# Triglyceride Composition of Tobacco Seed Oil

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## ABSTRACT

Fatty acid and triglyceride compositions of Indian tobacco seed oil (*Nicotiana rustica*) have been determined by combination of the techniques of systematic crystallization at low temperatures, pancreatic lipase hydrolysis, and gas liquid chromatography of methyl esters. The percentages of individual fatty acids were found to be linoleic 71.2, oleic 15.7, palmitic 8.4, and stearic 3.8. The special characteristic of the tobacco seed oil is its content of 37.4, 22.8, 4.9, 19.7, 9.3, and 3.3% of trilinolein, dilinoleoolein, dioleolinolein, dilinoleosaturated, linoleooleosaturated, and linoleodisaturated glycerides, respectively. In the present investigation, preference of linoleic acid over oleic acid for the 2-position of glycerol moiety was not observed.

## INTRODUCTION

India occupies an important position among the tobacco producing countries of the world. Tobacco seed (oil content 30-43%), a by-product from tobacco leaf industries, is collected from two species (*Nicotiana tabacum* and *Nicotiana rustica*) of the Solanaceae family (1). The acreage yields of seeds of the two species are ca. 160 kg and 500 kg, respectively. Tobacco seed oil is extremely rich in linoleic acid (> 70%) and may be a potential raw material for paint industries (2). Refined tobacco seed oil has also been used as an edible oil in a number of European countries (2). The oil shows good stability in accelerated oxidation tests in spite of its relatively high unsaturation (3,4). This oil has gained considerable importance in India as an article of trade and was sold at a price of Rs. 730.0 per quintal in 1974-75.

Tobacco seed oil, as reported by earlier workers (4-14), shows a wide variation in its fatty acid composition (linoleic 54-79%, oleic 30-8%, saturated 8-19.5%, and linolenic 0.6-1.8%) depending on the climatic and environmental conditions under which the plants develop. Most of these previous data are based on spectrophotometric determination of the polyethenoid acids preceded by low temperature crystallization of the mixed fatty acids. The composition reported by Gunstone and Qureshi (14) is, however, based on gas liquid chromatography (GLC). Triglyceride composition of tobacco seed oil was determined earlier by Venkatarao and co-workers (28) and also by Crawford and Hilditch (10), utilizing the low temperature crystallization technique, now considered to be inadequate for the purpose (15). Later, Gunstone and Qureshi (14) examined tobacco seed oil along with other linoleic rich oils by lipolysis (16,17) and by chromatographic separation on silica impregnated with silver nitrate. Results obtained in three ways (chromatography, lipolysis, and calculation of component glycerides on the basis of Mode I of the theory of Gunstone [18]) showed good agreement.

In our program of study on the triglyceride composition of indigenous linoleic rich oils (19-21), tobacco seed oil which shows promise to be used as an edible oil appeared to be of interest, more due to the demonstrations (22-24) of the dietary effect of linoleic rich oils like corn or safflower oils in lowering the serum cholesterol. Present communication reports the findings on the triglyceride composition of Indian tobacco seed oil determined by the combined techniques of low temperature segregation, selective enzymatic hydrolysis, thin layer chromatography (TLC), and GLC. This procedure was adopted with the idea that it may provide a better insight into the limitations of the technique of low temperature segregation as a method in the study of the triglyceride composition of linoleic rich seed oils.

TABLE I

Characterization of *Nicotiana rustica* Seed Oil and Its Mixed Fatty Acids

| Characteristics | Oil | Mixed fatty acids |
|---|---|---|
| Free fatty acids, % as oleic | 9.7 | - |
| Saponification equivalent | 294.0 | 278.3 |
| Iodine value (Wij's 30 min) | 141.1 | 144.1 |
| Reichert value | 2.9 | - |
| Polenske value | 0.3 | - |
| Unsaponifiable, % by wt | 2.1 | - |

## EXPERIMENTAL PROCEDURES

*N. rustica* seeds were procured from the local market. On extraction with petroleum ether (b.p. 40-60 C), the seeds yielded 37.3% of a light yellow colored oil. On analysis by standard procedures, the oil and its mixed fatty acids showed the characteristics given in Table I.

