

Positional distribution of fatty acids in the triglycerides of *Citrullus Colocynthis* seed oil growing in Algeria

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Abstract

Citrullus Colocynthis is one of the species of the *Cucurbitaceae* family which commonly found wild in the sandy lands of North West, the Punjab, Sind, and Central and southern India, and coromandal coast. Also found indigenous in Arabia, West Asia, and Tropical Africa and in the Mediterranean region. In this study, it was investigated as a new source of vegetable oil. The content of *Citrullus Colocynthis* seed oil of Algeria was 17.85 %, the predominant fatty acids were: palmitic acid (10.22 %), stearic acid (8.98 %), oleic acid (9.36 %) and linoleic acid (68.49 %). The distribution of fatty acids of TAG from *Citrullus Colocynthis* seed oil has been determined using Gunstone theory. The oil was found to contain TAG that were di-saturated (8.85%), mono-saturated (47.57%) and tri-unsaturated (45%). The results show that the major fatty acid entering the combination of most of the triglycerides is linoleic (L). The tri-linoleic LLL (30.39%) is the most TAG found followed by the two mono-saturated TAG: PLL (15.85%) and the SLL (13.92%).

1. Introduction

Fatty acids are carboxylic acids with a hydrophobic aliphatic chain saturated or unsaturated [1]. Belonging to the lipid category, they are subject to several nomenclatures: the standard international nomenclature, a nomenclature commonly called "omega" is a standard nomenclature [2].

Fatty acids are the major components of vegetable oils and fats. From saturated fatty acids, those C₁₂, C₁₆ and C₁₈ are the most widely distributed [3], while from unsaturated fatty acids, those C₁₈ provided with 1, 2 or 3 double bonds are the most important in the plant world and terrestrial animal. Fatty acids with 4 or more than 4 double bonds and 20-24 carbon atoms are in turn majority in the marine world [4].

The fatty acid profile of edible oils plays an important role in their stability and nutritional value. Mono-unsaturated (18:1) and poly-unsaturated (18:2) fatty acids have been found to be effective replacements for saturates as part of cholesterol-lowering diets [5]. However, it is also known that the oils with substantial amounts of unsaturation, particularly (18:2) fatty acids, are susceptible to oxidation and may produce products that contribute to arteriosclerosis and carcinogenesis. Some studies with experimental animals indicate that excessive amounts of linoleic acid promote carcinogenesis [6].

The main constituent include saturated fatty acids (SFA), mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA) that contribute in human physiology in different ways.

The distribution of the constituent fatty acids of fats has been the subject of much investigation. From this investigations, the theories and researches as Van Der Waals, Coleman, Fulton, Gunstone...[7-10], the most extensive work was done by Hilditch and coworkers, who discovered that some fats conform approximately to what they call the "rule of even distribution" [11]. In this study, Gunstone theory is used to calculate the percentage of the four groups of triglycerides: tri-saturated glycerides (GS₃), di-saturated glycerides (GS₂U), mono-saturated glycerides (GSU₂) and tri-unsaturated glycerides (GU₃) [12].

One of the possible alternative oils is the non-conventional oil from seeds of *Citrullus Colocynthis*. This plant is from *Cucurbitaceae* family [13-16], is a native of arid soils. It occupies the vast area extending from the west coast of northern Africa. It has a large, fleshy perennial root, which sends out slender, tough, angular, scabrid vine-like stems. These are usually on the ground for want of something to climb over, but which, if opportunity present, climb over shrubs and herbs by means of auxiliary branching tendrils [17-19].

The leaves are angular, lobed and, as already stated, almost the exact duplicate of watermelon leaves. The flowers are yellow, long-peduncle, and solitary in the axils of the leaves [20]. The fruit is globular, smooth, with a hard but thin rind, something like a gourd. It is filled with a soft, white pulp, in which are imbedded numerous seed. This pulp is the article used in medicine traditional [21-23] Figure 1.



Figure 1: *Citrullus Colocynthis*

2. Material and Methods

2.1. Plant material

The plant used in this study was collected in February 2013 in area "Al Mansura" in the Wilaya of Ghardaia, which is located in Algerian Sahara. The study station "Al Mansura", is located 70 km south of Ghardaia (Figure 2) [24, 25]. The identification of this plant was confirmed with the contribution of the members of the laboratory of Process Engineering, University of Laghouat.

