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Optical purity of (R)-(−)-1-octen-3-ol in the aroma of various species of edible mushrooms

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Abstract

The characteristic enantiomeric ratio of 1-octen-3-ol in various species of edible mushrooms was the subject of the investigation. The microdistillation–extraction apparatus of Likens–Nickerson was used for aroma isolation. The volatiles were separated on the usual GC capillary column and on the chiral GC column connected to an HP-6890 gas chromatograph. The following species of cultivated mushrooms were analysed: *Agaricus bisporus*, *Pleurotus ostreatus*, *Hericium erinaceum*, *Pholiota nameco* and *Lentinus edodes*, and wild growing: *Boletus edulis*, *Xerocomus badius* and *Macrolepiota procera.* Despite significant differences in the concentrations of 1-octen-3-ol, the optical purity of (R)-(−)-1-octen-3-ol in all the species was very high, the highest in *A. bisporus* (over 98.5%), the lowest in *X. badius* (over 82.1%). Several mushroom-like flavouring substances and food products of mushroom-like aroma were also tested. In all of them the racemic ratio of 1-octen-3-ol was determined; this suggests that the origin of the flavours was not natural.

Keywords: Mushrooms; 1-Octen-3-ol; Enantiometric ratio; Aroma nature identity

1. Introduction

The chiral nature of living systems has evident implications for biologically active compounds interacting with them. Metabolic and regulatory processes mediated by biological systems are sensitive to stereochemistry, and different responses can be observed when comparing the activities of a pair of enantiomers (Maier, Franco, & Lindner, 2001). But chiral discrimination has been also recognized as one of the important principles of odour perception (Mosandl, 1995). Several optical isomers have been described as having different odour qualities and/or different odour intensities for humans, possibly also small or even undetectable (Laska & Teubner, 1999). These results support the assumption that enantioselective molecular odour receptors may only exist for some, but not all the volatile enantiomers. It seems to be even more important that many natural flavour compounds have been detected with a characteristic size of the enantiomeric ratio; the evaluation of the origin specific enantiomeric ratios, despite some limitations, should be a valuable criterion for differentiating natural flavour compounds from those of synthetic origin (Marchelli, Dossena, & Palla, 1996; Mosandl, 1995).

Mushrooms have long been used as food or food flavouring materials because of their unique and subtle flavour which has been studied by many authors (Assaf, Hadar, & Dosoretz, 1997; Chen, Chen, Chen, & Wu, 1984; Chen & Wu, 1984; Fischer & Grosch, 1987; Lizárraga-Guerra, Guth, & Lopez, 1997; Maga, 1981; Mau, Beelman, & Ziegler, 1992; Mau, Chyau, Li, & Tseng, 1997; Wąsowicz, 1974; Venkateshwarlu, Chandravadana, & Tewari, 1999). The main odorants of the mushroom aroma are eight carbon atom (C8) compounds; the most important is 1-octen-3-ol. It occurs in two optically active forms (Bauer, Garbe, & Surburg, 1990; Chambers IV, Smith, Seitz, & Sauer, 1998; Mosandl, Heusinger, & Gessner, 1986). Studies of chemicals indicated that (R)-(−)-octen-3-ol had a fruity mushroom-like characteristic, whereas (S)-(+)-1-octen-3-ol had a mouldy, grassy note (Mosandl et al., 1986). The

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levorotatory antipode, formed by the enzymic oxidative breakdown of linoleic acid, has been described as the character impact flavour compound of mushrooms (Assaf et al., 1997; Mau et al., 1992).

The aim of this study was to determine the ratio of 1-octen-3-ol optical isomers in several species of edible mushrooms, as well as some commercial mushroom-like aroma products and a few food products of mushroom-like character in order to determine the origin of the aroma in these products.

2. Materials and methods

2.1. Mushrooms and chemicals

Samples of fresh mushrooms were taken mostly from the experimental cultivation plot at the Department of Horticulture, the August Cieszkowski Agricultural University of Poznań, in 2000, 2001 or 2002. The following species of cultivated mushrooms were analysed: *Agaricus bisporus* (two varieties), *Hericium erinaceum* (two varieties), *Pleurotus ostreatus*, *Lentinus edodes* and *Pholiota nameco*. Three samples of an unknown variety of *A. bisporus* were purchased in a street market in Poznań (Poland). Wild-growing mushrooms included in the study, i.e., *Boletus edulis*, *Xerocomus badius* and *Macrolepiota procera*, were picked in the forests of the Wielkopolska region in 2001 or 2002. Three mushroom-like aroma products used in the food industry, as well as three kinds of dehydrated mushrooms soups, were also tested.

