Natural Product Sciences
7(4) : 110~113 (2001)

Studies on the Lipid Classes of *Nicotiana tabacum* L. Seed Oil

Amran Waheed*, Shahid Mahmud, Muhammad Akhtar Javed and Muhammad Saleem

Applied Chemistry Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan

Abstract – The lipid classes constituents; hydrocarbons, wax esters, sterol esters, triacylglycerols, free fatty acids, 1,3-diacylglycerols, 1,2-diacylglycerols, free sterols, 2-monoacylglycerols, 1-monoacylglycerols, phosphatidylethanolamines, phosphatidylcholines, lysophosphatidylethanolamines and phosphatidylinositols of *Nicotiana tabacum* L. seeds oil were investigated by thin layer and gas chromatography. Palmitic, oleic and linoleic acids were the major components in all lipid classes studied.
Key words – *Nicotiana tabacum* L., fatty acids, triacylglycerol, Solanaceae.

Introduction

Nicotiana tabacum L. belongs to the genus *Nicotiana* of the family Solanaceae. The plant originally belongs to Bolivia and Argentina (William, 1972). The word tabacum originated from "Tamarakuta". Tamarakuta is a compound of Tamara "a red or copper colour" and kuta "deceitful or vile". *Nicotiana tabacum* L. (Murty *et al.*, 1989; Kashyab and Joshi, 1936) is a stout annual plant, about 3m high with a thick erect stem producing few branches. The seeds are nearly spherical or elliptic, light brown in colour.

A large number of papers have been available on *N. tabacum* dealing with smoking purpose (Comberloan *et al.*,1988; Blomberg and Windmark, 1975; Severson *et al.*,1978) and as a protein (Fantozzi *et al.*, 1982), but the aim of this publication is to widen knowledge of the chemical composition of the seeds and to apply the latest analytical techniques to supply the most up-to-date information.

Experimental

Extraction of lipids – The seeds of *N. tabacum* were ground and extracted with chloroform-methanol (2:1 v/v) to get lipids by shaking on a magnetic stirrer for half an hour at room temperature. The lipids thus obtained after separation of solid material by filtration were treated repeatedly with the solution of chloroform, methanol and sodium chloride (0.9%) in the ratio of (3:50:47 v/v/v) to remove non-lipid impurities (Javed *et al.*, 1991).

*Author for correspondence.

Preparative Thin-layer chromatography – Thin-layer chromatograms of 0.5 mm thickness (20×20 cm) were prepared for the separation and identification of lipids. The plates were activated by heating at 105°C for one hour. The solvent system used (Folch *et al.*, 1957] for the separation of the different classes of neutral lipids was hexane-diethyl ether-acetic acid (80:20:2 v/v) and the solvent used for the separation of the different classes of polar lipids was chloroform-methanol-ammonium hydroxide-water (60:35:5:2.5 v/v) (Folch *et al.*, 1957). The non-destructive locating agent 2,7-dichlorofluorescein was used which gave purple yellow coloured bands under an ultraviolet light at 366 nm.

Esterification of different lipid classes – The methyl esters of the total lipids and each lipid class except that of hydrocarbons and free sterols were prepared by the use of borontrifluoride-methanol solution (Raie *et al.*, 1989). The methyl esters of the fatty acids of lipids were purified by silica gel thin-layer chromatography using the solvent system hexane-diethyl ether (90:10) (Waheed *et al.*, 1992) prior to the use of gas chromatography for the identification of fatty acids.

Gas chromatography – The fatty acids composition of total lipids and its different classes were determined on Shimadzu GC-14A gas chromatograph equipped with a flame ionisation detector and capillary column (25 m×0.2 mm, i.d.) coated with polyethylene glycol. A temperature program for the column oven was 180°C-5 min-3°C/min-230°C while injector and detector temperatures were maintained at 250°C and 300°C, respectively. The peaks were recorded on Shimadzu C-R4A chromatopac and

identified by comparing their relative retention times with those of authentic sample run under the same parameters.

Results and Discussion

It has been found out that lipids, carbohydrates, proteins and alkaloids are widely distributed in the vegetables. The medicinal usefulness of a plant cannot be understood fully unless through investigation have been carried out.

The extraction of lipids is the first step to carry out further research on various classes of polar and non-polar lipids. The mixture of chloroform and methanol has been used so that the complete extraction of polar as well as non-polar lipids is possible. The seeds of *N. tabacum* contained 4.4% moisture and lipid contents were 41.3% as shown in Table 1. Further more the percentage of neutral lipids was 94.2% and of polar lipids was 5.8%.

