JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Storage on the Essential Oil Composition of *Piper nigrum* L. Fruits of Different Ripening States

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The qualitative and quantitative composition of the essential oil from black, green, and white pepper was determined by using a simultaneous distillation and extraction micromethod for oil isolation and gas chromatography (GC)/flame ionization detection (FID) and GC/mass spectrometry (MS) analysis techniques. The most abundant compounds in pepper oils were (*E*)-β-caryophyllene (1.4−70.4%), limonene (2.9−38.4%), β-pinene (0.7−25.6%), Δ-3-carene (1.7−19.0%), sabinene (0−12.2%), α-pinene (0.3−10.4%), eugenol (0.1−41.0%), terpinen-4-ol (0−13.2%), hedycaryol (0−9.1%), β-eudesmol (0−9.7%), and caryophyllene oxide (0.1−7.2%). Green pepper corn obtained by a sublimation drying method gave more oil (12.1 mg/g) and a much higher content of monoterpenes (84.2%) in the oil than air-dried green pepper corn (0.8 mg/g and 26.8%, respectively). The oil from ground black pepper contained more monoterpenes and less sesquiterprnes and oxygenated terpenoids as compared to green and white pepper oils. After 1 year of storage of pepper samples in a glass vessel at room temperature, the amount of the oils isolated decreased, the content of terpenes decreased, and the amount of oxygenated terpenoids increased. Differently from other pepper samples, 1 year storage of green pepper corn raised the oil amount more than twice of both drying methods.

KEYWORDS: ***Piper nigrum* L.; Piperaceae; black pepper; green pepper; white pepper; essential oil; GC/FID; GC/MS; effect of storage**

INTRODUCTION

Pepper is one of the oldest known spices, and it is the most widely used of all condiments. Black, green, and white peppers are all of the fruits of the *Piper nigrum* L. plant cultivated throughout the tropical world. The main countries of production are India, Indonesia, Brazil, Malaysia, and Sri Lanka. Black pepper is harvested before full maturity is reached, green pepper is produced from unripe green fruits, and fully ripe pepper fruits are used to make white pepper by removing the outer skin. Because of its unripeness, green pepper has a fresh and herbal flavor. The flower of white pepper is milder than that of black pepper. The pungent principle in black pepper is alkaloid piperine, only the trans,trans conformation contributes to pepper's pungency. The pungency is strongest in white pepper and weakest in green pepper (*1*, *2*). Black pepper contains about 3% of essential oil, which is the flavor characteristic of pepper. The volatiles of black pepper have been analyzed by several authors (*1*−*18*). Over 100 compounds have been identified in black pepper oil. Monoterpenoid (50−74%) and sesquiterpenoid (20−35%) hydrocarbons have been found to dominate in pepper oils, and oxygenated terpenoids were found only to form to 13% of total oil.

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Murthy et al. (*9*) have studied the grinding characteristics of black pepper in essential oil yield. They concluded that during grinding of pepper the loss of essential oil and grinding energy increases with the fineness of the particle. So far, no detailed work on the effect of storage to essential oil composition of *P. nigrum* L. fruits has been performed. The essential oil composition of green and white pepper has not received much attention (*1*, *2*, *5*, *7*, *10*, *14*, *19*).

The aim of this study was to determine the effect of storage on the essential oil amount and the composition of the oil isolated from black, green, and white pepper. Differences in the oil composition of green pepper corn obtained by air-drying and sublimation-drying methods were determined.

EXPERIMENTAL PROCEDURES

CAS Numbers. α-Pinene, 7785-70-8; camphene, 5794-04-71; β-pinene, 19902-08-0; Δ-3-carene, 498-15-7; sabinene, 2009-00-9; myrcene, 123-35-3; α-phellandrene, 4221-98-1; α-terpinene, 99-87-6; *p*-cymene, 99-87-6; limonene, 5989-27-5; γ-terpinene, 99-85-4; terpinolene, 586-62-9; linalool, 78-70-6; terpinen-4-ol, 562-74-3; α-terpineol, 7785-53-7; myrtenol, 19894-97-4; eugenol, 97-53-0; α-cubebene, 17699-14-8; α-copaene, 3856-25-5; (*E*)-β-caryophyllene, 87-44-5; α-humulene, 6753-98-6; caryophyllene oxide, 1139-30-6; α-farnesol, 4602-84-0; and α-bisabolol, 515-69-5.

