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COMPOSITION OF AMINO ACIDS, FATTY ACIDS AND DIETARY FIBRE MONOMERS IN KERNELS OF *ADANSONIA DIGITATA* AND *SCLEROCARYA BIRREA*

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ABSTRACT

There is increasing demand for sources of energy and non-meat protein with balanced amino acid profiles worldwide. Nuts are rich in protein and essential amino acids, and have a high energy value due to their high fat content. Kernels from two wild fruits in Mozambique, *Adansonia digitata* and *Sclerocarya birrea*, were selected for this study to determine their amino acid and fatty acid composition, as well as the monomeric composition of their dietary fibre. The fat content in *Adansonia digitata* kernels was around 32% and in *Sclerocarya birrea* kernels 49%. Both kernels were rich in unsaturated fatty acids. The dominating fatty acids in *Adansonia digitata* kernels were palmitic, linoleic and oleic acid; varying from 25.7% to 34.9% of the total fatty acids content. In *Sclerocarya birrea* kernels the main fatty acid was oleic acid, 72.4%. The *Adansonia digitata* kernels contained the two essential fatty acids linoleic (around 30%) and linolenic acid (2%). *Sclerocarya birrea* kernels contained linoleic acid (around 7%). The protein content in *Adansonia digitata* kernels was 35% and in *Sclerocarya birrea* kernels 29%. Glutamic acid was the most abundant amino acid, comprising more than 20% of the protein in both kernels. The contents of essential amino acids in the kernels were compared with the requirements stated by the WHO, and the findings indicate that *Adansonia digitata* and *Sclerocarya birrea* kernels can provide good, cheap sources of protein, especially when combined with foods with high lysine content. Regarding the dietary fibre fractions, the main constituent in the insoluble fraction of *Adansonia digitata* kernels was glucose, while in the soluble fraction arabinose was the dominating component. In kernels of *Sclerocarya birrea*, uronic acids constituted more than 90% of both dietary fibre fractions. The results of this study suggest that intake of these kernels can help providing a great part of the fatty acids and amino acids required in the daily diet, especially for people living in rural areas of Mozambique. The data could be used for intake estimates, and to encourage increased consumption and utilization of these kernels.

Key words: Wild fruit, consumption, seeds, essential, protein, fat, linoleic acid, linolenic acid, Mozambique

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INTRODUCTION

Wild fruits are major source of food for rural people in Southern Africa. The seeds and kernels especially add essential nutrients to the diet, and are available when other foods are scarce [1]. The main staple foods in Mozambique are cassava, maize, beans, sorghum and rice. Cereals and starchy roots provide almost 80% of the dietary energy supply, while the contribution from pulses (mainly beans), nuts and oil crops is 5%. However, the diet in rural areas is deficient in fat, protein and micronutrients, and does not supply enough nutrients to meet nutritional requirements [2].

Fat is an essential part of the diet as it provides the body with energy in a concentrated form. Also, dietary fats provide essential fatty acids and fat-soluble vitamins. Linoleic acid (an omega-6 fatty acid) and alpha-linolenic acid (an omega-3 fatty acid) are the essential fatty acids required in the diet [3]. These essential fatty acids have many functions in the body, and are of importance, for example, in the immune system, cell membranes and for the function of the brain and skin [4]. They may also reduce the risk of heart disease [5]. Among the food sources of essential fatty acids are fish, oils, kernels, and nuts. The consumption of foods rich in monounsaturated fatty acids has been associated with health promoting effects, for example decreased levels of low-density lipoprotein cholesterol and possibly increased high-density lipoprotein cholesterol [3]. Olive oil is regarded as having a good fat quality because of its high content of oleic acid [3]. The European Food Safety Authority (EFSA) and the World Health Organization (WHO) recommend an acceptable range of the intake of the essential fatty acid related to the energy intake [6, 7]. Other authorities, for example, the Food and Nutrition Board of the US Institute of Medicine, have established adequate intake (AI) levels in grams; around 10 g of omega-6 fatty acids and around 1 g of omega-3 fatty acids [5].

