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Roselle (*Hibiscus Sabdariffa*) Seeds and Kernels as a Potential Source of Oil, Protein and Minerals

O.M. Mabrouk [1] , Wafaa A. Amin[1], Ayat M. M. Youssef [2] and O.R Abou- Samaha[2]
[1]*Hort. Crop Processing, Res. Dept. Food Technol. Res. Inst., A.R.C, Alex. Lab., Egypt.*
[2]*Dept.of Food Sci. & Technol., Fac. of Agric., Univ. of Alex., El-Shatby, Egypt.*

THIS study aimed to evaluate Roselle seeds (RS) and Kernels (RK) as an unconventional source of oil , protein, and menirals.The results indicated that RS protein contain a high percentage of essential amino acids (39.47%) compared to that reported by FAO/WHO/UNU (1985) while, methionine and cystine were the limiting amino acids. The proteins were fractionated using SDS-polyacrylamide gel electrophoresis to 10 bands ranged in molecular weight, from 20 to 245 KDa and the main bands had molecular weight of 180, 135, 100, 63, 48 and 20 KDa. Thin layer chromatographic analysis of the crude RS oil indicated that total lipids consisted mainly of 8 lipid classes of glyceride and nonglyceride compounds in addition to the polar lipids located on the base line .The identified fractions were : polar lipids , monoacylglycerols , 1,2 and 2,3 diacylglycerols , sterol ,1,3 diacylglycerols , free fatty acids , triacylglycerols and hydrocarbons and sterol esters. Triacylglycerols were the predominant fraction. Gas Liquid Chromatographic "GLC" analysis of crude RS oil indicated that it composed of high percentage of unsaturated fatty acids (69.7%) in which oleic acid was 31.27%, while, linoleic acid was37.61%. Saturated fatty acids represented 30.3 % from the total fatty acids. Palmitic acid was the major fatty acid (22.44%) followed by stearic (5.77%). Physicochemical analysis of crude RS oil showed that it had a yellow colour and the Hunter colour components indicated that L*, a*, b* values of the oil were 29.85, 0.94 and 9.94, respectively. It had 1.4724 refractive index at 18°C, 0.919 g/cm^3 specific gravity, 99.47 iodine value, 194.4 saponification number, 7.52 peroxide value and 0.828 free fatty acids as % oleic acid. RS contained the following minerals: K, Mg, P, Ca, Fe, Cu, Zn. Potassium (1140 mg/100g) was the predominant element in the seeds, followed by phosphorus (482 mg/100g), magnesium (242 mg/100g) and calcium (239 mg/100g).Also, the effect of complete removing of RS hull through water soaking process for different time (10hr-15min) and temperatures (25-75 °C) to obtain Roselle kernel (RK) on the aforementioned analysis was considered. The results indicated that this process had some effects on the protein and mineral composition, while oil composition and it's characteristics did not affected by this treatment. It could be concluded that RS as a by-product could have potential for the functional food industry.

Introduction

Roselle (*Hibiscus Sabdariffa* L.) seeds are the waste that is left behind during processing of Roselle for juices or other Roselle related products. Disposing of wastes is highly undesirable both economically and environmentally (Nyam et al., 2010). Roselle seeds (RS), which until now do not have any commercial applications, can be considered a promising source of edible oil and protein (Tounkara et al., 2011and Elneairy, 2014).

Crude fat of RS reported in the literature varied between 16.0 and 27.78% (Mahmoud et al., 2008, Nzikou et al., 2011, Elneairy, 2014, Ghislain et al., 2014 and Islam et al., 2016). Some studies showed that RS oil is low in cholesterol and rich in other phytosterols and tocopherols and its composition is similar to edible oils such as cotton seed oil and corn oil (El- Sayed et al., 1998 and El- Sayed, 2001). However, there is a limited published_data on RS oil. Fiad (1991) reported that the triacylglycerols of RS oil composed

of 1.4% trisaturated"SSS", 16.4% disaturated-monounsaturated"SSU", 45.3% monosaturated-disaturated "SUU", and 36.7% triunsaturated «UUU». RS oil had high content of polyunsaturated fatty acids and the ratio of saturated to unsaturated fatty acids ranged between 1:2 to1:3 (Mahmoud et al. ,2008; Nakpong and Wootthikanokkhan ,2010 , Nzikou et al.,2011 and Elneairy,2014). Al-Wandawi et al. (1984) reported that oleic acid was the predominant unsaturated fatty acid (66.41-77.16%) and palmitic acid (17.25-28.46%) was the predominant acid of saturated fatty acids, but linoleic acid represented only 1% and stearic acid represented 2.27 to 4.93% of the total recovered fatty acids. Mohammed and Yagoub (2007) found small amounts of C14:0, C16:1, C17:0, C17:1, C17:2,C18:3, C19:1, C19:2, C20:0, C20:1, and C22:0. Mahmoud et al.(2008) and Nakpong and Wootthikanokkhan (2010) mentioned that the crude RS oil consisted of a high proportion of unsaturated fatty acids, comprised primarily of oleic (33.31%, and 38.46%, respectively) linoleic (38.17% and 33.25%, respectively) a lower proportion of saturated fatty acids, comprised mainly of palmitic(18.15% and 20.52, respectively). The other fatty acids were stearic (4.09% and 5.79%, respectively)and linolenic (2.09% and 1.69%, respectively). While, myristic, arachidic, behenic, lignoceric, palmitolice and erucic acids were found in amounts less than 1%. Nzikou et al. (2011) found that the major saturated fatty acids in Hibiscus sabdariffa L. seed oil were palmitic (20.84%) and stearic (5.88%) acids and the main unsaturated fatty acids were linoleic (39.31%) and oleic (32.06%). Tounkara et al.(2011) reported that the RS are a good source of unsaturated fatty acid (74.13%). Among the unsaturated fatty acids,linoleic (35.02%) and oleic (36.9%) were the highest fatty acids, however, α -linolenic acid and palmitoleic acid had the lowest level (1.85 and 0.36%, respectively). While, palmitic (19.21%) and stearic acid (5.13%) being the major saturated fatty acids. Elneairy (2014) found that the major saturated fatty acids in Egyptian and Libyan RS oil were palmilitic (18.15 and 12.70%, respectively) and stearic acid (4.09 and 15.9%, respectively), while the main unsaturated fatty acids were linoleic (38.17 and 17.50%,respectively) and oleic (33.31 and 16.50%,respectively) . Mohammed and Yagoub (2007), Mahmoud et al. (2008) and Nakpong and Wootthikanokkhan (2010) reported that the high content of unsaturated fatty acids in RS oil is consistent with low in cholesterol and rich in other phytosterols and tocopherols particularly β-sitosterols and γ-tocopherol and the linoleic-oleic fatty acid profile makes this oil healthier for human and very useful in some industrial applications like hydrogenation, butter production, and other applications in the food industry.

