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# Fatty acids compositional variations between the edible and non-edible fruit part of seven pomegranate varieties

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ABSTRACT

The biological importance of fatty acids in different metabolic routes and/or specific activities with medical, cosmetic, pharmaceutical interest makes it increasingly necessary to know in detail the lipid composition of foods. The objective of this work was to identify and characterize the fatty acids profile of seven pomegranate varieties with commercial interest, differentiating between its edible (seeds) and non-edible (peel plus carpellary membranes) parts, aiming to have a holistic and characteristic vision. The results confirmed the compositional fatty acids variations of the pomegranate, both between different varieties and the parts of the fruit. 29-101 variety presents a fatty acid profile with a higher potential for antifungal, antibacterial and antiviral properties. The content of punicic acid in the Kingdom variety makes it the most pomegranate varieties interesting for its nutraceutical, pharmaceutical, food and medical applications. The specific fatty acid content could define the best pomegranate variety depending on its potential use/application.

## 1. Introduction

Fatty acids (FA) could be defined as carboxylic acids with an aliphatic hydrocarbon chain. Generally, FA can be divided as short, medium and long-chain based on its number of hydrocarbons. Thus, the FAs short-chain are composed of between 4 and 6 carbon atoms, the medium-chain between 8 and 18 carbons and the long-chain contains >18 carbons (Ratnayake & Galli, 2009). In addition, FA can be saturated (SFA) or unsaturated (UFA), depending on whether they have single and/or double bonds, respectively. At the same time, UFAs, which are considered chemically more unstable, are classified based on whether they have a single double bond (monounsaturated - MUFA) or two or more double bonds (polyunsaturated - PUFA) (Lim, Singhal, Kachroo, & Kachroo, 2017).

From a nutraceutical point of view, PUFAs are considered essential for metabolism but cannot be synthesized by the body, so they must be supplied through external sources such as diet and/or nutritional supplements (Spector, 1999). Since the availability of FA depends on the diet, it is important to know and identify commercially viable sources (Laghari, Mahesar, Sherazi, Memon, & Sirajuddin, 2018).

On the other hand, the nutritional, scientific and industrial interest for the pomegranate (*Punica granatum* L.), its derivatives and by-products have increased in recent decades, due to the beneficial effects of its different biocompounds (Melgarejo-Sánchez et al., 2021; Tozzi et al., 2020).

The pomegranate fruit can be divided into two main parts, the edible and the non-edible part. The edible part is composed of the fleshy seeds contain inside the cotyledons and the embryo; while the peel and the carpellary membranes correspond to the non-edible part (Melgarejo, Núñez-Gómez, Legua, Martínez-Nicolás, & Almansa, 2020).

The present work aimed to identify and characterize the compositional spectrum of fatty acids of seven different varieties of pomegranate. The seven pomegranate varieties were selected based on their worldwide commercial interest. This work emphasized the FA characterization of the different pomegranate parts, the seeds (pomegranate edible part) and the peel with the carpellary membranes (pomegranate

*Abbreviations:* FA, Fatty acids; Nd, Not detected; SFA, Saturated fatty acid; UFA, Unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; POM, Plants with oily mesocarp.

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non-edible part), aiming to have a holistic and characteristic vision of both the pomegranate varieties and the parts of the fruits.

## 2. Materials and methods

### 2.1. Plant material

For this study, seven pomegranate varieties (*Punica granatum* L.) were chosen. The varieties were selected for their high commercial interest and relevance in both the Spanish and international markets (Tozzi et al., 2020). In addition, the varieties chosen represent the characteristic morphological diversity of the pomegranate fruits according to consumer preferences (hard and/or soft seeds and sweet, acidic and/or semi-acid varieties) and their maturation or marketing time (early, mid-season and/or late varieties). Thus, the early pomegranate varieties Acco (soft seed, semi-acid), Purple Queen (soft seed, sweet) and 29-101 (soft seed, sweet) were used; MR-100 (soft seed, sweet) as a mid-season pomegranate variety, and the late pomegranate varieties Wonderful (hard seed, acid), ME-17 (soft seed, sweet) and Kingdom (hard seed, semi-acid). The physicochemical and nutraceutical characterization (sugars and organic acids, content, antioxidant activity, etc.) of the seven pomegranate varieties considered in this work has already been carried out and published by the same authors (Tozzi et al., 2020).

The pomegranate trees used are located in Ojós (Community of Murcia) in the southeast of Spain, within an agricultural farm consolidated for the commercial pomegranate cultivation. All the trees were cultivated under homogeneous conditions and at the time of fruit harvesting the trees were in a good fitosanitary state.

For each pomegranate variety studied, 15 fruits from three different trees were manually collected according to the commercial maturity date. The pomegranate fruits number used in this work (n = 15) was defined according to the minimum sample size necessary for the sample to be representative and calculated following Eq. (1) (Ruiz-Maya, 1994).

$$n \geqslant \frac{(1.96xS)}{\left(\frac{\bar{y}}{10}\right)} \quad (1)$$

Where S represents the standard deviation of the sample and ŷ the sample mean.

In all cases, the fruits were collected from all directions of the tree, to maintain the representativeness of the samples. The selected fruits did not present any peel damage. The pomegranates were transported immediately after their collection to the laboratory, and their processing began the same day.

The pomegranate fruits of each cultivar (n = 15) were divided into three sub-samples (n = 3), with five fruits each, as standard replicates to carry out the subsequent fatty acid identification and quantification tests. All the fruits were carefully washed with tap water, and later lengthwise opened for the manual separation of the edible (seeds) and non-edible part (peel and carpellary membranes).

Once separated, the pomegranate samples for each variety (edible and non-edible parts) were immediately frozen and lyophilized (Alpha, 2–4, LSCplus, Martin Christ, Osterode am Harz, Germany) until their constant weight. Subsequently, the samples were carefully crushed and sieved (0.5 mm). All samples were stored in sterile polypropylene containers at constant temperature (−18° C) until their use.

