methyl esters were in the same order of magnitude or ethyl esters predominated (Schreier 1980; Young *et al.* 1995; Güntert *et al.* 1998). Higher concentrations of methyl esters compared to ethyl esters were previously observed in gooseberries (Hempfling *et al.* 2013b), jostaberries (Hempfling *et al.* 2013a), two other representatives of the family Grossulariaceae, and pineapple (Takeoka *et al.* 1989).

The third major group of volatile compounds, the terpenes, are the largest group of plant secondary metabolites with more than 40,000 different representatives. They are used as flavourings and fragrances in foods and cosmetics (Osorio *et al.* 2010). Large variations of the main hydrocarbon-terpenes (β-phellandrene: 56 - 3,787 µg/kg, sabinene: 8 - 2,436 µg/kg, Δ-3-carene: 44 - 1,945 µg/kg, limonene: 28 - 1,192 µg/kg, terpinolene: 44 - 1,189 µg/kg, (*Z*)-β-ocimene: 71 - 889 µg/kg, α-pinene: 16 - 727 µg/kg, (*E*)-β-ocimene: 26 - 610 µg/kg, and β-pinene: 4 - 560 µg/kg) as well as of the main oxygenated-terpenes (terpinen-4-ol: 26 - 1,588 µg/kg and 1,8-cineole: 17 - 377 µg/kg) were observed for the nine different investigated blackcurrant cultivars as depicted in Figure 9. Comparable variations were previously described in other studies on blackcurrant berries (Marriott 1986; Ruiz del Castillo and Dobson 2002b; Christensen and Pedersen 2006; Kampuss *et al.* 2008). Different cultivars were analysed in these previous studies, but the influence of the harvest year was not investigated. The present work demonstrated for the first time that the relative distributions of terpenes in fresh blackcurrant berries are stable over two consecutive harvest years for each investigated cultivar, as shown in Figure 9. As an example, the main terpene of 'Ometa' was sabinene in both harvest years, or the main terpene of 'Andega' and 'Tsema' was β-phellandrene in both harvest years.

The alcohol 2-methylbut-3-en-2-ol was identified as the main alcohol in each batch of fresh blackcurrant berries with concentrations between 38 µg/kg and 2,546 µg/kg. This alcohol was also detected by Andersson and von Sydow (1966a) in high amounts in frozen blackcurrant fruits. The alcohol 2-methylbut-3-en-2-ol was previously quantified in jostaberries (Hempfling *et al.* 2013a) and honey (Soria *et al.* 2008) in high concentrations.

Despite the large variability of individual volatile constituents, the investigated cultivars could be divided into three main categories (ester-type: 'Ben Sarek', 'Rosenthals', 'Titania'; terpene-type: 'Ometa', 'Andega', 'Silvergieters'; C_6-type: 8-Bona, 'Supernova', 'Tsema'), depending on the proportionately largest main volatile compound class present in each investigated cultivar (see Figure 10). Except for ‘Silvergieters’ and ‘Tsema’, these classifications were consistent for the two investigated years.

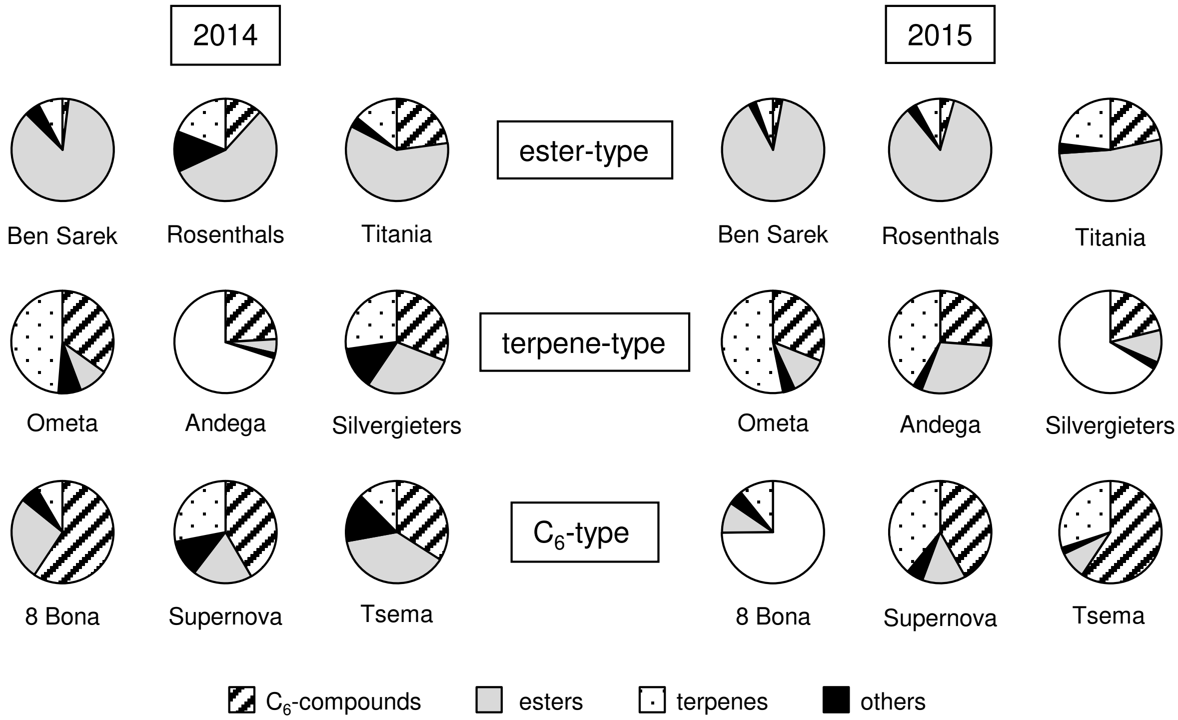

Figure 10: Classification of blackcurrant cultivars hand-picked in Deutenkofen, except for ‘Andega’ (Freising) in 2014 and 2015 based on the distribution of classes of volatile compounds.

In addition to the fruits grown at the same location and picked at the same state of ripeness, blackcurrant berries purchased at a local store were analysed (see Figure 11). For these fruits no consistent classification was possible. For example, the batch of cultivar ‘Supernova’ purchased in 2014 was an ester-type, whereas the batch purchased in 2015 was a terpene-type. Two batches of cultivar ‘Tenah’ purchased in 2014 were very different in composition; on the other hand, a batch bought in 2015 showed nearly the same distribution of compound classes as one of the batches from 2014. The actual dates of harvest of the purchased blackcurrant berries and their exact growing locations, as well as other preharvest and postharvest conditions, such as the state of ripeness, transportation, and storage, were not known. Assuming that the cultivars were correctly identified by the vendor, the impact of these parameters seems to be so decisive that without this information a varietal classification of blackcurrant fruits based on the volatiles is not possible.

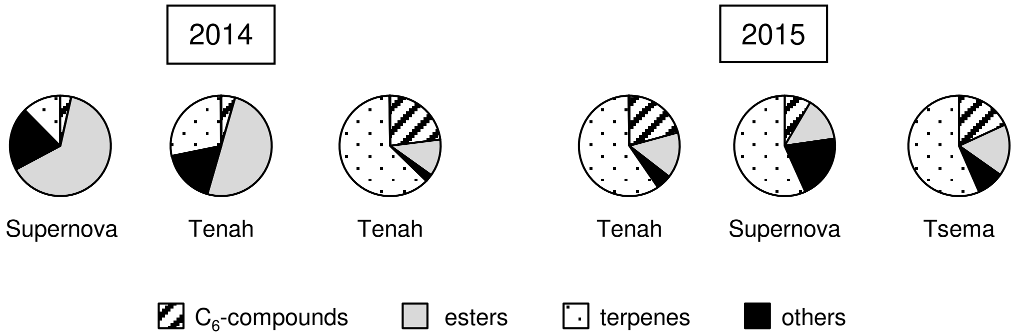

Figure 11: Distribution of the volatile compound classes in six purchased batches of blackcurrant berries in the years 2014 and 2015 (origin: Bühl, Gleinstätten and Oberkirch).

4.1.2.5 Impact of ripeness on the volatile compositions of blackcurrant berries

During ripening and maturation many biochemical and physical changes occur in fruits and vegetables. These changes include loss of chlorophyll, the generation of pigments, and the breakdown of carbohydrates, proteins, and lipids, which lead among other things to the formation of volatile compounds (Siegmund 2015). A change of the volatile profiles during ripeness has previously been observed for various fruits (Wan *et al.* 1999; Ménager *et al.* 2004; Soares *et al.* 2007; Serradilla *et al.* 2010; Sinuco *et al.* 2010; Hempfling *et al.* 2013a; Hempfling *et al.* 2013b).

As described in the previous section, different profiles of volatile constituents were determined for hand-picked (harvested at the same state of ripeness) and purchased blackcurrant berries. Motivated by these different results and the known effects of ripening, the influence of ripeness on the volatile composition of blackcurrant berries was investigated. To this end, the two cultivars ‘Rosenthal’ and ‘Titania’ were hand-picked from the same bushes at different degrees of ripeness in Deutenkofen. As illustrated in Figure 12 for the cultivar ‘Titania’, the colour of unripe berries was green to light-red and changed to black at the ripe stage. In addition, unripe berries were very hard and became softer during the maturation process.

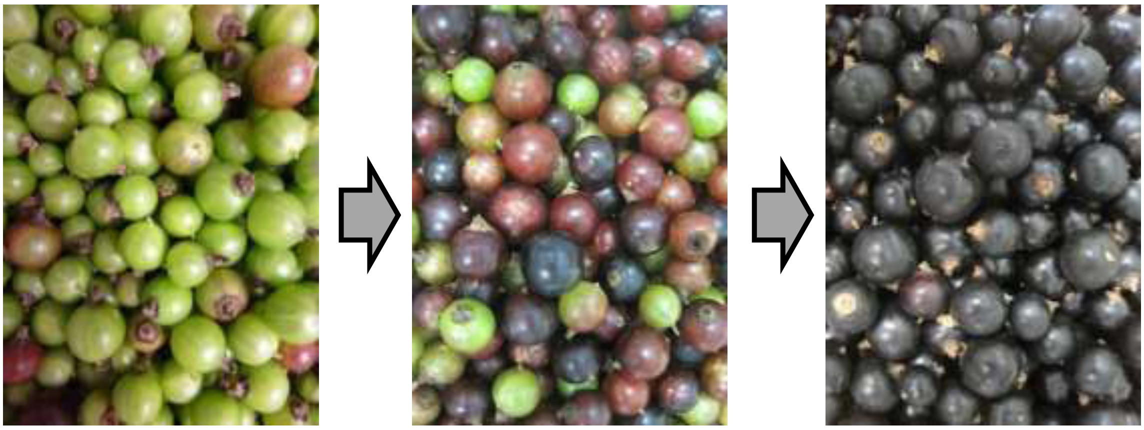

Figure 12: Blackcurrant berries (cultivar: ‘Titania’) at different degrees of ripeness.

During the ripening process, three main changes in the volatile profile of blackcurrant fruits occurred (see Figure 13). On the one hand, the concentrations of the C_6-compounds and the terpenes decreased and on the other hand, those of the esters increased.

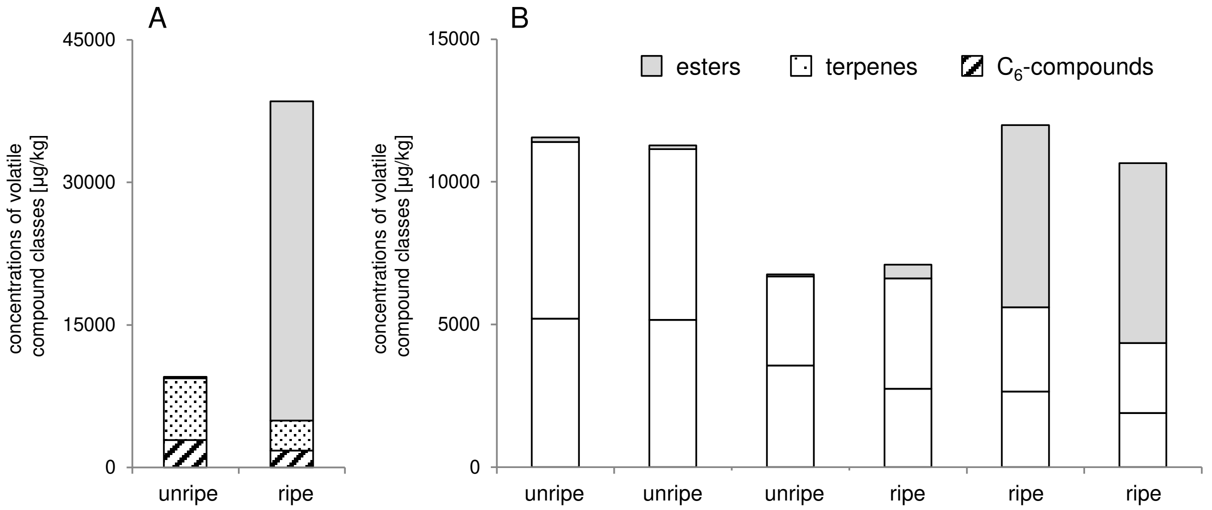

Figure 13: Concentrations of main volatile compound classes in blackcurrant berries at different stages of ripeness of the cultivars A: 'Rosenthals' (location: Deutenkofen; 17 June, 2015 and 3 July, 2015) and B: 'Titania' (location: Deutenkofen; 11 June, 2015, 17 June, 2015, 23 June, 2015, 29 June, 2015, 3 July, 2015, and 14 July, 2015).

As shown in Table 15, the most pronounced impact of the state of ripeness was seen for the esters. In unripe berries of the cultivar 'Titania' none of the investigated short-chained esters were present above the limit of the detection; in the cultivar 'Rosenthals' there were statistically significant increases in the concentrations of ethyl butanoate and methyl hexanoate. The increase of the concentrations of esters during ripening is in agreement with data reported for other fruits such as gooseberries (Hempfling *et al.* 2013b), guava (Soares *et al.* 2007; Sinuco *et al.* 2010), jostaberries (Hempfling *et al.* 2013a), and kiwi (Wan *et al.* 1999).

In the class of terpenes, there were statistically significant decreases of the concentrations of several monoterpene hydrocarbons upon ripening in the cultivar 'Titania'. However, due to the lack of normal distribution and equality of variance, respectively, they could hardly be confirmed in the cultivar 'Rosenthals'. The concentrations of the monoterpene alcohols terpinen-4-ol, sabinene hydrate, β-linalool, and citronellol decreased during ripening, whereas the data observed for 1,8-cineole and α-terpineol were inconsistent. The effects of ripening on the terpenes in blackcurrant berries were analysed in former studies with varying results (Andersson and von Sydow 1966a; Marriott 1986; 1987; Orav *et al.* 2002; Ruiz del Castillo and Dobson 2002a). Decreasing concentrations of terpene hydrocarbons upon ripening of blackcurrants were observed in two other studies (Marriott 1986; 1987). On the

Table 15: Concentrations of main volatile compounds in unripe and ripe blackcurrant berries.

	‘Rosenthals’[a]		‘Titania’[b]	
compound	unripe	ripe	unripe	ripe
	[µg/kg][c]		[µg/kg][c]	
C_6-compounds				
(*Z*)-hex-3-enal	1,520 (487 - 1635)	11 (9 - 13)	1,121 (1,095 - 1,200)	7 (6 - 8)
(*E*)-hex-2-enal	866 ± 381	1,240 ± 258	1,668 ± 288	1,317 ± 210
(*Z*)-hex-3-en-1-ol	371 ± 116	29 ± 4 (*)	1,059 ± 329	37 ± 2 (*)
(*E*)-hex-2-en-1-ol	333 ± 46	358 ± 38	1,122 ± 300	288 ± 52 (**)
hexan-1-ol	36 ± 3	40 ± 4	118 ± 28	84 ± 13
hexanal	27 ± 3	47 ± 13	66 ± 8	137 ± 39 (*)
(*E*)-hex-3-enal	7 ± 3	14 ± 3 (*)	11 ± 1	14 ± 3
(*Z*)-hex-2-enal	8 (2 - 8)	8 (7 - 9)	12 ± 4	10 ± 2
(*E*)-hex-3-en-1-ol	2 ± 1	2 ± 0	7 ± 2	2 ± 0 (*)
(*Z*)-hex-2-en-1-ol	2 ± 0	2 ± 1	4 ± 2	3 ± 0
esters				
methyl butanoate	6 ± 2	31,380 ± 13,468	n.d.[d]	4,064 ± 1,027
ethyl butanoate	3 ± 1	1,331 ± 478 (*)	n.d.	1,921 ± 259
methyl hexanoate	5 ± 2	634 ± 163 (*)	n.d.	155 ± 10
methyl octanoate	3 (3 - 4)	59 (52 - 68)	n.d.	21 ± 1
ethyl octanoate	n.d.	3 ± 1	n.d.	9 ± 1
alcohols				
2-methylbut-3-en-2-ol	4 (1 - 6)	1216 (635 - 1252)	2 (1 - 5)	214 (210 - 244)
terpenes				
sabinene	2,048 ± 872	480 ± 179 (*)	42 ± 17	36 ± 3
terpinen-4-ol	1,273 ± 177	944 ± 218	106 ± 23	56 ± 7 (*)
terpinolene	608 ± 271	277 ± 42	1,676 ± 282	617 ± 260 (**)
Δ-3-carene	453 (381 - 1,067)	296 (287 - 360)	2,137 ± 558	725 ± 336 (*)
sabinene hydrate	369 ± 98	106 ± 18 (*)	28 ± 11	11 ± 5 (*)
(*Z*)-β-ocimene	255 (231 - 524)	169 (165 - 189)	475 ± 94	261 ± 81 (*)
myrcene	240 ± 89	123 ± 15	329 ± 50	120 ± 43 (**)
(*E*)-β-ocimene	178 (165 - 368)	96 (90 - 117)	340 ± 63	125 ± 52 (*)
β-linalool	151 ± 32	58 ± 4 (*)	26 ± 4	9 ± 2 (**)
γ-terpinene	149 (130 - 374)	92 (89 - 174)	33 ± 7	18 ± 8
β-phellandrene	134 (121 - 323)	86 (83 - 105)	217 ± 51	174 ± 68
α-terpinene	99 (88 - 268)	70 (67 - 105)	79 ± 18	34 ± 15 (*)
limonene	69 (65 - 156)	42 (39 - 53)	109 ± 24	65 ± 26
citronellol	79 ± 9	35 ± 6 (**)	159 ± 27	36 ± 12 (**)
caryophyllene	34 (23 - 90)	29 (22 - 32)	132 ± 34	37 ± 12 (*)

Table 15: Continued.

compound	'Rosenthals'[a] unripe	'Rosenthals'[a] ripe	'Titania'[b] unripe	'Titania'[b] ripe
	[µg/kg][c]		[µg/kg][c]	
α-pinene	29 (26 - 64)	29 (24 - 31)	56 ± 15	30 ± 9
1,8-cineole	23 ± 3	157 ± 45 (*)	35 ± 3	24 ± 3 (*)
β-pinene	17 (16 - 38)	14 (11 - 16)	7 ± 3	18 ± 2 (**)
α-terpineol	15 ± 2	31 ± 7 (*)	16 ± 5	5 ± 1 (*)
α-phellandrene	12 (12 - 40)	10 (9 - 11)	32 ± 8	13 ± 6 (*)

[a] Deutenkofen (17 June, 2015 and 3 July, 2015). [b] Deutenkofen (11 June, 2015 and 14 July, 2015). [c] Triplicate analysis of unripe and ripe fruits; values are mean ± standard deviation where data are normally distributed; values are median (minimum maximum) where data are not normally distributed. Unpaired Student's t-test was used to test for equality of means between unripe and ripe blackcurrant berries of the same variety when normal distribution and equality of variances were assumed; Welch's t-test was performed when data were normally distributed but equality of variances was not assumed. Non-parametric Wilcoxon-Mann-Whitney U-test was used to compare medians when normality was not assumed. All tests were two tailed. [d] Below limit of detection (0.5 µg/kg).

other hand, there were also reports of increasing concentrations of Δ-3-carene, terpinolene, and β-caryophyllene during ripening (Andersson and von Sydow 1966b). Marriott (1986; 1987) also demonstrated that the concentrations of bound monoterpenes increased during ripening. The author suggested that the differing results with respect to Andersson and von Sydow can be explained by a hydrolysis of terpene glycosides during the distillation-step or an increase of monoterpene olefins resulting from acid catalysed dehydration of the corresponding alcohols (Andersson and von Sydow 1966b; Marriott 1986; 1987).

