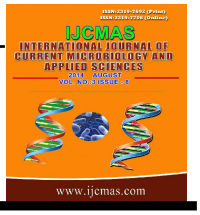
Int.J.Curr.Microbiol.App.Sci (2014) 3(8) 778-792

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 8 (2014) pp. 778-792**
http://www.ijcmas.com



Original Research Article

Natural conjugated and *trans* fatty acids in seed oils and phytochemicals in seed extracts issued from three Tunisian pomegranate (*Punica granatum*. L) cultivars

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Keywords

Pomegranate seed oil; conjugated fatty acids; *trans* fatty acids; phytochemicals

A B S T R A C T

Fatty acid profiles of pomegranate seed oils (PSOs), phytochemicals and antioxidant activities of pomegranate seed extracts (PSE) and pomegranate seed residue extracts (PSRE) of three Tunisian (*Punica granatum*. L) cultivars were investigated. PSOs lipid characterization showed 32 fatty acids (FA). *trans* MUFAs represent more than 50% of total MUFAs and exceptionally characterised by the presence of elaidic acid C18:1 n9 (*trans*) that significantly differed among the studied cultivars ($p<0.05$). However, PUFAs that seemed most important (83.77%) were mainly characterised by the presence of conjugated fatty acids (CFAs) including conjugated linoleic acids (CLAs) and conjugated linolenic acids (CLnAs), essentially, punicic acid that was the major fatty acid in PSOs ranging from 35.27 to 38.64%. The highest total phenolic content (TPC) was determined as 1.17 mg GAE /g DW of PSE in Tounsi cultivar. Acceptable antioxidant activity of PSE and PSRE were found in Tounsi cultivar with 38.57 and 21.51 % respectively.

Introduction

Pomegranate (*Punica granatum L.*) is one of the oldest edible fruits widely spread in Mediterranean countries, Iran, India and to some extent in the U.S. (California), China, Japan and Russia (Fadavi et al., 2006). In Tunisia, the cultivation of pomegranate has taken a great development in recent years. The pomegranate is cultivated in all regions with a concentration in the southern oases (Gabes) and the northwest region (Testour). Other production centers are located in the coastal stations (Mars and Gaaliche, 1993). Pomegranate seed as a byproduct of pomegranate processing is about 20% (w/w) of the whole fruit depending on cultivar (Tehranifar et al., 2010). Recently, it has been shown that pomegranate seed could potentially be a good source of nutrients and antioxidants in association with the presence of bioactive compounds, particularly polyphenols. Recent studies showed significant levels of phenolic content in pomegranate seeds (Elfalleh et al., 2011). Moreover, the lipid composition of pomegranate seeds is attracting more attention (Fadavi et al., 2006). Consumers

are more likely concerned with the saturated/unsaturated fatty acids ratio in the diet due to their beneficial biologic effects (Koji and Teruyoshi, 2005) and are notably interested to essential fatty acids, with emphasis on the health potential of unsaturated fatty acids (UFAs). However, UFAs should not be lumped together. With rare exceptions, almost all edible fats and oils of plant origin contain UFAs in the cis conformation (Wolff et al., 1998). *trans* fatty acids (TFAs) are UFAs containing one or more double bonds in the *trans* configuration. The physicochemical, nutritional, biochemical and biological properties of the TFA isomers are different from those of the corresponding *cis* isomers (Ledoux et al., 2000). Conjugated linoleic acid (CLAs) are a family of positional and geometric isomers of linoleic acid 18:2n-6 (c9,c12) in which all carbon–carbon double bonds are conjugated. The major naturally-occurring isomer of CLA is rumenic acid 18:2 (c9, t11) and it is found in plant oils and dairy products (Chin et al., 1992). Conjugated linolenic acid (CLNA) is a collective term for the positional and geometric isomers of octadecatrienoic fatty acid (FA). Natural seed oils of certain plants include conjugated trienoic fatty acids that are isomers of α-linolenic acid (LnA, 9c12c15c-18:3). Pomegranate seed oil contains punicic acid (PA, 9c11t13c-18:3). Suzuki et al (2001) reported a strong cytotoxic effect of CLNA from catalpa, tung, and pomegranate on human monocytic leukemia cells.

The extracts of pomegranate seeds were reported with antidiarrhoeal and antioxidant bioactivities (Singh et al., 2002). Most of these functional components were detected in pomegranate seed oil. However, the antioxidant and chemical constitutes of pomegranate seed residue (defatted pomegranate seed), which are considered as agricultural wastes, have not been reported except one investigation conducted by Li et al (2011).

To our knowledge, no comparable study has been carried out previously on Tunisian-pomegranate geometric and positional FA isomers. The present study highlights the characterization of the fatty acid profile, the antioxidant compounds, and the antioxidant properties in order to investigate the potential conversion of the polar and lipid fractions of pomegranate into value-added products.

Materials and Methods

Mature pomegranates from "Tounsi, Nabli, Gabsi and Chelfi" cultivars were manually picked from Testour (governorate of Beja) in the northwest region of Tunisia (36.55°N, 9.45°E) during the autumn campaign (October 2011). Samples were immediately peeled and seeds were carefully separated by hand from the pulp, and dried in oven at 40°C until constant weight. Then, seeds were crushed and sieved to obtain fine powders and were extracted with methanol according to the method of Mau et al (2001). The pomegranate seed extracts (PSE) were kept at -20°C in the dark for further use.

