Amel *et al.*, Sudan Academy of Sciences Journal, No. 10, 2015, 80-92
ISSN 1816-8272

Physiochemical Properties of Mango (*Mangifera indica* L.) Seed Kernel's Oil

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Abstract

Mango (*Mangifera indica* L.) is one of the important fruits in the Sudan. After consumption of the mango fruit, seed kernels usually remain as waste. With the objective of making use of the remains, we extracted seed kernels of five Sudanese mango cultivars and evaluated their physiochemical properties. The extracted oil content was ranged between 15.2% and 2.73% and their melting point more than 40.1°C. High peroxide value (3.31mg/g) was reported in Abu-samaka cultivar. The saponification value exceeded 190 mg/g in all cultivars while their un-saponifiable matters were found to be in the range of 1.2% to 2.3%. The extracted oils were found to be rich in K, Na, Mg, Ca, Co, Fe and Mn. The main unsaturated fatty acids found were stearic acid (60.88%-67.41%) and palmatic acid (13.78%-16.95%), while the saturated oleic acid was also found (8.32% to 11.43%). All the studied oils showed free radical scavenging and iron chelating activities. The highest DPPH level was observed in Abu-samaka cultivar (67%) whereas the lowest was of Elphons (40%). Phenolic, flavonoid and tannin compounds were also observed. These results suggested that the mango seed kernel oils could be used as a potential composition in industries rather than to left as waste.

***Keywords**:* Mango Seed Kernel Oil, Phenolics, Favonoids, Radical Scavenging Activity.

Introduction

Mango fruit (*Mangifera indica* L.) is a delicious seasonal fruit. It is one of the most nutritionally rich fruits with unique flavor, fragrance, taste and heath promoting qualities making it one of the popular functional foods. The tree is grown in the tropics and believed to be originating from sub-Himalayan plains of Indian subcontinent (Jasim *et al.*, 2005). Flesh of fresh mango fruit was found to be an excellent source of minerals, vitamins and flavonoids which have been known as antioxidant and of essential importance in human nutrition (Violeta *et al.*, 2013).

Mango fruits are very popular and gaining increase consumption, hence considerable amounts of seeds kernels (about 17-22% of the fruit size) usually resulted and discarded as a waste (Abdalla *et al.*, 2007). Since there is no any kind of utilization for the mango seed kernels their accumulation may turn to be a source of pollution (Soong and Barlow, 2004). This waste could be treated as a specialized residue for production of appreciable amount of oil that rich in useful compounds (Maisuthisakul and Gordon, 2009). In this article, we studied the

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physicochemical properties of oils extracted from the seed kernels of some Sudanese mango cultivars.

Materials and Methods

Oil extraction

Fruits of five mango (*M. indica*) cultivars, which are commonly known as Abu samaka, Baladia, Doctor Night (D. Night), Elphons, and Sennaria, were bought from the local market. Fruits were selected according to their homogenous, color and freedom from physical defects.

The kernels were manually removed from their coats, air dried and finely grinded. Oil extraction was carried out according to the method described by Nzikou *et al.*, (2010), in which 30 g of the kernels fine powder was fed into a Soxhelt fitted with a 500 ml round-bottom flask and a condenser. Extraction was executed in a water bath for 8 h with 200 ml of *n*-hexane. Further, the solvent was distilled off under vacuum in a rotary evaporator equipped with an aspirator and digital water bath. The obtained oil fractions were weighted and stored under refrigeration to be used in further analysis.

Physical and Chemical Properties

Physical properties included refractive index, acid value, density and melting point was determined according to AOCS (1989). Determinations for peroxide value, saponification value, unsaponifiable matter were carried out using Pena *et al*., (1992) standard analytical methods.

Determination of mineral elements in oil sample

Minerals were determined using the method described by Nunes *et al*. (2011). In the method, sample was prepared by microwave digestion. The digestions were performed by adding mixture of 3.5 ml of concentrated HNO_3 and 1.0 ml H_2O_2 (30%) to 0.5 g of the oil sample followed by microwave oven heating till a clear solution was formed. After cooling, the mineral contents (Mg, Ca, Co, Fe and Mn) of the sample were analyzed by ICP/MS). Sodium and potassium was determined using flame photometer.

