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Compositional and structural analysis of epicarp, flesh and pitted sample of Doum fruit (*Hyphaene thebaica* L.)

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Abstract

Epicarp, flesh and pitted samples of doum fruit *(Hyphaene thebaica)* were analyzed for proximate, fatty acid and amino acid (essential and non-essential) composition. Microstructure of the samples surface was observed using scanning electron microscopy (SEM). Total dietary fiber (TDF) and insoluble dietary fiber (IDF) content was found higher in the epicarp (61.48 and 57.31 g/100g respectively) compared to the other samples. On the other hand, flesh, pitted fruit and epicarp samples contained (6.92 g/100g), (6.01 g/100g) and (4.17 g/100g) SDF, respectively. The saturated fatty acids of epicarp, pitted sample and flesh were dominated by palmitic acid (C16:0) 75.10%, 64.47% and 34.14% respectively. The results showed flesh sample had higher percentages ($p<0.05$) of unsaturated fatty acids; specifically, in oleic acid (25.66%) and linoleic acid (21.39%) compared to the other samples. The highest amount of essential amino acids in each samples were phenylalanine and leucine. While glutamic and aspartic acids were found higher among nonessential amino acids in each samples. SEM images showed that the hollows and voids were more obvious on the surfaces of each samples.

Introduction

Doum palm (*Hyphaene thebaica* L.) is a desert palm belonging to the family of Arecaceae. It is widespread in the Sahel and tends to grow in areas where groundwater is present and is found along the Nile River in Egypt and Sudan. It is registered as one of the beneficial plants of the world (Fletcher, 1997). The trunk of the palm is used for construction, as well as for manufacture of various domestic utensils and the leaves used to make mats, bind parcels and writing paper. The oblong, yellow-orange apple sized fruit has a red outer skin, a thick, spongy and rather sweet, fibrous fruit pulp that tastes like gingerbread and a large kernel. The covering of the fruit is edible and can either be pounded to form a powder or cut off in slices; the powder is often dried then added to food as a flavoring agent (Orwa, 2009). Several studies have emphasized that the doum fruit contain high levels of essential minerals such as potassium, sodium, calcium, magnesium, and phosphorus. As well as, doum fruit contains B-complex vitamins, carbohydrates, and fiber, which is essential for good nutrition (Admassu *et al*., 2013; Aboshora *et al*., 2014; Seleem, 2015). A number of studies have shown that the doum fruit extracts contain high levels of phenols and flavonoids, and possess significant antioxidant and antimicrobial activities (Hsu *et al*., 2006; Mohamed *et al*., 2010; Aboshora *et al*., 2015). Recently, dietary fiber has attracted great interest by researchers as many studies have proven that numerous health benefits are associated with an increased intake of dietary fiber including a reduced risk of coronary heart disease, diabetes, obesity, some forms of cancer and also health promotion of consumers through a reduction in cholesterol and fat (Anderson *et al*., 2009; Brownlee, 2011). Keeping in view the above rationale, various studies have applied doum fruit in food products (Hussein *et al*., 2010; Seleem, 2015; Aboshora *et al*., 2016).

The composition of fatty acids and amino acids of any food material are important factor to evaluate the quality of raw materials apart from their carbohydrate and mineral constituents. The factors responsible for the quality and nutritional value of proteins are amino acid composition, ratio of essential amino acids to non-essential amino acids, susceptibility to hydrolysis during digestion, source, and the effect

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of processing (Schuster-Gajzago *et al*., 2006; Radha and Prakash, 2007).

The advanced technique of scanning electron microscopy (SEM) was used for the investigation of surface structures of mollicutes. The electrons interact with atoms in the sample, producing various signals that can be detected and contain information about the sample's surface structures of topography and composition. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. This method provides a great depth of field, which means, the area of the sample that can be viewed in focus at the same time is actually quite large (Stanley *et al*., 1993). To the best of our knowledge, this is the first study for the determination of the soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) in doum fruit parts. Moreover, no previous reports are available for structural analysis of doum fruit parts through SEM. The objective of this study is to assess and compare the proximate composition, dietary fiber, amino acids and fatty acids of epicarp, flesh and pitted sample of doum fruit (*Hyphaene thebaica* L.). Furthermore, this study is aimed at assessing and comparing the structure of different parts of the doum fruit through SEM.