The protein content of RS was higher than that seen in commonly consumed cereals like jowar, wheat, maize and rice but lower than those for pulses like black gram, chickpea , pigeon pea soybeans, and groundnuts (FAO, 2001, Bengaly et al., 2006 and Elneairy,2014). Tounkara et al. (2013) extracted protein fractions from defatted RS. These fractions were globulin, albumin, glutelin and prolamin. They showed that globulin was the major protein fraction in RS comprised of 31.18% of total protein, followed by albumin (16.47%), glutelin (10.20%) and prolamin (5.57). El-Sayed (2001) indicated that RS protein contained 8 essential amino acids besides 10 non essential amino acids. The major essential amino acids presents in RS protein were leucine (7.29), phenylalanine (5.25) , lysine (5.46), histidine (1.91) and arginine (10.65) as g /16 g N. The limiting amino acid in RS flour was tryptophan (0.34 g) .The non essential amino acids found in RS protein were aspartic acid (10.5), glutamic acid (21.38), alanine(5.10) and arginine (10.49) as g / 16 g N . Besides, moderate amounts were found such as serine (4.65), threonine (4.66), proline (4.14),glycine (4.27), histidine (2.59) and cystine (2.78) as g / 16g N. In Malaysia, Hainida et al. (2008) found that the protein of RS contained lysine, arginine, leucine, phenylalanine and glutamic acid, while cystine (4.04 – 5.32 g/100 g protein) and methionine (2.50–3.96 g/100 g protein) were the limiting amino acids .They stated that all the essential amino acids were higher than those found in wheat grain and rice as well as lysine content of raw and processed seeds was found to be higher and adequate for human requirement. Shaheen et al. (2012) studied the amino acid composition of three RS cultivars in Egypt (Aswan, Sewa and Sudan-1) .Their results showed that significant differences were found between the studied cultivars in essential and non- essential amino acids. RS especially Aswan cultivar, could be used as a rich source of amino acids especially lysine, arginine, leucine, phenylalanine, and glutamic acids. Tounkara et al. (2013) and Elneairy (2014) suggested that Roselle protein fractions and its isolates have good nutritional quality and could be used as a

good source of protein fortification for a variety of food products for protein deficient consumers as well as a potential food ingredient. Accordingly, RS could be used as a supplement food or as diet enrichment especially in the low protein diets. Therefore, its consumption as supplement material for poor food in lysine could balance the amino acid intake.

It was of interest to note that the most prevalent mineral elements in *Hibiscus sabdariffa* L. seeds were potassium, magnesium, calcium, sodium and phosphorus as found by Hainida et al.(2008), Tounkara et al.(2011) , Nzikou et al. (2011) and Ghislain et al.(2014). While, the minor elements were zinc, cupper, manganese, iron and aluminum as mentioned by Mohammed and Yagoub (2007) and Tounkara et al. (2011).The contents of the minerals in RS were 20341.µg/g for potassium as predominant element , followed by magnesium (5433.33µ / g) and calcium (2282.82 µ/g). RS were found to contain 489.33 µ/g sodium, 146.67 µ/g manganese, 46.90 µ/g cupper and 93.76 µ/g iron, but aluminum and phosphorus were relatively low(2.71 and 8.81 µ/g) , respectively, as found by Tounkara et al. (2011). Another arrangement was found by Nzikou et al. (2011) who mentioned that potassium is as high as 1329±1.47 mg/100 g, followed in descending order by sodium (659±1.58 mg/100 g), calcium (647±1.21mg/100 g), phosphorus (510±1.25 mg/100 g) and magnesium (442.8±1.80 mg/100 g). Ghislain et al. (2014) found that the content of Ca, Mg, K, Na, P, Fe and Zn ranged from 1054 to 1920, 1670 to 2083, 26.45 to 272.7, 14 to 22, 10.58 to 90.78, 137.3 to 169.22 and 10.13 to 70.11 mg/kg, respectively.

Although seeds of Roselle represented high percentage of the flower capsule and have high nutritional value, limited research has been carried out and no attention has been paid on exploitation and utilization of these seeds. Therefore, this work aimed to evaluate the protein, oil and minerals of RS. Also, the effect of hull removing from the seeds on the aforementioned components was considered.

Materials and Methods

Materials

Roselle (*Hibiscus sabdariffa* L.) var «Sabahia 17» flowers were used in this investigation. Flowers were obtained from Medicinal and Aromatic Plants Department, Horticulture Research Institute, ARC, Sabahia Horticulture Research Station, Alexandria, Egypt in 2010. All chemicals used were of analytical grade, and were purchased from El-Gomhouria Co. for Chemical and Medical Requisites, Alexandria, Egypt except chemicals used in electrophoresis that were purchased from Bio-Rad chemical Co. ,CA, And U.S.A.

Methods

Technological methods

Figure 1 shows the preparation steps of Roselle seed (RS) , Roselle Kernel (RK) and their flowers (RSF,RKF) from Roselle flowers as described previously by Mabrouk et al. (2016).

Analytical methods

Oil analysis

Extraction and determination: Oil was extracted from RS and RK using petroleum ether (40-60 C°) by Soxhlet apparatus for 16 hr as described in the AOAC (2000). The obtained oil was kept in brown glass bottles in refrigerator at 4°C until analysis. The resultant meals (RSM, RKM) were dried in an air oven at 60°C to evaporate the residual solvent, then sieved to pass through 100 mesh and used for protein and mineral analysis.

Fractionation of oil classes: Crude extracted oil was fractionated into different classes using a TLC technique according to the method of Mangold and Malins (1960).

