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# DISTRIBUTION OF FATTY ACIDS IN THE TRIGLYCERIDES OF *CITRULLUS VULGARIS*

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The triglycerides separated from *Citrullus vulgaris* 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 ∝, ∝' and β-position was determined by the use of pancreatic lipase and gas chromatography. The unsaturated $C_{18}$ acids occupy the β-position depending upon the comparatively higher percentage of the respective acid.

***Key words:*** *Citrullus vulgaris,* Triglycerides, Fatty acids.

## Introduction

*Citrullus vulgaris* (watermelon) belongs to the family *Cucurbitaceae* (Chopra 1970) and is cultivated all over Pakistan. It is used as fruit and its seed kernels are used as brain tonic since time immemorial. No exhaustive work has been carried out on the lipids of the seeds. The triglycerides being the major fraction (73.0%) (Javed *et al* 1991) were separated from the oil and an effort was made to determine the structure of its triglycerides in the present paper.

Triglycerides' composition and structure are important from the stand point of nutrition, oil stablility and possible physiological effects. The pure quantitative separation of triglycerides, the nature and type of the fatty acids attached at their ∝, ∝' and β positions are necessary for the determination of the structure of triglycerides.

The argentation thin layer chromatography is used for the separation of pure tiglycerides into a number of fractions depending upon their saturated/unsaturated nature (Ahmad *et al* 1992). The fractionated and unfrac-tionated triglycerides are hydrolysed to liberate the fatty acids at ∝,∝' positions by lipolytic enzymes (Luddy *et al.* 1969). Panceratic lipase liberates 2-monoglyceride which is later on separated by borax impregnated silica gel thin layer chromatography (Thomas *et al* 1965). and then methylated. The fatty acids at β-position can be determined by gas chromatography.

The range of fatty acids in the triglyceride molecules is ($C_{14:0}$- $C_{18:3}$). The survey of literature usually reflects the distribution of $C_{18:1,}$ $C_{18:2}$ and $C_{18:3}$ at β-position of the triglycerides but they do not infact compete equally (Gunstone *et al* 1965 and Daneshrad 1978).

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## Materials and Methods

The seeds of *Citrullus vulgaris* were powdered in an electric grinder and its oil was extracted with chloroform at room temperature by magnetic stirrer. The triglycerides were separated from the oil by the application of silica gel thin layer chromatography using hexane, diethyl ether and acetic acid (80:20:2) as a developing solvent (Javed *et al* 1991). The silver nitrate (20%) impregnated thin layer chromatography was used for the fractionation of pure triglycerides (Ahmad *et al* 1992) into six bands depending upon their saturation and unsaturation. The plates (20 cm x 20 cm) of 0.25 mm silica gel thickness (Helmat 1964) were prepared and developed in a solvent mixture of benzene and diethyl ether (9:1) (Daneshrad 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 fractions. 30 mg of pure triglycerides and each of fraction were taken separately in the stoppered 10 ml glass tubes and hydrolysed for 1 h in a water bath shaker at 50°C using 20 mg pancreatic lipase, 0.5 ml diisopropyl ether and 5 µl distilled water (Akhtar *et al* 1992) for each sample. These were cooled, diluted with 0.5 ml diisopropyl ether and then centrifuged at 2000 r.p.m. 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-monoglycerides, 2-monoglycerides, free fatty acids, 1,2-diglycerides, 1,3-diglycerides and unreacted triglycerides by the application of 4.3% sodium tetraborate impregnated silica gel thin layer chromatography (Thomas *et al* 1963) using benzene, diethyl ether, ethyl alcohol and glacial acetic acid (50:40:2:0.2) as the developing solvent (Javed *et al* 1992).

The fraction 2-monoglycerides either from the total triglycerides or from the six fractionated triglycerides was converted into methyl esters by treating with boron trifluoride-methanol reagent (Javed *et al* 1992).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 diethylene glycol succinate 15% coated on Shimalite AW 201 (60-80 mesh) was used and kept at 200°C. Injector and detector temperatures were 250 and 300°C respectively. Nitrogen was used as a carrier gas with a flow rate of 40 ml $min^{-1}$. 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 and reported (Table-1).

## Results and Discussion

The triglycerides separated from *Citrullus vulgaris* seed oil were fractionated into six bands by silver nitrate impregnated thin layer chromatography. The fractions (Thomas 1963; Gunstone 1965; Ludy *et al* 1968; Chopra 1970; Javed 1991; Ahmad 1992) from the solvent front to the base line were 15.2%, 16.2%, 18.0%, 22.0% 15.8% and 12.8% respectively depending upon their degree of unsaturation (Table 2). The fraction I, close to the solvent front showed the highest percentage of saturated acids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ (80.7%) and then decreased from 80.7-2.7% in II -VI fractions. The fractions III-VI showed higher percentage of unsaturated acids ($C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$) and increased from 65.7-97.3% This is also supported by the previous workers (Jurriens and Kroesen 1965). $C_{18:3}$ was only present in factions V-VI which is in accordance with the random theory (Colman 1961).