Lipid extraction and Fatty Acid Methyl Esters (FAMEs) analysis

About 10 g of powdered seeds were used. Lipid extraction was carried out with a Soxhlet extractor with petroleum ether and then the solvent was removed by evaporation at 40°C. FAMEs were prepared, following the procedure described by Mekni et al (2013) (a) . FAMEs were identified by comparing their relative and absolute retention times to those of authentic *cis*-fatty acid and *trans* fatty acids standards. The pomegranate seed residues issued from

soxlhet extraction undergo same methanolic extraction as PSE to finally obtain pomegranate seed residue extracts (PSRE) which were also kept in same conditions as PSE.

Determination of total phytochemicals

Total phenolic contents and O-diphenols of methanolic fractions were determined according to the method of (Montedoro et al., 1992) with minor modifications (Mekni et al., 2013) (b). Results were expressed on a dry weight (DW) basis as mg gallic acid equivalents (GAE) /g of sample. Total flavonoid contents of the extracts were determined according to the colorimetric assay developed by (Zhishen et al., 1999). The results were also expressed on a dry weight (DW) basis as mg catechin equivalents (CEQ)/ g of sample. Determination of condensed tanins was based on the procedure reported by Sun et al (1998) with slight modification (Mekni et al. 2013) (b). The amount of tannins was expressed as mg tannic acid /g of dry weight (mg TA/g DW).

Reducing power, ABTS and DPPH radical scavenging assay

The reducing powers of the studied extracts were determined according to the method described by Chung et al (2005). The reducing powers of the tested samples increased with the absorbance values and expressed as mg/ml of Ascorbic acid (AA). The ABTS+ radical cation scavenging activity of each sample was determined according to the literature (Yvonne et al. 2005). The antioxidant capacity was expressed as % of ABTS+ reduction.The DPPH method (Kontogiorgis and Hadjipavlou-Litina, 2005) was used to determine antioxidant activity of studied sample extracts. The % reduction values were determined and compared to appropriate standards. Inhibition of the free radical DPPH was calculated in percent (%).

Statistical analyses

All data were subjected to analyses of variance (ANOVA one-way) using SPSS.17.0. (SPSS, Chicago, IL, USA). The data shown are mean values (n = 3) and the significance of the differences was compared using Duncan multiple range test at $P < 0.05$ probability level. The Hierarchical Cluster Classification (HCC) of the fatty acids of each cultivar, was carried out using the same software. Simple associations between variables were calculated as the Pearson correlation. Principal component analysis (PCA) was carried out and the antioxidant components as well as the antioxidant activity of each cultivar were used as variables in PCA using XLStat-Pro version 7.5.2 for Windows (Addinsoft, New York, USA).

Results and Discussion

Total phenolics, O-diphenols, flavonoids and tannins content

Results concerning the differences between the amounts of phenolic compounds (including total polyphenols, O-diphenols, flavonoids and tannins) in PSE and PSRE are registered in Table 1. Indeed, our findings showed that PSRE had obviously lower levels of phenolic compounds than amounts found in PSE ($p<0.05$), which suggest that the major part of phenolic compounds is probably located in the oil fraction. In almost cases, significant divergences among cultivars were stated ($p<0.05$). The TPC observed for the cultivars involved in the present study slightly decreased after oil extraction. The Tounsi cultivar exhibited the highest level of

total phenolic content TPC (1.17 and 0.68 mg GAE/g DW, in PSE and PSRE respectively) followed by Nabli and Gabsi varieties. However, O-diphenol amounts have been obviously reduced from 0.31 in PSE to 0.06 mg GAE/g in PSRE for the Nabli cultivar which exhibited the highest total O-diphenols content among all the tested seeds. The other cultivars also registered a strong reduction in their amounts after lipid extraction.

Total flavonoid content (TFC) of PSE among the studied cultivars ranged from 1.72 to 1.31 mg CEQ/g DW and was exhibited in Nabli cultivar. TFC was nearly eight folds higher before lipid extraction compared to that of defatted seeds, where values decreased to 0.15 and 0.19 mg CEQ/g DW. According to Table 1, it could be stated that TFC and total O-phenols contents have been most affected by oil extraction. Indeed their amounts after removing the fatty portion decreased strongly.

Researches focusing on the pomegranate seeds flavonoid levels are rare. For instance, we could mention those conducted by Jing et al (2012). They have found values lower than ours ranging from 0.42 to 0.62 mg CEQ/g DW in Jingpitian and Suanshiliu varieties respectively. The data suggested that these pomegranate seeds could potentially serve as natural source of dietary bioactive compounds.

Total tannins content (TTC) significantly varied from 1.78 to 1.49 mg TA/mg DW in the three varieties. We have also noticed highly significant correlations between levels of TTC and TPC ($r = 0.891$, $p < 0.01$), O-diphenols ($r = 0.812$, $p < 0.01$) and those of TFC ($r = 0.808$, $p < 0.01$). This result has been previously confirmed through many publications which reported that tannins are one of the most potent polyphenols having the highest antioxidant activity among different organs of pomegranate. As reported by Seeram et al (2006), pomegranate seeds could be considered as the richest pomegranate part in tannins.

