Sterols in Pumpkin Seed Oil

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ABSTRACT AND SUMMARY

The sterol fraction of unsaponifiable matter obtained from a Yugoslav pumpkin seed ripening was investigated by gas liquid chromatography on a glass capillary column. It contained at least 14 different sterols ten of which were identified primarily by combined gas chromatography-mass spectrometry as cholesterol, brassicasterol, campesterol, stigmasterol, 24-methylcholest-7-en-3β-ol, $\Delta^{7,22,25}$-stigmastatrien-3β-ol, α-spinasterol, $\Delta^{7,25}$-stigmastadien-3β-ol, Δ^{7}-stigmastenol, and Δ^{7}-avenasterol. It was shown that the unidentified sterols in the oil obtained from a Chinese pumpkin seed were $\Delta^{7,25}$-stigmastadien-3β-ol and $\Delta^{7,22,25}$-stigmastatrien-3β-ol. There was practically no difference in the composition of Yugoslav and Chinese pumpkin seed oil, the main characteristic of which was the presence of Δ^{7}-sterols as was already stated by Sucrow.

INTRODUCTION

Edible oil of pumpkin seed has been used for a long period in Yugoslavia, Austria, Hungary, and the southern part of the USSR where it was highly appreciated and considered very good for health (1). However, little work has been conducted on the unsaponifiable material. Sucrow and Reimerdes (2) identified in the seed of the Cucurbita pepo, a European pumpkin species, α-spinasterol, $\Delta^{7,25}$-strigmastadien-3β-ol, and $\Delta^{7,22,25}$-stigmastatrien-3β-ol. Sucrow (3) found later, as an impurity in, $\Delta^{7,25}$-stigmastadien-3β-ol, a small quantity of a $\Delta^{7,24(28)}$ stigmastadien-3β-ol, (probably Δ^{7}-avenasterol). Jeong et al. (4) analyzed the sterol fraction of an oil obtained from a Chinese pumpkin seed and found ten different sterols. They identified, in trace amounts, cholesterol and brassicasterol and in larger quantities campesterol (6%), stigmasterol (2%), Δ^{7}-stigmastenol (28%), Δ^{7}-avenasterol (12%), 24-methylcholest-7-en-3β-ol (3%), and α-spinasterol (39%). They were not able to identify two sterols one of which was isolated together with Δ^{7}-stigmastenol (28%) and the other (10%) whose peak in the gas chromatogram was located between α-spinasterol and Δ^{7}-stigmastenol. There was no evidence of the 25-ene-sterols identified by Sucrow and Reimerdes (2) in the European species.

In the present study the sterol fraction of an oil obtained from Yugoslav pumpkin seed ripening without husk known as "golica" or "Cermakova beskorka" has been investigated. The capillary column gas chromatogram showed the presence of at least 14 different sterols, the structure of which had been determined by mass spectrometry (MS), nuclear magnetic resonance (NMR), and infrared (IR) analysis. Only four sterols present in traces were left unidentified. Also it was possible to show the structure of the unknown components which had been isolated by Jeong et al. (4).

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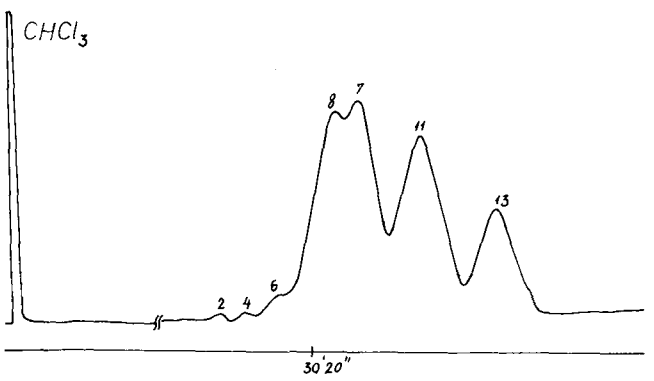

FIG. 1. Gas chromatogram of sterol fraction on 1.5 m OV-17 glass column.