Determination of fatty acid composition

Determination was carried out according to Lee *et al*. (1998). A gas chromatograph from Shimadzu hyphenated to a mass spectrometer QP 2010 plus (Tokyo, Japan) equipped with an auto-sampler (AOC-20S) and auto-injector (AOC-20i) was used). After methylation with hexan and sodium methoide, to separate out the pure solution of fatty acid methyl ester, sample was carefully collected and injected in the column of GC/MS. Identification of the compounds were carried out by comparing the obtained mass spectra with those of standard mass spectra of the NIST library (NIST 05).

Determination of radical DPPH scavenging activity

Free radical scavenging capacity of betel leaf extract was evaluated according to Masuda *et al*. (2007), procedure using the stable 2,2-diphenyl -1-picrylhydrazyl radical (DPPH). The final concentration was 100 μM for DPPH. The absorbance at 517 nm was measured against a blank of pure methanol after 30 min of reaction, and used for estimation of the remaining radical levels. The DPPH

ISSN 1816-8272

activity was expressed in terms of EC50 (µg.ml/1) and the antiradical activity was reported in the form of 1/EC50.

Iron chelating activity

The chelation of ferrous ions by extracts was estimated by method of Dinis *et al*. (1994). Briefly, 50 µl of 2 mM $FeCl_2$ was added to 1 ml of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine - Fe^{+2} complex formation was calculated as [(A0- As)/ As] ´ 100, where A0 was the absorbance of the control, and As was the absorbance of the extract/ standard. Na_2EDTA was used as positive control.

Antioxidants screening

Alkaloid detection

Presence of alkaloid (Phenolic compounds) was determined, as described by Rahman *et al*. (2011), by stirring 0.2 g of the extracts in 10 ml of 2N HCl while stand for heating on water bath for 10 minutes. The mixture was cooled filtered and divided into two test tubes. To one of the test tubes few drops of Mayer's reagent were added while to the other tube few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the tow test tubes was taken as presumptive evidence for the presence of alkaloids.

Flavonoid detection

The flavonoid content of each oil extract was determined following the colorimetric method of Chang *et al*. (2002). 0.2 g of each extract was dissolved in 30 ml of 80 % ethanol and filtered. The filtrates were used for following tests:

A- Three ml of the filtrate was mixed with 1ml of 1% methanolic solution of aluminum chloride. Formation of a yellow color indicated the presence of flavonoids (flavones or and chalcone).

B- Three ml of the filtrate was mixed with 1ml of 1% methanol solution of potassium hydroxide in was added. Development of a dark yellow color indicated the presence of flavonoids compounds (flavones or flavonenes and chalcone or flavonols).

Tannin detection

Ten ml of hot saline solution was added to with 0.2 g of each extracts and stirred thoroughly. The mixture was cooled, filtered and the volume of the filtrates was adjusted to 10 ml with more saline solution. 5 ml of solution was treated with few drops of gelatin salt reagent. Formation of immediately precipitate was taken as an evidence for the presence of tannins in the plant sample. To another portion of the solution, few drops of ferric chloride were added. The appearance of blue, black or green color was taken as an evidence for the presence of tannins (Pankaj, *et al.*, 2013).

Results and Discussion

There were variations in size shape and weight of the kernels obtained from different cultivars of mango. These variations could be attributed to the genetic variations of the varieties and the differences in environmental conditions of the

ISSN 1816-8272

origins such as soil, water and temperature which have an impact on nutritional contents of the plants.

The percentage of mango seed kernels oil

The extracted mango seed kernel oil was pale yellow in color and waxy solid at the room temperature. The percentage oil of mango seed kernels was presented in table (1). Results indicated the existence of variation in the extracted oil contents. Variation might be due to the differences in plant cultivars, cultivation climate, ripening stage and the harvesting time of the seeds. Among oil extractions, the cultivar D. Night showed the highest total oil yield (15.2%), followed by Sennaria (8.53%). Comparatively, moderate amount of oil were found in the cultivars; Elphons, and Baladia, while the least amount of the extracted oil was found Abu-samaka cultivar. The interaction between solvent and solutes, as well as composition of mango kernel may contribute on the extraction efficacy and yield. Most of the values fall within the ranges published in the literature (Khammuang and Sarnthima, 2011).