Fatty acid composition: Preparation of fatty acid methyl esters (FAMEs) of crude oil was performed according to the procedure of Radwan (1978). The obtained FAMEs were separated using Shimadzu gas chromatograph (GC4-CM, PFE) under the following conditions: column, 10% Silar CP on 80/100 chromosorb Q, detector , FID; column temperature .,190-240º C, detector temp., 270º C ; flow rate , 20ml /min. Gas flow, N2 and chart speed, 5 mm/min. Standard fatty acid methyl esters were used for identification. The area under each peak was measured by the triangulation method and percentage of each fatty acid was expressed with regard to the total area.

*Physicochemical properties:*Refractive index (RI) of the oil sample was determined according to the AOAC (2000) using Abbe refractometer (Leica Mark II NARP 79190).Specific gravity (SG) of the oil sample was determined as described in the AOAC (2000). Colour of Roselle seed oil was measured by Hunter system. After calibration of

the instrument, the oil was placed in a small glass cylinder container. The glass container was placed into the measurement chamber and covered with black cylinder. The measurements were recorded after pushing the start-operating key.

The values L*, a* and b* were recorded as colour indicators where:-

L* express value varied from 0 (black) to 100 (white) colour indicator (brightness).

a* express value varied from -100 (green) to +100 (red) colour indicator.

b* express value varied from -100 (blue) to + 100 (yellow) colour indicator**.**

The oil viscosity was determined at 40°C using a Brookfield digital viscometer (model DV-II + Pro; Brookfield Engineering Labs., Inc., Middleborough, MA, USA).

Identity characteristics

Some properties of Roselle seed oil including saponification value (SV), peroxide value (PV), iodine number (IN) and free fatty acids (FFA) were determined according to the AOAC (2000).

Protein analysis

Samples of the obtained meals (RSM and RKM) after oil extraction were used for protein analysis. Protein content of RS and RK meals were determined according to the method of the AOAC (2000).

Fractionation

Sample preparation: Samples of RSM and RKM were dissolved in phosphate buffer pH 8. They were shaked for one hr at room temperature, then centrifugated at 5000 xg for 10 min to remove any insoluble material causing streaking during electrophoresis. The obtained supernatant was collected and kept for analysis.

An appropriate volume of the protein sample (1%) was mixed with an equal volume of the sample buffer (0.5M Tric-HCl, pH 6.8, 2% SDS, 10% glycerol, and 0.002% bromophenol blue, with 5% β -mercaptoethanol) and submitted to heat treatment for 5 min in a boiling water bath prior to be applied to the gel. Samples were allowed to cool to room temperature, finally centrifuged at 1000x g for 5min to remove any insoluble material causing streaking during electrophoresis.

Sodium dodecyl sulphate (SDS-PAGE) technique was carried out using the discontinuous buffer system described by Laemmli (1970), and mentioned by Hames and Rickwood (1990).

Gel preparation

A 10% slab gel was prepared. The resolving gel was prepared as follows: acrylamide – bisacrylamide, 10 ml; resolving gel buffer stock, 3.75 ml; SDS 10%, 300 µl; freshly prepared 1.5 % ammonium persulphate , 1.5 ml ; distilled water, 14.45 ml and TEMED, 15 µl. Stacking gel was prepared using the following reagents ; acrylamide – bisacrylamide, 2.5 ml; stacking gel buffer stock, 5ml; SDS 10%, 200µl; freshly prepared 1.5% ammonium persulphate, 1ml; distilled water, 11.3 ml and TEMED, 15 µl.

Electrophoresis conditions

Electrophoresis separation was performed using Mini-PROTEANII (Bio-Rad) at 75 V through the stacking gel followed by 125 V to the end of electrophoresis (2 hr).

Staining and destaining

Both were carried out according to the method of Hames and Rickwood (1990), using 0.1% Coomassie blue R-250 in water for staining (Bio-Rad), water: methanol:
glacial acetic acid (5:5:2 by volume). The staining period was 30 min. A ratio of (1:3:6 by volume) glacial acidic acid, methanol and distilled water was prepared and used as a distaining solution.

Amino acid composition

Duranti and Cerletti (1979) method was used to determine the amino acid composition using Beckman Amino Acid Analyzer Model 119 CL.

Mineral analysis

Minerals were determined according to the method of AOAC(2000). Ca, Mg,Fe, Cu and Zn were determined by atomic absorption spectrophotometric (Perkin-Elmer Instrument Model 2380). K was determined by flame photometer, while P was determined colourimetrically (Spekol 11, Carlzelss Jena) at 630 nm (Jacobs, 1959).

Results and Discussion

Roselle seed oil

Oil classes

Figure 2 represented the chromatogram of crude ether extract classes extracted from RS comparing with that extracted from cotton seed as a reference. The fractionations were according to their differences in polarity. The results indicated that RS oil contained 8 lipid fractions as follows; from origin to front, polar lipids (phospholipids), monoacylglycerols, 1,2 and 2,3 diacylglycerols , sterol , 1,3 diacylglycerols, free fatty acids, triacylglycerols, and hydrocarbons and sterol esters.

