

Physico-chemical Characteristics of *Washingtonia robusta* Fruit Oil

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Abstract: The fruit seed kernel oil from *Washingtonia* (*W. robusta*) locally in Aswan governorate, Egypt were evaluated for their physicochemical properties, and composition of fatty acids, tocopherols and phytosterols. The contents of carbohydrates, fiber, protein and ash for *W. robusta* palm fruits were 47.00, 6.50, 5 and 4.2%, respectively. The oil content in the seed kernels was 32.80%. The extracted oil had as refractive index (at 20°C) 1.46, specific gravity (at 20°C) 0.92, colour (yellow, 3.5; red 0.09), average iodine value (g of I/100g oil) 87.68; acid value (% oleic acid) 0.22%; saponification value (191.66 mg of KOH/g oil; unsaponifiable matters 0.70, the oxidation stability was 30. The major fatty acids detected in oil were noted to be oleic acid (64.80%) followed by palmitic acid (14.40%), linolenic acid (5.20%), arachidic acid (5.10%), linoleic acid (4.50), stearic acid (4.30) and myrctic acid (1.70%). The observed data showed that the total saturated fatty acids 25.50%, whereas the amounts of total unsaturated fatty acids 74.50%. The content of total tocopherols in oil was 97.4 mg/100g, with α -tocopherol 116.0, γ -tocopherol 131.4, and δ -tocopherol (50 mg/100g). β -sitosterol (11.331 mg/100 g) the main phytosterols in oil. The results of this study revealed that the *Washingtonia robusta* fruit could be explained as a potential source of high-oleic oil for the local oil industry as well as the seeds is a nutritive value.

Keywords: *Washingtonia robusta* oil, physicochemical properties, tocopherols unsaponifiable matters

INTRODUCTION

Palm trees represent the third most important plant family (Arecaceae) with repeat to human use and human edible products are obtained from palms, including the familiar date palm fruits, coconut palm nuts and various palm oil (Johnson, 1998).

Most of palm products are available commercially and valuable for trading (oily palm seeds). Fan palm (*Washingtonia* spp.) fruits are not used commercially all over the world. The genus *Washingtonia* is commonly cultivated as ornamental avenue plants and landscape foliage. It has two species, namely *Washingtonia filifera* and *Washingtonia robusta*. The California fan palm (*W. filifera*) and the Mexican fan palm (*W. robusta*) are relatively similar (Anonymous, 2010). *W. robusta* (Mexican Fan Palm) is a fast-growing palm with a thick, reddish trunk and big, dark green leaves. Long inflorescences of small fleshy flowers are produced in the late spring, followed by black-brown berry-like, small fruits that have a thin, sweet pulp that tastes somewhat like dates or butterscotch. Each fruit contains a single seed. *W. robusta* fruits have high content of sugar and oils (Mazmanci, 2011).

Fruits have a very large, brown seed surrounded by thin and sweet pulp, that taste likes dates (Turner and Wasson, 1997). Fruits of fan palms are used by Native Americans, eaten raw or cooked, and dried for later use (Watson and Preedy, 2009) or ground up as flour for making breads and cakes. They also use soaked fruits to produce a sweet beverage and make jelly from the berries (Facciola, 1990).

W. robusta palms provide many useful products in general; they are rich in oils, terpenoids and phenolic compounds. Mesocarp and endocarp oils from many palms include a range of volatile compounds and other terpenoids that are reported beneficial for health, such as phytosterols, carotenoids and pro-vitamin A, tocopherols and

vitamin E and triterpene pentacyclics. Among the phenolic compounds, phenolic acids, resveratrol and other stilbenes, anthocyanins, flavones, flavonols, dihydroflavonoids, flavan-3-ol, procyanidins and lignans have been described in different tissues of this palm, especially in fruit pulp, seeds and leaves (Agostini-Costa, 2018).

This investigation was carried to evaluate physico-chemical characteristics on oil extracted from *W. robusta* palm fruits.

