Plant Foods for Human Nutrition **61:** 51–56, 2006.

DOI: 10.1007/s11130-006-0010-z

Amino Acid, Mineral and Fatty Acid Content of Pumpkin Seeds (*Cucurbita spp*) and *Cyperus esculentus* Nuts in the Republic of Niger

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Published online: 13 June 2006

Abstract. Dried seeds and nuts are widely consumed by local populations of the western Sahel, especially those who inhabit rural areas. In light of the need for quantitative information regarding the content of particular nutrients in these plant foods, we collected dried pumpkin (*Cucurbita spp*) seeds and nuts of *Cyperus esculentus* in the Republic of Niger and analyzed them for their content of essential amino acids, minerals and trace elements, and fatty acids.

On a dry weight basis, pumpkin seed contained 58.8% protein and 29.8% fat. However, the lysine score of the protein was only 65% relative to the FAO/WHO protein standard. The pumpkin seed contained useful amounts of linoleic (92 μg/g dry weight) and the following elements (on a μg per g dry weight basis): potassium (5,790), magnesium (5,690), manganese (49.3), zinc (113), selenium (1.29), copper (15.4), chromium (2.84), and molybdenum (0.81), but low amounts of calcium and iron. Except for potassium (5,573 μg/g dry weight) and chromium (2.88 μg/g dry weight), the *C. esculentis* nuts contained much less of these same nutrients compared to pumpkin seeds.

In conclusion, pumpkin seeds represent a useful source of many nutrients essential to humans. The data in this report should of practical value to public health officials in rural areas of sub-Saharan Africa.

Key words: Amino acids, *Cyperus esculentus*, Fatty acids, Niger, Nutrition, Pumpkin seeds, Trace minerals

Introduction

Nuts and seeds contribute significantly to the nutrition of human populations in many parts of the world. Consumed together with other food items, in sauces for example, or alone as snacks, these edible plants can be dried and stored for convenient use during the cold, dry season in West Africa. Some such seeds and nuts, like pumpkin seeds (*Cucurbita spp*), are derived from cultivated plants whereas others, such as the nuts of *Cyperus esculentus* [1, 2], are produced by both cultivated and uncultivated plants. Pumpkin seed is valued from a nutritional standpoint, in part at least, for its high protein content [3–5] and the useful amount of the essential fatty acid, linoleic acid, it provides [6, 7].

We have had a longstanding interest in plant foods of the western Sahel [8–12] because of their year-round importance to the nutrition of the inhabitants of this part of Africa and because they assume special significance during periods when inadequate rainfall or locust invasions cause serious shortfalls in the harvest of cereals such as millet. Our goal, like that of other investigators, has been to provide quantitative information about the content of various nutrients in the diverse plant foods of the western Sahel which could be used by nutritionists, medical personal and public health workers to improve the diets of populations in this part of the world. Pumpkin seed and the nut of *C. esculentus* are two such plant foods of interest to us and which are widely consumed in Niger, Nigeria and other countries in the region.

There are few reports in the literature that address the nutrient composition of *C. esculentus* nuts [1, 2], but many which describe the amounts of various essential nutrients in pumpkin seed [3, 7–13]. However, since the content of a particular nutrient may vary considerably depending on soil conditions, climate and genetic factors, we deemed it worthwhile to also analyze pumpkin seeds from Niger and northern Nigeria for their content of essential amino acids, fatty acids and minerals and trace elements. This report presents the results of that study.

Materials and Methods

Collection of Plant Foods

Dried seeds of pumpkin (*Cucurbita spp*) and *Cyperus esculentus* were obtained in July 2004 from vendors in the central market (Kasuwar Dole) in the town of Zinder, Niger. The purchased samples were sealed in individual plastic bags for transport to the United States.

Amino Acid Analysis

Plant specimens were ground to a fine powder with the aid of a stainless steel mill and dried under vacuum at room temperature until a constant weight was reached. Each sample was analysed in duplicate. Five to nine mg of each specimen were weighed and placed in 2-ml ampoules, to which the internal standard (norleucine) and 0.45 ml of 6 N HCl were added. Norleucine was used as internal standard

