

First report on fatty acids composition, total phenolics and antioxidant activity in seeds oil of four fig cultivars (*Ficus carica* L.) grown in Morocco[☆]

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Abstract – With the progresses of oilseed industry, an important interest is currently being focused on exploiting novel and underutilized sources for vegetable oils. Being so far the less studied part in fig fruits, seeds separated from four fig cultivars were assessed for their oil content, fatty acids identification, total phenolics and invitro antioxidant analysis. A one-way Anova yielded statistically significant differences for all parameters, with the exception of pentadecylic, margaric and arachidic acids besides the total saturated fatty acids. Fig seeds presented a yellow colored oil, of which the content ranged from 21.54 ± 1.71 to $28.52 \pm 0.62\%$. Gas liquid chromatography analysis of the seed oil showed high percentages of linolenic acid in the four cultivars ranging from 38.43 ± 0.01 to 43.57 ± 0.04 , followed by linoleic acid (28.9 ± 0.06 – $34.5 \pm 0.04\%$). Palmitic acid and stearic acid were the dominating saturated fatty acids in all samples, where the amounts were in the range from 8.54 ± 0.04 to $9.05 \pm 0.06\%$ and from 2.59 ± 0.13 to 3.3% respectively. The efficiency of the desaturation from oleic acid to linoleic acid estimated within desaturation pathway, was higher among all cultivars than the efficiency of the desaturation from linoleic acid to linolenic acid. This explains the large increase of 18:3 concentration in all samples. The local cultivar ‘C11A21’ exhibited the highest total unsaturated fatty acids and the lowest level of saturated fatty acids, while the cultivar ‘White Adriatic’ combined the most relevant phenolics content, antioxidant activity and half maximum inhibitory concentration. All sampled oil possessed an important phenolics content that displayed variable levels of antioxidant activity. The objective of this study is to bring new data on the biochemical attributes of fig seeds as a new source oil that can be used for nutritional, pharmaceutical and cosmetic purposes.

Keywords: fatty acids / antioxidant activity / figs seeds / total phenolics / *Ficus carica* L

Résumé – Premier rapport sur la composition en acides gras, composés phénoliques totaux et l’activité antioxydante de l’huile de graines de quatre cultivars de figes (*Ficus carica* L.) cultivés au Maroc. Le progrès que connaît actuellement l’industrie des oléagineux a suscité un intérêt important à l’exploitation de nouvelles sources d’huiles végétales. Étant jusqu’à présent la partie de la figue la moins étudiée, les graines ont fait objet d’une extraction de l’huile où le profil d’acides gras, la teneur en polyphénols totaux et l’activité antioxydante sont analysés. L’Anova à un seul facteur et la comparaison multiple du test Duncan ont révélé des différences très significatives pour l’ensemble des paramètres à l’exception des acides pentadécylique, margarique et arachidique en plus des acides gras saturés totaux. Les

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graines de figes présentent une huile de couleur jaune, dont la teneur oscille selon les cultivars entre $21,54 \pm 1,71$ et $28,52 \pm 0,62$ %. L'analyse par chromatographie en phase gazeuse révèle des pourcentages élevés d'acide linoléique dans les quatre cultivars allant de $38,43 \pm 0,01$ à $43,57 \pm 0,04$, suivi d'acide linoléique dont les teneurs varient entre $28,9 \pm 0,06$ et $34,5 \pm 0,04$ %. Les acides palmitique et stéarique sont les acides gras saturés dominants, où les concentrations sont situées respectivement entre $8,54 \pm 0,04$ et $9,05 \pm 0,06$ % et entre $2,59 \pm 0,13$ et $3,3$ %. La désaturation de l'acide oléique en acide linoléique estimée par la voie de la bioconversion est plus élevée chez tous les cultivars par rapport à la désaturation des acides linoléiques en acides linoléiques. Ceci explique les fortes concentrations de 18:3 révélées dans toutes les huiles. Le cultivar local « C11A21 » présente le taux le plus élevé d'acides gras insaturés totaux et le taux le plus bas d'acides gras saturés, tandis que le cultivar « White Adriatic » combine la teneur en polyphénols et l'activité de piégeage des radicaux libres les plus importantes en plus de la concentration minimale inhibitrice de 50 % des radicaux libres (IC50) la plus faible. L'objectif de cette étude est d'apporter de nouvelles données sur la qualité biochimique des graines de figue en tant que nouvelle source d'huile pouvant être exploitée pour des fins industrielles, nutritionnelles, pharmaceutiques et cosmétiques.

Mots clés : acides gras / activité antioxydante / graines de figes / composés phénoliques totaux / *Ficus carica* L