Plant Material. The following samples of *Piper nigrum* L. fruits were obtained from Paulig Baltic LC in spring 2001: air-dried black

10.1021/jf030635s CCC: $27.50
Published on Web 03/30/2004

Table 1. Composition of the Essential Oil from Pepper Corn Samples[a]

			concentration (%)							
	RI		black pepper[b]		green pepper[b]		green pepper[c]		white pepper[b]	
compound	NB-30	SW-10	2001	2002	2001	2002	2001	2002	2001	2002
α-thujene[MS]	923	1028	0.2	0.1	0.8	0.7	4.2	4.9	0.1	tr
α-pinene[MS]	931	1028	7.3	6.6	2.1	2.1	8.3	9.0	9.9	10.4
camphene	945	1072	0.2	0.2	0.1	tr	0.2	0.2	0.3	0.2
sabinene[MS]	968	1123	1.4	0.8	4.6	3.7	4.1	7.5	0.4	0.4
β-pinene[MS]	973	1115	19.0	15.6	3.7	3.8	18.3	13.3	24.2	25.6
myrcene[MS]	986	1160	2.6	2.5	0.5	0.4	2.5	1.7	3.6	3.5
α-phellandrene*	1002	1165	2.2	1.8	0.8	0.7	3.4	1.2	2.1	1.9
Δ-3-carene[MS]	1008	1146	10.6	8.7	2.4	4.2	19.0	11.3	9.8	9.6
α-terpinene	1010	1180	0.1	0.1	1.5	0.9	1.4	3.4	0.1	tr
p-cymene	1016	1272	0.5	0.7	0.9	tr	1.0	5.2	0.5	0.6
β-phellandrene*[MS]	1024	1213	0.3	0.2	1.5	1.3	4.5	1.7	0.3	tr
limonene[MS]	1024	1202	29.7	23.9	5.0	4.9	14.1	18.3	38.4	38.0
γ-terpinene	1051	1246	0.2	0.1	2.3	2.2	2.1	1.7	0.2	
trans-sabinene hydrate*	1057	1460	tr	0.1	0.2	tr	tr	tr	0.1	tr
terpinolene[MS]	1080	1280	0.6	0.5	0.6	0.6	1.1	0.6	0.6	0.6
linalool[MS]	1089	1545	2.1	3.9	2.9	2.0	0.5	0.6	0.7	0.6
terpinen-4-ol[MS]	1166	1593	0.3	0.7	13.2	8.8	2.9	2.6	0.2	tr
α-terpineol[MS]	1177	1692	0.5	0.8	1.7	0.9	0.7	0.7	0.6	0.7
myrtenol	1185	1781	tr	0.2	0.1	0.3	0.1	0.2		
dihydrocarveol*	1188	1620	tr	0.3	tr	0.3	tr	0.1		
eugenol[MS]	1336	2162	0.2	0.5	0.8	12.1	tr	0.8	0.1	2.0
α-copaene[MS]	1375	1484	1.6	0.7	1.1	0.3	0.5	0.5	0.1	
α-cubebene[MS]	1377									
β-elemene*[MS]	1386		0.3	0.1	0.6	tr	0.1	0.1		
(*E*)-β-caryo-phyllene[MS]	1419	1587	14.0	10.3	13.1	2.7	2.1	1.4	4.1	2.1
α-quaiene*[MS]	1434				0.5	tr	tr	tr		
(*E*)-β-farnesene*[MS]	1453	1662	0.7	0.7	1.5	0.3	0.2	0.1	0.3	0.2
α-humulene[MS]	1455	1660								
germacrene D*[MS]	1478	1690	tr	tr	0.7	tr	tr	0.3		
β-selinene*[MS]	1484	1710	0.3	0.3	0.1	0.5	tr	tr		
zingiberene*[MS]	1488				1.3	tr	0.3	tr		
α-selinene*[MS]	1493	1706	0.3	0.3	1.1	tr	0.2	0.2	0.1	0.2
α-farnesene*[MS]	1503	1745	0.2	0.1	3.1	0.6	0.4	tr	tr	
β-bisabolene*[MS]	1512	1737	0.2	0.3	0.1	tr	tr	tr		
δ-cadinene*[MS]	1519	1749	0.6	0.6	1.3	tr	0.2	0.2	0.1	
hedycaryol*[MS]	1541	2077	0.4	0.5	6.7	9.1	0.7	1.2		
(*E*)-nerolidol*[MS]	1552	2033			2.4	1.8	0.3	0.2		
caryophyllenol*	1561				tr	1.1	tr	0.1	0.2	
caryophyllene oxide[MS]	1575	1967	0.8	3.4	5.5	7.2	0.1	0.6	1.3	1.1
ledol*	1600	2100	tr	0.8	0.4	0.6	tr	tr		
β-eudesmol*[MS]	1620		1.1	2.9	0.4	0.9	1.9	tr		
δ-cadinol*[MS]	1627	2184	tr	0.5	tr	1.8	tr	tr	0.1	0.3
T-cadinol*	1629	2156			2.6	4.7	1.1	3.0	0.2	
T-muurolol*	1635	2170	0.1	0.2	1.0	3.1	tr	0.5	0.7	0.9
α-cadinol[MS]	1644	2218	tr	0.3	0.7	1.3	tr	1.4		
α-farnesol	1661	2255	tr	0.3	0.2	tr	tr	0.2	0.2	
α-farnesol	1667	2347	tr	0.4	tr	2.4	-	-	0.2	0.4
α-bisabolol[MS]	1673	2205	0.2	tr	1.3	2.3	0.1	0.1		
total (%)			98.8	91.0	91.4	90.6	96.6	95.1	99.8	99.3
monoterpenes			74.9	61.8	26.8	25.5	84.2	80.0	90.5	90.8
oxygenated monoterpenes			3.1	6.5	18.9	24.4	4.2	5.0	1.7	3.3
sesquiterpenes			18.2	13.4	24.5	4.4	4.0	2.8	4.7	2.5
oxygenated sesquiterpenes			2.6	9.3	21.2	36.3	4.2	7.3	2.9	2.7
total amount of oil (mg/g)			0.6	0.5	0.8	2.1	12.1	24.3	2.1	1.7

