EXTRACTION AND COMPOSITION OF LIPIDS FROM POMEGRANATE SEED OIL FROM WEST ALGERIA

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Pomegranate trees (Punica granatum L.) are originally from Asia. They spread gradually to various regions [1], including the Mediterranean basin. In Algeria, pomegranates flourish throughout the country. The one that caught our attention is that of Beni-Snouss,a mountainous region of 835 m altitude at 34° 38′ 35″ North and 1° 33′ 41″ West , characterized by a Mediterranean climate. In this area, the Atmi variety stands out for its low consumption due to its very acidic juice.

Figure 1. *Punica granatum L.* flowers and fruits from the Beni-Snouss region

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While pomegranate seeds have traditionally been considered waste in the pomegranate juice industry, recent reports suggest that seeds offer various benefits, such as antidiabetic and anticancer activities, particularly against hormonal cancers, as described by several groups [2- 4]

Figure 2. *Punica granatum L.* Arils with seeds

The studied pomegranate variety, originating from Beni-Snouss, is non-edible due to its highly acidic juice and large-sized seeds. Therefore, the product's commercial viability relies solely on its waste, which serves as a valuable bio-source for exploration. This prompted our unique study of Algerian pomegranate seed oil (PSO) for the first time, utilizing various extraction techniques and conducting multiple analyses

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Soxhlet extraction

Two Protocols were used for such an extraction:

Method A: 20g of *Punica Granatum* seeds are placed in the extraction cartridge, which is then placed in the top part of the Soxhlet. Then, 200 mL of the solvent is charged in the bottom part and refluxed for 8 hours, before a transfer to the rotary evaporator for full solvent evaporation.

Method B: In this protocol, in order to reduce the extraction time, 20g of *Punica Granatum* seeds are placed in the extraction cartridge, which is then placed into a flask containing 200 mL of solvent. After refluxing for 30 min, both solvent and cartridge are transferred into a Soxhlet and refluxed for an additional 60 min. Solvent removal in the rotavapor.

Solvent of extraction	Solvent's boiling point (° C)	Yield in oil (%)	
		Method A	Method B
n-Hexane	68.7	15.62	16.25
Cyclohexane	80.7	14.89	13.05
Petroleum Ether	40 – 65	13.68	11.84
Chloroform	62	13.3	16.5
Isopropanol	83	17.21	17.11

Table 1: Total lipid yields of Atmi's oil extraction from seeds with method A and B

PSO Physico-Chemical Indexes

The physico-chemical analysis of oils is crucial as it reflects their nutraceutical and pharmaceutical value. This is often achieved by measuring important indices[5]. For example peroxide index measurement for Atmi PSO yielded an average value of 1.69, indicating excellent stability against oxidation and longer preservation .

Physico-chemical Property	PSO of Atmi
Refractive Index (25°C)	1.5178
Density at 20°C	0.943
Iodine Index (g I_2/100 g oil)	153.04
Acid Index (mg KOH /g)	2.24
Peroxide Index (meq O_2 /kg)	1.69
Saponification Index (mg KOH /g)	196.40
Viscosity(Pa.s)	0.191

Table 2. *Physico-chemical characteristics of the Atmi PSO.*

The measured indexes (Table 2) for the PSO from *Atmi's* variety indicate good oil quality .

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Chemical Composition of the PSO

PSO extracts were analyzed using (HPTLC) in order to determine the various lipid classes[6]

Analysis of the extracted oils revealed the following:
•Triglycerides (TAGs) constitute over 99% of the total lipids.
•Diacylglycerides (DAGs) are present in only trace amounts, approximately 0.59-1% (see Figure 3).
•Our findings align closely with those previously reported by the Kaufman team [7].

Saturated Fatty Acids (SFA)	**4.16%**
Mono-Unsaturated Fatty Acids (MUFA)	6.12 %
Poly-Unsaturated Fatty Acids (PUFA)	89.72%
Σ Unsaturated Acids	95.84%
Ratio SFA/(PUFA + MUFA)	0.043

