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Study of the chemical composition of argan oil according to the shape of the fruit

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The aim of this study was to investigate the effect of phenotypic diversity of argan fruit with different morphological characteristics (fusiform, oval, apiculate and spherical) on fat and protein content, inflexibility and fat chemical composition, oil acids and sterols. To investigate the links of argan fruit shape with the chemical composition of argan oil, with the help of native communities, 4 different fruit shapes (fusiform, apiculate, spherical and oval) were selected, which were harvested from the same place (Tamanar) in Essaouira province (South Plain region, Western Morocco). After harvesting the fruit of the argan tree, 100 samples were taken from each form. They were crushed to destroy the core. After extraction of hexane with Soxhlet, fat content, protein level, unsaponifiable content, composition of fatty acids and sterols in fat were determined. The results showed that the oval shape is the best shape of argan fruit because their kernels contain more than 50% fat and a higher percentage of unsaponifiables. The results on fatty acids and sterols showed that argan oil contained 80% of unsaturated fatty acids. The results also showed that the main products of the sterol composition in argan oil were schottenol (or Δ -7-stigmasterol) (42.8 and 46.4%) and spinasterol (39.8 and 45.6%). The study of the chemical composition showed that there was no correlation between the shape of the fruit of the argan tree and the composition of fatty acids. Depending on the shape of the argan fruit, fatty acids and sterols were not only related to the shape but also to the nature of the soil and its altitude, longitude and distance from the sea.

Keywords: argan oil, chemical composition, fatty acid profile, fruit shape, sterol

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Изучение химического состава арганового масла в зависимости от формы плода

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Целью данного исследования было изучение связи фенотипического разнообразия плодов аргании с различными морфологическими характеристиками (веретеновидных, овальных, остроконечных и шаровидных) с содержанием жира и белка, изменчивостью и химическим составом жирных кислот и стеролов в масле. Для исследования влияния формы плодов аргании на химический состав арганового масла с помощью местных общин были отобраны плоды четырех разных форм (веретеновидная, остроконечная, шаровидная и овальная), которые собирали в одном месте (Таманар) в провинции Эс-Сувеира (регион Южной равнины, Западное Марокко). После сбора плодов арганового дерева от каждой формы отбирали по 100 образцов. Их измельчали, чтобы разрушить ядро. После экстракции гексаном по Сокслету определяли жирность, уровень белка, неомыляемых веществ, состав жирных кислот и стеролов в масле. Результаты показали, что плоды аргании овальной формы являются лучшими, потому что их ядра содержат более 50% жира и более высокую долю неомыляемых веществ. Данные по жирным кислотам и стеролам свидетельствовали, что аргановое масло содержит 80% ненасыщенных жирных кислот. Результаты также показали, что основными продуктами стерольного состава арганового масла были шутенол (или Δ -7-стигмастерол) (42,8 и 46,4%) и спинастерол (39,8 и 45,6%). Изучение химического состава показало отсутствие корреляции между формой плодов арганового дерева и составом жирных кислот. В зависимости от формы плодов аргании жирные кислоты и стеролы были связаны не только с формой, но и с характером почвы, высотой над уровнем моря, долготой и расстоянием от моря.

Ключевые слова: аргановое масло, химический состав, жирнокислотный профиль, форма плода, стерол

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Introduction

The argan tree (*Argania spinosa* (L.) Skeels) is a tree endemic in Morocco, where it constitutes the second forest species of the country, after the holm oak and just before the thuya. It is a tree that can live up to 200 years. Some studies have observed 250-year-old trees. The argan forest covers approximately 800,000 ha, and has more than 20 million trees (Chamich, 2013). This tree from the Sapotaceae family is particularly resistant to the dry and arid conditions of Southwest Morocco. It can in fact withstand temperatures ranging from 3 to 50°C and be satisfied with very low rainfall (Mateille et al., 2016).

The argan tree grows wild and in abundance in the arid zone of Southwest Morocco, where it plays an irreplaceable role in the ecological balance and in the preservation of biodiversity. Thanks to its powerful root system, it helps to maintain the soil and fight against water and wind erosion, which threatens much of the region with desertification (Fig. 1).