[a] tr, trace (<0.5%). *, tentatively identified. MS, identification by GC/MS. [b] Air-dried samples. [c] Sublimation-dried samples.

pepper corn and ground black pepper (<0.2 mm), Malaysian origin; air-dried green pepper corn and ground green pepper (<0.2 mm), Indian origin; sublimation-dried green pepper corn, Indian origin; and air-dried white pepper corn and ground green pepper (<0.2 mm), Indonesian origin. The samples were stored in glass vessels (from Paulig Baltic LC) at room temperature in a dark place for 1 year.

Isolation of the Essential Oil. Essential oil was isolated from black pepper fruits samples (10 g) by simultaneous distillation and extraction (SDE) with *n*-hexane (Fluka, >99.0%) as the solvent (0.5 mL) by using a Marcusson type microapparatus (*20*). The SDE process was carried out for 2 h. The oil amount (mg/g) was determined using *n*-tetradecane (Reachim, >99.9%) as internal standard (2 μL). The reproducibility of three parallel SDE procedures with a single pepper sample showed the variation coefficients below 20%.

Gas Chromatography (GC)/Flame Ionization Detection (FID) Analysis. The SDE extracts were analyzed using a Chrom-5 chromatograph with FID on two fused silica capillary columns (50 m × 0.20 mm) with two stationary phases: nonpolar poly(dimethylsiloxane) (NB-30, Nordion, Finland) and polar poly(ethylene glycol) (SW-10, Supelco, Switzerland). The film thickness of both stationary phases was 0.25 μm. The carrier gas was helium with the split ratio of 1:150, and a flow rate of 17−25 cm/s was applied. The temperature program

Table 2. Composition of the Essential Oils from Ground Pepper Samples[a]

