Pak. J. Biotechnol. 11 (1) 33 – 40 (2014) ISSN Print: 1812-1837
www.pjbt.org ISSN online: 2312-7791

DETERMINATION OF FATTY ACIDS AND ELEMENTS FROM COCONUT *(COCOS NUCIFERA)* SHELL

Nasreen K. Thebo[1], Altaf A. Simair[2]*, Wazir A. Sheikh[1], Sher M. Mangrio[1], Pirkash L. Nagni[3], Sughra G. Mangrio[2] and Hassan M. Nizamani[4].

[1]Mycology Research laboratory, Institute of Plant Sciences, [2]Institute of Biotechnology and Genetic Engineering and [3]Free Radical Research Laboratory, National Centre of Excellence in Analytical Chemistry University of Sindh, Jamshoro, Sindh, Pakistan. [4]Al-Mafraq Hospital, Dubai. e-mail*: altafsimair@hotmail.com

ABSTRACT:

GCMS analysis showed twenty eight fatty acids, 12 n-octanoate, dodecanotic acid, n-hexadecanoic acid, n-heptaecenoate, tridecatrienoate acid methyl-2-tridecynote and essential elements were isolated from the shell of *Cocos nucifera* viz., along with calcium, cadmium, chromium, copper, iron, lead, potassium, magnesium, manganese, nickel and zinc. Present study revealed that accumulation of tetradeconic acid was higher among saturated fatty acids among metals magnesium and iron 593.06 ± 42.17, 997.9 ± 52.25 respectively were highest following by low accumulation of lead (1.46 ± 0.21) was recorded in coconut shell.

Keywords: *Cocos nucifera*, fatty acids elemental analysis, GCMS.

INTRODUCTION

Composition of nut consumption (Gonzales, 1970) and lipid lowering effect of fatty acids have been demonstrated in experimental studies in almonds, peanuts, pistachios and walnuts (Sara *et al.*, 2007). Most of these beneficial effects are due to the presence of antioxidant (free radical scavengers) and antioxidant activity (Gulcin *et al.*, 2010). Nuts are considered one of the most nutritious human foods due to its high content of protein, carbohydrates, unsaturated fatty acids, vitamins and minerals (Welna *et al.*, 2008). Nut consumption reduces the risk of cardiovascular heart diseases (CHD) and also posse's cholesterol-lowering effect (Fraser *et al.*, 1992) such as pistachios and walnuts (Hyson *et al.*, 2002). Such health-promoting properties of the nuts could be linked to the presence of bioactive compounds such as flavonoids, isoflavones, synthetic anti-oxidants such as butylated hydroxyl-anisole (BHA), butylated hydroxyl-toluene (BHT) and tertiary butyhydro-quinone (TBHQ) and other phenolic compounds (Siriwardhana and Shahidi, 2002). Coconut (*Cocos nucifera* L.) belongs to Prunus species of Arecaceae family. Coconut is valuable food product in terms of nutritional and medicinal properties.

The shells of almond, peanut and coconut can be used for fuel as a source of carbon (Periasamy *et al.*, 1994). In some Asian countries like India, Srilanka and Thailand coconut shells are used as utensils besides in the manufacture of various handi-

crafts, Coir (the fiber cover the coconut shell) is used in the preparation of doormats, brushes, pocket, caulking boats and as stuffing fiber for mattresses. It is used in gardening as potting compost, especially in orchid mix (Horsfall *et al.,* 2005). Coconut shells are mainly used around enrich potting soil or covering around small plants in a garden setting (Prieto *et al.,* 2010).

MATERIALS AND METHODS

Collection of plant material: *Cocos nucifera* shells were collected from the Tower Market Hyderabad Sindh, Pakistan. Samples were identified through the literature of the flora of Pakistan (Nasir and Ali, 1990) and washed with tap water, followed by distilled water and dried in the shade at room temperature for 25 days.

Extraction: The dried samples were cut into small pieces and immersed in 5 L of ethanol (EtOH) for about one month at room temperature. The ethanol extract was filtered and evaporated under reduced pressure below 40°C using a rotary evaporator, which produced thick dark green residue. The extract was partitioned with ethyl acetate (EtOAc) and water, and this procedure was repeated 3 times.