MATERIALS AND METHODS

Materials:

W. robusta fruits, samples were collected from Plants Garden, Aswan governorate, Egypt, during March 2018. The fruits were cleaned manually and all foreign material, such as dirt, smashed and broken seeds, was removed and dried, then the mature and healthy fruit was ground and stored in glass bottles at 4°C for physico-chemical analysis. Experiments were replicated three times and the average values were reported. The palm fruits were smashed with a hammer, ground using a blender and sieved through a 30 mesh stainless steel screen. The residue was blended again until all the ground powder passed through the screen to obtain uniform powder.

Methods:

Proximate analysis

Analysis of samples was done for moisture, protein (% N \times 6.25), ash, fat, and fibre according to methods described in AOAC (2010). Total carbohydrates were calculated by difference [100 - (protein + fat + ash)].

Oil extraction

The seed kernels were crushed using a commercial blender. The crushed material, packed in a

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thimble, was placed in Pyrex glass Soxhlet apparatus connected with a water condenser and a 500 ml round bottom flask. The extraction was carried out with a-hexane on water-bath for 6 h. after extraction; the excess solvent was removed under pressure using a rotary evaporator.

Analysis of extracted oil

Analysis of physical and chemical parameters including density, refractive index, iodine value, acid value, saponification and unsaponifiable matter of the oil were made according the standard AOAC methods (2010).

Colour Scale and Intensity

Using Colour Difference Meter and Spectrophotometer by transfer 5 g *W. robusta* fruit oils to Petri dishes for colour scale analysis, colour scale was measured by a colour difference meter (CR-300, Konica Minolta Inc., Tokyo, Japan). Each of L^* (0/100 darkness/lightness), a^* (\pm , redness/greenness), and b^* (\pm , yellowness/blueness) in the Hunter scale were measured three times, from which mean values were calculated. Values of the standard plate, used as a reference, were $L^* = 93.59$, $a^* = 2.62$, and $b^* = 1.88$. To measure brown color intensity that is related with browning of seed oils, 200 seed oils were added to a 96-well plate and OD was measured at 420 nm using a spectrophotometer (Multiskan Go, Thermo Fisher Scientific, and Waltham, MA, USA). A higher OD value was considered as a higher brown color intensity of oil (Kim *et al.*, 2017).

Oxidative Stability by Rancimat Measurements

The oxidative stability was determined with 743 Rancimat apparatus from Metrohm, Herisau, Switzerland, according to (ISO, 2016), utilizing a sample of 2.50 ± 0.01 g. All the samples were studied at the same temperatures of 120°C under a constant air flow (20 L/h). The induction times [h] were printed automatically by the apparatus software with the accuracy of 0.005. During the Rancimat test, oil samples were taken and content of primary oxidation products (PV) generated during heating and aeration was tested. On the basis of the results, it was determined after what time the PV exceeded the value of 10 m Eq O_2/kg oil, that is the maximum PV value presented in CODEX STAN 210, (1999) for refined oil used for consumption.

Gas chromatographic Fatty acids (FA) analysis

The fatty acid composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950 μl of n-hexane 50 mg of oil followed by 50 μl of sodium methoxide using the method of Nzikou *et al.* (2009). The blends were vortex for 5 s and permitted

to make due for 5 min. The top layer (1 μl) was injected into a gas chromatography Perkin Elmer (model 8700, Newark city), fitted with a non-bonded biscyanopropyle cyclohexane stationary phase, polar capillary column SP-2340 (602×0.25 mm), 0.2 μm film thickness and a Flame Ionization Detector. Nitrogen was utilized as a mobile phase at a stream rate of 3.5 mL min⁻¹. Different conditions were: beginning stove temperature, 130°C ; slope rate, $4^\circ\text{C}/\text{minute}$; last temperature, 220°C ; injector temperature, 260°C ; finder temperature, 270°C ; temperature hold, 2 min before the pursue and 17 min the run. A sample volume of 1.0 μL was injected. FAMES were recognized by looking at their relative and outright maintenance times to those credible guidelines of FAMES acquired from Sigma Chemicals Company. The majority of the measurement was finished by implicit information taking care of program given by the maker of the gas chromatograph. The fatty acids compositions structure was accounted for as an overall level of the absolute peak area.

