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EFFECTS OF STORAGE ON THE VOLATILE COMPOSITION OF NUTMEG

K. JEAN SANFORD and D. E. HEINZ*

Department of Consumer Sciences, University of California, Davis, California, U.S.A

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Abstract—The volatile constituents of nutmeg (*Myristica fragrans*) were isolated and identified using GLC techniques and I r. and u.v. spectral data In addition to the previously reported volatile constituents, α-thujene, Δ^3-carene, 1(7),2-*p*-menthadiene, and *trans* and *cis*-sabinene hydrate were identified Prolonged storage of nutmeg resulted in changes in the volatile composition as determined by gas chromatography. The variation in composition of these nutmeg constituents appears to be mainly a function of their volatilization. The percentage of free myristic acid may serve as an indication of the age of ground nutmeg.

INTRODUCTION

PREVIOUS investigators[1–4] have reported the volatile chemical constituents of nutmeg, the dried ripe seed of *Myristica fragrans* Houttuyn (Myristicaceae), to be mainly monoterpene hydrocarbons and alcohols, and substituted benzene derivatives. However, the composition of nutmeg as determined by a number of authors varies, and Shulgin *et al.*[3] have reported variations in the composition of nutmeg from different geographical regions. In addition, although unpublished results of a questionnaire survey[5] indicated that most consumers purchased ground nutmeg and stored it near heat for at least 12 months, no studies have been published concerning changes occurring in this spice during storage. The present study was done to characterize further the volatile substances of nutmeg and to determine to what extent prolonged storage affects these constituents.

RESULTS

A typical gas chromatogram of the volatile components of nutmeg, isolated from a single seed, is shown in Fig. 1. The previously reported volatile constituents as determined by retention or spectral data are given in Table 1. In addition to these compounds, α-thujene, Δ^3-carene, β-phellandrene, and *trans* and *cis*-sabinene hydrate were identified by their spectral properties (Table 1).

To determine to what extent individual nutmegs vary in the amounts of their volatile constituents, individual seeds were extracted and analysed separately. Considerable variability in volatile compositions was observed (Table 2). For example, peak 33 (methyleugenol) constituted only 0·3% of the volatiles of nutmeg A, but this peak constituted 17·9% of the volatiles of nutmeg C. Peak 38 (myristicin) varied from 0·2% in nutmeg A to

* Present address: Chemistry Department, De Anza College, Cupertino, California, U S A.

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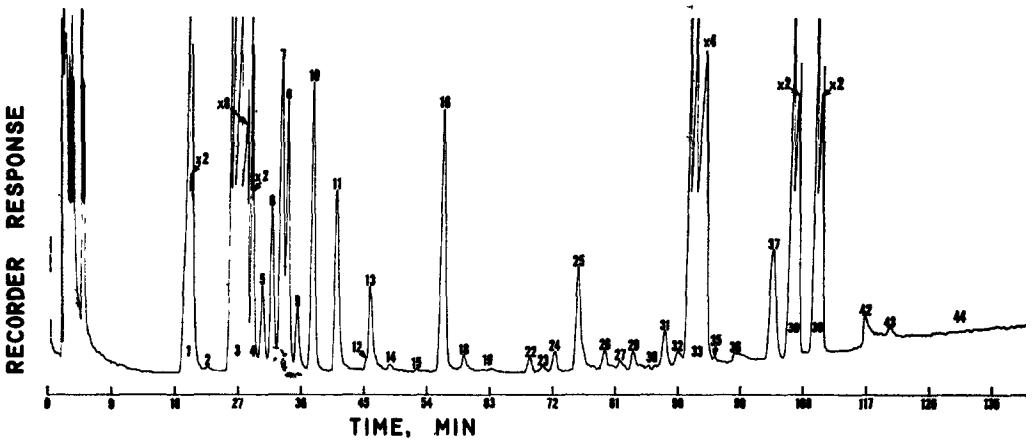

FIG. 1. A GAS CHROMATOGRAM OF A PENTANE–ETHER NUTMEG EXTRACT (200 μl) THE CHROMATOGRAM WAS OBTAINED USING A 762 × 0·64 cm STAINLESS STEEL COLUMN CONTAINING 1% OV-17 LIQUID PHASE ON CHROMOSORB G SOLID SUPPORT