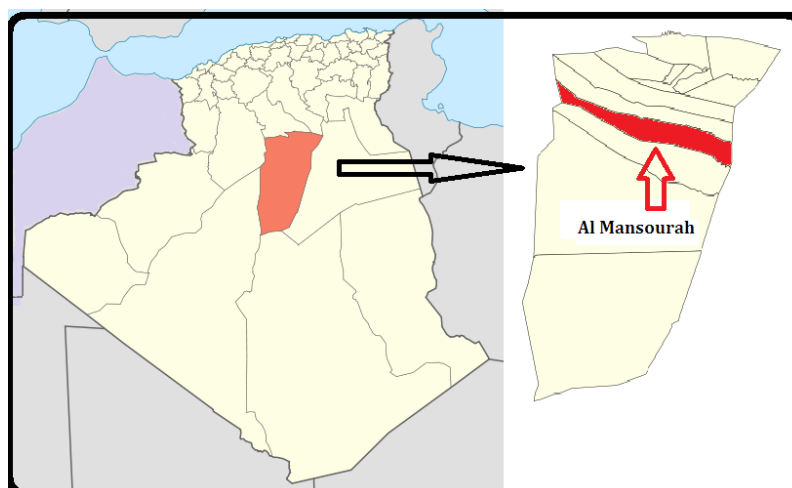


Figure 2 : Map of Algeria with the location of the Wilaya of Ghardaia and the area of Al Mansoura.

2.2. Lipid extraction

Mature fruits (68,37 g) were cut to recover seeds were finely ground and then conducted to extraction using soxhlet and hexane as solvent. After three hours, the recovered hexane is evaporated under reduced pressure at 50 ° C with pressure using a rotavapor [26], A viscous yellow oil (12,2 g) having a foul odor was obtained.

2.3. GC-MS analysis of Seed oil

The fatty acid methyl ester (FAME) composition was determined by converting them to methyl esters prepared by the method of Christie [27] and analysed by HP (Agilent technologies) 6800 Plus Chromatograph coupled by HP (Agilent technologies) MSD 5973 Mass spectrometer. The chromatograph is equipped with an FID detector and column HP-5MS (long 30 m, D int 0.25 mm) with a film thickness 0.25 μ m.

The temperatures of the injector and the detector are fixed respectively at 250°C and 280 ° C. Programming the column temperature varied from 90 ° C to 250 ° C in flow rate of 4 ° C / min and then maintained at 2 and 5 min. The carrier gas was Helium with a flow rate of 1ml/min. The peaks obtained, by injecting of methyl esters, were identified by running a standard fatty acid mixture and comparing the retention indices values [28].

2.4. Calculation of the distribution of fatty acids

Following the results of the GC/MS analysis and the composition of fatty acids and by applying the equations of Gunstone, we can determinate the composition of the triglycerides of the four groups (GS3, GS2U, GSU2 and GU3) in the fatty acid.

3. Results and discussion

The seed oil had an amber yellow colour and a very characteristic nutty flavour. The oil has also liquid state at ambient temperature with a yield (17.85%). The fatty acid compositions of the seed oils are given in Table 1.

Table1: Fatty acid (%) compositions of *Citrullus Colocynthis* seed oils and retention indices (min).

Fatty acids	<i>Citrullus Colocynthis</i> (%)	Retention indices (min)
C 14:0	0.07	24,02
C 16 : 1	0.10	28.67
C ₁₆ : 0	10.22	29.35
C 17 : 0	0.08	31.66
C 18 : 2	68.49	33.73
C 18 : 1	9.36	33.79
C 18 : 0	8.98	34 .20
C 20 : 0	0.30	38.40
C 22 : 0	0.12	42.44

From the chromatogram of the FAMES, we have set the Table 1 which indicates the relative proportions of the different fatty acids methyl esters obtained by saponification of the *Citrullus Colocynthis* oil. We notice that the *Citrulluscolocynthis* oil contains nine fatty acids commonly observed in vegetable oils, namely: Margaric acid, Palmitic acid, Linoleic Acid, Oleic Acid, Stearic Acid, Arachidic Acid, Behenic Acid, Myristic Acid and Palimtolic Acid. Unsaturated fatty acids represent 77.96% of the total fatty acids. The most present fatty acid in our study is the Linoleic Acid (C 18:2) with a percentage of 68.49%. We notice additionally a rather important value of the rate of saturated fatty acids with 19.51%; the most present saturated fatty acid is Palmitic Acid (C 16:0) with a percentage of 10.22%. If we compare our results with the ones published, we notice that the content of fatty acids are almost identical and close to the values found [30-32]. From the values of the calculated TAGs following Gunstone method, we have drawn the Table 2 which regroups the different TAGs of the majority found in the oil. The values in Table 2 were used to plot the histogram of Figure 3

The glyceride composition of *Citrullus Colocynthis* fat determined by Gunstone procedure is given in Table 3.

Based on the above results it is clear that the tri-linoleic (LLL) is the major compound triglyceride with a percentage of 30.39%, this value is responsible of the high percentage of UG₃ group (45%).

The GSU₂ group presents the highest value (47.57%), this is due to the presence of both triglycerides: PLL (15.85%) and SLL (13.92%).

The high proportion of Linoleic Acid (68.49%) accounts for the predominance of the triglyceride LLL. This acid combines with all the other saturated and unsaturated fatty acids that have the majority, to form all the TAGs which constitute the three groups trisaturated (GU_3), disaturated GS_2U and monosaturated GSU_2 and in a particular case the three fatty Acids (Oleics, Stearics and Palmitics) which form the majority triglycerides PLL, SLL and OLL. *Citrullus Colocynthis* oil does not contain a tri-saturated triglyceride.