Tocopherols analysis

The analysis of the tocopherols has been achieved by HPLC in normal phase (Shin *et al.*, 2009). 20 mg of the oil was dissolved in hexane and isopropanol (99:1) mixture. After filtering, it was injected at a flow rate of 1 mL/mn in Agilent Series 1100 (France) device equipped with a quaternary pump, a manual injector with 20 μL buckle of injection. The apparatus was coupled with UV DAD (set in $\lambda = 295$ nm) detectors. The column was Luna Si 60 Phenomenex (France) (5 μm , 4.6x 250mm) type. The picks have been identified by injection of standards of tocopherols (Sigma Aldrich, USA). The curves of standardization were drawn while proceeding to a range of dilution of 0.3-8 $\mu\text{L}/\text{mL}$.

RESULTS AND DISCUSSION

Table (1) shows the data related to proximate analysis of *W. robusta* fruits. The carbohydrates content in total fruit was 47.00% followed by fat 32.80%, fiber 6.50%, protein 5% and Ash 4.2%. Carbohydrates and fats content were highest in fruits. Fruit composition gave us an overall idea about the chemical composition which has an effect on the sugar, oil and protein yield of the product therefore; *W. robusta* fruits have a high nutrition value. This results are in agreement with Besbes *et al.* (2004), Al-Farsi and Lee (2008) whose reported that fruits from *W. robusta* palms are edible and have been a food source for ancient cultures; in Sonora inhabitants at San Carlos Bay, used the seeds for food.

Table (1): Chemical composition of *Washingtonia robusta* fruits

Items	% Moisture	% crude Protein	% crude Fat	% Fiber	% Total ash	Carbohydrate*
%	4.50	5.00	32.80	6.50	4.20	47.00

*Calculated by difference

Physico-chemical properties of *Washingtonia robusta* fruit oil

The results for physico-chemical parameters of the extracted oil from *W. robusta* fruits are presented in Table (2).

Table (2): Physicochemical properties of *Washingtonia robusta* fruits oil

Physicochemical parameters	Results
Refractive index at (20°C)	1.46
Colour	yellow unit
	red unit
Specific gravity at (20°C)	0.92
Acid value % (as oleic acid)	0.22
Iodine value (g/100g of oil)	87.68
Oxidation stability	30
Saponification value (mg KOH/g of oil)	191.66
Unsaponifiable matters (%)	0.70

Refractive index

Data in Table (2) shown the value of refractive index (at 20°C) of *W. robusta* fruits oil extracted is 1.465; this result is comparable to those of many different varieties of melon seeds oil (Mabaleha *et al.*, 2007). The high refractive index of oil also explained that the fatty acids in the oil contain a large number of carbon atoms (Bello and Olawore, 2012).

Colour values

The colour values for the investigated *W. robusta* fruits oil are (3.5Y/ 0.09 R), these result meant that extracted oil is light and clear. The development of colour in vegetable oils is mainly linked with the presence of some colouring matters/pigments, for example, chlorophyll and carotenoids which are extracted along with the oil during the process of oil seed extraction. Such colouring components have to be thoroughly removed through a step known as oil bleaching. Lighter coloured oils, being valuable from a technical view-point, are thus more acceptable for edible or oleochemical applications (Anwar *et al.*, 2012).

Specific gravity

The specific gravity of *W. robusta* fruits oil is 0.92 (Table 2). The density of palm fruits oil is less than water (1.00) and the differences between the oils are very small, especially among the common vegetable oils (Davis *et al.*, 2008).

Acid value

The free fatty acids present of *W. robusta* fruits oil is 0.22 (Table 2). Free fatty acids can excite

oxidative deterioration of oils by enzymatic and or chemical oxidation to form off flavour components. Free fatty acid value is a manifestation of lipase activity (Ukhun, 1986). Free fatty acids (FFA) are mainly the product of hydrolysis and their presence in oil can promote development of objectionable flavour and odours. FFA contents harshly extracted crude vegetable oil is generally below 1% (Oderinde *et al.*, 2009).

