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REGULAR ARTICLE

Chemical compositions of the Tunisian *Ziziphus jujuba* oil

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Abstract

Thirteen fatty acids were identified from the pulps of four Tunisian *Ziziphus jujuba* ecotypes (Sfax, Choutrana, Mahres and Mahdia), using capillary gaseous chromatography method. These oils presented 8.31% to 12.35% of dry weights. Compared to the other ecotypes, Mahres and Choutrana were the richest of the oleic acid. The palmitic acid was the most important compound of the Sfax ecotype. Unsaturated fatty acids ranged from 62.63% to 72.40% of the total fatty acids of each ecotype. So a ratio of the unsaturated/saturated (U/S) varied from 1.68 to 2.37. The β-sitosterol and the Stigmasterol were identified as major sterols. The β-sitosterol was the prominent component in the all ecotypes and the highest level (10.65 mg/100g) was noted in Choutrana ecotype. Stigmasterol was more abundant in Sfax (16.12 mg/100g) and Choutrana (4.67 mg/100g) ecotypes. The campesterol sterol was identified only in Sfax and Choutrana ecotypes being more important (2.4 mg/100g) in Choutrana pulps.

Key words: Fatty acids, Linoleic acid, Oleic acid, β-sitosterol, *Ziziphus*

Introduction

Jujube (*Zizyphus* spp.), are cultivated economically in several countries. It is a species of about 40 species and grows mainly in tropical and subtropical parts of the world (Mukhtar et al., 2004; Laamouri, 2009). Three species are known in Tunisia: *Z. lotus*, *Z. spina-christi* and *Z. jujuba*. The *Z. jujuba* also known as “anneb” is the most popular specie. This tree, with 10 cm of length and 50 cm of diameter, offer a delicious read fruit (jujube) that was consumed fresh, dried and processed (jams, loaf, cakes, etc.). This jujube has many medicinal and pharmaceutical fields. In fact, it was widely used to treat chronic hepatitis or distress and fullness in the chest (Yamaoka et al., 1996; Sheng et al., 2009). Oil extracted from different *Z. jujuba* organs has been widely used to treat immunity deficiency (Zhao et al., 2006; Goncharova et al., 1990; Peng et al., 2000). The fatty acid profiles of jujube were influenced by their developmental stage (Guil-Guerrero et al., 2004). The oleic and the palmitic acids are defined as the major compound of oil pulps extracts (Guil-Guerrero et al., 2004). San et al. (2010) added the linoleic and the palmitolic acids to this composition.

Z. jujuba oil was also rich on unsaturated fatty acids that constitute the important cell membranes components and provide protection effects against heart disease, diabetes, certain types of cancer and some other diseases (Lunn and Theobald, 2006).

Phytosterols had been also detected in different part of *Z. jujuba*. They had been used to lower plasma total cholesterol and lipoproteins (Aioi et al., 1995; Owen et al., 2000; Das et al., 2003). In fact, and according to Bonanome et al. (1988), the sterols ration had been envisaged as cosmetic ingredient. However, there is a little information detailed fatty acids, and sterols compositions of *Z. jujuba*.

In Tunisia, *Z. jujuba* is located especially in the southern country (Mahdia, Gafsa Kébili, Sfax, Mahres). In the north, some trees are present in private gardens (Ariana, Choutrana). In the previous study, four *Z. jujuba* ecotypes: Sfax, Choutrana, Mahres and Mahdia were selected (Elaloui, 2013). The objectives of the present study

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were to characterize and to compare the chemical composition of fatty acids and sterols in the pulp oils.

Materials and Methods

Plant material

Pulps were collected from plants cultivated in the tunisian experimental station of "Rouhia" (northwestern Tunisia; 35° 40'-15.39" N; longitude 9° 0.3 - 15.29 E; altitude 636 m). The fruits (Figure 1) of *Ziziphus zizyphus* were collected in September 2009 and identified by (Laamouri, 2009). A voucher sample is deposited at the Herbario of National Institute for Research in Rural Engineering, Water and Forests (INRGREF) in Tunisia.