### 2.2. Pomegranate lipids content

The total lipids content were determined both the edible and the non-edible part according to the Soxhlet method (da Cruz et al., 2021). For this, 4 g of the lyophilized samples were individually packed in filter paper cartridges (26 mm × 60 mm, Whatman ™, Kent, UK). Diethyl ether stabilized with 6 ppm BHT (PanReac AppliChem® ITW Reagents, Barcelona, Spain) was used as a solvent. The samples were kept for 2 h in the Soxhlet Selecta DET-Gras 6 (JP Selecta, Abrera, Sapin) at boiling temperature. After that, the samples were kept in an oven (60° C) for at least 24 h until constant weight as a way of guaranteeing solvent elimination. The total lipids content was calculated by weight difference. For each pomegranate part of each variety, three samples were used (n = 3). The results are presented as the mean values obtained and their standard deviation.

### 2.3. Pomegranate fatty acids (FA) composition

The FAs present in the pomegranate fruit parts were determined by Gas Chromatography (GC) using an HP-6890 chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a 100 m × 0.25 mm diameter HP-88 capillary column. internal and 0.2 μm thick (Agilent Technologies, Santa Clara, USA) and automatic injector. For all samples, the assays were carried out following the methodology and experimental conditions described by Ferrara et al. (2014). Supelco 37-Component FAME Mix reagent (Sigma Aldrich, St. Louis, USA) was used as a standard reference to identify fatty acids by comparing their retention times. The results were processed and analyzed with the G2072AA Rev. A.05.02 Chemstation software (Agilent Technologies, Santa Clara, USA). The tests for FA characterization in the pomegranate fruit parts were carried out in replicates (n = 3) and the results are presented as the average total percentage of fatty acids.

### 2.4. Statistical analysis

An analysis of variance (ANOVA) for comparisons of means was performed on the experimental data obtained. The discrimination of means, multiple range tests, was performed using Fisher's Least Significant Difference (LSD) procedure with a confidence level of 95.0%. For the treatment of statistical data, OriginLab version 2020b OriginPro software was used.

## 3. Results

For the pomegranate varieties studied, the edible part (seeds) represented between 53% and 63% of the total fruit weight, while the non-edible part (peel and carpellary membranes) was between 37 and 47% of the total weight of the fruit. (Table 1). Based on that, it is evident that the characterization and identification of the fatty acids in each fruit parts are fundamental for the efficient use, functionalization and reuse of the fruit in different pharmacological, medical, cosmetic and nutritional processes, among others.

### 3.1. Pomegranate lipid content

As mentioned above, the total lipids content was determined for the seven pomegranate varieties, differentiating between the edible and the non-edible parts of the fruit. The results obtained are shown in Table 2. The total lipids in dry matter ranged between 1.28 and 4.47 g 100 $g^{-1}$ in the non-edible part, and between 5.54 and 9.76 g 100 $g^{-1}$ in the

**Table 1**
Representativeness in % of the total fruit weight, of the edible part (seeds) and non-edible part (peel and carpellary membranes) of each of the seven pomegranate varieties studied.

| Pomegranate variety | Non-edible part (%) | Edible part (%) |
|---|---|---|
| Wonderful | 42% | 58% |
| ME-17 | 44% | 56% |
| Acco | 43% | 57% |
| Kingdom | 47% | 53% |
| 29-101 | 46% | 54% |
| MR-100 | 37% | 63% |
| Purple Queen | 42% | 58% |

**Table 2**
Total lipid content (g 100 $g^{-1}$) identified in the edible (seeds) and non-edible (peel and carpellary membranes) of the seven pomegranate varieties studied. The values correspond to the mean (n = 3) and standard deviation. Within the same column, different letters mean statistically significant differences according to the Fisher test ($\rho \leq 0.05$).

| | Total lipid content (g 100 $g^{-1}$) | |
|---|---|---|
| **Pomegranate variety** | **Non-edible part** | **Edible part** |
| Wonderful | 2.83 (0.10)b | 6.98 (0.05)c |
| ME-17 | 1.70 (0.22)c | 5.54 (0.30)d |
| Acco | 2.83 (0.48)b | 9.04 (0.23)d |
| Kingdom | 4.47 (0.23)a | 9.76 (0.04)a |
| 29-101 | 2.46 (0.17)b | 5.67 (0.18)d |
| MR-100 | 2.77 (0.17)b | 6.45 (0.26)c |
| Purple Queen | 1.28 (0.32)c | 5.73 (0.27)d |

pomegranate edible part. The results showed statistically significant differences between the varieties.

The semi-acid pomegranate varieties, Kingdom and Acco, showed higher lipid content in the edible part (9.76 g 100 $g^{-1}$ Kingdom and 9.04 g 100 $g^{-1}$ Acco) with values considerably higher than the other varieties that remained in the range between 5 and 6 g 100 $g^{-1}$, while the sweet pomegranate varieties, ME-17, 29-101 and Purple Queen, presented the lowest lipid content with values between 5.54 and 5.73 g 100 $g^{-1}$.

In the non-edible and edible pomegranate part, the higher lipid content was identified for Kingdom variety (4.47 g 100 $g^{-1}$), followed by Wonderful, Acco, MR-100, and 29-101 which lipid content values between 2.83 g 100 $g^{-1}$ and 2.46 g 100 $g^{-1}$ without statistical significant differences, while the Purple Queen variety (1.28 g 100 $g^{-1}$) and ME-17 (1.70 g 100 $g^{-1}$) showed the lowest values in the non-edible pomegranate part respectively.

If compared the total lipid content between the edible and non-edible pomegranate fruit parts for each variety, Acco and Kingdom showed the greatest differences between their fruit parts, with a total lipid content in the edible part five times higher than that identified for its non-edible part. For Purple Queen and Wonderful was four times greater, while the rest of the varieties (ME-17, MR-100 and 29-101) was only three times greater.

### 3.2. Fatty acids characterization in the pomegranate fruits parts

#### 3.2.1. Fatty acid profile of edible parts

For the pomegranate seeds, 30 different FA were identified and quantified. Although most of these FA were present in all pomegranate varieties seeds, some presented significant and differentiating characteristics between them, either due to their presence and/or absence (Table 3). The Wonderful edible part, with 26 FA identified, was the variety with the greatest diversity of fatty acids, while for the ME-17 edible part only 21 FA were identified. Thus, the classification, in relation to the number of fatty acids identified, was Wonderful > Acco > Purple Queen = Kingdom > MR-100 > 29-101 > ME-17. However, the 29-101 edible part showed the highest percentage of unidentified fatty acids (5.48%). A significantly higher percentage compared to the other pomegranate varieties that oscillated between 0.04%−0.29%. For the Wonderful edible part, all its FA were identified.

