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Composition in fatty acids, sterols and tocopherols of vegetable oil extract from kernels of Zizyphus lotus L.

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Abstract

Cosmetology often uses extracts of natural substances, in particular vegetable oils, which are used as main material of massage or as a diluent of the essential oils, whose formulation obtained is dedicated to relieve the rheumatic pains and the muscular traumatisms. With the aim of extracting, an unconventional vegetable oil from the kernels obtained by peeling the kernels of the Zizyphus Lotus fruit harvested in September 2016.The cores are then cold mechanically pressed by means of a mill without the use of an organic solvent. The oil obtained with a yield of 8,1% is then analyzed by gas and liquid chromatography to determine its chemical composition of fatty acids, tocopherols and sterols. Analysis of the chromatograms shows that this oil is rich in unsaturated fatty acids (80%), of which oleic acid (C18: 1 ω-9) represents 66.8% followed by linoleic acid (C18: 2 ω-6) with 13.6%, that it contains only γ-tocopherol with a level of 32.9 mg / kg of total tocopherols and reveals a predominance of β-sitosterol with 71.7% of total sterols.

1. Introduction

Plant biodiversity in Morocco, gives it an inexhaustible subject in terms of raw materials, among which we quote the Zizyphus lotus [1]. It is a species that grows in several arid and semi-arid regions biotopes [2]. Zizyphus lotus, also known as the jujube, belongs to the angiosperm, the Rhamnaceae family. This family includes about 135 to 170 species of Zizyphus [3]. It forms clumps of a few meters in diameters and reach up to 2 m long. Its leaves are short petiolate, glabrous, deciduous alternate, oval with entire margins. Each leaf contains at its core two stipules transformed into thorns uneven and vulnerable. The flowers are yellow, pentameters and grouped in cymose inflorescence.

The fruits are drupes to welded nuclei. The mucilaginous endocarp, called "nbag", is sweet and edible [4]. This plant offers a delicious fruit that can be eaten fresh, dried consumed by local people [5, 6]. Different species of the jujube are widely used in the treatment of some diseases such as inflammatory diseases [7] digestive disorders, disorders of the liver, symptomatic benign prostatic [8] urinary disorders, diabetes, and insomnia [9]. Research has focused on the extraction of oil from the kernels of the Zizyphus lotus fruit to study its chemical composition [10, 11].

Often the grains are thrown away as waste, in order to valorise the latter, we extracted the kernels of the grains which we used as raw material to extract a vegetable oil, by cold mechanical pressure [12], without using any organic solvent, for the purpose to study its quality and its chemical composition in fatty acids, tocopherols and sterols, to determine its use in particular in the cosmetics and / or therapeutic field [13].

2. Material and Methods

2.1. Plant material

The fruit of the Zizyphus lotus L. was harvested in Moulay Bouazza, rural commune of Khénifra province, in the Meknès-Tafilalet region, during the month of September 2016. The fruits were dried out of the Sun, and then the pulp was separated from the shells that contained one or two kernels. The shells were broken without damaging the kernels that have been properly conditioned to 25 °C for a subsequent extraction of oil.

2.2. Determination of humidity (H %)

The kernels obtained by peeling the seeds are then ground finely. 2.00 g of the powder obtained was placed in a dry and calibrated crucible. The whole is placed in a desiccator preheated to 102 ± 3 ° C for 3 hours. The moisture content is determined according to the following relationship:

$$\boldsymbol{H\% = ts - m/ts} \qquad (1)$$

ts: test sample(2g); m: mass of dried test

2.3. Extraction of oil [11]

The extraction of unconventional oil of Zizyphus lotus was obtained from kernels of the jujube by mechanical pressure [14] using an oil extractor model YD-ZY-O1C. A funnel makes it possible to introduce the kernels at the level of the extractor. The pressure is obtained by gradually reducing the volume generated by the rotation of the screw in a cage consisting of spaced bars, which allows the passage of the oil. The cake escapes at the end of the screw. Then the oil obtained is filtered and packaged in a sterile bottle and stored in a cool place away from light.