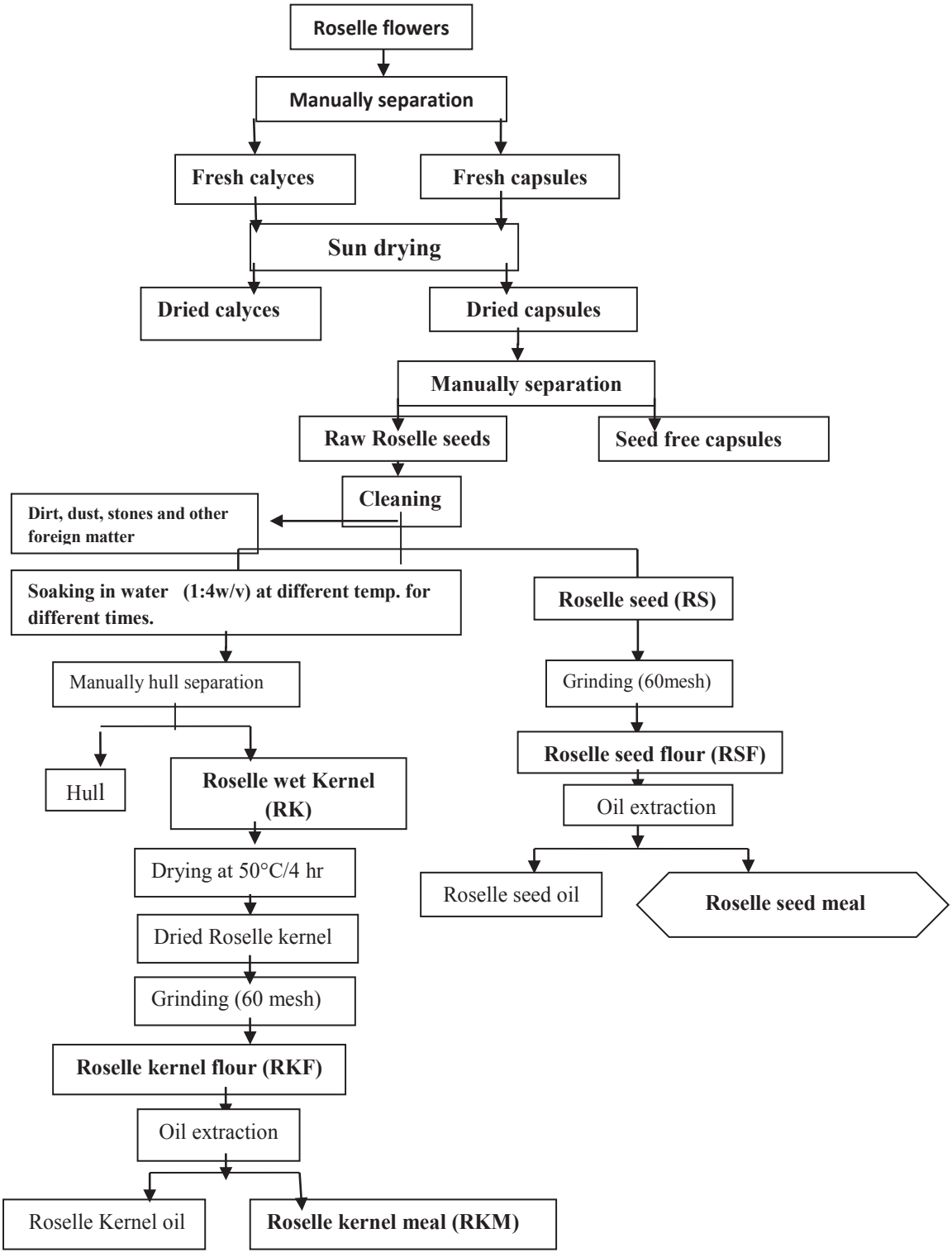


Fig. 1. Preparation steps of RS,RK and there flours (RSF, RKF) from Roselle flowers.

Fatty acid composition

Quantitative determination of FAMEs of the crude RS oil determined by GLC technique was shown in Table 1. The obtained data indicated that RS oil consisted of 30.31 % saturated and 69.7 % unsaturated fatty acids. Among the saturated fatty acids, palmitic was found to be the major acid (22.44 %) followed by stearic acid (5.77%) while, myristic had a minor percentage (0.71 %).

Concerning the identified unsaturated fatty acids, the obtained data showed that RS oil contained high percentages of such fatty acids; mainly linoleic(37.61%) followed by oleic (31.27%). However, linolenoic acid was detected at a minor percentage (0.11%) . These results are in accordance with those reported by Mahmoud et al. (2008) and Elneairy (2014) . The characteristic of linoleic – oleic fatty acid profile makes RS oil very useful in some industrial applications like butter production.

The proportion of unsaturated fatty acids (69.7%) was greater than the saturated fatty acids (30.31%). The ratio of saturated to unsaturated fatty acids was 1**:** 2.3. This ratio is closed to that mentioned by Elneairy (2014) and Rehab and Ayman (2017) and lower than that obtained by Nzikou et al. (2011) and Tounkara et al. (2011).

Physicochemical characteristics of RS oil

The data of physicochemical characteristics of crude oil extracted from RS are presented in Table 2 .Refractive index of RS oil was 1.4724. This figure is within the range for most common vegetable oils. This value is in accordance to that stated by Nzikou et al. (2011), and Elneairy (2014) and higher than that reported by Bamgboye and Adejumo (2010) and Eltayeib and Abd Elaziz (2014). Specific gravity at 25ºC was 0.9197.

This finding is comparable to that found by Elneairy (2014) in Libyan seed and Eltayeib and Abd Elaziz (2014) but higher than that found by Elneairy (2014) in Egyptian seeds and lower than that found by Bamgboye and Adejumo (2010). The viscosity value was 30 cp at 40ºC. This value is higher than that reported by Betiku and Adepoju (2013) and Eltayeib and Abd Elaziz (2014).

Crude RS oil is a liquid of yellow colour at room temperature. Hunter colour components indicated that L*, a*, b* values of the oil sample were 29.85, 0.94 and 9.49, respectively (Table 2). Iodine value of RS oil was 99.47 g of I_2/100 g .This value showed that the oil has a high content of unsaturated fatty acids and could be considered as a semi-drying oils (100 –130).

Peroxide value of RS oil was 7.52 meq O_2 / kg oil. This value is comparable to that found by Elneairy (2014) in Libyan seeds and higher than that stated by Nzikou et al. (2011) and Eltayeib and Abd Elaziz (2014). In contrast, it is lower than that found by Mohammed and Yagoub (2007). Saponification value of RS oil was 194.4. This value is within the range of 188-196 for most oils of plant origin. The value obtained in the present

TABLE 1. Fatty acid composition of crude RS oil (% of total fatty acid content).

Fatty acids	Value
Lauric C12:0	0.64
Myristic C14:0	0.71
Pentadecanoic C15:0	0.32
Pentadecnoic C15:1	0.11
Palmitic C16:0	22.44
Palmitoleic C16:1	0.6
Heptadecanoic C17:0	0.32
Stearic C18:0	5.77
Oleic C18:1	31.27
Linoleic C18:2	37.61
Linolenoic C18:3	0.11
Arachidic C20:0	0.11
Total saturated fatty acids (S)	**30.31**
Total unsaturated fatty acids (U)	**69.7**
S/U ratio	**1:2.3**

TABLE 2. Physicochemical properties of crude RS oil.