TABLE 1. VOLATILE CONSTITUENTS OF NUTMEG

Peak No. in Fig. 1	Name of compound	Evidence for	Others reporting, Refs.
1a	α-Pinene	i r	1, 2, 3, 4
1b	α-Thujene	i r.	None
2	Camphene	r t.	1, 3, 4
3a	Sabinene	i r.	3
3b	β-Pinene	i r	1, 2, 3, 4
4	Myrcene	i r	4
5a	Δ^3-Carene	i r.	None
5b	1,5-*p*-Menthadiene (α-Phellandrene)	r t	4
6	1,3-*p*-Menthadiene (α-Terpinene)	i r., u v	4
7	1,8-*p*-Menthadiene (Limonene)	i r.	1, 2, 3
8	1(7),2-*p*-Menthadiene (β-Phellandrene)	i r, u v.	None
9	*p*-Cymene	i r	3, 4
10	1,4-*p*-Menthadiene	i r	3
11a	1,4(8)-*p*-Menthadiene (Terpinolene)	r t	3, 4
11b	*trans*-Sabinene hydrate	i r, r t	None
12	Linalool	r t	1, 2, 3, 4
13	*cis*-Sabinene hydrate	i r, r t	None
15	8-*p*-Menthen-1-ol (β-Terpineol)	r t	4
16a	1-*p*-Menthen-4-ol (1-Terpineol-4)	i r	1, 3, 4
16b	Borneol	r t	1
18	1-*p*-Menthen-8-ol (α-Terpineol)	i r.	1, 3, 4
19a	Citronellol	r t	4
19b	Linalyl acetate	r t	2
22a	Bornyl acetate	r t	2
22b	Geraniol	r t	1, 4
24	Geranyl acetate	r t	3
25	Safrole	i r	1, 2, 3, 4
26	β-Caryophyllene	r t	4
30	Eugenol	i r	1, 3
33	Methyleugenol	i r, u.v.	3, 4
36	*trans*-Isoeugenol	i r	1, 3
37	*trans*-Methylisoeugenol	i r, u v	3
38	Myristicin	i r, u v	1, 2, 3, 4
39	Elemicin	i r, u v	3
42	Methoxyeugenol	i r, u v.	3
43	*trans*-Isoelemicin	i r, u v	3, 4
44	Myristic acid	i r	1, 3

i r. = Infrared spectra; r t = retention data (enrichment technique), u v = ultraviolet spectra.

TABLE 2. VARIABILITY AMONG VOLATILE COMPOSITIONS OF SIX WHOLE NUTMEGS. PEAK HEIGHTS ARE EXPRESSED AS PERCENTAGES OF TOTAL HEIGHT FOR EACH CHROMATOGRAM