Standards of 1-octen-3-ol and other chemicals used for the identification of volatile compounds were purchased from Sigma Chemical Co. and the other reagents, such as pentadecane, ethyl ether and pentane were of analytical grade for GC.

2.2. Isolation of volatiles by microdistillation–extraction procedure

The volatiles from mushrooms and mushroom-like products were isolated by the microdistillation–extraction procedure in a Likens–Nickerson apparatus (Bouseta & Collin, 1995), with ether–pentane (1:1 v/v) as the extraction solvent. Seventy five grams of fresh mushrooms were used for isolation, or 1–2 g of aroma products. Pentadecane as the internal standard was added (0.6 mg) before distillation. Fresh mushrooms were cut into pieces and homogenized with 150 ml of distilled water for 5 min. The homogenate was left for 15 min to maximize the enzymatic production of flavour (Venkateshwarlu et al., 1999) and subjected to a simultaneous distillation and solvent extraction under reduced pressure in a Likens–Nickerson apparatus. The flavour extract was dried over anhydrous sodium sulphate and concentrated to 0.1 ml at room temperature. The recovery of added 1-octen-3-ol realized in this method was 76%.

2.3. Solid phase microextraction

To prepare the sample of the aroma product, 0.5 g of it was placed in 10-ml headspace vials, followed by the addition of 2.5 ml of deionized water, and the vial was capped with a teflon-linked cap. The SPME fibre 100 μm PDMS (Supelco Inc. Bellefonte, PA) was preconditioned in the GC port at 260 °C for 2 h. The optimum exposure time was 15 min at 50 °C, while 4 min desorption time was used, so no compounds were present when fibre was reinjected after 4 min.

2.4. Gas chromatography analysis

A Hewlett-Packard HP 6890 gas chromatograph with a split/splitless injector and FID detector was used for the analyses. Compounds were separated using a capillary column Innowax (Hewlett-Packard, 30 m × 0.25 mm × 0.25 μm) and a chiral capillary column RtβDex sa (Restek, 30 m × 0.32 mm × 0.25 μm). The identity of separated compounds was confirmed on a Hewlett-Packard HP 5890 II gas chromatograph coupled to an HP 5971MSD quadrupole mass spectrometer. The injection of volatiles was done in the split mode. Analysis parameters on the Innowax column were as follows: initial temperature 60 °C, then 30 °C/min to 200 °C, while on the chiral column: initial temperature 60 °C, then 3 °C/min to 150 °C and 10 °C/min to 200 °C. The flow of hydrogen, used as a carrier gas, was 1.6 ml/min. The concentrations of volatiles were calculated on the basis of the known amounts of the internal standard added to the sample prior to distillation. The enantiomeric ratio was calculated as the ratio of the peak areas of the authentic sample.

3. Results and discussion

Agaricus bisporus, the most popular cultivated species of edible mushrooms, was the main species investigated in this study. The study was focussed on the concentration and enantiomeric ratio of 1-octen-3-ol. The two varieties of *A. bisporus*: Korona-7 and Euromycel-12, originating from two independent cultivations, were analysed separately in two size classes: small (2–2.5 cm of diameter) and big (3–3.5 cm of diameter) caps. Three samples of *A. bisporus* purchased on the market, of an unknown variety, were also included in the experiment. The resolution of volatiles performed with the Innowax column is presented in Fig. 1. The most abundant aroma compound was 1 octen-3-ol; other compounds of importance, such as 3-methyl-butanol, 3 octanone,

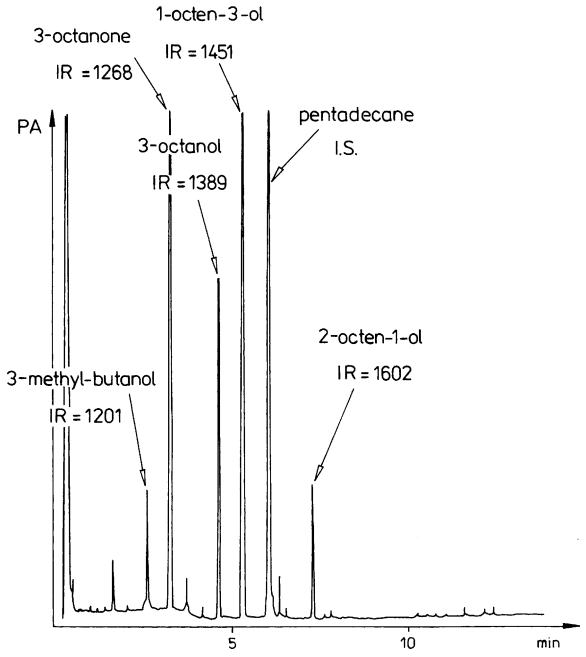

Fig. 1. Gas chromatographic resolution of volatiles from *A. bisporus* on Innowax column. IR, Kovats' index of retention; IS, internal standard.