	RI		concentration (%)					
			black pepper		green pepper		white pepper	
compound	NB-30	SW-10	2001	2002	2001	2002	2001	2002
α-thujene[MS]	923	1029	0.3	0.1	0.7	2.3		
α-pinene[MS]	932	1029	2.6	0.8	1.2	3.8	0.3	0.9
camphene	945	1074	0.1	tr	tr	0.1		
sabinene[MS]	969	1125	3.8	0.8	4.5	12.2		
β-pinene[MS]	973	1116	5.9	1.5	1.8	4.7	0.7	1.7
myrcene[MS]	984	1161	1.8	0.4	0.4	1.0	0.4	1.1
α-phellandrene*[MS]	999	1165	2.8	0.5	0.6	1.6	0.4	1.1
Δ-3-carene[MS]	1009	1149	13.8	3.0	2.1	6.4	1.7	4.3
α-terpinene	1012	1180	0.1	0.1	0.2	0.3	0.1	tr
p-cymene	1015	1273	0.5	0.2	0.1	tr	0.3	0.9
β-phellandrene*	1024	1213	0.1	tr	1.0	2.2	tr	0.1
limonene[MS]	1026	1204	14.3	3.0	3.0	7.7	2.9	6.9
γ-terpinene[MS]	1051	1246	0.2	0.1	0.4	1.2		
trans-sabinene hydrate*[MS]	1058	1460	0.1	tr	0.1	tr		
terpinolene[MS]	1080	1282	0.8	0.1	0.2	0.5	0.2	0.3
linalool[MS]	1089	1547	0.5	0.6	0.8	0.4	0.7	0.5
terpinen-4-ol[MS]	1166	1592	0.2	0.3	1.0	1.6		
myrtenal	1173	1630	tr	tr	tr	tr	0.2	tr
α-terpineol[MS]	1176	1692	0.1	0.1	0.2	0.3		
myrtenol	1185	1784	tr	tr			0.1	tr
eugenol[MS]	1336	2162	1.7	2.8	1.1	18.6	2.5	41.0
α-cubebene	1346	1453	0.2	0.2			0.1	tr
α-copaene[MS]	1375	1485	3.1	4.6	1.9	0.2	1.5	tr
β-cubebene*[MS]	1377							
β-elemene*[MS]	1388		0.8	1.4	1.1	0.1	0.2	tr
(*E*)-β-caryophyllene[MS]	1418	1589	38.1	63.0	46.3	4.4	70.4	3.5
α-quaiene*[MS]	1434		0.1	0.1	1.4	tr	0.2	tr
(*E*)-β-farnesene*[MS]	1450	1661	2.1	3.1	2.7	0.4	4.2	0.2
α-humulene[MS]	1452	1658						
germacrene D*[MS]	1478	1690	0.2	0.2	1.0	tr	0.3	tr
γ-muurolene*[MS]	1482	1688	0.8	1.4			0.1	tr
β-selinene*[MS]	1484	1709	0.1	tr	tr	0.4	0.4	tr
zingiberene*[MS]	1488				3.4	0.2		
α-selinene*[MS]	1492	1705	0.8	1.1	4.1	0.3	tr	1.0
α-farnesene*[MS]	1503		0.2	0.2	3.9	0.5	0.4	tr
β-bisabolene*[MS]	1512	1736	0.2	0.4	tr	0.4	0.1	tr
δ-cadinene*[MS]	1517	1748	1.1	1.6	1.8	0.2	1.1	0.5
hedycaryol*[MS]	1534	2079	0.1	0.1	1.3	2.2		1.3
(*E*)-nerolidol*[MS]	1550	2035	0.1	0.1	1.8	1.4	0.2	1.4
caryophyllenol*	1562		tr	0.5	tr	1.1	tr	0.7
caryophyllene oxide[MS]	1575	1972	0.4	3.7	0.7	2.6	2.8	3.1
ledol*	1600	2100	tr	0.1	0.1	0.5	tr	0.7
β-eudesmol*[MS]	1619		0.6	2.7	tr	3.6	3.5	9.7
δ-cadinol*[MS]	1626	2186	tr	0.2	tr	1.5	0.7	
T-cadinol*	1629	2156			1.1	4.6	tr	1.5
T-muurolol*	1636	2172	0.2	0.5	0.1	2.6	tr	3.8
α-cadinol*[MS]	1643	2218	tr	tr	0.1	0.4	0.1	1.7
α-farnesol	1660	2254	tr	tr	0.1	0.3	0.1	0.6
α-farnesol	1667	2348	tr	tr	tr	1.1	0.1	1.7
α-bisabolol[MS]	1675	2206	tr	tr	0.8	2.7	tr	0.8
(*E*,*E*)-farnesol*	1703						0.2	0.4
total (%)			98.9	99.6	93.1	96.6	97.2	91.4
monoterpenes			47.1	10.6	16.2	44.0	7.0	17.3
oxygenated monoterpenes			2.6	3.8	3.2	20.9	3.5	41.5
sesquiterpenes			47.8	77.3	67.6	7.1	79.0	5.2
oxygenated sesquiterpenes			1.4	7.9	6.1	24.6	7.7	27.4
total amount of oil (mg/g)			21.8	10.6	7.6	4.2	5.4	1.6

[a] tr, trace (<0.05%). *, tentatively identified. MS, identification by GC/MS.

was from 50 to 250 (NB-30) and 70 to 230 °C (SW-10) at 2 °C/min, and the injector temperature was 200°C. A Hewlett-Packard model 3390A integrator was used for data processing.