The total amounts of C_6-compounds decreased upon ripening; this was mainly due to decreasing concentrations of (*Z*)-hex-3-enal and the corresponding alcohol (*Z*)-hex-3-en-1-ol. In unripe berries of the cultivar 'Rosenthals', (*Z*)-hex-3-enal was the main compound. It also occurred in high concentrations in the cultivar 'Titania'. However, the percentage of (*E*)-hex-2-enal increased during ripening and it was the dominating compound in ripe berries of both cultivars. A possible explanation for this phenomenon could be an increase of isomerase activity during ripening. The decrease of the total C_6-compounds could be attributed to the potential increase of the C_6-precursor compounds linoleic acid and linolenic acid during ripening or a change in the activity of the enzymes involved in the generation of C_6-compounds, such as HPL or LOX. A change in HPL and LOX activity during ripening was previously observed for strawberries (Pérez *et al.* 1999; Leone *et al.* 2006). The importance of substrate supply for the generation of esters was shown in banana and strawberries (Wyllie and Fellman 2000; Pérez *et al.* 2002). In gooseberries (Hempfling *et al.* 2013b) and jostaberries (Hempfling *et al.* 2013a), which are two other representatives of the family Grossulariaceae*,* a decrease of total C_6-compounds during ripening was also demonstrated, especially for (*Z*)-hex-3-enal. Decreases of the concentrations of C_6-compounds during ripening were also detected in guava (Soares *et al.* 2007; Sinuco *et al.* 2010), nectarine (Engel *et al.* 1988), and strawberries (Ménager *et al.* 2004). On the other hand, in kiwi (Wan *et al.* 1999) no changes were observed, and in cherries (Serradilla *et al.* 2010) an increase of C_6-compounds was shown.

4.1.3 Sensory evaluations of volatile compounds of fresh blackcurrant fruits

4.1.3.1 Objectives

As described in section 4.1.2, a large number of volatile constituents was identified in blackcurrant berries. However, it was shown that only a limited number of volatile constituents may be sufficient for the characteristic aroma profile of foods (Reineccius 2010). In mango, for example, more than 300 different volatile compounds were identified, but only 15 were necessary to imitate the authentic aroma profile (Pino *et al.* 2005; Munafo *et al.* 2016).

A first evaluation of the odour properties of frozen blackcurrant berries was assessed by von Sydow and Karlsson in 1971 via GC/O (von Sydow and Karlsson 1971b). In 1982, the important contribution of methyl butanoate, ethyl butanoate, 1,8-cineole, diacetyl, and a 'catty urine odour' to the typical blackcurrant aroma was demonstrated via GC/O (Latrasse *et al.* 1982). In the present work, the unambiguous evidence of the presence of 4-methoxy-2-methyl-2-butanethiol, a sulphur-containing compound with a 'catty urine odour' note, in fresh blackcurrant fruits was demonstrated (see section 4.1.1). Additionally, OAVs were calculated and indicated that this sulphur-containing compound contributes to the aroma of blackcurrant berry. Besides Latrasse *et al.* (1982), odour-active compounds were identified in two other investigations in frozen blackcurrant berries via GC/O (Mikkelsen and Poll 2002; Tiitinen *et al.* 2004). Since it was demonstrated in the present work that the original profile of volatile compounds changes during freezing, it is important to emphasise that the sensory contributions of the previously identified odour-active compounds were determined on frozen fruits in all of those studies. Furthermore, no OAV-concept or reconstitution experiments were realised.

Thus, the objectives of this part of the present work were to prove the importance of 4-methoxy-2-methyl-2-butanethiol for the characteristic aroma of fresh blackcurrant fruits and to assess the contributions of single volatile compounds to the overall aroma of those fresh fruits.

4.1.3.2 Results

The aqueous condensates containing volatile compounds extracted by means of VHS from fresh blackcurrant berries featured a typical blackcurrant-like odour. The contributions of single volatile constituents, which are responsible for this odour, were evaluated by AEDA. For this purpose, a pooled and concentrated extract resulting from the isolation of volatiles via VHS from 3 kg of fresh blackcurrant berries of the cultivar 'Titania' (see section 3.2.5), was diluted gradually with a mixture of diethyl ether and *n*-pentane (1:1; v/v) and subjected, as a first step, to AEDA and assessed by two panellists via GC/O.

Twenty-six odour-active volatile compounds were identified by comparison of chromatographic, mass spectrometric, and sensory data with those of authentic reference compounds (see Table 16). Both panellists detected ethyl butanoate, 2-isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, (Z)-octa-1,5-octadien-3-one and 4-methoxy-2-methyl-2-butanethiol as the volatiles with the highest FD factors.

As a second step, the OAV concept, developed by Rothe and Thomas (1963), was used. Based on the fact that blackcurrant berries mainly consist of water (Souci *et al.* 2015), the OAVs were calculated with odour thresholds determined in water and the concentrations of volatile compounds determined in the cultivar 'Tsema' (origin: Bühl; 4 August, 2015). In section 4.1.1, it was shown, that 4-methoxy-2-methyl-2-butanethiol contributes to the aroma of blackcurrant berries, and its concentration was determined in seven cultivars. Except for the cultivar 'Andega' which showed an outstandingly high concentration of this sulphur-containing aroma compound, the concentration in the other investigated cultivars ranged from 0.16 µg/kg to 0.72 µg/kg. The concentration of 4-methoxy-2-methyl-2-butanethiol determined in the cultivar 'Tsema' (0.35 µg/kg) was nearly identical to the average concentration of 4-methoxy-2-methyl-2-butanethiol (0.34 µg/kg) determined in six cultivars. Therefore, the concentrations of aroma-active compounds determined in this cultivar were used as basis for the calculation of the OAVs.

However, several compounds with high FD factors, such as 2-isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, and oct-1-en-3-one, were present in the extract below the respective limits of quantification using GC/FID as detection mode. To circumvent that problem, ten extracts obtained from a total of 5 kg of blackcurrant berries of the cultivar 'Tsema' via VHS were pooled and quantifications were realised in SIM mode.

For a total of 20 compounds OAVs ≥ 1 could be calculated (see Table 16). Among them are compounds of each major chemical class such as the C_6-compounds (*Z*)-hex-3-enal (475), hexanal (18), and (*E*)-hex-2-enal (8), the short-chained esters ethyl butanoate (96) and methyl butanoate (13), as well as the monoterpenes 1,8-cineole (22) and α-pinene (14). Additionally, the sulphur-containing compound 4-methoxy-2-methyl-2-butanethiol (350), the lipid-oxidation product oct-1-en-3-one (48) as well as the pyrazines 2-isopropyl-3-methoxypyrazine (13), 2-isobutyl-3-methoxypyrazine (12), and 2-*sec*-butyl-3-methoxy-pyrazine (10) were identified as odour-active compounds with high OAVs. For the lipid-oxidation product (*Z*)-octa-1,5-dien-3-one no quantification was possible and no OAV was calculated.

In previous studies on frozen blackcurrant berries, the C_6-compounds hexanal, the esters ethyl butanoate and methyl butanoate, the terpenes 1,8-cineole, citronellol, geraniol, linalool, α-pinene, rose oxide, and α-terpineol, as well as the lipid oxidation product oct-1-en-3-one

Table 16: Concentrations and sensory data of key aroma compounds of fresh blackcurrant berries.

aroma compound	RI[a]	odour quality[b]	FD factor[c]		odour threshold		conc.	OAV
			A	B	in water [µg/L]	remark	[µg/kg][d]	
(*Z*)-hex-3-enal	1128	grassy	-	4	0.6	*e*	285	475
4-methoxy-2-methyl-2-butanethiol	1199	sulphur, sweat	64	31	0.001	*f*	0.35[g]	350
ethyl butanoate	1028	pineapple, fruity	1024	256	2.5	*e*	240	96
oct-1-en-3-one	1289	mushroom, metallic	64	16	0.005	*h*	0.24[i]	48
1,8-cineole	1194	terpeny, forest	32	-	2	*e*	44	22
hexanal	1067	grassy, green	32	8	4	*e*	70	18
α-pinene	1010	forest, wood	1	2	6	*j*	85	14
2-isopropyl-3-methoxypyrazine	1416	musty	256	32	0.004	*h*	0.05[i]	13
methyl butanoate	973	sweat, buttery, sweet	32	8	63	*e*	795	13
2-isobutyl-3-methoxypyrazine	1512	green pepper, mushroom	512	32	0.005	*h*	0.06[i]	12
2-*sec*-butyl-3-methoxypyrazine	1482	musty, earthy	4	2	0.004	*h*	0.04[i]	10
(*E*)-hex-2-enal	1200	apple, marzipan	32	2	77	*e*	632	8
pent-1-en-3-one	1008	solvent	32	-	1	*e*	4	4
(*E*)-non-2-enal	1517	cucumber	-	2	0.15	*h*	0.57[i]	4
(*Z*)-rose oxide	1341	flowery	1	8	0.5	*j*	1.59[i]	3
(*Z*)-hex-3-en-1-ol	1373	green	1	-	28	*k*	90	3
decanal	1485	citrus	2	-	5	*h*	6	1
citronellol	1755	flowery	8	4	40	*h*	52	1
linalool	1538	flowery, citrus, sweet,	8	-	5	*h*	5	1
methional	1426	potato	32	2	0.2	*h*	0.29[i]	1
methyl pentanoate	1072	musty	32	8	20	*l*	0.19[i]	< 1
(*E*)-hex-3-enal	1123	grassy, green	32	8	160	*m*	14	< 1

Table 16: Continued.

aroma compound	RI[a]	odour quality[b]	FD factor[c] A	FD factor[c] B	odour threshold in water [µg/L]	odour threshold remark	conc. [µg/kg][d]	OAV
bornyl acetate	1563	citrus, flowery, fruity	32	8	75	*n*	8	< 1
geraniol	1834	citrus	64	-	5	*h*	3[i]	< 1
α-terpineol	1683	fruity, sweet	4	-	182	*o*	11	< 1
(*Z*)-octa-1,5-dien-3-one	1354	geranium, metallic, green	256	16	0.0012	*h*	n.d.[p]	

[a] Linear retention index on a DB-Wax-column (see section 3.2.2.1 and section 3.2.3.1). [b] Assessed by gas chromatography/olfactometry (GC/O). [c] GC/O and aroma extract dilution analysis (AEDA) were performed by two panellists (A and B) using a concentrated vacuum-headspace extraction (VHS)-extract corresponding to 3 kg of cultivar ‘Titania’ (location: Deutenkofen; season 2015). [d] Concentrations determined in commercially obtained cultivar ‘Tsema’ (purchased: 4 August, 2015; origin: Bühl). [e] Hempfling *et al.* (2013a). [f] Odour thresholds were determined in water and in a blackcurrant-type matrix, respectively (see section 3.2.5.2). [g] Concentrations determined after enrichment on mercurated agarose gel (see section 3.2.1.3). [h] Rychlik *et al.* (1998). [i] Concentrations were determined with pooled extracts from ten batches of cultivar ‘Supernova’ (purchased: 4 July; 2016; origin: Oberkirch). [j] Leffingwell & Associates. [k] Hempfling *et al.* (2013b). [l] Takeoka *et al.* (1989). [m] Tamura *et al.* (2001). [n] Buttery *et al.* (1968). [o] Schrade (2014). [p] Below limit of detection, tentative identification by comparison of retention indices and odour description with literature data (Rychlik *et al.* 1998; Baba and Kumazawa 2014).

were also detected as odour-active compounds via GC/O, but no OAVs were calculated. Furthermore, a 'catty urine note' and isopropyl-methoxypyrazine were also determined in two previous investigations (Latrasse *et al.* 1982; Mikkelsen and Poll 2002; Tiitinen *et al.* 2004). The 20 compounds listed in Table 16 with an OAV ≥ 1 were used as a basis for reconstitution experiments to confirm their importance to the fresh blackcurrant berry aroma. To imitate a blackcurrant berry environment, these compounds were dissolved in a blackcurrant-type matrix containing the natural occurring organic acids and sugars (Haila *et al.* 1992; Bordonaba and Terry 2008; Milivojević *et al.* 2009; Nour *et al.* 2011; Mikulic-Petkovsek *et al.* 2012b; Vagiri *et al.* 2013). According to the panellists, the recombinate was reminiscent of blackcurrant berries. The aroma profile was also in fairly good agreement with that of fresh blackcurrant berries; however, the descriptor apple-like was rated higher in the recombinate (Figure 14).

The discrepancy in the descriptor apple-marzipan-like indicates that the time-dependent enzymatic formation of the aroma-active C_6-components seems to present a considerable challenge for the recombination of the blackcurrant berries aroma. As shown in Figure 6 (see section 4.1.2.3) a higher concentration of (*E*)-hex-2-enal was quantified in non-inhibited berries. Hence, the concentration of the apple-marzipan-like smelling compound (*E*)-hex-2-enal may have been overestimated. Based on this supposition, the influence of (*E*)-hex-2-enal was investigated in a second recombination experiment. The concentration of this apple/marzipan-like smelling compound was reduced to 22 µg/L. This corresponded to the concentration determined in the experiment with the cultivar 'Tenah' involving the inhibition of enzymes after 30 s (see Figure 6). Although this concentration was lower than the odour threshold of 77 µg/L reported for (*E*)-hex-2-enal in water (Hempfling *et al.* 2013a), the difference in perception of this descriptor between the fresh fruits and the recombinant remained. This indicates that there might be rather narrow ranges regarding the concentrations and/or specific proportions of aroma-active C_6-compounds that are required for the blackcurrant berry aroma. The demonstrated time-dependent dynamics in the enzymatic formation of C_6-compounds upon crushing of blackcurrant berries renders the elucidation of this phenomenon even more difficult. Additionally, matrix effects may have an impact on the aroma, as already shown for different classes of non-volatile compounds, such as alcohol, carbohydrates, lipids, proteins, polyphenols, and salts (Paravisini and Guichard 2016). In this reconstitution experiment, phenolic compounds like for example anthocyanins were not included in the recombinant, but may have an influence on the aroma.

Nevertheless, these reconstitution experiments confirmed, for the first time, the importance of the 20 investigated odour-active compounds to the aroma of fresh blackcurrant berries.

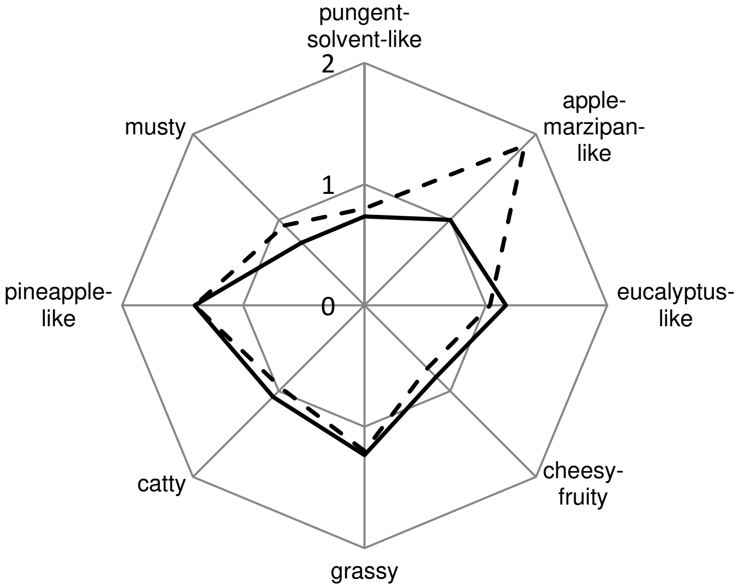

Figure 14: Aroma profiles of fresh blackcurrant berries (continuous line) and of the reconstitution model (broken line) based on concentrations of odour-active compounds in the cultivars ‘Tsema’ from Bühl (4 August, 2015) and ‘Supernova’ from Oberkirch (4 July, 2016).

4.1.4 Conclusion

By means of VHS and GC/MS, 150 volatile constituents were isolated from and identified in fresh blackcurrant fruits. Fifty-two of these compounds were reported for the first time. In agreement with previous studies with frozen fruits, short-chain esters and terpenes were major compound classes. However, rather high concentrations of C_6-compounds (e.g. (*E*)-hex-2-enal, (*Z*)-hex-3-enal) constituted a striking difference to data reported for frozen fruits. The alcohol 2-methylbut-3-en-2-ol also occurred in high concentrations.

Frozen storage of blackcurrant berries was shown to result in drastically reduced concentrations of C_6-compounds. As the concentrations of esters and 2-methylbut-3-en-2-ol also decreased upon freezing, the effect of freezing causes a shift in the distribution of volatile compounds in favour of the terpenes. These findings indicate that the original volatile profiles of blackcurrant berries cannot be evaluated by analysing frozen fruits.

Freezing resulted in an increase of hexanal and a decrease of compounds generated from linolenic acids; in addition, enzymatic reactions particularly affected the distributions of (*Z*)-hex-3-enal and (*E*)-hex-2-enal. Enzyme inhibition experiments showed that (*Z*)-hex-3-enal is the main compound at the beginning of homogenisation. Without inhibition, the content of (*Z*)-hex-3-enal decreased in favour of the isomer (*E*)-hex-2-enal and the corresponding alcohols (*E*)-hex-2-en-1-ol and (*Z*)-hex-3-en-1-ol.

Investigations of hand-picked fruits from nine different blackcurrant cultivars showed large variations of the main compounds in dependence of the cultivar, whereas the growing year had no effect. Additionally, the influence of ripeness was investigated and a decrease of C_6-compounds and terpenes as well as an increase of esters were shown. Nevertheless, blackcurrant cultivars can be classified according to their contents of C_6-compounds, esters, and terpenes in the fresh fruits, if prerequisites, such as the same growing location and the same state of ripeness, are met.

Furthermore, for the first time, unambiguous evidence for the presence of 4-methoxy-2-methyl-2-butanethiol in fresh berries of seven blackcurrant cultivars was provided. Among the investigated blackcurrant cultivars, ‘Andega’ exhibited by far the highest concentration of this sulphur-containing compound. The determination of OAVs revealed that 4-methoxy-2-methyl-2-butanethiol is a major contributor to the aroma of fresh blackcurrant berries.

The aqueous condensates obtained via VHS from fresh blackcurrant berries featured a typical blackcurrant-like odour, and the contributions of the different volatiles to the odour of fresh blackcurrant berries were evaluated by AEDA, determination of FD factors, and calculation of OAVs. In addition to 4-methoxy-2-methyl-2-butanethiol, representatives of the major chemical classes, i.e. C_6-compounds, short-chained esters, and monoterpenes, turned out to be odour-active constituents. The lipid-oxidation product oct-1-en-3-one and the pyrazines 2-isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, and 2-*sec*-butyl-3-methoxypyrazine were further identified as compounds with high OAVs. A recombination of the aroma of fresh blackcurrant fruits could not be fully achieved in the present work. Further investigations of the dynamics of the enzymatic formations and isomerisations of the C_6-compounds might be the key to solve this issue.

4.1.5 Comparison of the volatile profiles of blackcurrant berries to those of gooseberry and jostaberry fruits

Jostaberry (*Ribes x nidigrolaria* Bauer) is a hybrid of blackcurrant (*Ribes nigrum* L.) and gooseberry (*Ribes uva crispa* L.) (Bauer 1978). Recently, the volatile profiles of the fruits of these two other *Ribes* species (gooseberry and jostaberry) were analysed via VHS (Hempfling *et al.* 2013a; Hempfling *et al.* 2013b; Schrade 2014). In a preliminary screening, the concentrations of selected main volatile compounds in jostaberry fruits were compared to the corresponding concentrations in blackcurrant and gooseberry fruits (Hempfling *et al.* 2013a; Schrade 2014). In addition, the volatile profile of blackcurrant fruits, isolated from a single purchased batch, was compared to the volatile profiles of gooseberry and jostaberry fruits by Schrade (2014). Furthermore, the identified odour-active compounds of gooseberry and jostaberry fruits were compared by Schrade (2014) to the sensory evaluations of frozen blackcurrant fruits from the literature.

The results of the present work allow, in addition to a comparison of the volatile profiles isolated from a high number of batches via VHS, a comparison of the odour-active compounds of fresh fruits isolated via the same isolation method.

In the following, the results of the present work are compared to the results of gooseberry and jostaberry fruits (Hempfling *et al.* 2013a; Hempfling *et al.* 2013b; Schrade 2014).

In blackcurrant berries, 150 volatile compounds were identified (see section 4.1.2) and 85 of these compounds were also reported in gooseberry and/or jostaberry fruits (Hempfling *et al.* 2013a; Hempfling *et al.* 2013b; Schrade 2014). The volatile profile of gooseberry fruits is dominated by C_6-compounds and short-chain esters with the three main compounds (*Z*)-hex-3-enal, (*E*)-hex-2-enal, and methyl butanoate (Hempfling *et al.* 2013b; Schrade 2014). In jostaberry fruits, the C_6-compounds and esters were also identified as the main compound classes (Hempfling *et al.* 2013a; Schrade 2014). However, in blackcurrant fruits the terpenes represent, besides C_6-compounds and esters, a major compound class. Only two representatives of this chemical class (1,8-cineole and terpinen-4-ol) were present at high concentrations in jostaberry fruits. 2-Methylbut-3-en-2-ol, the main alcohol identified in blackcurrant berries, was quantified in similar concentrations in jostaberry fruits (Hempfling *et al.* 2013a; Schrade 2014).