EXPERIMENTAL PROCEDURES

Materials

Crude pumpkin seed produced in 1974 was obtained from the area surrounding Koprivnica (West Slavonia). The following sterols were used as reference samples: cholesterol (Fluka AG, Buch SG), a sterol fraction consisting of campesterol, stigmasterol, and β-sitosterol supplied by Riken Vitamin oil Co., Tokyo, Japan, and brassicasterol obtained from rapeseed oil and supplied from Prof. Matsumoto, College of Science and Technology, Nihon University, Japan. Solvents were refined in the usual way and finally distilled through a 600 mm x 8 mm column packed with "Heli-pak-packing."

Saponification

A mixture of 20 g oil in 200 ml 1.0 N alcoholic KOH was refluxed for 1.5 hr under nitrogen. The reaction mixture was diluted with 400 ml distilled water, and unsaponifiable material was extracted with three 400 ml portions of diethyl ether. The extracts were combined, washed in turn with 100 ml each 3% aqueous NaOH and water, and dried over anhydrous sodium sulfate. After evaporation of diethyl ether under nitrogen, 1.06% unsaponifiable matter was obtained.

Thin Layer Chromatography

Alkaline modification (0.2 N KOH) of Silica Gel G was used for separation of unsaponifiable matter. Development was performed with hexane-diethyl ether mixture (1:1).

TABLE I

Relative Retention Times (RRT) of Sterols of Pumpkin Seed

Components number[a]	RRT[b]	RRT[c] from authentic samples
2	0.81	0.81 Campesterol
4	0.88	0.88 Stigmasterol
6	0.94	0.95 24-Methylcholest-7-enol
8	1.04	1.03 α-Spinasterol
7	1.09	1.09 Unidentified
11	1.18	1.18 Δ^{7}-Stigmastenol
13	1.31	1.32 Δ^{7}-Avenasterol

[a]Numbering is done on the basis of the numbering In Figure 2 and Table II so that the identical components have the same number throughout this study.

[b]Relative retention times as compared with β-sitosterol (RRT = 1.00), whose retention time under experimental conditions was 30.33 min.

[c]Published retention times of certain sterols as compared with β-sitosterol, whose retention time under experimental conditions was 30 min (4,5).

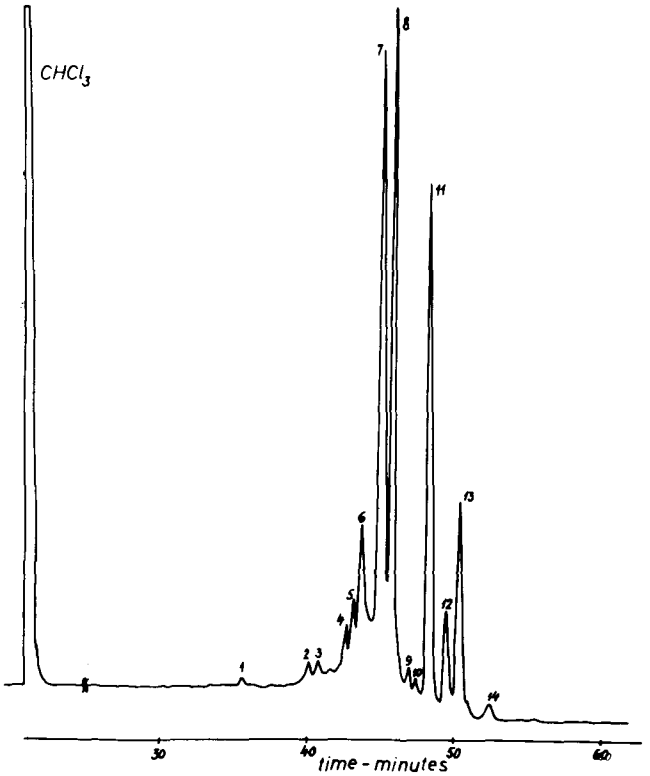

FIG. 2. Gas chromatogram of sterol fraction on 40 m SE 30 glass capillary column.