Antioxidant activity

Reducing power

During the current study, no significant differences among the three studied pomegranate seed varieties have been found, neither before nor after lipid extraction process, except for Tounsi cultivar which showed a higher content (0.59 mg AA/ml; $p<0.05$) (Table 1). Nevertheless, we found that the reducing power of the studied seeds was strongly associated with their phenolic content. Indeed, acceptable significant correlations were observed with the reducing power and the TPC ($r = 0.521$, $p < 0.05$), O-diphenols ($r = 0.535$, $p < 0.05$), TFC ($r = 0.518$ $p < 0.05$) and TTC ($r = 0.710$, $p < 0.01$) indicating that the antioxidant capacity of pomegranate seed might be attributable to total phenolic content. Our results are in accordance with those of Jing et al (2012) who found significant correlation between phenolics and ferric reducing power.

$ABTS^+$ scavenging activity

As shown in Table 1, all the studied samples endowed with an ability to scavenge the $ABTS^+$ cation, showing a range between 58.09 to 66.44 % for PSE and 40.67 to 48.13 % for PSER ($p<0.05$). Once again, Gabsi and Nabli exhibited the highest percentages for both pomegranate seeds and defatted pomegranate seeds. This antioxidant capaicty was found to be highly correlated with TPC, TFC, O-diphenols and TTC ($r=0.670$, $r=0.777$, 0.755 and $r=0.693$, $p<0.01$, respectively). These results are in

accordance with previous works reporting high antioxidant power in pomegranate (Xu et al., 2011 ; Jing et al., 2012).

DPPH radical scavenging capacity

According to our findings (Table 1), we could emphasize that all the tested PSE exhibited acceptable antioxidant power where percentage of inhibition ranged from 34.21 to 38.57 % with no significant differences. However, after lipid extraction, radical scavenging capacity decreased significantly in the extracted residues. We noticed that the "Nabli" and "Gabsi" varieties possessed the highest percentages followed by "Tounsi" variety. Our results were similar to those found by Jing et al (2012). Compared to previous works dealing with other plants such as red grapes (Bozan et al., 2008) and soybean (Slavin et al., 2009), it might be suggested that pomegranate seeds could be considered as a promising source of antioxidant compounds. Differences among the DPPH radical scavenging values could be attributed to the genotypes and growing conditions which might influence the antioxidant activity in pomegranate seeds (Jing et al., 2012). Indeed, there was a clear interdependence between the percentages of DPPH inhibition and TPC ($r = 0.811$, $p < 0.01$), TFC ($r = 0.805$, $p < 0.01$), total O-diphenols content ($r = 0.759$ $p < 0.01$) and TTC ($r = 0.804$, $p < 0.01$) indicating that the antioxidant capacity of pomegranate seed could be associated with phenolic compounds. In addition, a significant correlation was stated between DPPH radical scavenging activity and the reducing power of pomegranate seeds ($r = 0.605$, $p<0.01$) and with ABTS scavenging ability (0.582, $p<0.05$). These results could be comparable to those of Zaouay et al (2012) who found significant correlation between analytical methods DPPH, ABTS and reducing power used to determine the antioxidant power of pomegranate cultivars. They suggested that the antioxidant activity is proportional to the amount phenolic compounds. Madrigal-Carballo et al (2009) also considered the presence of good correlation between the DPPH scavenging ability and the reducing power. Nevertheless, it is important to mention the significant correlation between ABTS and reducing power tests in our research work.

Chemometric Analysis

The principal component analysis (PCA) (Fig 1) was carried on the database of total phenolic compounds, DPPH scavenging activity and the reducing power of the three studied cultivars of pomegranate seeds. PCA accounted for 77.84% of the total variance (88.46%) on the first component while the second component accounted 10.59%. Fig. 1 shows the presence of two distinctive groups. The first group is composed of seed samples prior to lipid extraction process. While the second group is characterized by the same samples but after being extracted with soxlhet. The first group is positively associated with PC1 while the last group is negatively associated with PC1 which was dominated by the following variables: total phenolics, total O-diphenols, total flavonoids, total tannins, the reducing power and finally DPPH scavenging activity. However, this study showed that these components are totally absent at PC2. These results suggest that the major amount of phenolic compounds of pomegranate seeds is concentrated in the lipid fraction. It seemed that these compounds act synergistically, to contribute to the reducing power and to the radical scavenging activity of the seeds. Hence, could it be possible to suggest that this antioxidant power might be generated by the fatty acids present in the pomegranate seeds ?

Total lipid contents

Oil contents within the studied samples of variety "Nabli" and" "Tounsi displayed the highest lipid content which were respectively of 21.88 and 19.37 % (data not shown), while the lowest content was that of the variety "Gabsi" (15.57 %). By comparing our results with those previously found, we noticed that the total lipid content of our samples were much higher than those found by Hernandez et al (2000) and Fadavi et al (2006) but were similar to those found by Kýralan et al (2009). Therefore, we could refer these variations to several factors such as the genetic variability and environmental differences additionally to the use of different extraction process and parameters (Eikani et al., 2012). It is also important to mention that the seed oil yields of our samples were very low compared to other plants, which could partly explain the high price of pomegranate seed oils.