Almost all the edible pomegranate parts of the studied varieties presented a similar compositional trend for saturated and unsaturated FA. However, while the 29-101 edible part presented the highest

**Table 3**
Fatty acids composition (relative abundance %) in the edible fruit part (seeds) of seven pomegranate varieties cultivated in the southeast of Spain. The values represent the mean (n = 3). The different letters within the rows indicate significant differences according to the Fisher test ($\rho \leq 0.05$).

| | | POMEGRANATE VARIETY | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Fatty acid (FA) (%)** | | **Acco** | **Wonderful** | **Purple Queen** | **Kingdom** | **MR-100** | **ME-17** | **29-101** |
| C10:0 | Capric acid | Nd | 0.03a | 0.05a | 0.03a | 0.36b | 0.04a | 6.59c |
| C11:0 | Hendecanoic acid | 0.57a | 1.23b | 1.67c | 0.88d | 1.29b | 1.55c | 3.87e |
| C12:0 | Lauric acid | 0.17a | 0.17a | 0.18a | 0.13a | 0.15a | 0.11a | 0.51b |
| C13:0 | Tridecylic acid | Nd | 0.02a | Nd | Nd | Nd | Nd | Nd |
| C14:0 | Myristic acid | 0.09a | 0.14b | 0.06a | 0.03c | 0.08a | 0.10a | 0.19d |
| C14:1 | Myristoleic acid | 0.05a | 0.08b | 0.11b | 0.05a | 0.06a | 0.08b | 0.22c |
| C15:0 | Pentadecylic acid | 0.06a | 0.20b | 0.28b | 0.20b | 0.48c | 0.57d | Nd |
| C15:1 | – | 0.02a | 0.02a | 0.03a | Nd | Nd | Nd | 0.10a |
| C16:0 | Palmitic acid | 3.80a | 3.69a | 4.69b | 3.14c | 4.15a | 5.12b | 10.87d |
| C16:1 | Palmitoleic acid and derivates | 0.08a | 0.35b | Nd | Nd | Nd | Nd | 0.14c |
| C17:0 | Margaric acid | 0.06a | 0.05a | 0.08a | 0.05a | 0.14b | 0.08a | 0.26c |
| C17:1 | Margaroleic acid | Nd | 0.03a | Nd | Nd | Nd | Nd | 3.27b |
| C18:0 | Stearic acid | 2.45a | 1.71b | 1.44c | 1.67b | 1.85b | 1.94b | 4.71d |
| C18:1n9t | Elaidic acid | 9.67a | 7.72b | 5.34c | 5.63d | 5.05c | 5.03c | 21.33d |
| C18:1n9c | Oleic acid | 5.90a | 11.80b | 14.08c | 8.44d | 9.42d | 11.73b | 29.38e |
| C18:1n7 | *cis*-Vaccenic acid/asclepic | 0.26a | 0.22a | 0.25a | 0.21a | 0.23a | 0.24a | Nd |
| C18:2cis9,12 | Linoleic acid | 0.64a | 0.57b | 0.57b | 0.45c | 0.66a | 0.73a | 1.81d |
| C20:0 | Arachidic acid | 0.58a | 0.60a | 0.56a | 0.59a | 0.44b | 0.45b | 0.72c |
| C18:3cis6,9,12γ | γ-Linolenic acid | 0.29a | 0.27a | 0.45c | 0.23b | 0.23b | 0.40d | 0.51e |
| C20:1cis11 | – | 0.05a | 0.05a | Nd | 0.02a | 0.04a | 0.12b | 0.20c |
| C18:3cis9,12,15α | α-Linolenic acid | 0.18a | 0.15a | 0.27b | 0.14a | 0.32b | 0.46c | 0.73d |
| C20:2 | Eicosadienoic acid | Nd | Nd | Nd | Nd | 0.13a | Nd | Nd |
| C22:0 | Behenic acid | 0.05a | 0.08a | 0.06a | 0.10b | Nd | 0.22c | Nd |
| C18:3 | Punicic acid and derivates | 74.30 | 68.69 | 68.68 | 77.28 | 72.34 | 70.30 | 6.38 |
| C20:3 | DGLA. Dihomo-γ-Linolenic | Nd | 0.29a | Nd | Nd | Nd | Nd | Nd |
| C22:1cis13,16 | – | 0.21a | 0.21a | 0.19a | 0.10b | 0.11b | 0.06b | Nd |
| C24:0 | Lignoceric acid | Nd | 0.91a | 0.30b | 0.12c | 1.77d | Nd | Nd |
| C24:1cis5 | Nervonic acid/Selacholeic acid | 0.04a | 0.35b | Nd | 0.04a | Nd | Nd | 2.33c |
| *Unidentified fatty acid* | | *0,04a* | *0.04a* | *Nd* | *0.05a* | *0.16b* | *0.29c* | *0.09a* |
| | **∑ SFA** | **7.84** | **8.84** | **9.38** | **6.93** | **10.73** | **10.18** | **27.71** |
| | **∑ UFA** | **91.70** | **90.79** | **89.97** | **92.58** | **88.58** | **89.15** | **66.41** |
| | ∑ MUFA | 16.25 | 20.47 | 20.00 | 14.44 | 14.90 | 17.26 | 54.64 |
| | ∑ PUFA | 75.42 | 69.97 | 69.97 | 78.10 | 73.55 | 71.90 | 9.43 |
| | Ratio UFA/SFA | 11.69 | 10.23 | 9.59 | 13.36 | 8.26 | 8.76 | 2.31 |
| | Ratio SFA/UFA | 0.09 | 0.10 | 0.10 | 0.07 | 0.12 | 0.11 | 0.43 |

Nd: Not detected; SFA: Saturated fatty acid; UFA: Unsaturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

percentages of saturated FA (SFA) with 27.71%, for the Kingdom edible part it did not exceed 7% (6.93%). The other pomegranate edible parts presented intermediate values, but in all cases with significant differences.

The unsaturated FA (UFA) content in 29-101 edible part shown significant differences when compared with the edible parts of the other varieties of pomegranate. In this way, while for the 29-101 edible part the UFA represented 66.08% of the identified FA, for the other varieties the percentage was higher than 88% with Kingdom > Acco > Wonderful > Purple Queen > ME-17 > MR−100.