Protein is another important component in the diet. Inadequate supply of protein is considered to be responsible for malnutrition among people living in developing countries. Different beans, walnuts and peanuts are common sources of non-meat protein [8]. According to the Food and Nutrition Board of the US Institute of Medicine, the AI levels of protein range from 19 g for children 4 to 8 years up to 71 g for pregnant women [5]. The content of essential amino acids determines the protein quality [9]. The WHO has defined essential amino acid profiles required for different age groups; it should be noted that histidine is regarded as being essential for children, but not for adults [10].

Several studies have shown that dietary fibre components in fruits and seeds are beneficial to human health. Information on the chemical composition of dietary fibre fractions is important for technological, nutritional and physiological studies [11, 12].

Kernels from wild fruits are commonly consumed in the southern and central parts of Mozambique, where a variety of edible fruits can be found. The kernels offer a convenient and cheap means of fat, protein, minerals and other health-promoting components to people living in rural areas [13]. A previous study on the content of fat, protein, dietary fibre and minerals in some wild fruits and kernels from Mozambique

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showed that kernels of *Adansonia digitata* (*A. digitata*, also called baobab) and *Sclerocarya birrea* (*S. birrea*, also called marula) had high contents of fat, protein and dietary fibre [14, 15]. Kernels of *A. digitata* are highly appreciated in the diet, and are eaten roasted as a snack, or used for oil extraction. They are also used in soups or mixed with wild spinach or other food [16]. Kernels of *S. birrea* can be dried and eaten alone, or cooked and, for example, eaten together with a mixture of dried peanut extracts, red pepper, salt and other spices [17]. Literature and data from Mozambique on the nutritional components of the kernels of *A. digitata* and *S. birrea* are scarce. This study is part of a programme between universities in Mozambique and Sweden with the overall aim to increase the utilization and consumption of wild fruits. Information on the nutritional composition of the kernels is thus important for estimations of how these kernels can contribute to the nutrient intake. The aims of the present study were, therefore, to measure the contents of amino acids, fatty acids and dietary fibre components in the kernels.

MATERIALS AND METHODS

Samples

Kernels from two wild fruit species were studied: *Adansonia digitata* (Fam. *Bombacaceae*, local name n´buyu or malambe), and *Sclerocarya birrea* (Fam. *Anacardiaceae,* local name n´canhi). Seeds were collected in 2013 in districts where they are commonly consumed. Seeds (5 kg) from *A. digitata* were collected in family orchards in the Changara district, 95 km from the city of Tete. *S. birrea* seeds (1 kg), shade-dried for two to three months, were obtained from a small family orchard in the Manhiça district, 50 km from Maputo. The seeds were crushed and the shells removed and the kernels inside were vacuum-packed in plastic bags and stored at -18ºC in a freezer. Before analysis, *A. digitata* kernels were milled in a coffee grinder (TEFAL, Type 8100, Prep'Line, China) and sieved (500 µm mesh). *S. birrea* kernels were ground with a mortar and pestle.

Chemicals

All chemicals were of analytical grade. Water was passed through a Milli-Q purification system. Amino acid standards, DL-norleucine (internal standard) and phenol were purchased from Sigma Chemical Co. (St. Louis, USA), and lithium buffers and ninhydrin from Biochrom Ltd (Cambridge, UK). Sodium di-hydrogen phosphate, di-sodium hydrogen phosphate, sodium hydroxide, ethanol and acetone were purchased from Fluka (Sigma-Aldrich, Steinem, Germany). For the analysis of dietary fibre, pepsin was obtained from Merck (Darmstadt, Germany), pancreatin from Fluka (Sigma-Aldrich), and galacturonic acid, chloroform, monohydrate and myo-inositol (internal standard) from Sigma Chemical Co. The standards rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose were obtained from Merck, together with hydrochloric acid, sulphuric acid, acetic acid, acetic acid anhydride, ammonium hydroxide, potassium hydroxide, sodium sulphate, potassium borohydride and 1-methylimidazol.

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Laboratory Analysis

Fat and Fatty Acids

Fat and fatty acids in the kernels were analysed in an authorised laboratory. For determination of fatty acid gas chromatography was used [18]. The determinations were performed in duplicate. The results of the fatty acid analysis are expressed in g/100 g fat.