	%*					
Peak No. in Fig. 1	A†	B	C	D	E	F
1	11·1	18 3	5·5	14 2	12 8	8·8
2	0 2	0·4	0 1	0 3	0 3	0 2
3	25 2	20 6	27 1	28 7	28·5	29 6
4	4 9	4 0	2 6	5 1	5 4	6 9
5	1 2	1·3	1 3	1 2	2 4	2 9
6	3 3	3 2	2 4	2 9	2 7	2·5
7	4 8	4 9	4 5	5 8	5 3	5·3
8	4 0	2 7	3 9	3 1	4 1	4 5
9	2 8	1 5	0 9	2 4	1 6	1 3
10	5 4	4 9	4 2	4 5	4 4	4 4
11	3 0	2 3	2 6	2 2	2 6	3 0
12	0 2	0 3	0 2	0 3	0 4	0 3
13	1 5	0 8	1 2	0 9	0·9	1·1
14	0 2	0 1	0 1	—	—	0 2
15	0 1	—	—	—	—	0·1
16	6 4	2·7	3 8	3 4	3 8	3·5
17	—	0 1	—	—	0·1	—
18	0 4	0 3	0 3	0 3	0 3	0·5
19	—	—	—	—	—	—
20	—	—	0 1	—	—	—
21	—	—	—	—	—	—
22	0 4	0 4	0·2	0 2	0 5	0 3
23	0 1	0 1	0·1	0 1	0·1	0·1
24	1·4	1 3	0·3	0 1	1·8	0 5
25	0·3	4 6	1·5	3 1	1·7	4 2
26	0 2	0 4	0 3	0 4	0 2	0 1
27	0 1	0 2	0 1	0 4	0 2	1 0
28	0 1	—	—	—	—	—
29	0 4	0·6	0 3	0 1	0 4	—
30	—	0 2	—	—	—	0·2
31	0·6	0 4	0 6	—	0 9	0·7
32	0·3	0 5	0 2	0 3	0 5	0 2
33	0 3	0 5	17 9	0 5	0 3	0·7
34	0 5	0 6	—	—	0 7	0·3
35	0 1	0 2	0 1	0 1	0 1	0·1
36	0·1	1 1	0·1	1 0	—	0 1
37	0 3	0 2	1·6	0 2	0 1	0 1
38	0 2	14 6	7·6	14·5	14 0	10 5
39	5 4	1 3	7·5	0 9	1 0	5 4
40	0·2	—	—	—	—	0 2
41	0 2	0 1	—	—	—	0 1
42	2 9	3 2	0 5	2·7	2 1	0 2
43	0·2	0 2	0 3	—	—	—
44	10 9	1·2	—	—	—	—

* Dash indicates a peak height value of 0 00–0·04%. Peak heights were used without correction factors to estimate the composition.

† Nutmeg A was smooth and light brown with no dark flecks. Nutmeg B was small, wrinkled, and light brown. Nutmeg C was medium sized, wrinkled, and medium brown with dark brown flecks. Nutmeg D was small and wrinkled, with dark brown ridges. Nutmeg E was large, smooth, and light brown. Nutmeg F was long, medium smooth, and light brown with slight dark brown flecks.

14·6% in nutmeg B. Peak 44 (myristic acid) was 10·9% in nutmeg A, 1·2% in nutmeg B, but missing in the other nutmegs tested. In duplicate analyses illustrating the reproducibility of the extraction, chromatographic, and calculation procedures, peak height percentages in 75% of the cases varied only from 0·0 to 0·2%. In the remaining cases, the largest deviation was 1·4%. Thus the extreme variability among individual nutmegs could not be attributed to lack of reproducibility in procedures.

There appeared to be no correlation between the external appearance of a particular nutmeg and its volatile composition. For example, although nutmegs A and E had similar external characteristics (Table 2), their volatile compositions were strikingly different. In contrast, nutmegs D and E had similar volatile compositions but very different external morphology. There are numberous possible reasons for variability among whole nutmegs, such as differences in cultivation practices, maturity at harvest, pre-shipping storage conditions, and genetic mutations. There is a need for further work in this area.

All six nutmegs used in studying variability were taken from one canister of Indonesian nutmegs supplied by Spice Islands of Leslie Foods, Inc. Thus the variability observed was even more striking when compared to that reported by Shulgin *et al.*[3] in their study of geographical variations. Variability of selected volatiles in individual whole nutmegs from one source was about three to five times greater than variability reported to occur in nutmeg samples from different geographical regions.[3]

Because of variability among individual nutmegs, studies concerning changes in the volatile constituents during storage could not be accomplished using extracts of individual nutmegs. Therefore, all storage studies were done with samples of 16 or more nutmegs, ground in a mortar, and mixed before storing.

Freshly ground nutmeg stored in open containers up to 48 hr at 40° changed in its composition of volatile constituents (Table 3). The more volatile fractions, peaks 1–10, decreased in percentages, while those of the higher boiling compounds increased. Peak 44 (myristic acid) increased from 1·9 to 22·8% of the total height of peaks 1–44. Peak 33 (methyleugenol) increased from 6·9 to 15·0%, peak 38 (myristicin) from 12·1 to 27·1%, and peak 39 (elemicin) from 7·2 to 15 5% of the total height of peaks 1–43. During these same 48 hr, peak 1 (α-pinene and α-thujene) decreased from 11·2 to 3·5%, peak 3 (β-pinene and sabinene) from 28·3 to 11·0%, and peak 7 (1,8-*p*-menthediene) from 5 0 to 1·1% of the total height of peaks 1–43.

