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Unsaponifiable matter and fatty acid composition of pea oil

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Dry peas (Pisum sativum) contain a low value of oil (1.4–1.6 %) with a large quantitiy of unsaponifiable matter (11.9 %) (1). Unsaponifiable matter of the oil obtained by extraction is higher than that of the oil obtained by pressing (2). Unsaponifiable matter consists mostly of sterols, tocopherol, and small amounts of other high alcohols and hydrocarbons (3). Phytosterols consist mostly of β-sitosterol, which proved to possess a relatively high esterogenic acitivity (4). Certain steroids are found associated with unsaponifiable matter in nature, either by reason of mutual solubility or owing to some actual metabolic relationship. Steroids include precursors of D vitamins and certain hormones of several types that have physiological properties. Tocopherols are precursors of E vitamins, and have antioxidant properties that prevent rancidity in oils and fats, while hydrocarbon carotenoids are provitamins A (5).

The present study was undertaken to investigate the composition of the unsaponifiable matter fractions of pea oil with gas liquid chromatography (GLC) and column chromatography along with thin-layer chromatography (TLC). The work has been extended to determine the fatty acid contents of pea oil by GLC.

Materials and methods

Materials

Dry peas variety Little Marvel was supplied from 1980 crop of the experimental plots of Vegetable Research Section, Horticulture Research Center, Giza (Egypt). The powdered seeds were exhaustively extracted by soaking with purified hexane till the solvent was colourless. The oil was obtained after evaporating the solvent under vacuum at 60 °C. Four references of sterols – cholesterol, campesterol, stigmasterol and B-sitosterol – as well as three ones of hydrocarbons, C_{30}, C_{32} and squalene, were used. The reference specimens of sterols, hydrocarbons, and fatty acids were supplied by B.D.H. Chemical Ltd., England. α-tocopherol reference was supplied by Fluka Co., Switzerland.

Separation of unsaponifiable matter

The oil (22 g) was saponified with 120 ml alcoholic KOH 50 % for 48 h (6). The

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reaction mixture was diluted with 50 ml distilled water, and the unsaponifiable matter was then extracted with petroleum ether (b.p. 40–60 °C). The combined extracts were washed with 100-ml portions of distilled water till neutrality, dried over anhydrious sodium sulphate, and evaporated under vacuum to dryness. The unsaponifiable matter (2.5 g) was subjected to GLC and column chromatographic analysis.

Preparation of methyl esters of fatty acids

The methyl esters of fatty acids of pea oil were prepared in dry methanol containing sodium methoxide (7). The methyl esters were extracted with petroleum ether (b.p. 40–60 °C), dried over anhydrious sodium sulphate, and concentrated under vacuum to 2 ml for GLC analysis.

Gas liquid chromatography

The unsaponifiable fractions and fatty acid contents were analysed with a Pye Unicam gas chromatograph equipped with a flame ionization detector. The chromatograph was coated with 1.5 % OV-17 for unsaponifiable matter and coated with 10 % polyethyleneglycol adipate for fatty acids. The column was operated at 270 °C for unsaponifiable matter and 190 °C for fatty acids, with nitrogen at 30 ml/min as carrier gas. Detector temperature was 300 °C for unsaponifiable matter and 250 °C for fatty acids. Under these conditions the retention time of B-sitosterol was 10.6 min and of oleic acid was 11 min.

Column chromatography

The unsaponifiable matter (2.5 g) was dissolved in 5 ml chloroform and mixed with silica gel D (2.5 g). It was fractionated on column (35 × 2.5 cm), plugged with glasswool, and packed with sand[1]) for 0.5 cm height, then with the same silica gel for 35 cm height (25 g). The less polar compounds (hydrocarbons, aliphatic alcohols, etc.) were eluted with 250 ml petroleum ether (b.p. 60–80 °C) to give 414 mg of these compounds. The sterols were eluted with benzene-ethylacetate-acetic acid (12 : 1 : $^1/_2$), and 5-ml fractions were periodically examined on thin-layer chromatoplates along with authentic sample of β-sitosterol, and they had the same R_f value (0.53). The plates were developed with toluene-ethylacetate-acetic acid (12 : 4 : $^1/_2$) for 30 min and sprayed with chlorosulphonic acid acetic acid (1 : 3). Fractions containing β-sitosterol were combined together to give 591 mg of colourless crystalline plates (from methanol) which had positive Liebermann-Burchardt test (8). The fractions eluted from the column using the same solvent mixture before isolating β-sitosterol were found to behave the same on the chromatoplates (R_f 0.66). They were collected together and crystallised from methanol to give 100 mg of colourless crystalline needles, which were positive to Liebermann-Burchardt test for sterols (8), and were confirmed by the following analysis.