The neutral lipids were classified into ten classes while polar lipids into four classes by thin-layer chromatography. These classes were identified by comparison their R_f values (Table 2) with those of standard ones. Among the neutral lipids, the presence of free sterols and sterol esters were also confirmed by spraying the reagent of antimony trichloride (Raie *et al.*, 1983) on thin-layer chromatogram. These compounds showed red violet colour after heating in the oven at 100°C for ten minutes. Similarly, hydroxylamine ferric chloride reagent was sprayed to confirm the presence of different types of acylglycerols, which show purple colour under the above-mentioned similar conditions. Among the polar lipids it was also observed that molybdenum blue (Colowick *et al*, 1969a) reagent gave blue spots on spraying and heating the thin-layer chromatogram where phospholipids have been spotted. Later on these phospholipids were further differentiated as phosphatidylethanolamine and its lyso derivative, which showed red violet spots with ninhydrin (Colowick *et al*, 1969b) whereas phosphatidylcholine and phosphatidylinositol gave orange and yellow colouration with the spray of Dragendorff and periodate Schiff reagent (Colowick *et al*, 1969c), respectively.

There are fourteen lipid classes separated from the oil of *Nicotiana tabacum* L. comprising ten neutral lipids such as hydrocarbons, wax esters, sterol esters, triacylglycerols, free fatty acids, 1,3-diacylglycerols, 1,2-diacylglycerols, free sterols, 2-monoacylglycerols, 1-monoacylglycerols and four polar lipids such as phosphatidylethanolamines, phosphatidylcholines, lysophosphatidylethanolamines and phosphatidylinositols. The percentage compositions of these fourteen lipid classes have been shown in Table 3.

According to Table 3, the percentages of various lipid classes of *N. tabacum* are hydrocarbons (1.4%), wax esters (1.7%), sterol esters (2.4%), triacylglycerols (69.3%), free fatty acids (6.2%), 1,3-diacylglycerols (4.6%), 1,2-diacylglycerols (3.5%), free sterols (2.3%), 2-monoacylglycerols (2.1%), 1-monoacylglycerols

Table 1. Moisture and Lipid Contents of *Nicotiana* Species

Moisture	4.4%
Lipids	41.3%
Neutral lipids	94.2%
Polar lipids	5.8%

Table 2. R_f Values of Lipids of *Nicotiana tabacum*

No.	Lipid classes	R_f value
	(A) Neutral lipids	
1	Hydrocarbons (HC)	0.96
2	Wax esters (WE)	0.93
3	Sterol esters (SE)	0.71
4	Triacylglycerols (TG)	0.60
5	Free fatty acids (FFA)	0.41
6	1,3-diacylglycerols (1,3-DG)	0.33
7	1,2-diacylglycerols (1,2-DG)	0.29
8	Free sterols (S)	0.22
9	2- monoacylglycerols (2-MG)	0.18
10	1-monoacylglycerols (1-MG)	0.14
	(B) Phospholipids	
1	Phosphatidylethanolamines (PE)	0.68
2	Phosphatidylcholines (PC)	0.60
3	Lysophosphatidylethanolamines (LPE)	0.53
4	Phosphatidylinositols (PI)	0.17

Table 3. Percentage of Various Lipid Classes Present in *Nicotiana tabacum* L.

Lipid classes	%
Hydrocarbons (HC)	1.4
Wax esters (WE)	1.7
Sterol esters (SE)	2.4
Triacylglycerols (TG)	69.3
Free fatty acids (FFA)	6.2
1,3-diacylglycerols (1,3-DG)	4.6
1,2-diacylglycerols (1,2-DG)	3.5
Free sterols (S)	2.3
2-monoacylglycerols (2-MG)	2.1
1-monoacylglycerols (1-MG)	1.8
Phosphatidylethanolamines (PE)	1.7
Phosphatidylcholines (PC)	0.9
Lysophosphatidylethanolamines (LPE)	1.5
Phosphatidylinositols (PI)	0.6

(1.8%), phosphatidylethanolamines (1.7%), phosphatidylcholines (0.9%), lysophosphatidylethanolamines (1.5%) and phosphatidylinositols (0.6%).

