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Studies on the Lipids of Kinnow Orange Seeds

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Abstract. The seeds of *Citrus reticulata* var. Kinnow orange contained 26.0% lipids. The seed oil was examined for its lipids and fatty acids composition. The lipids were fractionated into neutral lipids (96.3%) and polar lipids (3.7%) by thin layer chromatography. The neutral lipids were identified as hydrocarbons (1.2%), wax esters (1.1%), sterol esters (3.2%), triacylglycerols (70.5%), free fatty acids (2.3%), 1,3-diacylglycerols (4.0%), 1,2-diacylglycerols (4.2%), glycolipids (1.6%), sterols (2.1%), 2-monoacylglycerols (3.1%), and 1-monoacylglycerols (3.0%). The polar lipids were identified as phosphatidyl ethanolamines (1.2%), phosphatidyl cholines (0.8%), lysophosphatidyl ethanolamines (0.6%), and phosphatidyl inositols (1.1%). The fatty acids range of all the esterified lipids was $C_{14:0} - C_{18:2}$, showing higher percentage of unsaturated fatty acids. The major fatty acids were palmitic, stearic, oleic and linoleic acids.

Keywords: *Citrus reticulata*, lipids, fatty acids, triacylglycerols, Kinnow orange, polar lipids, Rutaceae

Introduction

Citrus is among the most widespread aboreal plants in the world. Citrus plants are cultivated in over 130 countries, between 40 °N and 40 °S, extending to four million hectares (Starrantino, 1992). Orange and the inter-varietal hybrid Kinnow orange are the most cultivated fruits among the citrus group, followed by lemon, lime, grapefruit, Feutral and 'mitha' (sweet orange). For a long time, the main commercial outlet of citrus fruits has been the fresh fruit market as table fruit. However, during more recent years a higher proportion of citrus fruits has been processed, mainly for juice production (FAO, 2000). Seeds and peels are the wastes generated during juice production. Extensive research work has been done on the fatty acids composition of citrus seed oils from different kinds of citrus fruits (*C. reticulata* varieties Feutral, Tangerine and 'Sangtra', *C. limenta, C. acida*, *C. grandis*) (Mahmud *et al.,* 2001; Sattar *et al.,* 1991; 1988; 1987; Nordby and Nagy, 1974; Kefford and Chandler, 1970). However, studies on lipids of Kinnow orange have not yet been reported. The present work is on the investigations of fatty acids composition of lipid classes of Kinnow orange seeds. Kinnow orange is a hybrid variety of *Citrus reticulata,* family Rutaceae (Nasir and Ali,1972). The present study reports the separation, purification and identification of lipids of *Citrus reticulata* var. Kinnow orange. The separated lipids were then hydrolyzed and methylated to determine their fatty acids composition with the help of gas liquid chromatography.

Materials and Methods

Extraction of lipids. Fresh, mature Kinnow orange fruits were collected from a citrus orchard near Pattoki, Pakistan, and cut into two halves. 100 g seeds were picked, washed and dried in an oven at 105 °C for one h. The dried seeds of Kinnow orange were ground and extracted with chloroform and methanol (2 : 1, v/v) by shaking on a magnetic stirrer for 30 min at room temperature. The lipids thus obtained, after removing the solid material by filtration, were treated repeatedly with the solvent mixture of chloroform, methanol and sodium chloride (0.9%) in the ratio of (3 : 48 : 47, v/v) to remove non-lipid impurities (Waheed *et al.,* 2001).

Preparative thin layer chromatography. Thin layer chromatograms (20 x 20 cm) of 0.5 mm thickness were prepared for the separation and identification of lipids. The plates were activated by heating at 105 °C for one h. The solvent systems used for the separation of the different classes of neutral as well as polar lipids were hexane-diethylether-acetic acid (80 : 20 : 2 v/v) and chloroform-methanol-ammonium hydroxide-water (60 : 35 : 5 : 2.5, v/v), respectively (Javed *et al.,* 2000). The non-destructive locating reagent 2,7-dichlorofluorescein was used which gave purple yellow colour bands under an ultraviolet light at 366 nm.

The neutral lipids were separated into eleven classes while polar lipids into four classes by TLC. These classes were identified by comparison of their R_f values with the corresponding standards (Table 1). Among the neutral lipids, the presence of sterols and sterol esters was also confirmed on TLC by spraying antimony trichloride reagent (Raie *et al.,* 1983). These compounds showed red violet colour after heating the plates in oven at 100 °C for 10 min. Similarly, hydroxylamine ferric chloride reagent was sprayed to confirm the presence of different types of acylglycerols, which showed purple colour under the above-mentioned conditions

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(Javed *et al.,* 2002). Molybdenum blue reagent gave blue spots on spraying and heating the TLC plates where phospholipids were spotted. Later on, these phospholipids were further differentiated as phosphatidylethanolamine and its lyso derivative, which showed red violet spots with ninhydrin whereas phosphatidylcholine and phosphatidylinositol gave orange and yellow colouration with the spray of Dragendorff and periodate Schiff reagent, respectively (Ahmed *et al.,* 1994).