Preparation of the acetate derivatives of sterols

The acetate derivative of the isolated sterols and B-sitosterol reference was prepared by mixing 50 mg of the sample with 5 ml acetic anhydride and 2 drops of pyridine. The mixture was left overnight and distilled under vacuum till dryness. The obtained acetate derivative was crystallised from methanol.

Infrared spectroscopy of sterols and their acetate derivatives

Infrared spectra of the isolated sterol and their acetate derivatives were measured on a Unicam Sp 1000 Infrared spectrophotometer using 10 mg of KBr pellets for each 1.0 mg sample, while β-sitosterol reference and its acetate were measured

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[1]) Washed with conc. H_2SO_4, 10 % NaOH, and ethanol respectively, then dried.

on a Perkin Elmer Infra Cord 137 spectrophotometer, using the same amounts of KBr and sample.

Optical rotation of sterols and their derivatives

Optical rotation $(\alpha)_D$ was measured on a polyrimeter Carl Zeiss using 10 mg of the sample dissolved in chloroform and tube 1 decimeter at room temperature.

Results and discussion

Unsaponifiable matter composition of pea oil

The pattern of unsaponifiable fraction in pea oil analysed by GLC and their percentage yield are shown in table 1. Hydrocarbons C_{30}, and C_{32} squalene, α-tocopherol, cholesterol, campesterol, stigmasterol, and β-sitosterol were identified by comparing their RRT with those of the reference specimens. The predominant unsaponifiable fraction was found to be β-sitosterol. These results agreed with those of Miyazawa et al. (9) who found that the sterols in pea oil were campesterol, stigmasterol, and β-

Table 1. Unsaponifiable matter composition of pea oil.

Fraction	Relative retention*) time to β-sitosterol	Percent of total
Unknown	0.16	1.15
Unknown	0.17	11.61
C_{30} hydrocarbon	0.24	14.56
Squalene	0.32	0.83
C_{32} hydrocarbon	0.37	0.43
Unknown	0.41	0.43
α-Tocopherol	0.53	8.12
Cholesterol	0.67	0.39
Campesterol	0.82	2.46
Stigmasterol	0.92	0.46
β-sitosterol	1.00	54.75
Unknown	1.15	3.24
Unknown	1.33	0.73
Unknown	1.500	0.85

*) Relative retention time for β-sitosterol (retention time 10.6 min) was taken as 1.00.

Table 2. Melting point (m. p.) and optical rotation $(\alpha)_D$ of β-sitosterol and its acetate of pea oil.

Sample	β-sitosterol m. p. °C	$(\alpha)_D$*)	β-sitosterol acetate m. p. °C	$(\alpha)_D$
β-sitosterol of pea oil	136–137	−36 °	122–123	−39 °
β-sitosterol reference	136–137	−36 °	125–126	−39 °
Reported values (13)	137–137,5	−37 °	127–128	−41 °