Protein and Amino Acids

About 5 g of each type of kernel was used for the analysis of protein and amino acids. The samples were defatted using acetone in the proportions 1:10 (w/v), stirred for one hour and ultra-centrifuged at 18 000 rpm for 20 minutes. The samples were left to evaporate overnight at room temperature. Total protein content in the defatted samples was determined using an elemental analyser (Flash EA 1112 Series, Thermo Fisher Scientific, Sweden) by combustion of 25 mg samples. Aspartic acid was used as the standard. Total amount of protein was calculated by multiplying the amount of nitrogen by a factor 5.7. Determinations were performed in triplicates.

Aliquots (35 mg) of the sample residue were put into glass tubes, 5 ml of 6 N HCl containing 0.1% phenol was added, and the samples were hydrolysed at 110ºC for 24 hours [19]. The samples were then centrifuged at 4000 rpm for 10 minutes after which 2 ml of each supernatant was transferred to round-bottomed flasks and 100 µl of the internal standard (DL-norleucine 22.9 µmol/ml) was added. The samples were evaporated at 40ºC to dryness [19]. Lithium citrate buffer (5 ml 0.2 M, pH 2.2) was then added, and the solutions were mixed for a few minutes, transferred to Eppendorf tubes with a 0.45 micron inner vial, and centrifuged at 13 000 rpm for 10 minutes. The samples were analysed using an amino acid analyser (Biochrom 30 series Amino Acid Analyser, Biochrom Ltd). The method [19] is based on ion-exchange chromatography with post-column derivatization using ninhydrin and adopted from the manufacturer: The injection volume was 20 µl. The column (Physiological Fluid High Performance, 200 x 4.6 mm, Biochrom Ltd) was eluted with lithium citrate buffers of pH 2.80, 3.00, 3.15, 3.50 and 3.55 at a flow rate of 25 ml/hour. Post-column derivatization was performed with ninhydrin at a flow rate 20 ml/hour. The temperature of the reaction coil was 135ºC. In the reaction coil, ninhydrin reacts with the amino acid present in the eluent and forms conjugated compounds with absorbance maxima at 570 and 440 nm. The internal standard was used to calibrate the amino acid response. Identification was confirmed by comparing the retention times of the peaks with those of a standard solution of amino acids run under the same conditions. The calibration curve was linear for the concentration range used in the analysis. The results of the amino acid analysis are reported as g amino acid/100 g protein.

Dietary fibre and dietary fibre monomers

Dietary fibre in the kernels was separated into soluble and insoluble fractions using a gravimetric method [20]. Then the contents of monosaccharides and uronic acids in the fractions were determined. The monosaccharide composition was analysed with gas chromatography (HP6890 Hewlett-Packard, with a flame ionization detector) and the content of uronic acids was determined with spectrophotometry (Pharmacia Biotech Novaspec II model 80-2088-64, Sweden) using D-galacturonic acid monohydrate as a

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reference [12, 21]. The determinations were performed in duplicates. The calibration curves were linear for both monosaccharides and uronic acids for the concentration ranges used in the analysis.

Dry Matter

The dry matter content was determined after drying 2 g samples in an oven at 105ºC until constant weight [18]. The determinations were performed in triplicates.

Statistical analysis

Microsoft® Excel was used for the statistical evaluation. Student's t-test was performed to determine significant differences. A value of $p<0.05$ was considered to indicate statistical significance.

RESULTS

The total fat content in the kernels was 31.7 ± 0.0% for *A. digitata* and 49.4 ± 3.7% for *S. birrea*. The results of the determination of the fatty acid compositions of the kernels are presented in Table 1. The unsaturated fatty acids constituted about 68 and 80% of the total fat content in *A. digitata* and *S. birrea* kernels, respectively. The most abundant fatty acids in *A. digitata* kernels were palmitic acid, linoleic acid and oleic acid; their contents varying from 25.7 to 34.9%. In *S. birrea* kernels, oleic acid constituted 72.4% of the fatty acids. *A. digitata* kernels contained linolenic acid (2.1%) which was not detected in *S. birrea* kernels. The difference in fatty acid composition between the kernels was significant for palmitic acid, oleic acid and linoleic acid ($p<0.05$).