The argan tree is also of great economic interest because it is a multipurpose tree. Each part of the tree is usable and serves as a source of income or food for the user: the wood is used as fuel, the leaves and fruits constitute fodder for goats and camels, and the oil extracted from the almond is used in human food and in traditional medicine (Justamante et al., 2017).

The argan tree therefore plays a socio-economic and environmental role (Justamante et al., 2017) of prime importance in these geographical areas. It got particular legislative status in Dahir on March 4, 1925 and specifications related to agrarian practices under the argan tree in July 20, 1983. This makes it a state forest whose right of use dedicated to local communities is very extensive: right to harvest fruit and collect wood for domestic use, with free fees. Unfortunately, its agricultural overexploitation, soil erosion, and the advance of the desert are all attack on this unique heritage. In less than a century, more than half of the forest has disappeared and its average density has increased from 100 to 300 trees per ha (Le Polain de Waroux, Lambin, 2012).

Despite all these interests, an alarming decline has been observed in argan groves both in acreage and density. In less than a century, more than 2/3 of the forest has disappeared and each year there are losses of 600 ha (Khayati et al., 2018). The argan tree is a multipurpose tree. Each part of the tree constitutes a source of income or food for the user. The argan tree plays an essential role in the fight against rain erosion by fixing the soil of the hills that it populates. It sets up a rampart against the desertification of the pre-Saharan areas of the Souss plain. This is how the uses of the argan tree are multiple. The importance of its environmental (brake against erosion and desertification) and socio-economic (grazing, argan oil, construction and firewood) roles now requires the development and implementation of a national and international strategy to safeguard this unique, very slowly growing species.

Argan oil has remarkable nutritional qualities indeed. It is an oil rich in monounsaturated fatty acids (43.15% of oleic acid: omega 9) and polyunsaturated ones (38.86% of linoleic acid: omega 6). The level of linoleic acid (vitamin F) is three times higher in argan oil than in olive oil (10.83% of linoleic acid). The tocopherol content of argan oil is 717 mg/kg (320 mg/kg in olive oil). Argan oil is very rich in gamma tocopherol (631 mg/kg) (30 mg/kg in olive oil) which gives it a protective effect against free radicals. Argan oil is rich in phytosterols. These belong to the delta 7 stigmastane family. The major sterols in argan oil are schottenol and spinasterol.

Schottenol is shown to be anticarcinogenic with a pronounced cytotoxic potential.

Faced with this problem, the Laboratory of Plant Chemistry and Organic and Bioorganic Synthesis, Faculty of Sciences of Rabat University, has set before itself the objective of promoting the products of the argan tree for the benefit of rural communities, so that they become more motivated to protect and replant the argan tree.

This work was therefore part of the continuation of the series of research carried out by the Laboratory of Plant Chemistry and Organic and Bioorganic Synthesis, Faculty of Sciences of Rabat University, on the argan tree to improve and enhance the products of the argan tree to preserve and develop the argan diversity.

Materials and methods

Preparation of samples

Weight study of samples

To carry out this work, we selected the region of Tamanar because this region is full of the argan tree. This region is located in the southwest of Morocco on a plain. Then we selected four trees of the argan tree, each tree having a different fruit shape (each tree yields a single shape) and then we took 100 fruits from each argan tree. Then we determined the fat content, protein rate, humidity and unsaponifiable content in the almond of the argan fruit.

Sample preparation

The extraction of argan oil is done in several stages (Hilali et al., 2020c):

- Pulping: the skin is removed from the fruit using two stones; the pulp and the nut are separated as the pulping is carried out.
- Crushing, or hulling: it is done with the same stones as the pulping, the nut is crushed by crushing it strongly. Sorting is done at the end of the operation.
- Roasting of almonds: it is done in earthen containers on a soft wood fire.
- Almond pressing: pressing is carried out by a KOMET D85-type worm screw press. Its output varies from 6 to 8 liters of oil per hour.

