

# Fatty Acids Composition of Red and Purple Pomegranate (*Punica granatum* L) Seed Oil

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## Abstract

The aim of this investigation was to determine the content and composition of fatty acid in seed oil of red and purple pomegranate (*Punica granatum* L). The extraction process was performed by Soxhlet extractor with petroleum ether as solvent. The separation and identification of pomegranate seed oil was done by using GCMS. The total oil content of red and purple pomegranate were 128 g/kg d.w and 103 g/kg d.w respectively. Both showed the same major fatty acids as palmitic, stearic, oleic, linoleic and punisic acid. Oleic acid ( 19-21% ) and linoleic acid ( 20-21% ) were found as the most dominant fatty acids in red pomegranate, whereas purple pomegranate seed oil was dominated by oleic acid ( 41-43% ) and punisic acid ( 0-25% ). Neutral lipid fraction of red and purple pomegranate seed oils was more dominant than glycolipid and phospholipid. Neutral lipid fraction of red and purple pomegranate seed oil were 89 % and 91% respectively. Glycolipid fraction of red and purple pomegranate seed oil were 8 % and 5 %, whereas phospholipid fraction of red and purple pomegranate seed oil were 3 % and 4 %. The punisic acid content of total lipid of purple pomegranate seed oil (PPSO) (0-25%) was higher than red pomegranate (RPSO) (9-16%). On the contrary neutral lipid of red pomegranate showed higher punisic acid content (54-75%) than the purple pomegranate (14-55%). Glycolipid of red pomegranate contained punisic acid (0-42%). The punisic acid content of the phospholipid fraction of red pomegranate was higher (0-22 %) than the one of purple pomegranate (0-2%).

**Keywords:** fatty acid, pomegranate, *Punica granatum*, punisic acid, seed oil.

## INTRODUCTION

Pomegranate (*Punica granatum* L) which has a lot of seeds 50-70% per fruit, is a rich source of antioxidant. It has been linked to improve heart health and also indicates to protect against cancer (Hernandez, *et al.*, 2002; Lansky and Newman, 2006). According to Sing *et al.*, 2002, methanol extract of pomegranate seed oil showed the strong antioxidant effect.

The seed oil of pomegranate contain conjugated fatty acid (Conjugated Linolenic Acid/CLA or punisic acid), which is showed a potential to prevent cancer and atherosclerosis (Boussetta, *et al.*, 2009). The antioxidant effect was exhibited by its CLA are being potential used in cosmetics as anti aging. The punisic acid containing seed oil can be obtained by Soxhlet extraction by using non polar solvent.

Based on fruits color there are three kinds of pomegranate (*P. granatum*) were found in Indonesia, which are white, red and purple pomegranates. But in this study only red and purple pomegranate seed were used as the samples. The aim of this study is to determine the content and composition of fatty acid in seed oil of red and purple pomegranate (*P.granatum*), especially the punisic acid content.

## METHODS

Samples of red and purple pomegranate seeds were collected from Tegal, Bandungan and Kopeng Salatiga, Central Java The seeds were crushed and oven-dried at 60°C for two days.

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Seed oil extraction process was performed by Soxhlet extractor using petroleum ether as solvent for 6 hours. The extracted oil was dried by  $\text{Na}_2\text{SO}_4$  anhydrous, filtered and weighed to obtain the rement.

Column chromatography was used to separate neutral lipid, glycolipid and phospholipid fraction with silica gel as stationary phase and chloroform, acetone and then methanol as mobile phase. The separated samples were evaporated and weighed, so that the percentage of neutral lipid, glycolipid and phospholipid fraction can be obtained.