because it is an amino acid not commonly found in proteins. The ampoules were evacuated, sealed and placed in an oven for 24 h at 110°C. After hydrolysis, 20 μl aliquots of the hydrolysates were dried, mixed with 10 μl of redry solution (ethanol:water:triethylamine, 2:2:1), dried again, and finally derivatized with 20 μl phenylisothiocyanate reagent (ethanol :water: triethylamine: phenylisothiocyanate, 7:1:1:1) for 20 min at room temperature (14). Excess reagent was removed with the aid of a vacuum at room temperature. Derivatized samples were dissolved in 0.1 ml of 0.14 M sodium acetate that had been adjusted to pH 6.4 with dilute acetic acid. A 20 μl aliquot was injected onto the column. Quantitation of amino acids was performed using a Waters C18 column (3.9 × 150 mm) with gradient conditions as described elsewhere (15). Derivatized amino acids were eluted from the column with increasing concentrations of acetonitrile. The eluate was monitored at 254 nm and the areas under the peaks were used to calculate the concentrations of the unknowns using a Pierce Standard H amino acid calibration mixture (Rockford, IL). A sample of eggwhite lysozyme, analysed in duplicate, served as the control protein.

Samples intended for the determination of cysteine were first oxidized with performic acid (80% formic acid and 30% hydrogen peroxide, 9:1) for 18 h at room temperature (16). The oxidizing reagent was removed with the aid of an evaporative centrifuge and the samples were hydrolysed with 6 N HCl as described above.

The tryptophan content was determined in a separate analysis. The weighed samples were placed in polypropylene tubes and after the addition of the internal standard (norleucine) they were hydrolysed in 4.67 M KOH containing 1% (w/v) thiodiglycol for 18 h at 110°C (17). After hydrolysis, the KOH was neutralized with 4.2 M perchloric acid, and the supernatant was adjusted to pH 3.0 with acetic acid. A 20 μl aliquot of the hydrolysed specimen was subjected to derivatization as described above. The solution of amino acid standard standards was supplemented with tryptophan. Quality control assurance for the tryptophan determination was obtained by demonstrating that the method yielded the correct number of tryptophan residues for egg white lysozyme. Tryptophan analysis was performed using a Waters C18 reversed-phase column (3.9 × 150 mm) (Waters, Milford, MA) and the solvents and gradient conditions were as described by Hariharan et al. [18]. Use of this elution protocol was necessary in order to adequately separate tryptophan from ornithine which results from the alkaline hydrolysis of arginine.

Mineral Analysis

Two replicate aliquots (50–500 mg) from each of the dried, powdered plant specimens were weighed, then wet-ashed by refluxing overnight with 15 ml of concentrated HNO_3 and 2.0 ml of 70% $HClO_4$ at 150°C. The samples were dried at 120°C and the residues were dissolved in 10 ml of 4.0 N HNO_3-1% $HClO_4$ solution. The mineral content of each sample solution was determined by inductively coupled argon plasma atomic emission spectroscopy (ICP-AES, Jarrel-Ash as described elsewhere [8, 19]. The mineral contents of the samples were quantified against standard solutions of known concentrations which were analysed concurrently.

Fatty Acid Analysis

The dried specimens were extracted with chloroform:methanol (2:1, v/v) and the solid, non-lipid material was removed by filtration. The total extracted lipid material was recovered after solvent removal in a stream of nitrogen. The samples were then redissolved in anhydrous chloroform/methanol (19:1, v/v) and clarified by centrifugation at 10,000 × *g* for 10 min. Transmethylation was performed using 14% (w/v) boron trifluoride (BF_3) in methanol [20]. Fifty nanograms of heptadecanoic acid (internal standard) and a 1 ml aliquot of each sample were transferred to a 15 ml Teflon-lined screw-cap tube. After removal of solvent by nitrogen gassing, the sample was mixed with 0.5 ml of BF_3 reagent, placed in a warm bath at 100°C for 30 min and cooled. After the addition of saline solution, the transmethylated fatty acids were extracted into hexane. A calibration mixture of fatty acid standards was processed in parallel.

Aliquots of the hexane phase were analysed by gas chromatography. Fatty acids were separated and quantified using a Hewlett-Packard gas chromatograph (5890 Series II) equipped with a flame-ionization detector. One or two microliter aliquots of the hexane phase were injected in split-mode onto a fused-silica capillary column (Omegawax; 30 m × 0.32 mm I.D., Supleco, Bellefonte, PA). The injector temperature was set at 200°C, detector at 230°C, oven at 120°C initially, then 120–205°C at 4°C per min, 205°C for 18 min. The carrier gas was helium and the flow rate was approximately 50 cm/sec. Electronic pressure control in the constant flow mode was used. The internal standard (heptadecanoic acid, C17:0) and calibration standards (NuCheck, Elysian, MN) were used for quantitation of fatty acids in the lipid extracts. The fatty acids reported represent the average of three determinations.