To study the relation between the shape of the argan fruit and the chemical composition of argan oil, with the help of indigenous populations 4 different fruit shapes (fusiform, apiculate, spherical and oval) (Hilali et al., 2020c) were selected among those harvested from the same place (Tamanar) in the province of Essaouira (the plain area in Southwest Morocco), knowing that the argan tree gives a single form of fruit.

After the harvest of the fruit of the argan tree, of each form, 100 fruits were selected, pulped and crushed to remove the kernel. After the hexane extraction with Soxhlet, the fat content, protein level, unsaponifiable content, and the compositions of fatty acids and sterols were determined.

Physicochemical analyses of oil

All the analyses were done in the Official Laboratory of Chemical Analysis and Research (LOARC) in Casablanca, Morocco. Percentage of fat, unsaponifiable content, percentage of protein, sterols and cis-fatty acids were measured according to the standardized methods of reference.

Determination of ISO 659 fat content

The lipid content was determined according to the AOAC method: 25 g of a sample was added to the filter paper cartridges, then placed on the Soxhlet. Then 250 mL of hexane was poured into a flask. The flask was heated for 4 hours. After removal of the solvent by distillation, the flask was dried at

a temperature of 70-80°C, then weighed after cooling in a desiccator.

The fat content was determined according to the following formula:

$$L (\%) = ((P2 - P1) / P3) \times 100,$$

where P1 is the weight of the empty flask (g);
P2 is the weight of the flask with the extracted oil (g);
P3 is the weight of the test portion (g)

Determination of the protein content

The protein content was determined according to the Kjeldahl method. This method was based on the quantification of the nitrogen content, then the protein content was calculated by multiplying the total nitrogen content N (%) by the coefficient of 6.25. This method was applied in two stages: 1 g of a sample was mixed with 1 g of the Kjeldahl catalyst (copper and potassium sulfate) and 15 mL of sulfuric acid, the mixture was prepared in a mineralization flask by applying progressive heating. When the solution became clear, it was cooled with 100 mL of distilled water. The second stage was the distillation which consisted in solubilizing the mineral nitrogen in the form of ammonia; this stage was carried out in a Rota vapor by adding 20 mL of soda to 35–102% in the flask and 25% of boric acid in a 250 mL flask. The ammonia was recovered in a solution of boric acid. The last step was titration: carried out by adding a few drops of the Tachiro indicator (mixture of methylene blue and methyl red) to the flask containing ammonia and boric acid. The excess ammonia was then dosed with 0.05 N sulfuric acid by simple titration.

The total nitrogen content has been determined according to the following formula:

$$N (\%) = (Co \times 2 \times V \times 14) / P$$

$$P (\%) = N (\%) \times 6.25,$$

where N is the percentage of nitrogen (%);
P is the percentage of protein (%);
Co is the normality of sulfuric acid (0.05);
V is the volume of sulfuric acid poured (mL);
P is the weight of the test portion (g).

Determination of unsaponifiable content

The unsaponifiable content is defined as the percentage of the substances present in the product which, after saponification thereof with potassium hydroxide and extraction with a specified solvent, are not volatile under the specified operating conditions.

Procedure

In a 250 mL flask, weigh 5 g of the argan oil and add 50 mL of the KOH (1N) solution (ethanolic). Bring to a gentle boil for an hour. Then add 100 mL of distilled water from the top of the condenser, and allow to cool. The reaction mixture is transferred to a separating funnel and then extracted three times with 100 mL of diethyl ether.

The diethyl ether extracts are combined and then washed 3 times with 40 mL of distilled water while gently rotating the separatory funnel. The water from the last wash should not give a pink color by adding a drop of the phenolphthalein solution. The ethereal phase is transferred to a 500 mL flask which has been dried and tared beforehand. The solvent is evaporated off with a rotary steamer and the residue is dried in an oven at $103 \pm 2^\circ\text{C}$ for 15 min until the difference between two successive weighings is less than 0.00015 g, or P1 (the mass of the residue).

After weighing the residue, it is dissolved in 4 mL of diethyl ether. Then 20 mL of preneutralized 95° ethanol and a few drops of phenolphthalein are added.