Property	*Value
(at 18ºC)Refractive index	0.15 ± 1.472
at 25ºC Specific Gravity	0.12± 0.9197
(at 40ºC (cp Viscosity	30.00 0.5 ±
: Colour	
*a	0.94
*b	9.94
*L	29.85
(g 100 / Iodine value (g of I_2	0.7 ± 99.47
(kg oil / meq O_2) Peroxide value	0.27± 7.52
Saponification value	0.3± 194.4
(oleic acid %) Free fatty acids	0.03 ± 0.83

Mean ± standard deviation (SD)*.
Each value represents the average of three determinations.

study is quite close to that stated by Nzikou et al. (2011) and Elneairy (2014). On the contrary, it is very high comparing with that found by Bamgboye and Adejumo (2010) and this suggest the use of such oil in the production of liquid soap, shampoos and lather shaving creams as mentioned by Nzikou et al. (2011).

Free fatty acids of RS oil as % oleic acid was 0.828%. This value confirmed well with the obtained fraction by TLC as shown in Fig. 2. This value is very low and showed that this oil is stable as mentioned by Nzikou et al. (2011).

Mahmoud et al., (2008) found a comparable value to that found in the present study. This value indicated that the oil did not subjected to any serious rancidity during storage of the seeds before extraction. From the above results of RS oil composition and properties it can be concluded that RS oil is similar to other edible vegetable oils such as cotton seed oil and corn oil and therefore, it can be used in many industrial applications which will add value for cultivation of this plant.

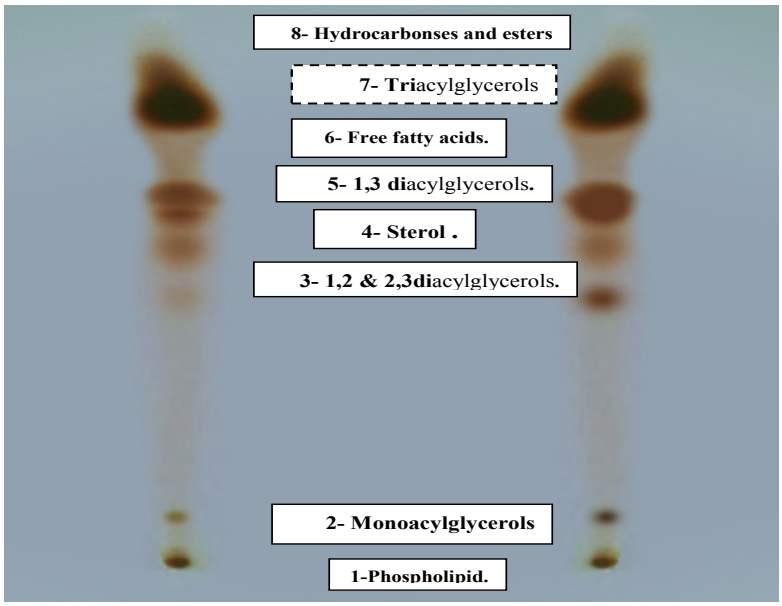
Roselle proteins

Fractions

SDS polyacrylamide gel electrophoresis SDS-PAGE was carried out to demonstrate the main protein fractions of RS. The separation of the protein with SDS PAGE technique was based on charge and molecular weight (M.W) using protein marker with a molecular weight ranged between 17 to 245 KDa. Fig. (1a) illustrates the SDS PAGE pattern of RS protein. From this Fig., it can be noted that pattern consisted of ten bands which ranged between 20 to 245 KDa with different colour intensity. The main bands have molecular weight of 180,135, 100, 63, 48 and 20 KDa. Tran-Thi et al. (2013) reported the SDS-PAGE patterns of RS protein in the range from 95 kDa to a little smaller than 22 kDa and can be divided into 6 fractions, while Tounkara et al. (2013) mentioned that molecular weight ranged from 55,000 Da to below 14,300 Da.

The results in Fig. 3 (b and c) show the patterns of proteins of RK comparing with those for RS which revealed between the two patterns in the numbers and intensity of bands, according to the conditions of soaking process for dehulling. Protein of "RKA" (which produced from soaking RS in water at room temp. for 10 hr) contained 8 bands ranging in apparent MW from 35 to 245 KDa. The main bands had apparent molecular weight 35 to 135 KDa.. The decreasing in band numbers comparing with RS pattern may be due to solubility of low molecular weight bands (17, 25 KDa).

RKB (which produced from soaking RS in water at 45°C for 2hr) exhibited 11 subunits of MW ranged between 17 to 245 KDa. The main bands were 135,100,75,48,35 and 17 KDa. The increment in band numbers comparing with RS and RKA as shown in Fig. 3 (c) may indicate breakdown of some proteins to give a number of low molecular weight subunits.



RS oil **Cotton seed oil**

Fig. 2. TLC chromatogram of total lipid classes of RS oil.

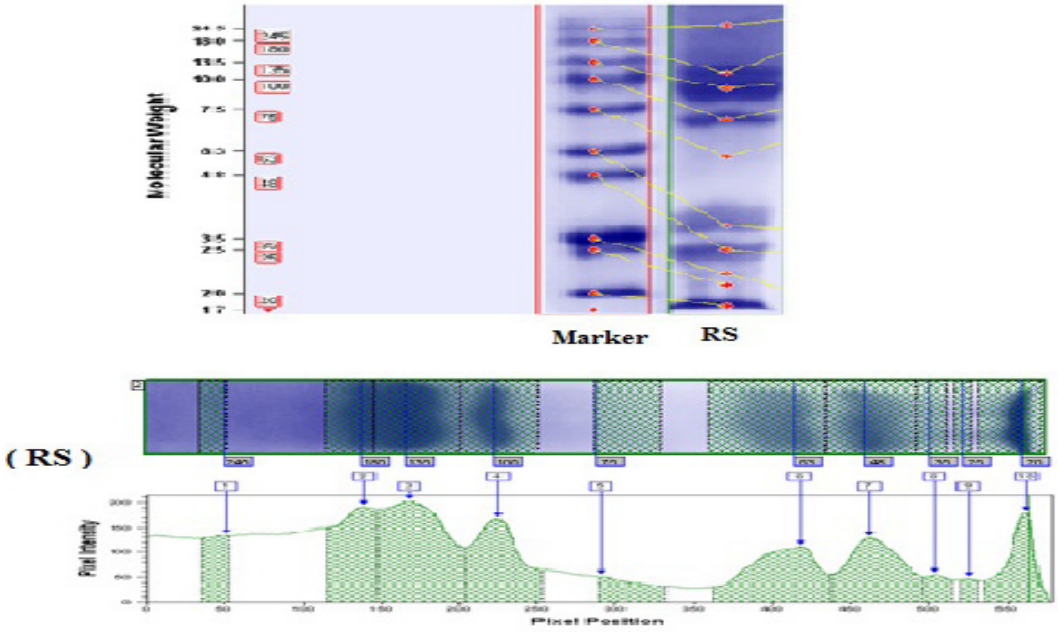


Fig. (3a): Electrophoretic pattern (SDS-PAGE) of RS protein.