Fatty acids composition of pomegranate seed oils (PSOs)

Saturated fatty acids (SFAs)

The different SFAs in the seeds of *Punica granatum* L. represent 8.27% of total fatty acids (TFA) (Table 2). Among the different components of this fraction, we found that the Palmitic acid (C16: 0) was the major SFA with 3.28 % for the variety "Tounsi", 3.37 % for variety "Gabsi" and 2.84 % for the variety" Nabli", followed by Stearic acid (C18:0) ranging from 1.93 (Nabli and Tounsi) to 1.49% (Gabsi). The Arachidic acid (C20:0) was present in lesser amount as well as Behenic acid (C22:0) and Lignoceric acid (C24:0). It is important to emphasize that Lauric acid and Myristic acid were present in trace (p<0.05). Our results were not consistent with those suggested by El-Nemr et al (1990) who found that PSO exhibited 83.6% of SFA Some studies have not identified the presence of behenic acid in their oils (El-Nemr et al., 1990 ; Yücel, 2005). Melgarejo and Artes (2000) demonstrated that SFAs such as palmitic acid, the most abundant fatty acid in the human diet, causes oxidative DNA damage, necrosis and apoptosis in human cells *in vitro*, but when consumed with other fatty acids, like PUFAs which were detected in pomegranate seed oils, is unlikely to have any significant impact on human health.

Unsaturated fatty acids (USFAs)

Monounsaturated fatty acids (MUFAs)

The detailed investigation of the fatty acids in PSOs showed 7 *cis* MUFAs having low proportions with 4.24% of total FAs in Tounsi (Table 2). According to our data, we noticed that there were significant differences in the composition of MUFAs (p<0.05) including Myristoleic acid (C14:1); Palmitoleic acid (C16:1 *cis*); Margaroleic acid (C17:1); Vaccenic acid (C18:1 n7 *cis*) and Nervonic acid (C24:1), except for Gadoleic acid (C20: 1). The content of oleic acid (C18:1 n9 *cis*) ranged from 0.68 to 1.56 % (Table 2). Our results did not coincide with those reported by Melgarejo and Artes (2000) in which they showed a level of 3.7 to 20.3% for C18:1. Similarly, Fadavi et al (2006) found highly variable levels for 25 different pomegranate cultivars (0.4 to 17.4%). Furthermore, other researchers have not detected the presence of Gadoleic acid in their samples (El-Nemr et al., 1990 ; Yücel, 2005; Fadavi et al., 2006)**.**

For all studied PSO cultivars, *trans* MUFAs presenting more than 50% of total MUFAs. Elaidic acid (C18:1 n9 *trans*) was found to be the major *trans* MUFA with 5.35 % (Tounsi), 4.09 % (Nabli) and 3.16 % (Gabsi) (Table 3**).** *trans* MUFA isomers with 18-carbon chain length (*trans*-18:1) are some of

the predominant TFAs present in the human diet. In observational studies utilizing biomarkers of TFAs consumption, both 18:1 and 18:2 isomers appear to contribute to risk of CVD (Uauy et al., 2009). A recent study provides evidence for a direct effect of TFAs on liver dysfunction causing the disturbances in liver lipid metabolism that result in NAFLD that is a key component of the cardiometabolic syndrome. This suggests that TFAs may influence risk factors for CVD (Dhibi et al., 2010).

Polyunsaturated fatty acids (PUFAs)

Although unsaturated fatty acids (UFA) represent 91.38% of total FA, the *cis* form of this fraction did not exceed the 12% in all studied pomegranate cultivars. The omega-3 PUFAs were represented by the α-linolenic acid (C18: 3 n-3) and eicosatrienoic acid (C20: 3 n-3). Indeed, the amounts of α-linolenic acid were in the order of 0.08% for Tounsi seeds and 0.09% for Nabli and Gabsi seeds with no significant difference as well as for the eicosatrienoic acid **(**Table 2**)**. The omega 6 PUFAs family was characterized by the presence of linoleic acid C18:2 n6 (*c*9, *c*12), γ-linolenic acid (C18:3 n6); Eicosadienoic acid (C20:2 n6) and dihomo-γ-linolenic acid (C20:3 n6). The most important PUFA omega 6 as shown in Table 2, was the C18: 2 n-6 (*c*9, *c*12) having a level of 7.28 to 5.88% in Tounsi and Nabli, respectively. Boden et al (2005) suggested that linoleic acid and linolenic acids belonging to ω6 and ω3 family, respectively, are essential for normal growth, health promotion, and disease resistance in man. Both have been considered to have very important roles in physiology, especially during fetal and infant growth, in particular in the formation of the central nervous system and retina and for the prevention of cardiovascular diseases, having antithrombotic and anti-inflammatory proprieties (Carvalho et al., 2011). In addition we could consider that they might have antioxidant potential since we found good correlation between some DPPH scavenging activity and some ω6 fatty acids including C18 :2 n6 (*c*9, *c*12), C18:3 n6 with r=0.767 and r=0.718, p<0.05, respectively.