The titration is carried out with an ethanolic KOH solution titrated at 0.1 N to determine the free fatty acids.

Calculation of the unsaponifiable content

The percentage unsaponifiable content is calculated using the following formula:

$$[P1 - (0.28 \times V \times T)] \times 100 / P,$$

where P is the mass in g of the test sample;
P1 is the mass in g of the residue;
T is the exact normality of the KOH solution (0.1 N);
V is the volume of the KOH solution (0.1N) in cm³.

Determination of composition and nature in total sterols

Operating mode

Argan oil was weighed in a 250 mL flask and then 25 mL of a solution of potassium hydroxide (1N ethanol) was added. The flask was heated at reflux for 30 min until the solution became clear. Finally, to stop the reaction, 25 mL of distilled water was added.

Extraction of the unsaponifiable was carried out using 75 mL of hexane or petroleum ether. The organic phase was subjected to a series of washings with 15 mL of the mixture (water/ethanol 95°) (90/10) in a separatory funnel.

The hexane phase was transferred from the top of the ampoule into a 100 mL flask. After evaporation of the solvent using a rotary evaporator, the unsaponifiable material was recovered.

The unsaponifiable agent, diluted with 300 µL of hexane or petroleum ether, was filtered on a silica column (25 cm × 4 mm). The HPLC device was equipped with a 205–254 nm UV detector. The eluent was an isooctane/isopropanol (99/1) mixture whose flow rate was 1.2 mL/min. The duration of the analysis was 15 min, the sterol fraction, recovered according to standard NF 12228 May 1999, was evaporated to dryness.

The sterols were converted to silylated derivatives (TMS) using a mixture of pyridine, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS), (9/1/1), (v/v/v). The pyridine was evaporated to dryness and the silylated derivative was diluted with 60 µL of heptane or hexane.

The TMS sterols were analyzed by gas chromatography (GC) on an apolar column (Chroma pack) (30 m × 0.32 mm, DI: 0.25 µm, phase: CPSIL8CB).

The HP Hewlett Packard 6890 GC Series Chromatograph was equipped with a FID detector (T: 300°C). The carrier gas was nitrogen and its flow rate was 1 mL/min (PE: 8.6 bar). The analysis was performed in temperature programming (200°C up to 270°C with a speed of 10°C/min and an isotherm at 270°C for 35 min).

Analysis of cis-fatty acids

Operating mode

The test sample of argan oil (1 g) was supplemented with 0.5 mL of methanolic KOH for HPLC (minimum 98%) and 10 mL of methanol in a 100 mL flask. The mixture was refluxed for 15 min until the solution became clear. Then 1 mL of heptane was added to the reaction mixture after cooling.

The heptanic phase containing the methyl esters was transferred to a test tube and then a solution of sodium carbonate Na₂CO₃ was added. This neutralized all free acids by giving sodium salts with a release of carbon dioxide.

The methyl esters, which were in the organic phase, were removed using a 2 mL cone pipette and placed in a test tube.

The methyl esters underwent a series of washings. 20 mL was taken from the esters, which was placed in a tube of the nominal capacity of 2 mL and then filled with heptane. The fatty acid was analyzed by GC gas chromatography. The HP Hewlett Packard 6890 GC Series GC chromatograph was equipped with a divider (T: 240°C) and a FID (T: 260°C) injector. The carrier gas was nitrogen (PE: 12.4 bar). The analysis was carried out in temperature programming (140°C to 200°C with a speed of 10°C/min and an isotherm at 200°C for 40 min) on a capillary column (polyethylene glycol) (30 m × 0.32 mm, DI: 0.25 µm).

Results and discussions

Our field investigation revealed four different argan-tree fruit shapes: fusiform, oval, apiculate, and spherical (Gharby et al., 2013). With the help of the indigenous communities, an area located in the plain was selected. So, there were 4 samples containing different fruit shapes and in places to see the relationship between the shape and the chemical composition (Table 1).

It emerged from these results that the argan kernel was very rich in fat (54%) (Table 1) and in lipid extract; the values found for the fat of the almond varied from 49% for the apiculate shape to 54% for the oval shape. Moreover, the unsaponifiable rate varied from 0.22% to 0.60% for the oval shape.