1 Introduction

Demand for novel sources of oil in pharmaceutical, cosmetic and food industries is gaining an increasing importance, since they are valuable natural sources of nutritional bioactive compounds (Górnas and Rudzińska, 2016). Simultaneously, an increasing amount of the by-products generated by the fruit processing industry or after human consumption, partly in the form of seeds, are usually discarded and may otherwise be exploited as a newer source of oils (Raihana *et al.*, 2015; Górnas and Rudzińska, 2016). Despite the fact that not all fruits are processed by industry, the amount of seeds that can be recovered from the by-products and utilized as a natural source of lipophilic compounds is significant (Górnas and Rudzińska, 2016). The worldwide production of seed oils knows an exponential growth, which encouraged exploiting newer plant resources for production of oil and to provide the raw material to meet the increasing demand (Chai *et al.*, 2018). With the new developments in the oilseed industry, the seeds oil quality depends mainly on their fatty acid, triacylglycerol compositions and antioxidant attributes (Chielle *et al.*, 2016). Consumption of unsaturated fatty acids is linked to improve human health. A number of studies have investigated the relationship between the dietary intake of unsaturated fatty acids and coronary heart disease (Chougui *et al.*, 2013). Apart from their nutritive role in human diet, lipids are also utilized as food ingredients in delivering better texture, imparting flavor and mouth feel in new formulation (Rosianski *et al.*, 2016). Recently, the research has been directed towards essential fatty acids and natural anti-oxidants in the fruits' seeds (Chougui *et al.*, 2013). For this reason, many fruits' seeds have been studied for potential industrial or food uses such as newer sources of bioactive compounds. Particularly, a novel source for industrial oils, such as *Opuntia ficus-indica*, papaya, honeydew, mangosteen, rambutan, durian, date palm, etc. (Raihana *et al.*, 2015).

As, one of the most important fruits in the Mediterranean diet providing high concentrations of phytochemicals and antioxidant compounds (Hssaini *et al.*, 2019), figs (*Ficus carica* L.) are known to contain considerable amounts of seeds, that differ in their number and size and contribute to the taste

and flavor of fresh and dry figs (Gaaliche *et al.*, 2011). This aspect is strongly dependent to the varietal profile, ripening stage and caprification (Solomon *et al.*, 2006; Rosianski *et al.*, 2016; Mahmoudi *et al.*, 2018). Figs were given a particular interest by researchers. They were largely studied for their primary and secondary metabolites (Del Caro and Piga, 2007; Slatnar *et al.*, 2011; Veberic and Mikulic-Petkovsek, 2016), the biological activity and antidiabetic properties of the whole fruit (El-Shobaki *et al.*, 2010; Jun *et al.*, 2012; Mopuri and Islam, 2016) and, more generally, the fig nutritional significance (Aljane *et al.*, 2007; Vemmos *et al.*, 2013). However, among all these reports, seeds have been so far the less studied part. Therefore, there is a very few data on their oil fatty acids composition and almost no reports on their antioxidant properties. To date, only one study identified fatty acids contained in dried figs of Mission variety (Jeong and Lachance, 2001). The latter reported unsaturated fatty acids as being predominant, such as linolenic, linoleic and oleic acids. However, the fatty acids were reported as dried fig rather than oil extracted from seeds. The same results were stated by Nakilcioglu-Tas (2019), who also reported some major minerals in fig seeds such as Ca, K and P. Nevertheless, there is no report regarding the varietal effect on these aspects. Furthermore, to our knowledge, so far nobody has investigated the phenolics amounts and free radical scavenging activity of *Ficus carica* L. seeds oil.

Determining oil content, fatty acids, total phenolics and antioxidant activity in fig seeds can serve as a basis for their nutraceutical use, and thus lead to value-added utilization of fig seed and enhance the profitability of the fruit production and processing industries and of the fruit seed oil manufacturers. For this aim, we extracted oil from fig seeds of four cultivars planted in the experimental station of the National Institute for Agriculture Research (INRA) of Meknes in Morocco. The present study is an attempt to shed light on the chemical composition of the new oil extracted from seeds of four fig cultivars grown in Morocco, and to determine its nutritive and industrial uses. This work was carried out to:

- quantify the oil content in figs seeds;
- determine the fatty acids composition of these seeds;
- assess phenolic attributes;
- evaluate the combination of these parameters within the phenotypic variation.

2 Material and methods

2.1 Plant material

2.1.1 Seeds extraction

Fruits from four fig cultivars were collected separately from trees growing in the experimental station of the national institute for agricultural research (INRA Meknes, Morocco). These comprised two white cultivars (var. 'White Adriatic' and the local clone 'C7A14') and two dark cultivars (var. 'Bourjassote Noir' and local clone 'C11A21'). All fruits were collected at their full ripening stage in August of 2018. Fruits were peeled manually and their pulps were put in 10% of technical ethanol after 5 min, the mixture were agitated till the seeds separated from the pulp by decantation. The seeds were washed abundantly with distilled water and then dried at room temperature for 24 hours. Being round in shape and yellowish in color, the average thousand seed weight was about 1.14 ± 0.01 g. The seeds were then reduced into a fine powder using IKA A11 Basic Grinder (St. Louis, MO).

2.1.2 Oil extraction

Fig. seeds oil (FSO) was extracted using the Soxhlet apparatus following ISO (1999) method 659:199. Thus, twenty grams of each powder were mixed with 150 mL of n-hexane (99%) as an oil extracting solvent using cellulose extraction thimbles (123×43 mm i.d.; Whatman International, Brentford, UK). The solvent was evaporated at 40 °C using a rotavapor. The oil weight was determined as follows: oil weight (%) = $[(M1 - M0)/M2] * 100$, where M0 is the weight of the empty flask (g), M1 the weight of the flask after evaporation (g) and M2 the weight of the seeds powder (g). The resulting oils were stored in darkness at 4 °C until analysis.