Column chromatography (CC): The residue which contains the fatty acid fraction separated by chromatography on silica gel (Merck 70-230 mesh) column. The column was eluted first with n-hexane and then chloroform in order to increase polarity. First fraction “A” was eluted with hexane: chloroform (75:15), fraction "B" from hexane:chloroform (70:20), the fraction of "C" from hexane:chloroform (85: 25) and the fraction "D" of hexane: chloroform (80:30).

Esterification: All fractions (A to D) were etherified with diazomethane. The each fraction amount 0.5 mg was dissolved in MeOH and 0.5 ml of diazomethane. The reaction mixture was kept overnight at room temperature (26°C) and then evaporated. The fractions of methylated fatty acids were first analyzed by GC-MS technique.

Identification

Gas chromatography- mass spectrometry (GC-MS): The fatty acid analysis was performed on JEOL JMS 600H Agilest 68 g EN, equipped with 30 mx 0.32 MMHP-5 column 0.50µm stationary phase coating. The column temperature was maintained at 250°C for 2 min. With increase at 5°C per minute to the inlet temperature of 250°C, split ratio 1:35, the carrier gas (helium) flow rate was 1.8ml / min.

Conventional digestion method (CDM)

Elementary test: The samples were investigated for the elemental analysis using an atomic absorption spectrophotometer (Hitachi 180-50.S.N5721 Ltd). Appropriate working standard solutions were prepared for each element. Calibration curves were obtained for the concentration vs absorbance. Data were statistically analyzed by the appropriate use of the straight line by the least squares method. All elements were determined

in the medicinal plants under the inquiry procedure reported by (Kazi and Kazi, 1993) and a blank reading was also taken.

RESULTS AND DISCUSSION

The GC-MS of fatty acid methyl Lated (FAS) revealed the presence of 13 saturated and 15 unsaturated fatty acids UFA and two different compounds 9SS, SS4 19 cycle-4, 4, 14, x 5ά trimethyl-cholestan-3SS-ol Ethyl 24R-cholest-5-en, 3β-ol were isolated (Table 1). The saturated fatty acids were present in a higher amount (67.5% 4) than unsaturated fatty acids UFA (30.15%) as shown in Table 2. The other compounds were isolated in very small amounts (2.3%). Most SFA is 14.5% of n-heptadecanoate, hexa-decanoic acid 13.00% and n-nonade-canoate, 6.1% of n-octadecanoic acid, 5.00% N-Methyl-pentacosanoic 4.45%, 4.2% n-tetradecanoate, n-heptacosanoate 4.1% while n-nacosanate 3.09%, Methylpenta-decanonic2.6%, 1.92% nonanoic, Nonacosatrienoic acid 1.77% and 1.56% n-nonanoate.

The 1-octanoate Sfac, dodecanotic acid, hexadecanoic acid, hexanoic acid, eicosanoic acid, tetradeconic acid, stearic acid, tetracosanoic acid, carboxylic acid, hexadecanoic acid, stearic acid, eicosanoic acid and docosanoic acid, belongs to pamitoleic unsaturated fatty acid. The linoleic acid, erucic acid, lignoceric acid, palmitoleic acid, oleic acid, gadoleic acid, hiragonate stearidonic acid were also present in Coconut shell samples. The lowest and the highest percentage of fatty acid present in *Cocos nucifera* ranges tetracosgnoic acid (0.2%) of hexadecoric (5.43%) acid, and the lowest and the highest in UFA were present in the range of gamma linolenic acid (0.27%), 9-enoic acid octadec (11.89%). The total acidity expressed as the acid value taken into account the contribution of all the constituent fatty acids in the oil (Ekpa and Ekpe, 1995). This is a quality control parameter used by paint manufacturers to monitor the concentration of acids in resins. Both oils contain appreciable amounts of free fatty acids and this explains their applications as free fatty acid values were 6.1mg KOH/7.8mg KOH g/g for BRZ and AFR varieties respectively. Oils such as *Telfaria occidentalis* (1.10 ± 0.2), have been reported *Chryosophyllum albidum* (1.81 ± 0.1), groundnut seed (0.44 ± 0.14), palm kernel (0.57 ± 0.15) to have lower values of fatty acids and oils are edible (Cindric *et al.,* 2007).