Iodine value (IV)

The iodine value is an important parameter which reflects the magnitude of unsaturation and potential oxidative of oils. The iodine value of extracted oil was 87.68g/100g as shown in Table (2). It is well recognized that IV is the measurement of the degree of unsaturation of the fatty acids present in oil, principally the oleic and linoleic acids. A high iodine value means that the oil is more unsaturated and could be used for edible purposes. This value is helpful in determining the quality of the oil, whether as a drying, semi-drying or non-drying oil. The iodine value is also related to the melting point or hardness of an oil or fat. The iodine value of oil is reported to decrease as a result of unfavorable storage conditions, especially temperature, due to the oxidation of unsaturated fatty acids (Rossell, 1991).

Oxidative stability

The result of the oxidative stability is shown in Table (2). It is well recognized that vegetable oils mainly contain unsaturated fatty acids (USFA). Under unfavourable storage conditions, upon oxidation of USFA, primary oxidation products mainly hydroperoxides, are formed which undergo further breakdown to generate aldehydes and ketones etc. these aldehydic products affect the nutritive quality of oils adversely by developing rancid and bad odour, discolouration and hence nutritional loss. Therefore, measurement of oxidation stability is taken as a key factor to assess and evaluate their stability for edible purposes (Anwar *et al.*, 2014). The results were summarized in Obtained oxidative stability was 30, this result is similar to Symoniuk *et al.*, 2017).

Saponification value

The saponification value of *W. robusta* fruits oil (Table 2) was found 192.88 mg KOH/g. The saponification value of *W. robusta* fruits oil was in acceptable range of most vegetable edible oils. A saponification value of 200 mg KOH/g refers to a high proportion of fatty acids of low molecular weight and chain length. This Saponification value isn't in line with those reported (200.00 mg KOH/g of oil) by Baboli and Kordi (2010) for watermelon seed oil; however comparable with those (193.80 mg KOH/g of oil) investigated for different melon seed oils (Mabaleha *et al.*, 2007). Such differences in saponification value might be attributed to the replacement of long chain (C₂₂) fatty acids by shorter chain (C₁₈) fatty acids of the oils analyzed (Anwar *et al.*, 2006).

Unsaponifiable matter

Unsaponifiable matters can be used to predict the contents of non-triglyceridic minor components such as tocopherols and colouring pigments etc., which cannot

be saponified by an alkali under specified set of the test conditions. The amount unsaponifiable matter (USM) of the investigated of *Washingtonia robusta* fruits oil was 0.70%. This value was agreement with reported by Anwer *et al.* (2006). Higher oil USM reflects the presence of higher concentration of valuable minor components such as tocopherols, carotenoids, squalene and phytosterols which not only impart oxidative stability to the oils but also have potential medicinal value. Mostly the concentration of USM constitutes up to 0.50 to 2.50%, however in exceptional cases 5 to 6% of vegetable oils (Bockisch, 1993; Gordon and Magos, 1983; Malecka, 1994).

The quantification of tocopherols

Data presented in Table (3) indicated the total tocopherols content in *W. robusta* fruits oil (97.4 mg/100g). Concentrations of α -tocopherol, γ -tocopherol, and δ -tocopherol were 16.0, 31.4 and 50 mg/100g respectively; the present δ -tocopherol level is considerably the highest than other (50 mg/100 g). On the other hand, the present α -tocopherol content was found to be lowest (16.1) mg/100gm (Stevenson *et al.*, 2007), while β -tocopherol were not detected in the present analysis of *W. robusta* fruits oil. A considerable amount of α -tocopherol was detected in the present analysis oil was found comparable with those reported for other palm oil (0.4-18.5 mg/100 g), coconut oil (1.7 mg/100 g), soybean (0.9-35.2 mg/100g) and maize (2.3-5.73 mg/100 g) (Rossell, 1991). The δ -tocopherol contents (50.0 mg/100g), which was higher in that of maize (2.3-7.5 mg/100g), and close agreement with soybean (15.4-93.2 mg/100g).