2.4. Yield of extraction

The performance of extraction was determined according to the following relationship:

$$\mathbf{Rt\%} = \left[\frac{\mathbf{Wo}}{\mathbf{Ws(1-H)}}\right]\mathbf{x100} \qquad (2)$$

Wo: the oil obtained mass

Ws: mass of kernels

H: humidity content of kernels

2.5. Organoleptic characteristics

After extraction of the oil, we examined its organoleptic characteristics, especially the colour, smell, appearance and taste.

2.6. Physicochemical parameters

To characterize this unconventional oil, we have determined the values of some physicochemical parameters according to standard methods by AFNOR, especially the acid index, the saponification index, the index of esters, iodine, peroxide index and the index of refraction.

2.6.1. Acidity index

The free acidity of organic oil of Zizyphus lotus, expressed as a percentage of oleic acid, is determined through titration in alcoholic environment of free fatty acids of Zizyphus lotus oil, by an ethanol solution of hydroxide of potassium. A control test was performed under the same conditions. The dosage was done twice.

2.6.2. Saponification index

Saponification of oil index is the necessary weight of potassium hydroxide (KOH) expressed in milligrams to neutralize the fatty acids from hydrolysis of 1 g of this oil. It indicates the amount of total fatty acids in the fat material. It is a dosage in return. We react too hot a solution of fatty acids with an excess of KOH. This excess is then dosed in return by a solution of hydrochloric acid of known normality.

2.6.3. Index of ester

The index of esters of a lipid is the mass of potassium hydroxide (KOH) (in mg) required to saponify esterified fatty acids in 1 g of oil. It allows us to determine the molar mass of glycerides. The index of the ester is calculated by:

Index of ester = saponification index - index of acid (3)

2.6.4. Iodine number (In)

We classify the oils into three categories: drying (In > 150), semi-drying (110 < In < 150) or not drying (0 < In < 110). These categories are defined according to their iodine. The iodine value expresses the degree of the establishment of a fat material. It can be determined by the double bonds by the iodine dosage, and then corresponds to the mass of iodine, expressed in grams, fixed for 100 g of fat. To facilitate the addition reaction, the Wijs reagent is used preferentially to iodine [15].

2.6.5. Peroxide index (IP)

The peroxide value, expressed in milliequivalents of active oxygen per kg of oil (milliequivalent O_2 / kg of oil), was determined by dissolving a mass of jujube oil in a mixture of acetic acid / chloroform (3: 2 V / V). The reaction is triggered in the dark, in the presence of a saturated solution of potassium iodide. The liberated iodine is titrated with a 0.01 N solution of sodium thiosulphate in the presence of starch and a control test (without fat) is carried out under the same conditions.

2.6.6. Refractive Index

The index of refraction noted n_D^T (measure temperature T and D Ray sodium) is the ratio between the celerity of the speed of light c in a vacuum on the speed v of the in the resulting product transparent. In practice, this measurement is carried out by an Abbe refractometer essentially for liquid compounds.

2.7. Determination of the composition oil extracted from the kernels of the grains of Zizyphus lotus L. [16]

The determination of the chemical composition [17] of the oil extracted from the kernels of Zizyphus lotus L. to evaluate its quality and to foresee its use in the food, cosmetics and therapeutic field.

2.7.1. Preparation of methyl esters of fatty acids

Composition of fatty acid was determined by gas chromatography followed the ISO draft standard method [18]. 25 mµL of 2M solution of sodium methanolate in methanol were added to 10 mg of oil dissolved in 1 mL of petroleum ether. To this mixture are introduced 20 mµL of water. After centrifugation, the aqueous phase was separated. Then, 20 µL of methyl orange in 0.1 N hydrochloric acid was added as a pH indicator. The mixture was stirred well and various derivatives were analyzed by GC Clarus 580 GC_G12086, equipped with a N2 PFloW type column whose characteristics are:

- The length of 30 m, 0.32 mm internal diameter and a film of thickness 0.25 µm,
- the injector was in split, ratio 1/80 at the temperature of 260 °C,
- the carrier gas was helium its flow is 1.5 ml/min,
- the flame ionization detector (IDF) is increased to 280 ° C,
- The programming of the oven temperature was as follows: 100 °C for 2 minutes followed by an increase of 6 °C/min to 240 °C.