The plates were sprayed with 2,7-dichlorofluorescein, and the bands were observed under UV light. Five separate zones containing sterols, aliphatic alcohols, triterpene alcohols, and 4-methyl-sterols, tocopherols, and hydrocarbons with squalene, respectively, were cut off and extracted with diethyl ether. For two-dimensional thin layer chromatography plates spread with 250 μ layer of Silica Gel G impregnated with $AgNO_3$ were used. The plates were developed with a chloroform-acetone mixture (1:1), sprayed with 2,7-dichlorofluorescein, and the bands were observed under UV light.

Gas Liquid Chromatography

The sterol fraction was analyzed on the Varian 1400 gas chromatograph equipped with a flame ionization detector on a 1,5 m x 4 mm ID silanized glass column packed with 3% OV-17 and 1% OV-1 on Chromosorb W 80/150 mesh. Nitrogen was used as carrier gas. Glass capillary column gas liquid chromatography (GLC) was performed on a 2101 AC Carlo-Erba instrument with the glass capillary column of 40 m x 0.3 mm ID coated with SE 30, programmed at 2°/min from 250-300°, with He as carrier gas (1.5 ml/min) and with the flame ionization detector (H_2 10 ml/min, air 30 ml/min). The injection temperature was 280 C.

Combined Gas Chromatography — Mass Spectrometer — Computer

Analyses were performed on the LKB 2091 gas chromatograph-mass spectrometer combination (GC-MS) equipped with LKB 2130 computer system. The gas chromatograph was fitted with a 30 m x 4 mm ID glass capillary column coated with SE 30. Working conditions: temperature program 150-300°/2°/min, carrier gas He, ionization source 275 C, ionization voltage 70 eV, total ionic current 20 eV.

RESULTS AND DISCUSSION

The sample of the unrefined oil obtained from a Yugoslav pumpkin seed contained 0.3% sterols. The relative retention times (RRT) for the most abundant sterols in vegetable oils have been determined by GLC on an OV-17 column using β-sitosterol with a retention times of 30 min as a standard (4,5). For this work similar GLC conditions were chosen (RT of β-sitosterol 30.33 min) and the chromatogram obtained of the sterol fraction is shown in Figure 1. (Numbering of components in Figure 1 and Table I is done on the basis of the numbering of components in Figure 2 and Table II so that the identical components have the same number throughout this study.) The presence of at least seven different sterols is obvious, and in Table I their RRT values together with sterols of the same RRT values are presented (4,5).

Following the GLC analysis on a 1.5 m OV-17 column the sterol fraction was analyzed on a 40 m, SE 30 glass capillary column. The gas chromatogram in Figure 2 shows the presence of at least 14 components.

Table II shows their RRT values based on RT of β-sitosterol (46.16 min under the conditions used), methylene indices, contents in the sterol fraction, and the determined structures.

Combination GC-MS with a 30 m, SE 30 glass capillary column gave good mass spectra for components, 1, 2, 3, 4, 6, 7, 8, 11, 12, and 13. Mass spectra of components 5, 9, 10, and 14 wer of poor quality and inadequate for interpretation. On the basis of the comparison of mass spectra and RRT values with those of authentic samples, components 1, 2, 3, and 4 were identified as cholesterol, brassicasterol, campesterol, and stigmasterol, respectively.