Figure 1. Fruits of *Ziziphus jujuba* (experimental station-Rouhia).

Pulps, hand removed from fruits, were grounded by a mill equipped with a grid whose holes are 1.00 mm in diameter.

Reagents and standards

All solvents used in our experiments: Tert-Butyl-Methyl Ether (TBME), cyclohexane; KOH; N-methyl-N-trimethylethylsilyl-heptafluoro butyramide (MSHFBA); dihydrocholesterol; chloroform the homologous fatty acids and sterol standards were purchased from Sigma Aldrich (Steinheim, Germany).

Lipids extractions

Ground *Ziziphus* pulps were extracted with cylohexane in a soxhlet apparatus for 6 h. The extract was concentrated under reduced pressure using a rotary evaporator at 60°C. The extracted oil was kept in obscurity at 4°C in waiting for analysis. The extractions were carried out in duplicate.

Fatty acids extraction

For FAs extractions, the procedure of Macherey Nagel was adopted. We dissolve 20 mg of oil with 1 ml of a suitable solvent (TBME: Tert Butyl Metyl Ether). Then, we Add 50 µL reagents to 100 µL of this solution. A methylation with TMSH is recommended for free acids, chlorophenoxycarboxylic acids, their salts and derivatives as well as for phenols and chlorophenols (Butte, 1983). One great advantage is the simplification of the sample preparation. Lipids or triglycerides can be converted to the corresponding fatty acid methyl esters (FAMEs) by a simple transesterification. This reaction is very elegant and convenient, because it is just necessary to add the reagent (0.2 M in methanol) to the sample solution. Removal of excess reagent is not required, since in the injector of the gas chromatograph at 250°C pyrolysis to volatile methanol and dimethylsulfide will occur. Due to the high reactivity, complete derivatization is often obtained at ambient temperature. However, heating (10 min at 100°C) in a closed sample vial may be necessary.

Sterols extraction

Unsaponifiables and sterols extractions were carried out according to (Sriti et al., 2009). A mixture was prepared by adding 100 µg of dihydrocholesterol (internal standard dissolved in chloroform) to 140 mg of oil and mixed to 3 ml of a solution of KOH (1 M in ethanol). After heating the mixture at 75°C for 30mn, 1ml of distilled water and 6 ml isohexane were added. The isohexane phase was allowed to isolate unsaponifiable fraction which was analyzed by GC (Sriti et al, 2011).

For silylation, 160 µl of the organic phase containing the sample was added to 40 µl of silylation reagent (1 ml N-methyl-N-trimethylsilyl-heptafluorobutyramide (MSHFBA)) mixed with 50 µl of 1-methylimidazole and heated for 5 min at 103°C before GC analysis.

Analysis conditions

FAs analyses were done on a capillary gaseous chromatography Varian (CPG). The mixture is injected directly. The temperature of the injector must be at least 250°C. The column used was Select CB for FAME fused silica WCOT (50 m x 0.25 mm; film thickness 0.25 mm). The temperature gradient was185°C for 40 min, then at 15°C/min to 250°C, and 250°C for 10 min. The analysis time was 55 min. The detector FID was set up at 250°C. The helium was the carrier gas at 1.2 mL/min.

Sterols analyses were performed by GC using a flame ionization detector (FID) Perkin Elmer (Waltham, MA, USA) chromatograph. A CP-SIL 8 CB capillary column (30 m; 0.25 mm; 0.25 µm) was used. Chromatograph worked under suitable programs: in the first time 160°C during 0.5 min, then increased from 160°C to 260°C at a rate of 20°C min^{-1}, 2°C min^{-1} to 300°C and 45°C min^{-1} to 350°C respectively. The carrier gas was helium with a flow rate of 1 ml min^{-1} (on column injection was used). The detector was set up at 360°C. The injection volume was 1 µL. The identification of the compounds was done by comparison to the commercial standards. Sterols quantification was done by internal calibration with the addition of cholestanol. It is estimated that analyzed sterols and cholestanol reply the same way.