Table 2: Triglyceride composition of the *Citrullus Colocynthis* seed oil

TAG	%
LLL	30.39
PLL	15.85
SLL	13.92
OLL	12.47
PLO	4.33
POL	4.33
SLO	3.81
SOL	3.81
SLP	3.63
PPL	2.35
LOO	1.70
SSL	1.59
Others	1.76

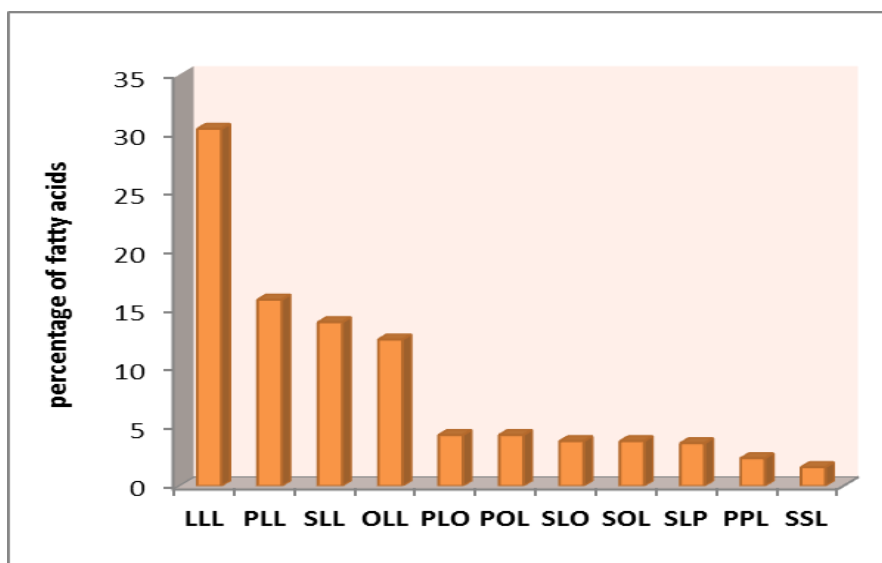


Figure 3: The main triglyceride content in the *Citrullus Colocynthis* seed oil

Table 3: The (%) of four categories of total TAGs

Categories*	% TAG
GS_2U	8.65
GSU_2	46.82
UG_3	44.52
GS_3	-

(*) GS_3 , tri-saturated glycerides; GS_2U , di-saturated glycerides; GSU_2 , mono-saturated glycerides; GU_3 , tri-unsaturated glycerides.

The extrapolation of percentage of saturated fatty acids (19.51 %) on graph published by Gunstone (Figure 4) [29] gives probably the same results found by the theory of distribution position GS₂U (9%), GSU₂ (43%), UG₃ (45.5%) and GS₃ (undetermined)

Below $s = 66.7\%$, GS₃ (SSS) do not exist, while more than this value, $s = 66.7\%$, GU₃ (UUU) and GSU₂ (SUU) are non-existent.

Gunstone theory provides that the saturation lower to ($s = 66.7\%$), the fats consist only of UUU, SU and SUS, whereas above this value, they will contain only SUS and SSS (Figure 4).

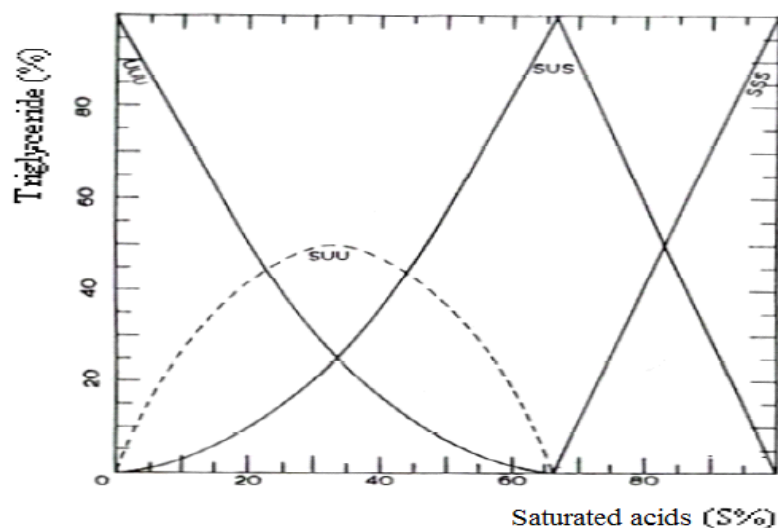


Figure 4: Glyceride composition for the positional distribution (Gunstone) [29]

Conclusion

The experimental results reported in this paper revealed that seeds of *Citrullus Colocynthis* could be potential sources of nutrient mainly of essential fatty acids ($C_{18:2}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$). The seed oil contains linoleic acid, as a major fatty acid in 68.49 %.

The distribution of TAG by Gunstone theory allowed us to establish the triglyceride structure of *Citrullus Colocynthis* oil. Triglycerides are found in oil that results from the combination of palmitic, oleic, stearic and linoleic LLL, PLLs, SLL, and OLL.

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