The sensory evaluations of these three *Ribes* species revealed that (*Z*)-hex-3-enal is the compound with the highest OAV in each species. The other odour-active C_6-compounds of blackcurrant berries, i.e. (*E*)-hex-2-enal, (*Z*)-hex-3-en-1-ol, and hexanal were also identified in gooseberry and/or jostaberry fruits as compounds with an OAV > 1. Ethyl butanoate, the compound with the third highest OAV in blackcurrant berries, was also detected in gooseberry and jostaberry fruits as the compound with the third highest OAV. Furthermore, methyl butanoate, an odour-active compound in blackcurrant berries, was also identified in these two other *Ribes* species as an odour-active compound (Hempfling *et al.* 2013a; Hempfling *et al.* 2013b). In addition to blackcurrant fruits, 1,8-cineole was characterised in jostaberry fruits as a key aroma compound, whereas the mushroom-like smelling compound oct-1-en-3-one was only identified via GC/O in jostaberry fruits (Hempfling *et al.* 2013a).

To summarise, the comparison demonstrates that several identical compounds contribute to the aroma of blackcurrant, gooseberry, and jostaberry fruits. However, some important odour-active compounds, which contribute significantly to the aroma of blackcurrant berries, such as the ‘catty’ note of 4-methoxy-2-methyl-2-butanethiol and the musty smelling pyrazines, were not detected in these two other *Ribes* species.

4.2 Investigation of volatile compounds in redcurrant berries

4.2.1 Identification and quantification of volatile compounds in redcurrant berries

Analytical investigations of the volatile profiles of redcurrant berries are limited. Mehlitz and Matzik (1956a, b) identified free and bound acetic acid and formic acid in juice of redcurrant fruits. In addition, 38 volatile compounds were determined by Schreier *et al.* (1977) via LLE in fresh redcurrant fruits and in redcurrant juice. Furthermore, Boschetti *et al.* (1999) monitored the postharvest aging of redcurrant berries by the detection of acetaldehyde, acetic acid, ethyl acetate, methyl acetate, ethanol, and methanol. However, in these three studies the variability in the volatile profiles of fresh redcurrant berries and the impact of these compounds on the overall aroma were not investigated.

Therefore, the objectives of this part of the thesis were to identify and quantify volatile compounds in fresh redcurrant berries and to investigate the influence of cultivar, location, season, and state of ripeness on the volatile profiles of fresh redcurrant berries. Furthermore, the time dependency of the enzymatic generation of C_6-compounds and the effects of freezing on the volatile profiles should be analysed.

In a first attempt, volatile compounds of redcurrant berries were isolated in analogy to other *Ribes* species, i.e. blackcurrant berries (see section 4.1), gooseberries (Hempfling *et al.* 2013b), and jostaberries (Hempfling *et al.* 2013a) via VHS. However, the extracts resulting from this isolation protocol did not exhibit a redcurrant berry-like aroma and were reminiscent of green beans. Furthermore, the GC/FID and GC-MS chromatograms were rather ‘empty’. As a consequence, VHS was not considered to be useful for the isolation of volatile compounds from redcurrant berries.

The overall aroma of redcurrant berries exhibits an acidic note. Considering that LLE is an isolation method which isolates acids more efficiently than VHS (Schrade 2014), this technique was applied as alternative approach to isolate volatile compounds of redcurrant berries. However, the resulting LLE extracts also exhibited a green bean off-flavour. Therefore, enzymatic activities in redcurrant fruits were inhibited before isolation via LLE with an inhibition approach adapted from Buttery *et al.* (1987) by addition of saturated aqueous calcium chloride solution to crushed berries 180 s after the homogenisation had started. The extracts obtained with this procedure exhibited a fruity and acidic note without the green bean off-flavour.

4.2.1.1 Volatile profiles of fresh redcurrant berries

One part of the redcurrant berries was hand-picked at four locations in Southern Germany (Deutenkofen, Fahrenzhausen, Freising, and Weinsberg). In addition to these fruits with known origin and time of harvest, commercially available redcurrant berries were investigated. Quantification was based on the use of heptan-1-ol as internal standard; a preceding screening had shown that this compound is not present in redcurrant fruits. For the final calculations, the relative recovery rates and response factors were taken into account. Recovery rates were determined for selected substance in water or a redcurrant-type matrix, containing sugars and organic acids (see section 8.2, Table 25).

After isolation via LLE, 139 compounds (29 tentatively) were identified in 39 batches of redcurrant berries. One-hundred-and-seventeen of these compounds (28 tentatively) were identified for the first time in redcurrant berries. The distributions of volatile compounds determined in the cultivars ‘Rovada’, ‘Jonkheer van Tets’, and ‘Roodneus’ (locations: Deutenkofen and Freising, year: 2015) are shown as example in Table 17. Compounds identified in redcurrant berries in addition to those listed in Table 17 are summarised in Table 18, and a typical example chromatogram of an LLE-extract is shown in Figure 15.

Table 17: Volatile compounds isolated via liquid-liquid extraction (LLE) from berries of three hand-picked batches of redcurrant cultivars.

no.[a]	compound	RI[b]	‘Rovada’[c] [µg/kg][f]	‘Jonkheer van Tets’[d] [µg/kg][f]	‘Roodneus’[e] [µg/kg][f]	remark
	C$_6$-compounds					
23	(*E*)-hex-2-enal	1199	985 ± 74	2,449 ± 216	3,356 ± 739	*g, i*
13	(*Z*)-hex-3-enal	1128	33 ± 4	97 ± 17	279 ± 216	*g, i*
21	(*Z*)-hex-2-enal	1185	13 ± 2	32 ± 4	31 ± 7	*h, k, m*
12	(*E*)-hex-3-enal	1123	n.q.[o]	5 ± 1	13 ± 8	*g, k, l*
51	(*E*)-hex-2-en-1-ol	1394	35 ± 4	50 ± 4	49 ± 20	*g, i*
47	(*Z*)-hex-3-en-1-ol	1373	23 ± 4	22 ± 5	99 ± 9	*g, i*
6	hexanal	1067	11 ± 2	39 ± 11	47 ± 13	*g, i*
42	hexan-1-ol	1346	3 ± 0	5 ± 1	7 ± 1	*g, i*
	acids					
57	acetic acid	1430	1,426 ± 214	2,933 ± 1,127	1,655 ± 840	*g, i*
68	propanoic acid	1515	23 ± 1	27 ± 1	49 ± 13	*g, i, l*
84	butanoic acid	1607	10 ± 3	6 ± 3	7 ± 1	*g, i, l*
98	pentanoic acid	1717	n.d.[p]	n.d.	2 ± 1	*g, i, l*
106	hexanoic acid	1825	8 ± 1	12 ± 4	20 ± 3	*g, i, l*
114	heptanoic acid	1933	11 ± 2	5 ± 2	21 ± 5	*g, j, l*
122	octanoic acid	2039	4 ± 0	3 ± 1	9 ± 1	*g, j, l*
126	nonanoic acid	2143	2 ± 0	5 ± 0	3 ± 1	*g, j, l*
127	decanoic acid	2237	4 ± 2	3 ± 1	9 ± 2	*g, j, l*
136	hexadecanoic acid	2855	162 ± 11	168 ± 38	136 ± 38	*g, i, l*

Table 17: Continued.

no.[a]	compound	RI[b]	‘Rovada’[c]	‘Jonkheer van Tets’[d]	‘Roodneus’[e]	remark
				[µg/kg][f]		
137	octadecanoic acid	3097	77 ± 6	88 ± 32	135 ± 64	*g, i, l*
71	2-methylpropanoic acid	1548	15 ± 1	30 ± 3	22 ± 5	*g, i, l*
89	2-methylbutanoic acid	1651	18 ± 1	118 ± 8	38 ± 6	*g, i, l*
116	(*E*)-hex-2-enoic acid	1943	n.d.	24 ± 2	n.d.	*g, i, l*
112	(*E*)-hex-3-enoic acid	1914	6 ± 1	9 ± 9	28 ± 9	*g, i, l*
129	furan-2-carboxylic acid	2327	58 ± 15	44 ± 17	98 ± 36	*g, i, l*
130	benzoic acid	2329	6 ± 3	2 ± 1	8 ± 5	*g, i, l*
128	levulinic acid	2284	33 ± 5	34 ± 11	n.d.	*g, i, l*
	terpenes					
28	(*E*)-β-ocimene	1239	n.d.	n.q.	n.d.	*g, j, l*
27	(*Z*)-β-ocimene	1224	n.d.	n.q.	n.d.	*g, j, l*
32	terpinolene	1266	n.c.[q]	n.c.	n.d.	*g, l*
20	limonene	1181	6 ± 2	6 ± 2	n.d.	*g, i*
17	α-terpinene	1162	3 ± 2	3 ± 1	n.d.	*g, i, l*
30	*p*-cymene	1251	3 ± 1	3 ± 2	n.q.	*g, j, l*
8	limetol	1098	18 ± 1	2 ± 1	4 ± 1	*g, j, l*
22	1,8-cineole	1194	n.d.	n.q.	n.d.	*g, i, l*
46	α-pinene oxide	1361	n.c.	n.c.	n.d.	*g, l*
56	(*E*)-linalool oxide	1428	59 ± 6	30 ± 2	91 ± 9	*g, i, l*
60	(*Z*)-linalool oxide	1428	24 ± 3	13 ± 1	32 ± 3	*g, i, l*
77	dill ether	1573	3 ± 0	3 ± 0	4 ± 0	*h, k, m*
70	linalool	1538	n.q.	4 ± 1	n.q.	*g, i, l*
91	(*E*)-ocimenol	1667	4 ± 2	5 ± 0	n.d.	*h, k, m*
87	(*Z*)-ocimenol	1644	4 ± 1	4 ± 0	n.q.	*h, k, m*
82	hotrienol	1597	42 ± 5	7 ± 2	3 ± 0	*h, k, m*
123	terpin	2081	7 ± 1	11 ± 6	n.d.	*h, k, m*
97	γ-terpineol	1687	n.d.	2 ± 0	n.d.	*g, j, l*
78	terpinen-4-ol	1588	n.q.	2 ± 0	n.q.	*g, j*
95	α-terpineol	1683	78 ± 52	66 ± 44	25 ± 19	*g, i, l*
96	borneol	1685	n.d.	n.d.	2 ± 2	*g, j, l*
90	neral	1658	3 ± 2	3 ± 1	2 ± 1	*g, j, l*
80	*p*-menth-1-en-9-al (or isomer)	1593	52 ± 14	43 ± 14	18 ± 4	*h, k, m, n*
81	*p*-menth-1-en-9-al (or isomer)	1596	58 ± 10	53 ± 10	41 ± 4	*h, k, m, n*
92	*p*-menth-1-en-9-al (or isomer)	1671	85 ± 17	88 ± 5	15 ± 2	*h, k, m, n*

Table 17: Continued.

no.[a]	compound	RI[b]	‘Rovada’[c]	‘Jonkheer van Tets’[d]	‘Roodneus’[e]	remark
				[µg/kg][f]		
102	*p*-menth-1-en-9-al (or isomer)	1768	6 ± 2	6 ± 0	n.q.	*h, k, m, n*
104	*p*-menth-1-en-9-al (or isomer)	1791	6 ± 3	6 ± 3	n.d.	*h, k, m, n*
65	camphor	1490	n.d.	2 ± 1	4 ± 0	*g, i, l*
	others					
4	propan-1-ol	1030	n.d.	n.q.	n.d.	*g, j, l*
29	pentan-1-ol	1242	3 ± 1	3 ± 0	4 ± 1	*g, i*
11	pentan-2-ol	1116	8 ± 2	6 ± 1	11 ± 2	*g, i*
72	octan-1-ol	1549	n.q.	n.d.	2 ± 0	*g, j, l*
100	decan-1-ol	1758	3 ± 1	2 ± 0	n.d.	*g, j, l*
110	undecan-1-ol	1855	3 ± 0	2 ± 0	n.d.	*g, j, l*
76	propan-1,2-diol	1572	n.c.	n.c.	n.c.	*g, l*
69	butan-2,3-diol	1565	n.d.	5 ± 1	n.d.	*g, j, l*
2	2-methylbutan-2-ol	1010	64 ± 23	49 ± 10	76 ± 14	*h, k*
63	2-ethylhexan-1-ol	1482	3 ± 1	n.q.	13 ± 1	*g, i, l*
16	pent-1-en-3-ol	1151	15 ± 2	24 ± 1	39 ± 8	*g, i*
38 39	(*Z*)-pent-2-en-1-ol 3-methylbut-2-en-1-ol	1311	26 ± 6	31 ± 0	41 ± 7	*g, i, l*
59	oct-1-en-3-ol	1442	6 ± 1	12 ± 1	8 ± 0	*g, i*
5	2-methylbut-3-en-2-ol	1034	93 ± 9	90 ± 15	106 ± 20	*g, i, l*
50	2-butoxyethanol	1388	14 ± 0	10 ± 0	22 ± 3	*h, k, m*
41	1-butoxypropan-2-ol	1331	4 ± 0	4 ± 0	8 ± 1	*h, k, m*
103	1-(2-butoxyethoxy)ethanol	1778	4 ± 1	6 ± 1	4 ± 2	*h, k, m*
109	benzyl alcohol	1852	12 ± 0	15 ± 1	23 ± 4	*g, i, l*
111	2-phenylethanol	1886	n.d.	n.d.	6 ± 1	*g, j, l*
133	allylphenol	2478	3 ± 2	5 ± 1	6 ± 2	*h, k, m*
19	heptanal	1170	n.d.	n.q.	n.q.	*g, j, l*
48	nonanal	1379	4 ± 1	7 ± 3	6 ± 1	*g, i*
64	decanal	1485	2 ± 1	4 ± 2	3 ± 0	*g, i*
10	(*E*)-pent-2-enal	1109	22 ± 12	32 ± 2	15 ± 3	*g, i*
37	(*E*)-hept-2-enal	1304	n.d.	37 ± 4	n.d.	*g, i, l*
53	(*E*)-oct-2-enal	1411	n.d.	5 ± 1	4 ± 0	*g, j, l*
134	vanillin	2515	8 ± 3	16 ± 3	49 ± 15	*g, i, l*
18	heptan-2-one	1165	n.d.	n.q.	n.q.	*g, j, l*
9	pent-3-en-2-one	1107	19 ± 2	5 ± 1	14 ± 2	*g, i, l*
31	3-hydroxybutan-2-one	1262	n.d.	n.c.	n.d.	*g, l*

Table 17: Continued.

no.[a]	compound	RI[b]	'Rovada'[c]	'Jonkheer van Tets'[d]	'Roodneus'[e]	remark
				[µg/kg][f]		
33	cyclohexenone	1268	4 ± 1	2 ± 0	2 ± 0	*h, k, m*
86	acetophenone	1640	10 ± 3	2 ± 0	11 ± 2	*g, j, l*
119	3,4-dehydro-β-ionone	1984	5 ± 1	n.d.	7 ± 1	*h, k, m*
105	(*E*)-β-damascenone	1800	n.d.	n.q.	n.q.	*g, i, l*
132	4-methyl itaconate	2469	3 ± 1	5 ± 2	4 ± 0	*h, k, m*
99	methyl salicylate	1746	3 ± 1	n.d.	3 ± 1	*g, j, l*
135	benzyl benzoate	2608	n.d.	3 ± 0	n.d.	*g, j, l*
79	γ-butyrolactone	1593	n.c.	n.c.	n.d.	*g, l*
93	γ-hexalactone	1673	n.d.	n.d.	n.q.	*g, j, l*
120	pantolactone	1999	56 ± 19	42 ± 3	131 ± 14	*g, i, l*
26	2-pentylfuran	1217	n.d.	6 ± 3	19 ± 3	*g, i*
34	2-(2-pentenyl)-furan	1285	n.q.	3 ± 1	3 ± 1	*h, k, m*
58	furfural	1437	21 ± 2	21 ± 1	28 ± 5	*g, i, l*
131	HMF	2446	n.d.	60 ± 89	10 ± 2	*g, i, l*
94	citraconic anhydride	1673	n.c.	n.c.	n.c.	*g, l*
117	glutaconic anhydride	1959	29 ± 7	32 ± 8	59 ± 9	*h, k, m*
49	2-*sec*-butylthiazole	1381	2 ± 1	3 ± 1	4 ± 1	*g, i, l*
55	*p*-α-dimethylstyrene	1416	18 ± 5	22 ± 2	4 ± 1	*g, j*
139	methyl indole-3-acetate	3276	124 ± 34	417 ± 104	195 ± 76	*g, i, l*
138	methyl indole-3-pyruvate	3125	392 ± 42	1,575 ± 381	109 ± 4	*h, k, m*

[a] Numbers correspond to the chromatogram shown in Figure 15. [b] Linear retention index on a DB-Wax-column (see section 3.2.2.1 and section 3.2.3.1). [c] Freising (July 13, 2015). [d] Deutenkofen (June 29, 2015). [e] Freising (July 16, 2015). [f] Triplicate analysis of redcurrant berries: mean ± standard deviation. [g] Identification based on comparison of GC and mass spectral data with those of authentic reference compounds. [h] Tentatively identified by comparison of mass spectral data with those from database (National Institute of Standards and Technology (NIST). [i] Quantitation on the basis of recovery rate and response factor. [j] Quantitation on the basis of response factor, no recovery rate considered. [k] No recovery rate and response factor were considered. [l] Identified for the first time in redcurrant berries. [m] Reported for the first time in redcurrant berries. [n] For the corresponding mass spectrum of the compound see section 8.3. [o] Below limit of quantification (1.5 µg/kg). [p] Below limit of detection (0.5 µg/kg). [q] Detected but concentration not calculated because of too low recovery via LLE.

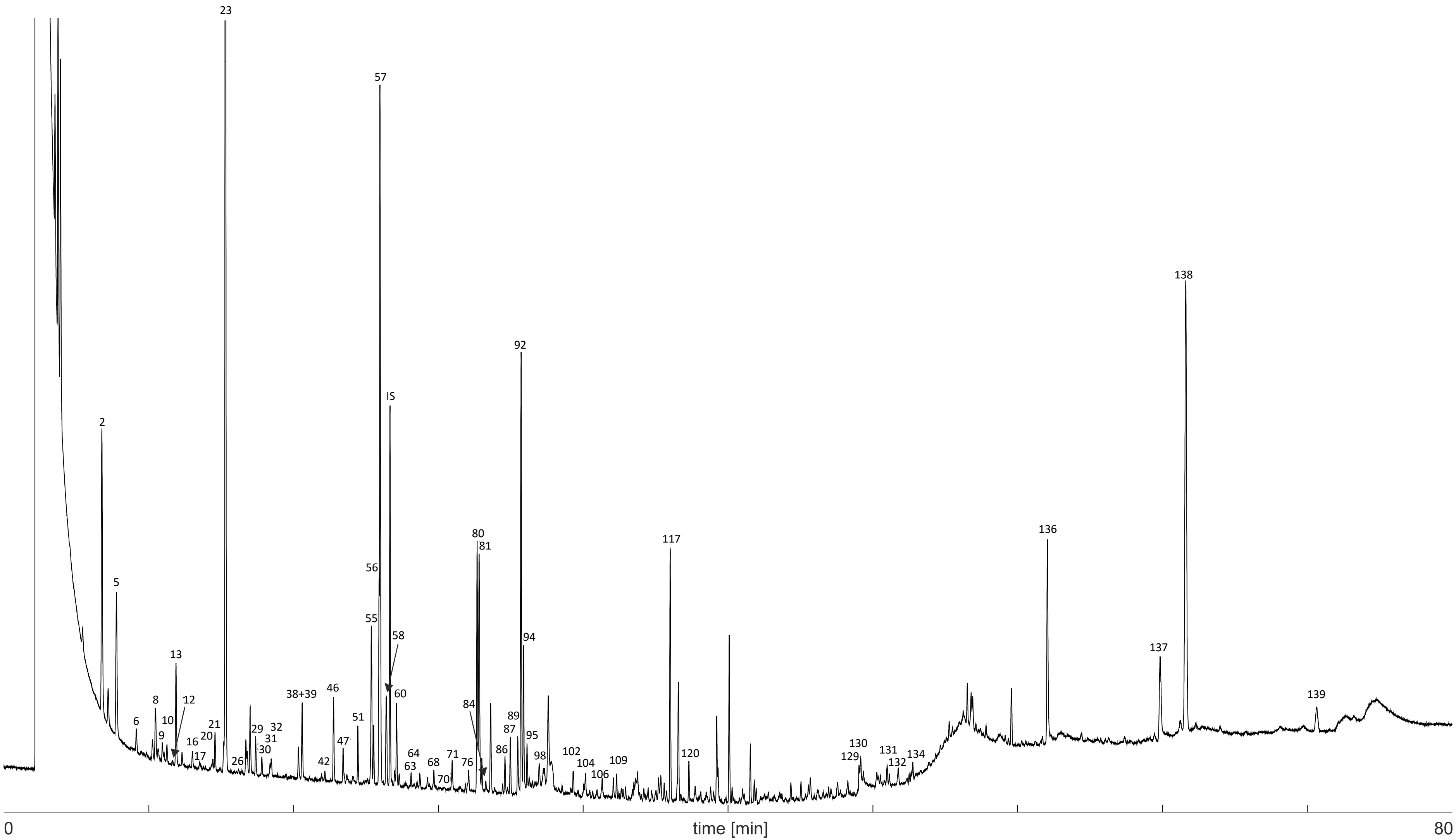

Figure 15: Typical gas chromatographic separation of volatile compounds isolated from fresh redcurrant berries (‘Rovada’, Gleinstätten, 12 August, 2013) by liquid-liquid extraction (LLE). Peak numbers correspond to Table 18; for gas chromatography (GC) conditions see section 3.2.3.1; IS: internal standard heptan-1-ol.