Statistical analysis

Data were subjected to a statistical analysis using the program package STATISTICA. Total volatile compounds are means ± SD of three experiments. The one-way analysis of variance (ANOVA) followed by Duncan multiple range test was employed.

Results and Discussion

Oil yields

Yields of oils obtained from *Z. jujuba* pulps is shown in Figure 2. Oils yield varied from an ecotype to another and flow from 8.31% to 12.35% based on the dry weight of each ecotype.

The pulps of ecotype Sfax had the highest oil content (12.35%) followed by Choutrana and Mahres (10%) whereas the ecotype Mahdia presented the lowest oil yield (8.31%).

Fatty acid profile

In this section, we focused on the analysis of fatty acids (FA) extracted from *Z. jujuba* pulps oils grown in an experimental station. The typical GC profile (Figure 3) showed the existence of large variation between the four ecotypes (Sfax, Choutrana, Mahres and Mahdia).

The major FA observed was the oleic acid (omega-9) at a level of 50.68% and 42.82% of the total oil in Mahres and Choutrana ecotype, respectively. Then its composition decreased slightly to reach the proportions of 38.67 and 32.37% in pulps from ecotype Sfax and Mahdia respectively.

On the other hand and as shown in table 1, palmitic acid (C16:0) was a major compound of the Sfax subjects (18.67%). The linoléic acid (C 18: 2n6) was present in all ecotype at 18.13% and 10.88% in Choutrana and Sfax ecotype respectively. The pulps oil were also rich on C18:0 FAs ranging from 7.29% in Choutrana to 8.43% in Sfax. The *Ziziphus* pulps composition was more important than those confirmed in *Z. mauritiana* pulps (Guil-Guerrero et al., 2004). In fact, they adopt this composition C12 : 0 (18,3%), C10 : 0 (12,5 %), C18 : 2 (9,27%), C16 : 1 (8,50%), C16 : 0 (7,25%), et C18 : 1 (5,34%). San et al. (2010) have qualified *Ziziphus* oil as a mixture of linoleic, oleic, palmitic and palmitoliec acid.

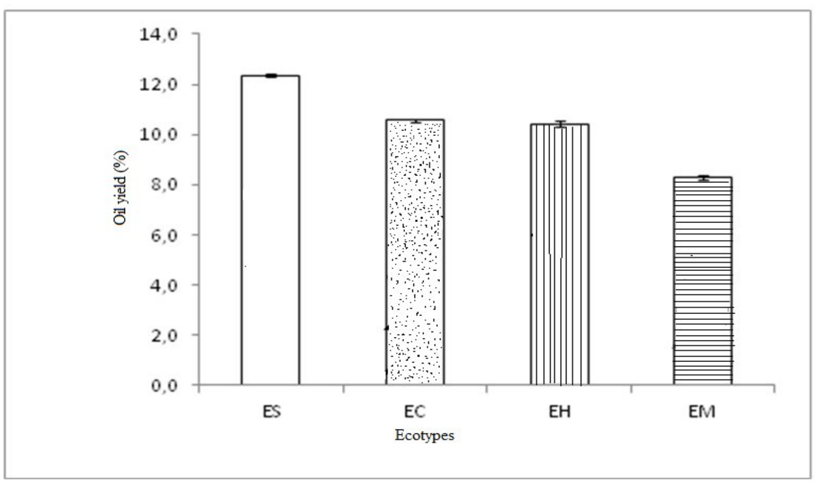

Figure 2. Oil yield (%) of four tunisian *Ziziphus zizyphus* ecotypes (Sfax, Choutrana, Mahres and Mahdia). The data are means values of three measurements. The confidence intervals were calculated at the threshold of 5%.

