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Research Article

COMPOSITION IN FATTY ACIDS, STEROLS AND TOCOPHEROLS OF VEGETABLE OIL EXTRACT FROM RED ONION AMPOSTA OF TETOUAN REGION

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ABSTRACT

This work aims to determine the physicochemical characteristics and the fatty acid, sterols and tocopherols from unconventional vegetable oil obtained by extraction with hexane from Amposta red onion seeds *Allium cepa* L. in the Tetouan region in northern Morocco. GC analysis of this oil in fatty acid shows a predominance of linoleic acid (62.3 %) and oleic acid (27.7 %) and sterol a predominance of β -sitosterol (61.9%) with a overall rates of sterols 96.1 mg /100g. Finally, analysis the tocopherols of this oil by HPLC give a rate of 1666 mg/Kg the tocopherols with 47.44% of γ-tocopherols.

Keywords: Red onion Amposta, fatty acids, sterols, tocopherols, linoleic acid, β-sitosterol

1. INTRODUCTION

The red onion Amposta is an herbaceous species [1], perennial by its unique bulbous, grown as an annual or biennial plant. This is a plant that reaches a height of 40-80 cm. Its green leaves are cylindrical and hollow. Its upright flower stem is hollow and has a bulge at its base. Its bulb is moderately large, spherical, sometimes more or less flattened. Its small flowers (4 to 5 mm wide), white in color, are grouped into a spherical umbel terminal position on the rod. The flowers have trimer symmetry, three sepals, three petals and six stamens. The single ovary is divided into three cells. The fruit is a capsule opening by three valves, releasing each usually two seeds. This plant has a bulb that allows it to reproduce.

The onion has been consumed since long ago by humans. In Morocco about 400000 tones are produced out of which 10% is red onion Amposta [2]. Medicine has shown better benefits [3] of onion in general and red onion Amposta in a particular way. More recently, many studies confirm the extraordinary powers of this vegetable plant. Today we recognize onions have very strong antioxidant properties [4]. It is very rich in a variety of protective agents against cancer. It is particularly rich in quercetin [5], a powerful protective antioxidant in the prevention of digestive tract cancers [6].

Onion is particularly indicated for the patient bloated or puffy edema, dyspepsia, diabetes and prostate [7 - 9]. It has anti-inflammatory, antiallergic properties [10] and helps prevent some cancers. Its antiseptic and anticatarrhal activity [11] makes it useful for colds. Its antiplatelet activity seems insufficient *in vivo*. It is also neuroprotective [12] and inhibits osteoclast activity. The onion's medical value is due to its properties that prevent proliferation of cancer cells but also because it destroys harmful bacteria that may trigger a stomach cancer [13]. Milled red Amposta onion seeds are used in Moroccan traditional medicine to treat asthma attacks. Nevertheless, the data is insufficient as regards the use and study of vegetable oil seeds of onion which is still not recovered. This work aims at the valuation, determination of the composition of fatty acids, sterols and tocopherols from unsaponifiable fraction [14, 15] and the study of physico-chemical characteristics of the oil from the seeds of red onion from Amposta in Tetouan region.

2. MATERIELS AND METHODS

2.1. Plant Material

The lot of the seeds of red onion Amposta was harvested in the month July 2012 at Bnihssaine located in the region of Tetouan in northern Morocco. The seeds were separated from their envelopes, freed of all impurities, dried in the sunlight, and then they are placed in an oven for 6 hours at 55°C, then they were finely ground and conditioned at 25°C in a sealed vial before extracting the oil.

2.2. Extraction of oil

The Alternative seed oil of *Allium cepa* L. Amposta red was obtained by maceration [16] of the powder finely ground seeds. 100g of the powder in a ground glass 500 ml flask and 400 ml of hexane were added. The mixture was heated to reflux for 20 minutes and then left to stir for 12 hours. The filtrate was then evaporated under reduced pressure to remove traces of hexane. The resulting oil was packaged in a dark bottle under an inert atmosphere.

2.3. Physico-chemical characterization of the oil

The acid index, the refractive index, the index of peroxide, iodine number and the ester number were determined according to standard methods by AFNOR .

2.4. Determination of fatty acid composition by Gas Chromatography

2.4.1. Preparation of methyl esters of fatty acids

According to the protocol of standard NF T60-233, methyl esters were prepared to determine the fatty acid composition of the seed oil red onion Amposta.

2.4.2. Fatty acid composition

One µl of a hexane solution of methyl esters was injected into a chromatograph Clarus 580 GC_G12086 equipped with a N2 Pflow-type column of length 30 m, internal diameter 0.32 mm and film thickness of 0,25µm. The injector was split mode, ratio 1/80 made 260 °C. The carrier gas was helium flow rate 1.5 ml / min. The flame ionization detector is increased to 280 °C. Programming of the oven temperature was 100 °C for 2 min followed by an increase of 6°C/min to 240°C. The peak identification was made by comparison of methyl esters retention times of fatty acids of vegetable oils such as olive oil, sunflower oil, argan oil, injected into the same operating conditions.