2.2 Seed oil analysis

2.2.1 Identification of fatty acids by gas liquid chromatography

The fatty acid profile was determined according to EU standard methods (Annexes II and IX of European Community Regulation EEC/2568/91). The methyl esters (FAME) were prepared using cold alkaline transesterification method with methanolic potassium hydroxide solution and extracted with n-heptane. The fatty acid profile was determined with gas liquid chromatography (GLC) Varian CP 3380 Chromatograph, equipped with a capillary column (CP-Wax 52 CB: L=30 m; $\Phi=0.25$ mm; Ft=0.20 μ m), an injector split-splitless equipped with CP-8400 auto-sampler and FID detector. The temperature of the injector, the detector and the oven were held at 220, 230 and 180 °C, respectively. Hydrogen was used as carrier gas at an internal pressure of 110 kPa. The oven temperature was set at 70 °C during the first 4 min, increased to 110 °C at a rate of 8 °C/min, then increased up to 170 °C at 5 °C/min and holding for 10 min, finally increasing to 250 °C at a rate of 4 °C/min and hold for a further 15 min. The injected volume was 1 μ L with a split ratio of 1:50. The results are expressed as the relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area. The fatty acids methyl ester reference standard mixture (C4–C24, FAME Mix 37) was used

for used for calibration and for the identification of the FAME by their retention times.

2.2.2 Phenolic extraction

Extraction of phenolic compounds was carried out according to the method described by Tsimidou *et al.* (1992), with slight modifications. Two grams of oil sample were dissolved in 10 mL of hexane, then were added to 4 mL of methanol 60% (V/V). The mixture was subjected to an agitation during 2 h at room temperature in darkness and then filtered through Whatman No.1 filter paper. A second extraction was performed following the same conditions. The two filtrates were combined, concentrated under vacuum using a rotary evaporator and finally reconstituted in 10 mL of pure methanol and stored at 20 °C.

2.2.3 Total phenolics

Total phenolics content were determined based on Folin-Ciocalteu colourimetric method described by Favati *et al.* (1994). The reaction mixture contains 50 μ L of extract, 3 ml of distilled water, 250 μ L of Folin-Ciocalteu reagent and 750 μ L of sodium carbonate (7%). The mixture was stirred for 8 min at ambient temperature and then 950 μ L of distilled water was added. After 1 h of incubation in darkness, the absorbance was measured at 760 nm against a blank. Gallic acid was used as standard for the calibration curve. The analysis was performed in triplicate and results were expressed as mg equivalent of gallic acid (GAE) per 100 g of oil.

2.2.4 *In-vitro* antioxidant activity

Antioxidant activity of oil samples was determined following DPPH (2,2-diphenyl-1-picrylhydrazyl) and cationic radical ABTS⁺ (3-ethylbenzothiazoline-6-sulfonic acid) essays described by Brand-Williams *et al.* (1995), and Re *et al.* (1999) respectively, with a slight modifications. All tests were carried out in triplicate.

2.2.4.1 DPPH assay

50 μ L of each oil extract were added to 950 μ L of DPPH methanolic solution (0.030 mg. mL⁻¹). The mixture was first incubated in darkness for 60 min, then the absorbance was read at 515 nm. The control was prepared as above without oil extract.

2.2.4.2 ABTS assay

10 μ L of extract or distilled water (control) was mixed with 990 μ L of diluted ABTS^{•+} solution, and after 30 min of incubation in the dark absorbance of this mixture was measured at 734 nm.

ABTS^{•+} solution was produced by the reacting of 7 mM ABTS (3,6 g.L⁻¹) water solution with 2.45 mM potassium persulfate (0.662 g.L⁻¹) followed by an incubation of the mixture in darkness for 18 h at room temperature. The methanol was used to dilute the stock solution of ABTS^{•+} until an absorbance of 0.70 ± 0.05 at 734 nm was reached.

For the both essays, the antioxidant activity results were expressed as mg Trolox equivalent /g of oil using a linear

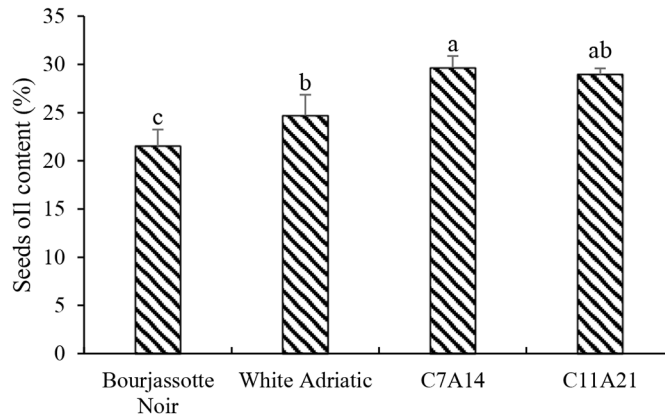


Fig. 1. Fig seeds oil content (%) of studied cultivars (average values \pm standard deviation). Statistically significant differences among cultivars are presented over error bars (a–c).

regression equation calculated with the Trolox solution concentrations as the independent variable (X) and the percentage of scavenging effect on the DPPH and ABTS radicals, independently as the variable (Y). The results were obtained using the following equation:

$$\text{mg trolox eq} = \frac{\left(\left(I(\%)_{\text{sample}} - b \right) / a \right) (\text{mg} \cdot \text{mL}^{-1}) \times 10^3}{C_{\text{sample}} (\text{mg} \cdot \text{mL}^{-1})},$$

where “a” and “b” correspond respectively to the slope and the constant of the linear equation related to the standard curve of each assay. And $I(\%)$ is the percentage of inhibition calculated as follow:

$I(\%)_{\text{sample}} =$

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100.$$

2.2.4.3 Half maximal concentration inhibitory (IC50)

IC50 value (mg extract/mL) was the inhibitory concentration at which DPPH and ABTS radicals were scavenged by 50%. It was calculated from the graph plotting inhibition percentage against oil extract dilutions.