Table 18: Compounds identified in fresh redcurrant berries in addition to those listed in Table 17.

no.[a]	compound	RI[b]	n[c]	remark
	C_6-compounds			
44	(*E*)-hex-3-en-1-ol	1353	4	*f, k, m*
52	(*Z*)-hex-2-en-1-ol	1404	1	*d, j, l*
	acids			
113	2-ethylhexanoic acid	1932	4	*d, j, l*
	terpenes			
15	α-phellandrene	1148	1	*d, j, l*
61	nerol oxide	1466	20	*f, k, m*
108	geraniol	1834	- [e]	*i, l*
107	p-cymen-8-ol	1830	13	*d, j, l*
118	perillyl alcohol	1982	10	*d, i, l*
85	β-terpineol	1609	1	*d, j, l*
73	fenchol	1563	10	*d, j, l*
	others			
14	butan-1-ol	1136	1	*d, j*
3	butan-2-ol	1020	5	*d, j*
69	butan-2,3-diol	1565	4	*d, j, l*
7	2-methylpropan-1-ol	1083	2	*d, j, l*
25	2-methylbutanol	1200	13	*d, k, l*
24	3-methylbutanol	1200	10	*d, k, l*
36	(*E*)-pent-2-en-1-ol	1301	6	*f, k, m*
83	oct-2-en-1-ol	1600	2	*f, k, m*
88	furfuryl alcohol	1646	5	*d, j, l*
121	furaneol	2001	1	*h, l*
62	(*E*,*E*)-2,4-heptadienal	1480	3	*d, j, l*
66	benzaldehyde	1506	9	*d, i*
1	pent-1-en-3-one	1008	20	*d, i, l*
35	oct-1-en-3-one	1289	- [e]	*i, l*
45	(*Z*)-octa-1,5-dien-3-one	1354	1	*g, m*
40	6-methylhept-5-en-2-one	1322	1	*d, j, l*
101	*p*-methylacetophenone	1759	3	*d, j, l*
124	γ-decalactone	2092	- [e]	*i, l*
125	sotolon	2125	1	*h, l*
75	mesifurane	1568	1	*d, j, l*

Table 18: Continued.

no.[a]	compound	RI[b]	n[c]	remark
43	2-methoxy-3-methylpyrazine	1347	-[e]	*i, l*
54	2-isopropyl-3-methoxypyrazine	1416	-[e]	*i, l*
67	2-isobutyl-3-methoxypyrazine	1512	-[e]	*i, l*
115	benzothiazole	1935	3	*d, j, l*

[a] Numbers correspond to the chromatogram shown in Figure 15. [b] Linear retention index on a DB-Wax-column (see section 3.2.2.1 and section 3.2.3.1). [c] Identified in *n* of 39 batches. [d] Identification based on comparison of mass spectral and capillary gas chromatographic data with those of authentic reference compounds. [e] Identified in a pooled extract resulting from a total of 6 kg redcurrant berries, based on comparison of mass spectral and GC data with those of authentic reference compounds in SIM mode. [f] Tentatively identified by comparison of mass spectral data with those from database (National Institute of Standards and Technology (NIST)). [g] Tentatively identified by comparison of retention time and odour with literature data (Rychlik *et al.* 1998; Baba and Kumazawa 2014). [h] Tentatively identified by comparison of retention time and odour with authentic reference compounds. [i] Quantification on the basis of recovery rate and response factor. [j] Quantification on the basis of response factor, no recovery rate considered. [k] No recovery rate and no response factor were considered. [l] Identified for the first time in redcurrant berries. [m] Reported for the first time in redcurrant berries.

The major classes of volatile compounds isolated from fresh redcurrant fruits were C_6-compounds and acids. In contrast to blackcurrant berries (see section 4.1.2), terpenes constituted only a minor compound class. In addition, various other volatile compounds, such as alcohols, aldehydes, ketones, and lactones, were isolated.

In total, ten C_6-compounds were determined in the 39 analysed batches of redcurrant fruits. The C_6-profiles were dominated by (*E*)-hex-2-enal. This compound was previously reported in redcurrant fruits by Schreier *et al.* (1977). High concentrations of (*E*)-hex-2-enal were quantified in other fruits, such as blackcurrant berries (see section 4.1.2), jostaberries (Hempfling *et al.* 2013a), kiwis (Talens *et al.* 2003), and nectarines (Engel *et al.* 1988). The constitutional isomers of (*E*)-hex-2-enal (e.g. (*E*)-hex-3-enal), the corresponding alcohols ((*E*)-hex-2-en-1-ol and (*Z*)-hex-3-en-1-ol) and C_6-compounds originating from linoleic acid (i.e. hexanal and hexan-1-ol) were quantified in much lower concentrations compared to (*E*)-hex-2-enal.

Acetic acid, the major acid determined in redcurrant berries in this work, has previously been identified in redcurrant juice (Mehlitz and Matzik 1956a, b) and in redcurrant fruits (Boschetti *et al.* 1999) as well as in many other fruits and juices (Hirvi *et al.* 1981; Franco and Janzantti 2005; Qian and Wang 2005; Serradilla *et al.* 2010; Meret *et al.* 2011). In addition to acetic acid, 18 other acids were determined in redcurrant fruits. Rather high concentrations were observed for hexadecanoic acid and octadecanoic acid. These long-chain fatty acids were recently identified in oils of redcurrant seeds together with other fatty acids, such as linoleic acid (Šavikin *et al.* 2013).

In addition to C_6-compounds and acids, 34 terpenes and 75 substances of other chemical classes were determined in redcurrant fruits after isolation via LLE. High concentrations were determined for the two indole derivatives methyl indole-3-acetate and methyl indole-3-pyruvate. Methyl indole-3-acetate was previously identified in different matrices, such as *arabidopsis* (Pan *et al.* 2010) and *isatis tinctoria* leaves (Hartleb and Seifert 1994) and honey (Kuś *et al.* 2013). It is a derivative of indole-3-acetic acid, a potent auxin phytohormone, and serves as a storage form of this compound. Indole-3-acetic acid can be biosynthesised via different pathways, such as the indole-3-pyruvate pathway (Korasick *et al.* 2013). Methyl indole-3-pyruvate was tentatively identified in redcurrant berries by comparison of mass spectral data with those from the NIST database. To the best of the author's knowledge, methyl indole-3-pyruvate was not identified in plant matrices so far. As the two indole derivatives, methyl indole-3-acetate and methyl indole-3-pyruvate are no volatile compounds, they are not further considered in this study.

4.2.1.2 Variability of volatile compounds isolated from fresh redcurrant berries depending on cultivar, location, and season

To investigate the natural variability in the volatile profiles of fresh redcurrant berries in dependence of cultivar, location, and season, batches of fruits of different redcurrant cultivars were hand-picked at the same state of ripeness (assessed by colour and firmness of the hand-picked berries) at four locations (Deutenkofen, Fahrenzhausen, Freising, and Weinsberg) in two consecutive years (2014 and 2015, except Fahrenzhausen and Weinsberg). In addition, seven batches of the cultivar 'Rovada' were purchased at local grocery stores in 2013 and 2014. In contrast to the hand-picked berries, the transport and storage conditions as well as the time of harvest were unknown for these batches.

The volatiles were isolated from redcurrant berries that had been homogenised for 30 s; then the enzyme activities were inhibited by addition of saturated calcium chloride solution after additional 150 s. Consequently, the resulting volatile profiles reflect the C_6-compounds generated within this defined time period.

In Figure 16 and Figure 17 the distributions of the concentrations of selected representatives of the two compound classes C_6-compounds and acids are depicted for four cultivars harvested in Deutenkofen ('Rovada', 'Jonkheer van Tets', 'Red Lake', and 'Rosetta') and three cultivars harvested in Freising ('Rovada', 'Red Poll', and 'Roodneus').

The concentrations of the four C_6-compounds (*E*)-hex-2-enal, (*Z*)-hex-3-enal, (*E*)-hex-2-en-1-ol, and (*Z*)-hex-3-en-1-ol, generated from linolenic acid, are shown in Figure 16. The aldehyde (*E*)-hex-2-enal was identified in all batches as the quantitatively dominating C_6-compound, varying in concentrations between 985 µg/kg and 5,047 µg/kg. The constitutional isomer (*Z*)-hex-3-enal was identified as the second most abundant C_6-compound in most batches of analysed redcurrant fruits. The profiles of the C_6-compounds were similar in two different harvest years (2014 and 2015).

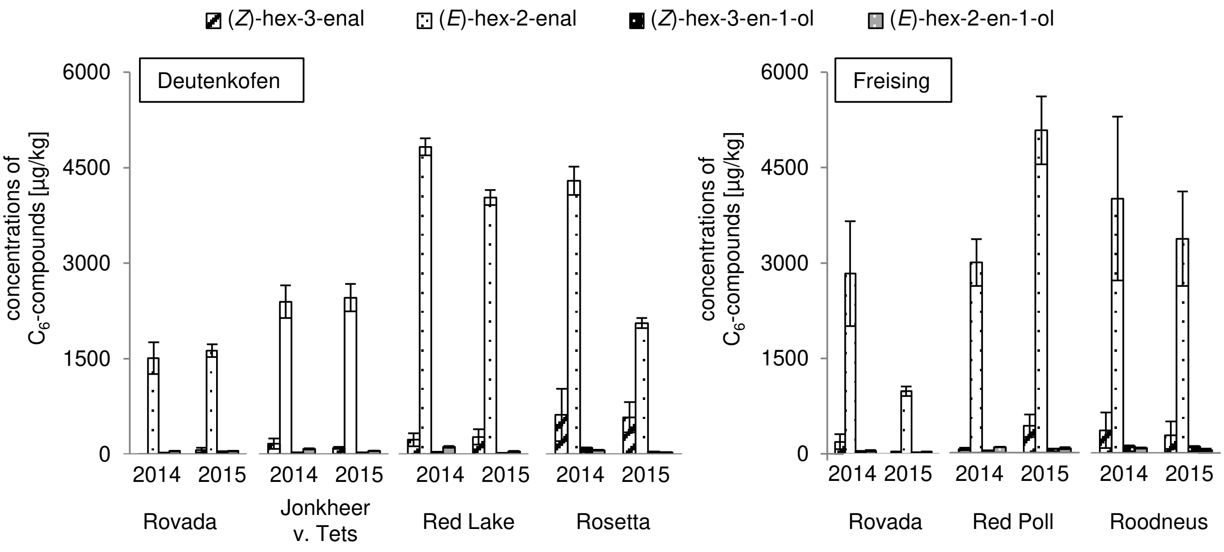

Figure 16: Concentrations of major C_6-compounds isolated via liquid-liquid extraction (LLE) from ripe, fresh redcurrant berries hand-picked at the locations Deutenkofen and Freising in 2014 and 2015.

The concentrations of the main acid, acetic acid, varied between 625 µg/kg and 2,933 µg/kg, while 113 - 832 µg/kg hexadecanoic acid and 57 - 489 µg/kg octadecanoic acid were quantified (Figure 17). The profiles of these acids were similar in each analysed cultivar for both harvest years (2014 and 2015).

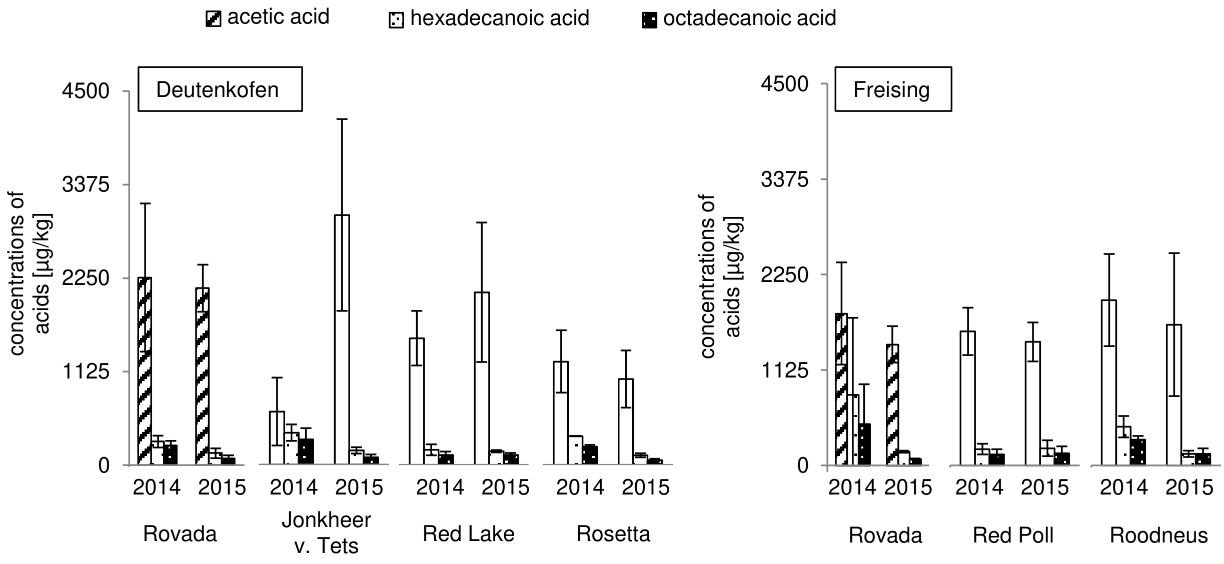

Figure 17: Concentrations of major acids isolated via liquid-liquid extraction (LLE) from ripe, fresh redcurrant berries hand-picked at the locations Deutenkofen and Freising in 2014 and 2015.

In Figure 18, the distributions of volatile compound classes of nine different redcurrant cultivars, hand-picked at four different locations in 2014 and 2015, are depicted.

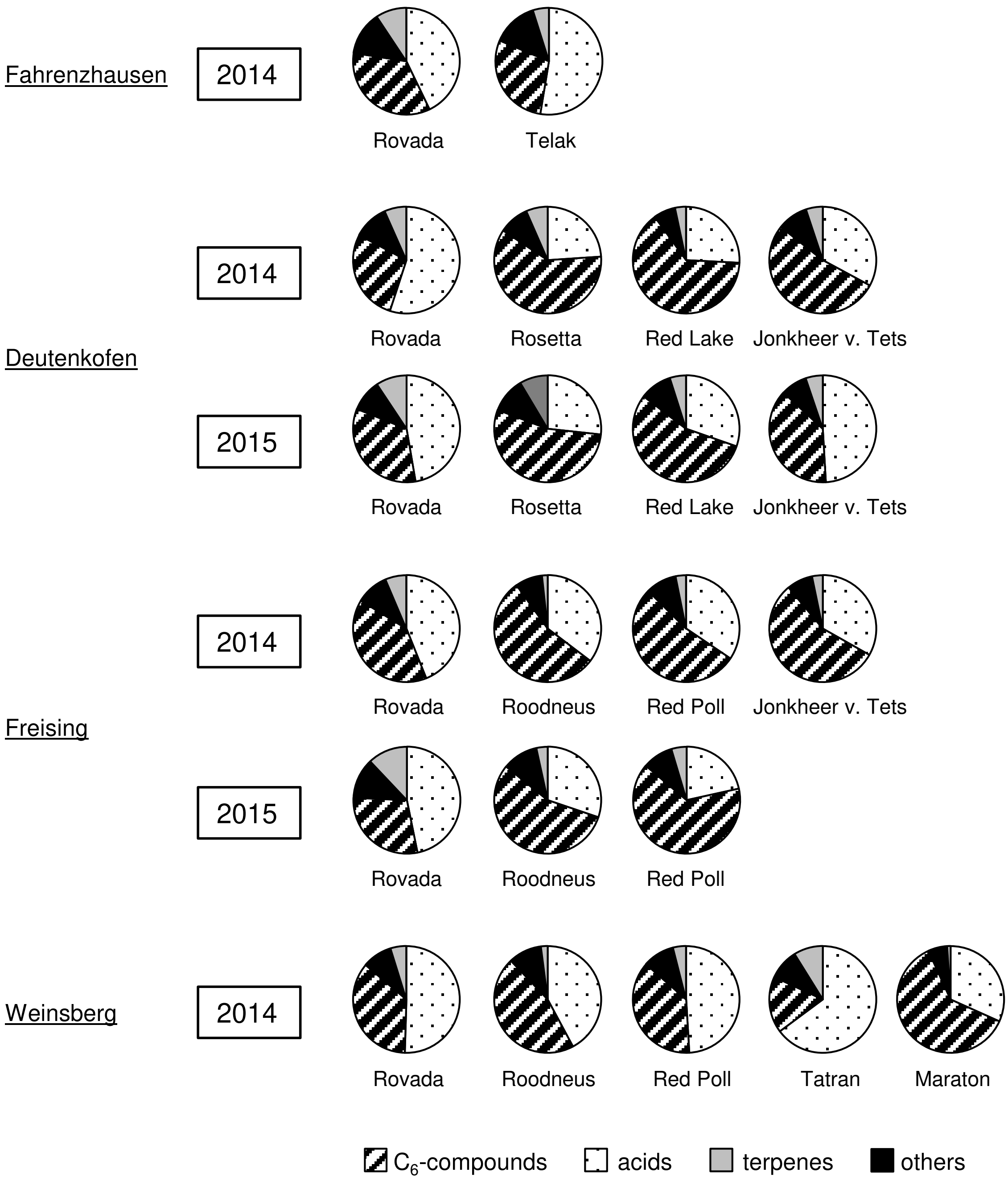

Figure 18: Distributions of volatile compound classes determined in berries of redcurrant cultivars, hand-picked at different locations in 2014 and 2015.

C_6-compounds and acids were the main compound classes in each analysed batch. The percentage distributions of these two compound classes varied from 17 % to 65 % for C_6-compounds and from 24 % to 64 % for acids. The volatile profiles of the cultivars ‘Rovada’, ‘Telak’, and ‘Tatran’ were dominated by acids, while C_6-compounds were the dominating compound class in the cultivars ‘Rosetta’, ‘Red Lake’, ‘Roodneus’, and ‘Maraton’.

For the cultivar ‘Rovada’, acids were the quantitatively dominating compound class in each batch of berries regardless of growing location and harvest year. For the cultivars ‘Jonkheer van Tets’ and ‘Roodneus’ similar volatile profiles could be found for berries from batches harvested at different growing areas in the harvest 2014. For the cultivar ‘Red Poll’, however, the volatile profiles for berries from hand-picked batches harvested at different growing areas in the harvest year 2014 were not as similar as for the cultivars ‘Jonkheer van Tets’, ‘Roodneus’, and ‘Rovada’.

When comparing different harvest years (2014 and 2015), the profiles of volatile compound classes determined in batches of berries hand-picked at the same location were similar in the cultivars ‘Rosetta’, ‘Roodneus’, ‘Red Lake’, and ‘Red Poll’. For the cultivar ‘Jonkheer van Tets’, however, the percentage distributions of volatile compound classes were not as similar as for the other cultivars.

In addition to batches of hand-picked fruits, seven different batches of purchased fruits of the cultivar ‘Rovada’, one of the commercially most important cultivars in Germany, were analysed; the percentage distributions of the volatile compound classes are shown in Figure 19.

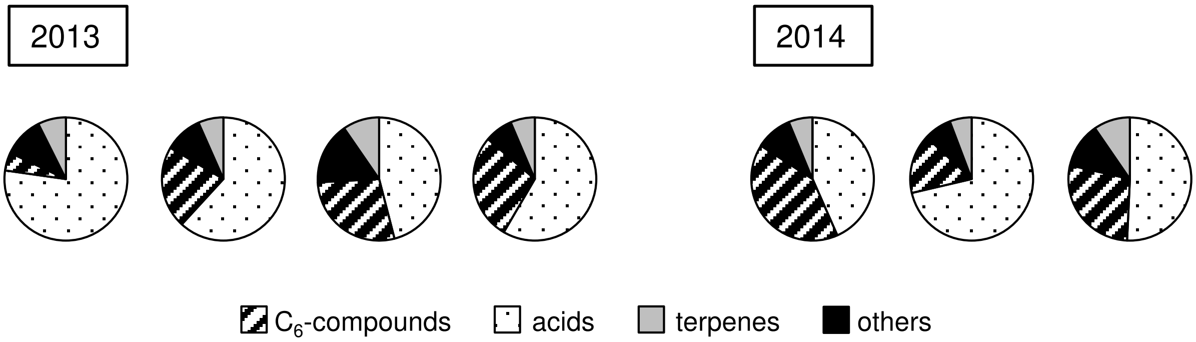

Figure 19: Distributions of volatile compound classes in seven batches of purchased berries of the redcurrant cultivar ‘Rovada’ in the years 2013 and 2014.