Esterification of different lipid classes. The methyl esters of 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 so formed were purified by silica gel TLC using the solvent system hexane-diethyl ether (90 : 10) (Mahmud *et al.,* 2004), prior to gas chromatography for the identification of fatty acids.

Gas chromatography. The fatty acids composition of different classes was determined on Shimadzu GC-14A gas chromatograph, equipped with a flame ionization detector and capillary column (25 m x 0.2 mm, i.d.) coated with polyethylene glycol. A temperature programme for the column oven was 180 °C-5 min-3 °C/min-230 °C, while the 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 samples run under similar conditions.

Results and Discussion

The Kinnow orange seeds were found to contain moisture and lipid, 5.6% and 26.0%, respectively. The percentage of neutral lipids was very high (96.3%) and of polar lipids was low (3.7%), which was usual. This composition was comparable with the lipids composition of *Sesamum indicum,* having similar composition of neutral and polar lipids (Javed *et al.,* 2000).

Fifteen lipid classes were separated from the oil of *C. reticulata* var. Kinnow orange, comprising 11 neutral and 4 polar lipids. The percentage composition of these 15 lipid classes is shown in Table 1, which comprised of hydrocarbons (1.2%), wax esters (1.1%), sterol esters (3.2%), triacylglycerols (70.5%), free fatty acids (2.3%), 1,3-diacylglycerols (4.0%), 1, 2-diacylglycerols (4.2%) glycolipids (1.6%), sterols (2.1%), 2-monoacylglycerol (3.1%), 1-monoacylglycerol (3.0%), phosphatidyl ethanolamines (1.2%), phosphatidyl cholines (0.8%), lysophosphatidyl ethanolamines (0.6%), and phospatidyl inositols (1.1%). This composition is very close to the composition of *Citrullus colocynthis* having 71.0% of tricylglycerols, as the predominant fraction, but fatty alcohols and lysophosphatidyl cholines were absent in the Kinnow orange seeds, which however were present in minute quantities in *C. colocynthis* (Javed *et al.,* 1992).

Table 1. Lipid classes in the Kinnow orange seeds

Lipids	R_f value	%
Neutral lipids		
Hydrocarbons	0.95	1.2
Wax esters	0.94	1.1
Sterol esters	0.73	3.2
Triacylglycerols	0.61	70.5
Free fatty acids	0.42	2.3
1,3-Diacylglycerols	0.34	4.0
1,2-Diacylglycerols	0.28	4.2
Glycolipids	0.24	1.6
Sterols	0.20	2.1
2-Monoacylglycerols	0.19	3.1
1-Monoacylglycerols	0.17	3.0
Polar lipids		
Phosphatidyl ethanolamines	0.70	0.18
Phosphatidyl cholines	0.51	0.8
Lysophosphatidyl ethanolamines	0.54	0.6
Phosphatidyl inositols	0.18	1.1

All lipid classes, except hydrocarbons and sterol fractions, were converted into their methyl esters by BF_3-methanol reagent and fatty acids composition was determined by gas chromatography. The fatty acids range was $C_{14:0} - C_{18:2}$, containing saturated and unsaturated fatty acids in all the lipid classes. The oleic acid ($C_{18:1}$) was found as the predominant fraction (30.3 – 39.3%) in neutral as well as in polar lipids (Table 2). This pattern of fatty acids composition resembles the composition of family Umbellifereae (Ahmad *et al.,* 1994). The other fatty acids were myristic, palmitic, stearic and linoleic acids. Unsaturated fatty acids were higher, as compared to saturated fatty acids in all the lipid classes (Table 3), which is the characteristic of vegetable oils. Palmitic acid ($C_{16:0}$), which was highest in the saturated acids profile may be the precursor of higher fatty acids. The linoleic acid ($C_{18:2}$), which is an essential fatty acid and the precursor of prostaglandins plays a definite role in human health by its existence in lung tissues, seminal plasma and accessory genital glands. It may be concluded that the fatty acids composition of the oil showed that it may be classified as a moderately unsaturated oil resembling other edible oils.

Table 2. Fatty acids composition (%) of different lipid classes of Kinnow orange seeds

Lipid class	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
Wax esters	3.5	24.6	3.3	20.2	33.4	15.0
Sterol esters	3.0	22.5	4.0	18.8	35.6	16.1
Triacylglycerols	2.0	25.4	6.2	17.0	30.3	19.1
Free fatty acids	3.6	24.6	4.4	13.1	36.6	17.7
1,3-Diacylglycerols	4.5	27.4	5.2	14.9	34.4	13.6
1,2-Diacylglycerols	2.3	31.2	3.5	10.7	33.2	19.1
Glycolipids	3.0	28.2	6.4	11.1	35.3	16.0
2-Monoacylglycerols	3.0	27.4	6.0	7.5	38.8	17.3
1-Monoacylglycerols	4.1	29.7	5.0	7.9	39.3	14.0
Phosphatidyl ethonalamines	5.4	24.4	4.7	19.2	33.2	13.1
Phosphatidyl cholines	5.0	22.8	6.5	15.9	35.6	14.2
Lysophosphatidyl ethanolamines	6.1	31.5	5.6	9.4	36.6	10.8
Phosphatidyl inositol	4.3	28.7	5.3	12.1	38.4	11.2