2.3 Statistical Analysis

Data analysis was performed using SPSS v22. Analysis of variance was performed to test significant differences among the samples collected. The differences in the contents levels of the analyzed components were estimated with Duncan new multiple range (DMRT) test.

3 Results and discussion

3.1 Oil content

Oil yield is usually the main goal of oil seeds management, due to economic reasons; therefore, this measurement was the first stage of the present study. All sampled fig seeds had light

yellow colored oil, which the content varied significantly among samples [$F(3, 8) = 37.06, p < 0.001$]. The highest oil content was recorded by the local clones ‘C7A14’ ($29.65 \pm 1.21\%$) and ‘C11A21’ ($28.96 \pm 0.62\%$), whereas the varieties ‘Borjassoute Noir’ and ‘White Adriatic’ had the lowest value, that were 21.54 ± 1.71 and $24.71 \pm 2.14\%$, respectively. The oil levels found in fig seeds were generally comparable to those reported in other fruits seeds such as pumpkin (27.83%) (Alfawaz, 2004), honeydew (25%) (Yanty *et al.*, 2008) and Mangosteen (21.18%) (Ajayi *et al.*, 2007). However, they are higher than dose found in seeds of guava (16%) (Prasad and Azeemoddin, 1994) and durian (1.8%) (Berry, 1980). Compared to *Opuntia ficus-indica* seeds that presented a range of oil content of 5.4–9.9% (Taoufik *et al.*, 2015), the fig seeds oil (FSO) content was found much higher (Fig. 1).

For several fruit seeds, the macro quality components such as oil yielding are found to vary due to either varietal differences, geographical variations or pollination factor (Raihana *et al.*, 2015). However, in our study, since the cultivars belong to an ex-situ collection conducted under the same conditions, the large variation observed are mainly related to the cultivar. Nonetheless, some studies indicate clearly, that genetic factors have a greater impact than environmental factors on oil yield and quality (Górnas and Rudzińska, 2016). Showing an important oil yield, fig seeds may be economically attractive for industrial exploitation similar to other fruits’ seeds oil such as pumpkin, guava and kiwi. Besides, encouraging commercialization of these oils can have a great economic impact for local communities and significantly contribute to household economy.

3.2 Fatty acids composition of seeds oil

Figure 2 shows the GC chromatogram of fig seeds oil of the variety ‘White Adriatic’, which confirms that the extracted oil from all samples were composed mainly by total unsaturated fatty acids (TUFA) where the average content was 88.12 ± 0.15 , including mono-unsaturated fatty acids (oleic, eicosanoic, heptadecenoic and palmitoleic acids) and the poly-unsaturated fatty acid (linoleic and linolenic acids) which the average contents were 15.18 ± 0.18 and $72.95 \pm 1.10\%$, respectively (Tab. 1). Indeed, mono (MUFA) and polyunsaturated