TABLE II

Composition of Sterol Fraction of Pumpkin Seed Oil

Component number	RRT[a]	Methylene indices	Content % of sterol fraction	Content % of oil	Name
1	0.76	31.30	tr	tr	Cholesterol
2	0.86	32.55	tr	tr	Brassicasterol
3	0.88	32.75	tr	tr	Campesterol
4	0.92	33.35	0.9	0.003	Stigmasterol
5	0.94	33.45	1.4	0.004	Unidentified
6	0.95	33.55	6	0.02	24-Methyl-cholest-7-en-3β-ol
7	0.98	34.00	29.4	0.09	$\Delta^{7,22,25}$-Stigmastatrien-3β-ol
8	0.99	34.13	27.2	0.08	α-Spinasterol
9	1.01	34.20	tr	tr	Unidentified
10	1.03	34.55	tr	tr	Unidentified
11	1.05	34.83	21.5	0.06	$\Delta^{7,25}$-Stigmastadien-3β-ol
12	1.07	35.20	3.8	0.01	Δ^{7}-Stigmastenol
13	1.09	35.46	9.6	0.03	Δ^{7}-Avenasterol
14	1.13	---	tr	tr	Unidentified

[a]Relative retention time (RRT) for β-sitosterol (retention time: 46.16 min) taken as 1.00.

Component 6 showed the following mass spectrum considering m/e values above 200 (the base peak in the entire spectrum was the fragment m/e 55 instead of fragment m/e 255 which had the relative intensity 38%). m/e: 400 (62%, M^+), 385 (31%), 382 (10%), 367 (17%), 273 (39%), 255 (100%), 246 (25%), 231 (51%), 229 (60%), 213 (53%). Basically the same mass spectrum with greater relative intensities (up to 10%) was given for 24-methylcholest-7-en-3β-ol (6). This structure was also suggested for a sterol isolated from a Chinese pumpkin seed (4) which showed the same fragmentation patterns and RRT values (Table I) as component 6. Consequently it was identified as 24-methylcholest-7-en-3β-ol.

Component 7 showed following mass spectrum considering m/e values above 200. Only peaks present in the spectrum above m/e 300 are given. In the range between m/e 200 and m/e 300 only fragments with relative intensities greater than 10% are given. The base peak in the entire spectrum was fragment m/e 81 instead of fragment m/e 271 which had the relative intensity 90%. m/e: 410 (15%, M^+), 395 (10%), 392 (2%), 381 (10%), 377 (4%), 326 (5%), 314 (3%), 311 (3%), 300 (17%), 273 (36%), 271 (100%), 269 (16%), 257 (15%), 255 (65%), 203 (18%), 246 (10%), 231 (10%), 229 (19%), 227 (10%), 213 (22%), 201 (15%). The mass spectrum of $\Delta^{7,22,25}$-stigmastatriene-3β-ol which was isolated from a European pumpkin species showed the same fragments (7) as component 7. By two-dimensional thin layer chromatography the component 7 was isolated preparatively. Its NMR and IR spectra were in agreement with the structure of $\Delta^{7,22,25}$-stigmastatrien-3β-ol (7). On the basis of the above information, component 7 was identified as $\Delta^{7,22,25}$-stigmastatrien-3β-ol.

Component 8 showed following mass spectrum considering m/e values above 200 (the base peak in the entire spectrum was the fragment m/e 271 together with m/e 55; above m/e 300 all peaks present in the spectrum are given; in the range between m/e 200 and m/e 300 only fragments with relative intensities greater than 10% are given). m/e: 412 (30%, M^+), 397 (12%), 394 (3%), 383 (3%), 369 (23%), 351 (3%), 327 (2%), 314 (3%), 306 (2%), 300 (19%), 299 (12%), 273 (37%), 271 (100%), 257 (14%), 255 (49%), 246 (26%), 231 (19%), 229 (19%), 213 (16%). Basically the same fragmentation with variations in peak intensities which were not greater than 10% are obtained for a sterol to which the structure of α-spinasterol was assigned (6). By two-dimensional thin layer chromatography the component 8 was isolated preparatively. Its NMR and IR spectra showed the same characteristics as α-spinasterol (6,8). On the basis of the above information, component 8 was identified as α-spinasterol which is in agreement with their practically identical RRT values (Table I).