Acids were also the quantitatively dominating compound class in the purchased batches of ‘Rovada’. The percentage distributions of the volatile compound classes in the purchased batches, however, showed a stronger variability than in the hand-picked batches (Figure 18). The percentage distributions of C_6-compounds and acids in the hand-picked batches of ‘Rovada’ were 28 - 40 % and 43 - 55 %, respectively, while the percentage distributions of C_6-compounds and acids in the purchased batches were 5 - 41 % and 43 - 77 %, respectively. As already discussed for blackcurrant fruits in section 4.1.2.4, preharvest and postharvest conditions could have an influence on the volatile profiles of redcurrant fruits. For the purchased berries, these factors (state of ripeness, transportation, and storage) were not as defined as for the hand-picked berries.

In summary, the volatile profiles of redcurrant berries are quantitatively dominated by C_6-compounds and acids. (*E*)-Hex-2-enal and acetic acid are the two main volatile compounds in redcurrant berries, if enzyme activities are inhibited after 180 s. In each of the 39 analysed batches, the percentage distributions of these two compounds were 4 - 58 % and 12 - 61 %, respectively. Furthermore, it was shown that the distributions of volatile compound classes for hand-picked redcurrant berries are mainly influenced by the cultivar. In contrast, location and year showed no pronounced influence on the volatile profiles.

4.2.1.3 Impact of ripeness on the volatile compositions of redcurrant berries

As shown in the previous section, the profiles of volatile compound classes in commercially available fruits of the cultivar 'Rovada' showed much larger variability than those determined in hand-picked fruits of this cultivar. Based on these findings and the known effects of ripening on the volatile profile of blackcurrant berries (see section 4.1.2.5) and other fruits (Engel *et al.* 1988; Wan *et al.* 1999; Soares *et al.* 2007; Serradilla *et al.* 2010; Sinuco *et al.* 2010; Hempfling *et al.* 2013a; Hempfling *et al.* 2013b), the influence of ripening on the volatile composition of redcurrant berries was analysed. To this end, berries originating from the two cultivars 'Rovada' and 'Rosetta' were hand-picked from the same bushes in the unripe and the ripe state in Deutenkofen. The colour of unripe berries was green to light-red and changed to red at the ripe state (see Figure 20). Furthermore, unripe berries were very hard and became softer during maturation.

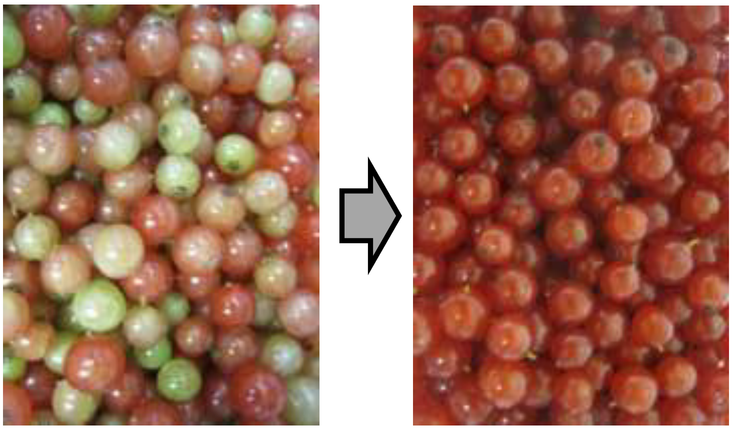

Figure 20: Redcurrant berries (cultivar: 'Rovada') depicted in the unripe and ripe state.

The concentrations of each volatile compound class decreased during ripening, expect for the acids in the cultivar 'Rovada' (Figure 21).

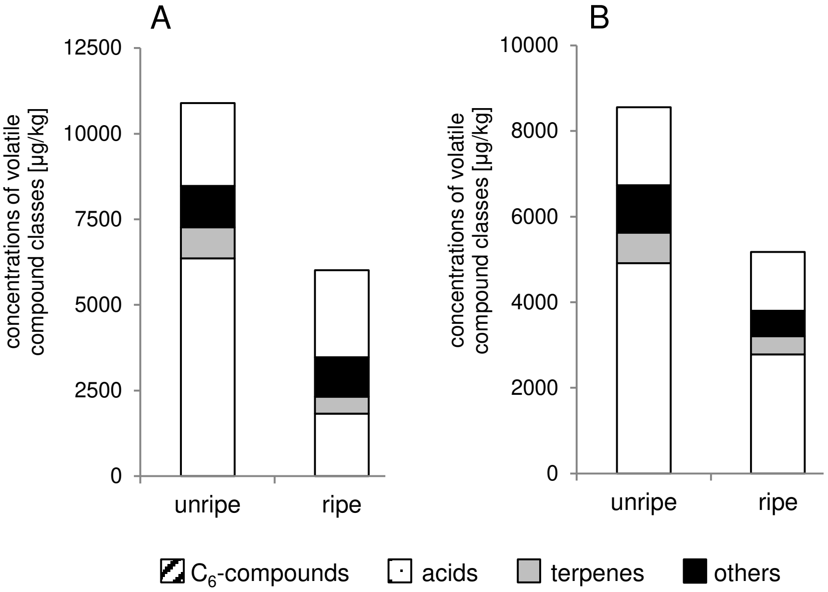

Figure 21: Concentrations of volatile compound classes in unripe and ripe redcurrant berries of the cultivars A: ‘Rovada’ and B: ‘Rosetta’ (location: Deutenkofen; season: 2015).

As shown in Table 19, the most pronounced impact of the state of ripeness was seen for the C_6-compounds. All individual C_6-compounds decreased during ripening and most of the decreases were statistically significant. (*E*)-Hex-2-enal represented the main C_6-compound in unripe as well as ripe redcurrant fruits. In unripe fruits, it accounted for 73 % (‘Rovada’) and 78 % (‘Rosetta’) of the total C_6-compounds, respectively. (*E*)-Hex-2-enal has been previously identified as the major C_6-compound in other immature fruits, such as nectarines (Engel *et al.* 1988) and guava (Soares *et al.* 2007). The percentage distribution of (*E*)-hex-2-enal increased during ripening to 82 % (‘Rovada’) and 89 % (‘Rosetta’) of the total C_6-compounds, respectively. In contrast, (*Z*)-hex-3-enal decreased in percentage distribution upon ripening from 14 % (‘Rovada’) and 21 % (‘Rosetta’) to 4 % (‘Rovada’) and 13 % (‘Rosetta’) of the total C_6-compounds, respectively. A possible increase of isomerase activity during ripening could explain the shift in the percentage distribution of the C_6-compounds generated from linolenic acid.

For the acids, there were sporadic statistically significant differences, i.e. decreases of the concentrations of 2-methylbutanoic acid and (*E)*-hex-2-enoic acid as well as a statistically significant increase of the concentrations of furan-2-carboxylic acid upon ripening in the cultivar ‘Rosetta’.

For the terpenes, statistically significant decreases of α-terpineol, (*E*)-linalool oxide, and (*Z*)-linalool oxide upon ripening were observed for both cultivars, while a decrease of *p*-menth-1-en-9-al (or isomer, KI: 1593 and 1671) was only statistically significant for ‘Rosetta’.

Statistically significant decreases upon ripening were also observed for the group of other volatile compounds, e.g. for the alcohols pent-1-en-3-ol and oct-1-en-3-ol as well as for the aldehyde (*E*)-pent-2-enal. For most of these volatile compounds, the increases were, however, only observed in one cultivar.

Table 19: Concentrations of volatile compounds in unripe and ripe redcurrant berries.[a]

	‘Rovada’[b]		‘Rosetta’[b]	
compound	unripe	ripe	unripe	ripe
	[µg/kg][c]		[µg/kg][c]	
C_6-compounds				
(*E*)-hex-2-enal	4,979 ± 1,085	1,626 ± 100 (*)	3,586 ± 403	2,053 ± 79 (**)
(*Z*)-hex-3-enal	927 ± 361	64 ± 36	1,031 ± 426	577 ± 237
(*Z*)-hex-2-enal	62 ± 16	21 ± 1 (*)	44 ± 5	30 ± 4 (*)
(*E*)-hex-2-en-1-ol	136 (135 - 153)	50 (49 - 52)	54 ± 10	28 ±43 (*)
(*Z*)-hex-3-en-1-ol	194 ± 21	40 ± 6 (***)	116 ± 10	38 ± 4 (***)
hexanal	40 ± 15	13 ± 3 (*)	58 ± 14	44 ± 12
acids				
acetic acid	1,750 ± 450	2,129 ± 282	1,491 ± 392	1,008 ± 335
propanoic acid	41 ± 17	30 ± 11	37 ± 5	24 ± 6
heptanoic acid	17 ± 3	7 ± 1	13 ± 2	10 ± 4
hexadecanoic acid	208 (126 - 624)	156 (80 - 196)	84 ± 27	175 ± 28
octadecanoic acid	n.d.	80 ± 39	n.d.	57 ± 15
2-methylpropanoic acid	29 ± 11	13 ± 3	12 ± 1	9 ± 2
2-methylbutanoic acid	39 ± 14	16 ± 3	28 ± 2	18 ± 2 (**)
(*E*)-hex-2-enoic acid	36 ± 3	n.d.[d]	26 ± 3	13 ± 0 (*)
(*E*)-hex-3-enoic acid	59 (52 - 60)	14 (13 - 18)	54 (52 - 55)	14 (13 - 14)
furan-2-carboxylic acid	n.d.	69 ± 22	30 ± 3	54 ± 6 (**)
levulinic acid	35 ± 26	24 ± 12	n.d.	27 ± 6
terpenes				
limetol	38 ± 15	14 ± 4	45 (44 - 46)	21 (20 - 21)
(*Z*)-linalool oxide	49 ± 16	21 ± 3 (*)	31 ± 4	19 ± 2 (**)
(*E*)-linalool oxide	122 ± 37	54 ± 7 (*)	79 ± 10	48 ± 3 (**)
hotrienol	48 ± 18	30 ± 6	32 ± 3	22 ± 2
terpin	11 ± 5	6 ± 2	30 (24 - 84)	12 (7 - 25)
γ-terpineol	3 ± 1	n.d.	10 ± 1	24 ± 29
α-terpineol	38 (23 - 142)	94 (7 - 408)	94 ± 18	41 ± 10 (*)
p-menth-1-en-9-al (or isomer; KI: 1593)	63 (47 - 202)	51 (18 - 51)	64 ± 8	48 ± 1
p-menth-1-en-9-al (or isomer; KI: 1596)	121 ± 48	52 ± 20	103 ± 23	55 ± 14 (**)

Table 19: Continued.

compound	'Rovada'[b] unripe	'Rovada'[b] ripe	'Rosetta'[b] unripe	'Rosetta'[b] ripe
	[µg/kg][c]		[µg/kg][c]	
p-menth-1-en-9-al (or isomer; KI: 1671)	219 ± 74	77 ± 11	140 ± 20	77± 14 (**)
others				
2-methylbutan-2-ol	125 ± 72	69 ± 19	82 ± 12	70 ± 11
pent-1-en-3-ol	85 ± 25	20 ± 3 (*)	69 ± 8	20 ± 1 (**)
(*Z*)-pent-2-en-1-ol 3-methylbut-2-en-1-ol	71 ± 20	26 ± 3 (*)	64 ± 9	21 ± 5 (**)
oct-1-en-3-ol	37 ± 5	12 ± 1 (***)	159 (154 - 161)	41 (41 - 43)
2-methylbut-3-en-2-ol	117 ± 40	83 ± 5	112 ± 9	70 ± 12 (**)
benzyl alcohol	24 ± 9	14 ± 2	28 ± 2	12 ± 1 (***)
(*E*)-pent-2-enal	148 ± 47	35 ± 6 (*)	123 ± 42	31 ± 3
(*E*)-hept-2-enal	29 ± 15	n.d.	29 ± 2	n.d.
vanillin	39 ± 11	16 ± 2 (*)	12 ± 2	8 ± 1
pent-3-en-2-one	23 (20 - 49)	19 (17 - 20)	20 ± 4	9 ± 2 (**)
pantolactone	52 (45 - 152)	59 (23 - 60)	63 ± 13	67 ± 12
furfural	38 ± 13	17 ± 4	25 ± 1	17 ± 2 (**)
HMF	102 ± 65	48 ± 45	53 ± 17	33 ± 28
glutaconic anhydride	56 (45 - 191)	27 (14 - 28)	80 ± 10	41 ± 10 (**)
p-α-dimethylstyrene	41 ± 21	15 ± 3	30 ± 7	19 ± 3

[a] All compounds with concentrations ≥ 20 µg/kg in at least one of the analysed batches are considered. [b] Deutenkofen (3 July, 2015 and 14 July, 2015). [c] Triplicate analysis of unripe and ripe fruits; values are mean ± standard deviation where data are normally distributed; values are median (minimum maximum) where data are not normally distributed. Unpaired Student's t-test was used to test for equality of means between unripe and ripe redcurrant berries of the same variety when normal distribution and equality of variances were assumed; Welch's t-test was performed when data were normally distributed but equality of variances was not assumed. Non-parametric Wilcoxon-Mann-Whitney U-test was used to compare medians when normality was not assumed. All tests were two tailed. (***), $p < 0.001$; (**), $p < 0.01$; (*), $p < 0.05$. [d] Below limit of detection (0.5 µg/kg).

4.2.1.4 Impact of freezing on the volatile compositions of redcurrant berries

Redcurrant fruits are commercially available in frozen form. Several studies demonstrated that the storage temperature influences the volatile profile and the aroma of numerous foods (de Ancos *et al.* 2000; Siegmund *et al.* 2001; Talens *et al.* 2003; Perez-Cacho and Rouseff 2008). This was also shown in the current work for blackcurrant fruits (see section 4.1.2.2). Hence, the influence of freezing on the volatile profiles of fruits (two batches) of the cultivar 'Rovada' was investigated after storage at -20 °C for three, six, nine, and twelve months. The frozen fruits had an acid-like and fruity odour character, however, less intensive compared to the fresh fruits. The concentrations of volatile compound classes determined in batches of the aforementioned cultivar analysed either directly after purchase or after storage at -20 °C for up to twelve months, are depicted in Figure 22.

In particular, two main effects could be observed. After three months of storage at -20 °C, almost a complete decrease of the C_6-compounds was observed, whereas the concentration of acids was increasing, slightly varying over the storage period. A possible explanation for these variations may be the inhomogeneity of the purchased redcurrant fruits. Furthermore, the concentrations of terpenes and others were relatively constant or a slight decrease over the storage period at -20 °C was observed.

In a previous study by Douillard and Guichard (1990), the influence of freezing (-18 °C, one month) on the volatile profile of strawberries was analysed. Apart from an increase of C_6-compounds, no significant influence was observed for the total concentration of acids. However, for two single compounds, i.e. hexanoic acid and octadecanoic acid, a slight increase was detectable. In contrast, in another study by Hirvi (1983) on strawberries (-18 °C, five months) an increase of all individual acids (butanoic acid, hexanoic acid, and 2-ethylbutanoic acid) was shown.

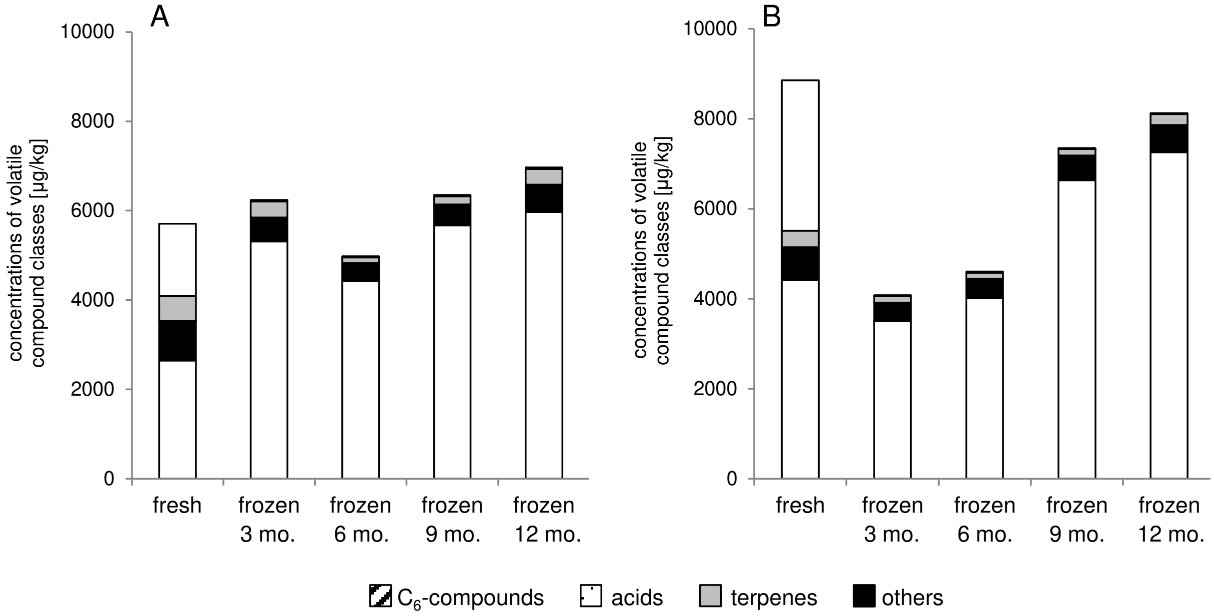

Figure 22: Concentrations of volatile compound classes in fresh and frozen (-20°C) redcurrant berries of the cultivar ‘Rovada’ (purchased) from A: Gleinstätten and B: Tettnang. mo.: months.

The concentrations of single volatile compounds, determined in the batches analysed directly after purchase or after nine months of storage at -20 °C, are listed in Table 20. Analogous to blackcurrant fruits, the largest impact of freezing was observed for the C_6-compounds generated from linolenic acid. The concentrations of the C_6-compounds generated from linolenic acid after storage for nine months fell under the limits of detection. On the other hand, the concentrations of known autoxidation products, such as decanal, hexanal, heptanal, nonanal, and 2-pentylfuran (Grosch 1987), were increased after freezing.

The increase of the total acid concentration upon freezing was mainly caused by an increase of the three main acids acetic acid, hexadecanoic acid, and octadecanoic acid. However, the differences in concentrations could only be shown to be statistically significant for hexadecanoic acid and octadecanoic acid in the batch originating from Gleinstätten. A large increase of propanoic acid was additionally detected in this batch. The difference in concentration, however, was not statistically significant due to the lack of normal distribution.

For the terpenes, statistically significant decreases of (*Z*)-linalool oxide upon freezing were observed for both cultivars, while decreases of *p*-menth-1-en-9-al (or isomer; KI: 1593 and 1596), limetol, and neral were only statistically significant for the batch originating from Gleinstätten. On the other hand, the concentration of hotrienol increased upon freezing.

For the group of other volatile compounds, statistically significant decreases were observed for various substances, such as the alcohols 2-methylbutan-2-ol and pent-1-en-3-ol as well as for the aldehyde furfural.

Table 20: Concentrations of volatile compounds in fresh and frozen redcurrant berries of the cultivar 'Rovada'.[a]

	Gleinstätten[b]		Tettnang[b]	
compound	fresh	frozen[c]	fresh	frozen[c]
	[µg/kg][d]		[µg/kg][d]	
C_6-compounds				
(*E*)-hex-2-enal	1,349 ± 247	n.d.[e]	3,150 ± 564	n.d.
(*Z*)-hex-3-enal	199 ± 121	n.d.	58 ± 14	n.d.
(*Z*)-hex-2-enal	18 ± 4	n.d.	40 ± 4	n.d.
(*E*)-hex-2-en-1-ol	21 ± 7	n.d.	48 ± 6	n.d.
hexanal	17 ± 1	26 ± 4 (*)	19 ± 7	19 ± 4
acids				
acetic acid	2,009 ± 155	3,983 ± 1,438	3,900 ± 1,555	5,485 ± 833
propanoic acid	17 (16 - 17)	252 (250 - 254)	13 ± 3	26 ± 6
pentanoic acid	33 ± 15	13 ± 2	44 ± 19	12 ± 1
hexanoic acid	13 ± 0	33 ± 3 (**)	16 ± 7	26 ± 5
hexadecanoic acid	271 ± 86	676 ± 122 (**)	242 ± 126	615 ± 261
octadecanoic acid	180 ± 110	643 ± 261 (*)	97 (96 - 119)	356 (210 - 702)
2-methylpropanoic acid	25 ± 2	19 ± 6	7 (4 - 75)	9 (9 - 12)
2-methylbutanoic acid	31 ± 2	20 ± 6 (*)	3 ± 1	12 ± 5
(*E*)-hex-2-enoic acid	n.d.	n.d.	28 ± 4	n.d.