The identification of the oil components was accomplished by comparing their retention indices (RI) on two columns with the RI values of reference standards, our RI data bank, and literature data (*21*−*24*). The results obtained were confirmed by GC/mass spectrometry (MS). The amount of the oil (mg/g) isolated by SDE from pepper samples was determined using an internal standard method.

The percentage composition of the oils was calculated in peak areas using a normalization method without using correction factors. The relative standard deviation of percentages of oil components of three repeated GC analyses of a single oil sample did not exceed 5%.

GC/MS Analysis. The mass spectrometric analysis was carried out on a Hitachi M-80 B gas chromatograph double focusing mass spectrometer using a AT-5 (5% phenyl, 95% dimethyl polysiloxane, 30 m × 0.32 mm) fused silica capillary column from Alltech. The column temperature was 5 min at 60 °C and then 60−280 °C at 2 °C/min.

RESULTS

Fifty-one compounds were identified and quantitatively evaluated in the SDE extracts from *P. nigrum* L. fruits of different ripening states, representing more than 90% of the total oil **(Tables 1** and **2)**. Mono- and sesquiterpene hydrocarbons

(51−95%) predominated in all oil samples analyzed, and oxygenated terpenoids were found to form from 4 to 40%. Quantitatively the most important compounds in pepper oils before storage were (*E*)-β-caryophyllene (2.1−70.4%), limonene (2.9−38.4%), Δ-3-carene (1.7−19.0%), β-pinene (0.7−24.2%), and α-pinene (0.3−9.9%). The main oxygenated terpenoids in oils were caryophyllene oxide (0.1−5.5%), hedycaryol (0.1−6.7%), β-eudesmol (0.4−3.5%), and eugenol (0.1−2.8%). The same main components in *P. nigrum* L. oils have been reported previously; however, hedycaryol was not mentioned in the literature (*1*−*18*).

Essential Oil from Pepper Corn. The essential oil amounts isolated by SDE from air-dried black, green, and white pepper corn were very small (0.6−2.1 mg/g) but much higher from green pepper corn obtained by sublimation drying (12.1 mg/g). The quantitative composition of the oils from green pepper corn of different drying methods was also quite different (**Table 1**). The oil from air-dried pepper corn contained all component groups of terpenoids in similar amounts (18.9−26.8%), but the oil of sublimation dried corn was rich in monoterpenes (84.2%). These results show the advantages of sublimation drying over the air-drying method.

The comparison of the oil composition of three air-dried pepper corn samples of different ripening states showed that white pepper oil contained more monoterpenes (90.5%) than black pepper oil (74.9%) and green pepper oil (26.8%). The content of sesquiterpenes was the highest in green pepper oil (24.5%), smaller in black pepper oil (18.2%), and the smallest in white pepper oil (4.7%). Green pepper oil was rich in oxygenated terpenoids (40.1%) as compared to black and white pepper oils (5.7 and 4.6%, respectively).

The amounts of the oil isolated by SDE from pepper corn samples decreased only a little after 1 year of storage of black pepper (from 0.6 to 0.5 mg/g) and white pepper (from 2.1 to 1.7 mg/g). The oil amount rose after 1 year of storage of green pepper (from 0.8 to 2.1 mg/g). This fact may be caused by postmaturation of green pepper.

The results of GC analysis showed small changes in the composition of pepper corn oils after 1 year of storage of air-dried white and black pepper corn samples and sublimation-dried green pepper sample. The relative amount of mono- and sesquiterpenes decreased, and the share of oxygenated terpenoids increased due to evaporation of more volatile compounds and due to the oxidation process. These effects in the oil composition after storage were greater in air-dried green pepper corn, where the content of sesquiterpenes decreased after storage from 24.5 to 4.4% (among them the content of β-caryophyllene from 13.1 to 2.7%). The content of oxygenated terpenoids in air-dried green pepper corn increased after storage from 40.1 to 60.7% (among them the content of eugenol from 0.8 to 12.1%). The amount of monoterpenes in the oils after storage of air-dried and especially of sublimation-dried green pepper corn increased.