Table 1. Oil content in the mango seekernel of Sudanese cultivars

Mango cultivar	Oil content (%)
Abu-samaka	2.73
Baladia	3.89
D. Night	15.2
Elphons	4.55
Sennaria	8.53

Physical properties of mango seed kernels oil

The physical characteristics of mango seed kernel oil is given in table (2). The obtained refractive index values were ranging between 1.434 and 1.457. They were in close agreement with values reported for conventional oils (De Bussy, 1975) as for soybean (1.466-1.470) and palm kernel (1.449-1.451). The high refractive index of mango seed kernel oil seems to confirm the high number of carbon atoms in their fatty acids (Falade *et. al*., 2008). Refractive index also increases as the double bond increases (Eromosele and Paschal, 2003).

The density is important physical characteristic as indicative of the handling and storage of vegetable oils. It is usually measured as the ratio of the mass of a given volume of oil to the mass of an equal volume of water; it is also related to the fatty acid content of the oil and the average molecular weight or number of carbon atoms of the fatty acids. The density values for mango seed kernels are shown in table 2. In this study and except for density of Sennaria cultivar (0.937), the densities of the extracted oils (ranging between 0.763 and 0.841and for Elphons Abu- samaka cultivars respectively) were less than the density values of edible

ISSN 1816-8272

oils such Canola oil, cottonseed, olive, corn, soybean and ground nut oils. (Barnwal and Sharma, 2005). The values could suggest that mango seed kernel oil may find outlets in the manufacture of lubricants and other purposes like biodiesel production.

The melting points of the different cultivars were varied from the least value of 29.2°C for the Abu-samaka cultivar to highest 40.1°C for the Elphons (table2). In general the melting point is temperature where the solid fat transited into a liquid form. Fats that obtained from plants are commonly unsaturated fats (e.g. soybean oil, mustard oil and sunflower oil). From the observed results, the melting points of mango seed kernel oils are close to the Sudan room temperature and, to some extent, it could be considered as un-saturated fatty acids. These figures could help in determining the level of un-saturation of the fatty acids in oils and their nutritive quality of interest (Nagre *et al.*, 2011).

Table 2. Physical properties of mango seed kernel oil

Mango cultivar	Refractive index	Density (g/ml)	Melting point (°C)
Abu-samaka	1.445 ± 0.02	0.84 ± 0.01	40.1 ± 0.2
Baladia	1.444 ± 0.02	0.772 ±0.01	39.76 ± 0.4
D. Night	1.434 ± 0.02	0.82 ±0.02	30.2 ± 0.3
Elphons	1.457 ± 0.03	0.75 ± 0.01	29.2 ± 0.3
Sennaria	1.456 ± 0.02	0.94 ± 0.01	36.40 ±0.8

* Values are means of the triplicate determination

Chemical properties of mango seed kernels oil

The peroxide, acidity, iodine, saponification un-saponifiable values are the major chemical characterization parameters for oil quality. The chemical properties of mango seed oil are given in table 3. Peroxide value is one of the most widely used tests for oxidative rancidity in oils and fats, peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. The results showed that the peroxide values in the extracted oils, ranging from 3.31 mg/g for Abu-samaka to 2.29 mg/g for Elphons cultivar. In general, the lower peroxide value reflects better quality and more stable oil.

The good peroxide value should not exceed 10 mg/g in the fresh oils (Borchani *et al.*, 2010). Ojeh (1981) reported that oils with high peroxide values are unstable and easily become rancid. From the results it could be conclude that all the extracted oils are stable.