3-octanol, 2-octen-1-ol, known from the other data published (Fischer & Grosch, 1987; Maga, 1981; Mau et al., 1992; Venkateshwarlu et al., 1999), were also identified.

The concentrations of 1-octen-3-ol in the analysed samples of *A. bisporus* ranged, depending on the variety and origin of the sample, from 1.90 to 5.05 mg/100 g of fresh mushrooms. The relative percentage of this compound was about 60–74%. The totals of 78% (Wąsowicz, 1974) and 56.6% (Venkateshwarlu et al., 1999) were reported previously. It was interesting that, in small caps, higher concentrations of 1-octen-3-ol were observed in comparison to those bigger in size, but the differences were not statistically significant in every case. Despite the differences in the 1-octen-3-ol concentration, its enantiomeric ratios were very similar in both analysed varieties regardless of the cultivation plot, the size of caps and also in the samples of an unknown variety, purchased at a street market. The predominant optical form (R)-(−)-1-octen-3-ol was of very high optical purity, not lower than 98.5%. It is known that chiral flavours are generally characterized by a specific distribution of enantiomers, which may differ, depending on several phenomena (Marchelli et al., 1996). The enantiomeric ratios of 1-octen-3-ol were estimated from the chiral separation of volatiles performed with an enantioselective GC-column, Restek Rt-βDex sa. The chiral separation of the 1-octen-3-ol standard and the distillate from *A. bisporus* is illustrated in Figs. 2 and 3, while the data estimated for the concentration of 1-octen-3-ol and its enantiomeric ratio are presented in Table 1.

Two varieties of *H. erinaceum*, D-5 and H-1, grown on four kinds of media: beech, birch, adler and oak sawdust, were analysed in terms of the same parameters. The C8 compounds were found as in the case of *A. bisporus*; among them 1-octen-3-ol was predominant. Relatively significant amounts of benzaldehyde were also observed in these mushrooms. The relative percentage of 1-octen-3-ol ranged from 40% to 65% of total volatiles, its concentration varied significantly between the samples, and the enantiomeric purity of (R)-(−)-1-octen-3-ol was at the same high level (not lower than 96.7%). The data obtained for the samples of *H. erinaceum* are presented in Table 2.

At the next stage of the experiment, three other species of cultivated edible mushrooms were tested: *P. ostreatus*, *L. edodes* and *P. nameco.* The flavour profiles of these mushrooms are rather different. In *P. ostreatus* the same compounds were found as in the case of *A. bisporus* and even in similar amounts. The relative percentage of 1-octen-3-ol accounted for 78% of total volatiles. Similar data are published for *Pleurotus florida* (Venkateshwarlu et al., 1999). *Pleurotus* is an important

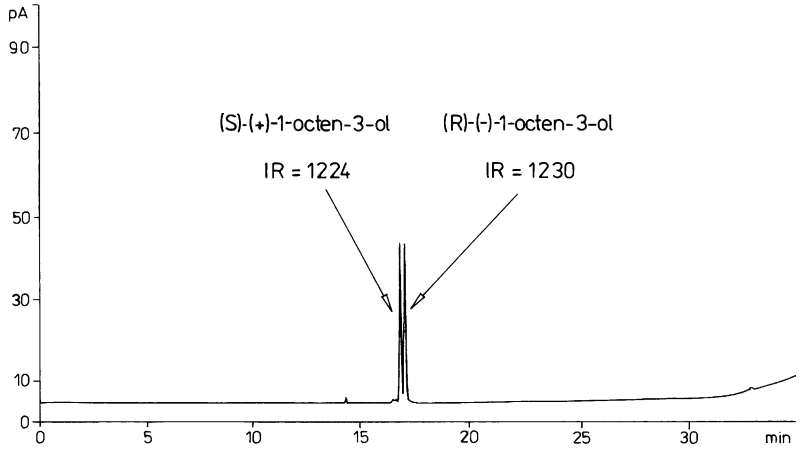

Fig. 2. Enantioselective gas chromatographic resolution of 1-octen-3-ol standard on Rt-βDex sa column. IR, Kovats' index of retention.

