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THE GLYCERIDES STRUCTURE OF *CITRULLUS COLOCYNTHIS*

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The triacylglycerols separated from *Citrullus colocynthis* seed oil were fractionated by silver nitrate impregnated thin layer chromatography into six fractions with respect to their degree of unsaturation. The composition and nature of the fatty acids at their 1,3- and 2-positions were determined by the use of pancreatic lipase and gas chromatography. The unsaturated C_{18} acids occupy the 2-position depending upon the comparatively higher percentage of the respective acid.

Key words: *Citrullus colocynthis* , Fatty acids, Triacylglycerols, Thin layer chromatography.

Introduction

Citrullus colocynthis (colocynth) belongs to the family Cucurbitaceae (Chopra 1970) and grown as a wild perennial in desert region of the world. It is also found in the sandy lands of Pakistan. It is used since the time of immemorial for different ailments. Investigations on its seeds, roots, fruit and oil have been carried out previously (Khatri *et al* 1993; Rao and Udaysekhara 1994; Gawarikar and Mehta 1994; Agrawal *et al* 1994; Wasfi *et al* 1995; Al-Khalifa 1996; Moussata and Akoh 1997; El-Gengaihi *et al* 1998; Manorajani *et al* 1999; Javed *et al* 1999; Igwe and Ogbobe 2000; Nimla *et al* 2000) but its glycerides structure is reported for the first time in this paper. The triacylglycerols being the major fraction (71.0%) (Javed *et al* 1992) were separated from the oil and an effort was made to determine the structure of its triacylglycerols in the present studies.

Triacylglycerol composition and structure are important from the stand point of nutrition, oil stability and possible physiological effects. The pure quantitative separation of triacylglycerols, the nature and type of the fatty acids attached at their 1,3- and 2-positions are necessary for the determination of the structure of triacylglycerols.

The argentation thin layer chromatography is used for the separation of pure triacylglycerols into a number of fractions depending upon their saturated/unsaturated nature (Ahmad *et al* 1992). The fractionated and unfractionated triacylglycerols are hydrolysed to liberate the fatty acids at 1 and 3-positions by lipolytic enzymes (Luddy *et al* 1964). Pancreatic lipase liberates 2-monoacylglycerols which are later on separated by borax impregnated silica gel thin layer chromatography (Thomas *et al* 1965) and then methylated. The fatty acids at 2-position can be determined by gas chromatography.

The range of fatty acids in the triacylglycerols molecules is ($C_{12:0}$- $C_{18:3}$). The survey of literature usually reflects the distribution of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ at 2-position of the triacylglycerols but they do not infact compete equally (Gunstone *et al* 1965; Deneshrad 1978).

Materials and Methods

The seeds of *Citrullus colocynthis* were powdered in an electric grinder and its oil was extracted with chloroform at room temperature by magnetic stirrer. The triacylglycerols (695mg) were separated from the oil (1g) by application of silica gel thin layer chromatography using hexane, diethyl ether and acetic acid (80:20:2 v/v) as a developing solvent (Javed *et al* 1999). The silver nitrate (20%) impregnated thin layer chromatography was used for the fractionation of pure triacylglycerols (600mg) into six bands depending upon their saturation and unsaturation (Ahmad *et al* 1992). The plates (20 cm x 20 cm) of 0.25 mm silica gel thickness were prepared and developed in solvent mixture of benzene and diethyl ether (9:1) (Deneshrad 1978). Locating reagent 2,7 dichlorofluorescein was sprayed to make the six bands visible in pinkish colour under UV light at 366 nm. Each fraction was scraped and eluted with chloroform separately. However, the enrichment technique was used for the accumulation of the above six fractions. The amount of the separated fractions (I-VI) from the highest to the lowest R_f

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values were 70 mg, 100 mg, 110 mg, 140 mg, 90 mg and 80 mg, respectively. Pure triacylglycerols 30 mg and each of the fraction was taken separately in the stoppered 10ml glass tubes and hydrolysed for 1 h in a water bath shaker at 50°C using 20mg pancreatic lipase, 0.5 ml diisopropyl ether and then centrifuged at 2000 rpm for 2 min. The supernatant of each tube was separated and the solvent was removed to obtain the hydrolysed material. These were further fractionated into 1-monoacylglycerols, 2-monoacylglycerols, free fatty acids, 1, 2-diacylglycerols, 1, 3 diacylglycerols and unreacted triacylglycerols by the application of 4.3% sodium tetraborate impregnated silica gel thin layer chromatogrphy (Thomas *et al* 1965) using benzene, diethyl ether, ethyl alcohol and glacial acetic acid (50:40:2:0.2v/v) as the developing solvent (Javed *et al* 1998).