The mango seed kernel oil was found to contain saponins that form soap lather when mixed with water. The saponification values were found to be high and

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more than 190 mg/g for all the cultivars. The highest saponification value was obtained by the cultivar Sennaria (196.3 mg/g) followed by Elphons (193.3mg/g), and the least value (190.87mg/g) was observed with Abu-samaka cultivar. High saponification values are recommended in detergents manufacturing and possibly provide some cleansing, thus it could be used in the production of soaps, shampoos and lather shaving creams and some other cosmetics (Auwal *et al.*, 2010).

The mango seed kernel oil contains low (1.2-2.3 %) unsaponifiable matter. As shown by (Khammuang and Sarnthima, 2013), unsaponifiable matter content are known to affect the physical properties of oils related to the melting-points and probably responsible for high refining losses (Abayeh, *et al.*, 2013).

Table 3. Chemical properties of mango seed kernel oil

Cultivar	Peroxide value (meq /Kg)	Saponification value (mg KOH/g)	Unsaponifiable matter
Abu-samaka	3.31 ± 1.1	190.9 ± 2.6	1.23 ± 0.19
Baladia	2.43 ± 0.43	192.0 ± 4.2	1.20 ± 0.25
Elphons	2.29 ± 0.08	193.3 ± 0.4	1.60 ± 0.22
D. Night	2.93 ± 0.3	192.3 ± 0.3	2.30 ± 0.31
Sennaria	3.2 ± 0.03	196.3 ± 0.2	1.56 ± 0.23

* Values are means of the triplicate determination

Elemental nutrient composition of mango seed kernel oil

The mineral contents of mango seed oil are shown in table 4. Results showed great variations among the different cultivars in the mineral elemental composition of their seed kernel oils. It is of interest to note that the oil seed kernel of Baladia cultivar is the richest in mineral element especially for calcium which is a high as 141.9 mg/100g) Also, it has appreciable amount of magnesium and potassium (36.39 and 37.51 mg/100g respectively).

In general, the extracted mango seed kernel oils were found to be rich in some useful microelements (iron, cobalt, and manganese) and minerals that make it attractive as a natural source. Potassium is an essential nutrient and identified as the synonym for health insurer. It contains qualities for maintaining a high level of human well-being and a cheerful lifestyle. Apart from acting as an electrolyte, this mineral is required for keeping heart, brain, kidney, muscle tissues and other important organs of human body in good condition. It has an important role is the synthesis of amino acids and proteins (Heyka, 2009). Calcium and magnesium plays a significant role in, bone building mineral for enzyme activity and nucleic acids metabolism (Heaney, 2001).

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Table 4.Mineral elements compositions of mango seed kernel oil

Cultivar	Element						
	K	**Na**	**Mg**	**Ca**	**Co**	**Fe**	**Mn**
Abu-.samaka	29.16	19.99	4.84	25.40	0.478	3.33	0.18
Baladia	36.39	23.74	37.51	141.90	2.34	26.6	0.65
D. Night	5.64	3.66	2.97	8.70	0.28	1.75	0.076
Elfons	7.195	2.03	1.30	3.205	0.185	0.864	0.024
Sennaria	29.365	3.62	25.61	29.41	0.179	2.197	0.236
Tymor	13.01	4.023	3.63	14.99	0.479	1.245	0.063

*Analysis was carried out by using ICP/MS; values are in (mg/100g)