The protein content was 35.0 ± 0.0% in *A. digitata* and 29.2 ± 0.0% in *S. birrea*.
A chromatogram from the analysis of amino acids *in A. digitata* is shown in Figure 1.

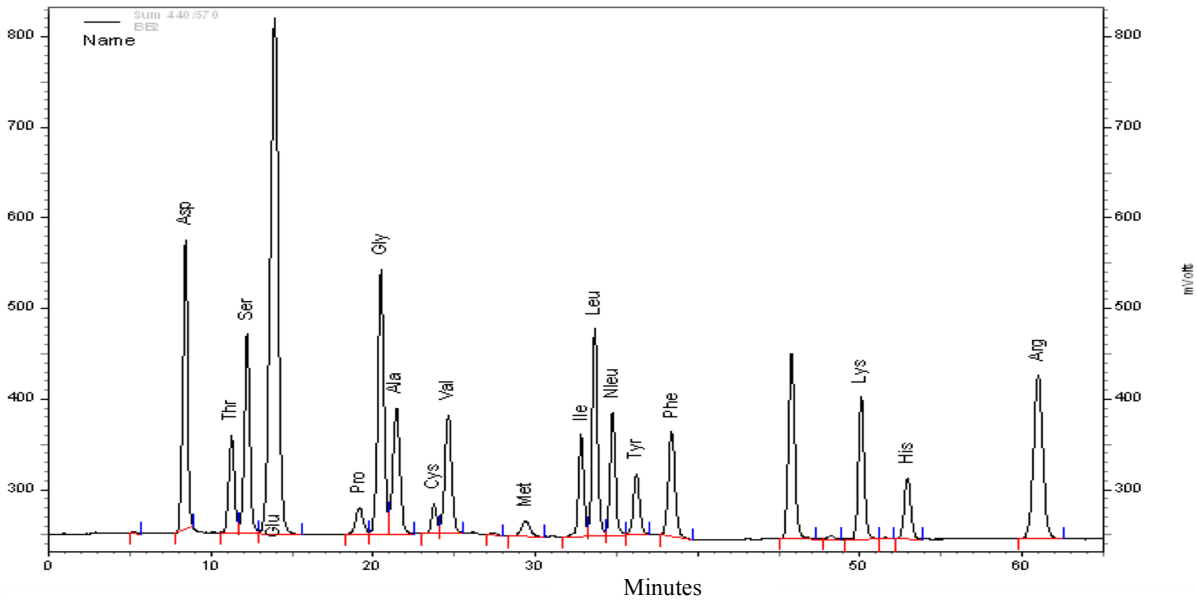

Figure 1: Chromatogram of amino acid analysis of *Adansonia digitata* kernels. The peaks corresponding to the different amino acids are indicated

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Table 2 gives the amino acid compositions of the *A. digitata* and *S. birrea* kernels. Glutamic acid was the most abundant amino acid in both types of kernels, comprising more than 20% of the total amino acid content, followed by arginine. The differences between the contents of the various amino acids in the kernels were not significant.

The contents of monosaccharides and uronic acids in the in the dietary fibre fractions from the kernels are presented in Table 3. The compositions differed between the kernels. The main constituent in the insoluble fraction of *A. digitata* kernels was glucose, followed by arabinose, while in the soluble fraction arabinose was the dominating component, followed by uronic acids. In kernels of *S. birrea*, uronic acids constituted more than 80% of both dietary fibre fractions.

The dry matter content was found to be 92.8 ± 0.0% for *A. digitata* kernels and 95.8 ± 0.1% for *S. birrea* kernels.

DISCUSSION

Data on fatty acids, amino acids and dietary fibre monomers have been obtained for two commonly consumed kernels of wild fruits in Mozambique. The dry matter content of the two kernels corresponds well with results from a previous study [14, 15].