Table (3): Tocopherols content in of *Washingtonia robusta* fruits oil

Tocopherol	mg / 100 g
Total Tocopherols	97.4
α -tocopherol	16.1
β -tocopherol	-
γ -tocopherol	31.3
δ -tocopherol	50

Tocopherols are minor components that contribute significantly to the oxidative stability of vegetable oils during storage and processing. They are naturally present in vegetable oilseeds and extracted along with the oils, however, their concentration usually decreases as result of oil processing. In vegetable oils, their original concentration may vary from a few mg/kg to a thousand mg/kg or even more depending on the oil type and oil fatty acid composition (Rossell, 1991). On the different isomers of α -tocopherol reported so far, an

isomer has the greatest vitamin E efficacy, while δ -tocopherol exhibits the highest antioxidant activity (Matthaus and Ozcan, 2009).

Fatty acid composition

The fatty acids composition of oil gives good information about the commercial usefulness of their oils. Hence, analysis of fatty acid composition of oil is a decisive parameter for the evaluation of nutritional or technical applications of the oils (Anwar *et al.*, 2014).

Saturated fatty acid

The fatty acid composition of *W. robusta* fruits oil is reported in the Table (4). Saturated fatty acids content (myristic, palmitic, stearic and arachidic acids) were 1.7, 14.4, 4.3 and 5.1%, respectively. The total amounts of these saturated fatty acids (TSFA) were 25.50%, the present TSFA levels were less than in the watermelon (30.0%) and comparable with those in var. Sugar Baby (26.2%); and higher than those reported (17.8%) earlier peanut varieties and variety Colocynthoide (Ziyada and Elhaussien, 2008). These TSFA of *W. robusta* fruits oil in agreement with varied those investigated for different species of musk melon seed oils (Tilak *et al.*, 2006).

Unsaturated fatty acids

The *W. robusta* fruits oil tested contained relatively higher levels (74.5%) of unsaturated fatty acids with oleic acid (64.8%) as the major component followed by Linolenic (5.2%) and linoleic acid (4.5 %). The contents of TUSA in the present analysis were found to be lower than those reported by Baboli and Kordi (2010) (81.6%). However, these levels of TUSA were in line with those detected (67.93-82.36%) in some species of melon by Mabaleha *et al.* (2007) and comparable with those reported (64.6-88.2%) for different species of muskmelon (Tilak *et al.*, 2006).

Table (4): Fatty acid composition of *Washingtonia robusta* fruits oil

Fatty acid	Area (%)
Myristic acid (C _{14:0})	1.70
Palmitic acid (C _{16:0})	14.40
Stearic acid (C _{18:0})	4.30
Arachidic acid (C _{20:0})	5.10
Oleic acid (C _{18:1})	64.80
Linoleic acid (C _{18:2})	4.50
Linolenic acid (C _{18:3})	5.2
TSFA*	25.50
TUSFA**	74.50
SFA/ USFA	0.34

*Total saturated fatty acid, ** Total unsaturated fatty acid

Overall the fractionation of fatty acids content present in *W. robusta* fruits oil is in agreement with Nehdi (2011) who reported that the oil extracted from *W. filifera* seeds contains high levels of oleic acid (40.60%) followed by lauric acid (17.87%), linoleic acid (16.26%), myristic acid (11.43%) and palmitic acid (9.23%).

Fractionation of unsaponifiable matters in *Washingtonia robusta* fruits oil

The phytosterols composition of *W. robusta* fruits oils gives in Table (5). Total sterols content was 21.4 mg/100 g of unsaponifiable matters. Several sterols (Stigmasterol, Campesterol, Cleostrol, D₇-Stigmasterol) were found in 11.331, 3.267, 3.045, 1.344 and 1.236% of total sterols of unsaponifiable matters, the maximum concentration of 11.331 mg/100 g in the case of β -sitosterol in palm oil.