Results

Amino Acid Analyses

Table 1 contains the results of amino analysis of the pumpkin seeds and seeds of *C. esculentus* following HCl hydrolysis to release amino acids from proteins. Whereas

Table 1. Amino acid composition of two plant foods from Niger

Amino acid	*Cyperus esculentus*		Pumpkin seed	
	(mg/g dry weight)	(% of protein)	(mg/g dry weight)	(% of protein)
Alanine	3.70 (0.10)	5.7	23.4 (0.46)	3.7
Arginine	6.37 (0.41)	11.1	93.2 (2.60)	16.4
Aspartic acid	6.21 (0.14)	10.4	52.8 (1.12)	9.0
Cysteine	0.77 (0.05)	1.3	6.73 (0.21)	1.1
Glutamic acid	10.1 (0.09)	17.2	104 (2.68)	17.9
Glycine	2.74 (0.11)	4.0	28.3 (0.86)	4.2
Histidine	1.44 (0.05)	2.5	13.8 (0.30)	2.4
Isoleucine	2.50 (0.06)	4.2	23.0 (0.72)	3.9
Leucine	4.01 (0.08)	6.7	40.9 (0.88)	6.9
Lysine	3.48 (0.17)	5.9	22.0 (0.58)	3.8
Methionine	1.41 (0.19)	2.4	12.4 (0.26)	2.1
Phenylalanine	2.33 (0.05).	4.0	31.4 (0.55)	5.5
Proline	2.70 (0.10)	4.4	20.2 (0.56)	3.4
Serine	2.62 (0.04)	4.2	31.7 (0.49)	5.2
Threonine	2.89 (0.05)	4.8	18.4 (0.68)	3.1
Tryptophan	1.47 (0.07)	2.6	15.3 (0.02)	2.7
Tyrosine	1.61 (0.03)	2.8	22.1 (0.2)	3.9
Valine	3.38 (0.10)	5.6	28.2 (0.92)	4.7
[a]Total protein (mg/g)	51.4		508.5	

Note. The values reported represent the average of three determinations. The number in parentheses is the standard deviation.
[a]Total protein was calculated using the anhydrous weights of the amino acids.

the protein content of pumpkin seed was high (50.9%), that of *C.esculentus* was relatively low (5.14%). However, the overall quality of the protein in the pumpkin seeds was compromised by its low lysine content (3.8% of the amino acid total). In fact, the lysine content of the pumpkin seeds was only 65% of the FAO/WHO standard for children (Table 2) (21). The proportion of threonine in pumpkin seed (92%), another essential amino acid, was also below the WHO/FAO standard for children (20) (Table 2). In contrast, the percentages of all eight of the essential amino acids or amino acid pairs in the *C. esculentus* seeds met or exceeded the percentages of these amino acids in the WHO/FAO standard. Noteworthy is the fact that both kinds of seeds contained remarkably high proportions of the essential amino acid tryptophan (Table 1).

Mineral Analyses

ICP-AES allowed us to determine the content of 32 minerals in the plant foods, many of which are required in man and others which are toxic. Table 3 summarizes the results of these analyses. The pumpkin and *C.esculentus* seeds both contained relatively large amounts of potassium (5,573 and 5,790 μg/g dry weight, respectively), and chromium (approx. 3 μg/g dry weight). However, the sodium content of pumpkin and *C. esculentus* seeds was low (<100 μg/g dry

Table 2. Essential amino acid composition of two plant foods from Niger compared to the WHO "ideal protein"

Amino acid	WHO ideal protein) (% of total protein)	*Cyperus esculentus*		Pumpkin seed	
		% of total amino acid	% amino acid / ideal × 100	% of total amino acid	% amino acid / ideal × 10
Isoleucine	2.8	4.2	149	3.9	140
Leucine	6.6	6.7	102	7.0	105
Lysine	5.8	5.8	101	3.7	65
Methionine + Cysteine	2.5	3.6	146	3.3	130
Phenylalanine + Tyrosine	6.3	6.6	105	9.1	145
Threonine	3.4	4.8	143	3.1	92
Tryptophan	1.1	2.5	225	2.6	237
Valine	3.5	5.6	161	4.8	137