The studied cultivars presented SFA/UFA ratio of 0.091, 0.084 and 0.095 in "Tounsi", " Nabli and "Gabsi", respectively indicating that these varieties contained high levels of UFA, making them more suitable to the consumer wishing to ingest healthy fats. At the contrary, Fadavi et al (2006) found higher ratio (0.05 to 0.37). These differences seemed to be attributed to several factors including genetic diversity (Galli and Marangoni, 2006). By contrary, more attention might be paid to the *trans/cis* ratio which seems very high for MUFA fraction reaching the 1.34 for Nabli cultivar (Table 3).

For PUFAs geometric configurations, two *trans* linoleic acid isomers were detected in the three studied cultivars C18:2 n6 (*t*9, *c*12) and C18:2 n6 (*c*9, *t*12)]. The C18:2 n6 (*c*9, *t*12) represent more than 90% of total *trans* linoleic acid for Nabli and Gabsi cultivars that significantly (p<0.05) differs from those detected in Tounsi PSO with 27% for the C18:2 n6 (*t*9, *c*12) isomer (Table 3).

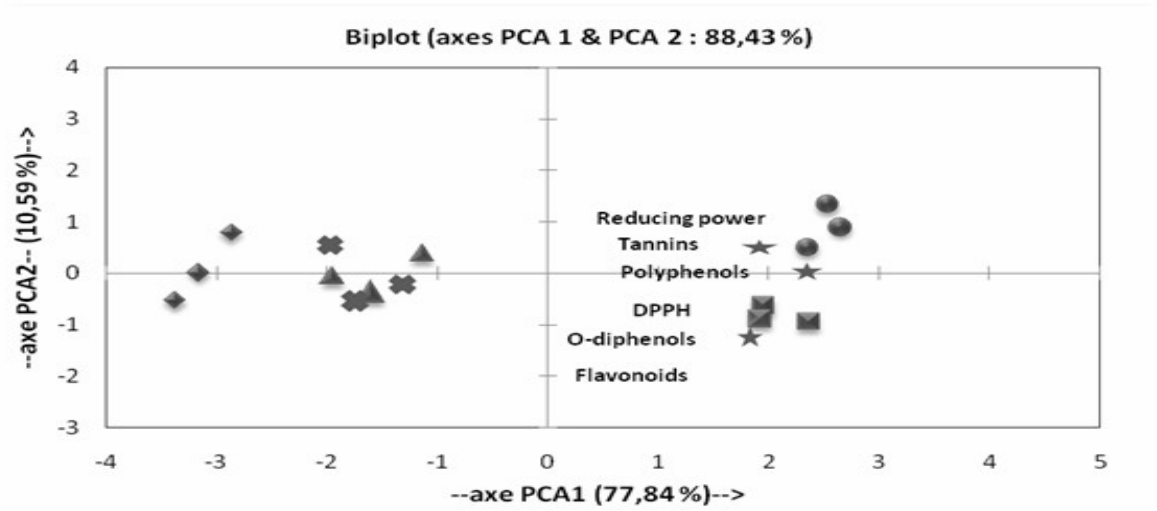
As illustrated in Fig. 2, eight different Conjugated fatty Acids (CFAs) were identified in the PSO, including three Conjugated Linoleic Acids (CLAs) namely **:** [C18:2 (*t*9, *t*11)-CLA]; [C18:2 (*c*11, *t*13)-CLA] and [C18:2 (*t*10, *c*12)-CLA]; and five Conjugated Linolenic Acids (CLnA) namely Punicic acid C18:3 (*c*9, *t*11, *c*13); Jacaric acid C18:3 (*c*8, *t*10, *c*12); α-Eleostearic acid C18:3 (*c*9, *t*11, *t*13); Catalpic acid C18:3 (*t*9, *t*11, *c*13); β-eleostearic acid C18:3 (*t*9, *t*11, *t*13). A typical chromatogram of the studied CLnAs is illustrated in Fig. 3.

Table.1 Phytochemicals (mg/g DW) and antioxidant capacities of PSEs and PSREs in three Tunisian pomegranate cultivars

	Polyphenols	O-diphenols	Flavonoïds	Tannins	Reducing power (mg AA/ml)	DPPH radical scavenging assay (I%)	$ABTS^+$ cation scavenging (%)
Pomegranate Seed Extracts (PSE)							
Tounsi	1.17 ± 0.001^{e}	0.25 ± 0.03^{c}	1.31 ± 0.22^{b}	1.78 ± 0.06^{d}	0.59 ± 0.06^{b}	38.57 ± 4.62^{c}	58.09±5.93$^{b.c}$
Nabli	1.09 ± 0.02$^{d.e}$	0.31 ± 0.04^{d}	1.72 ± 0.06^{d}	1.49 ± 0.19^{c}	0.49 ± 0.05^{a}	37.10 ± 5.73^{c}	64.22±11.01^{c}
Gabsi	1.04 ± 0.04^{d}	0.26 ± 0.07^{c}	1.49 ± 0.09^{c}	1.58 ± 0.19$^{c.d}$	0.5 ± 0.09^{a}	34.21 ± 7.72^{c}	66.44±10.77^{c}
Pomegranate Seed Residue Extracts (PSRE)							
Tounsi	0.68± 0.02^{a}	0.08 ± 0.00$^{a.b}$	0.15 ± 0.02^{a}	0.54 ± 0.08^{a}	0.43 ± 0.03^{a}	21.51 ± 3.43^{a}	40.67±8.39^{a}
Nabli	0.97± 0.05^{c}	0.06 ± 0.01^{a}	0.15 ± 0.08^{a}	1.03 ± 0.23^{b}	0.43 ± 0.03^{a}	27.29 ± 3.14$^{a.b}$	48.13±3.37$^{a.b}$
Gabsi	0.89 ± 0.04^{b}	0.1 ± 0.00^{b}	0.19 ± 0.04^{a}	1.04 ± 0.06^{b}	0.45 ± 0.04^{a}	27.13 ± 6.15$^{a.b}$	47.41±6.36$^{a.b}$