The fraction 2-monoacylglycerols either form the total triacylglycerols or from the six fractionated triacylglycerols was converted into methyl esters by treating with boron trifluoride-methanol reagent (Javed *et al* 1999). The fatty acids as methyl esters were identified on Shimadzu GC 14A gas chromatograph with flame ionization detector. Glass column 1.6 m x 3 mm(i.d) packed with 15% diethylene glycol succinate coated on Shimalite AW 201 (60-80 mesh) was used and its temperature was programmed from 150°C to 200°C with a rise of 5°C per minute. Injector and detector temperatures were 250°C and 300°C respectively. Nitrogen was used as a carrier gas with a flow rate of 40 ml/min. The methyl esters were identified by comparing their retention times with those of authentic methyl esters under the same conditions (Rie *et al* 1989). The percentage of the various fatty acids was determined by Shimadzu C-R4A chromatopac computing integrator in duplicate and reported in Table 1.

Results and Discussion

The triacylglycerols separated from *Citrullus colocynthis* seed oil were fractionated into six bands by silver nitrate impregnated thin layer chromatography. The fractions one to six from the solvent front to the base line were 11.7%, 16.9%, 19.3%, 24.2%, 14.4% and 13.5% respectively depending upon their degree of unsaturation (Table 1). The fraction I, close to the solvent front showed the highest percentage of saturated acids, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ and then decreased from 76.9-1.4% in II-VI fractions. The fractions II-VI showed higher percentage of unsaturated acids ($C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$) and increased from 51.5-98.6%. This is also supported by the previous workers (Jurriens and Kroesen 1965). $C_{18:3}$ was only present in fractions V-VI which is in accordance with the random theory (Coleman 1961).

The argentation thin layer and gas chromatographic studies of fractionated and unfractionated triacylglycerols show the distribution of fatty acids with regard to saturated, monoenoic, dienoic and trienoic and their abbreviations used are given below:

S = Saturated fatty acids($C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$)
O = Monounsaturated fatty acids($C_{12:1}$, $C_{14:1}$, $C_{16:1}$, $C_{18:1}$)
L = Diunsaturated fatty acid ($C_{18:2}$)
Le = Triunsaturated fatty acid($C_{18:3}$)

In fraction I, which is monounsaturated fatty acid of the triacylglycerols consisted mainly of palmitic, oleic and stearic

Table -1

Fatty acid composition (%) of triacylglycerols fractions separated by $AgNO_3$, TLC and unfractionated triacylglycerols of *Citrullus colocynthis*

Fatty Acids	Unfractionated TG	Fraction I	Fraction II	Fraction III	Fraction IV	Fraction V	Fraction VI
$C_{12:0}$	0.3	6.2	3.7	-	-		
$C_{12:1}$	0.2	-		-	2.1		
$C_{14:0}$	0.4	6.5	4.9	4.6	3.4	-	
$C_{14:1}$	0.2	-		-	3.9	2.3	-
$C_{16:0}$	11.8	49.7	27.3	16.3	6.7	2.5	1.4
$C_{16:1}$	0.2	-	2.2	2.0	0.9	0.3	-
$C_{18:0}$	10.6	14.5	12.6	3.2	1.3	0.8	-
$C_{18:1}$	24.6	21.8	33.1	43.5	34.5	31.0	20.2
$C_{18:2}$	50.2	1.3	16.2	30.4	47.2	53.9	56.1
$C_{18:3}$	1.5			-	-	9.2	22.3
Proportion of fractions (%)	_	11.7	16.9	19.3	24.2	14.4	13.5