Table (5): Fractionation of unsaponifiable matters of *Washingtonia robusta* fruit oil

Contents	% Total sterols of unsaponifiable matter
Cholesterol	0.057
Brassicasterol	0.036
24-Metilen cholesterol	0.014
Campesterol	3.045
Campestanol	0.047
Stigmasterol	3.267
D ₇ -Compesterol	0.168
D ₇ -Stigmasterol	1.236
D ₅ -Adrennasterol	0.753
Cleostrol	1.344
β -Sitosterol	11.331
Sitostanol	0.122
Total sterols	21.42

The results were in agreement with that recounted for many vegetable oils where β -sitosterol (82.60%) constitutes the major phytosterol follow-up by stigmasterol (13.05%) (Kris-Etherton *et al.*, 1999; Awadand Fink, 2000). In the same way, the total phytosterols (141 mg/100g) is similar to those of other edible oils (Akpambang *et al.*, 2008; CODEX, 1993). Plant phytosterols have been described as anti-inflammatory and anti-cancer compounds (García-Llatas *et al.*, 2008).

β -sitosterol has been established as the most common and major plant sterol distribution in vegetable seed oils. The health benefits of phytosterols are also gaining recognition, especially towards lowering incidence of prostate cancer and cholesterol, as well as in modulating and improving immune functions of the body (Anwar *et al.*, 2008b). As phytosterols are not synthesized in the human body, they need to be provided through diet. The human body has an extremely low level of phytosterol in the blood, which is because of a low intestinal absorption rate of phytosterols.

CONCLUSION

The results of the present analytical study show that *W. robusta* fruits oil could be have a high nutrition value and helpful for the nutritionist in the selection of the best foods and food products. The presence of high amounts of unsaturated fatty acids as compared to saturated fatty acids, favours the suitability of the investigated *W. robusta* fruits oil for nutritional applications.

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الخصائص الفيزيوكيميائية لزيت ثمار الواشنطنونيا

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أجرى هذا البحث على ثمار نخيل الواشنطنونيا المنزرعة في محافظة أسوان، مصر وتم تقدير الخصائص الفيزيوكيميائية، تركيب الأحماض الدهنية، التوكوفيرولات والفيتوستيرولات للزيت المستخلص. قد أظهرت النتائج أن محتوى الثمار من الكربوهيدرات، الألياف، البروتين والرماد كان ٤٧، ٦.٥، ٥، ٤.٢٪ على الترتيب. والزيوت المستخلص كان ٣٢.٨٪. وكذلك أظهرت الخصائص الفيزيوكيميائية للزيت المستخلص أن معامل الانكسار ١.٤٦ (عند درجة حرارة ٢٠م°)، الوزن النوعي للزيت ٠.٩٢ (عند درجة حرارة ٢٠م°)، درجة امتصاص اللون الأصفر ٣.٥ والأحمر ٠.٠٩، متوسط الرقم اليودي ٨٧.٦٨ جم/١٠٠جم، ورقم الحموضة ٠.٢٢٪، رقم التصبن ١٩١.٦٦ جم هيدروكسيد بوتاسيوم/جم زيت، المواد غير المتصبة ٠.٧٠، الثبات ضد الأكسدة ٣.٠. كان الحامض الدهني السائد في الزيت المستخلص كان حمض الأوليك ٦٤.٨٪ متبوعا بالبالمتيك ١٤.٤٠٪، واللينولينك ٥.٢٪، والاراكيدك ٥.١٠٪، واللينوليك ٤.٥٠٪، والاستياريك ٤.٣٠٪ والميرستيك ١.٧٠٪. وأن نسبة الأحماض الدهنية المشبعة كانت ٢٥.٥٠٪، ونسبة الأحماض الدهنية الغير مشبعة من ٧٤.٥٠٪. وأشارت النتائج المتحصل عليها أن محتوى زيت الثمار من التوكوفيرولات الكلية كان ٩٧.٤ مجم/١٠٠جم، وكانت نسبة الألفا والبيتا والسيجما توكوفيرول ١٦، ٣١، ٤، ٥٠، ٥٠ مجم/١٠٠جم. وسجلت النتائج أن بيتاستوستيرول ١١.٣٣١٪ أعلى نسبة من الستيرولات الكلية الموجودة في الزيت.