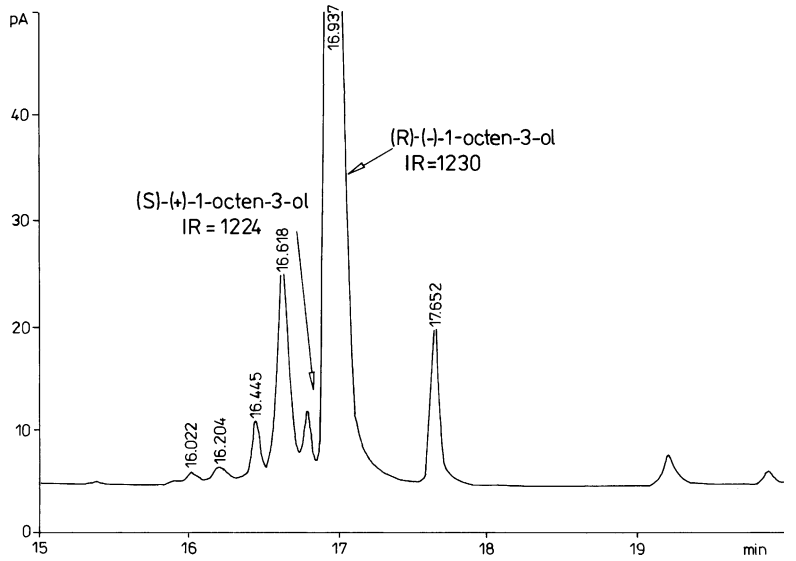

Fig. 3. Enantioselective gas chromatographic resolution of volatiles from *A. bisporus* – fraction with 1-octen-3-ol on Rt-βDex sa column. IR, Kovats' index of retention.

Table 1
Concentration and enantiomeric ratio of 1-octen-3-ol in *A. bisporus*

Sample description (variety)	Concentration of 1-octen-3-ol (mg/100 g)[a]	Enantiomeric ratio (−)/total compound (%)[b]
Variety Korona		
Cultivation plot 2000		
B	4.87	99.1
S	5.23	99.2
Cultivation plot 2001		
B	1.64	99.0
S	2.63	98.9
Variety Euromycel		
Cultivation plot 2001		
B	1.80	99.3
S	2.41	99.3
Cultivation plot 2002		
B	1.53	98.9
S	2.33	98.9
Unknown variety		
I	3.72	98.9
II	2.30	99.1
III	1.91	99.0

B, big caps; S, small caps.
[a] The values represent means of four repetitions, coefficient of variation <5.5%.
[b] The values represent means of three repetitions.

Table 2
Concentration and enantiomeric ratio of 1-octen-3-ol in *H. erinaceum*

Sample description		Concentration of 1-octen-3-ol (mg/100 g)[a]	Enantiomeric ratio (−)/total compound (%)[b]
Media	Variety		
Beech	D-5	3.46	96.8
	H-1	1.18	97.2
Birch	D-5	2.55	96.9
	H-1	1.72	97.3
Adler	D-5	2.23	96.7
	H-1	1.57	96.9
Oak	D-5	2.61	97.0
	H-1	1.52	97.0

[a] The values represent means of four repetitions, coefficient of variation <5.8%.
[b] The values represent means of three repetitions.

edible mushroom and its flavour has been attributed to the C8 compounds, mainly 1-octen-3-ol (Lizárraga-Guerra et al., 1997). In *L. edodes*, the shiitake mushroom, C8 compounds: 3-octanone, 3-octanol and 1-octen-3-ol were detected (1-octen-3-ol in the highest amount), as well as dimethyl disulphide and dimethyl trisulphide. The formation of C8 compounds, such as 1-octen-3-ol, and sulfurous compounds, such as dimethyl disulphide and dimethyl trisulphide, is affected by the pH value, while the characteristic aroma of shiitake is related to a cyclic sulfurous compound, lenthionine (Chen et al., 1984). *P. nameco* was recognized as a less aromatic mushroom with higher concentrations of hexanal and benzaldehyde; the relative percentage of 1-octen-3-ol was about 15%. In all the three analysed species, high purity of minus 1-octen-3-ol was observed (Table 3).