Table 2
Fatty acid composition (%) of 2-monoacylglycerols (2-MG) of *Citrullus colocynthis* and the percentage of fatty acids esterified in the position-2(P-2)

Fatty Acids	Un-fractionated Triacylglycerols		Fraction I		Fraction II		Fraction III		Fraction IV		Fraction V		Fraction VI	
	2MG	% P-2	2MG	% P-2	2MG	% P-2	2MG	% P-2	2MG	% P-2	2MG	% P-2	2MG	% P-2
$C_{12:0}$	0.2	22.2	8.6	46.2	3.2	28.8	-	-	-	-	-	-	-	-
$C_{12:1}$	0.1	16.6	-	-	-	-	-	-	1.1	17.5	-	-	-	-
$C_{14:0}$	0.3	25.0	10.5	53.8	3.7	25.2	1.3	9.4	0.9	8.8	-	-	-	-
$C_{14:1}$	0.2	33.3	-	-	-	-	-	-	2.8	23.9	1.3	18.8	-	-
$C_{16:0}$	12.6	35.6	15.3	10.2	7.8	9.5	3.7	7.5	1.3	6.5	0.4	5.3	0.1	2.4
$C_{16:1}$	0.1	16.6	-	-	1.6	24.2	1.2	20.0	0.5	18.5	0.1	11.1	-	-
$C_{18:0}$	9.8	30.8	32.4	74.5	21.4	56.6	3.1	32.3	1.2	30.8	0.4	16.7	-	-
$C_{18:1}$	19.2	26.0	33.2	50.8	51.5	51.9	68.3	52.3	37.2	35.9	27.0	29.0	11.4	18.8
$C_{18:2}$	57.0	37.8	-	-	10.8	22.2	22.4	24.6	55.0	38.8	67.0	41.4	74.2	44.1
$C_{18:3}$	0.5	11.1	-	-	-	-	-	-	-	-	3.8	13.7	14.3	21.4

acids. The fraction may contains the triacylglycerols of the types SSO and SOS (Mattson and Volpenhein 1961). In fractions II-VI, the amount of saturated fatty acids decreased while unsaturated fatty acids increased. The types of the triacylglycerols in these fractions are di-, tri-, and tetraunsaturated, respectively. The principal constituents of fraction II may be SOO and OSO; fraction III, OOO, SLO, SOL and fraction IV, OLO, OOL and SLL. In fractions V and VI oleic acid decreases while linoleic acid and linolenic acid increase. The possible configurations of fraction V are OLL, LOL and of fraction VI are LLL and OLe L.

The fatty acid composition of 2-monoacylglycerols is reported in Table 2. The percentage of these fatty acids was also calculated according to Mattson and Volpenhein (1961) as follows

$$\% \text{ of fatty acid in the middle position} = \frac{\% \text{ of fatty acids in 2-monoacylglycerols}}{3 \times \% \text{ of this fatty acid in the triacylglycerols}} \times 100$$

In fraction I, oleic and stearic acids are esterified in the middle position. Inspite of the fact that almost half of the fatty acid composition of triacylglycerols (Table 1) is composed of palmitic acid (49.7%) and only a small amount of this acid (15.3%) is esterified in position 2, it means that normally it is esterified in positions 1 and 3.

In fractions II-IV, almost all the middle positions are occupied by unsaturated fatty acids (oleic and linoleic). In fractions IV to VI, the amount of oleic acid decreases and linoleic acid increases. In fraction V, fatty acids esterified in the 2-position consist of 27.0% oleic acid, 61.0% linoleic acid, 3.8% linolenic acid and in fraction VI, 11.4% oleic, 74.2% linoleic and 14.3% linolenic acids respectively.

The distribution of 18:1 depends upon total unsaturation of triacylglycerols. When the degree of unsaturation is less than three double bonds, 18:1 is more likely to be esterified in the middle position. When unsaturation of triacylglycerols is more than three double bonds, it will be esterified in the external position and the internal position will be esterified by 18:2 and 18:3. It seems that with equal chain length, the degree of unsaturation determines the type of distribution.

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