When the whole nutmegs were ground in a mortar and stored at 37°–40° for 11 months in closed containers, similar results were observed. By 4 months, peak 44 (myristic acid) had become the largest peak (41·0% of peaks 1–44); and peak 38 (myristicin) was larger than peak 3 (sabinene and β-pinene), a reversal from the proportions in the control sample (freshly ground nutmeg).

The commercially ground nutmeg stored in closed containers at 37°–40° for up to 1 yr, also changed in volatile composition. The fact that myristic acid was already at 27·4% in the commercially ground sample when purchased indicated that the nutmeg had aged between the times of grinding and purchase. In both freshly ground and commercially ground samples, myristic acid had achieved similar percentages (47·2 and 49·7) after 8 months storage.

In general, all storage conditions resulted in losses of the more volatile fractions and consequent increases in the relative concentrations of the less volatile fractions. However, some other changes observed may have been the result of chemical degradation. The formation of free myristic acid (peak 44) may have been due to hydrolysis of the esterified

TABLE 3. VOLATILE COMPONENTS OF WHOLE NUTMEGS GROUND IN A MORTAR AND STORED IN OPEN JARS AT 40°

Peak No. in Fig. 1	%*					
	0 Time	2 hr	4 hr	7 hr	24 hr	48 hr
1	11·2	8 4	7·4	5 8	3·1	3·5
2	0 1	0 2	0 1	0 1	—	—
3	28·3	25·6	24 5	23·1	11 6	11 0
4	4·3	3·7	3 2	3 0	0 6	0·4
5	1 6	1 5	1·4	1 3	0·4	0·3
6	1·8	2·1	2 1	2·1	1 3	1 2
7	5·0	4·9	4 7	4·5	1 8	1 1
8	4·3	4·1	4 1	3·8	1·4	0·9
9	1·2	1·3	1·3	1·3	0·5	0 3
10	3·2	3·8	4 0	3 8	2 6	2 1
11	2·0	2 4	2 5	2·6	1 9	1 2
12	0·3	0·3	0 2	0 4	0 4	0·3
13	0 7	0·7	0·6	1 0	1·0	0 8
14	0·1	0·1	0 1	0·2	0·1	0·1
15	—	0 1	—	—	—	—
16	2·7	3 5	3·7	3·7	4 8	3 6
17	—	—	—	—	—	0·1
18	0 2	0 2	0 2	0 3	0 5	0 4
19	—	—	—	—	0 1	0 1
20	—	—	—	—	0·1	—
22	0·3	0·2	0 2	0·2	0 4	0 3
23	0·2	0·1	0·1	0 1	0 1	0·1
24	0·6	0·6	0·6	0 7	1·0	0 8
25	2·1	2·6	2·8	2·8	3 9	3·3
26	0·3	0·4	0 4	0·4	0·6	0·7
27	0 3	0·3	0 4	0·5	0 6	0 9
29	0 1	0 2	0 1	0·2	0·2	0·3
31	0 1	0·1	0·2	0·3	0·4	0·4
32	0 1	0·2	0·2	—	0·5	—
33	6·9	7 8	8 1	8·8	14·1	15 0
34	0 3	0 3	0 3	0 4	0 7	0·7
35	—	—	0·1	0 1	0 2	—
36	0 2	0 3	0 6	0·7	0·7	1·1
37	1 3	1·5	1·5	1 6	2 9	2·9
38	12·1	13 2	13·6	14 7	23·9	27 1
39	7 2	8 2	8 5	9·4	15·6	15 5
41	—	—	—	1·5	0 1	0·3
42	0 4	0·9	1 7	0 3	1·5	2·1
43	0 2	0·2	0·3	0 5	0 6	0 7
44	1 9	4·6	7 6	8 7	16 0	22·8

* Values for peaks 1–43 are percentages of the total height of peaks 1–43. The value for peak 44 is the peak height per cent of the chromatogram total (1–44). The dash indicates a peak height value of 0·00–0·04%. Peak heights were used without correction factors to estimate the composition.

acid. Sammy and Nawar[4] reported myristic acid to be 90·4% of the total fatty acids of nutmeg, as determined by inter-esterification followed by gas chromatographic analysis.