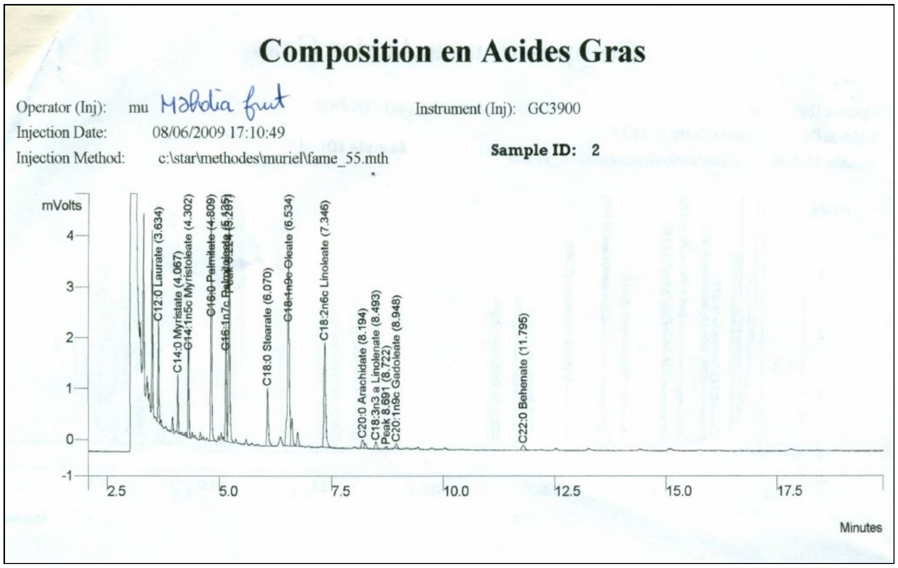

Figure 3. Fatty acids compositions of *Ziziphus jujuba* pulps.

On the other hand, the results showed an assessment of saturated, monounsaturated and polyunsaturated fatty acids in the pulp of *Ziziphus* values 29.62% to 37.3%; 48.17% to 59.89% and 12.51% to 15.05% the most important saturated fatty acids were palmitic, stearic, behenic and arachidic acid. Oleic and gadoleic acids were the major monunsaturated fatty acids. But the polyunsaturated acids are formed essentially by linolic and linolenic acid. This abundance of unsaturated fatty acids (68.5% to 72.4%) was also noticed in jujube fruits and 26.4 to 30.2% for saturated fatty acid (San et al., 2010).This composition can give preventive action against cardiovascular disease by lowering levels of cholesterol (Watkins et al., 2003; Tapiero et al., 2002; Hachicha et al., 2006).

Table 1. Fatty acids compositions of the pulp of four Tunisian *Ziziphus jujuba* ecotypes (Sfax, Chautrana, Mahres and Mahdia).