Free fatty Acid profile using GC/MS

Table 4, shows the fatty acid composition of *Mangifera indica* L. seed kernel oil. The main unsaturated fatty acids found were oleic acid (monounsaturated) and linolenic acids (polyunsaturated), which observed only in the oil extracted from Abu-samaka cultivar, while the major saturated fatty acids in the seed oil of all varieties were stearic and palmitic acids (table 5). The presence of high amounts of the essential oleic suggests that the mango seed oil could be to be used in some as nutrient rich food (Kittiphoom and Sutasinee 2013). The available stearic acid could be used in the production of detergents, soaps, and cosmetics such as shampoos and shaving cream products. Good levels of oleic were observed in the mango seed kernel oil (11.43-10.99% as in Abu-samaka and D. Night respectively). In exception of the oil extracted from Abu-samaka cultivar no linoleic acid was detected. Also, no margric acid was detected in D. Night. Oleic acid (omega-6) is a mono-unsaturated fatty acid found high concentrations in some vegetable oils such as olive oil (55-80%). High concentrations of oleic acid help in lowering the levels of blood cholesterol and lower the risk of heart problems (Tine, *et al.*, 2005). The recent discovery of the fact that oleic acid is responsible for protection from breast cancer has stimulated renewed interest in oleic acid. Oleic acid blocks the action of a cancer-causing oncogene, called HER-2/neu, which is found in about 30% of breast cancer patients (David, 2005). Similarly, Linoleic acids was unequivocally proved to reduce the incidence of tumors and inhibit carcinogenesis and in a number of experimental animal models in experimental animals (Clement *et al.*, 2014).

DPPH radical scavenging activity

The 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) radical scavenging activity (RSA) evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the RSA of specific fats. As shown in table (6), majority of the tested compounds in these series showed moderate interaction with the DPPH radical at 100-1000 µg/ml concentration. The maximum DPPH - RSA was observed in Abu-samaka cultivars (67% and), followed by Baladia and D. Night

ISSN 1816-8272

(63% 62% repectivly).The respective DPPH free radical scavenging Elfons and Sennaria cultivar were found to be 41% and 40%. The DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) is a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by antioxidant (Lu and Yeap, 2001). It has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Anna *et al*., 2008). On the other hand, iron as an essential mineral for normal physiology may form reactive hydroxyl radicals and thereby contribute to oxidative stress (Hippeli and Elstner, 1999). It poses an important mechanism of antioxidant activity as the ability to chelate/deactivate transition metals, through its ability to catalyze hydroperoxide decomposition reactions. Therefore, it is important to screen the iron chelating ability of the seed kernel oils. In this study all the extracts demonstrated different levels of ability to chelate metal ions (table 6). The Sennaria cultivar was found to have the maximum chelating ability (7%) while Baladia and D. Naght were equal in their ions chelating activity (4%).

The scavenging potential and metal chelating ability of the antioxidants are dependent upon their unique phenolic structure and the number of hydroxyl groups (Pazos *et al*., 2005). The major role of phenolics compounds as scavengers of free radicals was highlighted in several reports (Adedapo *et al*., 2008). The results obtained in the present study showed that the oil of mango seed kernel possessed good reducing power as determined via metal chelating and DPPH for this reason the extracted oils could be employed as a functional food ingredient or as a nutraceutical component for disorders caused by oxidative stress (Khammuang and Sarnthima, 2011).

Occurrence of polyphenolics and antioxidants in mango seed kernel oil

The studied mango seed kernel oil extracts revealed the presence of phenolic compounds in all cultivars except for Elphons. While flavonoids were absent in Baladia and Elphons. The Baladia cultivar was also found to be poor in tannins (table7). The detected phytochemical compounds (phenolic compounds, flavonoids and tannins) are known to have beneficial importance in industrial and medicinal products.

Oxygen is necessary for human beings and other living organisms to function and the oxidative mechanism is necessary for the cells to survive in the body. However, when oxygen is transformed to free radicals and reactive oxygen species (ROS), which then act as oxidants that have a harmful role in the human biological activities (Karakaya *et al*., 2001; Fernández-Pachón *et al*., 2004). ROS and free radicals could also cause oxidation that induces deterioration of food, resulting in rancidity, changes in color, and declines in nutritional quality, flavor, texture and safety (Antolovich *et al*., 2000).