Materials and Methods

Materials

Doum fruit (*Hyphaene thebaica* L.) was purchased from local market in Nyala, Sudan. The preparation of the raw material (Figure 1) was carried out according to our previous study (Aboshora *et al*., 2014). Analytical reagents and HPLC-grade solvents were obtained from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China.

Proximate composition

The proximate composition of the powder (epicarp, flesh and pitted sample) was carried out according to AOAC (1995). Soluble dietary fiber (SDF), insoluble dietary fiber (IDF) and total dietary fiber (TDF) contents in epicarp, flesh and pitted sample powder were determined according to AOAC methods (AOAC, 1996; AOAC, 2005).

Determination of fatty acid composition

Fat was extracted from the respective epicarp, flesh and pitted sample of doum fruit by the Soxhlet method. The fatty acid profile was determined in triplicate by GC–MS after conversion of samples into fatty acid methyl esters (FAME) according to

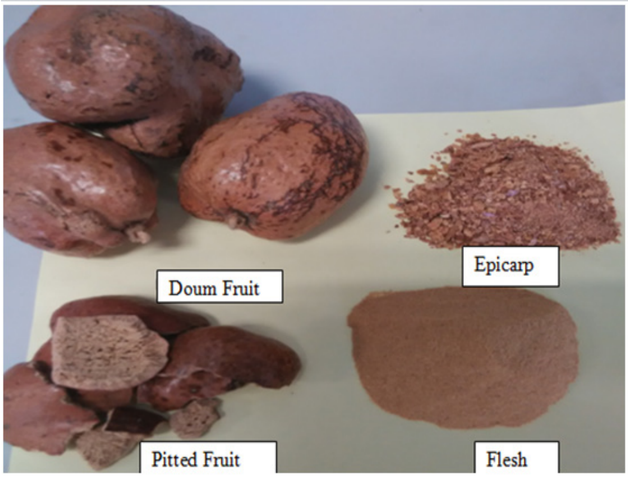

Figure 1. Doum fruit (*Hyphaene thebaica* L.), epicarp, pitted fruit and flesh samples

Shi *et al.* (2009). In brief, 1 μL of FAME sample was injected into the gas chromatograph (Series PEG30M) equipped with a flame ionization detector. GC separation was conducted on a capillary column PEG30 M (30 m × 0.32 mm × 0.50 μm). Nitrogen was used as carrier gas (with a constant flow rate of 1 ml/min). Initially, the oven temperature was calibrated at 190°C for 1 min, then increased to 230°C at a rate of 3°C min^{-1} and finally maintained at 230°C for 10 min. The temperatures of the injection port and detector were 240 and 250°C, respectively. The peaks were identified on the chromatogram according to the retention data of analyzed standard samples and the results were reported as relative percentage (%) calculated on the chromatographic area of each peak.

Amino acids analysis

Dried samples were digested with 6 M HCl at 110°C for 24 h under nitrogen atmosphere. Reversed phase high performance liquid chromatography (RP-HPLC) analysis was carried out in an Agilent 1100 (Agilent Technologies, Palo Alto, CA, USA) assembly system after pre-column derivatization with ortho-phtaldialdehyde (OPA). Each sample (1 μL) was injected into a Zorbax 80 A C18 column (4.6 ×180 mm, Agilent Technologies) at 40°C with detection at 338 nm. Mobile phase A was 7.35 mM/L sodium acetate/ triethylamine/ tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted at pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mM/L sodium acetate/ methanol/ acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as mg of amino acid per 100 g of protein.

Scanning electron microscopy (SEM)

Dried samples of epicarp, flesh and pitted sample powder were mounted on a scanning electron microscope (SEM) (S-4800, Hitachi Metals, Ltd., Japan). The samples were coated before loading to the scanning electron microscopy. The coated samples were loaded into the system and the image was viewed under 2.0 KV potential using secondary electron image. The image was captured using 7.7 mm Ricoh Camera of 500x Mag.