Free myristic acid may be useful as an indicator of aging in this spice. Analysis of a sample of commercially ground nutmeg, obtained from a consumer who dated it as approximately 15 yr old, revealed only very small amounts of any lower boiling compounds, while myristic acid increased to 80% of the volatiles remaining.

EXPERIMENTAL

Samples were chromatographed on a Varian Aerograph Model 202 gas chromatograph equipped with a thermal conductivity detector and a linear temperature programmer A 762 × 0 64 cm O D stainless steel column having 1% OV-17 (w/w) as liquid phase and 70–80 mesh Chromosorb G (acid washed and DMCS treated) as solid support was used When necessary for identification purposes, further purification of samples collected from the OV-17 column was accomplished by rechromatographing each fraction on a different column [Carbowax 20M, SF96-50, or 1,2,3,-Tris (2-cyanoethoxy) propane]. All analytical runs were chromatographed under the following conditions· injector temperature, 235°; detector temperature, 250°; carrier gas, helium at a flow rate of 50 ml/min Operating procedure was as follows: isothermal at 50° for 6 min after injection of sample, then linearly programmed at 2°/min to 250°, then isothermal for the duration of the run I r spectra were obtained as films between NaCl plates; u v spectra were obtained in methanol.

Storage of Nutmeg Samples

Six whole nutmegs stored at room temp were separately analysed to determine variability among individual seeds

Sixteen whole nutmegs (Spice Islands of Leslie Foods, Inc.) were ground and mixed in a mortar Portions (3 g) were placed in each of 20 glass tubes having Teflon-lined screw caps, and then were stored in an incubator at 37°–40° Two tubes were transferred from the incubator to −20° storage at zero time, 8 days, and 1, 2, 4, 6, 8, and 11 months.

Commercially ground nutmeg was divided into 18 samples of 3 g each and stored at 37°–40° in glass tubes having Teflon-lined screw caps Two tubes were transferred from the incubator to −20° storage at 8 days and 1, 2, 4, 6, 8, and 12 months

Sixteen whole nutmegs were ground and mixed in a mortar. Fresh samples weighing 2 5 g each were placed in open 'Virtis' jars in the 37°–40° incubator. Samples were analysed at zero time and at 2, 4, 7, 24 and 48 hr

Extraction of Nutmeg Samples

A 2·50 g sample of ground nutmeg and 0 50 g of nuchar attaclay (a solid adsorbant) were macerated for 2 min in a Virtis blender with 10 ml pentane–ether (1:1, v/v) while in a dry ice–ethanol bath. The resultant slurry was centrifuged for 5 min A 200 μl portion of the clear supernatant liquid was used for both analytical and preparative gas chromatographic studies.

Calculations

Peak height percentages, as described by Essential Oil Association of U S A.,[6] were used to measure the relative changes in the volatile constituents. Each peak on the chromatogram was compared with the identical peak on chromatograms of different samples which had been run under the same chromatographic conditions However, peak heights were used to estimate the percentage composition only as no correction factors were used in the calculations.

Identification Procedures

The i r spectra obtained from the purified constituents were compared with known or reported spectra for authentication. The spectra reported by Mitzner *et al.*[7,8] were used for comparison for the monoterpene hydrocarbons and alcohols, with the exception of Δ^3-carene and *trans* and *cis*-sabiene hydrate. The spectra of *trans* and *cis*-sabinene hydrate were compared to authentic samples supplied by Russell and Jennings [9] Myristic acid and Δ^3-carene were substantiated by comparing their spectra with the spectra of samples purchased from Aldrich Chemical Company. Identification of the aromatic compounds was based upon comparison of their i.r spectra with those of authentic samples supplied from the work of Shulgin *et al.*[3]

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