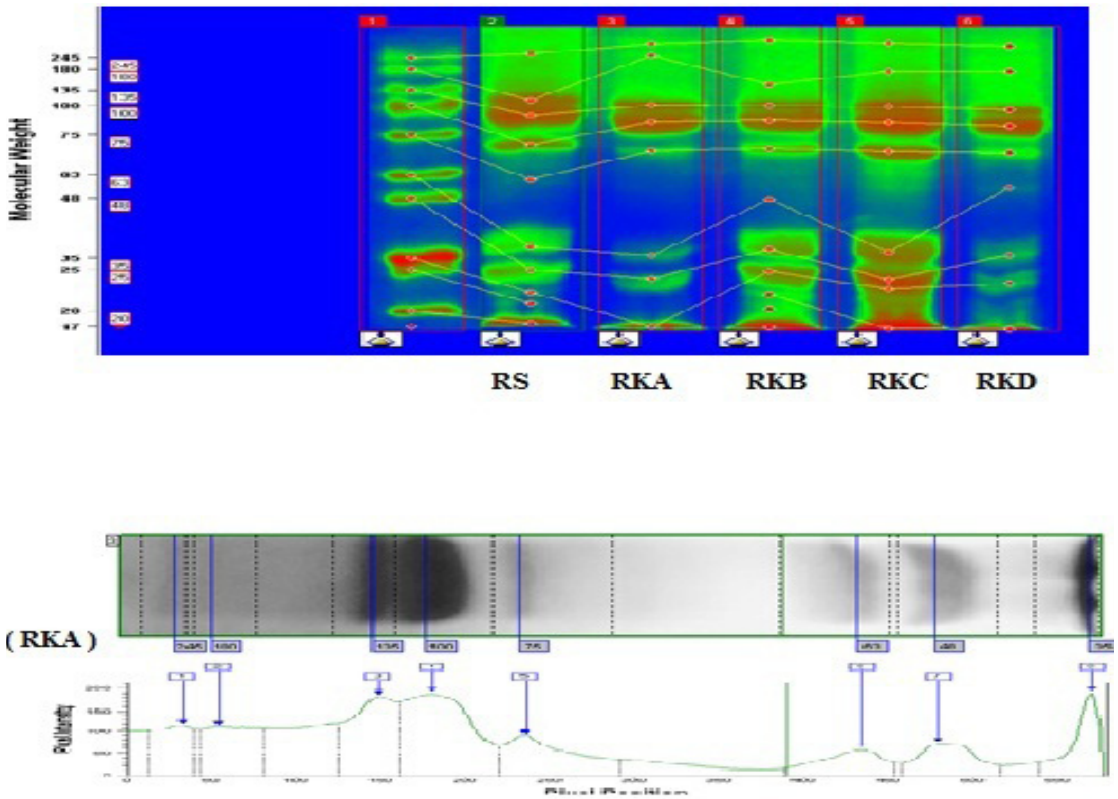


Fig. (3b): Electrophoretic pattern (SDS-PAGE) of RK and RKA protein.