Statistical analysis

All results in this study were expressed as mean ± standard deviation of three replicates. Data in triplicate were analyzed by one-way analysis of variance using the SPSS statistical software, version 16.0 (SPSS, Chicago, Illinois, USA).

Results and Discussion

Proximate composition

Proximate composition of the epicarp, flesh and pitted sample of doum fruit was presented in table 1. The moisture content of the epicarp and pitted sample (5.96 and 5.79 g/100g respectively) was higher ($p< 0.05$) than the flesh (5.54 g/100g); these results were generally within the range expected from most of the doum fruits samples (Admassu *et al*., 2013; Aboshora *et al*., 2014; Seleem, 2015). The data showed that the flesh and pitted sample powder had higher levels of protein (4.12% and 3.88% respectively) than epicarp (2.18%). These results were similar to those reported in literature (values ranged between 2.17% and 7.05%) (Hussein *et al*., 2010; Aboshora *et al*., 2014; Seleem, 2015). The ash content of the doum fruit samples ranged between 4.00 and 6.55 g/100g; the highest value was found in flesh followed by pitted sample and epicarp (Table 1). Moreover, the results showed that the flesh and pitted sample have higher fat content (0.97 g/100 g and 0.91g/100g respectively) when compared to the epicarp (0.41g/100g). These results of fat content on different parts of doum fruit were comparable with the findings of Admassu *et al*. (2013) and Aboshora *et al*. (2014).

Table 1 also shows total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) content of the epicarp, flesh and pitted sample as well as the ratio between IDF and SDF. Significant differences ($p<0.05$) in the fruit parts were observed for TDF, IDF and SDF. The total dietary fiber (TDF) of the doum fruit samples ranged between 61.19 and 41.992 g/100g; the highest value was found in epicarp followed by pitted sample and flesh (Table 1).The highest amount ($p< 0.05$) of IDF was noticed for the epicarp (57.31 ± 0.888 g/100g) whereas flesh sample possessed the highest amount of SDF (6.92 ± 0.121). There was no literature concerning any study conducted about the dietary fiber of doum fruit. Fruits and vegetables rich in dietary fiber can be used as functional ingredients in order to provide numerous health benefits including reduced risk of coronary heart disease, diabetes, obesity, reduce hyperlipidemia and hypertension. Furthermore, these contribute to gastrointestinal health, reduction in cholesterol and fat and also some forms of cancer (Anderson *et al*., 2009; Viuda-Martos *et al*., 2010; Brownlee, 2011).

Fatty acid composition

Six different fatty acids were detected in epicarp, flesh and pitted sample of doum fruit and their percentages were presented in (Table 2). Doum fruit samples were found to be a rich source of palmitic acid. Palmitic acid (C16:0) ranged between 75.10% and 34.14%; the highest value was found in epicarp followed by pitted sample and flesh. Arachidic acid (C20:0) was not detected (ND) in the epicarp while it was found at low level in flesh (3.76%) and pitted sample (2.69%). Conversely, the highest Steric acid (C18:0) (8.57%) was detected in epicarp sample as compared with other samples. Data analysis also showed that the flesh had high content of unsaturated fatty acid especially oleic acid (C18:1 trans-9) 25.66% and linoleic acid (C18:2 cis-9–12) 21.39%. Among the unsaturated fatty acid linolenic (C18:3 cis-9, 12, 15) was detected only in flesh sample (3.28%). According to the statistical analysis, there was a significant difference of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids among the doum fruit samples (Table 2). The highest level of MUFA was obtained for flesh sample followed by pitted sample and epicarp. The ratio of PUFA/SFA is generally used to evaluate the nutritional value of the fat. Chang and Huang (1998) Studied the effects of monounsaturated fatty acids (MUFA) and ratio of the sum of polyunsaturated and monounsaturated fatty acids to saturated fatty acids (PUFA+MUFA)/SFA on plasma and liver lipid concentrations in rats. They concluded that there were many prerequisites for keeping low plasma and liver lipid concentration such as high PUFA/MUFA ratio, low MUFA/SFA ratio and the ratio of (PUFA+MUFA)/SFA must be ≤ 2. In our study, the value of MUFA/SFA was 0.12 in epicarp, 0.62 in flesh and 0.2 in pitted sample; it was interesting to note that these results of MUFA/SFA for all samples were very low. In contrast the value of PUFA/MUFA was high in flesh (0.96) comparing to epicarp and pitted sample. (PUFA + MUFA)/SFA ratio was 1.21 for flesh, 0.35 for pitted sample and 0.20 for epicarp. Interestingly, the value of (PUFA +