Table 20: Continued.

compound	Gleinstätten[b] fresh	Gleinstätten[b] frozen[c]	Tettnang[b] fresh	Tettnang[b] frozen[c]
	[µg/kg][c]		[µg/kg][c]	
terpenes				
limetol	29 ± 5	8 ± 2 (*)	13 ± 3	10 ± 3
(*Z*)-linalool oxide	44 ± 4	17 ± 4 (**)	107 ± 22	19 ± 9 (**)
hotrienol	n.d.	27 ± 12	n.d.	9 ± 6
neral	25 ± 2	8 ± 2 (**)	9 (7 - 12)	7 (6 - 7)
p-menth-1-en-9-al (or isomer; KI: 1593)	108 ± 35	9 ± 6 (*)	21 (16 - 160)	15 (6 - 26)
p-menth-1-en-9-al (or isomer; KI: 1596)	104 ± 19	22 ± 6 (**)	54 ± 15	26 ± 12
p-menth-1-en-9-al (or isomer; KI: 1671)	162 ± 15	48 ± 14	57 ± 16	18 ± 2
others				
propan-1-ol	n.d.	42 ± 27	n.d.	10 ± 6
pentan-1-ol	14 ± 3	20 ± 4	17 ± 4	19 ± 4
2-methylbutan-2-ol	188 ± 64	18 ± 9 (*)	129 ± 36	25 ± 16 (**)
pent-1-en-3-ol	15 ± 1	10 ± 2 (*)	27 ± 2	9 ± 2 (***)
(*Z*)-pent-2-en-1-ol	34 ± 9	9 ± 1 (*)	42 (21 - 46)	13 (12 - 14)
2-methylbut-3-en-2-ol	141 ± 12	68 ± 12 (**)	116 ± 22	93 ± 14
benzyl alcohol	6 ± 2	12 ± 6	3 (3 - 8)	25 (24 - 25)
allylphenol	5 ± 3	21 ± 5 (*)	n.d.	n.d.
(*E*)-pent-2-enal	32 ± 13	7 ± 3 (*)	48 ± 30	n.d.
heptanal	n.d.	3 ± 1	n.d.	3 ± 1
nonanal	n.d.	28 ± 10	n.d.	27 ± 19
decanal	n.q.	n.d.	n.d.	6 ± 4
(*E*)-hept-2-enal	22 ± 5	6 ± 1 (*)	31 ± 9	15 ± 4 (*)
pantolactone	109 ± 31	38 ± 8 (*)	47 (39 - 224)	46 (38 - 65)
2-pentylfuran	3 ± 0	7 ± 1 (**)	6 ± 4	7 ± 4
furfural	82 ± 9	14 ± 5 (***)	50 ± 9	28 ± 13
HMF	48 ± 26	25 ± 9	10 ± 4	11 ± 5
glutaconic anhydride	104 ± 14	49 ± 18	68 ± 18	101 ± 23
p-α-dimethylstyrene	46 (40 - 49)	6 (6 - 6)	14 ± 3	5 ± 1 (*)

[a] All compounds with concentrations ≥ 20 µg/kg in at least one of the analysed batches are considered, except for 2-pentylfuran, decanal, and heptanal. [b] Fruits were purchased at 12 August, 2013. [c] Redcurrant berries were stored for nine months at -20 °C. [d] Triplicate analysis of unripe and ripe fruits; values are mean ± standard deviation where data are normally distributed; values are median (minimum maximum) where data are not normally distributed. Unpaired Student's t-test was used to test for equality of means between unripe and ripe redcurrant berries of the same variety when normal distribution and equality of variances were assumed; Welch's t-test was performed when data were normally distributed but equality of variances was not assumed. Non-parametric Wilcoxon-Mann-Whitney U-test was used to compare medians when normality was not assumed. All tests were two tailed. (***), $p < 0.001$; (**), $p < 0.01$; (*), $p < 0.05$. [e] Below limit of detection (0.5 µg/kg).

4.2.1.5 Impact of enzymatic reactions on profiles of C_6-compounds

In section 4.1.2.1 to 4.2.1.4 the volatile profiles of redcurrant berries were analysed after the enzyme activities were inhibited by addition of saturated aqueous calcium chloride solution to crushed berries 180 s after the homogenisation had started, in accordance with an approach adapted from Buttery *et al.* (1987). Therefore, the resulting volatile profiles contained C_6-compounds which were generated within this defined time period. In this part of the work the time-dependency of the enzymatic generation of C_6-compounds until 180 s was analysed. To this end, enzyme activity was inhibited by addition of saturated aqueous calcium chloride solution 30 s, 60 s, 90 s, and 180 s after the homogenisation had started, respectively.

As shown in Figure 23, the total amounts of C_6-compounds increased over time in two batches, while in a third batch the total amount of C_6-compounds remained relatively constant. However, the spectrum of C_6-compounds shifted over time in each batch due to isomerase and alcohol dehydrogenase activities. The content of (*Z*)-hex-3-enal decreased in favour of the isomer (*E*)-hex-2-enal and the corresponding alcohols (*Z*)-hex-3-en-1-ol and (*E*)-hex-2-en-1-ol.

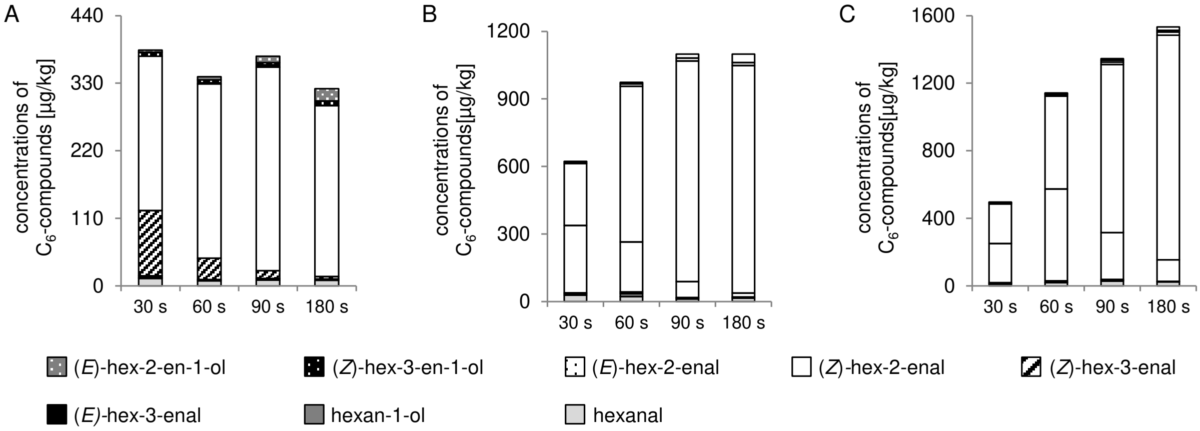

Figure 23: C_6-compounds isolated via liquid-liquid extraction (LLE) from fresh redcurrant berries of the cultivar (A) 'Rovada' (Weisenheim, 10 August, 2015) and two unknown cultivars (B + C) (unknown location, 3 August, 2015 + Marbach-Rheinheim, 27 July, 2015), after enzyme inactivation with calcium chloride at defined time points (30 s, 60 s, 90 s, and 180 s after homogenisation had started, respectively).

Schreier *et al.* (1977) also investigated the influence of enzymatic reactions on C_6-compounds in redcurrant juice after production. Enzyme inhibition was carried out with methanol after 1.5 min, 5 min, and 20 min. They also observed an increase of the concentrations of total C_6-compounds over time, especially for the alcohols (*Z*)-hex-3-en-1-ol, (*E*)-hex-2-en-1-ol, and hexan-1-ol. The aldehydes (*Z*)-hex-3-enal, (*E*)-hex-2-enal, and hexanal dominated the C_6-profile after 1.5 min. Decreases of the concentrations of these aldehydes in favour of the corresponding alcohols (*Z*)-hex-3-en-1-ol, (*E*)-hex-2-en-1-ol, and hexan-1-ol were observed after 20 min. Due to the different time points chosen for enzyme inhibition, a generation of these alcohols in high concentrations was not detectable in the present study. In general, the C_6-aldehydes (*Z*)-hex-3-enal and hexanal can be generated from linolenic acid and linoleic acid after distribution of the cell structure and can be further metabolised to the corresponding aldehyde (*E*)-hex-2-enal and the corresponding alcohols (*Z*)-hex-3-en-1-ol, (*E*)-hex-2-en-1-ol, and hexan-1-ol via isomerisation and reduction reactions (Matsui 2006; Stumpe and Feussner 2006).

4.2.2 Sensory evaluations of volatile compounds of redcurrant fruits

Until now, no sensory evaluation of volatile compounds in redcurrant fruits has been conducted. Therefore, the objectives of this part of the work were to identify the odour-active volatiles in redcurrant fruits and to assess their contributions to the overall aroma.

The sensory evaluation of volatile compounds in redcurrant fruits was performed, as a first step, by means of AEDA of a pooled and concentrated extract of 3 kg of redcurrant berries of the cultivar 'Rovada' (see section 3.2.5). Twenty-two odour-active compounds were identified by comparison of chromatographic, mass spectrometric, and sensory data with those of authentic reference compounds (see Table 21).

The highest FD factors were determined for the green pepper-like smelling pyrazine 2-isobutyl-3-methoxypyrazine, for the vanilla-like smelling vanillin, and for sotolon, a seasoning-like smelling lactone.

In the next step, OAVs were calculated by dividing the concentrations of the individual substances by their odour thresholds. As redcurrant berries mainly consist of water (Souci *et al.* 2015), odour thresholds determined in water were used. Based on the OAV-concept, ten substances turned out to be odour-active (OAVs ≥ 1). (*E*)-β-Damascenone (395), a compound with a low odour threshold of 0.002 µg/L (Rychlik *et al.* 1998), had the highest OAV, followed by the C_6-compound (*Z*)-hex-3-enal (87). Other lipid oxidation products with high OAVs were oct-1-en-3-one (20) and one of the quantitatively major compounds, (*E*)-hex-2-enal (16). Acetic acid, the other quantitatively major compound, also had a high OAV (23). In addition, the two pyrazines 2-isobutyl-3-methoxypyrazine (28) and 2-isopropyl-3-methoxypyrazine (10) were identified as volatile compounds with high OAVs. As last step, a reconstitution experiment was conducted with the ten identified odour-active substances, obtained by GC/O and subsequent OAV analysis, in order to confirm their importance to the redcurrant berry aroma. Besides the composition of the odour-active volatile profile, the overall-aroma of redcurrant berries can be influenced by the interaction of aroma compounds with other food constitutions. For example, an interaction of aroma compounds and different classes of non-volatile compounds, such as alcohols, carbohydrates, lipids, proteins, polyphenols, and salts were observed (Paravisini and Guichard 2016). In order to imitate a redcurrant berry-like matrix, aroma compounds were dissolved in an aqueous solution of sugars and organic acids naturally occurring in the berries (Haila *et al.* 1992; Nour *et al.* 2011; Mikulic-Petkovsek *et al.* 2012b).

A first reconstitution experiment was based on the concentrations of odour-active volatile compounds (OAV ≥ 1), which are listed in Table 21. The reconstitution model of the

Table 21: Concentrations and sensory data of key aroma compounds in redcurrant berries.

			FD factor[c]		odour threshold		conc.	OAV
aroma compound	RI[a]	odour quality[b]	A	B	in water [µg/L]	remark	[µg/kg][d]	
(*E*)-β-damascenone	1800	fruity, canned peach	2	8	0.002	*e*	0.79[f]	395
(*Z*)-hex-3-enal	1128	grassy	16	16	0.6	*g*	52	87
2-isobutyl-3-methoxypyrazine	1512	green pepper	32	4	0.005	*e*	0.14[f]	28
acetic acid	1430	vinegar	16	2	70	*h*	1597	23
oct-1-en-3-one	1289	mushroom, metallic	8	2	0.005	*e*	0.10[f]	20
(*E*)-hex-2-enal	1199	apple, marzipan	4	2	77	*g*	1236	16
2-isopropyl-3-methoxypyrazine	1416	musty	4	4	0.004	*e*	0.04[f]	10
pent-1-en-3-one	1008	solvent	2	-	1	*g*	1.27[f]	1
(*Z*)-hex-3-en-1-ol	1373	fruity, sweet	-	2	28	*i*	31	1
vanillin	2515	vanilla	128	64	20	*e*	20	1
(*E*)-hex-3-enal	1123	green	2	1	160	*j*	2	< 1
2-methoxy-3-methylpyrazine	1347	musty, hazelnut	2	-	3	*k*	0.91[f]	< 1
linalool	1538	flowery, citrus	2	-	5	*e*	3	< 1
2-methylbutanoic acid	1651	sweaty, vinegar	2	1	50	*e*	14	< 1
pentanoic acid	1717	pungent, fruity	16	-	2,100	*e*	2	< 1
geraniol	1834	citrus	1	-	5	*e*	0.17[f]	< 1
γ-decalactone	2092	fruity, flowery, sweet	16	1	5	*e*	0.20[f]	< 1
sotolon	2125	seasoning	32	16	2	*l*	n.d.[m]	< 1
(Z)-octa-1,5-dien-3-one	1354	geranium, metallic, green	16	8	0.0012	*e*	n.d.[n]	
2-*sec*-butylthiazole	1381	musty	-	2			2	
citraconic anhydride	1673	seasoning, fruity	8	-			n.c.[o]	

Table 21: Continued.

aroma compound	RI[a]	odour quality[b]	FD factor[c] A	FD factor[c] B	odour threshold in water [µg/L]	odour threshold remark	conc. [µg/kg][d]	OAV
furaneol	2001	cotton candy, berry	16	4	60	p	n.c.	

[a] Linear retention index on a DB-Wax-column (see section 3.2.2.1 and section 3.2.3.1). [b] Assessed by gas chromatography/olfactometry (GC/O). [c] GC/O and aroma extract dilution analysis (AEDA) were performed by two panellists (A and B) using a concentrated liquid-liquid extract (LLE)-extract corresponding to 3 kg of redcurrant berries of the cultivar 'Rovada' (location: Deutenkofen and Freising; season: 2015). [d] Concentration determined in the cultivar 'Rovada' (location: Deutenkofen and Freising; season: 2015). [e] Rychlik *et al.* (1998). [f] Concentrations were determined with pooled extracts from twelve batches of cultivar 'Rovada' (purchased: 1 August, 2016; origin: Oberkirch). [g] Hempfling *et al.* (2013a). [h] Yang *et al.* (2010). [i] Hempfling *et al.* (2013b). [j] Tamura *et al.* (2001). [k] Leffingwell & Associates. [l] Pons *et al.* (2008). [m] Below limit of detection, tentative identification by comparison of retention indices and odour description with authentic reference compounds. [n] Below limit of detection, tentative identification by comparison of retention indices and odour description with literature data (Rychlik *et al.* 1998; Baba and Kumazawa 2014). [o] Detected but concentration not calculated because of too low recovery via LLE. [p] Buttery *et al.* (1995).

redcurrant berry aroma was compared by panellists to the aroma of fresh redcurrant fruits. The comparison by the panellists confirmed the importance of the ten identified aroma compounds. As shown in Figure 24, the reconstructed aroma, however, did not fully reflect the original aroma profile.

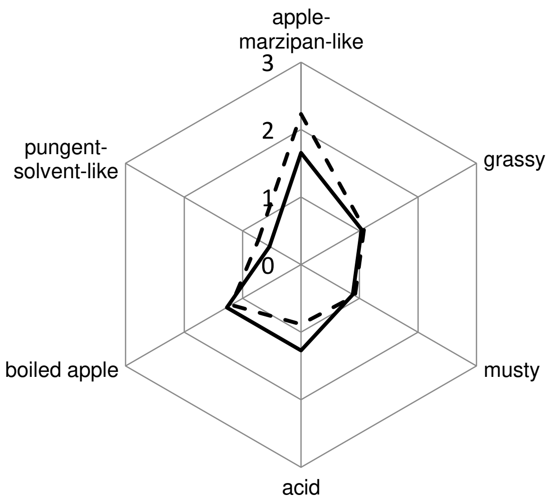

Figure 24: Aroma profiles of fresh redcurrant berries (continuous line) and of the reconstitution model (broken line) on the basis of concentrations of odorants in the cultivar ‘Rovada’ from Freising (13 July, 2015), Deutenkofen (14 July, 2015) and Oberkirch (1 August, 2016).

Differences were recognisable especially for the apple-marzipan-like and the acid note. The concentrations of odour-active volatile compounds in redcurrant berries were determined after the enzyme activity was inhibited (180 s after homogenisation had started). Accordingly, the resulting concentrations reflect the C_6-compound profile generated within this defined time period. However, as shown in Figure 23 (section 4.2.1.5), lower concentrations of the apple-marzipan-like smelling (*E*)-hex-2-enal were quantified in extracts derived from extraction processes involving enzyme inhibition initialised 30 s, 60 s, and 90 s after the homogenisation had started. As a consequence, the concentration of the apple-marzipan-like smelling C_6-compound (*E*)-hex-2-enal may have been overestimated, which might explain the deviation in the apple-marzipan-like note. Additionally, not all odour-active compounds detected by GC/O were identified. Furthermore, the matrix of redcurrant berries was not completely imitated. For example, phenolic compounds, such as anthocyanins, which were not included in the reconstitution experiment, may have an impact on the aroma of redcurrant fruits.

4.2.3 Volatile profiles of white currant berries

White currant berries are a colour variant of the redcurrant species (Barney and Hummer 2005). Up to now, volatile profiles of these berries have not been analysed.

Therefore, the objectives of this part of the work were to identify and to quantify volatile compounds in white currant fruits and to compare the volatile profiles of white currant fruits to those of redcurrant fruits.

Berries of the two white currant cultivars 'Weißer Versailler' and 'Zitavia' were hand-picked in 2015 at Deutenkofen. The concentrations of volatile compounds isolated in analogy to the procedure applied for redcurrant fruits are listed in Table 22.