If differenced within the UFA, the polyunsaturated FA (PUFA) and monounsaturated (MUFA), the difference between the pomegranate varieties becomes more significant. Since while the 29-101 edible part presented 9.43% for PUFA and 54.64% for MUFA, the other six pomegranate varieties presented completely inverse results, that is, higher proportions of PUFA, with values between 78.10% and 69.97% (Kingdom > Acco > MR-100 > ME-17 > Wonderful > Purple Queen), than of MUFA whose results were between 20.47%−14.44% (Wonderful > Purple Queen > ME-17 > Acco > MR-100 > Kingdom). In all cases, the results were shown significant differences between them.

Palmitic acid (C16: 0), stearic acid (C18: 0), elaidic acid (C18: 1n9t), oleic acid (C18: 1n9t), nonadecanoic acid (C19: 0), and punicic acid and its derivatives (C18: 3) were the main FA identified in edible fruit samples of all pomegranate varieties. The rest of FA can be considered a minority because they represent mean values lower than 1% (Sidorov & Tsydendambaev, 2014).

For six of the seven pomegranate varieties studied in this work, punicic acid and its derivatives (C18: 3) were the main fatty acid identified in the edible part, with values between 77.28% and 68.69% (Kingdom > Acco > MR-10 > ME-17 > Wonderful > Purple Queen). These values show a very significant difference when compared with the 29-101 edible part result, which presented a percentage of punicic acid and its derivatives of 6.38%.

A similar trend between the pomegranate varieties was observed for oleic acid and elaidic acid content. Therefore, 29-101 edible part presented the highest amounts for both FA (29.39% and 21.33% respectively). These results were much higher when compared to the content in the other varieties quantified in the range between 9.67% (Acco) and 5.03% (ME-17) for elaidic acid, and between 14.08% (Purple Queen) and 5.90% (Acco) for oleic acid. All pomegranate varieties except Acco presented a concentration of oleic acid between 1.3 and 2.6 times higher than elaidic acid.

Capric acid (C10: 0) was significantly higher in 29-101 edible part in contrast to the other pomegranate varieties studied, where it did not represent >1% of the FA spectrum. It was not identified in the Acco edible part. The amount of hendecanoic acid (C11: 0) was highly variable among the edible pomegranate parts with values of 3.87% for 29-101 or 0.58% for Acco.

Palmitic acid (C16:0) also showed significant differences between the values obtained. While the highest percentages were quantified (10.87%) in the 29-101 edible part, the Kingdom edible part presented the lowest amounts (3.14%). The results were not shown significant differences between the edible parts of the pomegranate varieties Acco (3.80%), Wonderful (3.69%) and MR-100 (4.15%) and between the edible parts of the Purple Queen (4.69%) and ME-17 (5.15%) varieties. Palmitic acid was the most abundant SFA in all pomegranate edible part. On the other hand, palmitoleic acid and its derivatives (C16: 1) were only identified in Acco (0.08%), 29-101 (0.14%) and Wonderful (0.35%) with significant differences between them and with values < 1 %.

The highest content of margaric acid (C17: 0) was identified for 29-101 (0.27%) with a value much higher than in the other six pomegranate varieties (between 0.05% and 0.14%). Margaroleic acid (C17: 1) was only detected, with significant differences, in the edible parts of Wonderful and 29-101, 0.03% and 3.27% respectively. 29-101 edible part also presented the highest content for linoleic acid, both for α-Linoleic (C18: 2cis9,12,15α) and γ-Linoleic (C18: 2cis9,12γ). The higher content of the γ-Linoleic (C18: 2cis9,12γ) was identified for 29-101 and Purple Queen edible parts (0.51% and 0.45% respectively), while for the other varieties that did not exceed 0.30%. In addition, the (C13: 0) and (C20:2) acids were only quantified in Wonderful and MR-100, respectively. The Wonderful edible part was the only one in which the presence of DGALA (Dihomo-γ-Linolenic) was identified, although in low proportions (0.29%), which may be of interest for its identification, at least among the varieties studied.

### 3.2.2. Fatty acid profile of non-edible parts

A total of 23 FA were identified for the pomegranate non-edible parts (peel and carpellary membranes) (Table 4). The Kingdom non-edible part was the variety with the most compositional diversity of FA, with a total of 22, while Purple Queen and Acco only presented 16 and 17 FA, respectively. On the other hand, 1.50% of the FA for 29-101 non-edible part were not identified. This percentage is much higher when compared to the results for Kingdom, MR-100 and ME-17 (with values between 0.24% and 0.46%) and Wonderful and Acco (between 0.11% and 0.08%). All the FA of the Purple Queen were identified.

The hendecanoic (C11: 0), palmitic (C16: 0), elaidic (C18: 1n9t), oleic (C18: 1n9c) and γ-Linoleic (C18: 3cis6,9,12γ) acids were the most abundant FA in the pomegranate non-edible part for all the varieties studied with percentages > 90%. The results for hendecanoic acid (C11: 0) showed significant differences between the varieties. Therefore, the non-edible parts of Kingdom and 29-101 quantified the highest proportions with values > 5%, while for Purple Queen, Wonderful and ME-17 the percentage was close to 4%. The highest palmitic acid (C16: 0) content was detected for Wonderful (9.39%) followed by the MR-100 (8.98%) and Purple Queen (8.75%), and the lowest content was for Acco (6.69%) and Kingdom (6.47%). The results showed significant differences. The minimal and maximal content of elaidic acid (C18: 1n9t) were identified for Kingdom (8.90%) and Purple Queen (16.25%) respectively. The non-edible parts of Kingdom (68.71%) and Acco (66.85%) showed the highest values of oleic acid (C18: 1n9c), while in Wonderful, MR-100 and Purple Queen the results were fixed around 57%. In all the non-edible parts studied, the oleic acid content was between 3.5 and 7.7 times higher than elaidic acid content.

The highest percentages for γ-Linolenic acid (C18: 3cis6,9,12γ) were identified for the Wonderful non-edible part (6.75%), while for Kingdom it was barely 4.33%. The non-edible part characterization results indicated that, for all the pomegranate varieties studied, the content of γ-Linolenic acid was much higher than the α-Linolenic acid content. Related to punicic acid and its derivatives (C18: 3), the 29-101 non-edible part (0.40%) presented the lowest value, while Purple Queen and ME-17 represented between 2.88% and 4.31%, respectively.