*) in chloroform

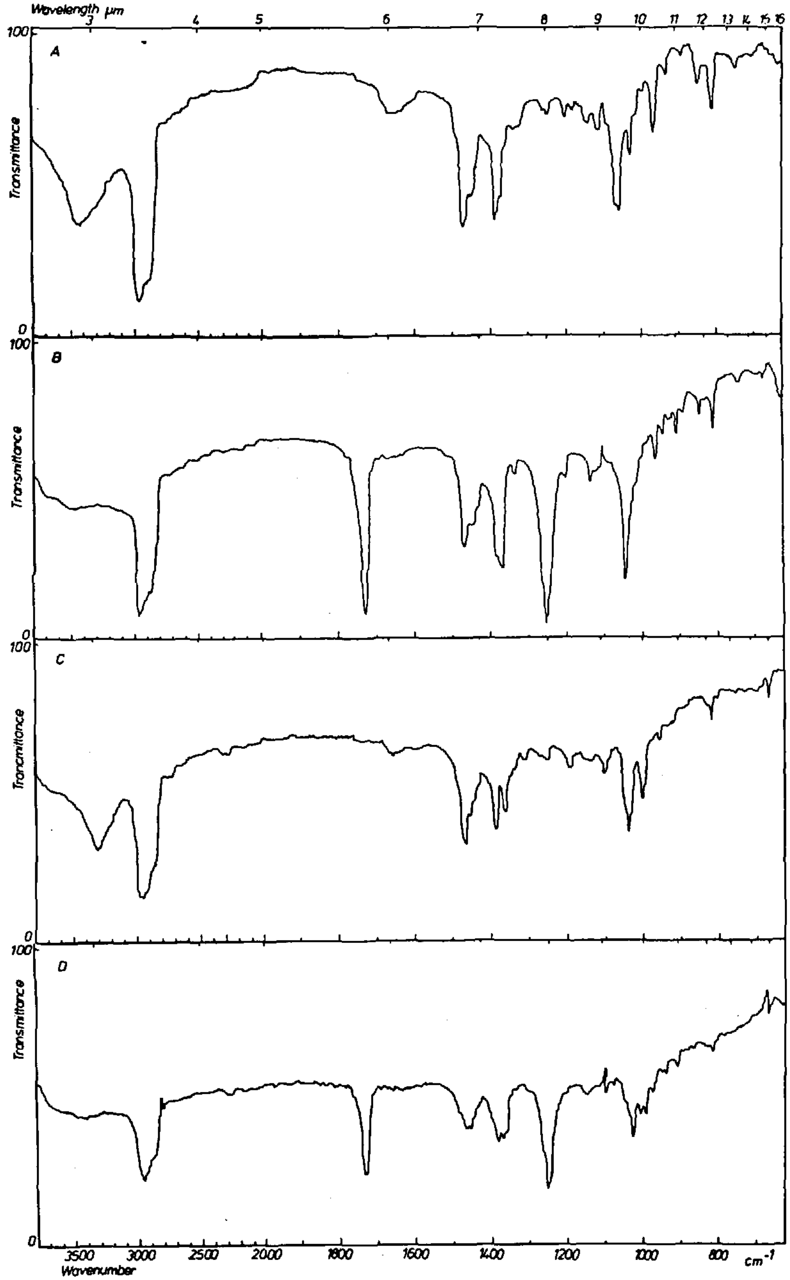

Fig. I Infrared spectra (in KBr) of sterols isolated from peas oil.

A-β-sitosterol. B- Acetate derivative of β-sitosterol.

C- Unidentified sterol. D-Acetate derivative of this unidentified sterol.

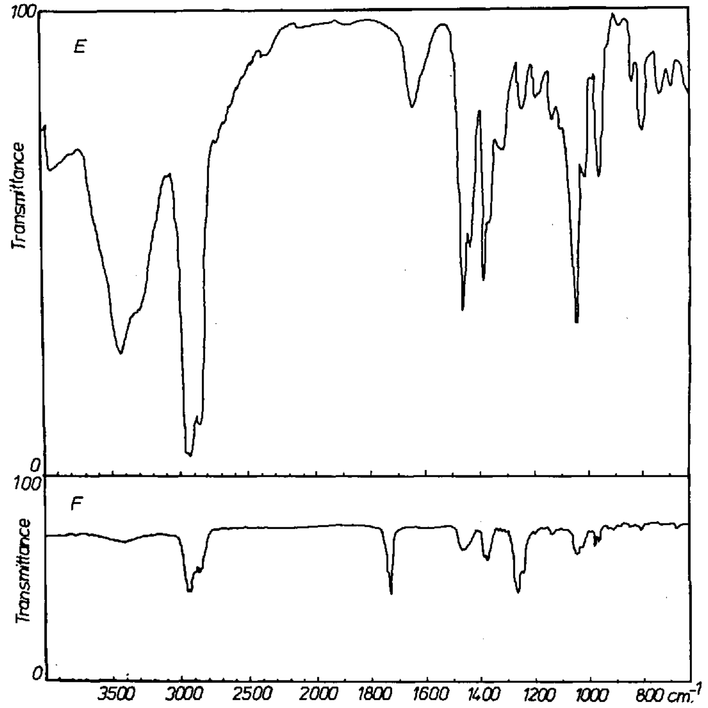

Fig. II Infrared spectra (in KBr) of reference β-sitosterol.
E - β-sitosterol.
F - Acetate derivative of β-sitosterol.