The identification of the peaks was made by comparison of retention times of methyl esters of fatty acids of oil plants such as olive oil, sunflower oil and palm oil, injected under the same operating conditions. In this study, we used olive oil as a standard .Each injection was repeated three times in the same operating conditions.

2.7.2. Determination of total sterols of Zizyphus lotus oil:

2.7.2.1. Determination of the unsaponifiable content:

The unsaponifiable content was determined according to the IUPAC method [19].

2.7.2.2. Preparation of the sterol fraction:

To determine the total sterols, 0.5 g of oil, 1 mL of cholesterol and 5 mL of an alcoholic solution of potassium hydroxide were introduced in a balled ball with 2 grains of pumice stone. The obtained mixture is carried to reflux for 15 minutes, and then 5 mL of ethanol is then introduced into the ball from the top of the refrigerant. After cooling, the mixture is introduced into a chromatography column full of aluminium oxide (0.063 < I < 0.2 mm). The elution made successively with 5 mL of ethanol and 30 mL of diethyl ether. The solvent is then evaporated and the sterol fraction obtained was dissolved in 1 mL of chloroform.

2.7.2.3. CCM preparation of the sterol fraction:

20 µL for a standard of cholesterol and 400 µL of the unsaponifiable of the oil fraction have been successively deposited using a depositor Linomat IV - Y CAMAG (Merck, no. 022-786) on a plate of silica 60 (Alltech, 20 x 10 cm, thickness 250 µm). Elution was made by a mixture of chloroform/diethylether (90/10, %v/v). The part containing the cholesterol deposit was revealed by nebulization of a mixture Cu^{++} / H_3PO_4 (1/1, %v/v) and a stint in the oven at 180 °C for 10 min.). The band of sterols for cholesterol spot has been scraped and sterols have been desorbed in chloroform (10 mL/g of silica) at ambient temperature with magnetic agitation for 5 minutes. Once become transparent, silica filtration on Millipore filters (0.45µm, Ref. SLFH 013 NL) allowed recovering total sterols without solid contaminants.

2.7.2.4. Composition and sterol content

1µL of this sterol fraction has been injected to determine the oil sterols content. The analysis of sterols was made in isothermal conditions (280 °C) in a chromatograph 6890 GC equipped with a column of type Agilent 19091J-413, whose characteristics are: 30 m long, 0.32 mm diameter internal and 0.25 µm of the film thickness). The temperature of the flame ionization detector is maintained at 300 ° C and that of the injector in split, ratio 1/100 to 325 ° C. The carrier gas was helium (2.0 ml/min). To identify the peaks, we injected cholesterol standards, β-sitosterol and stigmasterol (Sigma, concentration of 1 mg/mL quality products). In order to check the reproducibility of the results, each injection was repeated three times in the same operating conditions. Total sterols were calculated in the following way:

$$\text{Total sterols (mg/g)} = \sum_{x} (A_x \, x \, m_s \, x \, K \, x \, 100) / (A_x \, x \, m)$$

A_x= Area of the peak of the sterol
A_s= area of the peak of cholesterol
m_s= mass of added cholesterol;
m = mass of oil
K = response factor of the sterol calculated based on the area of the standard internal for the same concentration.

2.7.3. Determination of the composition and the content of tocopherols by HPLCUV

The tocopherols of oil analysis was conducted by HPLC in normal phase [20-23]. A solution to 20 mg of oil per mL of hexane and isooctane (99%) / propanol-2 (1%) has been filtered using a 0.45 µm in diameter millipore filter. The device and its accessories (pump, injector and detector) are products of Dionex RS 2000, containing a Quaternary pump, a manual injector with a loop of 20 µL injection and a fluorimeter sensor. The column was type C18.5 µm, 4.6 x 250 mm (K. romasil100 SIL). The mixture of solvents in isocratic conditions consisted of hexane and isopropanol for HPLC (99:1, % v: v).The flow of the column was 1 mL/min and pressure of 33 bar with a detector fluorimeter to the wavelength of 290 - 330 nm. The peaks were identified by injection of standards of tocopherols (Sigma aldrich products). Calibration curves were plotted using the range of dilution from 0.3 to 8 µL/mL.

3. Results and discussion

The moisture content, extraction yield and organoleptic characteristics of the oil of the kernels of Ziziphus Lotus kernels are summarized in Table 1.