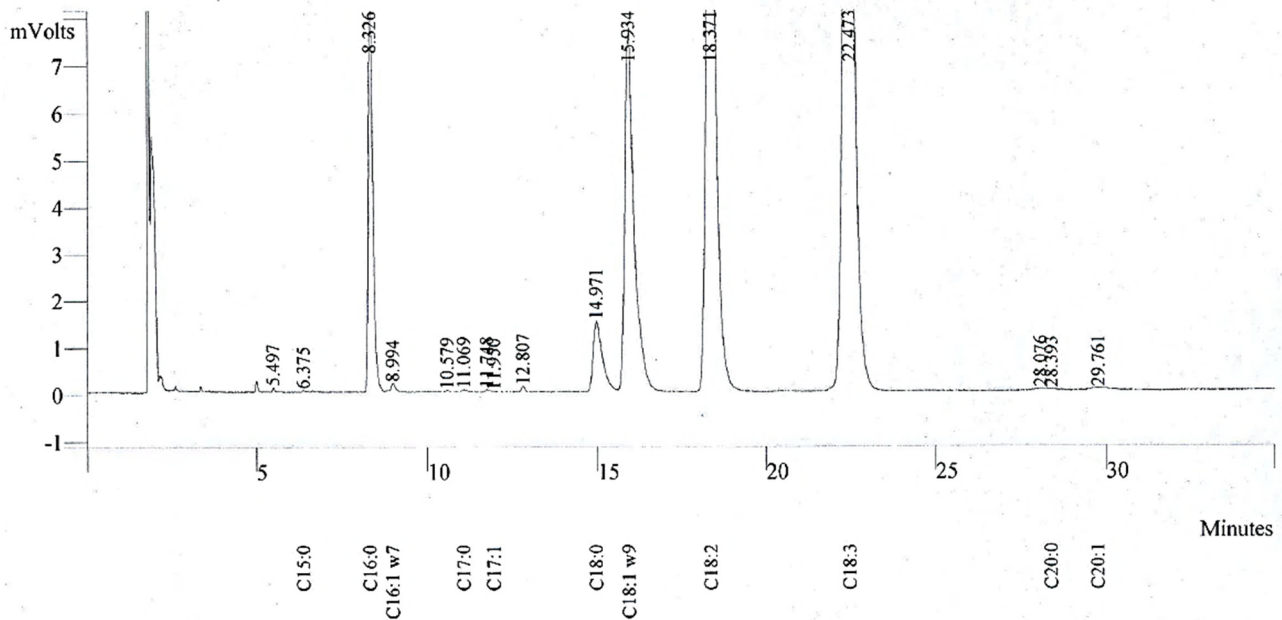


Fig. 2. Typical GC-chromatogram of the fatty acids of fig seeds oil. Example of variety “White adriatic” (C15:0: pentadecylic acid; C16:0: palmitic acid; C16: ω1: palmitoleic acid; C17:0: margaric acid; C17:1: heptadecenoic acid; C18:0: stearic acid; C18:1 ω9: oleic acid; C18:0: linoleic acid; C18:3: g-linolenic acid; C20:0: arachidic acid; C20:1: gondoic acid).

fatty acids (PUFA) ratio varied sensitively among samples. Hence, it was higher in ‘Bourjassotte Noir’ (0.22), while it was lower in ‘C11A21’ (0.18).

A one-way Anova yielded a statistically significant differences between the means on the fig seeds fatty acids ($p < 0.001$), but a non-significant difference on minor fatty acids namely C15:0, C17:0 and C20:0. Moreover, TSFA and its ratio with TUFA did not show significant difference among oil samples. This may be related to these very low percentages that was closely similar across oil samples. Taken together, these results suggest that these fatty acids may not be much useful in specific screenings of large samples based on these components.

The fatty acid composition data of fig seed oil of the studied cultivars showed the presence of 11 identified fatty acids. Post-hoc comparisons using Duncan test indicated that the mean score for total unsaturated fatty acids (TUFA) ($M = 72.95$, $SD = 1.1$) was significantly different than total saturated fatty acids (TSFA) ($M = 11.91$, $SD = 0.12$). In fact, TSFA were mainly dominated by palmitic (C16:0), and stearic (C18:0) fatty acids, and were quantified at lower rates (8.75 ± 0.21 and $3 \pm 0.28\%$, respectively), while arachidic (C20:0), margaric (C17:0) and pentadecylic (C15:0) acids were detected at very minor levels (0.09 ± 0.04 , 0.046 ± 0.01 and $0.02 \pm 0.03\%$). These results are in agreement with those found in “Sarılop” seeds (Nakilcioglu-Tas, 2019) and some other fruits seeds oil, such as guava (87.3% and 11.8% for TUFA and TSFA, respectively) (Vosoughkia *et al.*, 2012) and *Opuntia ficus-indica* (83% of TUFA and 16-17% of TSFA) (Taoufik *et al.*, 2015). Unlike FSO, fatty saturated acids were the predominant acids in mangosteen seeds (59.6% of TSFA and 35.3% of TUFA).

In all the samples, linolenic acid (C18:3) was the dominating fatty acid ranging from 38.43 ± 0.01 (C7A14) to

$43.57 \pm 0.04\%$ (White Adriatic) followed by linoleic acid (C18:2), which the average contents were in the range of 28.9 ± 0.06 (White Adriatic)- $34.5 \pm 0.04\%$ (C11A21). Being the third important fatty acid in FSO, oleic acid (C18:1) was in range of 13.44 ± 0.12 (C11A21)- $15.64 \pm 0.02\%$ (Bourjassotte Noir). Among above unsaturated fatty acids, oleic acid was reported to have an effective plasma cholesterol-lowering activity, whereas linolenic and linoleic acids are known for their ability to convert to hormone like eicosanoids which may be involved in physiological reactions such as blood clotting and immune response (Oomah and Mazza, 1999).

As the predominant saturated fatty acid, C16:0 was higher in C11A21, while C7A14 heled the highest level of C18:0. However, the other fatty acids (C15:0, C16:1, C17:0, C17:1 and C20:1) were detected in minors traces. C17:0 was not detected in ‘C11A21’ and ‘Bourjassotte Noir’. The fatty acids contents obtained in this study were quite similar to those reported by Icyer *et al.* (2017). Total unsaturated (87.3%) and saturated fatty acids (11.8%) in guava seeds oil are also similar to those found in FSO. The same composition was also found on pumpkin and papaya seeds (Raihana *et al.*, 2015; Chielle *et al.*, 2016). Since the cultivars studied are planted at the same edaphoclimatic conditions and fruits were collected at their full maturity stage, we estimate that the differences in concentrations could be mainly due to cultivars.

Nowadays, there is a growing nutritional interest for healthier oil from newer and underutilized sources with higher proportion of polyunsaturated fatty acids. The latter are particularly known for playing a preventive role in cardiovascular diseases and promoting the reduction for both total and LDL cholesterol (Ajayi & Ajayi, 2009). The high proportion of unsaturated fatty acids would make fig seeds oil a potential substitute for other seeds with highly unsaturated oils. Owing to this fact, fig seeds have the potential to become an

Table 1. Fatty acids profiles of fig seeds oil of studied cultivars determined by gas liquid chromatography (% w/w).