The carbohydrate content was calculated by difference: 100 - (% crude protein + % moisture + % ash + % total lipid). The energy content of time (1 cal = 1000 cal = 1 kcal) of the sample was calculated as the sum of the percent crude protein and carbohydrate total factor of 4 times (Cal. g-1) added to total times lipid content Factor 9 (Cal.g-1), that is, the energy value, Cal = [(4 x protein content) + (4 x carbohydrate content) + (9 x total fat content) as reported by De Sousa *et al.,* (2010).

The FA identified in the sample was determined in mg.g-1 sample

(Welna *et al.,* 2008). The sum of the SFA in Brazil nuts were highest (13, G.100 34 g-1 of sample, 23% of the total lipid), a value which was statistically different from those of the other samples. Macadamia nuts were 9.08 G.100g-1 samples SFA with significant differences from other seeds. SFA represents 14% of total lipids in samples of pistachios, cashews and macadamia nuts, and 7% in hazelnuts, walnuts, almonds and peanuts from Europeans. The sum of PUFA was lower in hazelnuts (G.100-1 4.72 g sample) and macadamia nuts (1.15 g G.100-1 sample), which were significantly different from each other and the values of the other samples.

Table 2 also shows the PUFA/SFA ratio, which gives information about the quality of this FA in foodstuffs. Value less than 0.45 is considered unhealthy, particularly in relation to cardiovascular diseases, as recommended by the Department of Health and Social Security, England (Baydar *et al.,* 1999).

Table- 1: *Saturated Fatty acid of Cocos nucifera L. shell analyzed* methyl ester

S. #	SYSTEMATIC NAME	COMMON NAME	MOLECULAR FORMULA	MOL.Wt	Rel.% ag
1.	n-octanoate acid	caprylate	$C_9H_{18}O_2$	158	3.22
2.	dodecanotic acid	lauric acid	$C_{12}H_{24}O_2$	200	0.89
3.	n-hexadecanoic acid	palmilate acid	$C_{17}H_{34}O_2$	270	4.43
4.	n-hexadecanoate	margorate	$C_6H_{12}O_2$	116	3.11
5.	n-eicosanoic acid,	arachidic acid	$C_{32}H_{64}O_2$	480	2.21
6.	tetradeconic acid	myristic acid	$C_{18}H_{36}O_2$	284	5.33
7.	n-octadecanoic acid	stearic Acid	$C_{18}H_{36}O_2$	284	3.84
8.	tetracosanoic acid	lignoceric Acid	$C_{24}H_{48}O_2$	368	0.30
9.	n-docosanoate	behenic Acid	$C_{21}H_{42}O_2$	326	4.29
10.	hexadecanoic acid	palmitic acid	$C_{19}H_{38}O_2$	298	3.89
11.	n-hexocosanoate acid	cerotate	$C_{23}H_{46}O_2$	354	0.48
12.	10-octadecenoate	oleate acid	$C_{20}H_{40}O_2$	312	0.05
13.	nonanoate	laurate acid	$C_{21}H_{42}O_2$	326	0.04
				Total	32.08