Three wild-growing species of mushrooms picked in the forests of the Wielkopolska region in 2002, *X. badius*, *B. edulis* and *M. procera*, also showed different concentrations of 1-octen-3-ol. The highest concentration of 1-octen-3-ol was found in *B. edulis* (Table 3), as well as its relative percentage which accounted for 86%

Table 3
Concentration and enantiomeric ratio of 1-octen-3-ol in different species of edible mushrooms

Species	Concentration of 1-octen-3-ol (mg/100 g)[a,b,c]	Enantiomeric ratio (−)/total compound (%)[d]
Cultivated		
Pleurotus ostreatus		
2001	6.48[a]	97.2
2002	6.75[a]	97.3
Lentinus edodes – shiitake	1.21[b]	95.5
Pholiota nameco	0.11[c]	92.5
Wild growing		
Xerocomus badius		
2001	2.53[b]	83.3
2002	2.15[b]	82.1
Boletus edulis	15.60[a]	96.7
Macrolepiota procera	0.57[c]	98.3

[a–c]The values represent means of four repetitions, coefficients of variation: a < 2%, b < 5%, c < 6.5%.
[d]The values represent means of three repetitions.

of total volatiles. The totals of 50% or 82% of 1-octen-3-ol were found in *B. edulis* by other authors (Maga, 1981). The lowest concentration of 1-octen-3-ol, with a relative percentage of only about 14% was observed in *M. procera* ; hexanal was the predominant compound in this case. The enantiomeric purity of (R)-(−)-1-octen-3-ol was high in all three species: however, it was significantly lower in *X. badius.*

1-Octen-3-ol is a characteristic aroma compound of mushrooms, occurring in most of them in huge amounts; in some species it occurred in smaller quantities, but in every case with a specific enantiomeric ratio and optical purity of the minus form, which means that it can be used for mushroom aroma authenticity control. From that point of view, mushroom-like aroma products of known brands as well as some food products (dehydrated soups) of mushroom-like aroma were tested. The solid phase microextraction was applied instead of the traditional distillation to evaluate the enantiomeric ratio of 1-octen-3-ol in aroma products because, in control systems, rapid methods are beneficial. SPME has been successfully used for the separation of volatile organic compounds in foodstuffs, such as apples (Song, Gardner, Holland, & Beaudry, 1997), coffee (Roberts, Pollien, & Milo, 2000), beer (Jeleń, Wlazły, Wąsowicz, & Kamiński, 1998), mushrooms – truffles (Diaz, Senorans, Reglero, & Ibanez, 2002; Pelusio et al., 1995) and vegetable oils (Jeleń, Obuchowska, Zawirska-Wojtasiak, & Wąsowicz, 2000), also combined with enantioselective gas chromatography (Fuchs, Beck, Sandvoss, & Mosandl, 1999). This technique was used for the fast analysis of dill seed odorants and their enantiomers (Zawirska-Wojtasiak & Wąsowicz, 2002). The data obtained for the tested material are presented in Table 4. In all of the products, the predominant compound was 1-octen-3-ol; it was sometimes the only aroma component; however, it was found in different concentrations. The enantiomeric ratio of 1-octen-3-ol was measured by both methods, which gave the same results. In all the analysed products presented in Table 4, the racemic ratio of 1-octen-3-ol was determined. The same was done for a few other aroma products tested. The results suggested that most mushroom-like aromas produced were of synthetic origin. This is probably because of huge losses during the preparation processes of 1-octen-3-ol, naturally occurring in mushrooms (Maga, 1981). On the other hand, the aromas are usually regarded as nature-identical, so they contain the same compound as in the natural aroma. Nevertheless, the sensory characteristics of the two optical isomers of 1-octen-3-ol are significantly different, so the racemic ratio may alter sensory properties in comparison with the natural specific enantiomeric ratio of 1-octen-3-ol in mushrooms, which sometimes means almost optically pure (R)-(−)-1-octen-3-ol.

Table 4
Concentration and enantiomeric ratio of 1-octen-3-ol in different aroma and food products

Sample	Concentration of 1-octen-3-ol (mg/100 g)[a,b]	Enantiomeric ratio (−)/total compound (%)	
		Distillation[c]	SPME[c]
Aroma products			
I	133[a]	49.5	49.5
II	1.51[a]	50.0	50.0
III	69.0[a]	50.2	50.2
Dehydrated soups			
A	0.12[b]	50.2	50.2
B	0.09[b]	50.1	50.0
C	0.18[b]	51.6	51.8

[a]The values represent means of four repetitions, coefficient of variation <3%.
[b]The values represent means of four repetitions, coefficient of variation <5%.
[c]The values represent means of three repetitions.

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