3.1. Determination of physicochemical parameters

The results obtained by calculating the physicochemical parameters of this oil are summarized in Table 2. The value of the ester number obtained is 111.26 mg KOH / 1 g of oil. This value is between 80 and 180, which

allows us to say that this oil is of good quality. Depending on the value of the iodine number obtained: 86 mg I_2 / 100 g of oil, it can be concluded that Zizyphus lotus oil is non-drying, therefore does not form a film in contact with air , making it a base oil for massage and any other use in cosmetics. The value of the peroxide value found is 6 mEq / kg is in standard (15 mEq / kg of oil).

Table 1: moisture content, extraction yield and organoleptic characteristics of the oil of the kernels of Ziziphus Lotus

Humidity level	Performance of extraction	Organoleptic characteristics			
6,13%	8,1%	Color	appearance	odor	taste
		Light yelow	fluid	pronounced	characteristic

It can be concluded that the oil obtained by cold mechanical pressure from the kernels of the seeds of Ziziphus Lotus is of good quality.

Table 2: physicochemical parameters of kernels of Zizyphus lotus oil

Physico-chemical data	Zizyphus lotus oil
Index of acidity (mg KOH / 1 g of oil)	10.74
Index of saponification value (mg KOH / 1 g of oil)	122
Index of ester (mg KOH / 1 g of oil)	111.26
Refractive index (20 ° C)	1.46
Peroxide index (meq/kg)	6.0
Index of iodine (mg I_2 for 100g of oil)	86

3.2. Determination of chemical composition

3.2.1. Composition of fatty acids of kernels oil of the wild jujube

The chromatogram analysis presented by figure 1 shows the existence of particularly saturated fatty acids palmitic acid (C16:0) about 9.2% and stearic acid (C18:0) at a rate of 4.9%, and unsaturated fatty acids are more than 84% mainly oleic acid (C18: 1; ω-9) 66.84%, linoleic acid (C18: 2;) ω-6) 13.64% and a low amount of 0.5% of linolenic acid (C18: 3; ω-3) (Table 3).

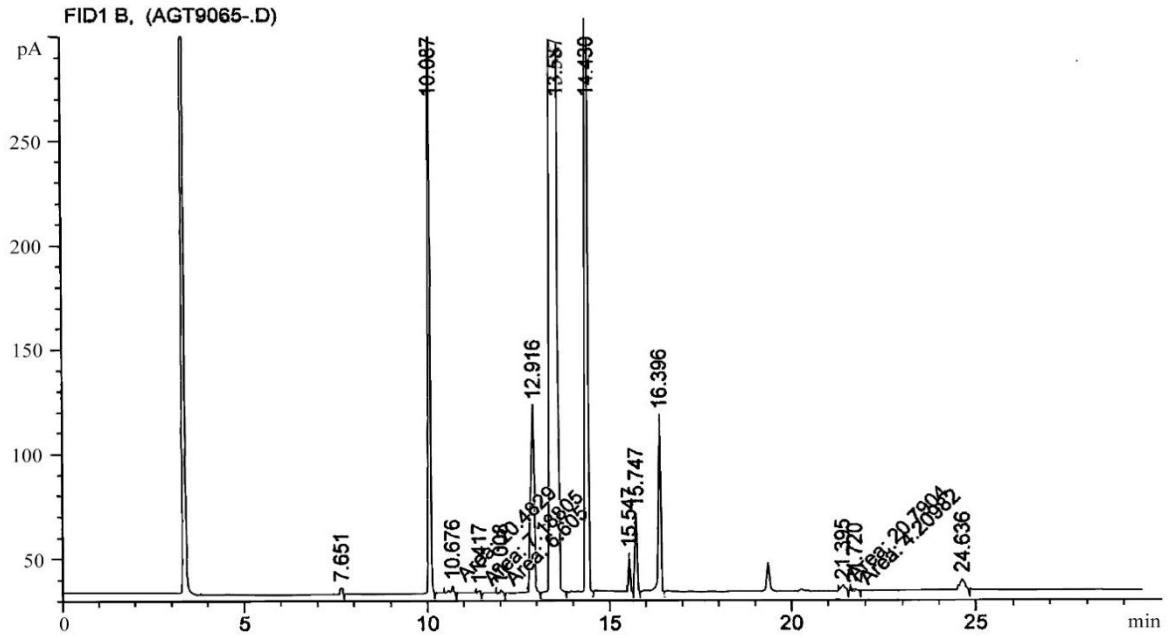

Figure 1 : Chromatogram of the major fatty acids of kernel oil of Zizyphus lotus L.