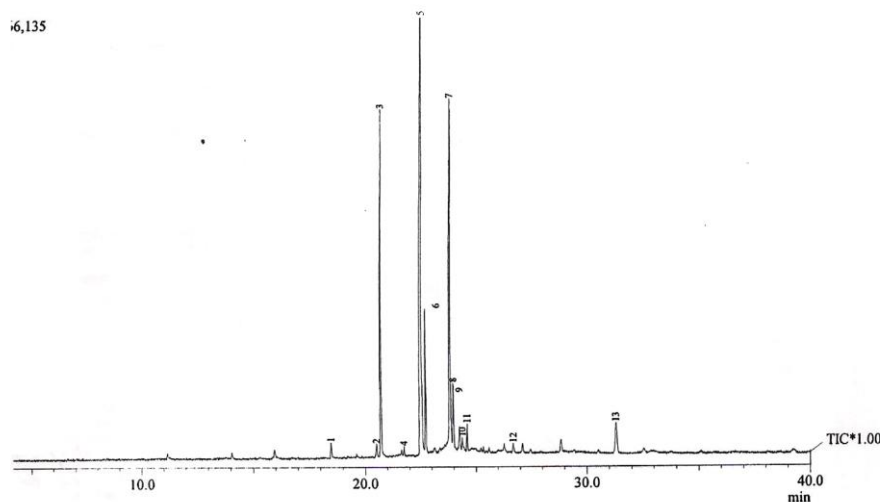
The fatty acid identification of pomegranate seed oil was done by using GCMS (SHIMADZU QP2010S) after esterification to FAME. GCMS conditions were as follows: column Rtx-5MS (30 m, ID 0.25 mm,  $80^\circ\text{C} - 5'$ ; injector:  $300^\circ\text{C} - 34'$ ; Helium as carrier gas, 16.5 Kpa, flow rate 0.50ml/min. MS: detector temperature  $300^\circ\text{C}$ , 3.00 min.

## RESULTS

The lipid content of red and purple pomegranate seed oil were 128 g/kg d.w and 103 g/kg d.w respectively. Total fatty acid of RPSO was higher than the one of PPSO. The oil color was clear yellow.

The total oil amount of neutral lipid, glycolipid and phospholipid fractions in both red and purple pomegranate showed similar pattern (Table 1). Neutral lipid was the highest and followed by glycolipid and phospholipid fraction.

The fatty acid composition of Red Pomegranate Seed Oil (RPSO) and Purple Pomegranate Seed Oil (PPSO) were compared and reported in Table 2. The fatty acid composition of both showed six major fatty acids (Figure 1)



**Figure 1. GC-MS Chromatogram Purple Pomegranate Seed Oil**

**Note:** peaks 3: palmitic acid; 5: oleic acid; 6. stearic acid; 7. linoleic acid; 8. punnic acid; 1,2,4, , 9 – 13 : minor fatty acids

**Table 1. Lipid Content of Red and Purple Pomegranate Seed Oils (%w/d.w)**

Composition	Red Pomegranate	Purple Pomegranate
Neutral Lipid	89	91
Glycolipid	8	5
Phospholipid	3	4
Total	100.00	100.00

**Table 2. Fatty Acid Composition of Red and Purple Pomegranate Seed Oils (% w/d.w)**

Fatty acid	Red Pomegranate Seed Oil	Purple Pomegranate Seed Oil
Palmitic Acid (C16-0)	0-9	17
Stearic Acid (C18-0)	9-11	7-13
Oleic Acid (C18-1n-9)	19-21	41-43.
Linoleic Acid (C18:2n-6)	20-21	0-19
Punicic Acid (C18: 3n-5)	9-16	0-25
<b>Saturates</b>	<b>9-20</b>	<b>24-30</b>
<b>Mono-unsaturates</b>	<b>19-21</b>	<b>41-43.</b>
<b>Disaturates</b>	<b>20-21</b>	<b>0-19</b>
<b>Tri-unsaturates</b>	<b>9-16</b>	<b>0-25</b>
<b>Unsaturates</b>	<b>48-58</b>	<b>41-87</b>
<b>Saturates/unsaturates</b>	<b>0.18-0.34 (±0.26)</b>	<b>0.58-0.34 (±0.46)</b>