Table 1. Proximate composition of epicarp, flesh and pitted sample of doum fruit

Parameters	Epicarp	Flesh	Pitted sample
Moisture	5.96 ± 0.051[a]	5.54 ± 0.080[b]	5.79 ± 0.025[a]
Protein	2.18 ± 0.025[b]	4.12 ± 0.081[a]	3.88 ± 0.041[a]
Ash	4.00 ± 0.046[c]	6.55 ± 0.117[a]	4.60 ± 0.233[b]
Fat	0.41 ± 0.035[b]	0.97 ± 0.020[a]	0.91 ± 0.037[a]
TDF	61.48 ± 0.911[a]	41.93 ± 0.625[c]	48.83 ± 0.165[b]
IDF	57.31 ± 0.888[a]	35.01 ± 0.184[c]	42.82 ± 0.301[b]
SDF	4.17 ± 0.195[c]	6.92 ± 0.135[a]	6.01 ± 0.135[b]
Ratio IDF/SDF	13.74 ± 0.434[a]	5.05 ± 0.067[c]	7.12 ± 0.156[b]

Results were expressed as means ± standard deviation (n=3). Values in the same row with different letters are significantly ($p<0.05$) different. TDF: Total dietary fiber, IDF: insoluble dietary fiber, SDF: soluble dietary fiber.

Table 2. Fatty acid composition (%) of epicarp, flesh and pitted sample of doum fruit

Peak Name	Fatty acid Composition (%)		
	Epicarp	Flesh	Pitted sample
Saturated Fatty acid (SFA)			
C16:0 (Palmitic acid)	75.10 ± 0.314[a]	34.14 ± 0.183[c]	64.47 ± 0.221[b]
C18:0 (Steric acid)	8.57 ± 0.096[a]	3.53 ± 0.058[c]	6.77 ± 0.061[b]
C20:0 (Arachidic acid)	ND	3.76 ± 0.052[a]	2.69 ± 0.031[b]
Total SFA	83.67 ± 0.361[a]	41.43 ± 0.253[c]	73.93 ± 0.293[b]
Monounsaturated Fatty acid (MUFA)			
C18:1 trans-9 (Oleic acid)	10.00 ± 0.083[c]	25.66 ± 0.174[a]	15.00 ± 0.124[b]
Total MUFA	10.00 ± 0.157[c]	25.66 ± 0.123[a]	15.00 ± 0.172[b]
Polyunsaturated Fatty acid (PUFA)			
C18:2 cis-9–12 (Linoleic acid)	6.33 ± 0.112[c]	21.39 ± 0.207[a]	11.07 ± 0.143[b]
C18:3 cis-9,12,15 (Linolenic acid)	ND	3.28 ± 0.028[a]	ND
Total PUFA	6.33 ± 0.093[c]	24.67± 0.211[a]	11.07± 0.081[b]
PUFA/SFA ratio	0.08	0.60	0.15
MUFA/SFA ratio	0.12	0.62	0.20
PUFA/MUFA ratio	0.63	0.96	0.74
(PUFA + MUFA)/SFA ratio	0.20	1.21	0.35

All the data are expressed as mean ± SD (n=3). Means with the different superscript letters within the same line are significantly different ($p< 0.05$). ND= Not detected

MUFA)/SFA ratio of all samples remained below 2.