Fatty acids	Saturation	Percentages of fatty acids			
		Sfax	Choutrana	Mahres	Mahdia
Lauric	C 12 : 0	4.68 ± 0.13b	3.19 ± 01c	3.29 ± 0.02c	5.76 ± 0.99a
Myristic	C14 : 0	2.91 ± 0.37a	1.23 ± 0.02b	1.31 ± 0.01b	3.29 ± 0.41a
Myristoleic	C14:1n5	8.53 ± 0.79a	1.52 ± 0.14c	1.3 ± 0.01c	6.45 ± 1.19b
Palmitic	C16:0	18.67± 0.08a	18.59 ± 0.16a	15.58 ± 0.2b	18.36 ± 0.31a
Palmitoleic	C 16 :1n7	8.69 ± 0.79a	2.91 ± 0.08b	2.59 ± 0.01b	8.45 ± 1.6a
Stearic	C18 :0	8.43 ± 0.58a	7.29 ± 0.13b	8.13 ± 0.08ab	7.66 ± 0.72ab
Elaidic	C 18 : 1n9t	2.7 ± 0.01a	*	*	*
Oleic	C 18 : 1n9	38.67 ± 1.59b	42.82 ± 0.28b	50.68 ± 0.28a	32.37 ± 6.33c
Linoleic (oméga-6)	C 18 : 2n6	10.88 ± 0.89c	18.13 ± 0.51a	13.29 ± 0.04b	13.6 ± 1.76b
Arachidic	C20 : 0	1.59 ± 0.55a	1.06 ± 0.05b	0.73 ± 0.02b	1.64 ± 0.06a
Linolenic (oméga-3)	C18: 3 n3a	1.63 ± 0.07a	1.33 ± 0.05b	1.76 ± 0.04a	0.86 ± 0.29c
Gadoleic	C20 :1c	1.3 ± 0.15a	1.08 ± 0.08a	0.77 ± 0.04c	0.9 ± 0.13bc
Behenic	C22 : 0	0.56 ± 0.92a	0.85 ± 0.05a	0.58 ± 0.01a	0.67 ± 0.67a
Omega-6/Omega-3		6.67	13.63	7.55	15.81
∑polyunsat.		12.51	19.46	15.05	14.46
∑monunsat.		59.89	48.33	55.34	48.17
∑sat.		36.84	32.21	29.62	37.38
U/S		1.97	2.1	2.37	1.68

The first number indicates the length of the fatty acid chain and the second the number of double bonds (all *cis*) with signifying the location of the double bond(s). Saturated = 14:0 + 16:0 + 18:0 + 20:0 + 22:0. Monunsaturated = 16:1 + 18:1 + 20:1 Poly-unsaturated = 18:2 + 18:3. The data are means values of three measurement ± SE. for each column, values with the same letter indicate no-significant differences at 5%.

The ratio unsaturated/saturated (U/S) gives 1.68; 1.97; 2.1 and 2.37 in ecotypes Mahdia, Sfax Choutrana and Mahres respectively. This ratio is higher than other cited who had noticed equality between les mono and polyunsaturated acids.

This richness of unsaturated fatty acids can give preventive action against coronary, heart disease, diabetes and certain types of cancer (Berra et al., 1998; Jacot, 2001, Lunn and Theobald, 2006). Finally the ratio omega-6/omega-3 gives a large 5/1 (ES) to 14/1 (EM).in fact, that food enriched by jujubes can protect body against cancer (Jacot, 2001).

Sterols compositions

Oil extracted from pulps (dry matter) contains almost 14 mg/100g of total sterols (TSs). This quantitative richness differed from ecotype to another as (Figure 4).

As illustrate in Figure 4, all ecotypes (Sfax, Choutrana, Mahdia and Mahres) showed similar sterols constituents. In fact, all pulps oils were dominant on β-sitosterol and campesterol which β-sitosterol was the most prominent in these ecotypes.

Thus, the highest percentage (10.65 mg/100 g) was observed on Choutrana pulps. In contrast, the lowest level (3.02) was estimated in the pulp oil from Mahdia. However, the β-sitosterol level remained high for the species from Sfax (9.69 mg/100 g) of TSs (Table 2). Actually, this compound is the most intensively investigated with respect to its beneficial and physiological effects on health. The analyses of *Ziziphus* sterols pulps showed also the high level of the stigmaterol. In fact, pulps from Sfax were the most rich of this sterol with the rate of 16.12 mg/100 g oil, flowed by Choutrana pulps with the rate of 4.69 mg/100 g. The ecotype of Mahdia contains 1.81 mg/100 g. However the pulps of Mahres showed the poorer fraction of this sterol with only 1.35 mg/100 g oil. Campesterol was the third common sterol and exist only in Sfax and Choutrana selections. In fact, it was between 1.37 mg/100 g (Sfax) and 2.4 mg/100 g (Choutrana).