***Table-2**: Un-Saturated Fatty acid of Cocos nucifera L.shell analyzed as* methyl ester

S. #	SYSTEMATIC NAME	COMMON NAME	MOLECULAR FORMULA	MOL.Wt	Rel.%ag
1.	n-heptaecenoate	n-heptaecenoate	$C_{17}H_{32}O_2$	268	4.55
2.	tridecatrienoate	tridecatriecnoate	$C_{18}H_{32}O_2$	280	7.30
3.	Methyl-2-Tridecynote	decylacrylate	$C_{22}H_{42}O_2$	338	5.34
4.	Methyl tricosenoate	decylacrylate	$C_{14}H_{26}O_2$	226	5.2
5	2,4,5-tetradecatri-ecnoate	tetradecatrienoate	$C_{24}H_{42}O_2$	362	4.48
6.	7-Ethyl-3-Methyl-2, 6-undecadienoate	undecadienoate	$C_{16}H_{26}O_2$	250	4.37
7.	pentadecatrienoate	Pentadecatrienoate	$C_{18}H_{34}O_2$	282	3.89
8.	hexadecadienoate	hexadecadienoate	$C_{17}H_{26}O_2$	262	5.50
9.	n-hexadecanoate	palmitoleate	$C_{17}H_{28}O_2$	264	5.27
10.	heptadectrienoate	heptadectrienoate	$C_{18}H_{28}O_2$	276	3.22
11.	9,12,15, octadecatri-enoate	octadecatrienoate	$C_{19}H_{34}O_2$	294	3.13
12.	10-octadecenoate	Oleate	$C_{19}H_{36}O_2$	296	3.02
13.	n-octadecanoate	Stearate	$C_{19}H_{32}O_2$	292	4.6
14.	eicosatrienoate	Eicosatrienoate	$C_{20}H_{34}O_2$	306	4.83
15.	Methyl-17, 18-hexacosenate	Hexacosenote.	$C_{27}H_{52}O_2$	408	3.21
				Total	67.91
	TOTAL 13 Saturated, 15 Unsaturated, total compounds 28 Total % of Saturated + unsaturated fatty acid = 99.99%				
	(Mol.wt= molecular weight, Rel %= relative percentgage				

Elemental analysis:

Calcium 556.3mg/kg, copper 9.93 mg/ kg, cadmium 2.002 mg/kg, chromium 3.23mg/kg, iron 997.9mg/kg, potassium 1511.77mg/kg, magnesium 2593.06mg/ kg, manganese 8.67 mg/kg, nickel, 4.48 mg/kg., zinc 231.66 mg/kg and cobalt 1.46 mg/kg as shown in Table-3. Elemental supplementation was associated with high circulation of plasma but chromium and zinc improve antioxidant status in skin tissues. These changes were associated with an improved clinical outcome, including a reduced number of lung infections and wound healing.

Compared with others, *Cocos nucifera* has the lowest level of cadmium, but seems to be higher compared to a previous report of the same species (Peter, 1956). Previous studies reported high concentration of Cu in *A. vulgaris* 9.65 ppm, followed by *W. somnifera* 8.67 ppm, *M. pruriens* 8.43 ppm, *S. rebaudiana* 7.71 ppm, *G. aparine* 6.14ppm, *A . adscendens* 5.08 ppm and *C. tetragonoloba* 2.39 ppm. The concentration of Cu in selected herbs is high, but is beyond the plant level and discussed the possibility of relevance (Ozdemir, *et al.*, 2001; Ademoroti *et al.*, 1996). Zinc deficiency has been shown negative effect for healing on the outcome of infected conditions (Michaelsen, et al., 1994). The value of manganese in coconut shell was 8.67 mg/kg and this value is high in comparison to 1.56 mg/100g obtained from cocoa beans (Olaefe, 1987). The amount of magnesium in *Cocos nucifera* was found 2593.06 mg/kg and it was found more then cocoa beans 330 mg/100g (Olaefe, 1987). In present study, the result indicates that the concentration of K, zinc and magnesium is very high in nut shell, followed by the remaining elements in trace amounts.

Table-3: Elementology of *Cocos nucifera* shell Woodward 1897

S/No.	NAME OF ELEMENTS	FORMULA	AMOUNT mg/ Kg
1	Calcium	Ca	556.3± 18.9
2	Cadmium	Cd	2.002 ±0.3
3	Chromium	Cr	3.23±0.39
4	Copper	Cu	9.93±0.89
5	Iron	Fe	997.9±52.25
6	Lead	Pb	1.46±0.21
7	Potassium	K	511.77±89.18
8	Magnesium	Mg	593.06±42.17
9	Manganese	Mn	8.67±6.37
10	Nickel	Ni	4.48±1.40
11	Zinc	Zn	231.66±22.16

CONCLUSION

It is advisable that nut shells contain lot of fatty acids and different elements, which can be used in daily life for toxicity. The presence of low concentration of nickel and lead in *C. nucifera* do not lead to any undesirable effects and low value

indicates that plants are grown in pollution free fields because yield depends on uptake. The results of the analysis showed that all investigated agricultural residues may serve as precursors to activate carbon and elements. The potential of the fatty acid composition showed that the inherent each agricultural sample greatly effect on yields.