When it came to protein levels, almonds were rich in protein (Hilali et al., 2020a, 2020b).

In the almond, variations were from 21.5% for the fusiform shape to 25% for the apiculate shape (Fig. 1) (Hilali et al., 2020a, 2020b).

This result showed clearly that the shapes of the argan-tree fruits is connected with the percentage of fat, the protein and the unsaponifiable content in their argan fruit kernel and that the best shape is oval because it contains the highest percentage of fat and protein.

Analysis of fatty acids

The fatty acid composition of different oils was determined after methylation of argan oil and analysis of methyl esters by gas chromatography on a capillary column. Table 2 groups together the results obtained for the 4 samples.

The fatty acid composition corroborates with the data in the literature (Hilali et al., 2020a, 2020b) (Fig. 2).

The fatty acid analyses were made by gas chromatography (series Hewlett Packard 6890 GC). For the identification of fatty acid, we compared the time to remember these acids by the reference time.

Virgin argan oil contains 80% of unsaturated fatty acids. It is of the oleic-linoleic type and contains between 30 to 34% of essential fatty acids: linoleic acid (30 to 34%) (vitamin F) (Table 2).

Unsaturated fatty acids play an essential role in the prevention of cardiovascular disease, while the omega 6 family (like linoleic acid) is essential for the growth of a child (Dubey et al., 2020; Shivakumara et al., 2021).

Its oleic acid content makes argan oil particularly beneficial in regulating cholesterol (Shivakumara et al., 2021).

The other fatty acids present were: myristic acid C14:0 (0.12 to 0.18%), palmitic C16:0 (13 to 15%) and stearic C18:0 (4.7 to 6.4%) (Fig. 2). The percentage of linolenic acid (C18:3) in argan oil did not exceed 0.1%. The presence of long chain fatty acids, such as C20:0 (0.34%) and C22:0 (0.1%), was noted in virgin argan oil.

Table 1. Percentage of fatty matter, unsaponifiables and proteins in the almond of the argan fruit

Таблица 1. Процентный состав жиров, неомыляемых веществ и белков в ядре агранового плода

Lot	Sample No.	Form of the fruit	% of fat (almond)	% of unsaponifiable in oil	% of protein
Lot Plain	1	Apiculate	49	0,22	25
	2	Fusiform	51	0,36	21,5
	3	Spherical	50	0,36	23
	4	Oval	54	0,60	22

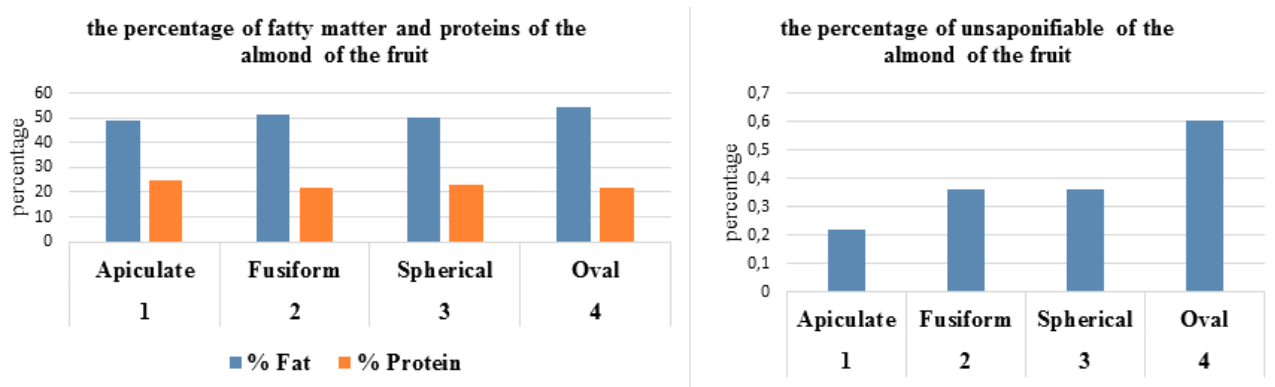


Fig. 1. Percentage of fatty matter, proteins and unsaponifiables of the almond of the argan tree