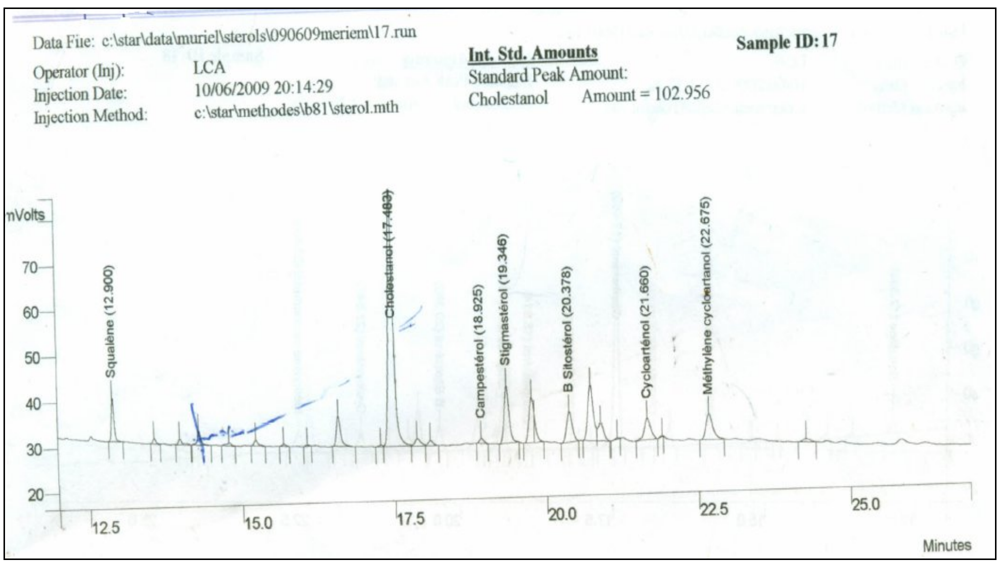

Figure 4. Sterols compositions of *Ziziphus jujuba* pulps.

Table 2. Sterols compositions (mg/100g) of four Tunisian jujube selections (Sfax,Choutrana, Mahres and Mahdia).

Ecotyps/ Sterols	Sfax	Choutrana	Mahres	Mahdia
Campesterol	1,37 ± 0,11b	2,4 ± 0,64a	*	*
Stigmasterol	16,12 ±0,87a	4,69 ± 1,18b	1,35 ± 0,9c	1,81 ± 0,16c
β-Sitosterol	9,69 ± 0,45a	10,65 ± 2,86a	4,14 ± 0,91b	3,02 ± 0,09b
d- 7- Stigmastenol	*	0,82 ± 0,01a	*	*

*Not identified.

The data are means values of three measurement ± SE. for each column, values with the same letter indicate no-significant differences at 5%.

As we can see in this Table, *Ziziphus* pulps were a big source of stigmasterol which is an excellent material for the synthesis of the progesterone hormone. This propriety gives a great importance to *Ziziphus* oil. Hamid et al. (2006) and Feng et al. (2011) adopted the importance of the β-sitosterol in olive oil, cannabis seed, in soja oil and *Z. spinosus* seeds respectively. The same result was observed on *Lens esculentus* (79.7%) and in *Arachis hypogaea* (72.0%) (Emile et al., 1983).

Conclusion

According to the described results, the *Z. jujuba* oil composition was dominated by oleic and palmitic acid with 32.4 - 50.7% and 15.6 - 18.7% respectively. The lipid of the jujube pulps was especially rich on omega-6, that human body is not capable of producing. Total sterol contents were 14 mg/100 g oil. The β-sitosterol was the prominent component in Sfax ecotype (16.12 g kg^{-1}) of the total sterols. The high rate of extraction insists on the importance of exploitation of this oil in cosmetic and pharmaceutics industries. Therefore jujube fruits can be recommended by nutritionists to be part of the diet.

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