Table 22: Volatile compounds isolated via liquid-liquid extraction (LLE) from berries of two hand-picked batches of the white currant cultivars 'Weißer Versailler' and 'Zitavia'.

compound	'Weißer Versailler'[a]	'Zitavia'[b]
	[µg/kg][c]	
C_6-compounds		
(*E*)-hex-2-enal	1,113 ± 143	1,633 ± 351
(*Z*)-hex-3-enal	17 ± 4	94 ± 38
(*Z*)-hex-2-enal	16 ± 2	22 ± 6
(*E*)-hex-3-enal	n.q.[d]	2 ± 1
(*E*)-hex-2-en-1-ol	72 ± 9	55 ± 5
(*Z*)-hex-3-en-1-ol	13 ± 3	24 ± 2
hexanal	14 ± 2	27 ± 2
hexan-1-ol	7 ± 0	5 ± 0
acids		
acetic acid	2,210 ± 711	1,558 ± 139
propanoic acid	22 ± 1	29 ± 8
butanoic acid	11 ± 3	73 ± 34
hexanoic acid	9 ± 1	12 ± 4
heptanoic acid	2 ± 1	n.d.[e]
octanoic acid	3 ± 0	3 ± 1
nonanoic acid	n.q.	6 ± 0
decanoic acid	3 ± 0	n.d.
hexadecanoic acid	194 ± 97	n.d.
octadecanoic acid	143 ± 112	n.d.
2-methylpropanoic acid	15 ± 1	43 ± 6
2-methylbutanoic acid	25 ± 4	75 ± 8
(*E*)-hex-2-enoic acid	31 ± 4	n.d.
(*E*)-hex-3-enoic acid	4 ± 2	4 ± 2

Table 22: Continued.

compound	'Weißer Versailler'[a]	'Zitavia'[b]
	[µg/kg][c]	
furan-2-carboxylic acid	58 ± 37	92 ± 18
benzoic acid	4 ± 5	5 ± 3
levulinic acid	29 ± 10	n.d.
terpenes		
(*E*)-β-ocimene	n.d.	3 ± 1
(*Z*)-β-ocimene	n.d.	2 ± 0
terpinolene	n.c.[f]	n.c.
limonene	n.d.	14 ± 4
α-terpinene	n.d.	17 ± 9
p-cymene	6 ± 2	20 ± 7
limetol	11 ± 3	71 ± 7
nerol oxide	n.q.	n.q.
1,8-cineole	n.d.	n.q.
α-pinene oxide	n.c.	n.c.
(*E*)-linalool oxide	68 ± 8	101 ± 7
(*Z*)-linalool oxide	28 ± 3	44 ± 4
dill ether	2 ± 1	5 ± 1
linalool	2 ± 0	19 ± 2
(*E*)-ocimenol	6 ± 1	49 ± 2
(*Z*)-ocimenol	5 ± 2	35 ± 3
hotrienol	6 ± 2	19 ± 6
terpin	10 ± 1	55 ± 13
γ-terpineol	n.d.	9 ± 5
terpinen-4-ol	n.q.	3 ± 0
α-terpineol	42 ± 40	119 ± 25
p-cymen-8-ol	2 ± 1	3 ± 1
neral	3 ± 1	10 ± 3
p-menth-1-en-9-al (or isomer; KI: 1593)	34 ± 21	128 ± 48
p-menth-1-en-9-al (or isomer; KI: 1596)	83 ± 22	189 ± 22
p-menth-1-en-9-al (or isomer; KI: 1671)	96 ± 12	286 ± 25
p-menth-1-en-9-al (or isomer; KI: 1768)	7 ± 2	23 ± 4
p-menth-1-en-9-al (or isomer; KI: 1791)	13 ± 0	38 ± 5

Table 22: Continued.

compound	‘Weißer Versailler’[a]	‘Zitavia’[b]
	[µg/kg][c]	
others		
propan-1-ol	2 ± 2	16 ± 5
pentan-1-ol	4 ± 0	3 ± 0
pentan-2-ol	15 ± 7	8 ± 0
undecan-1-ol	n.d.	4 ± 1
propan-1,2-diol	n.c.	n.c.
butan-2,3-diol	2 ± 1	8 ± 4
2-methylpropan-1-ol	20 ± 13	195 ± 68
2-methylbutan-2-ol	39 ± 13	82 ± 20
2-ethylhexan-1-ol	2 ± 1	8 ± 2
pent-1-en-3-ol	17 ± 1	23 ± 3
(*Z*)-pent-2-en-1-ol 3-methylbut-2-en-1-ol	46 ± 9	45 ± 4
oct-1-en-3-ol	4 ± 0	49 ± 3
2-methylbut-3-en-2-ol	206 ± 8	117 ± 17
2-butoxyethanol	10 ± 0	13 ± 3
1-butoxypropan-2-ol	3 ± 0	5 ± 1
1-(2-butoxyethoxy)ethanol	3 ± 1	5 ± 3
benzyl alcohol	12 ± 2	16 ± 1
2-phenylethanol	4 ± 1	n.d.
allylphenol	2 ± 1	20 ± 5
nonanal	2 ± 0	n.d.
decanal	2 ± 1	3 ± 0
(*E*)-pent-2-enal	n.d.	14 ± 5
vanillin	23 ± 1	22 ± 3
pent-3-en-2-one	20 ± 1	14 ± 2
3-hydroxybutan-2-one	n.c.	n.c.
cyclohexenone	3 ± 0	3 ± 0
acetophenone	4 ± 1	n.q.
(*E*)-β-damascenone	n.q.	n.q.
4-methyl itaconate	10 ± 1	28 ± 3
γ-butyrolactone	n.c.	n.c.
pantolactone	46 ± 20	50 ± 12
2-pentylfuran	n.d.	5 ± 2
furfural	14 ± 4	24 ± 4

Table 22: Continued.

compound	'Weißer Versailler'[a]	'Zitavia'[b]
	[µg/kg][c]	
HMF	22 ± 23	111 ± 51
citraconic anhydride	n.c.	n.c.
glutaconic anhydride	30 ± 10	143 ± 46
2-*sec*-butylthiazole	3 ± 0	n.d.
p-α-dimethylstyrene	12 ± 4	35 ± 8

[a] Deutenkofen (8 July, 2015). [b] Deutenkofen (3 July, 2015). [c] Triplicate analysis of white currant berries: mean ± standard deviation. [d] Below limit of quantification (1.5 µg/kg). [e] Below limit of detection (0.5 µg/kg). [f] Detected but concentration not calculated because of too low recovery via LLE.

In total, 92 volatile compounds (the two indole derivatives methyl indole-3-acetate and methyl indole-3-pyruvate are not considered) were identified (19 tentatively) in these two batches after isolation with LLE. Eight C_6-compounds, 17 acids, 28 terpenes, and 39 other substances were determined. The same major compounds as previously identified in redcurrant fruits were found in both batches. (*E*)-Hex-2-enal and acetic acid were the quantitatively dominating compounds in white currant fruits constituting more than 66 % of the total volatile compounds. Furthermore, the other minor volatile compounds detected in white currant fruits were also identified in redcurrant fruits in the current work.

In Figure 25, the distributions of the volatile compound classes are depicted for the two white currant cultivars 'Weißer Versailler' and 'Zitavia'.

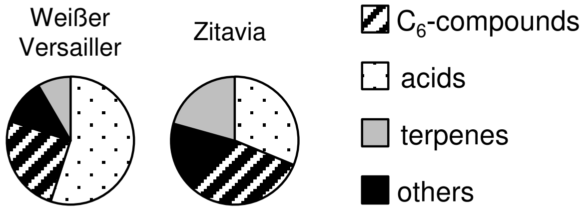

Figure 25: Distributions of the volatile compound classes determined in berries of two white currant cultivars, hand-picked at Deutenkofen in 2015.

In agreement with the findings in redcurrant fruits (see section 4.2.1.2), the compound classes C_6-compounds and acids were identified in the white currant cultivars as the main classes of volatile compounds with percentage distributions of 25 % and 31 %, respectively, for C_6-compounds as well as 55 % and 31 %, respectively, for acids. It can be seen that acids were the predominating class in fruits of 'Weißer Versailler', while the volatile profile of fruits originating from ‘Zitavia’ was not dominated by any volatile compound class. It is also striking that ‘Zitavia’ fruits contained a relatively high level of terpenes (21 %). The average percentage distribution of terpenes, taking into account the corresponding percentage distributions of all batches of redcurrant and white currant fruits except for ‘Zitavia’, were 6 %. However, in single batches of redcurrant fruits higher-than-average percentages of terpenes were also detected.

4.2.4 Conclusion

By means of LLE and GC/MS, 139 volatile constituents were isolated from and identified in fresh redcurrant fruits, and 117 of these compounds were reported for the first time. The volatiles were isolated from redcurrant berries that had been homogenised for 30 s; then the enzyme activities were inhibited by addition of saturated calcium chloride solution after additional 150 s. Thus, the resulting volatile profiles reflect the C_6-compounds generated within this defined time period. By applying this approach, the volatile profiles of redcurrant fruits were quantitatively dominated by C_6-compounds and acids, which represented at least 74 % of total volatile compounds in all investigated batches. (*E*)-hex-2-enal and acetic acid were identified as the two main compounds. In contrast to blackcurrant berries, terpenes were only a minor compound class. In addition, various other volatile compounds, such as alcohols, aldehydes, ketones, and lactones were isolated.

By analysing several hand-picked batches of nine different redcurrant cultivars it could be demonstrated that the distribution of volatile compound classes is mainly influenced by the cultivar. In contrast, the location and season showed no pronounced impact on the distribution of volatile compound classes. Though, this was only shown for hand-picked berries. A large variation in the distribution of volatile compound classes was demonstrated in seven batches of the commercially available cultivar ‘Rovada’, purchased in two consecutive years.

Additionally, the influence of ripeness was investigated, and for all volatile compound classes a decrease in concentrations during ripening occurred, expect for the acids in one cultivar.

The effect of freezing on volatile compounds in redcurrant fruits was also investigated and a strong decrease of C_6-compounds due to freezing was detected. In addition, the concentrations of acids increased with slight variations over the frozen-storage phase. The concentrations of all other classes of volatile compounds were relatively constant or a minor decrease was detectable.

Furthermore, the time-dependency of the enzymatic generation of C_6-compounds up until 180 s was analysed. This analysis showed that the primary profile of C_6-compounds is changed by isomerisation and reduction reactions. The percentage distribution of (*Z*)-hex-3-

enal decreased in favour of the isomer (*E*)-hex-2-enal and the corresponding alcohols (*Z*)-hex-3-en-1-ol and (*E*)-hex-2-en-1-ol through enzyme activity.

By means of sensory evaluation of a pooled and concentrated LLE-extract via AEDA, determination of FD factors, and calculation of OAVs, ten substances were identified as odour-active. Besides (*E)*-β-damascenone, the two main compounds acetic acid and (*E*)-hex-2-enal as well as two other lipid oxidation products ((*Z*)-hex-3-enal and oct-1-en-3-one), and two pyrazines (2-isobutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine) featured high OAVs. Reconstitution experiments could confirm the importance of the ten odour-active compounds to the aroma of redcurrant berries; however, the recombinant aroma model did not fully imitate the original aroma profile.

Furthermore, the volatile profile of white currant fruits was investigated and no significant difference of the volatile profile of this colour variant of the redcurrant species was detected with respect to the volatile profile of red coloured redcurrant species.

4.2.5 Comparison of the volatile profiles of fruits from different *Ribes* species

In the present work, the volatile profiles of fruits of two different *Ribes* species, i.e. blackcurrant and redcurrant, were investigated. Previously, the volatile profiles of fruits of two other *Ribes* species, i.e. gooseberry and jostaberry, were analysed (Hempfling *et al.* 2013a; Hempfling *et al.* 2013b; Schrade 2014). In section 4.1.5, the volatile profiles of blackcurrant, gooseberry, and jostaberry fruits were already compared. By analogy, the volatile profile of redcurrant fruits should be compared to the volatile profiles of fruits of the other three *Ribes* species (blackcurrant, gooseberry, and jostaberry).

In berries of redcurrant, 139 volatile compounds were identified and 86 of these compounds were also reported in blackcurrant, gooseberry, and/or jostaberry fruits after VHS extraction (Hempfling *et al.* 2013a; Hempfling *et al.* 2013b). The volatile profile of redcurrant was quantitatively dominated by the two main compounds (*E*)-hex-2-enal and acetic acid. The high concentration of acetic acid represents a distinctive feature compared to the other three *Ribes* species. Though, it has to be considered that the volatile constituents compared were not isolated with the same method. Volatile compounds were isolated from blackcurrant, gooseberry, and jostaberry fruits by means of VHS, while volatile compounds of redcurrant fruits were isolated via LLE. VHS is an isolation method with a low recovery rate for acids, such as acetic acid. Thus, acids were generally not quantifiable by means of VHS. Though, volatile compounds of gooseberry and jostaberry (Schrade 2014) fruits were previously also isolated via LLE. However, acetic acid was not quantified in high concentration. While the volatile profile of redcurrant berries was dominated by C_6-compounds and acids, high concentrations of C_6-compounds and esters were determined in the three other *Ribes* species (see section 4.1.2; Hempfling *et al.* 2013a; Hempfling *et al.* 2013b; Schrade 2014).

Furthermore, high concentrations of terpenes were quantified in blackcurrant berries (see section 4.1.2).

The sensory evaluations of the different *Ribes* species revealed that (*Z*)-hex-3-enal is an odour-active substance with a high OAV in fruits of each of the *Ribes* species. (*E*)-β-damascenone, the compound with the highest OAV in redcurrant berries, however, was not identified as an odour-active compound in fruits of any of the three other *Ribes* species and represents a distinguishing feature of the aroma of redcurrant fruits. Further, acetic acid, which was also identified as a compound with a high OAV in redcurrant berries, was only identified as an odour-active compound in gooseberry fruits via GC/O. Vanillin was not identified in the other *Ribes* species as an odour-active compound. The other odour-active compounds of redcurrant berries, i.e. (*E*)-hex-2-enal, (*Z*)-hex-3-en-1-ol, 2-isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, oct-1-en-3-one, and pent-1-en-3-one, were also identified in berries of blackcurrant, gooseberry, and/or jostaberry as odour-active compounds with an OAV > 1 (Hempfling *et al.* 2013a; Hempfling *et al.* 2013b; Schrade 2014).

To summarise, the volatile profile of redcurrant berries was characterised by significant differences compared to the volatile profiles of fruits of the three other *Ribes* species, i.e. blackcurrant, gooseberry, and jostaberry fruits. The sensory evaluations demonstrated that several compounds contribute to the aroma of fruits of all four *Ribes* species. Though, (*E*)-β-damascenone, the compound with the highest OAV in redcurrant berries, was not identified in fruits of one of the other three *Ribes* species.

5 SUMMARY

Volatile constituents of fresh blackcurrant berries (*Ribes nigrum* L.) were isolated via vacuum-headspace extraction (VHS) and analysed by capillary gas chromatography-mass spectrometry methods. The profiles of volatile compounds were dominated by three major compound classes, C_6-compounds (e.g. (*E*)-hex-2-enal and (*Z*)-hex-3-enal), esters (e.g. ethyl butanoate and methyl butanoate), and terpenes (e.g. Δ-3-carene, β-phellandrene, sabinene, and terpinen-4-ol). Additionally, high concentrations of the alcohol 2-methylbut-3-en-2-ol were quantified. It was demonstrated that freezing of blackcurrant berries prior to the investigation of volatile constituents results in overlooking an important class of compounds, that is, C_6-compounds formed upon crushing of fresh berries. In combination with lowered concentrations of esters and 2-methylbut-3-en-2-ol, this leads to a shift of the distribution of volatile constituents in favour of the terpenes in the frozen material. Therefore, the original volatile profile of fresh blackcurrant berries cannot be evaluated on the basis of frozen fruits.

The time-dependent enzymatic formation and isomerisation of C_6-compounds adds an additional element of variability to the spectrum of fresh blackcurrant volatiles. Enzyme inhibition experiments showed that (*Z*)-hex-3-enal, the main compound at the beginning of homogenisation, was converted to (*E*)-hex-2-enal by isomerisation and to the corresponding alcohols (*Z*)-hex-3-en-1-ol and (*E*)-hex-2-en-1-ol by reduction reactions.

Investigations of nine hand-picked blackcurrant cultivars revealed large variations in the distributions of the main compounds (C_6-compounds, esters, and terpenes) in dependence on the cultivar, whereas the growing year had no effect. Moreover, a decrease of C_6-compounds and terpenes, as well as an increase of esters was observed for fresh blackcurrant fruits in the course of fruit ripening. Nevertheless, blackcurrant cultivars can be divided into three main categories (C_6-, ester-, and terpene-type), if prerequisites, such as the same growing location and the same state of ripeness, are met.

For the first time, the occurrence of 4-methoxy-2-methyl-2-butanethiol was unambiguously proven in seven fresh blackcurrant cultivars by gas chromatography-mass spectrometry after enrichment on mercurated agarose gel. The cultivar ‘Andega’ (4.5 µg/kg) exhibited an approximately 13 times higher concentration of this sulphur-containing compound than the average concentration determined in the other cultivars (0.34 µg/kg). Calculation of the odour activity values (OAVs) indicated that 4-methoxy-2-methyl-2-butanethiol is an important contributor to the aroma of blackcurrant berries (OAV: 160 - 5,370).

The contribution of single volatiles to the overall odour was evaluated by gas chromatography/olfactometry in combination with aroma extract dilution analysis (AEDA) and calculation of OAVs. Besides 4-methoxy-2-methyl-2-butanethiol, representatives of all major volatile classes (C_6-compounds, esters, and terpenes) contribute to the overall aroma of fresh blackcurrant berries. Furthermore, the pyrazines 2-isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, and the lipid oxidation product oct-1-en-3-one were characterised by high OAVs. Reconstitution experiments confirmed the importance of these compounds for the overall aroma of fresh blackcurrant fruits; though, the natural aroma could not be completely imitated. Further investigations of the dynamics of the enzymatic formations and isomerisations of the C_6-compounds might be the key to solve this issue.

The volatile constituents of fresh redcurrant berries (*Ribes rubrum* L.) were isolated via liquid-liquid extraction (LLE) and analysed by capillary gas chromatography-mass spectrometry methods. The volatiles were isolated from redcurrant berries that had been homogenised for 30 s; then the enzyme activities were inhibited by addition of saturated calcium chloride solution after additional 150 s. Hence, the resulting volatile profiles reflect the C_6-compound profile generated within this defined time period. The spectrums of volatile compounds were particularly characterised by C_6-compounds and acids. (*E*)-Hex-2-enal and acetic acid were the two main compounds and constituted up to 79 % of the total volatile compounds. They were also detected in fresh white currant berries, a colour variant of the redcurrant species, as the main volatile compounds. Various other volatile compounds, such as alcohols, aldehydes, ketones, lactones, and terpenes, were additionally isolated from redcurrant and white currant fruits.

Investigations of several hand-picked batches of different redcurrant cultivars demonstrated an influence of the cultivar on the distribution of volatile compound classes. In contrast, location and year showed no pronounced influence on the volatile profiles. This observation, however, was not possible for commercially obtained batches of the cultivar ‘Rovada’. Furthermore, in the course of fruit ripening, a decrease of all compound classes was detected for redcurrant berries, except for the acids in one cultivar.

The concentrations of C_6-compounds were reduced in frozen-stored redcurrant berries, while an increase of the acids was observed. Additionally, the time-dependency of the enzymatic generation of C_6-compounds up until 180 s was analysed and showed that the primary C_6-compound spectrum was markedly changed by isomerisation of (*Z*)-hex-3-enal to (*E*)-hex-2-enal and by the reduction of (*Z*)-hex-3-enal and (*E*)-hex-2-enal to (*Z*)-hex-3-en-1-ol and (*E*)-hex-2-en-1-ol, respectively.

The ten substances (*E*)-β-damascenone, acetic acid, (*E*)-hex-2-enal, (*Z*)-hex-3-enal, (*Z*)-hex-3-en-1-ol, 2-isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, oct-1-en-3-one, pent-1-en-3-one, and vanillin were identified as odour-active compounds in fresh redcurrant fruits. However, a recombination of the aroma of fresh redcurrant fruits could not fully be achieved in the present work.

6 ZUSAMMENFASSUNG

Die flüchtigen Inhaltsstoffe frischer schwarzer Johannisbeeren (*Ribes nigrum* L.) wurden mittels Vakuum Headspace Technik (VHS) isoliert und mit kapillargaschromatographisch-massenspektrometrischen Methoden analysiert. Das Spektrum der flüchtigen Verbindungen wurde durch drei Hauptklassen, C_6-Verbindungen (z.B. (*E*)-Hex-2-enal und (*Z*)-Hex-3-enal), Ester (z.B. Ethylbutanoat und Methylbutanoat) und Terpene (z.B. Δ-3-Caren, β-Phellandren, Sabinen und Terpinen-4-ol), geprägt. Des Weiteren konnten hohe Konzentrationen des Alkohols 2-Methylbut-3-en-2-ol quantifiziert werden. Es konnte gezeigt werden, dass C_6-Komponenten, die beim Zerkleinern von frischen Beeren entstehen, durch den Gefrierprozess verloren gehen. In Verbindung mit verringerten Konzentrationen an Estern sowie 2-Methylbut-3-en-2-ol führte dies in gefrorenem Probenmaterial zu einer Verschiebung der flüchtigen Verbindungen zugunsten der Terpene. Das Profil flüchtiger Verbindungen frischer schwarzer Johannisbeeren ist daher nicht mit dem Profil gefrorener Früchte vergleichbar.

Die zeitabhängige enzymatische Bildung und Isomerisierung von C_6-Verbindungen stellt ein weiteres Phänomen dar, welches zur Variabilität des Spektrums flüchtiger Verbindungen frischer schwarzer Johannisbeeren beiträgt. Enzyminhibierungsexperimente zeigten, dass aus (*Z*)-Hex-3-enal, der Hauptkomponente zu Beginn der Homogenisierung, durch Isomerisierung und Reduktion (*E*)-Hex-2-enal und die korrespondierenden Alkohole (*Z*)-Hex-3-en-1-ol und (*E*)-Hex-2-en-1-ol gebildet wurden.

Durch die Analyse von neun selbst geernteten schwarzen Johannisbeersorten konnte eine große Variabilität der flüchtigen Hauptkomponenten (C_6-Verbindungen, Ester, und Terpene) in Abhängigkeit von der Sorte gezeigt werden. Das Anbaujahr zeigte hingegen keinen Einfluss. Zusätzlich konnte eine Abnahme der C_6-Komponenten und Terpene sowie eine Zunahme der Ester im Zuge der Fruchtreife festgestellt werden. Dennoch konnten die untersuchten schwarzen Johannisbeersorten unter bestimmten Voraussetzungen, wie z.B. derselbe Anbaustandort und vergleichbarer Reifegrad, in drei Grundtypen (C_6-, Ester- und Terpen-Typ) unterteilt werden.

Des Weiteren konnte 4-Methoxy-2-methyl-2-butanthiol erstmals eindeutig in sieben Sorten frischer schwarzer Johannisbeeren mittels Kapillargaschromatographie-Massenspektrometrie nach Anreicherung an mercurisiertem Agarose-Gel nachgewiesen werden. Die Sorte ‘Andega’ (4,5 µg/kg) wies eine ca. 13-mal höhere Konzentration dieser schwefelhaltigen Komponente auf als die durchschnittlich ermittelte Konzentration in den anderen Sorten (0,34 µg/kg). Die Berechnung von Aromawerten (OAVs) zeigte, dass 4-Methoxy-2-methyl-2-butanthiol von besonderer Bedeutung für das Aroma schwarzer Johannisbeeren ist (OAV: 160 - 5.370).