Amino acid composition

Amino acids are essential organic compounds for life and an important source of nitrogen (Amend and Shock, 2001; Ogawa and Tanoue, 2003). Essential amino acid composition of the epicarp, flesh and pitted sample of doum fruit was presented as mg/100g in Table (3). Nine essential amino acids were detected in the different parts of the doum fruit. The abundant essential amino acids in the studied samples were phenylalanine and leucine, with values ranging from 110.28 – 175.19 mg/100g and 134.89 –160.71 mg/100 g respectively. These were further followed by valine (89.54–144.54 mg/100g), isoleucine (77.30–104.06 mg/100g), threonine (86.53–90.33 mg/100g) and lysine (80.54–90.26 mg/100g). In the present study very low quantities of methionine (6.88–10.65 mg/100g) was observed in the doum fruit samples. It was worthwhile to notice that among the samples, the flesh contained the highest amount of essential amino acids except for threonine and lysine which were found in highest amount for pitted fruit sample.

Non-essential amino acid profile of the epicarp, flesh and pitted sample of doum fruit was reported in Table 3, expressed as mg/100g. In the flesh, higher amount of glutamic, aspartic, arginine, alanine and glycine were found in significant quantities (320.56

Table 3. Essential and non-essential amino acids of epicarp, flesh and pitted sample of doum fruit (mg/100g)

Essential amino acid (EAA)	Epicarp	Flesh	Pitted Sample
Histidine	40.67 ± 0.046ᵇ	49.72 ± 0.057ᵃ	41.19 ± 0.083ᵇ
Threonine	86.53 ± 0.141ᵇ	89.43 ± 0.123ᵃ	90.33 ± 0.149ᵃ
Valine	90.23 ± 0.212ᵇ	144.54 ± 0.283ᵃ	89.54 ± 0.194ᵇ
Methionine	6.91 ± 0.086ᵇ	10.65 ± 0.023ᵃ	6.88 ± 0.049ᵇ
Phenylalanine	110.28 ± 0.342ᵇ	175.19 ± 0.423ᵃ	112.93 ± 0.377ᵃ
Isoleucine	77.30 ± 0.146ᶜ	104.06 ± 0.221ᵃ	94.50 ± 0.172ᵇ
Leucine	134.89 ± 0.387ᶜ	160.71 ± 0.303ᵃ	156.22 ± 0.412ᵇ
Lysine	82.44 ± 0.139ᵇ	80.54 ± 0.113ᵇ	90.26 ± 0.157ᵃ
Non-essential amino acid			
Aspartic	215.86 ± 0.411ᶜ	267.89 ± 0.383ᵃ	223.99 ± 0.349ᵇ
Glutamic	247.88 ± 0.429ᵇ	320.56 ± 0.495ᵃ	246.49 ± 0.418ᵇ
Serine	119.65 ± 0.316ᵃ	95.63 ± 0.223ᶜ	111.25 ± 0.269ᵇ
Glycine	196.50 ± 0.297ᵃ	137.49 ± 0.198ᶜ	155.47 ± 0.273ᵇ
Arginine	104.37 ± 0.189ᵇ	160.62 ± 0.213ᵃ	89.54 ± 0.163ᶜ
Alanine	154.16 ± 0.225ᵃ	154.30 ± 0.329ᵃ	126.95 ± 0.231ᵇ
Tyrosine	40.33 ± 0.066ᶜ	52.65 ± 0.058ᵃ	46.32 ± 0.051ᵇ
Cysteine	11.88 ± 0.028ᵇ	18.47 ± 0.037ᵃ	3.03 ± 0.026ᶜ
Proline	127.9 ± 0.136ᵃ	95.86 ± 0.153ᵇ	127.57 ± 0.203ᵃ

All the data are expressed as mean ± SD (n=3). Means with the different superscript letters within the same line are significantly different ($p < 0.05$).