Component 11 showed following mass spectrum considering m/e values above 200 (the base peak in the entire spectrum was the fragment m/e 271 together with m/e 55; above m/e 300 all peaks present in the spectrum are given; in the range between m/e 200 and m/e 300 only fragments with relative intensities greater than 10% are given). m/e: 412 (23%, M^+), 397 (30%), 394 (3%), 379 (6%), 355 (2%), 328 (2%), 314 (2%), 300 (8%), 299 (11%), 271 (100%), 275 (12%), 255 (37%), 246 (17%), 231 (26%), 229 (16%), 227 (21%), 213 (30%). The mass spectra of $\Delta^{7,25}$-stigmastadien-3β-ol which was isolated from a European pumpkin species showed the same fragments (7) as component 11. Consequently component 11 was considered to have a structure of $\Delta^{7,25}$-stigmastadien-3β-ol.

Component 12 showed following mass spectrum considering m/e values above 200 (the base peak for the entire spectrum was fragment m/e 43 instead of fragment m/e 255 whifh had the relative intensity 90%); above m/e 300 all peaks present in the spectrum are given; in the range between m/e 200 and m/e 300 only fragments with relative intensities greater than 10% are given). m/e: 414 (92%, M^+), 399 (42%), 396 (3%), 381 (14%), 314 (9%), 301 (8%), 300 (6%), 273 (23%), 269 (17%), 255 (100%), 247 (18%), 246 (15%), 231 (40%), 229 (48%), 213 (56%), 201 (17%). Basically the same fragmentations with variation in intensities of peaks not greater than 10% were given for Δ^7-stigmastenol (6) which had identical RRT value as component 12. Hence the sterol 12 was identified as Δ^7-stigmastenol.

Component 13 showed following mass spectrum considering m/e values above 200 (the base peak in the entire spectrum was the fragment m/e 271; above m/e 271 all peaks with relative intensities greater than 2% are given; in the range between m/e 200 and m/e 271 only fragments with relative intensities greater than 5% are given. m/e: 412 (2%, M^+), 397 (6%), 379 (2%), 314 (31%), 299 (10%), 296 (2%), 286 (3%), 285 (5%), 281 (3%), 271 (100%), 257 (5%), 255 (10%), 253 (6%), 246 (10%), 231 (9%), 229 (5%), 228 (5%), 227 (6%), 213 (14%). Component 13 has the same RRT value as Δ^7-avenasterol (Table I). Fragmentation pattern of Δ^7-avenasterol is available from literature. The intensities of peaks of component 13 are in good agreement with those of Δ^7-avenasterol given by Brook et al. (9), except that the intensity of fragment m/e 314 was 57% instead of 31%. On the basis of the above information, the component 13 was identified as Δ^7-avenasterol. It should be mentioned, however, that the intensities of the fragments of Δ^7-avenasterol given by Itoh et al. (5) and Sucrow (3) differ considerably from peaks intensities of component 13.

In the oil from Chinese pumpkin seed analyzed by Jeong et al. (4), two sterols found in considerable amounts were not identified. They showed that one of those was admixed with Δ^7-stigmastenol representing 28% of the product, and the other with RRT value 1.09 was present in the quantity of 10% of the product. The mixture of Δ^7-stigmastenol and the unknown sterol had the same RRT value (1.18) as peak 11 in our work (Table I). By the analysis on the capillary column GLC (Fig. 2) and combined GC-MS, we showed it to be a mixture of Δ^7-stigmastenol (component 12) and $\Delta^{7,25}$-stigmastadien-3β-ol (component 11) in quantities of 3.8% and 21.5%, respectively. The other sterol unidentified by Jeong et al. had the same RRT value as component 7 found in Yugoslav pumpkin seed oil which we identified as $\Delta^{7,22,25}$-stigmastatrien-3β-ol.

The chromatograms in Figure 1 and 2 show that component 8 had a shorter retention time on a 1.5 m OV-17 column than component 7, but the reverse was true for a 40 m SE-30 capillary column.

Regarding the main components, it could be concluded that there was practically no difference in the composition between Yugoslav and Chinese pumpkin seed oils. The main characteristic of this oil is that all sterols found in quantites over 1% of sterol fraction had Δ^7-steroid nucleus.

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