2.5. Determination of total sterols

2.5.1. Determination of unsaponifiable material

The content of unsaponifiable material was determined according to IUPAC method [17].

2.5.2. Separation of the sterol fraction

In a grounded drum, 50 ml oil was successively introduced 0.5 g, 1ml cholesterol and 5 ml of an alcoholic solution of 2N potassium hydroxide. The resulting reaction mixture was refluxed for 15 minutes then 5 ml of ethanol was introduced through the top of the condenser. The mixture of fine reaction was cooled and then it was introduced into a chromatography column filled with aluminum oxide ($0.063 < I < 0.2$ mm). The elution was made successively with 5 ml of ethanol and 30 ml of diethyl ether. The solvent was then evaporated and the sterol fraction is dissolved in 1ml of chloroform.

2.5.3. TLC preparative of the sterol fraction

400 µl of the unsaponifiable fraction of the oil studied and 20 µl of a cholesterol standard solution are successively deposited on a silica plate 60 (Alltech , 20 x 10 cm, 250 mm thick) using a pinsetter Linomat IV -Y CAMAG (Merck , Ref. 022-786). Elution was done with a chloroform/diethyl ether (90/10, v/v%). The portion containing cholesterol deposition was revealed by spraying with a mixture Cu^{2+}/H_3PO_4 (1/1, v/v %) and a passage in the oven at 180°C for 10 min. The band corresponding to the sterol cholesterol spot was scraped and sterols was recovered in chloroform (10 ml/g of silica) at room temperature under magnetic stirring for 5 min. total sterols were recovered without contaminating solid by filtration on Millipore filter (0,45µm , Ref. 013 SLFH NL), once the silica became transparent.

2.5.4. Sterol Composition

To determine the total sterols content of the oil studied, 1µl of the sterol fraction was injected. The analysis of the sterols was carried out using isothermal conditions (280°C) by means of a GC6890 chromatograph equipped with a column of type Agilent 19091J-413 (Column: 30m long, 0.32 mm internal diameter and film thickness of 0,25µm). The temperature of the flame ionization detector was maintained at 300°C and that of the injector in split mode, ratio 1/100 to 325°C, the carrier gas was helium (2.0 ml / min). Cholesterol Standards, of β-sitosterol and stigmasterol (Sigma quality products, concentration of 1mg / ml) were injected in order to identify the corresponding peaks. Each injection was repeated three times in the same operating conditions In order to verify the reproducibility of the results. The total sterols contents are calculated using the following formula:

Total sterols (mg / g) = $\Sigma((Ax.ms.K.100)/ As.m)$ with

Ax = area of the sterol peak X (sample)

As = area of the standard cholesterol peak

ms = mass of added standard cholesterol

m = mass of oil studied

K = the sterol response factor calculated based on the internal standard area for a same concentration.

2.6. Determination of the composition of tocopherols content by HPLCUV

The unsaponifiable fraction of the red onion Amposta oil was analyzed by normal phase HPLC [18, 19] to determine the tocopherols content. A solution prepared from 20 mg of oil per milliliter of hexane and isooctane (99%)/2-propanol (1%) filtered through a Millipore filter 0,45µm diameter. The manual injector is provided with an injection loop of 20 µl and the column is K.romasil100 SIL parameters (C18, 5µm, 4,6x250mm). The solvent mixture in the isocratic conditions consisted of hexane and isopropanol to HPLC (99: 1% v: v). The flow of the eluent was 1ml/min and pressure of 33 bar with a fluorescence detector at a wavelength of 290-330 nm. Peaks were identified by injection of tocopherols standards (Sigma Aldrich product). The calibration curves were plotted by taking a dilution range from 0.3 to 8 mg /ml.

3. RESULTS AND DISCUSSIONS

3.1. Physicochemical characteristics of oil from Amposta red onion seeds.

The physicochemical parameters of the oil obtained are summarized in the table 1.

According to JP. Wolff [20] there is a close relationship between the iodine number and the refractive index of which are two important criteria for determining the siccativity of oil. The results of analysis of this oil (1.55 acidity index and index of refraction 1.470) compared to literature indices (ID < 100 1.467 < IR <1.472) and therefore could not form any film contact with air , which could qualify to be a base oil for massage or cosmetics .