The lipid fractions of *N. tabacum* determined by earlier workers (Damain *et al.*, 1993) were hydrocarbons, sterol esters, triacylglycerols, diacylglycerols, monoacylglycerols and free sterols by using the hexane: diethyl ether: acetic acid (90:10:1 v/v) as eluent. In the present work the results are almost similar except that we also found out free fatty acids and two isomers of diacylglycerols such as 1,3-diacylglycerols and 1,2-diacylglycerol and of monoacylglycerols as 2-monoacylglycerols and 1-monoacylglycerols. These fractions were obtained after a slight change in the composition of developing mixture hexane: diethyl ether: acetic acid (80:20:2 v/v). The presence of free fatty acids may be due to difference in agronomical factors and higher activity of lipase present in the oil.

The fractionated neutral and polar lipids are mostly in the esterified form. The major role in the structure of these esters is of fatty acids. The importance of fatty acids in lipids is ever realized. Each fraction either neutral or polar except hydrocarbons and free sterols is hydrolysed and esterified into methyl ester, with methanol in the presence of borontrifluoride.

The methyl esters of fatty acids are purified and identified by the application of thin layer chromatography and gas chromatography, respectively. The results of fatty acid composition of seed oils are given in Tables 4 and 5. The results of fatty acid composition shown in Table 4, reflect the highest percentage (7.3%) of lauric acid is found in phosphatidylinositols and the lowest (0.3%) in sterol esters. Myristic acid is the maximum (7.5%) in lysophosphotidylethanolamines and minimum (0.6%) in wax esters. The lipid fraction phosphatidylcholines shows the highest percentage (17.3%) of palmitic acid whereas 2-monoacylglycerols contains lowest percentage (4.3%) of palmitic acid. Wax esters show the highest percentage (6.3%) of stearic acid and 1-monoacylglycerols contain the lowest (1.2%) of stearic acid. The highest percentage (28.2%) of oleic acid is found in phosphatidylethanolamines and the lowest (16.5%) in triacylglycerols. Linoleic acid is maximum (74.5%) in 2-monoacylglycerols and minimum (42.6%) in phosphatidylethanolamines. The lipid fraction wax esters show the highest percentage (3.5%) of linolenic acid whereas 1,2-diacylglycerols and phosphatidylinositols contain lowest percentage (0.3%) of linolenic acid. Wax esters show the highest percentage (1.1%) of arachidic acid and triacylglycerols contain the lowest percentage (0.3%) of arachidic acid.

The fatty acid range in the lipid classes of *N. tabacum* seeds has been found to be $C_{12:0}$ to $C_{20:0}$ containing saturated and unsaturated fatty acids. The percentage of saturated and unsaturated fatty acids of

Table 5. Percentage of Saturated and Unsaturated Fatty Acids in Different Lipid Classes of *Nicotiana tabacum*

Lipid fraction	Saturated fatty acids (%)	Unsaturated fatty acids (%)
WE	20.6	79.4
SE	18.6	81.4
TG	15.2	84.8
FFA	17.5	82.5
1,3-DG	14.1	85.9
1,2-DG	22.1	77.9
2-MG	6.7	93.3
1-MG	10.8	89.2
PE	28.5	71.5
PC	33.0	67.0
LPE	32.7	67.3
PI	34.9	65.1

Table 4. Fatty Acid Composition (%) of Different Lipid Classes in the Seeds of *Nicotiana tabacum*

Lipids	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
WE	–	0.6	12.6	6.3	25.6	50.3	3.5	1.1
SE	0.3	2.7	16.3	2.3	23.2	54.1	1.1	–
TG	0.5	0.9	9.6	3.9	16.5	67.1	1.2	0.3
FFA	–	–	12.5	4.2	20.3	60.1	2.1	0.8
1,3-DG	1.2	1.7	8.5	2.7	22.3	61.7	1.9	–
1,2-DG	3.6	5.3	11.2	1.3	20.4	57.2	0.3	0.7
2-MG	–	0.7	4.3	1.7	18.2	74.5	0.6	–
1-MG	0.4	1.3	7.5	1.2	17.9	70.1	1.2	0.4
PE	4.5	3.9	15.6	4.5	28.2	42.6	0.7	–
PC	6.7	5.3	17.3	3.7	23.4	43.1	0.5	–
LPE	6.3	7.5	14.7	4.2	20.6	46.2	0.5	–
PI	7.3	6.9	16.2	4.5	19.7	45.1	0.3	–

each lipid fraction is shown in Table 5. This Table reflects that the highest percentage (34.9%) of saturated fatty acids and lowest percentage of unsaturated fatty acids (65.1%) are found in phosphatidylinositols whereas 2-monoacylglycerols shows the highest percentage of unsaturated fatty acids (93.3%) and lowest percentage (6.7%) of saturated fatty acids.