**Table 3. Fatty Acid Composition, Neutral Lipid, Glycolipid and Phospholipid of Red and Purple Pomegranate Seed Oils (%w./d.w)**

Fatty Acid	Neutral Lipid		Glycolipid		Phospholipid	
	R	P	R	P	R	P
Palmitic Acid C16-0	0-10	6-12	0-6	11-24	0-25	35-38
Stearic Acid C18-0	3-6	4-9	0-10	4-10	10-12	12-14
Oleic Acid C18-1n-9	8-11	19-21	7-14	0-19	14-34	15-39
Linoleic Acid C18:2n-6	8-9	0-25	6-30	0-32	6-16	0-29
Punicic Acid C18: 3n-5	54-75	14-55	0-42	-	0-22	0-2

Notes : R : Red Pomegranate P : Purple Pomegranate

## DISCUSSION

In accordance with the purpose of this study in determining the total lipid content and fatty acid composition of seed oil in red and purple pomegranates, we found that PPSO had higher content of total lipid than the one of RPSO. RPSO extraction were  $\pm 128$  g/kg and PPSO was  $\pm 103$

g/kg. These values were higher than the range described by Hernandez *et al.* (2002), who reported the lipid content were approximately 70-80 g/kg and 105 g/kg. This might be caused by the different origin of the plants.

The relative abundance of fatty acids of the PPSO and RPSO were similar: palmitic, stearic, oleic, linoleic and punicic acids. The area percentage of the above mentioned fatty acids were

different between RPSO and PPSO. The major fatty acids in RPSO were linoleic acid (18:2) comprised 20-21 % of the total fatty acid. Oleic acid (C18:1) comprised 19-21 % and punicic acid (C18:3) comprised 9-16 % of the total. The remaining fatty acids were mainly stearic acid (C18:0) comprised 9-11 %, and palmitic acid (C16:0) 0-9 % from the total. The main fatty acids of PPSO oleic acid (C18:1) comprised 41-43 % of the total fatty acid, punicic acid (C18:3) comprised 0-25 %. The remaining fatty acids were mainly linoleic acid (18:2) comprised 0-19 %, palmitic acid (C16:0) 17 %, and stearic acid (C18:0) comprised 7-13 % from the total.

Table 2 also showed that the total lipid of PPSO contain a higher punicic acid (0-25 %) than RPSO (9-16 %). These percentage were far under percentage (66-79%) as reported by Hernandez *et al.* (2002). Many possibilities can influence to the result of experiment. In this study the differences of result were possible occurred due to the origin of the sample which used in this study come from e.g different place, climate and soil condition. Different places of sample will influence against plants compound composition (Inchbald, 2000). Another reason was probably different extraction methods gave different result.

The neutral lipid fraction of RPSO and PPSO showed higher punicic acid (54-75 % and 14-55 %) than glycolipid and phospholipid. These value indicates that punicic acid can be extracted more than 50 % in the neutral lipid fraction.

Concerning the ratio of saturated fatty acid/unsaturated fatty acid (Table1) were very different with the figure given by Hernandez *et al.* (2002). The differences could be understood because of the different punicic acid content, which was very important for the calculate of Saturated/unsaturated ratio.

## CONCLUSION

1. The seed oils of red and purple pomegranate showed a similar main fatty acid composition such as palmitic acid, stearic acid, oleic acid, linoleic acid, punicic acid and eicosanoic acid.

2. Total lipid of red pomegranate seed oil was dominated by linoleic acid (20-21%), oleic acid (19-21%) and followed by punicic acid (9-16%), stearic acid (9-11 %), palmitic acid (0-9 %), whereas the purple pomegranate seed oil showed oleic acid (41-43 %), punicic acid (0-25 %), followed by linoleic acid (0-19 %), palmitic acid (17 %), and stearic acid (7-13 %).

3. Total lipid of purple pomegranate seed oil have punicic acid content was higher than red pomegranate seed oil. Based on the below following fact, it could be concluded that seed oil of the purple variety was better than the red one.

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