Values are expressed as means ± standard deviation (n = 3). Means with different letters in the same column were significantly different at the level of $p < 0.05$

Fig 1: Principal component analysis based on phenolic compounds, DPPH radical scavenging activity and reducing power in PSEs* and PSREs** obtained from three varieties of pomegranate seeds



*PSEs : Tounsi ( ) Nabli ( ) Gabsi ( )
**PSREs : Tounsi ( ) Nabli ( ) Gabsi ( )

Table.2 Saturated and cis unsaturated Fatty acids composition (%) in Pomegranate Seed Oils (PSO) of three Tunisian cultivars

		Tounsi	***Nabli***	***Gabsi***
Saturated fatty acids (SFAs)	-	**8.51 ± 1.60**	**7.80 ± 1.43**	**8.51 ± 1.27**
Lauric acid	C12:0	0.18±0.02^{c}	0.12±0.01^{b}	0.08±0.02^{a}
Myristic acid	C14:0	0.23±0.11^{b}	0.18±0.05^{ab}	0.06±0.01^{a}
Palmitic acid	C16:0	3.28±0.57^{a}	2.84±0.05^{a}	3.37±0.21^{a}
Stearic acid	C18:0	1.93±0.38^{a}	1.93±0.7^{a}	1.49±0.14^{a}
Arachidic acid	C20:0	1.22±0.26^{a}	1.12±0.25^{a}	1.66±0.47^{a}
Behenic acid	C22:0	0.64±0.05^{a}	0.62±0.06^{a}	1.32±0.22^{b}
Lignoceric acid	C24:0	1.03±0.17^{b}	0.97±0.3^{ab}	0.52±0.19^{a}
Cis monounsaturated fatty acids (MUFAs)	-	**4.27 ± 0.71**	**3.1 ± 0.43**	**2.54 ± 0.44**
Myristoleic acid	C14:1	0.24±0.01^{b}	0.25±0.00^{b}	0.13±0.04^{a}
Palmitoleic acid	C16:1 (*cis*)	0.12±0.03^{b}	0.26±0.04^{c}	0.05±0.00^{a}
Margaroleic acid	C17:1	0.23±0.01^{c}	0.1±0.01^{b}	0.07±0.01^{a}
Oleic acid	C18:1 n9 (*cis*)	1.56±0.42^{b}	0.89±0.03^{a}	0.68±0.06^{a}
Vaccenic acid	C18:1 n7 (*cis*)	0.08±0.01^{b}	0.04±0.01^{a}	0.04±0.01^{a}
Gadoleic acid	C20:1 n9	1.9±0.57^{a}	1.29±0.22^{a}	1.42±0.31^{a}
Nervonic acid	C24:1	0.14±0.02^{a}	0.27±0.06^{b}	0.15±0.01^{a}
cis polyunsaturated fatty acids (PUFAs)	-	**10.39 ± 1.77**	**8.59 ±0.64**	**9.1±0.17**
Linoleic acid (LA)	C18 :2 n6 (*c*9, *c*12)	7.28 ± 0.44^{b}	5.88 ± 0.24^{a}	6.03 ± 0.3^{a}
γ-Linolenic acid	C18:3 n6	0.24 ± 0.31^{b}	0.09 ± 0.01^{a}	0.085±0.01^{a}
Eicosadienoic acid	C20:2 n6	0.35 ± 0.02^{a}	0.49 ± 0.08^{b}	0.46±0.06^{ab}
Dihomo-γ-linolenic acid	C20:3 n6	1.64 ± 0.86^{a}	1.59 ± 0.3^{a}	1.96 ± 0.6^{a}
Eicosatrienoic acid	C20:3 n3	0.80 ± 0.12^{a}	0.45 ± 0.01^{a}	0.48 ± 0.02^{a}

Values are expressed as means ± standard deviation (n = 3). Means with different letters in the same column were significantly different at the level of $p < 0.05$.