REFERENCES:

Ademoroti,C., Environmental Chemistry and Toxicology (1st ed), Folude Press Ltd, Ibadan, Nigeria (1996).

Baydar,H., R. Marquard and I. Turgut, Pure line selection for improved yield, oil content and different fatty acid composition of sesame, *Sesamum indicum*. J. Plant Breeding 11: 462-464 (1999).

Cindric,J., Z. Michaela, and I. Steffan, Trace elemental characterization of edible oils by ICP–AES and GFAAS. Microchem. J. 85, 136–139 (2007).

De Sousa, D. A., E. De Oliveira, and M.C.Nogueira, and B. P. Esposito, Development of a heavy metal sorption system through the functionalization of coconut (*Cocos nucifera*) fibers J. Bioresource Technol. 101: 138-143 (2010).

Ekpa,O.D. and U. J. Ekpe, The effects of coconut oil concentration and air exposure to the total acidity of palm oil. Global J. Pure and Appl. Sci. 12: 51-58 (1995).

Fraser, G. E., Sabate, J., Beeson, W. L. and Strahan, M. A. A possible protective effect of nut consumption on risk of coronary heart disease, Arch Intern Med. 152: 1416-1424 (1992).

Gonzales, P., Analysis and pulping of coir dust R.Emata (Ed.), J. Coconut Research and Development 12: 163-173 (1970).

Gulcin, I., Antioxidant properties of resveratrol: A structure-activity insight. Innov. Food Sci. Emerg. 11: 210-218 (2010).

Horsfall, M. and A. I. Spiff, Effects of temperature on the adsorption of Pb^{2+} and Cd^{2+} from aqueous solution by Caladium bicolor (Wild Cocoyam) biomass. Electronic J. Biotechnol. 8: 162-169 (2005).

Hyson, D. A., B. O. Schneeman and P. A. Davis, Almonds and almond oil have similar effects on plasma lipids and ldl oxidation in healthy men and women. J. Nutr. 132: 703-707 (2002).

Kazi,T. G. and S. A. Kazi, Absorption and Distribution of Micronutrients and trace elements in Pea and Carrots in Sludge amended soil. J. Env.Anal.Chem. 2: 41-46 (1993).

Michaelsen, K. F., G. Samuelson and T. W. Graham, Zinc intake, zinc status and growth in a longitudinal study of healthy Danish infants. J. Acta Paediat. 83: 115-121 (1994).

Nasir, E. and S. Ali, Flora of Pakistan, University of Karachi. (1970-1990).

Olaefe, J. O., Nigerian tress. Annual of Bot. 14: 159-161 (1987).

Ozdemir, M., F. Ackurt, M. Kaplan, M.Yildiz, M.Loker, T.Gurcan, G. Biringen, A. Okay and G. Seyhan,

Evaluation of new Turkish hybrid hazelnut (*Corylus avellana* L.) varieties: fatty acid composition, α-tocopherol content, mineral composition and stability. Food. Chem. 73: 411-415 (2001).

Periasamy and C.Namasivam, Process development for the removal and recovery of calcium from waste water by a Low cost adsorbent. Industrial & Eng. Chem. Res. 33: 317-320 (1994).

Peter,A.O., West African J. Biological and Appl. Chem. 14: 120-130 (1956).

Prieto, W. H., I. A. Santana and P. A. Freitas, Kinetic study of adsorption of indigo blue dye at bark of green coconut fiber (*Cocos nucifera* L.). J. Nat Prod. 20: 72-84 (2010).

Sara,A., J.Perez-Jimenez and F. Saura-Calixto, Antioxidant capacity of walnut (*Juglans regia* L.). J. European Food Research and Technology 227: 425-31 (2007).

Siriwardhana, S. S. K. W. and F. Shahidi, Antiradical activity of extracts of almond and its by-products, J. Am. Oil Chem. Soc., 79:903–908 (2002)

Welna, M., M. Klimpel and W. Zyrnicki, Investigation of major and trace elements and their distributions between lipid and non-lipid fractions in Brazil nuts by inductively coupled plasma atomic optical spectrometry, Food Chem. 111: 1012-1015 (2008).