Table 3 : Mass Composition of the major fatty acids of Zizyphus Lotus L. oil

Fatty acids	Zizyphus lotus oil (%)	Retention time (min)	standard deviation(olive oil)	Retention time (min)	COI% (m/m) and Codex Alimentrius standard [24]
Myristic acid C14: 0	0.1	7.6	0.02	7.63	-
Palmitic acid C16: 0	9.2	10.08	11.9	10.058	7.5-20
Margaric acid C17:0	0.1	11.41	0.08	11.39	≤0.3
Stearic acid C18: 0	4.9	12.91	3.07	12.84	0.5-5
Arachidic acid C20: 0	1.1	15.74	0.38	15.71	≤0.6
Behinic acid C22:0	0.16	21.39	0.14	19.33	≤0.2
Lignoceric acid C24:0	0.38	24.63	0.05	24.62	≤0.2
Total saturated fatty acids	15.94	-	-	-	-
Palmitoleic acid C16 :1	0.2	10.67	1.02	10.65	0.3-3.5
Heptadecnoic acidC17:1	0.1	12	0.13	11.98	≤0.3
Oleic acide C18 :1	66.8	13.58	72.4	13.5	55-83
Linoleic acid C18 :2	13.64	14.43	9.9	14.36	3.5-21
Linolenic acidC18 :3	0.5	15.54	0.58	15.51	≤1
Gadoleic acid C20 :1	2.8	16.39	0.25	16.34	≤0.4
Erucic acid C22 :1	0.03	21.72	0.032	21.7	-
Total insaturated fatty acid	84	-	-	-	-

Oleic acid is the major component of this oil. Its unsaturated fatty acid composition is close to that of olive oil [25], which could make it a new food additive

The value of oleic acid (C18: 1) (66.8%) is significantly higher than that found by [11]which is of the order of 62.49% and the total saturated fatty acids found by mechanical pressure is 15.94%, it is lower than that found by (18.18%)[11].This difference is explained by the geographical area where the collection was made, the difference of the climate, the maturity of the plant also the mode of extraction can influence the values found.

There is a predominance of monounsaturated fatty acids in the order of 69.83%.a study conducted by [26] has shown that a mono-unsaturated oil has a low fluidity and during heating, it will have a great stability compared to that rich in polyunsaturated fatty acids. Another research proved that polyunsaturated fatty acids could solace symptoms of certain diseases such as coronary heart disease, stroke and rheumatoid arthritis [27].

The organic oil of Zizyphus lotus is unsaturated type can be categorized as oleic-linoleic oil. The food importance of this oil is based on its strong composition in unsaturated fatty acids: oleic acid known by its positive impact on human health. Regular consumption is therefore a source of essential fatty acids and produces particularly beneficial to the cardiovascular level by lowering circulating cholesterol levels, and its composition in linoleic acid, which is a polyunsaturated fatty acid in the omega-6 family. The organic oil of Zizyphus lotus of

kernels could have a cosmetic interest related to being rich in unsaturated fatty acids; these are known to oppose the activity of free radicals whose effect is harmful to the skin.

3.5.2. Composition of the sterol fraction

The table below shows the presence of sterols of Zizyphus lotus L. oil, mainly the β-sitosterol (71.66%), followed by stigmasterol (11.32%), the compesterol (8.48%) and the Δ5-avenasterol (4.24%). This oil has a content of total sterols 96.46 mg / 100 g of oil. The predominance of the β-Sitosterol is interested in this oil because it helps to combat cardiovascular disease by reduction of intestinal absorption of cholesterol [28], also, it has anti-inflammatory properties, antipyretics, antineoplastic and immunomodulating. Several studies show the interest of the β-sitosterol in the treatment of hyperplasia of prostate [29] it may have a beneficial effect on the overall health of a person with no diabetes.