Table.3 *trans* and conjugated fatty acid isomers in Pomegranate Seed Oils (PSO) of three Tunisian cultivars

	TOUNSI	**NABLI**	**GABSI**
Palmitelaidic acid	0.14±0.04[b]	0.07±0.01[a]	0.09±0.01[a]
Elaidic acid	5.35±0.45[c]	4.09±0.42[b]	3.16±0.03[a]
***trans* MUFA**	5.49 ± 0.85	4.16 ± 0.38	3.25 ± 0.48
***trans* /cis MUFA ratio**	1.28	1.34	1.27
trans Linoleic Acid C18 :2 (t9, c12)	0.14 ± 0.01b	0.03 ± 0.01a	0.03 ± 0.01a
trans Linoleic Acid C18 :2 (c9, t12)	0.38 ± 0.03b	0.31 ± 0.04a	0.29 ± 0.02a
***trans* PUFA**	0.52 ± 0.04	0.33 ± 0.05	0.32 ± 0.03
***trans/cis* PUFA ratio**	0.05	0.03	0.03
Conjugated linoleic acids	0.67 ± 0.13	0.59±0.06	0.63±0.09
Conjugated linolenic acids	73.34 ± 6.98	75.08 ± 4.49	73.63 ± 3.21

MUFA: monounsaturated fatty acids; PUFA polyunsaturated fatty acids

Fig.2 Conjugated Fatty Acids (CFAs) in the studied Pomegranate Seed Oils (PSOs)

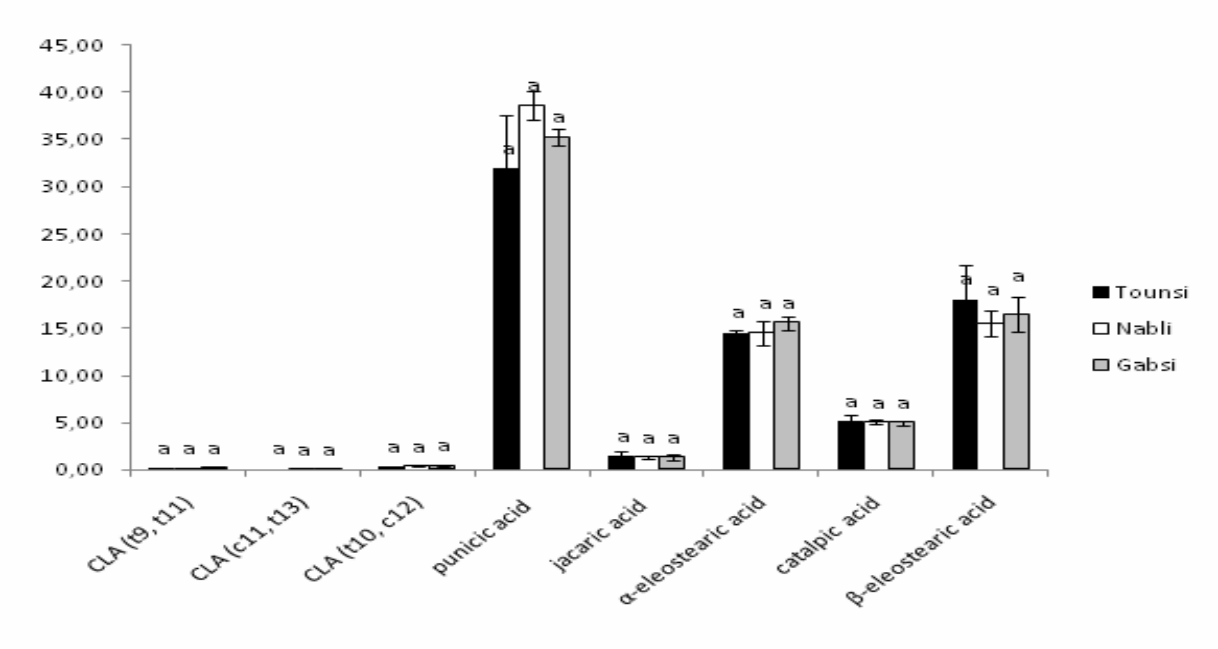


Fig.3 Typical chromatogram of Conjugated Linolenic Acids (CLnA) in Pomegranate Seed Oils (PSOs) of three Tunisian pomegranate seed cultivars