Lauric acid (C12: 0) was only identified in Kingdom (0.03%), Wonderful (0.06%) and 29-101 (0.07%). No significant differences were detected. On the other hand, palmitoleic acid and its derivatives (C16: 1) were quantified only in MR-100 (0.18%) and Kingdom (0.02%) with significant differences. (C24: 0) and (C20:2) acids were only detected in Acco and Kingdom non-edible parts, respectively, but with values lower than 1%.

The non-edible pomegranate parts results were uniform and presented the same trend of the predominance of UFA over SFA. In this sense, saturated FA (SFA) represented between 7% and 10% of the total FA, where 29-101 > Kingdom > Acco > Purple Queen > Wonderful > ME-17 > MR-100; while > 90% corresponded to unsaturated FA (UFA) with Purple Queen > ME-17 > MR-100 > Kingdom = Wonderful > Acco > 29-101. The richness of the pomegranate non-edible part in monounsaturated FA (MUFA), which represent >80% of the UFA, still stands out. The ME-17 non-edible part presented the highest values of polyunsaturated FA (PUFA) with 9.87%, this result contrast significantly with the 6.07% determined for 29-101.

### 3.2.3. Edible vs non-edible fraction

In addition, and in order to identify and study the variations in the

**Table 4**
Fatty acids composition (relative abundance %) in the non-edible fruit part (peel and carpellary membranes) of seven pomegranate varieties cultivated in the southeast of Spain. The values represent the mean (n = 3). The different letters within the rows indicate significant differences according to the Fisher test ($\rho \leq 0.05$).

| | | POMEGRANATE VARIETIES | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Fatty acid (FA) (%)** | | **Acco** | **Wonderful** | **Purple Queen** | **Kingdom** | **MR-100** | **ME-17** | **29-101** |
| C10:0 | Capric acid | 0.15b | 0.63a | 0.55a | 0.06d | 0.08c | 0.09c | 0.60a |
| C11:0 | Hendecanoic acid | 5.33b | 4.96c | 4.85c | 5.77a | 5.46b | 4.84c | 5.79a |
| C12:0 | Lauric acid | Nd | 0.06a | Nd | 0.03b | Nd | Nd | 0.07a |
| C13:0 | Tridecylic acid | 0.11d | 0.13d | 0.33c | 0.31c | 0.50a | 0.15d | 0.38b |
| C14:0 | Myristic acid | 0.47b | 0.35 cd | 0.37c | 0.48b | 0.34d | 0.40c | 0.52a |
| C16:0 | Palmitic acid | 6.69e | 9.39a | 8.75b | 6.47e | 8.98c | 7.65d | 7.63d |
| C16:1 | Palmitoleic acid and derivates | Nd | Nd | Nd | 0.02a | 0.18b | Nd | Nd |
| C17:0 | Margaric acid | 0.13b | 0.26a | Nd | 0.09bc | 0.10b | 0.07c | Nd |
| C17:1 | Margaroleic acid | Nd | Nd | Nd | 0.08b | 0.13ab | 0.27a | 0.04c |
| C18:0 | Stearic acid | 0.77e | 0.84d | 0.97c | 0.69ef | 1.01b | 1.30a | 0.67ef |
| C18:1n9t | Elaidic acid | 9.82d | 14.94bc | 16.25a | 8.90e | 15.36b | 9.54d | 12.58c |
| C18:1n9c | Oleic acid | 66.85b | 57.66d | 57.09d | 68.71a | 57.92d | 63.65c | 62.41c |
| C18:1n7 | *cis*-Vaccenic acid/asclepic | 7.34d | 10.84ab | 10.26b | 8.01 cd | 11.60a | 9.21c | 8.92c |
| C18:2cis9,12 | Linoleic acid | 0.32e | 0.54a | 0.42c | 0.35d | 0.58a | 0.47b | 0.51ab |
| C20:0 | Arachidic acid | Nd | 0.15c | 0.19b | 0.15c | 0.17bc | 0.17bc | 0.25a |
| C18:3cis6,9,12γ | γ-Linolenic acid | 6.11b | 6.75a | 6.14b | 4.33e | 6.04c | 5.34d | 5.27d |
| C20:1cis11 | – | Nd | 0.37a | 0.09c | 0.33ab | 0.26b | 0.37a | 0.08c |
| C18:3cis9,12,15α | α-Linolenic acid | 0.18e | 0.45a | 0.22d | 0.36c | 0.35c | 0.22d | 0.40b |
| C20:2 | – | Nd | Nd | Nd | 0.21a | Nd | Nd | Nd |
| C22:0 | Behenic acid | 0.18b | 0.19b | Nd | 0.11c | 0.09d | 0.21ab | 0.25a |
| C18:3 | Punicic acid and derivates | 1.66c | 1.26d | 2.88b | 1.55 | 0.91e | 4.31a | 0.40f |
| C24:0 | Lignoceric acid | 0.67a | Nd | Nd | Nd | Nd | Nd | Nd |
| *Unidentified fatty acid* | | *0.08d* | *0.08d* | *0.11e* | *Nd* | *0.42b* | *0.46a* | *0.24c* |
| | **∑ SFA** | **8.11** | **7.95** | **8.02** | **8.17** | **7.72** | **7.88** | **10.16** |
| | **∑ UFA** | **91.96** | **92.27** | **92.93** | **92.51** | **92.74** | **92.91** | **90.10** |
| | ∑ MUFA | 84.01 | 83.81 | 83.69 | 86.06 | 85.45 | 83.04 | 84.02 |
| | ∑ PUFA | 7.95 | 8.46 | 9.25 | 6.45 | 7.29 | 9.87 | 6.07 |
| | Ratio UFA/SFA | 11.34 | 11.61 | 11.59 | 11.33 | 12.01 | 11.80 | 8.87 |
| | Ratio SFA/UFA | 0.09 | 0.09 | 0.09 | 0.09 | 0.08 | 0.08 | 0.11 |

Nd: Not detected; SFA: Saturated fatty acid; UFA: Unsaturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

fatty acid profile identified for each variety and fruit part, a statistical analysis two-way ANOVA was carried out. The analysis was performed only considering the fatty acids present in all the varieties and in both parts of the fruit, in order to maintain the homogeneity of the results and guarantee their adequate interaction analysis. The analysis followed the same methodological principles described in materials and methods.