Each lipid class of *N. tabacum* shows the higher percentage of unsaturated fatty acids as compared to saturated fatty acids (Table 5) which is the characteristics of vegetable oils. The fatty acids found are $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$. Usually $C_{18:2}$ is the major fatty acid among the unsaturated fatty acids whereas $C_{16:0}$ is maximum among the saturated fatty acids moiety. These results are supported by previous workers (Frega *et al*, 1991) in the studies of tobacco seed oils.

References

Blomberg, L. and Windmark, G., Separation of fresh Tobacco smoke on a packed gas chromatographic column prior to online analysis by gas chromatography-mass spectrometry using a non-polar capillary column. *J. Chromatog.* **106**, 59-71 (1975).

Chamberlain, W.J., Schlotzhauer, W.S. and Chortyk, O.T., Chemical composition of non-smoking Tobacco product. *J. Agric. Food Chem.* **36**(1), 48-50 (1988).

Colowick, S.P. and Kaplon, N.O., *Methods in Enzymology*, Vol. XIV, p. 544, Academic press, London, 1969a.

Colowick, S.P. and Kaplon, N.O., *Methods in Enzymology*, Vol. XIV, p. 546, Academic press, London, 1969b.

Colowick, S.P. and Kaplon, N.O., *Methods in Enzymology*, Vol. XIV, p. 547, Academic press, London, 1969c.

Damain, M. and Carlos, A.G., Chemical composition of Tobacco seed from Argentina. *J. Sci. Food Agri.* **61**, 227-230 (1993).

Fantozzi, P. and Sensidoni, A. and Neri, M., Tobacco use as a smoking material and a possible food source. 1.-Deproteinized smoking material evaluation. *Ind. Aliment.*, **21**(4), 277-282 (1982).

Fantozzi, P. and Sensidoni, A., Use of tobacco as a smoking material and a possible food source. 2.-Food protein evaluation. *Ind. Aliment.*, **21**(5), 375-383 (1982).

Fantozzi, P., Garofolo, L. and Sensidoni, A., Tobacco utilization as a smoking material and a possible food source. 3. - Effect of some agronomical parameters on the content of structural and enzymic protein. *Ind. Aliment.*, **21**(6), 482-488 (1982).

Folch, J., Lees, M. and Stanley, S., A simple method for the isolation and purification of total lipid from animal tissues. *J. Biol. Chem.* **226**, 497-507 (1957).

Javed, M.A., Raie, M.Y. and Ali, N., Lipid studies of Citrullus vulgaris of the family Cucurbitaceae. *Pak. J. Sci. Ind. Res.* **34**, 181-83 (1991).

Kashyab, S.R. and Joshi, A.C. Lahore District Flora, p. 285, University of Punjab, Lahore, 1936.

Murty, A.V. and Subrahmanayam, N.S.A., A Textbook of Economic Botany, pp. 528-532, Wiley Eastern Ltd., New Delhi, 1989.

Raie, M., Ahmad, M., Ahmad, I. Khan, S.A. and Jafri, S.A., Chromatographic studies of cottonseed oils. *Fette Seifen Anstrichmittel.* **85**(7), 279-280 (1983).

Raie, M.Y., Ahmad, M., Khan, S.A. and Athar, S.A., *Fette Seifen Antrichmittl.* **7**, 279-280 (1983).

Severson, F.F., Ellington, J.J., Arrendala, R.F. and Snook, M.E., Quantitative gas chromatographic method for the analysis of aliphatic hydrocarbons, terpenes, fatty alcohols, fatty acids and sterols in Tobacco. *J. Chromatog.* **160**, 155-168 (1978).

Wahed, A. Sabir, A.W. and Sattar, A., Fatty acid composition of lipid classes from the seed oil of *Blepharis persica*.. *Pro. Pakistan Acad. Sci.* **29**(3), 237-243 (1992).

William, D., Phamacographia India, Vol. I, pp. 321-323, National Foundation Karachi, 1972.

(Accepted October 29, 2001)