Cultivars	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	MUFA	PUFA	TSFA	TUFA	MUFA/ PUFA	TSFA/ TUFA
C11A21	0.02 ±0.00	9.05 ±0.06 ^a	0.068 ±0.00 ^c	0.045 ±0.00	–	2.594 ±0.13 ^d	13.438 ±0.12 ^c	34.51 ±0.04 ^a	40.188 ±0.22 ^c	0.094 ±0.00	0.119 ±0.00 ^b	13.624 ±0.10 ^b	74.702 ±0.19 ^a	11.802 ±0.14	88.326 ±0.06 ^a	0.182 ±0.00 ^c	0.134 ±0 ^{–a}
Bourjassotte noir	0.017 ±0.00	8.658 ±0.02 ^c	0.086 ±0.00 ^b	0.036 ±0.01	–	3.148 ±0.07 ^b	15.642 ±0.02 ^a	29.465 ±0.03 ^c	42.837 ±0.07 ^b	0.099 ±0.00	0.038 ±0.00 ^d	15.766 ±0.01 ^a	72.302 ±0.11 ^b	11.959 ±0.10	88.068 ±0.09	0.218 ±0.00 ^a	0.136 ±0.00 ^b
C7A14	0.022 ±0.00	8.539 ±0.04 ^d	0.059 ±0.00 ^c	0.048 ±0.00	0.036 ±0.00	3.302 ±0.00 ^a	15.379 ±0.01 ^b	33.866 ±0.01 ^b	38.436 ±0.01 ^c	0.066 ±0.00	0.195 ±0.00 ^a	15.669 ±0.01 ^a	72.303 ±0.00 ^b	11.977 ±0.04	87.972 ±0.00 ^c	0.217 ±0.00 ^a	0.136 ±0.00 ^b
White adriatic	0.022 ±0.00	8.748 ±0.04 ^b	0.162 ±0.00 ^a	0.053 ±0.02	0.068 ±0.00	2.988 ±0.00 ^c	15.333 ±0.03 ^b	28.905 ±0.06 ^d	43.573 ±0.04 ^a	0.093 ±0.01	0.096 ±0.00 ^c	15.659 ±0.04 ^a	72.478 ±0.02 ^b	11.904 ±0.02	88.137 ±0.06 ^d	0.216 ±0.00 ^a	0.135 ±0.00 ^{ab}
Mean	0.02 ±0.03	8.75 ±0.21	0.09 ±0.04	0.046 ±0.01	0.052 ±0.01	3.0 ±0.28	14.95 ±0.94	31.69 ±2.70	41.26 ±2.21	0.09 ±0.04	0.11 ±0.06	15.18 ±0.18	72.95 ±1.10	11.91 ±0.12	88.12 ±0.15	0.21 ±0.01	0.135 ±0.002
Anova mean square	0 ns	0.143 ^e	0.007 ^e	0 ns	0.001 ^e	0.278 ^e	3.097 ^e	25.419 ^e	16.958 ^e	0.001 ns	0.127 ^e	3.234 ^e	4.132 ^e	0.019 ns	0.067 ^e	0.001 ^e	0 ns

MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TSFA: total saturated fatty acids; TUFA: total unsaturated fatty acids; ns: non-significant. Different letters (a–d) in columns represent statistically significant differences among cultivars (one-way Anova and Duncan test, $P < 0.05$).
^eDenote significant difference at level of 0.001.

Table 2. Variation in fatty acids desaturation and ω -6/ ω -3 ratios among cultivars.

Cultivars	ODR	LDR	ω -6/ ω -3
C11A21	0.85 ± 0.00 ^a	0.54 ± 0.00 ^c	0.86 ± 0.01 ^b
Bourjassotte noir	0.82 ± 0.00 ^c	0.59 ± 0.00 ^b	0.69 ± 0.00 ^c
C7A14	0.82 ± 0.00 ^b	0.53 ± 0.00 ^d	0.88 ± 0.00 ^a
White adriatic	0.83 ± 0.00 ^b	0.6 ± 0.00 ^a	0.66 ± 0.00 ^d
Mean	0.83 ± 0.00	0.565 ± 0.00	0.7725 ± 0.00
Anova mean square	0.000 ^e	0.004 ^e	0.038 ^e

$$ODR(\text{oleic desaturation ratio}) = \frac{\%C18:1 + \%C18:2}{\%C18:1 + \%C18:2 + \%C18:3};$$

$$LDR(\text{linoleic desaturation ratio}) = \frac{\%C18:3}{\%C18:2 + \%C18:3};$$

ω -6/ ω -3 = 18:2/18:3. Different letters (a–d) in columns represent statistically significant differences among cultivars (one-way Anova and Duncan test, $P < 0.05$).

^e Denote significant difference at level of 0.001.

important source of oil. Seeds can be separated from the pulp during fig processing, and used to extract oil that can be used in food products as well as supplement in dietary health.

3.3 Fatty acids ratios

Comparing individual fatty acids between fig cultivars may be associated with the pollination, that is required for seeds development. Thus, seeds are generally hollow, unless pollinated. Owing to this reason, several ratios were additionally used, such as oleic desaturation ratio (ODR), linoleic desaturation ratio (LDR) and ω -6/ ω -3. These ratios were largely used to emphasize a large number of other seeds oil species, in order to avoid breeding modification effect (Velasco *et al.*, 1998).