Only 10 of all the FA identified in the pomegranates were present in both all the varieties and in the two fruit parts. The statistical analysis results are presented in Table 5, where it can be observed that both factors (variety and part of the fruit) are significant in the FA content. The fruit part was highly significant ($p < 0.01$) for all FA except for palmitic and eladic acids ($p < 0.001$) and γ-Linolenic acid that was not significant. In the same line, the pomegranate variety factor was significative for almost all the FA at 99.9% confidence level ($p < 0.001$), except for stearic acid ($p < 0.01$), oleic acid ($p < 0.05$) and elaidic acid that was not significant.

**Table 5**
Statistical FA content relation between pomegranate variety and pomegranate fruit part. The different symbol within the rows indicate significance according to the two-way ANOVA test (*: $\rho < 0.05$; **: $\rho < 0.01$; and ***: $\rho < 0.001$).

| | | Pomegranate factor | |
|---|---|---|---|
| **Fatty acid (FA)** | | **Variety** | **Fruit part** |
| C11:0 | Hendecanoic acid | *** | *** |
| C14:0 | Myristic acid | *** | *** |
| C16:0 | Palmitic acid | *** | ** |
| C18:0 | Stearic acid | ** | *** |
| C18:1n9t | Elaidic acid | ns | ** |
| C18:1n9c | Oleic acid | * | *** |
| C18:2cis9,12 | Linoleic acid | *** | *** |
| C18:3cis6,9,12γ | γ-Linolenic acid | *** | ns |
| C18:3cis9,12,15α | α-Linolenic acid | *** | *** |
| C18:3 | Punicic acid and derivates | *** | *** |

Ns: Not significant.

## 4. Discussion

In relation to the pomegranate fruit parts representativeness, edible and non-edible, the results agree within the ranges reported in the bibliography but always considering the variations according to the genotype (Ferrara et al., 2014; Martínez, Melgarejo, Hernández, Salazar, & Martínez, 2006; Singh, Singh, Kaur, & Singh, 2018; Tozzi et al., 2020). On the other hand, the comparison of the pomegranate edible part percentages with the bibliography can be considered limited due to the methodological variability used in the different studies. This methodological variability is mainly based on botanical gaps related to the correct terminology of the pomegranate fruit (Melgarejo et al., 2020).

In recent years, there has been a notable increase in the interest of the scientific community in the identification and characterization of FA from fruits and/or vegetables, either for their direct use in the food industry or for their subsequent functionalization and application in other industries such as cosmetic, pharmaceutical, medical, agricultural, among others (Melgarejo-Sánchez et al., 2021). Although already have published studies focused on the FA characterization of the pomegranate seeds, to our knowledge the number of researches focused on the pomegranate non-edible part is still limited, but highly desirable due to its high content of bio compounds, which makes it a by-product with high commercial, nutraceutical, medical and/or pharmaceutical interest.

The results indicated a potential relationship between lipid content and pomegranate variety type (semi-acid > acid > sweet). Fadavi et al. (2006) observed the same relationship identified in the present study. Thus, the semi-acid varieties had the highest total fat content, followed by the acid and sweet varieties, which in this case received Kingdom and Acco > Wonderful > ME-17, MR-100, 29-101 and Purple Queen. No bibliographic references have been found that directly relate the total lipid content and the sugar content in plants, which can explain in some way this relationship in the pomegranate fruits.

However, and although the pomegranate is considered a fruit with

low aromatic intensity (Beaulieu & Stein-Chisholm, 2016), it has been identified, in other fruits, the relationship between FA on flavor volatiles compounds, mainly due to the activity of lipoxygenase (Ties & Barringer, 2012), which could indicate some type of correlation that explains this classification. Additional studies should be carried out aiming to identify metabolic interrelationships if any.

In general, the results agree with the bibliography, with ranges consistent with the data already published for pomegranates grown in warm climatic conditions (Garima & Akoh, 2009; Hajib et al., 2021; Melgarejo & Artés, 2000). However, the results are divergent when compared with American varieties of pomegranate grown in humid climatic conditions, for which a total lipid content, in its edible part, >18% was determined, even reaching, in some cases, at 21% (Garima & Akoh, 2009).

In this study, Garima and Akoh (2009) reported a total lipid content for the whole fruit between 0.2 and 0.3%, much lower than that reported in this study for the inedible part (>1.28%). These divergences could be attributed both to the methodology used by Garima and Akoh (2009), once they used a method of extraction and purification of total lipids specific for animal tissues and not for plant material, as well as to the cultivation conditions of pomegranates (Schwartz et al., 2009).

The FA identification and quantification in the pomegranate edible parts made it possible to confirm the compositional richness of all the varieties based on the number of FA identified (30). This compositional diversity, for all the pomegranate varieties studied, is higher than the results reported in seeds of other varieties as, for example, for Italian varieties, sixteen FA were identified, eleven in Turkish varieties, ten in Moroccan varieties, and only six in varieties grown in Mexico (Ferrara et al., 2014; Hajib et al., 2021; Kýralan, Gölükcü, & Tokgöz, 2009; Rojo-Gutiérrez et al., 2021).

Bar-Ya'akov et al. (2019) indicated that these differences in the content of FA could be mainly related to the genotype studied, however, the climatic and cultivation conditions seem to indicate a greater impact on the composition of the fruit. In this sense, Schwartz et al. (2009) evidenced and confirmed that pomegranates grown in dry and warm weather conditions contained higher amounts of FA in the seeds. These climatic conditions are the characteristics of southeastern Spain, the growing region of the varieties studied, so the results would confirm the significant compositional richness of the varieties grown in this area.