Рис. 1. Процентный состав жиров, белков и неомыляемых веществ в ядре агранового плода

Table 2. Fatty acid composition of argan oil in samples 1 to 4
Таблица 2. Жирнокислотный состав арганового масла у образцов 1–4

Fatty acid	Sample No., form			
	1 apiculate	2 fusiform	3 spherical	4 oval
Myristic C14 :0	0.15	0.12	0.16	0.18
Canoic pentade C15 :0	0.07	0.05	0.05	0.05
Palmitic C16 :0	14.52	13.69	15.11	14.15
Palmitoleic C16 :1	0.12	0.14	0.16	0.10
Heptadecanoic C17 :0	0.05	0.08	0.07	0.09
Stearic C18 :0	6.39	5.41	4.78	5.35
Oleic C18 :1	46.97	48.46	44.13	46.78
Linoleic C18 :2	30.75	31.02	34.56	32.32
Linolenic C18 :3	0.42	0.41	0.40	0.44
Arachidic C20 :0	0.34	0.35	0.36	0.34
Behenic C22 :0	–	0.10	0.08	0.08

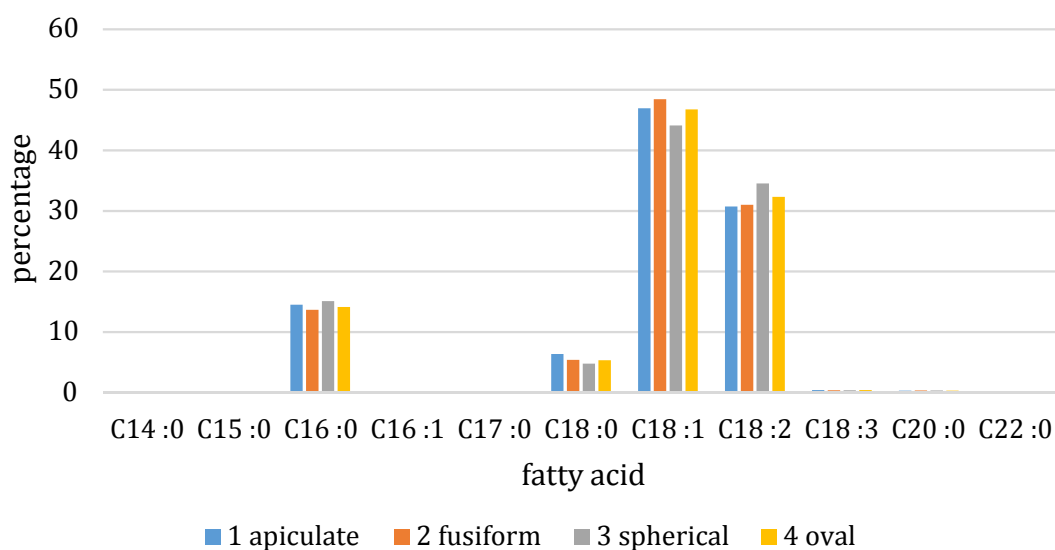


Fig. 2. Fatty acid composition of argan oil in samples 1 to 4
Рис. 2. Жирнокислотный состав арганового масла у образцов 1–4

It emerged from the study of the fatty acid composition of the fruits that there was no association between the shape of the fruit and the composition of fatty acids.

Analysis of sterols

The sterols in different samples of virgin argan oil were determined by gas chromatography after silylation of the sterol fraction. The latter was obtained by fractionating the unsaponifiable in virgin argan oil by HPLC on a normal phase. This analysis was carried out in the presence of an internal control: 0.2% α -cholestanol in chloroform.

The various sterols that we encountered were identified by gas chromatography coupled with mass spectrometry and by comparison with data from the literature. Their individual

and total assay was possible by GC using an internal standard: 0.2% α -cholestanol in chloroform.