Tobacco seed oil (100 g) was treated as 50% solution in hexane with 20° Be′ caustic soda to yield 86.3 g of refined oil (saponification equivalent 296.5; iodine value 140.1). Refined oil (70.2 g) was next segregated into seven fractions (A-G) with varying degree of unsaturation (iodine value 115.7, 130.8, 137.3, 142.9, 146.1, 147.3, and 145.0) by systematic crystallization at low temperature. Following the method of Hilditch and co-workers (25), the oil was crystallized from 10 vol of acetone at -60 C to -10 C, increasing the temperature by 10 C in each successive step. Aliquot portions from each of these fractions and native seed oil were next subjected to pancreatic lipase hydrolysis. Lipolysis was carried out as suggested by Coleman (26) at pH 8.5 and 37.5 C using a purified pancreatic lipase preparation with the addition of $Ca^{2+}$ ions and bile salts. The partial glycerides were separated on a thin layer (0.3-0.4 mm) of silica by developing with a solvent system of n-hexane, diethyl ether, and acetic acid (75:25:0.12). The monoglyceride fraction, detected with 2′,7′-dichlorofluorescein, was extracted with hot alcohol. The monoglyceride and the original triglyceride samples were converted to methyl esters by the semimicro method of Luddy et al. (27). GLC was carried out with the Hewlett Packard analytical gas chromatograph (model 700-R 12) equipped with flame ionization detector. The column (6 ft x 1/4 in.), packed with 10% polyester diethylene glycol succinate on 60-80 mesh Gas Chrom Z, was operated at 160 C with a carrier gas flow of 40 ml/min. Peak areas were determined as the product of peak height and the width at half height. The weight percentages obtained were converted to mol percentages. Results (mol%) are given in Table II.

Triglyceride compositions of *N. rustica* seed oil and of its seven fractions were next calculated from the fatty acid compositions of the triglyceride and the corresponding 2-monoglyceride using the assumptions of Vanderwal (28) and Coleman (26). While calculating, the fatty acids have been grouped as S - $C_{16:0}$, $C_{18:0}$, and $C_{20:0}$; O - $C_{18:1}$; and L - $C_{18:2}$ and $C_{18:3}$. The results are given in Table III.

## RESULTS AND DISCUSSION

Results of the present investigation along with some of the findings of the previous workers on the fatty acid composition of tobacco seed oil are shown in Table IV.

Present findings compare favorably with the fatty acid composition of tobacco seed oil reported earlier by Crawford and Hilditch (10) and Gunstone and Qureshi (14). Present findings also agree well with the fatty acid composition of one of the samples reported by Chakrabarty and Chakrabarty (12,13). But, in other cases, though there is good agreement in the content of linoleic acid, the deviation in the content of oleic acid is considerable. This may be due to the limitations of the method followed or to the climatic and environmental factors under which the seeds matured.

Present findings on the triglyceride composition of tobacco seed oil (*N. rustica*) and of its different fractions obtained by low temperature crystallization are shown in Table V, along with the findings of Gunstone and Qureshi (14) and of other previous workers (10,29). Triglyceride composition of tobacco seed oil determined in the present instance compares favorably with the composition reported by Gunstone and Qureshi (14) in the content of polyethenoid triglycerides. Disagreement between the triglyceride composition of Indian tobacco seed oil reported by Crawford and Hilditch (10) and that determined in the present instance, of course, may be accounted for by the limitations of low temperature crystallization process as mentioned by Hilditch and Williams (15). According to them, simple triunsaturated glycerides such as trilinolein, oleodilinolein, and dioleolinolein resist segregation by low temperature crystallization process, especially in the case of linoleic rich oils. Although the triglyceride composition of the native tobacco seed oil as determined in the present instance directly or as computed from the triglyceride compositions of the segregated fractions shows deviations from the findings of Crawford and Hilditch (10), it agrees well with the triglyceride composition reported by Gunstone and Qureshi (14). Results reported by Gunstone and Qureshi (14) provide support for the correctness of the assumptions adopted in handling lipolysis data. This general agreement between the findings of the present investigation and those by Gunstone and Qureshi (14) is important enough because it indicates that the limitations of the low temperature segregation process as pointed out by Hilditch and Williams (15) may not be critical enough. At least in the present case, triglyceride compositions of different fractions obtained by low temperature crystallization do not indicate that trilinolein, oleodilinolein, and dioleolinolein resist segregation.

Gunstone et al. (30) observed consistent preference of linoleic acid over oleic acid for the 2-position of the glycerine molecules of vegetable seed fats. Selective factors of these two acids for the 2-position were reported to be (1.0-1.2) and (0.8-1.0), respectively. In the present instance, fatty acid compositions of the 2-monoglycerides and of the corresponding triglycerides of different fractions (A-G) isolated from tobacco seed oil do not clearly show the preference of linoleic acid over oleic acid for the 2-position of the glycerol moiety, and in some cases reverse order is observed. This needs further confirmation from the study of other polyethenoid acid rich seed oils.