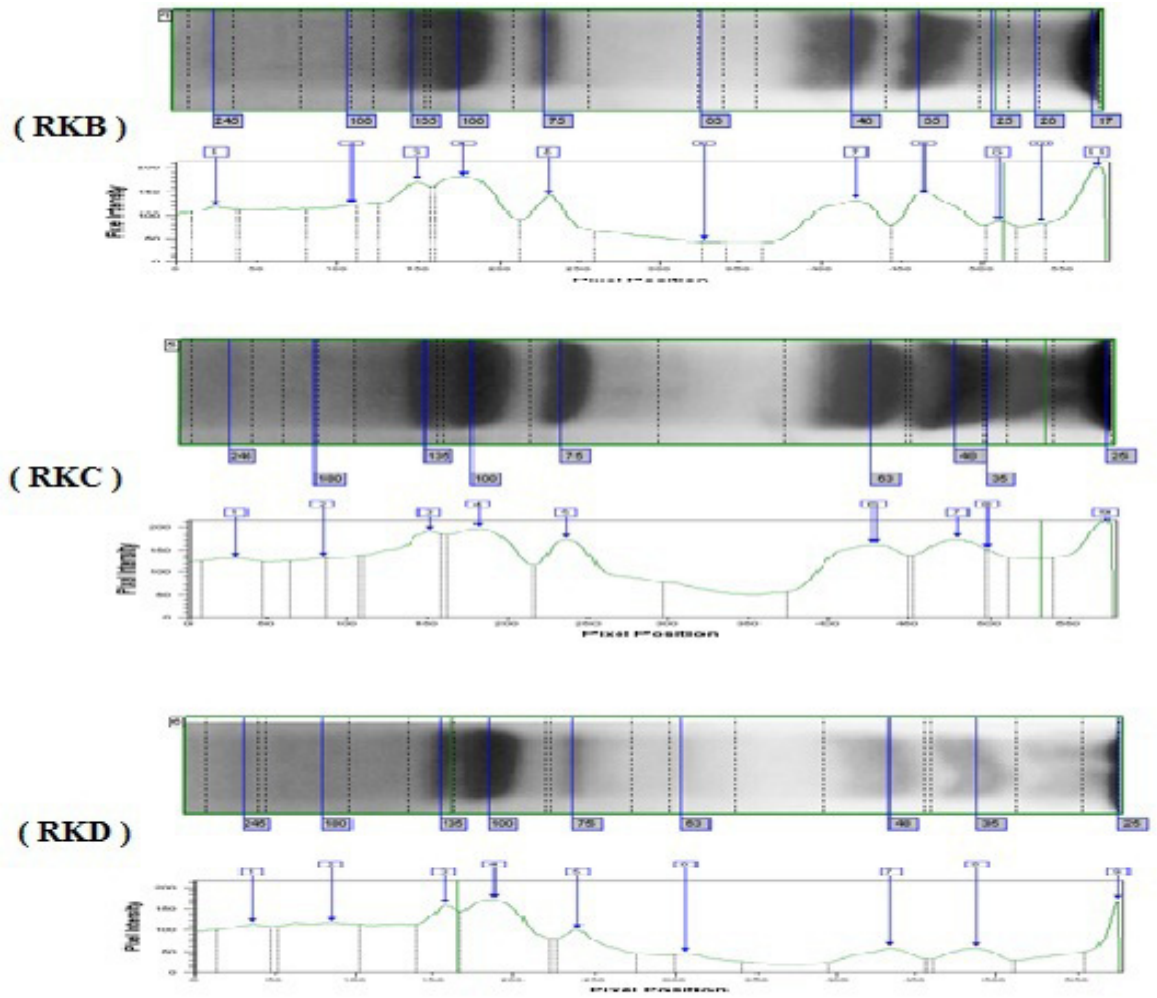


Fig. (3c): Electrophoretic pattern (SDS-PAGE) of RS and RK protein.

In case of RKC and RKD, the number of bands were similar (9 bands), but differing in their colour intensity. The bands of RKC were more intense than those of bands of RKD.

There were differences in number of bands and their intensity between RS and RK proteins. It means that the soaking treatments affected the dissociation of protein from other component such as carbohydrates and / or some of proteins became more soluble in water (Davidek et al., 1990).

Amino acid composition

Table 3 represents the amino acid composition of RS as g/100g protein. The results indicated that aspartic ,glutamic, arginine and aromatic amino acids were the major abundant amino acids in the RS. Sulphur containing amino acids are the limiting amino acids of the RS protein in the present study. The amino acid profile of the RS studied is comparable to those obtained by Shaheen et al. (2012) , Tran-Thi et al. (2013) and Ari et al. (2015). In a study by Elneairy (2014), isoleucine and valine were lower, while, leucine, methaionine , tyrosine and thereonine were higher than that found in the present study , while lysine and phenylalanine were comparable.

The total essential amino acids (39.47) were higher than the FAO/WHO/UNU (1985) reference. Isolucine, total aromatic amino acids, threonine, valine and histidine were much higher than FAO reference values. In contrast, leucine , lysine and total sulphur amino acids were lower than the FAO reference values. This means that the majority of essential amino acids of RS were higher than the FAO reference values. Therefore, RS could be considered as a good source of high

TABLE 3. Amino acid profile of RS and RK produced using different soaking conditions) g/100 g protein).

Amino acid	RS	RK				FAO* Pattern
		A	B	C	D	
Isoleucine	4.09	5.29	5.46	5.55	5.13	2.80
Leucine	6.28	6.35	5.67	7.58	8.57	6.60
Lysine	5.35	4.61	5.23	3.48	3.4	5.80
Methionine	0.95	1.51	1.52	1.63	2.43	-----
Cystine	0.11	0.31	0.37	0.17	0.15	----
Total sulphur amino acids	1.06	1.82	1.89	1.80	2.58	2.50
Tyrosine	3.17	4.23	4.28	3.89	3.51	----
Phenylalanine.	4.98	5.79	6.36	6.21	5.97	-----
Total aromatic amino acids	8.15	10.02	10.54	10.10	9.48	6.30
Threonine	3.76	3.65	2.55	2.02	2.11	3.40
Tryptophan	ND	ND	ND	ND	ND	1.10
Valine	6.16	6.86	6.70	6.82	6.22	3.50
Histidine	4.62	3.38	3.14	2.63	2.61	1.90
Total essential amino acids	39.47	41.98	41.18	39.98	40.1	33.9
Arginine	10.18	12.48	12.97	73.12	12.75	
Aspartic	11.66	8.71	8.97	93.8	8.11	
Glutamic	18.19	19.10	19.79	20.38	20.55	
Serine	4.61	2.64	2.36	2.07	1.53	
Proline	2.40	3.37	3.21	3.13	3.55	
Glycine	6.30	4.52	4.43	5.36	5.7	
Alanine	4.49	4.69	4.84	5.06	5.21	
Total non-essential amino acids	57.83	55.41	56.57	57.66	57.4	
Total amino acids	97.30	97.49	97.75	97.64	97.50	

ND= not determined. *FAO pattern: FAO/WHO/UNU (1985).

RS =Roselle seed. RK = Roselle kernel.

A°25 =C/10 hr. B= 45°C/2 hr. C= 65°C/1 hr .D= 75°C/15 min.

TABLE 4. Mineral composition of RSF and RKF produced using different soaking conditions.

*(mg/100g sample)Mineral	RSF	RKF			
		A	B	C	D
K	1145	1030	950	938	973
Mg	242	245	246	248	244
P	482	532	528	554	544
Ca	239	230	233	233	241
Fe	9.7	9.5	9.8	9.8	9.6
Cu	10.3	22.6	20.1	12.4	19.1
Zn	9.8	12.3	13.1	10.9	10.7

*On dry weight basis.

RSF=Roselle seed flour .RKF = Roselle kernel flour produced after soaking and dehulling RS at A°25 =C/10 hr. B= 45°C/2 hr. C= 65°C/1 hr. D= 75°C/15 min

quality protein. Also, Table 3 shows the effect of water soaking conditions (different times and temperatures) for seed dehulling to obtain RK on the amino acid composition of protein comparing to the same acids of RS and FAO. As in case of RS, glutamic, aspartic, arginine and aromatic amino acids were the highest among the determined acids. On the other hand, methionine, cystine, serine and proline were the lowest. Soaking and dehulling treatments had positive effect on the essential amino acids. Their content increased in the range of 1.29 to 6.35%. The highest increment was in RKA product (room temp/10 hrs) and the lowest one was in case of RKD product (65°C/ 1 hr). Sulphur containing amino acids were still the limiting amino acids but increased in their content than in case of RS and higher than that reported by FAO/WHO/ UNU (1985) reference. This increment ranged between 70 to 143%. The highest value was in RKD product (75°C/15 min) and the lowest in RKC product (65°C/1 hr) .On the other hand, RKD product contained the lowest content of aromatic amino acids.