The fatty acid composition differed between the two kernels but in both of them oleic acid was the most abundant fatty acid. For *A. digitata* kernels the results from the present study are in agreement with some reports [22, 23], but higher than in others [24, 25]. In addition, in one study, in which only four fatty acids were determined, the contents of those were in general at the same level as in the present study [26]. For *S. birrea* kernels, the results generally agree with literature data [26, 27, 28], but differ from the results in another [29]. Differences between reported analytical data are probably due to the use of different analytical methods, and variability in the raw materials in terms of growth location, weather conditions and maturity.

The fatty acid composition of *A. digitata* kernels and peanuts [30] correspond well, particularly with regard to oleic and linoleic acid. However, *A. digitata* kernels contained low amounts, approximately 2% of linolenic acid, which was not reported for peanuts. The fatty acid composition of *S. birrea* kernels and olive oil [30] are comparable; both having high contents of the monounsaturated fatty acid oleic acid (>70%). Estimations of the contribution to the AI of omega-6 and omega-3 fatty acids from the consumption of 40 g kernels show that 40 g *A. digitata* kernels can cover almost 40% of the AI of omega-6 fatty acids for those aged four to thirteen years.

There are a few reports in the literature on the amino acid profiles in the kernels of *A. digitata*. The data given in Table 2 agree for most amino acids with other reports [22, 23]. Some literature data for *S. birrea* kernels are also available and the results of the present study generally agree with them [28, 31]. However, our data for cysteine and methionine acids are somewhat lower for both kernels, which may be due to partial breakdown of these amino acids during hydrolysis [32]. Regarding the other essential

amino acids, the results for leucine and lysine in *S. birrea* kernels are lower than in one report [31], while the results for phenylalanine and valine are higher. It is interesting to note that the amino acid composition in the two kernels studied is comparable to that in peanuts and walnuts [33], which commonly form part of the diet.

Ideally, the protein in the diet should provide the body's requirement of all the essential amino acids, in the same relative proportions. The contents of essential amino acids in the kernels were compared with the amino acid requirement stated by the WHO for children aged 3-10 years [10]. For both kernels, the amounts of cysteine + methionine as well as lysine were lower than the requirements. However, the total amino acid compositions indicate that *A. digitata* and *S. birrea* kernels can provide good, cheap sources of protein, especially if combined with foods with higher contents of lysine.

For the dietary fibre monomers presented in Table 3, there is no literature data available for the kernels studied. However, the results from the two very different dietary fibre in this study may contribute to a database and also for evaluation of the consumption levels in a diet to beneficial human health.

CONCLUSION

In conclusion, data have been generated for the contents of amino acids, fatty acids and dietary fibre components in kernels of *A. digitata* and *S. birrea*. Regarding dietary fibre, the main monomer in the insoluble fraction of *A. digitata* kernels was glucose, and in the soluble fraction arabinose. In kernels of *S. birrea*, uronic acids constituted more than 90% of both dietary fibre fractions. The results of the amino acid analysis indicate that kernels of *A. digitata* and *S. birrea,* are good sources of protein and essential amino acids. The kernels are also good sources of fat and essential fatty acids and are especially rich in unsaturated fatty acid. The fatty acid profile differed between the two kernels. *A. digitata* kernels contained appreciable amounts of the essential fatty acids linoleic and linolenic acid, and *S. birrea* kernels contain linoleic acid. Taken together, the results show that the kernels of *A. digitata* and *S. birrea,* which form part of the diet in rural areas in Mozambique, are potential sources of essential nutrients and can contribute to the nutritional needs of various communities. Furthermore, the results can be used for the estimation of dietary intake and to encourage increased consumption and use of these wild fruit kernels.