TABLE II

Fatty Acid Composition (mol %) of Triglyceride and 2-Monoglyceride of Tobacco Seed Oil (*Nicotiana rustica*) and Its Different Fractions

| Component acids | Seed oil | | A (13.1%) | | B (10.4%) | | C (13.3%) | | D (9.9%) | | E (16.1%) | | F (4.6%) | | G (32.6%) | | Computed[a] values | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TG | MG | TG | MG | TG | MG | TG | MG | TG | MG | TG | MG | TG | MG | TG | MG | TG | MG |
| $C_{16:0}$ | 10.2 | 2.7 | 12.6 | 1.3 | 12.1 | 0.9 | 11.2 | 1.3 | 8.6 | 1.9 | 7.5 | 2.8 | 6.4 | 2.5 | 7.3 | 1.1 | 9.1 | 1.5 |
| $C_{18:0}$ | 4.8 | 1.1 | 8.3 | - | 4.7 | - | 3.7 | - | 2.7 | - | 2.4 | - | 2.5 | - | 2.7 | - | 3.7 | - |
| $C_{18:1}$ | 15.4 | 19.2 | 21.7 | 34.5 | 17.2 | 20.3 | 16.9 | 19.6 | 14.5 | 19.0 | 12.9 | 17.9 | 13.6 | 17.6 | 13.7 | 15.7 | 15.5 | 20.0 |
| $C_{18:2}$ | 68.8 | 77.0 | 56.5 | 64.2 | 65.4 | 78.8 | 67.5 | 79.1 | 73.0 | 79.1 | 76.0 | 79.3 | 76.4 | 79.9 | 75.6 | 83.2 | 70.8 | 78.5 |
| $C_{18:3}$ | 0.8 | - | 0.2 | - | 0.6 | - | 0.7 | - | 1.2 | - | 1.2 | - | 1.1 | - | 0.7 | - | 0.8 | - |
| $C_{20:0}$ | - | - | 0.7 | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.1 | - |

[a]Computed from the compositions of different segregated fractions.

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TABLE III

Triglyceride Composition (mol %) of Tobacco Seed Oil and Its Fractions (A-G)

| Triglycerides[a] | | Seed oil | A (13.1%) | B (10.4%) | C (13.3%) | D (9.9%) | E (16.1%) | F (4.6%) | G (32.6%) | Computed[b] values |
|---|---|---|---|---|---|---|---|---|---|---|
| SSS | SSS | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | - | 0.1 |
| SSU | SSO | 0.2 | 0.2 | 0.1 | 0.1 | - | 0.1 | 0.1 | - | 0.1 |
| | SSL | 1.0 | 0.4 | 0.2 | 0.4 | 0.4 | 0.6 | 0.4 | 0.3 | 0.4 |
| SUS | SOS | 0.8 | 3.4 | 1.2 | 0.9 | 0.5 | 0.3 | 0.3 | 0.3 | 0.9 |
| | SLS | 3.3 | 6.5 | 4.8 | 3.7 | 2.0 | 1.4 | 1.2 | 1.7 | 2.9 |
| USU[c] | OSL | 0.6 | 0.2 | 0.2 | 0.2 | 0.4 | 0.4 | 0.4 | 0.2 | 0.3 |
| | LSL | 1.7 | 0.4 | 0.3 | 0.5 | 1.0 | 1.6 | 1.5 | 0.6 | 0.8 |
| SUU | SOO | 1.0 | 3.4 | 1.6 | 1.3 | 0.8 | 0.6 | 0.5 | 0.6 | 1.1 |
| | SOL | 5.2 | 11.6 | 6.0 | 5.4 | 4.4 | 3.6 | 3.2 | 3.3 | 5.1 |
| | SLO | 4.2 | 6.2 | 6.1 | 5.3 | 3.1 | 2.2 | 2.2 | 3.0 | 3.9 |
| | SLL | 20.9 | 21.6 | 23.2 | 21.6 | 18.2 | 16.2 | 14.8 | 17.5 | 18.9 |
| UUU | OOO | 0.4 | 0.8 | 0.5 | 0.5 | 0.3 | 0.2 | 0.2 | 0.3 | 0.4 |
| | OOL | 3.4 | 5.6 | 3.8 | 3.8 | 3.2 | 2.8 | 3.2 | 2.9 | 3.5 |
| | LOL | 8.4 | 9.7 | 7.2 | 7.7 | 9.8 | 10.4 | 10.2 | 8.3 | 8.8 |
| | OLO | 1.4 | 1.5 | 1.9 | 1.9 | 1.2 | 0.9 | 1.1 | 1.4 | 1.4 |
| | OLL | 13.8 | 10.4 | 14.8 | 15.4 | 13.8 | 12.6 | 14.1 | 15.4 | 14.0 |
| | LLL | 33.4 | 18.0 | 28.0 | 31.2 | 40.8 | 46.0 | 46.5 | 44.2 | 37.4 |

[a]S - $C_{16:0}$, $C_{18:0}$, and $C_{20:0}$; O - $C_{18:1}$; L - $C_{18:2}$, $C_{18:3}$.