The differentiated composition of 29-101 edible part stands out, which presented higher proportions of Capric acid (C10: 0), Hundecanoic (C11: 0), Palmitic (C16: 0), Stearic (C18: 0), Elaidic (C18: 1n9t), Oleic (C18: 1n9c), Linoleic (C18: 2cis9,12) and Nervoric (C24: 1cis5), with significant differences with the other varieties. The highest abundance of Elaidic acid (C18: 1n9t) and Oleic (C18: 1n9c) identified in 29-101 edible part (21.33% and 29.38% respectively), when compared to the other pomegranate varieties (≥9.67% for elaidic acid and ≥ 14.0.8% for oleic acid) could indicate and confirm its potential applicability for antifungal, antibacterial and antiviral activities (Debbabi et al., 2017; Galbraith, Miller, Paton, & Thompson, 1971; Novak, Clark, & Dupuy, 1961). In addition, the content of Capric acid (C10: 0), Hundecanoic (C11: 0), would considerably increase its functionalization for antifungal and antimicrobial activities (Liu et al., 2008). Note that, although in most of the published works, carpic and hendecanoic acids are not normally identified, El-Nemr et al. (1990) quantified it as the majority SFA with an abundance > 36%, but mainly related to the juice. Value well above that determined for seed 29-101 (6.59%), as well as for the other varieties (<1%). On the other hand, the high content of Palmitic acid (C16: 0) could indicate its potential use in both medicine and industry (Deaver et al., 2020; Prasath, Tharani, Kumar, & Pandian, 2020; Wang, Zhu, Coomes, Haghseresht, & Lu, 2005). Based on that, and from a practical/applicable functional point of view related to the composition of the FA present in the edible part of 29-101, the suitability of this plant material could be indicated in preference to other varieties for antifungal and antibacterial activities among others (Liu et al., 2008).

In this study, the highest content of punicic acid and its derivatives (C18: 3) was identified for the Kingdom seed (77.28%), although all varieties were within an expected range (68–77%) and in accordance with the bibliography (Amri et al., 2017; Bar-Ya'akov et al., 2019; Melgarejo & Artés, 2000). 29-101 edible part showed considerably lower values (6.38%) but in agreement with those reported for pomegranate varieties cultivated in Indonesia (≥9%) (Soetjipto, Pradipta, & Timotius, 2010). No works have been found that can explain the variability of punicic acid and its derivatives among pomegranate varieties, so the most plausible explanation could be related to the specific genotype of each one.

The higher content of punicic acid and its derivatives in the Kingdom seed would indicate the suitability and prioritization in its pomegranate variety choice for nutraceutical, pharmaceutical, food and/or medical applications focused on the functional activities that punicic acid and its derivatives present (Beatty et al., 2021; Mouas, Kabouche, Benssuici, & Chaoui, 2021; Ngo Njembe et al., 2021). However, further research must be carried out in this field if it is intended to use and/or attribute objective medical and pharmaceutical effects to punicic acid and its derivatives extracted from the pomegranate.

Regarding the non-edible pomegranate parts, although the total number of FA was lower than that identified for their edible parts, with values between 22 and 16 FA, these results were also higher than those indicated for pomegranate varieties grown in Georgia. (USA) where only 11 FA were identified (Garima & Akoh, 2009).

The non-edible pomegranate parts showed higher UFA percentages than the edible parts in all varieties and whose values are according with those published for other varieties and/or growing conditions, such as the Turkish (89–93%), those grown in Mexico (ca. 86%), Tunisian (84–92%), Iranian (89–92%) and Chinese (87–89%) and even for different pomegranate varieties but grown in the same region, and therefore, subjected to similar environmental/climatic conditions (Momeni & Asadi-Gharneh, 2021; Rojo-Gutiérrez et al., 2021). Again, it should be noted that 29-101 seed, although within this line, presented the most divergent fatty acid profile, with a higher proportion of SFA compared to the other varieties studied and even with other Spanish varieties already studied (Melgarejo & Artés, 2000).

In general, SFA/UFA ratio presented values between 0.07 (Kingdom) and 0.43 (29-101) much higher than those previously reported (Melgarejo & Artés, 2000).

If it is considered that, at a biological level, SFAs are related to facilitating lipid adhesion in immunological and circulatory cells, and UFAs are attributed to the inhibition of aggregates, decreasing their levels (Balk et al., 2006; Connor, 2000), based on the results could be confirmed the potential of using the pomegranate, mainly the inedible part, as a relevant agent in the prevention of cardiovascular diseases since they mainly contain UFA. In this sense, the inedible part of variety 29-101 still stands out, where the differences between SFA and UFA were more significant.

Palmitic and stearic acids are common in pomegranate fruit regardless of the variety studied or the cultivation conditions, however, the reported values are highly variable depending on these parameters (Costa, Silva, & Torres, 2019; Laghari et al., 2018; Melgarejo & Artés, 2000). In general, they were found in higher proportions in the edible part than in the non-edible part, possibly due to their influence on the formation of the lipid membrane and triglycerides (Sidorov & Tsydendambaev, 2014).

On the other hand, relevant differences were observed when identifying MUFA and PUFA. Thus, while, in all the edible pomegranate part samples, except 29-101 as indicated above, PUFA was the predominant fatty acid type, in the non-edible pomegranate parts, without exceptions, the predominant FA were MUFA. This different behavior can be attributed to the high content of punicic acid and its derivatives in the seeds since it has not been detected in any sample of the non-edible part. Punicic acid and its derivatives are one of the most important and well-known FA present in pomegranate fruit, mainly due to its positive effects on human health and different metabolic pathways (Hajib et al., 2021;

Mphahlele, Fawole, Makunga, & Linus Opara, 2017; Nekooeian, Eftekhari, Adibi, & Rajaeifard, 2014). The absence of punicic acid in the non-edible pomegranate parts could suggest that the biological effects reported in studies carried out only with the pomegranate peel, would not be related to punicic acid and its derivatives, therefore, as has already been highlighted, they would be necessary additional research to clarify the results.

If analyze the results of the FA characterization (Fig. 1 for the same pomegranate variety, it can confirm that the acid and semi-acid pomegranate varieties (Acco, Wonderful and Kingdom) have a homogeneous FA spectrum between their parts, concerning the SFA and UFA, while the soft and sweet pomegranate varieties (Purple Queen, MR-100 and ME-17) show small compositional oscillations between these groups of FA. However, for all pomegranate varieties, significant differences are observed when MUFAs and PUFAs are considered, with a predominance of MUFAs in the non-edible part and PUFAs in the edible part, as has already been highlighted. This trend is not met for the 29-101 edible part. To our knowledge, no comparative bibliographic references have been found between the edible and non-edible parts of different pomegranate varieties in relation to their compositional FA profile, which would confirm and demonstrate the relevance and novelty of this work.