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Table 1: Fatty acid composition in kernels of *Adansonia digitata* and *Sclerocarya birrea* (mean ± standard deviation) n=2

Fatty acid		**g/100 g fat ± SD**	
No. of carbon atoms and double bonds	**Systematic name**	*Adansonia digitata*	*Sclerocarya birrea*
C14:0	Myristic	0.2 ± 0.0	n.d.[1]
C16:0	Palmitic	25.7 ± 0.2	12.1 ± 0.4
C16:1	Palmitoleic	0.2 ± 0.0	0.2 ± 0.0
C17:0	Margaric	0.2 ± 0.0	0.1 ± 0.0
C17:1	Margaroleic	0.2 ± 0.0	n.d.
C18:0	Stearic	4.6 ± 0.1	7.3 ± 0.1
C18:1	Oleic	34.9 ± 0.7	72.4 ± 0.6
C18:2	Linoleic[2]	29.9 ± 0.9	6.8 ± 0.4
C18:3	Linolenic[2]	2.1 ± 0.0	n.d.
C20:0	Arachidic	0.9 ± 0.1	0.6 ± 0.0
C20:1	Eicosenoic	0.2 ± 0.0	0.3 ± 0.0
C22:0	Behenic	0.3 ± 0.0	0.1 ± 0.0
C22:1	Erucic	0.1 ± 0.0	n.d.
C24:0	Lignoceric	0.2 ± 0.0	0.2 ± 0.0

[1] n.d. = not detected

[2] Essential fatty acid

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Table 2: Amino acid composition in kernels of *Adansonia digitata* and *Sclerocarya birrea* (mean ± standard deviation) n=3

Amino Acid	g/100 g protein ± SD		
	Adansonia digitata	*Sclerocarya birrea*	**Reference value**[1]
Histidine*[2]	2.2 ± 0.1	2.5 ± 0.2	1.6
Isoleucine*	3.4 ± 0.2	3.9 ± 0.3	3.0
Leucine*	6.0 ± 0.4	4.3 ± 1.3	6.1
Lysine*	3.4 ± 1.0	2.9 ± 0.2	4.8
Methionine*	0.8 ± 0.1	0.7 ± 0.1	2.3[3]
Phenylalanine*	4.5 ± 0.3	4.6 ± 0.3	4.1[4]
Threonine*	2.8 ± 0.1	2.8 ± 0.2	2.5
Valine*	4.5 ± 0.3	5.0 ± 0.4	4.0
Alanine	3.8 ± 0.3	3.7 ± 0.3	
Arginine	11.4 ± 0.6	13.9 ± 1.1	
Aspartic acid	8.1 ± 0.4	8.4 ± 0.5	
Cysteine*	0.5 ± 0.0	0.7 ± 0.0	
Glycine	4.4 ± 0.3	4.9 ± 0.3	
Glutamic acid	23.7 ± 1.3	25.7 ± 1.8	
Proline	3.2 ± 0.2	3.5 ± 0.2	
Serine	4.6 ± 0.3	2.9 ± 0.3	
Tyrosine	2.5 ± 0.1	4.7 ± 0.2	
Total	89.8	95.9	

* Essential amino acid

[1] Reference data for children 3-10 years; WHO [11]

[2] Essential for children

[3] Sum of Methionine and Cysteine

[4] Sum of Phenylalinene + Tyrosine

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Table 3: Monomeric composition of dietary fibre in kernels of *Adansonia digitata* and *Sclerocarya birrea* (mean ± standard deviation) n=2

Monomer	(% of dietary fibre) ±SD			
	Insoluble dietary fibre	Soluble dietary fibre	Insoluble dietary fibre	Soluble dietary fibre
	Adansonia digitata		*Sclerocarya birrea*	
Rhamnose	1.9 ± 0.2	2.6 ± 06	n.d.[1]	n.d.
Fucose	n.d.	n.d.	n.d.	n.d.
Arabinose	25.9 ± 2.5	37.6 ± 1.4	3.9 ± 0.5	1.3 ± 0.0
Xylose	11.1 ± 3.0	5.5 ± 0.5	3.2 ± 0.1	1.3 ± 0.1
Mannose	3.0 ± 0.8	0.4 ± 0.0	n.d.	n.d.
Galactose	5.6 ± 0.3	9.8 ± 0.4	n.d.	1.2 ± 0.0
Glucose	37.6 ± 3.2	11.1 ± 0.6	10.5 ± 0.4	n.d.
Uronic acids	14.8 ± 1.8	33.0 ± 3.0	82.4 ± 5.0	96.2 ± 2.2

[1]n.d. = not detected

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