[b]Computed from the composition of different segregated fractions.

[c]Seed oil also contains 0.1% OSO.

TABLE IV

Fatty Acid Composition (% by wt) of Tobacco Seed Oil

| Habitat | Saturated acids | | | | Unsaturated acids | | |
|---|---|---|---|---|---|---|---|
| | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{18:3}$ |
| Philippines (Ref. 6) | 0.1 | 7.2 | 3.1 | 0.4 | 27.2 | 62.0 | - |
| USA (Ref. 4) (12 variations) | - | 7.0 | 3.0 | - | 15.0 | 75.0 | - |
| Turkey (Ref. 10) | - | 8.3 | 4.1 | 0.4 | 12.0 | 74.6 | 0.6 |
| Rhodesia (Ref. 10) | - | 7.5 | 3.7 | 0.8 | 12.9 | 74.0 | 1.1 |
| England (Ref. 10) | ← | 11.4 | | → | 8.0 | 79.1 | 1.5 |
| India (Ref. 10) | - | 7.6 | 3.1 | 0.9 | 17.9 | 69.4 | 1.1 |
| India (Ref. 13) (15 species) | ← | (12.8-19.5) | | → | (9.3-19.3) | (64.7-72.6) | (1.1-2.0) |
| Unknown (Ref. 14)[a] | 0.1 | 11.2 | 4.6 | - | 14.1 | 68.5 | 0.9 |
| Present work | | | | | | | |
| India (West Bengal)[b] | - | 8.4 | 3.8 | 0.1 | 15.7 | 71.2 | 0.8 |

[a]Results, expressed in mol % (contains 0.6% of $C_{16:1}$ acid), were obtained by computation of the composition of nine fractions isolated by chromatography on a thin layer of silica gel impregnated with silver nitrate.

[b]Composition computed from those of seven fractions isolated from tobacco seed oil by low temperature crystallization.

TABLE V

Proportions (mol %) of Polyethenoid Glycerides in Tobacco Seed Oil[a]

| Authors | Component acids | | Component glycerides | | | |
|---|---|---|---|---|---|---|
| | Pe | X | $Pe_3$ | $Pe_2X$ | $PeX_2$ | $X_3$ |
| Venkatarao and Narasingarao (Ref. 29) | 54.6 | 45.4 | 7.0 | 50.0 | 36.0 | - |
| Crawford and Hilditch (Ref. 10) | 72.1 | 27.9 | 19.0 | 74.0 | 7.0 | - |
| Gunstone and Qureshi (Ref. 14) | 72.0 | 28.0 | 35.0 | 41.0 | 20.0 | 4.0 |
| Present work: | | | | | | |
| Native seed oil | 69.6 | 30.4 | 33.4 | 44.8 | 19.1 | 2.7 |
| Fraction A | 56.7 | 43.3 | 18.0 | 42.1 | 32.0 | 7.9 |
| Fraction B | 66.0 | 34.0 | 28.0 | 45.5 | 23.0 | 3.5 |
| Fraction C | 68.2 | 31.8 | 31.2 | 45.2 | 20.7 | 2.9 |
| Fraction D | 74.2 | 25.8 | 40.8 | 42.8 | 14.7 | 1.7 |
| Fraction E | 77.2 | 22.8 | 46.0 | 40.8 | 11.9 | 1.3 |
| Fraction F | 77.5 | 22.5 | 46.5 | 40.6 | 11.7 | 1.2 |
| Fraction G | 76.3 | 23.7 | 44.2 | 41.8 | 12.8 | 1.2 |
| Computed composition from the compositions of fractions (A-G) | 71.6 | 28.4 | 37.4 | 42.5 | 17.5 | 2.6 |

[a]Pe and X refer to polyethenoid and other (monoethenoid and saturated) acyl chains. Fractions (A-G) were obtained by systematic low temperature crystallization of tobacco seed oil.

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[Received April 19, 1976]