Essential Oil from Ground Pepper. The oil amount isolated from ground pepper samples was the highest from black pepper (21.8 mg/g), lower from green pepper (7.6 mg/g), and the lowest from white pepper (5.4 mg/g) (**Table 2**). The oil from ground black pepper contained more monoterpenes (47.1%) than the oils from ground green (16.2%) and white pepper (7.0%). The content of sesquiterpenes was highest in the oil from ground white pepper (79.0%), among them β-caryophyllene 70.4%. The oil from ground green pepper contained 67.6% sesquiterpenes (46.3% β-caryophyllene), and ground black pepper contained 47.8% sesquiterpenes (38.1% β-caryophyllene). The content of oxygenated monoterpenes was similar (2.6−3.5%) in the oils of three pepper samples of different ripening states. The oil from black pepper contained less oxygenated sesquiterpenes (1.4%) than the oils from green and white pepper (6.1 and 7.7%, respectively).

The amount of the oils from ground pepper samples after 1 year of storage decreased 3.4-fold for white pepper and about twice for black and green pepper. This greater loss of the oil of white pepper after storage may be caused by the lack of skin of white pepper.

After storage of powdered black pepper, the share of monoterpenes in the oil fell from 47.1 to 10.6%, the content of sesquiterpenes increased to 77.3% (β-caryophyllene 63.0%), and the share of oxygenated sesquiterpenes rose from 1.4 to 7.9%. The comparison of total amount of the component groups in the oils showed that only the amount of oxygenated sesquiterpenes increased after storage of black pepper.

After storage of powdered green pepper, the share of monoterpenes in the oil rose from 16.2 to 44.0%, and different from other pepper samples, the amount of monoterpenes increased obviously by postmaturation. A significant loss of content of sesquiterpenes (from 67.6 to 7.1%) and an increase of oxygenated terpenoids (from 9.3 to 45.5%, among them eugenol from 1.1 to 18.6%) was seen.

The significant changes were also found in the oil composition after storage of ground white pepper. The content of sesquiterpenes in the oil decreased after storage from 79.2 to 5.2%, among them the content of β-caryophyllene fell from 70.4 to 3.5%. The share and also the amount of oxygenated terpenoids increased. The highest changes were seen for eugenol (from 2.5 to 41.0%) and β-eudesmol (from 3.5 to 9.7%).

Some unidentified high-boiling compounds were found in the oil after 1 year of storage of ground white pepper, which was not noticed in the other pepper sample. These changes showed that evaporation of more volatile compounds, oxidation, and other processes of chemical changes obviously could take place during storage of ground pepper.

In conclusion, it may be marked that the composition of essential oil of black, green, and white pepper after 1 year storage of ground pepper significantly changed.

ACKNOWLEDGMENT

We are grateful to Paulig Baltic LC for obtaining the pepper samples.

LITERATURE CITED

(1) Lawrence, B. M. Major tropical spices—Pepper (*Piper nigrum* L). *Essential Oils* (*1979*−*1980*); Allured Publ. Corp.: Wheaton, MD, 1981; pp 140−228.

(2) Richard, H. M. J. Spices and condiments I. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Marcel Dekker, Inc.: New York, Basel, Hong Kong, 1991; pp 411−448.

(3) Debrauwere, J.; Verzele, M. New constituents of the oxygenated fraction of pepper essential oil. *J. Sci. Food Agric.* **1975**, *26*, 1887−1894.

(4) Debrauwere, J.; Verzele, M. Constituents of peppers: IV. The hydrocarbons of pepper essential oil. *J. Chromatogr. Sci.* **1976**, *14*, 296−298.

(5) Buckle, K. A.; Rathnawathie, M.; Brophy, J. J. Compositional differences of black, green and white pepper (*Piper nigrum* L.) oil from three cultivars. *J. Food Technol.* **1985**, *20*, 599−613.

(6) Gopalakrishnan, M.; Nirmala Menon, A.; Padmakumari, K. P.; Jayalekshmy, A.; Narayanan, C. S. GC analysis and odor profiles of four new Indian genotypes of *Piper nigrum* L. *J. Essent. Oil Res.* **1993**, *5*, 247−253.