There was a significant difference on desaturation pathway ratios across all cultivars at the $p < 0.001$ level. Thus, LDR varied significantly between 0.53 and 0.6, while ODR was in the range of 0.82–0.85. The ratio between ω -6 and ω -3 ranged sensitively among samples from 0.66 and 0.86. The efficiency of the desaturation from oleic to linoleic (ODR) acids estimated within desaturation pathway, was higher among all cultivars than the efficiency of the desaturation from linoleic to linolenic acids (LDR) (Tab. 2). The cultivar ‘C11A21’ and ‘White Adriatic’ had the highest ratio of ODR and LDR, respectively. This higher efficiency of the desaturation pathway explains the large increase of 18:3 content in all cultivars. In the case of ‘White Adriatic’, the combination of a high ODR and LDR led to a very high C18:3 content, the average value being 43.57%. Since, fatty acids are intercorrelated, elongation and desaturation pathways ratios are evidently a very useful indicators for assessment, breeding and management of oilseed species.

The balance between omega-6 (ω -6) and omega-3 (ω -3) which are two classes of essential fatty acids represented by linoleic acid and linolenic acids, respectively. They are an

important determinant of oil quality and a critical parameter in human diet (Simopoulos, 2016). Generally, all samples showed a very low ω -6/ ω -3 ratio, with an average value of 0.77. In blackberry seed oil, this ratio was reported about 3.63, which is three times higher than that in elderberry seed oil (1.19). It is worth noting that nutrition societies recommend a lower value of ω -6/ ω -3 ratio for a healthy diet (DACH, 2002). Growing evidence suggests that the reduction in n-6/n-3 ratio may be beneficial in reducing the risk of the development of cancer, enhancing bone health, and reducing the risk of cardiovascular disease (Aronson *et al.*, 2001; Hu, 2001). Therefore, an optimal ratio might improve the average human diet, since the n-6/n-3 ratio in most industrialized countries is generally as high as 15:1 (Simopoulos, 2002). For the local clones ‘C7A14’ this ratio was of 0.88, while it was of 0.86 for the cultivar ‘C11A21’. However, the ω -6/ ω -3 ratio was very low in the varieties ‘Bourjassotte Noir’ and ‘White Adriatic’, which the recorded values were 0.69 and 0.66, respectively. Several reports have shown that the biological availability and activity of Omega-6 fatty acids are inversely linked to the concentration of Omega-3 fatty acids in plant tissues (Simopoulos, 2016). Indeed, the healthy ω 6/ ω 3 ratio should be lower than 5 (WHO & FAO Joint Consultation, 1994); it has been recommended that the consumers evolve on a diet with a ratio of ω 6 to ω 3 of \sim 1 (Simopoulos, 2002). A lack of ω 3 FA dietary intake causes a high ratio in our daily diet, has been linked to some health issue such as blood lipid, autoimmune, cardiovascular and inflammatory diseases (Carvalho *et al.*, 2006).

3.4 Total phenolics and antioxidant activity

Total phenolics, antioxidant activity and IC50 are summarized in Figure 3. Total phenolics varied from 69.83 ± 13 (Bourjassotte Noir) to 100.99 ± 12.78 mg GAE/100 g of oil (White Adriatic). Seeds oil from local clones ‘C7A14’ and ‘C11A21’ exhibited average values of total phenolics that were respectively 80.18 ± 3.02 and 88.55 ± 11.29 mg GAE/100 g. These results were comparable with those reported in other fruits seeds oil such as *Opuntia ficus-indica* (48 ± 1–89.0 ± 5.1 mg GAE/100 g) (Chougui *et al.*, 2013). However, they were higher than others like mango, avocado and jackfruit (1.17 ± 0.13, 0.88 ± 0.02 and 27.7 ± 3.39 mg GAE/100 g, respectively) (Soong and Barlow, 2004).

DPPH free radical scavenging essay exhibited the highest total antioxidant activity, where the values ranged from 226.46 ± 10.95 (C7A14) to 294.36 ± 9.78 mg Trolox equivalent/g oil (White Adriatic). The cultivars ‘Bourjassotte Noir’ and ‘C11A21’ showed similar level of DPPH radical scavenging activity, which the average values were 246.27 ± 4.81 and 242.81 ± 8.18 mg Trolox equivalent/g oil, respectively (Fig. 3). The half maximum inhibitory concentration (IC50) was important in the variety ‘White Adriatic’ which recorded the lowest value (19.27 ± 1.15 mg/ml), followed by ‘Bourjassotte Noir’ where the average value was 60.7 ± 7 mg/ml. Local clones exhibited similar levels of IC50. Results of free radical scavenging activity based on ABTS essay, were largely lower than those of DPPH method. Similarly, ABTS IC50 values were, generally, lower than those recorded during DPPH essay. The variety ‘Bourjassotte Noir’ recorded the highest amounts of ABTS radicals’ inhibition (136.65 ± 91.00 mg Trolox equivalent/g oil) followed by ‘C11A21’ (34.29 ± 2.56 mg Trolox equivalent/g oil). The