In accordance with previous studies, the results confirmed the

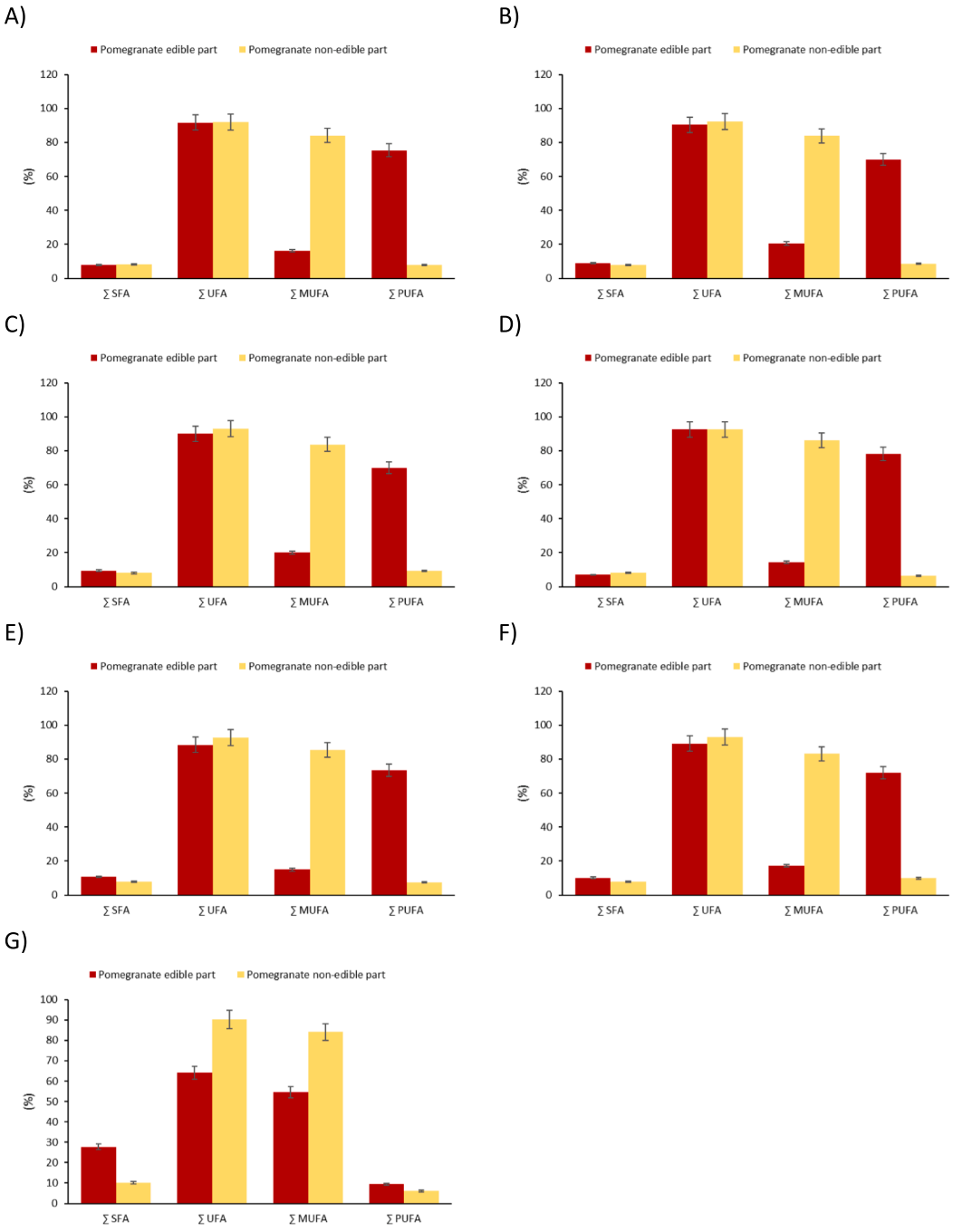


**Fig. 1.** Comparative representation of the FA compositional variations for the edible (seed) and non-edible (peel and carpellary membranes) parts of seven pomegranate varieties cultivated in the southeast of Spain, where a) represents the Acco pomegranate variety; b) Wonderful; c) Purple Queen; d) Kingdom; e) MR-100; f) ME-17; g) 29-101. SFA: saturated fatty acid; UFA: unsaturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

presence of γ-Linolenic, elaic and oleic acids in all pomegranate samples (Costa et al., 2019; Fadavi et al., 2006; Laghari et al., 2018; Melgarejo & Artés, 2000) with higher proportions in the non-edible parts compared to the edible parts.

Based on the results, the whole pomegranate fruit could be classified as "plants with oily mesocarp" (POM). POM are those plants in which significant amounts of oils are accumulated in other parts of the fruit other than the seeds (such as, for example, mesocarp, pericarp, etc.) (Sidorov & Tsydendambaev, 2014). This oil accumulation, together with other nutrients, make these parts of the fruit attractive to animals, promoting efficient seed dissemination by them (Berry, 1981).

## 5. Conclusions

- The number of pomegranate FA identified in this work, 30 FA in the edible part and 22 FA in the non-edible part, is much higher than those reported in the bibliography, resulting in a more complete fatty acid profile for pomegranate fruit.
- The genotype of each variety can be defined as the main responsible for the lipid composition of the edible and non-edible pomegranate parts.
- Among all the pomegranate varieties studied, the 29-101 edible part presents a fatty acid profile with a higher potential for its specific use due to its potential antifungal, antibacterial and antiviral properties. On the other hand, the content of punicic acid and its derivatives in the Kingdom makes it the most pomegranate varieties interesting for its nutraceutical, pharmaceutical, food and medical applications.
- The non-edible parts presented more MUFA percentages than the edible pomegranate parts, while the edible part has higher PUFA than the non-edible.
- The high pomegranate UFA content, both the edible and non-edible part, would confirm the great potential of this fruit in the prevention of cardiovascular diseases.
- Linoleic, elaic and oleic acids are present in higher proportions in the non-edible pomegranate parts compared to the edible parts.
- The results confirmed the compositional FA variations of the pomegranate, both between different varieties and between the parts of the fruit. Although most varieties do have a different compositional profile, it would be appropriate to periodically characterize the varieties, with and without modifications of the controlled growing conditions, to try to identify a "specific varietal footprint" of each of the varieties, which would facilitate its identification, conservation and application in different industrial, nutritional, pharmaceutical use, among others.

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