sitosterol and the main sterol was β-sitosterol. Sterols identified in pea oil were stigmasterol, campesterol, cholesterol, and 2.4-methylenecholesterol (10). Itoho et al. (11) reported that the sterol fraction was the major one in the unsaponifiabel matter, and cholesterol was found in traces or low amounts for most of the oils. α-tocopherol was found in considerable amount (table 1), which is known as good antioxidant material (12).

Sterol characterization of pea oil

The fractions collected from the column containing sterols (as evident from thin-layer detection) were separately combined together, and the residue was crystallised from methanol. One of the isolated sterols was identified as β-sitosterol, as its physical constants as well as its acetate derivative agreed with those of known β-sitosterol and its acetate derivative (table 2).

Infrared spectra of the isolated β-sitosterol from pea oil and its acetate derivative aggreed with those of known β-sitosterol (fig.1, 2). An absorption for OH group is detected at 3,430 cm^{-1} in the spectra of β-sitosterol, but not in that of its acetate. Strong absorptions at 2,940 and 2,860 cm^{-1} are based on CH_2 as well as absorptions at 1,470, 1,455, 1,390 and 1,370, cm^{-1} are based on methyl and methylene groups in the spectra of β-sitosterol.

Table 3. Fatty acid composition of pea oil (%).

Fatty acid	Relative retention time to oleic acid*)	Percent of total	Reported values % (17)	Reported values % (18)
10 : 0	0.13	4.58		
14 : 0	0.26	0.57	traces	0.14–0.25
14 : 1	0.29	0.97		
16 : 0	0.50	12.79	28.0	10.5–18.5
18 : 0	0.91	2.41	1.0	3.4–3.8
18 : 1	1.00	14.67	5.0	18.5–28.5
18 : 2	1.20	53.99	60.0	46.5–53.4
18 : 3	1.56	9.04	7.0	4.8–5.8

*) Relative retention time for oleic acid (retention time: 11 min) was taken as 1.00.

These results are in good agreement with those of Miyazawa et al. (9). Absorptions at 1,730, 1,440; 1,455; 1,390 and 1,255 cm^{-1} in the spectra of β-sitosterol acetate were detected. The other sterol isolated from pea oil was positive to Liebermann-Burchardt test (8), and its infrared spectra was identical with that of β-sitosterol in every detail (fig. 1, 2). Since its m.p. had 187–88 °C and the acetate derivative had m.p. 234–35 °C, it seems to be a complex with other constituents (not free sterol). Therefore this sterol is still under investigation.

Fatty acid composition of pea oil

The fatty acid pattern in pea oil was analysed by GLC and their percentage yield are presented in table 3. Linoleic acid was the major component. Agricultural products of divers varieties and grown in different locations have been reported to vary fatty acid pattern (14; 15, 16). Oil from peas grown in Bangladesh and in the Latvian SSR has somewhat different fatty acid pattern (17, 18) (table 3). The high content of polyunsaturated fatty acids in pea oil (63 %) indicated its good nutritive value, which agreed with other investigators (17).

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Summary

14 compounds were detected in the unsaponifiable matter of pea oil by gas liquid chromatography (GLC) and 8 of them were identified as hydrocarbons C_{30}, C_{32}, squalene, α-tocopherol, cholesterol, campesterol, stigmasterol and β-sitosterol. The predominant unsaponifiable fraction was β-sitosterol whereas the others were found in variable amounts. Two sterols were isolated by column chromatography. One of them was characterised as β-sitosterol by measuring the melting point, $[\alpha]_D$, and infrared spectra and those of its acetate. However, the other sterol was unidentified. The fatty acid contents in pea oil were determined quantitatively by GLC. They were present in variable amounts, whereas linoleic acid was the major one.

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