Table 3. *Chemical composition and relative amounts of PSO fatty acids*

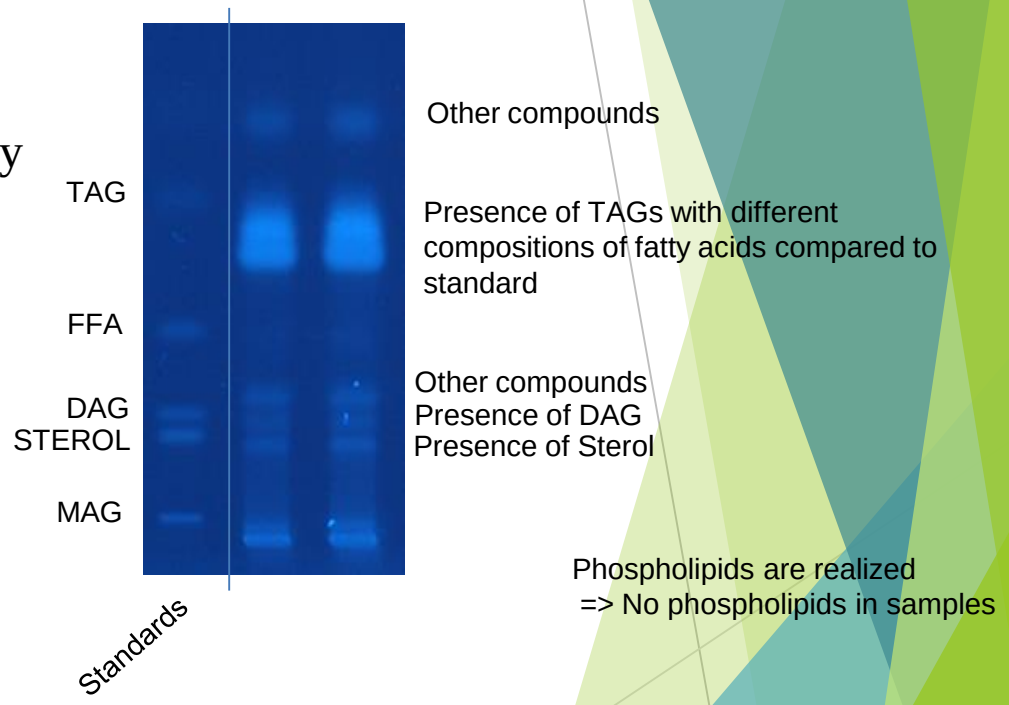

Figure 3. *Relative Profile of Lipid Classes by High Performance Thin Layer Chromatography*

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GC analysis of PSO highlighted two major compounds, namely punicic acid (C18:3) and linoleic acid (C18:2) (Figure.4). Together, they account for more than 89% of the total fatty acid composition in the extracted oils (Table 4). Oleic acid was the third most abundant fatty acid, constituting 5.69%.

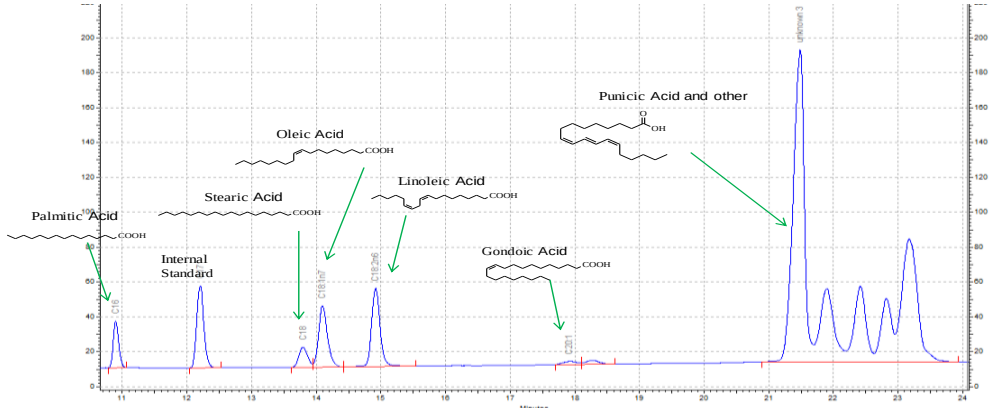

Figure 4. Profile of Fatty Acids from Oil

Fatty Acid		Percentage %
Saturated		
Palmitic Acid	$C_{16:0}$	2.43
Stearic Acid	$C_{18:0}$	1.73
Mono-unsaturated		
Oleic Acid (n-9)	$C_{18:1}$	5.69
Gondoic Acid (n-9)	$C_{22:1}$	0.43
Poly-unsaturated		
Linoleic Acid (n-6)	$C_{18:2}$	6.52
Punicic Acid (n-5)	$C_{18:3}$	83.20

Table 4. Yields (%) of fatty acid composition and lipid classes extracted from Atmi Pomegranate seeds using the Soxhlet method and hexane as solvent

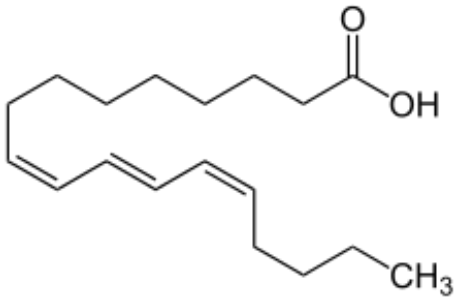

Figure 5. Structure of Punicic Acid

Punicic acid (PA) is, thus, found to be the most common ingredient in Atmi PSO. Its nutraceutical importance cannot be overstated. It improves the immune system through its anti-inflammatory and antioxidant properties [2,8]. Moreover, PA has demonstrated the ability to inhibit breast cancer cell proliferation in both estrogen-responsive and estrogen non-responsive cell lines [9]. Punicic acid severs the link between obesity and inflammation, making it anti-diabetic [10].

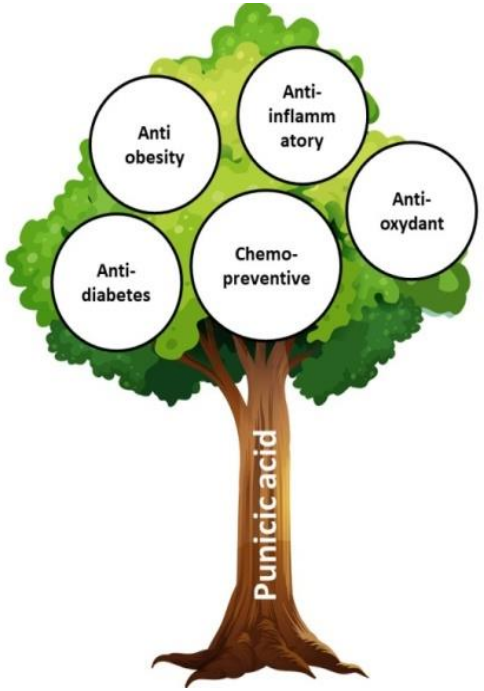

Figure 6. Biological properties of Punicic Acid[11]

The high levels of punicic acid and unsaturated fatty acids in Atmi PSO, as reported here, make this product highly valuable in the pharmaceutical and cosmetic industries

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