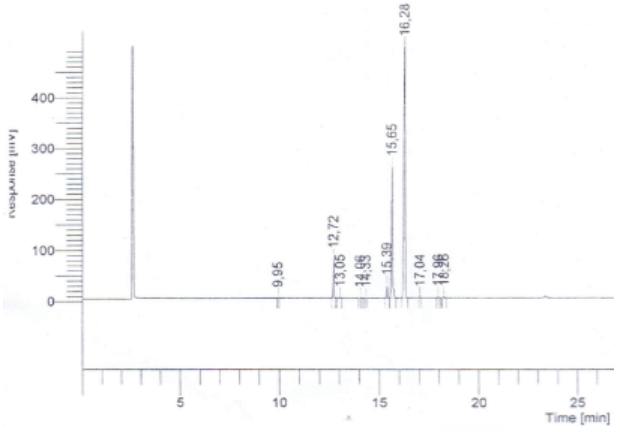
The acid value is 1.55 mg / 100g exceeds the standards of the Codex Alimentarius (… < 10). The strong acid value of this oil could be explained by a bad conservation.

Table 1: Physical and chemical oil onion seeds Total sterols (mg /100g of oil)

Physicochemical parameter	Oil red onion seeds Amposta
Acid value (mg / 100g)	1.55
réfraction index (at 20 ° C)	1.470
Peroxyde value (meq / kg)	2
Iodine value (mgd'I2 for 100g of oil)	94.33
Saponification number (mg KOH / 100 g of oil)	18.55
Total sterols (mg / 100g of oil)	96.10
Total tocopherols (mg / kg of oil)	1666

3.2. Fatty acid composition of the oil extracted from the seeds of red onion Amposta

The GC analysis of the fraction of the ester oil gave the chromatogram (Fig. 1) in which each peak corresponds to a fatty acid. The retention time of the peaks obtained are compared with the retention time of the chromatogram peaks standard thereby determining the composition of the sample fatty acid. The percentage for each peak was possible to be deduced from the percentage of each fatty acid. The result is summarized in Table 2.



Peak #	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1	9,947	2304,74	849,46	0,06
2	12,719	257008,29	77083,01	6,65
3	13,045	8525,54	1989,60	0,22
4	14,059	1461,26	433,85	0,04
5	14,332	1264,38	378,77	0,03
6	15,392	89525,13	24197,32	2,31
7	15,647	1071952,96	257534,51	27,72
8	16,278	2409576,23	565975,05	62,30
9	17,044	5800,74	1708,38	0,15
10	17,963	8775,67	2155,51	0,23
11	18,257	11389,87	2500,26	0,29
		3867584,81	934805,71	100,00

Fig.1: Chromatogram of the fatty acid composition of the seed red onion Amposta oil

Saturated fatty acids represent only 9.3% of total fatty acids of the oil (mainly the medium-chain fatty acids C16 and C18), while unsaturated fatty acids account for more than 90.7% mainly acid linoleic (C18: 2) from about 62 % oleic acid (C18: 1) 28 %, 0.2 % linolenic acid and gadoleic acid (C20: 1) 0.3 %, which allows to classify this oil in linoleic - oleic category. These acids are called essential fatty acids necessary for the growth and physiological activity of all tissues. They are involved in the regulation of cardiovascular disorders and cholesterol regulation.

However seed oil red onion Amposta could be a source of oleic acid (Omega -9) and linoleic acid (Omega-6).

Table 2: Composition of the main mass fatty acid red onion seeds Amposta oil

Major fatty acids	Retention time (min)	Percentage (%)
myristic acidC14 : 0	9.947	0.1
Palmitic acid C16 acid : 0	12.719	6.7
Stearic acid C18 acid : 0	15.392	2.3
Arachidinic acid C20 : 0	17.963	0.2
Total saturated fatty acid		9.3
Palmitoleic acide C16 : 1	13.045	0.2
Oleic acid C18 : 1	15.647	27.7
Linoleic acid C18 : 2	16.278	62.3
Linolenic acid C18 : 3	17.044	0.2
Gadoleic acid C20: 1	18.257	0.3
Total Unsaturated fatty acid		90.7

3.3. Total sterols composition of the red onion seeds Amposta oil

Table 4: Total sterol composition of oil from Amposta red onion seeds

Sterols main	Percentage (%)	Retention Time
Cholesterol	2.76	38.575
Brassicasterol	0.539	41.409
Campesterol	17.902	44.32
Stigmastérol	0.451	45.930
β - Sitostérol	61.948	50.292
Δ^5- Avenastérol	10.831	51.061
Δ^7- Stigmastérol	1.085	54.06
Δ^7- Avenasterol	0.606	55.209
Total Sterols	96.122	--

GC analysis of the seed oil of onion Amposta shows that the chromatogram is characterized by the presence of at least three sterols. The principal sterol is β-sitosterol, whose percentage is 61.9% followed by campesterol 17.9%, the percentage of Δ5-avenasterol 10.5% and a percentage amount of 2.8% cholesterol. The total sterol content was 96.1 mg /

100 g. This oil can be considered rich in β-sitosterol with a content of 59.6 mg / 100g. This sterol is known by its fight against cardiovascular disease by reducing the intestinal absorption of cholesterol and has anti-inflammatory, antipyretic, antineoplastic and immunomodulating properties. Several studies showed the interest of the β-sitosterol in the treatment of prostatic hyperplasia [21]. Campesterol representing 17.9% possesses anti-inflammatory properties.