**Table 1**

Fatty acid composition (%) of 2-monoglycerides of *Citrullus vulgaris* and the percentage of fatty acids esterified in the position-2

| Fatty Acids | Unfractionated triglycerides | | Fraction I | | Fraction II | | Fraction III | | Fraction IV | | Fraction V | | Fraction VI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 MG | % p-2 | 2MG | %p-2 | 2MG | %p-2 | 2MG | %p-2 | 2MG | %p-2 | 2MG | %p-2 | 2MG | %p-2 |
| $C_{14:0}$ | 3.1 | 31.3 | 15.3 | 52.0 | 7.5 | 34.2 | 2.1 | 20.0 | – | – | – | – | – | – |
| $C_{16:0}$ | 23.6 | 35.9 | 25.7 | 15.4 | 15.4 | 12.9 | 4.3 | 7.2 | 2.1 | 6.8 | 1.2 | 5.8 | 0.2 | 4.4 |
| $C_{16:1}$ | 2.7 | 27.3 | - | - | 4.3 | 38.7 | 0.7 | 3.8 | 0.3 | 3.1 | 0.2 | 2.6 | – | – |
| $C_{18:0}$ | 8.9 | 32.2 | 27.4 | 60.1 | 21.5 | 58.3 | 7.3 | 22.1 | – | – | 1.0 | 5.5 | 0.1 | 2.8 |
| $C_{18:1}$ | 9.7 | 20.2 | 31.6 | 72.6 | 38.9 | 78.6 | 57.5 | 94.4 | 46.0 | 93.5 | 36.9 | 81.4 | 17.8 | 50.7 |
| $C_{18:2}$ | 51.6 | 38.0 | – | – | 12.4 | 20.3 | 28.1 | 23.9 | 51.6 | 27.8 | 58.8 | 28.7 | 67.6 | 29.5 |
| $C_{18:3}$ | 0.4 | 12.1 | – | – | – | – | – | – | – | – | 1.9 | 48.7 | 14.3 | 63.5 |

**Table 2**

Fatty acid composition (%) of triglycerides fractions separated by Ag $NO_3$ TLC and unfractionated triglycerides of *Citrullus vulgaris*

| Fatty Acids | Unfractionated triglycerides | Fraction I | Fraction II | Fraction III | Fraction IV | Fraction V | Fraction VI |
|---|---|---|---|---|---|---|---|
| $C_{14:0}$ | 3.3 | 9.8 | 7.3 | 3.5 | - | - | - |
| $C_{16:0}$ | 21.9 | 55.7 | 39.8 | 19.8 | 10.3 | 6.9 | 1.5 |
| $C_{16:1}$ | 3.3 | 1.9 | 3.7 | 6.2 | 3.2 | 2.5 | 1.7 |
| $C_{18:0}$ | 9.2 | 15.2 | 12.3 | 11.0 | 8.3 | 6.0 | 1.2 |
| $C_{18:1}$ | 16.0 | 14.5 | 16.5 | 20.3 | 16.4 | 15.1 | 11.7 |
| $C_{18:2}$ | 45.2 | 2.9 | 20.4 | 39.2 | 61.8 | 68.2 | 76.4 |
| $C_{18:3}$ | 1.1 | – | – | – | – | 1.3 | 7.5 |
| Proprotion of fractions(%) | – | 15.2 | 16.2 | 18.0 | 22.0 | 15.8 | 12.8 |

The argentation thin layer and gas chromatographic studies of fractionated and unfractionated triglycerides show the distribution of fatty acids as regards to saturated, monoenoic, dienoic and trienoic and their abreviations used are given below

S = Saturated fatty acids ($C_{14:0}$, $C_{16:0}$, $C_{18:0}$)
O = mono unsaturated fatty acids ($C_{16:1}$, $C_{18:1}$)
L = diunsaturated fatty acid ($C_{18:2}$)
Le = triunsaturated fatty acid ($C_{18:3}$)

In fraction I, which is monounsaturated fatty acid of the triglycerides consisted mainly of palmitic, oleic, stearic and myristic acids. The fraction may contain the triglycerides of the types SSO and SOS (Mattson and Volpenhein 1963). In fractions II-VI, the amount of saturated fatty acids decreased while unsaturated fatty acids increased. The types of the triglycerides in these fractions are di-, tri-, and tetra-unsaturated 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 fraction V and VI oleic acid decreases while linoleic and linolenic acids 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-mono-glycerides reported (Table 1). The percentage of these fatty acids were also calculated according to (Mattson and Volpenhein 1963) as follows

$$\% \text{ of fatty acid in the middle position} = \frac{\% \text{ of fatty acids in 2monoglycerides.}}{3 \times \% \text{ of this fatty acid in the triglycerides.}} \times 100$$

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 triglycerides (Table 2) is composed of palmitic acid (55.7%) and only a small amount of this acid (15.3%) is esterified in position 2 that means that normally it is esterified in positions 1 and 3.

In fraction II-IV, almost all the middle positions are occupied by unsaturated fatty acids (oleic and linoleic). In these fractions the amount of oleic acid decreases and linoleic acid increases. In fraction V, fatty acids esterified in the 2-position consist of 36.9% oleic, 58.8% linoleic acids and in fraction VI, 17.8% oleic, 67.6% linoleic and 14.3% linolenic acids respectively.

The distribution of 18:1 depends upon total unsaturation of triglycerides. 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 triglycerides is more than three double bonds, it will be esterified in the externel 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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