(7) McCarron, M.; Mills, A.; Whittaker, D. Comparison between green and black pepper oils from *Piper nigrum* L. berries of Indian and Sri Lankan origin. *Flavour Fragrance J.* **1995**, *10*, 47−50.
(8) Martins, A. P.; Salgueiro, L.; Vila, R.; Tomi, F.; Canigueral, S.; Casanova, J.; Proenca, A.; Cunha, Da.; Adzet, T. Essential oils from four piper species. *Phytochemistry* **1998**, *49*, 2019−2023.
(9) Murthy, C. T.; Rani, M.; Srinivasa Rao, P. N. Optimal grinding characteristics of black pepper for essential oil yield. *J. Food Process Eng.* **1999**, *22*, 161−173.
(10) Nussbaumer, C.; Cadalbert, R.; Kraft, P. Identification of m-mentha-3(8),6-diene (Isosylveterpinolene) in black pepper oil. *Helv. Chim. Acta* **1999**, *82*, 53−58.
(11) Sumathykutty, M. A.; Madhusudana Rao, J.; Padmakumari, K. P.; Narayanan, C. S. Essential oil constituents of some *Piper* species. *Flavour Fragrance J.* **1999**, *14*, 279−282.
(12) Jagella, T.; Grosch, W. Flavour and off-flavour compounds of black and white pepper (*Piper nigrum* L.) I. Evaluation of potent odorants of black pepper by dilution and concentration techniques. *Eur. Food Res. Technol.* **1999**, *209*, 16−21.
(13) Jagella, T.; Grosch, W. Flavour and off-flavour compounds of black and white pepper (*Piper nigrum* L.) II. Odor activity values of desirable and undesirable odorants of black pepper. *Eur. Food Res. Technol.* **1999**, *209*, 22−26.
(14) Jagella, T.; Grosch, W. Flavour and off-flavour compounds of black and white pepper (*Piper nigrum* L.) III. Desirable and undesirable odorants of white pepper. *Eur. Food Res. Technol.* **1999**, *209*, 27−31.
(15) Ferreira, S. R. S.; Nikolov, Z. L.; Doraiswamy, L. K.; Maireles, M. A. A.; Petenate, A. J. Supercritical fluid extraction of black pepper (*Piper nigrum* L.) essential oil. *J. Supercrit. Fluids* **1999**, *14*, 235−245.
(16) Nirmala Menon, A.; Padmakumari, K. P.; Jayalekshmy, A.; Gapalakrishnan, M.; Narayanan, C. S. Essential oil composition of four popular Indian cultivars of black pepper (*Piper nigrum* L.). *J. Essent. Oil Res.* **2000**, *12*, 431−434.
(17) Tewtrakul, S.; Hase, K.; Kadota, S.; Namba, T.; Kamatsu, K. Fruit oil composition of *Piper chaba* Hunt., *P.longum* L. and *P.nigrum* L. *J. Essent. Oil Res.* **2000**, *12*, 602−608.
(18) Jirovetz, L.; Buchbauer, G.; Ngassoum, M. B.; Geissler, M. Aroma compounds analysis of *Piper nigrum* and *Piper guineense* essential oils from Cameron using solid-phase microextraction−gas chromatography, solid-phase microextraction−gas chromatography−mass spectrometry and olfactometry. *J. Chromatogr.* **2002**, *976*, 265−275.
(19) Nirmala Menon, A.; Chacko, S.; Narayanan, C. S. Free and glycosidically bound volatiles of pepper (*Piper nigrum* L.). *J. Essent. Oil Res.* **2001**, *13*, 166−169.
(20) Bicchi, C.; D'Amato, A.; Nano, M.; Frattini, C. Improved method for the analysis of small amounts of essential oils by microdistillation followed by capillary gas chromatography. *J. Chromatogr.* **1983**, *279*, 409−416.
(21) Davies, N. W. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *J. Chromatogr.* **1990**, *503*, 1−25.
(22) Zenkevich, I. G. Analytical parameters of essential oils for their GC and GC−MS identification. Mono- and sesquiterpenes. *Rastit. Resur.* **1996**, *32*, 48−58.
(23) Zenkevich, I. G. Analytical parameters of essential oils for their GC and GC−MS identification. Oxygencontaining derivatives of monoterpene and sesquiterpene hydrocarbons. *Rastit. Resur.* **1997**, *33*, 16−28.
(24) Zenkevich, I. G. Analytical parameters of essential oils for their GC and GC−MS identification. Acetates of terpenic alcohols. *Rastit. Resur.* **1999**, *35*, 30−37.

Received for review September 8, 2003. Revised manuscript received January 31, 2004. Accepted February 1, 2004. This work was supported by the Estonian Scientific Foundation Grant No. 4028.

JF030635S