mg/100g), (267.89 mg/100g), (160.62 mg/100g), (154.30 mg/100g), (137.49 mg/100g) respectively. The lowest quantity was shown by cysteine, tyrosine, serine and proline (18.47 mg/100g), (52.65 mg/100g), (95.63 mg/100g) and (95.86 mg/100g) respectively in the flesh of doum fruit. Epicarp resulted in providing a good amount of glutamic, aspartic, glycine and alanine in the quantities of (247.88 mg/100g), (215.86mg/100g), (196.50 mg/100g) and (154.16 mg/100g) respectively, while, the lowest quantities of cysteine (11.88 mg/100g), tyrosine (40.33 mg/100g), arginine (104.37 mg/100g), serine (104.37 mg/100g) and proline (127.9 mg/100g) were also discovered. Lastly, the pitted fruit sample was discovered to have highest amount of glutamic, aspartic, glycine, proline and alanine at the level of (246.49 mg/100g), (223.99 mg/100g), (155.47 mg/100g), (127.57 mg/100g) and (126.95 mg/100g) respectively. At the same time, the lowest quantities of cysteine, tyrosine, arginine and serine in the significant amount of (3.03 mg/100g), (46.32 mg/100g), (89.54 mg/100g) and (111.25 mg/100g) respectively were founded in the pitted fruit sample.

Scanning electron microscopy (SEM)

SEM was used to investigate the epicarp, flesh and pitted sample cell morphology of doum fruit (Figure 2). The epicarp, flesh and pitted sample particles were loosely dispersed by the high-speed shearing of grinding process. The epicarp (SEM) was showing irregular or prismatic shapes and this could be associated to the high amount of fiber present in epicarp (Figure. 2A) (Xu *et al.*, 2015). On the other hand, no specific shape was observed in the flesh and pitted sample of doum fruit; these seem to be related to the amount of starch (data not shown) present in these samples (Figure. 2B, 2C). Furthermore, epicarp sample showed some dark

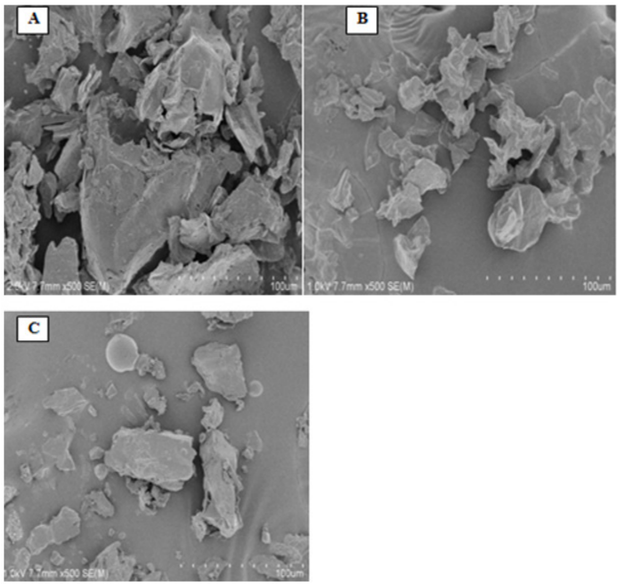

Figure 2. Scanning electron micrographs (SEM, ×3000). A: epicarp powder; B: flesh powder; C: pitted sample powder

"round" or "bar" depressions, might be caused by the gelatinized starch attaching to the fiber, implying that the presence of husk could decrease the surface connectivity between starch granules particularly in epicarp; these results were agreed with Song *et al.* (2013) and Xu *et al*. (2015).

Conclusion

The results of this study have revealed that doum fruits samples contain high level of dietary fiber, amino acids and fatty acids, which if properly utilized can improve human nutrition and health. Doum fruits samples have showed that TDF, IDF, SDF, amino acids and fatty acids differ significantly in the epicarp, flesh and pitted sample. The results showed that doum fruit is a good source of essential amino acids especially in the flesh such as phenylalanine, leucine, valine and isoleucine. Keeping in view the above mentioned results and data, it is needed to encourage the utilization of the doum fruits, especially in the areas of its availability, to ensure the health and nutrition of human kind. SEM images showed that the epicarp was more irregular and distorted surface which indicated a slight fragmentation and the formation of a deep groove. While flesh and pitted fruit samples showed no specific shape and appearance.

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