The sterolic composition was consistent with the data in the literature. They were essentially Δ -7-stigmasterols. The predominant products were schottenol (or Δ -7-stigmasterol) and spinasterol (Table 3; Fig. 3). Their proportion varied, respectively, between 42.8 and 46.4%, and 39.8 and 45.6% (Fig. 3) (Guillaume et al., 2019).

Commonly, schottenol and spinasterol are rarely found in vegetable oils, but were characteristic of this oil. Two minority sterols have been identified in argan oil. These were stigmastera-8,22-diene and stigmastera-7,24-28-diene (or Δ -7-avenasterol). Their proportion varied between 2.5% and 4.7% of the mixture of total sterols (El Kharrassi et al., 2014).

Table 3. Sterol composition of argan oil in samples 1 to 4
Таблица 3. Состав стеролов арганового масла в образцах 1–4

Sterol	Sample No., form			
	1 apiculate	2 fusiform	3 spherical	4 oval
Spinasterol :7,22diene-3b	39.88	45.63	41.03	43.18
Schottenol :7ene-3b	42.79	46.37	44.10	43.27
Stigmasta 8.22diene 3β ol	3.26	2.53	3.35	3.46
Stigmasta 7.24diene 3β ol	2.83	3.90	2.63	4.72

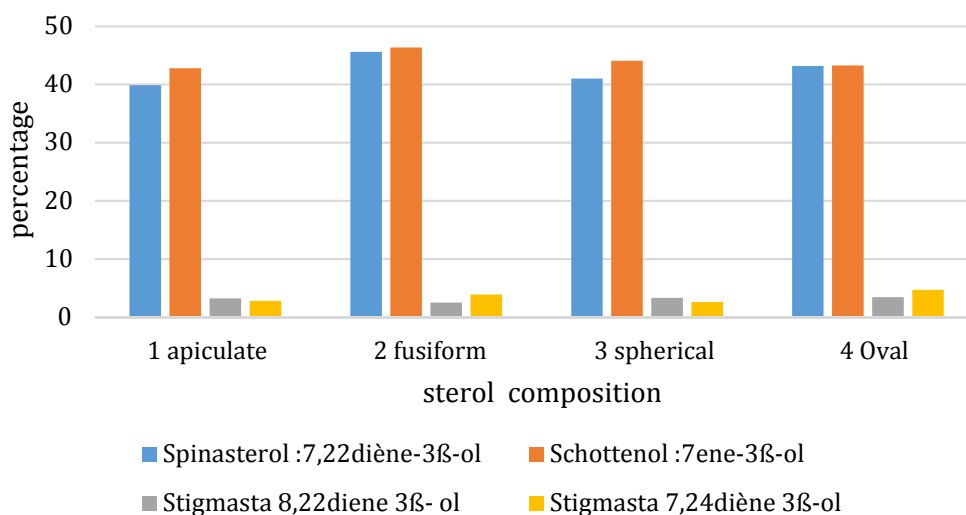


Fig. 3. Sterol composition of argan oil in samples 1 to 4
Рис. 3. Состав стеролов арганового масла в образцах 1–4

It was found that the campesterol content in argan oil is very low. It can be used as a parameter and as a marker to detect adulteration of argan oil.

The study of the sterolic composition of argan oil from 4 samples showed that there was no association between the shape of the argan fruit and the composition of sterols (El Abbassi et al., 2014).

Conclusion

For this study of the association between the argan fruit shape on the chemical composition of argan oil we selected, with the help of indigenous communities, 4 different shapes of fruit, collected from the same place (Tamanar) in the province of Essaouira, southern Morocco. The results of this work show that the oval shape represents the best shape of the argan-tree fruit because it contains a higher percentage of fat, protein and unsaponifiable, so it can be concluded that the oval shape is the best argan fruit shape compared with other shapes. There was no association between the shape of the fruit and the composition of fatty acids and sterols. The results obtained make it impossible to conclude that the fruit shape is associated with the compositions of fatty acids or sterols. The differences of these substances contents, observed in the study, were not associated only with the fruits' forms, but also depended on the nature of the soil and the altitude, as well as, the longitude and the distance from the sea.

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