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Table 5. Fatty acid composition (%w/w) of mango seed kernel oil

	Cultivar				
Fatty Acid	**Abu- samaka**	**Baladia**	**D. Night**	**Elphons**	**Sennaria**
Behenic acid	1.39	0.97	0.96	1.51	0.97
Oleic acid	11.43	9.81	10.99	8.32	9.57
Palmatic acid	16.95	13.78	15.57	16.32	15.95
Linoleic acid	1.75	-	-	-	-
Linolenic acid	1.75	0.76	0.89	0.79	1.31
Margric acid	0.33	0.41	-	0.41	0.35
Stearic acid	60.88	67.41	64.44	61.87	64.96

Table 6. DPPH free radical scavenging and iron chelating activity

Cultivar	DPPH RSA ±STD (%)	Iron chelating ±STD (%)
Abu- samaka	67 ± 0.45	02 ±0.01
Baladia	63 ± 0.35	04 ± 0.01
D. Night	62 ± 0.35	04 ± 0.04
Elfons	40 ± 0.56	03 ± 0.02
Sennaria	41 ± 0.56	07 ±0.03

Values are mean of threreplicate determinations standard deviation

Table 7. Antioxidant compounds in the extracted oil

Cultivar		Compound	
	Phenolic	**Flavonoids**	**Tannins**
Abu-samaka	+	+	+
Baladia	+	-	-
D. Night	+	+	+
Sennaria	+	+	+
Elfons	-	-	+

Note: '+' indicates presence '-' indicates absence

Conclusions

According to the physicochemical properties, fatty acid profile antioxidant of the mango seed kernel oil, it was found that the extracted oil is rich in the saturated oleic acid, and the unsaturated palmatic and stearic acids. Furthermore, results verified that mango seed kernel oil contained antioxidants such as phenolic compounds, falvonoids and tannins which have the capacity to reduce the possibility of several diseases such as cancer and cardiovascular diseases.

The study recommended the usage of mango seed kernel for oil production as a substitute for vegetable oil. Further, it has the potential to be utilized for extraction of ingredients that could be used for the development of some industries rather than to be discarded as a waste.

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ISSN 1816-8272

الخصائص الفيزيوكيميائية لزيت بذور المانجو
(*Mangifera indica* L)

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مستخلص البحث

تعتبر المانجو (.*Mangifera indica* L)، من أهم انواع الفاكهة في السودان. وبعد إستهلاك الثمار تبقى البذورة كمخلفات. وبغرض الاستفادة من المخلفات تم إستخلاص زيت البذور من خمسة أصناف للمانجو السودانية وتقييم خواصها الفيزوكيميائية. حيث وجد ان نسبة الزيت المستخلص تتراوح ما بين 15.2% الى2.73% وأن درجة إنصهار الزيوت أعلى من 40.1°C، فيما كانت أعلى قراءة لرقم البيروكسيد هى 3.31 مليجرام/جرام و ذلك فى صنف ابو سمكة. بينما نسبة التصبين كانت أعلى من 190 مليجرام/ جرام لجميع الأصناف. كذلك لوحظ أن نسبة المواد غير المتصبنة تتراوح بين 1.2-2.3%. كما وجد أن الزيت المستخلص غنى بالعناصر مثل البوتاسيوم، الصوديوم، الماغنيسيوم، الكالسيوم، الكوبالت، الحديد والمنجنيز. وعند تقدير الأحماض الدهنية كان المحتوي الرئيسي للاحماض الغير مشبعة بزيت نواة بذور المانجو هما حمضى الاستاريك والبالماتيك (60.88%-67.41%على التوالى). في حين أن حمض الأوليك المشبع كان في نطاق يتراوح بين 8.23%-11.43% وفقا للصنف. جميع الزيوت المستخلصة أظهرت نشاط خالب الحديد وكساح الجذور الحرة. كما سجلت أعلى مستويات (67،%) من DDPH فى صنف أبوسمكه فى حين أن أدنى المستويات (40%) كان فى صنف الفونس. و قد تم أيضا الكشف عن وجود مواد الفينول ومركبات الفلاڤونويد والتانين في بعض الأصناف و لم يلاحظ وجود للفلاڤونويد في الاصناف البلدية، كما لم يتم أيضا الكشف عن أيٍ من مركبات الفلاڤونويد والفينول في صنف الفونس. من خلال الخصائص التى تم التعرف عليها تشير الدراسة الى إمكانية إستخدام زيوت نواة بذور المانجو كمكونات فعالة فى بعض الصناعات فضلا عن تركها كمخلفات.

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ISSN 1816-8272