Table 3. Saturated and unsaturated fatty acids composition in different lipid classes of Kinnow orange seeds

Lipid class	Saturated fatty acid (%)	Unsaturated fatty acids (%)
Wax esters	48.3	51.7
Sterol esters	44.3	55.7
Triacylglycerols	44.4	55.6
Free fatty acids	41.3	58.7
1,3-Diacylglycerols	46.8	53.2
1,2-Diacylglycerols	44.2	55.8
Glycolipids	42.3	57.7
2-Monoacylglycerols	37.9	62.1
1-Monoacylglycerols	41.7	58.3
Phosphatidyl ethanolamines	49.0	51.0
Phosphatidyl cholines	43.7	56.3
Lysophosphatidyl ethanolamines	47.0	53.0
Phosphatidyl inositols	45.1	54.9

Conclusion

The current citrus processing industry in Pakistan can yield about 40,000 tonnes of citrus fruit seeds (Sattar *et al.,* 1991). Calculations indicate that even this minor source, if exploited, can provide almost 13.2 thousand tonnes (33%) of edible oil. With the increasing demand and better agricultural production techniques, more citrus fruits will become available for processing in the near future. It is, therefore, strongly recommended that the fruit seeds which are a waste at the present, must be exploited for obtaining fixed oils.

References

Ahmad, I., Raie, M.Y., Akhtar, M.W. 1994. Lipid studies of *Carum capticum* seed oil. *Pak. J. Sci. Ind. Res.* **37:** 150-152.

FAO. 2000. *Citrus Fruit, Fresh and Processed, Annual Statistics,* http://www.fao.org.

Javed, M.A., Akhtar, N., Jabbar, A. 2000. Fatty acid and lipid composition of *Sesamum indicum* DC. *Pak. J. Sci. Ind. Res.* **43:** 23-25.

Javed, M.A., Raie, M.Y., Ali, H. 1992. Lipid studies of *Citrullus colocynthis* of the family Cucurbitaceae. *Acta Sci.* **2:** 67-72.

Javed, M.A., Saleem, M., Waheed, S. 2002. Lipid composition of 'Maulsari' (*Mimusops elengilina*) seed oil. *Proc. Pak. Acad. Sci.* **39:** 89-92.

Kefford, J.F., Chandler, B.V. 1970. *The Chemical Constituents of Citrus Fruits*, Academic Press, New York, London, USA/UK.

Mahmud, S., Akbar, S., Sattar, A. 2001. The fatty acid composition of *Citrus sinensis* varieties Valencia and Salustiana. *Pak. J. Sci. Ind. Res.* **40:** 93-95.

Mahmud, S., Javed, M.A., Malik, M.S. 2004. Fatty acid composition of lipid classes of *Basella rubra* Linn. *Proc. Pak. Acad. Sci.* **41:** 79-80.

Nasir, E., Ali, S.I. 1972. *Flora of West Pakistan,* pp. 436-437, Fakhri Printing Press, Karachi, Pakistan.

Nordby, H.E., Nagy, S. 1974. Fatty acids of triglycerides and sterol esters from Duncan grapefruit, Dancy mandarin and their Tangelo hybrids. *Phytochemistry* **13:** 2215-2218.

Raie, M.Y., Ahmad, I., Javed, M.A., Waheed, I. 1989. Lipid

classes of *Apium graveolens* oil of the family Umbelliferae. *Proc. Pak. Acad. Sci.* **26:** 199-206.

Raie, M.Y., Ahmad, M.A., Ahmad, I., Khan, S.A., Jafri, S.A. 1983. Chromatographic studies of cottonseed oils. *Fette Seifen Antrichmittl* **85:** 279-280.

Sattar, A., Mahmud, S., Khan, S.A. 1987. Physicochemical studies on the seed oils of Feutral and Tangerine varieties of *Citrus reticulata blanco*. *Pak. J. Sci. Ind. Res.* **30:** 631-632.

Sattar, A., Mahmud, S., Khan, S.A. 1988. Studies on the seed oils of *Citrus limenta* and *Citrus acida. J. Sci. Res.* **17:** 11-15.

Sattar, A., Mahmud, S., Khan, S.A. 1991. Fatty acid composition of *Citrus grandis* and *Citrus reticulata* (var. 'Sangtra'). *Pak. J. Sci. Ind. Res.* **34:** 238-240.

Starrantino, 1992. Use of triploids for production of seedless cultivars in citrus improvement programme. In: *Proc. Int. Soc. Horticulture,* Acireale 8-13, March 1, 1992, pp. 117-121.

Waheed, A., Mahmud, S., Javed, M.A., Saleem, M. 2001. Studies on the lipid classes of *Nicotiana tabacum* L seed oil. *Nat. Prod. Sci.* **7:** 110-113.