Table 4: Total sterol composition of Zizyphus lotus oil

Sterols	Zizyphus lotus oil (%)	Retention time(min)	Standard deviation	Retention time(min)
Cholesterol	0.12	29.13	0.23	29.32
Campesterol	8.48	32.93	4	33.04
Stigmasterol	11.32	34.20	2.3	34.2
β-sitosterol	71.7	37.2	83.39	37.48
Δ 5-Avenasterol	4.24	37.51	5.46	37.81
Δ 7-Stigmastenol	0.3	39.18	1.69	39.49
Δ 7-Avenasterol	0.34	39.98	0.86	40.26
Total sterol (mg/100g)	96.5	-	-	-

3.5.3. Composition of the tocopherol fraction

The figure below shows the chromatogram of tocopherols of the Zizyphus lotus. The tocopherols known as vitamin E, are a family of phenolic compounds; they exist as α, β, γ or δ-tocopherol. The chromatogram analysis figure 2 shows the existence of a single peak corresponding to γ-tocopherol with a total content of 32.9 mg/kg. This oil contains only the γ-tocopherol, this form of vitamin E, has demonstrated anti-inflammatory and anticarcinogenic activity in the lung and colon [30]. Organic oil of Zizyphus lotus, thanks to the quantity in **γ**-tocopherol could exert a significant antioxidant activity.

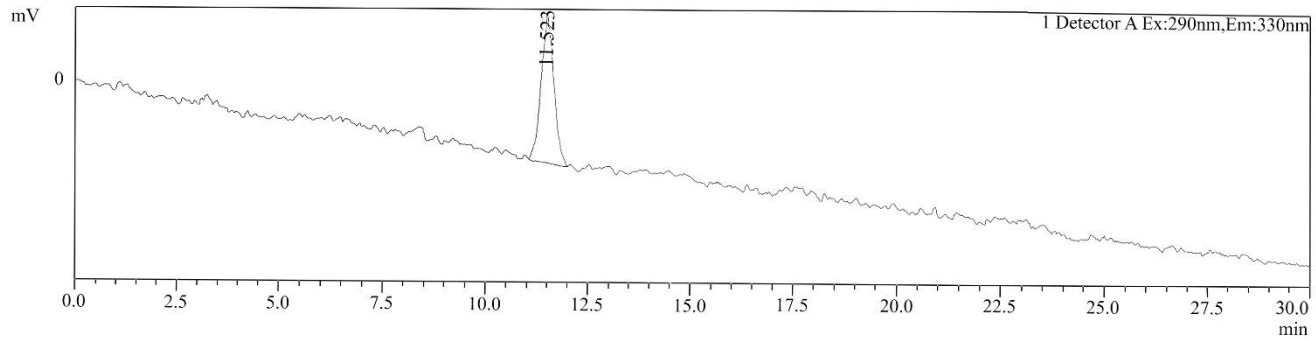

Figure 2 : Composition in the kernels of the Zizyphus lotus oil tocopherols

Conclusion

The present work allowed the extraction of an unconventional vegetable oil by mechanical cold pressing with a yield of 8,1% from almonds extracted from the kernels, which are generally discarded after the consumption of the fruit pulp.

This oil is characterized by the iodine value with a rate of 86 mg of I_2 / 100 g of oil which classifies this oil in the category of non-drying oils; on the one hand, and on the other hand ,by the peroxide value of 6 mEq O_2 / kg of oil below the standard established by the Codex Alimentarius (15 mEq O_2 / kg). The organoleptic examination of this oil shows that it is of acceptable quality.

The GC analysis of the fatty acid composition of the extracted oil shows that it contains more than 84% unsaturated fatty acids, or the majority fatty acid is oleic acid which allows it to classify this oil as oleic. The analysis of the unsaponifiable part shows a dominance of β-sitosterol, which accounts for 71.66% of the sterols present, and by HPLC, we note the presence of γ-tocopherol alone with the content of 32.9 mg /kg of oil.

Given these results obtained for the kernels oil of Zizyphus lotus fruit, we can conclude that this oil could be used as a massage oil, as a cosmetic raw material for the formulation of moisturizing creams and shampoos or as a food additive.

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