Die Beiträge einzelner flüchtiger Verbindungen zum Gesamtaroma wurden mit Hilfe von Kapillargaschromatographie/Olfaktometrie in Kombination mit Aromaextraktverdünnungsanalyse (AEDA) und der Berechnung von OAVs ermittelt. Neben 4-Methoxy-2-methyl-2-butanthiol tragen Vertreter aller Hauptklassen (C_6-Verbindungen, Ester und Terpenen) zum Gesamtaroma frischer schwarzer Johannisbeeren bei. Als weitere Verbindungen mit hohen OAVs konnten die Pyrazine 2-Isobutyl-3-methoxypyrazin, 2-Isopropyl-3-methoxypyrazin und 2-*sec*-Butyl-3-methoxypyrazin, sowie das Lipidoxidationsprodukt Oct-1-en-3-on identifiziert werden. Durch Rekombinationsversuche konnte die Bedeutung dieser Komponenten für das Aroma frischer schwarzer Johannisbeerfrüchte bestätigt werden, wobei jedoch das natürliche Aroma nicht vollständig nachgeahmt werden konnte. Weitere Untersuchungen zur zeitlichen Abhängigkeit der enzymatischen Bildung und Isomerisierung von C_6-Komponenten könnten zur vollständigen Nachahmung des Aromas frischer schwarzer Johannisbeeren beitragen.

Die flüchtigen Inhaltsstoffe frischer roter Johannisbeeren (*Ribes rubrum* L.) wurden durch Flüssig-Flüssig Extraktion (LLE) isoliert und mittels kapillargaschromatographisch-massenspektrometrischer Methoden analysiert. Die flüchtigen Verbindungen wurden aus roten Johannisbeeren isoliert, die für 30 s homogenisiert und deren Enzyme nach weiteren 150 s durch Zugabe gesättigter Calciumchloridlösung inhibiert wurden. Das ermittelte Spektrum flüchtiger Verbindungen spiegelt daher das C_6-Komponenten Profil wider, welches innerhalb dieses definierten Zeitraumes gebildet wurde. Das Spektrum der flüchtigen Verbindungen wurde vor allem durch C_6-Verbindungen und Säuren geprägt. Die beiden Hauptverbindungen (*E*)-Hex-2-enal und Essigsäure machten bis zu 79 % der gesamten flüchtigen Verbindungen aus und wurden ebenfalls in frischen weißen Johannisbeeren, einer Farbvariante der roten Johannisbeere, als flüchtige Hauptverbindungen detektiert. Zusätzlich wurden eine Vielzahl anderer Verbindungen, wie z.B. Alkohole, Aldehyde, Ketone, Lactone und Terpene, aus roten und weißen Johannisbeeren isoliert.

In Untersuchungen selbst geernteter Chargen unterschiedlicher roter Johannisbeersorten konnte ein Einfluss der Sorte auf die Verteilung der flüchtigen Stoffklassen gezeigt werden, während das Anbaugebiet und das Erntejahr geringeren Einfluss auf das flüchtige Profil hatten. Diese Korrelationen waren jedoch bei kommerziell erworbenen Chargen der Sorte ‘Rovada’ nicht nachvollziehbar. Weiter konnte im Zuge der Fruchtreife eine Abnahme der Konzentrationen aller Stoffklassen, mit Ausnahme der Säuren in einer Sorte, festgestellt werden.

Nach dem Gefrieren roter Johannisbeerfrüchte war vor allem die Bildung der C_6-Komponenten reduziert. Im Gegensatz dazu konnte eine Erhöhung der Säurekonzentrationen festgestellt werden. Des Weiteren wurde die zeitliche Abhängigkeit der enzymkatalysierten Bildung von C_6-Verbindungen in einer Zeitspanne von 180 s untersucht. Diese Untersuchung zeigte, dass das primär gebildete C_6-Spektrum durch die

Isomerisierung von (*Z*)-Hex-3-enal zu (*E*)-Hex-2-enal und die Reduktion von (*Z*)-Hex-3-enal und (*E*)-Hex-2-enal zu (*Z*)-Hex-3-en-1-ol und (*E*)-Hex-2-en-1-ol stark verändert wurde.

Als geruchsaktive Komponenten wurden die zehn Verbindungen (*E*)-β-Damascenon, Essigsäure, (*E*)-Hex-2-enal, (*Z*)-Hex-3-enal, (*Z*)-Hex-3-en-1-ol, 2-Isobutyl-3-methoxypyrazin, 2-Isopropyl-3-methoxypyrazin, Oct-1-en-3-on, Pent-1-en-3-on und Vanillin in frischen roten Johannisbeeren identifiziert. Das Aroma frischer roter Johannisbeerfrüchte konnte im Rahmen dieser Arbeit jedoch nicht vollständig nachgeahmt werden.

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8 APPENDIX

8.1 Recovery rates determined for the isolation of volatiles from blackcurrant berries via VHS

Table 23: Recovery rates determined for the isolation of volatiles via vacuum-headspace extraction (VHS).

compound	recovery [%][a]
C_6-compounds	
hexanal	86 ± 4[b]
(*E*)-hex-2-enal	85 ± 6[b]
hexan-1-ol	82 ± 2[b]
(*E*)-hex-2-en-1-ol	77 ± 7[b]
(*Z*)-hex-3-en-1-ol	76 ± 5[b]
(*Z*)-hex-3-enal	30 ± 10[c]
(*E*)-hex-3-enal	29 ± 7[c]
esters	
methyl salicylate	104 ± 2
ethyl benzoate	99 ± 4[b]
bornyl acetate	96 ± 6
(*Z*)-hex-3-enyl acetate	93 ± 5[b]
methyl octanoate	90 ± 10
hexyl acetate	88 ± 6
ethyl (*E*)-but-2-enaote	87 ± 3[b]
methyl benzoate	87 ± 13[b]
(*E*)-hex-2-enyl acetate	86 ± 7
methyl (*E*)-but-2-enoate	82 ± 5[b]
butyl acetate	81 ± 9
ethyl hexanoate	77 ± 6[b]
methyl pentanoate	77 ± 4
ethyl butanoate	76 ± 8[b]
methyl hexanoate	76 ± 6[b]
ethyl decanoate	71 ± 7[b]
methyl butanoate	63 ± 18[b]
alcohols	
octan-1-ol	104 ± 2
oct-1-en-3-ol	101 ± 0[b]
heptan-1-ol	64 ± 4[b]
(*Z*)-pent-2-en-1-ol	40 ± 2

Table 23: Continued.

compound	recovery [%][a]
2-methylbut-3-en-2-ol	40 ± 15[b]
2-methylpropan-1-ol	36 ± 2[b]
3-methylbut-2-en-1-ol	35 ± 9[b]
butan-1-ol	29 ± 1
propan-1,2-diol	1 ± 0
aldehydes	
neral	101 + 1
geranial	101 + 1
(*E*)-non-2-enal	97 ± 3
(*E*)-hept-2-enal	92 ± 7[b]
heptanal	85 ± 2[b]
(*E*)-pent-2-enal	73 ± 5
decanal	25 ± 2
ketones	
oct-1-en-3-one	92 ± 4
pent-1-en-3-one	48 ± 15
terpenes	
linalool	101 ± 2[b]
(*Z*)-rose oxide	100 ± 12
(*E*)-rose oxide	100 ± 12
citronellol	93 ± 10[b]
menthol	91 ± 1[b]
α-terpineol	90 ± 14[b]
1,8-cineole	86 ± 14[b]
terpinen-4-ol	83 ± 8[b]
geraniol	62 ± 8
germacrene D	48 ± 10
(*E*)-β-ocimene	48 ± 4[b]
(*Z*)-β-ocimene	48 ± 4[b]
γ-terpinene	48 ± 6
terpinolene	48 ± 4[b]
Δ-3-carene	47 ± 3[b]
caryophyllene	45 ± 5
myrcene	45 ± 4[b]
α-terpinene	44 ± 7
α-pinene	40 ± 2[b]
sabinene	34 ± 5

Table 23: Continued.

compound	recovery [%][a]
β-pinene	31 ± 7
limonene	30 ± 10[b]
β-phellandrene	30 ± 10[b]
α-phellandrene	29 ± 6
acids[d]	
hexanoic acid	31 ± 8[b]
butanoic acid	13 ± 0[b]
(*E*)-hex-2-enoic acid	13 ± 3[b]
(*E*)-hex-3-enoic acid	12 ± 1[b]
propanoic acid	4 ± 0[b]
acetic acid	2 ± 0[b]
others	
mesifurane	7 ± 1
methional	6 ± 1
pyrazines[e]	
2-*sec*-butyl-3-methoxypyrazine	144 ± 20
2-isobutyl-3-methoxypyrazine	104 ± 11
2-isoropyl-3-methoxypyrazine	97 ± 12

[a] Triplicate analysis: mean ± standard deviation. [b] Recovery rates were adopted from previous studies by Schrade (2014). [c] Average of a sixfold determination in a beery-type matrix by Schrade (2014). [d] Recovery rates for acids were determined in triplicate from buffer solutions (hydrochloric acid-sodium citrate buffer, pH 3.5) by Schrade (2014). [e] Recovery rates for pyrazines were determined with 2-methyl-5,6-diethylpyrazine as internal standard instead of heptan-2-ol.

Table 24: Recovery rates determined for the isolation of 4-methoxy-2-methyl-2-butanethiol via vacuum-headspace extraction (VHS) and enrichment on mercurated agarose (Affi-Gel® 10)

concentration	recovery [%][a]	
	VHS	Affi-Gel® 10
0.3 mg/mL	100 ± 3	92 ± 1
0.6 mg/mL	102 ± 3	91 ± 5
3 mg/mL	90 ± 15	76 ± 8
average	**98 ± 9**	**87 ± 9**

[a] Triplicate analysis: mean ± standard deviation.

8.2 Recovery rates determined for the isolation of volatiles from redcurrant and white currant berries via LLE

Table 25: Recovery rates determined for the isolation of volatiles via liquid-liquid extraction (LLE).

compound	recovery [%][a]
C_6-compounds	
(*Z*)-hex-3-enal	110 ± 4
hexan-1-ol	99 ± 1
(*E*)-hex-2-en-1-ol	99 ± 1
(*Z*)-hex-3-en-1-ol	97 ± 3
(*E*)-hex-2-enal	89 ± 3
hexanal	85 ± 6
acids[b]	
benzoic acid	116 ± 8
hexanoic acid	113 ± 8
butanoic acid	107 ± 0
furan-2-carboxylic acid	104 ± 13
2-methylbutanoic acid	103 ± 4
(*E*)-hex-2-enoic acid	100 ± 2
pentanoic acid	100 ± 3
2-methylpropanoic acid	99 ± 2
(*E*)-hex-3-enoic acid	99 ± 1
octadecanoic acid	96 ± 7
propanoic acid	91 ± 5
hexadecanoic acid	89 ± 16
acetic acid	64 ± 20
levulinic acid	41 ± 28
terpenes	
perillyl alcohol	102 ± 3
(*E*)-linalool oxide (furan)	102 ±1
(*Z*)-linalool oxide (furan)	101 ± 1
α-terpineol	100 ± 1
geraniol	97 ± 7
linalool	97 ± 2
camphor	91 ± 0
1,8-cineole	84 ± 1
limonene	50 ± 10
α-terpinene	29 ± 3

Table 25: Continued.

compound	recovery [%][a]
α-pinene oxide	2 ± 0
terpinolene	n.q[c]
others	
benzyl alcohol	100 ± 1
(*Z*)-pent-2-en-1-ol	98 ± 1
oct-1-en-3-ol	97 ± 1
pentan-1-ol	96 ± 1
3-methylbut-2-en-1-ol	93 ± 2
2-ethylhexan-1-ol	90 ± 0
pent-1-en-3-ol	85 ± 2
pentan-2-ol	80 ± 2
furaneol	1 ± 0
propan-1,2-diol	n.q.
decanal	108 ± 11
benzaldehyde	107 ± 3
(*E*)-β-damascenone	99 ± 3
vanillin	99 ± 2
furfural	90 ± 4
(*E*)-hept-2-enal	88 ± 2
(*E*)-pent-2-enal	79 ± 2
nonanal	68 ± 3
pent-3-en-2-one	73 ± 2
oct-1-en-3-one	60 ± 9
pent-1-en-3-one	56 ± 11
3-hydroxybutan-2-one	12 ± 1
γ-decalactone	102 ± 6
sotolon	39 ± 11
pantolactone	35 ± 7
γ-butyrolactone	18 ± 5
2-pentylfuran	36 ± 4
HMF	31 ± 12
2-methoxy-3-methylpyrazine[d]	97 ± 1
2-isobutyl-3-methoxypyrazine[d]	95 ± 1
2-isopropyl-3-methoxypyrazine[d]	92 ± 1
2-*sec*-butylthiazole	88 ± 0
citraconic anhydride	n.q.

Table 25: Continued.

compound	recovery [%][a]
methyl indole-3-acetate[b]	139 ± 8

[a] Triplicate analysis: mean ± standard deviation. [b] Recovery rates were determined in triplicate from a redcurrant-type matrix. [c] Below limit of quantification (1.5 µg/kg). [d] Recovery rates for pyrazines were determined with 2-methyl-5,6-diethylpyrazine as internal standard instead of heptan-1-ol.

8.3 Mass spectra

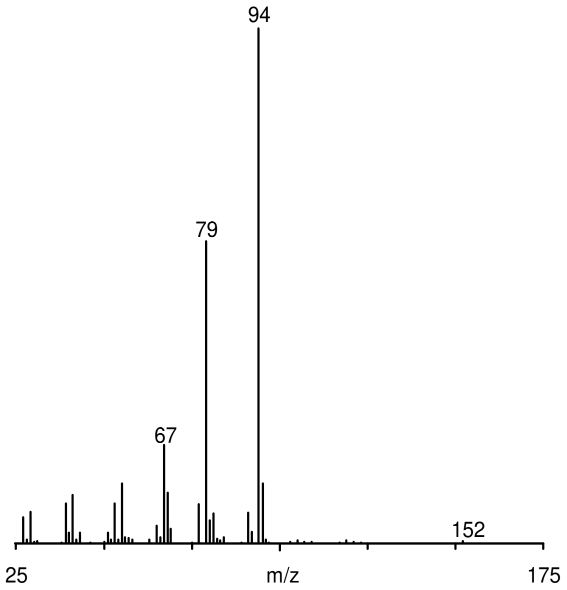

Figure 26: Mass spectrum of *p*-menth-1-en-9-al (or isomer); KI (DB-Wax): 1593

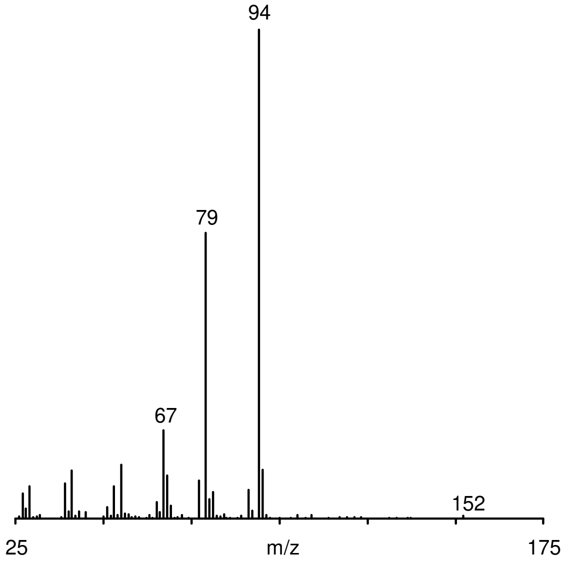

Figure 27: Mass spectrum of *p*-menth-1-en- 9-al (or isomer); KI (DB-Wax): 1596

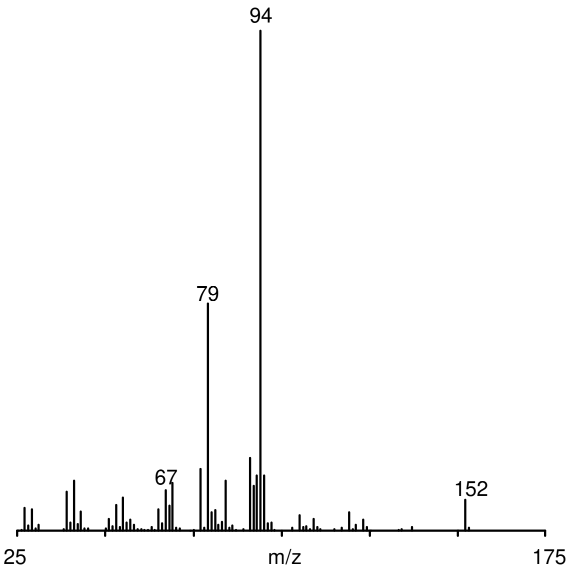

Figure 28: Mass spectrum of *p*-menth-1-en- 9-al (or isomer); KI (DB-Wax): 1671

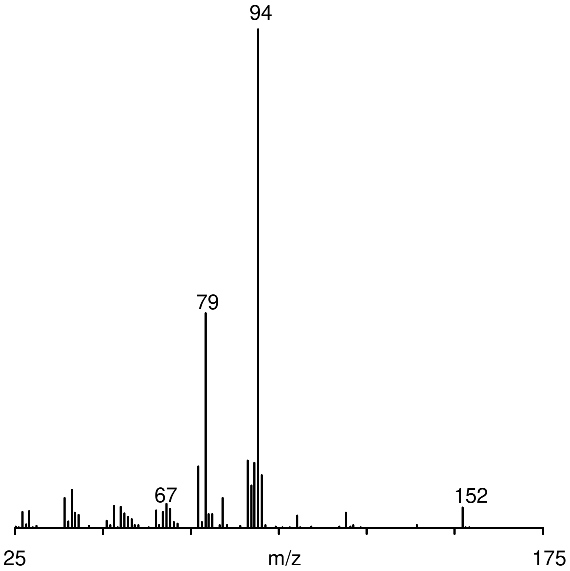

Figure 29: Mass spectrum of *p*-menth-1-en- 9-al (or isomer); KI (DB-Wax): 1768

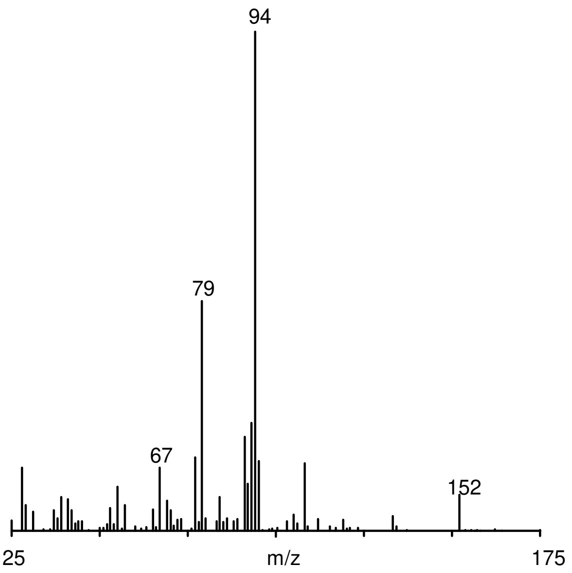

Figure 30: Mass spectrum of p-menth-1-en-9-al (or isomer); KI (DB-Wax): 1791

APPENDIX

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PhD thesis: ‘Analysis and sensory evaluation of volatile constituents of blackcurrant (*Ribes nigrum* L.) and redcurrant (*Ribes rubrum* L.) fruits’

ACADEMIC EDUCATION

10/2007 – 03/2013: Studies in Food Chemistry at the University of Kaiserslautern (Germany),
Degree: ‘Diplom’ and ‘Erstes Staatsexamen’

Diploma thesis: ‘Methodenetablierung zur Bestimmung der Anthocyankonzentration aus humanen Urin- und Plasmaproben nach Verzehr eines Heidelbeerextraktes (*Vaccinium myrtillus* L.)’

01/2012 – 04/2012: Research internship at the University of Barcelona (Spain),
Research topic: ‘Influence of sun exposure, irrigation and ripening on polyphenols in Albariño grapes’

09/2004 – 07/2007: Ernährungswissenschaftliches Gymnasium at Käthe-Kollwitz-Schule, Bruchsal (Germany)

PUBLICATIONS

Jung, K., Fastowski, O, Poplacean, I., Engel, K.-H. Analysis and sensory evaluation of volatile constituents of fresh blackcurrant (*Ribes nigrum* L.) fruits, Journal of Agriculture and Food Chemistry, 2017, *65*, 9475-9487.

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Jung, K.; Fastowski, O.; Engel, K.-H. Investigations of aroma compounds in blackcurrant (*Ribes nigrum* L.) berries, 11th Wartburg Symposium on Flavor Chemistry & Biology, Eisenach, 21.-24.06.2016.

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