The increment or decrement of amino acids due to the different treatments may be due to transamination and deamination reactions (Yagoub et al., 2008). Generally, removing hull from RS by using the aforementioned treatments improved the nutritional value of the obtained RK.

Mineral content of RS and RK

The mineral composition of RS is presented in Table 4. Potassium, phosphorus, magnesium and calcium were the major minerals of RS meanwhile, iron, copper and zinc were found to be low in their concentrations. Potassium was the predominant element (1145 mg/100 g) in the seeds, followed by phosphorus (482 mg/100 g), magnesium (242 mg/100g) and calcium (239 mg/100 g).

As seen from Table 4 different effects are noted on the mineral contents due to dehlling process. Generally, Mg, Ca and Fe were not affected, while P, Cu and Zn increased. On the other hand, K decreased but still the predominant element in RK. Increment or decrement in mineral composition of dehulled RS may be due to the removal of the hull, leaching out of the components in soaking water and due to the effect of temperature of soaking water (Adelakun et al., 2012).. Dehulling by soaking in water resulted in the greatest retention of some minerals.

Conclusion

The results obtained in the present study showed that RS as a by-product have good nutritional quality and could be used as an economic and novel promising source of protein fortification for a variety of food as well as a potential food ingredient. Oil of RS can be used for covering a part of lack in edible oils and can be used in some food industrial applications as cotton seed oil and corn oil. Also, RS as a whole or it's flour after oil extraction can be considered as a good source of major minerals especially K, ph, Mg and Ca.

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بذور ولب بذور الكركديه كمصدر جيد للزيت والبروتين والمعادن

أسامه محمد مبروك[١]،وفاء على أمين[١]، ايات محمد مصطفى[٢] ،أسامه راشد أبوسماحة[٢]
[١]قسم بحوث تصنيع الحاصلات البستانية –معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – مصر
[٢]قسم علوم و تقنية الأغذية – كلية الزراعة – الشاطبى – جامعة الاسكندرية – مصر

أستهدفت هذه الدراسة تقييم بذور الكركديه الكاملة (RS) والبذور المقشورة (الحبة) (RK) كمصدر غير تقليدي للزيت ، والبروتين ، و المعادن . وأشارت النتائج إلى أن بذور الكركديه ((RS أحتوت على نسبة عالية من الأحماض الأمينية الضرورية (٣٩,٤٧ ٪) مقارنة بما ذكر بواسطة FAO/WHO/UNU (٥٨٩١) ، بينما كان الميثيونين والسيستين هما الأحماض الأمينية الحدية . فصلت البروتينات باستخدام الهجرة الكهربية الى ١٠ أقسام مختلفة التركيز و كانت أوزانها الجزيئية ما بين ٢٠ الى ٢٤٥ كيلو دالتون . و أن البروتينات الاساسية ذات أوزان جزيئية ١٨٠, ١٣٥ ، ١٠٠ ، ٦٣ ،٤٨، ٢٠ كيلو دالتون .أوضح فصل الزيت الخام للبذور(RS) باستخدام كروماتوجرافيا الطبقة الرقيقة أنه يشمل على ٨ أقسام أساسية من المركبات الجلسريدية وغير الجلسريدية بالأضافة الى الليبيدات القطبية التى تظهرعند خط البداية. و كانت الأقسام المتعارف عليها هى الليبيدات القطبية ، الجلسريدات الأحادية ، ١-٢ و ٢-٣ جلسريدات ثنائية ، اسيترولات ، ١-٣ جلسريدات ثنائية ، أحماض دهنية حرة و جلسريدات ثلاثية ثم هيدروكربونات مع استرات استيرولات ، و ان الجلسريدات الثلاثية هى المكون الاساسى . أشار تحليل كروماتوغرافيا الغاز السائل لزيت بذور الكركديه الخام احتوائه على نسبة عالية من الأحماض الدهنية غير المشبعة (٦٩,٧٪) حيث مثل حامض الأوليك ٣١,٢٧٪ بينما مثل حامض اللينوليك ٣٧,٦١٪ من اجمالى الاحماض الدهنية. فى حين مثلت الأحماض الدهنية المشبعة ٣٠,٣ ٪ من إجمالي الأحماض الدهنية حيث كان حامض البالمتيك هو الحامض السائد (٢٢,٤٤ ٪) يليه استياريك (٥,٧٧ ٪). أظهر التحليل الفيزيائى الكيميائى لزيت بذور الكركديه (RS) أن اللون أصفر بقيم (a* 0.94 ، b* 9.94، L* 29.85) و له معامل انكسار ١,٤٧٢٤ عند ١٨ ° م , و كثافة نوعية ٠,٩١٩.جم/ سم٣ و رقم يودى ٩٩,٤٧ و رقم تصبن ١٩٤,٤، رقم بيروكسيد ٧,٥٢ مليمكافىء اكسجين/كجم زيت ، النسبة المؤية للحموضة ٠,٨٢٨ % كحامض أوليك . أحتوت بذور الكركديه على المعادن التالية :- بوتاسيوم ، ماغنسيوم ، فوسفور ، و كالسيوم بجانب الحديد ،و النحاس و الزنك و كان البوتاسيوم هو المعدن السائد (١١٤٠ ملجم/١٠٠جم) يلية الفوسفور (٤٨٢ ملجم /١٠٠جم) ثم الماغنسيوم (٢٤٢مجم/١٠٠جم) و الكالسيوم (٢٣٩ ملجم /١٠٠جم) . أوضحت النتائج وجود تأثير واضح لعملية ازالة القشرة بالكامل للحصول على لب بذور الكاركادية (RK) عن طريق النقع فى الماء لأزمنة مختلفة (١٠ساعات الى ١٥دقيقة) ودرجات حرارة (٢٥-٧٥°م) حيث أوضحت النتائج بعض التاثيرات على تركيب البروتين والمعادن بينما لم يتاثر تركيب الزيت وخصائصه بتلك المعاملة.