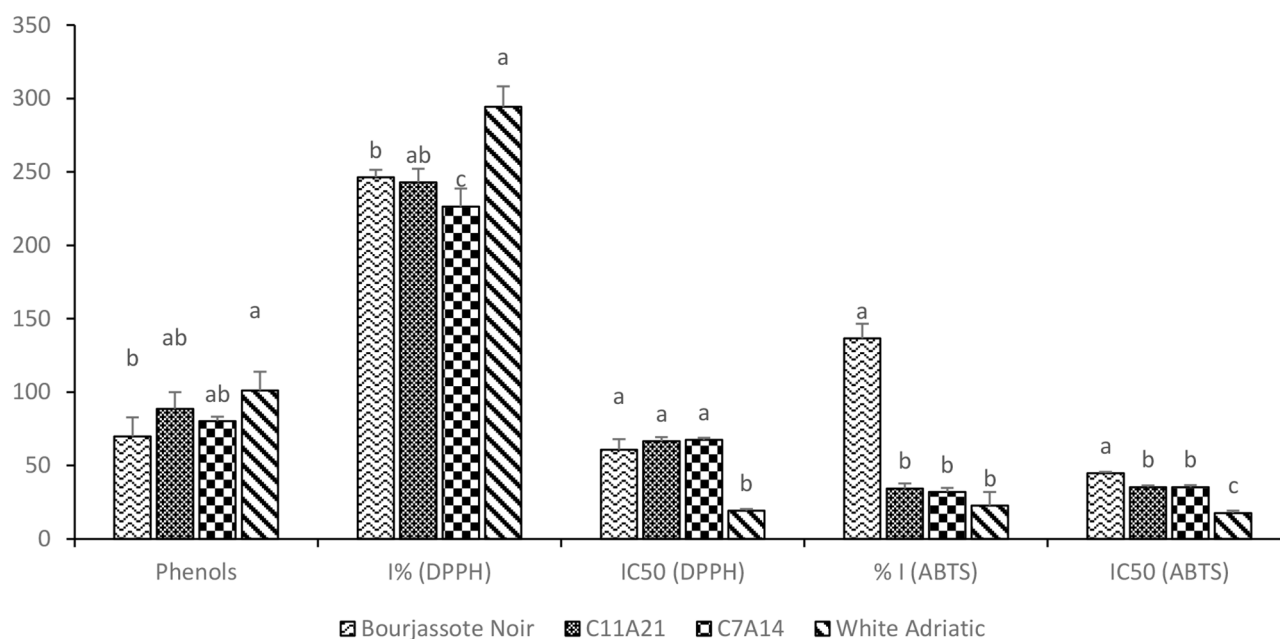


Fig. 3. Variation of total phenolics (mg GAE/100 g), DPPH and ABTS antioxidant activity (%) and the half maximum inhibitory concentration IC50 (mg/ml) among cultivars (average values \pm standard deviation). Statistically significant differences among cultivars are presented over error bars (a–c).

lower concentrations were recorded respectively by ‘White Adriatic’ (22.65 ± 1.18 mg Trolox equivalent/g oil) and ‘C7A14’ (31.87 ± 1.39 mg Trolox equivalent/g oil). IC50 of ABTS assay followed the same trend among cultivars as the one recorded for DPPH method. Thus, the variety ‘White Adriatic’ had the lowest value (17.60 ± 1.52 mg/ml) followed by ‘C7A14’ (35.27 ± 1.31 mg/ml). Whereas, ‘Bourjassote Noir’ and ‘C11A21’ had the highest values (44.65 ± 0.90 and 35.30 ± 1.10 mg/ml) (Fig. 3). Generally, compared to DPPH, IC50 was most important in ABTS assay. These differences in antioxidant activity and the IC50 may, particularly, depend on the composition and phenolic profile rather than total phenols level. In fact, because, phenolic extracts always contain a mixture of different chemical compounds, it is very difficult to attribute the radicals scavenging activity of a total extracts to one or a few active principles (Wu *et al.*, 2009). Besides the major constituents, minor constituent may also significantly contribute to the antioxidant activity of extracts. Following the results above, FSO is shown to be rich in polyphenols and present an important free radical scavenging activity compared to results reported in *Opuntia ficus-indica* oil (Chougui *et al.*, 2013).

Taken together, these results strongly suggest that seed have a significant contribution to the antioxidant proprieties of the whole fig. However, phytochemical investigation of fig seeds oil is needed to screen for the active components more involved in the antioxidant activity of FSO. The antioxidant hypothesis stresses that ‘as antioxidants can prevent oxidative damages, increased intakes from the diet will also reduce the risks of chronic diseases’ (Stanner *et al.*, 2004). This explains the intensive researches attempting to link diets rich in natural antioxidants with degenerative disease.

4 Conclusion

Recently, the research has been directed towards the possibilities of exploiting newer sources of oils. Being so far the less studied part of fig fruit, the seeds were found to be an important source of unsaturated oil with important antioxidants activity. A one-way ANOVA showed a highly significant differences ($p < 0.001$) between cultivars based on their oil rate, fatty acids composition and biochemical attributes. The results showed high levels of unsaturated acids (87.9–88.3%). Linolenic acid was the predominant, followed by linoleic acid. Palmitic and stearic were the most relevant saturated fatty acids. Considerable amounts of total phenolics were found among all samples (69.83 ± 13 – 100.99 ± 12.78 mg GAE/100 g). Sampled seeds oils, exhibited a high antioxidant potency. These characteristics, may encouraged oil industry to more explore the natural antioxidants contained in fig seeds oil. Its targeted utilization may be exploited for economic and health benefits. However, further studies are needed in order to reinforce this conclusion. Furthermore, the most relevant concentrations of IC50, total phenolics, unsaturated fatty acids were combined by the cultivar ‘White Adriatic’. As far as we know, this is the first study that investigated, fatty acids, total phenolics and antioxidant on fig seeds oil. The data obtained may be of great importance as an indication of the nutraceutical and economic utility of fig seeds that can be suggested as a new source of fruit oils and functional foods as well as supplement in dietary health. Evidently, there is no single oil that can fulfill all the requirements or standards of the growing oil sector. Since there has been a renewable nutritional interest for unsaturated oils with higher antioxidant capacity.

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