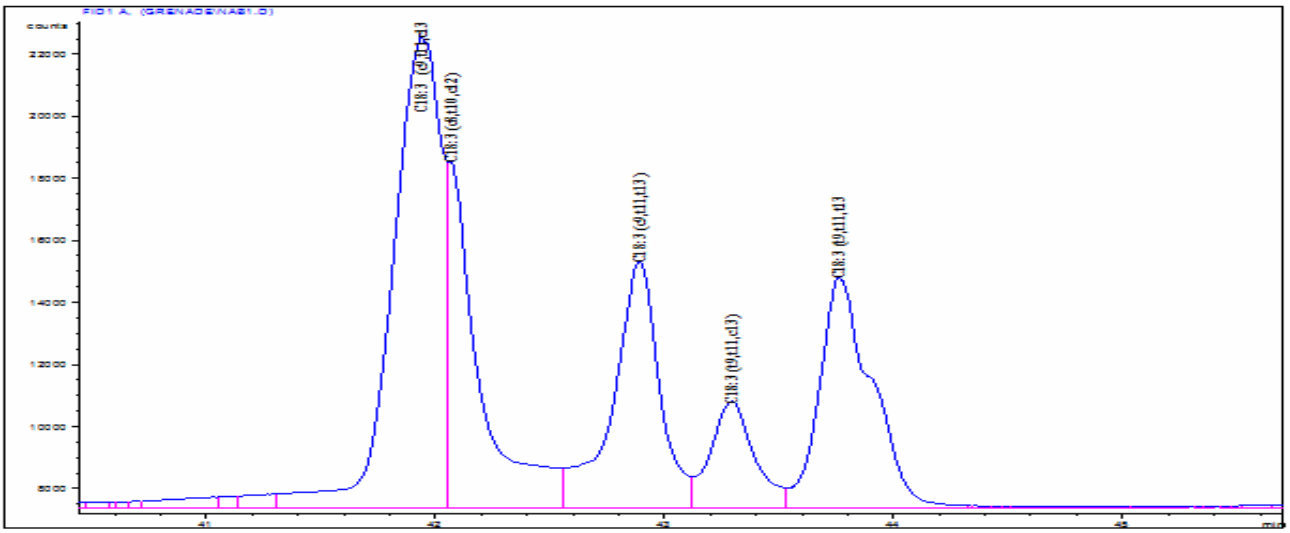
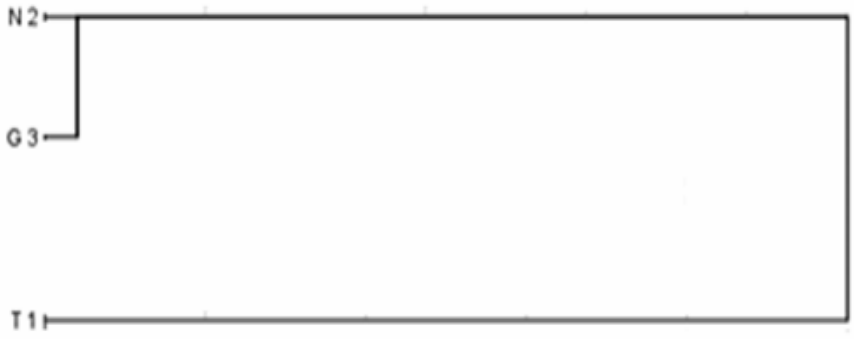


Fig.4 Hierarchical Cluster Analysis of pomegranate seeds based on the fatty acid profiles of the three studied varieties



Punicic acid presented the higher level of TFAs (31.97 in Tounsi, 35.27 in Gabsi and 38.64 % in Nabli) followed by β-eleostearic acid and α-eleostearic acid. However, the catalpic acid and the jacaric acid were the lowest with proportions from 4.97 to 5.16% and from 1.31 to 1.52%, respectively. Our results were not consistent with some previous works. According to Kýralan et al (2009), punicic acid is the most frequent fatty acid in PSO ranging from 70.42 to 76.17 % followed by the α-eleostearic acid with values of 5.94 and 6.85 %, the Catalpic acid and the β-Eleostearic acid. Idem, Yücel (2005) have also suggest that PSO is rich in punicic acid, catalpic acid and β-eleostearic acid but could not detect the presence of α-eleostearic acid. We also stated that DPPH scavenging activity of pomegranate seeds was significantly well correlated with [C18:2 (*t*9,*t*11)-CLA] in

one hand, and β-eleostearic acid [C18:3 (*t*9,*t*11,*t*13)-CLnA] in the other hand (r=0.717 and r=0.800, p<0.01, respectively). The antioxidant property of a-eleostearic acid from bitter gourd also has been reported (Li et al., 2011). Previous studies have demonstrated that many fatty acids have the ability to act as antioxidants or prooxidants (Geneive et al., 2002).

It is very important to emphasize that the presence of conjugated fatty acid groups in pomegranate seed oils could be very promoting being in a perspective of nutritional consumption in human diet.

Hierarchical clustering analysis of pomegranate seeds based on fatty acids composition

According to Fig. 4**,** it seemed that the seeds of "Nabli" cultivar had relatively similar composition of fatty acids with seeds of "Gabsi". However, hierarchical clustering analysis allowed a clear distinction of seeds of Tounsi variety, which showed a different composition compared to the two other varieties. The synthesis of the obtained results concerning fatty acid profiles clearly showed that the lipid fraction composition might be a specific trait for each variety. Indeed, we noticed that the "Tounsi" variety provided higher proportions in *cis* and *trans* MUFA and a relatively important *trans/cis* PUFA ratio. However, the "Nabli" cultivar presented relatively high levels of conjugated fatty acids and similar proportions of MUFAs and PUFAs in both the form *cis* and *trans*. We could attribute these differences to the variability of genetic heritage of the varieties.

In conclusion, the results showed that a positive correlation between the content of total polyphenols and antioxidant activities, thus pointing the richness of this seed in antioxidants, which might explain their utility especially in food processing industries. Also, results presented in this work highlighted the interests of the pomegranate seeds as a natural source of edible oil containing natural conjugated fatty acids which seemed to be involved in the antioxidant proprieties of pomegranate seeds. These characteristics suggest its exploitation in the field of nutrition. Therefore, more investigations should be conducted to make a process of creating purified CLnA from pomegranate seeds.

Acknowledgements

This research was supported by a grant from the 'Ministère de l'Enseignement Supérieur et de la Recherche Scientifique" LR12ES05 " Nutrition-Aliment Fonctionnel et Santé Vasculaire LR-NAFS " and 'DGRST-USCR-Spectrométrie de masse. We are also grateful to Mr "Rabah Essid" for providing pomegranate samples.

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