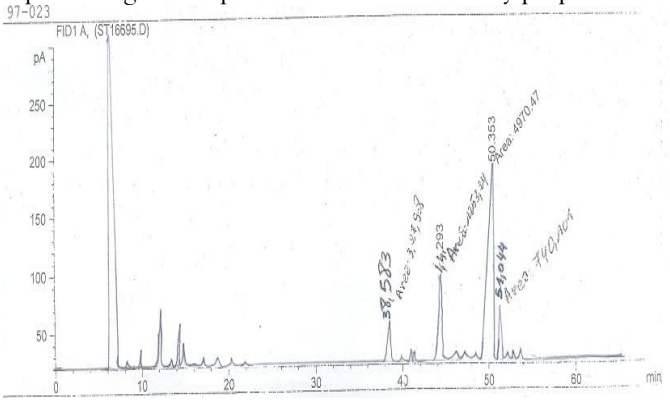


Fig. 2: Chromatogram of the sterol fraction of the oil from Amposta red onion seeds

3.4. Composition of the tocopherols oil from Amposta red onion seeds

The chromatogram of Fig. 3 contains main peaks corresponding to the tocopherols. Tocopherols content is 1666 mg /kg, higher than that of olive oil (110-183mg / kg). The main tocopherols present in the sample are the β-tocopherol 19.58 %, the γ-tocopherol 47.44 %, and the δ -tocopherol 4.85 % with the absence of α -tocopherol (Table 3).

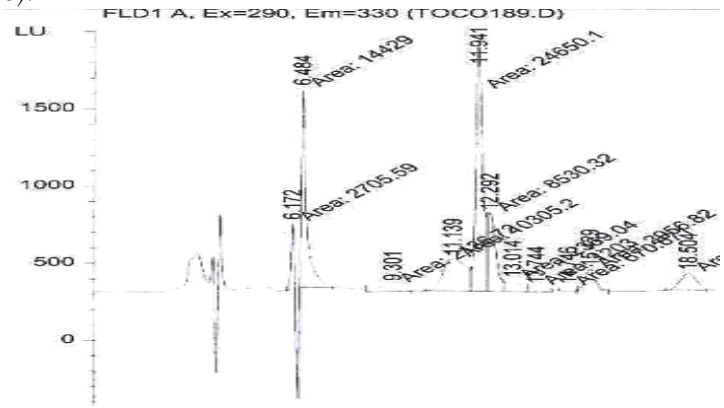


Fig. 3: Chromatogram of the composition of total tocopherols in the Amposta red onions seed oil.

The γ-tocopherols are the most abundant form which has antioxidant properties and participates in the protection of all cell membranes in the body by fighting against free radicals. Its presence in the oil limits fat oxidation responsible for cardiovascular diseases and strengthens the immune system.

Table 5: Composition of total tocopherols in the seed oil of red onion Amposta

Tocophérols	(%)
α-tocopherol	--
β-tocopherol	19,58
γ-tocopherol	47,44
δ-tocopherol	4,85
Total tocopherols (mg/kg)	1666

4. CONCLUSION

The oil extracted from Amposta red onion is obtained with a yield of 17%. Analysis of the fatty acid fraction by GPC shows that the oil is rich in essential unsaturated fatty acids in particular linoleic acid (62.3) and oleic acid (27.7%).

The unsaponifiable fraction that was analyzed by GC revealed the presence of the β-sitosterol in a percentage of 62%, campesterol with a percentage of 17.9 and the Avenasterol Δ5- with a percentage of 10.8 which corresponds to 90.7% of total sterols. The composition of tocopherols in the unsaponifiable fraction determined by HPLC shows that γ-tocopherols are the majority with 47.44% and β-tocopherols monitoring of 19.58% with an absence of α- tocopherols.

The composition of this vegetable oil is rich in linoleic acid (omega-6) which is of interest in food, rich in beta-Sitosterol known by its controlling effect against cancer, diabetes, and arthritis and Cardiovascular diseases and also rich in γ-tocopherols in particular β-tocopherol and tocopherols known for their antioxidant activity.

We can conclude that this oil could be used as an additive in food and ingredient in cosmetics

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