Table 3. Mineral content (average SD) of two plant foods from Niger

	μg/g dry weight	
Mineral	*Cyperus esculentus* Average (SD)*	Pumpkin seed Average (SD)
Aluminum, Al	69.5 (2.74)	9.21 (0.6)
Arsenic, As	ND	0.45 (0.05)
Barium, Ba	2.68 (0.03)	1.16 (0.02)
Beryllium, Be	0.01 (0.002)	ND
Calcium, Ca	188 (4.38)	346 (5.51)
Cadmium, Cd	0.19 (0.009)	ND
Cobalt, Co	0.23 (0.004)	0.29 (0.003)
Chromium, Cr	2.88 (0.38)	2.84 (0.05)
Copper, Cu	2.44 (0.46)	15.4 (0.08)
Iron, Fe	52.9 (0.96)	106 (3.36)
Potassium, K	5573 (207)	5790 (124)
Magnesium, Mg	763 (12.7)	5690 (286)
Manganese, Mn	11.9 (0.28)	49.3 (0.46)
Molybdenum, Mo	0.055 (0.013)	0.805 (0.004)
Sodium, Na	82.1 (8.0)	6.9 (10.2)
Nickel, Ni	1.4 (0.04)	0.53 (0.03)
Phosphorus, P	1937 (64.3)	15700 (802)
Lead, Pb	ND	ND
Selenium, Se	0.28 (0.095)	1.29 (0.096)
Strontium, Sr	1.45 (0.03)	1.83 (0.03)
Titanium, Ti	0.56 (0.07)	ND
Vanadium, V	0.09 (0.002)	ND
Yttrium, Y	0.09 (0.005)	ND
Zinc, Zn	11.2 (0.23)	113 (1.30)
Zirconium, Zr	0.14 (0.03)	ND

Note. ND, not detected. The following elements were not detected: Ag, La, Li, Pb, Sb, Te, and Tl (silver, lanthanum, lithium, lead, terillium, thallium.). The values reported represent the average of three determinations.
*SD, standard deviation.

weight). Pumpkin seeds contained relatively large amounts of magnesium, zinc, copper, molybdenum and selenium, but the amounts of these same elements in *C. esculentus* seeds were relatively low. Noteworthy are the low amounts of calcium in the two edible seeds.

Fatty Acid Analyses

Fatty acids accounted for 29.8% of the dry weight of pumpkin seeds, and 15.5% of the dry weight of *C. esculentus* seeds (Table 4). The fatty acid composition of the crude lipid fraction of both seeds was relatively simple (Table 5), with four fatty acids accounting for >97% of the fatty acid total: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9) and the essential fatty acid linoleic acid (18:2n-6). On a percentage basis, in each case, oleic acid was the predominant fatty acid. Whereas linoleic acid accounts for nearly one-third of the fatty acid total in pumpkin seeds, α-linolenic acid contributed insignificantly to the fatty acid profile of either pumpkin seeds or *C. esculentus* seeds.

Table 4. Fatty acid composition of two plant foods from Niger

	% of total fatty acids*	
Fatty acid	*Cyperus esculentus*	Pumpkin seed
12:0	0.02 (0)	0.02 (0)
14:0	0.12 (0.01)	0.16 (0.01)
14:1	0.09 (0.01)	0.07 (0.01)
15:0	0.02 (0)	0.02 (0)
16:0	14.5 (0.02)	13.0 (0.01)
16:1n-7	0.33	0.17 (0.01)
18:0	5.6 (0.01)	7.8 (0.06)
18:1n-9	64.8 (0.02)	45.4 (0.08)
18:1n-7	1.3 (0.02)	0.98 (0.04)
18:2n-6	11.8 (0.01)	31.0 (0.09)
18:3n-6	0.02 (0)	0.09 (0.05)
18:3n-3	0.21 (0)	0.19 (0)
20:0	0.69 (0)	0.58 (0)
20:1	0.20 (0)	0.13 (0)
20:2n-6	ND	ND
20:3n-6	ND	ND
22:0	0.15 (0.01)	0.16 (0)
22:1	0.07 (0.02)	0.08 (0.01)
24:0	0.25 (0.01)	0.15 (0.01)
24:1	ND	0.09 (0.02)

*Number in parentheses is the difference between the means of two determinations.
Note. ND, not detected (<0.005 mg/g dry weight).

Discussion

In this study we analyze two edible seeds that are widely available in the semi-arid regions of West Africa and present data on their nutritional content. Though the literature does

Table 5. Total Fatty acid composition of two plant foods from Niger

	(mg/g dry weight)*	
Fatty acid	*Cyperus esculentus*	Pumpkin seed
12:0	0.03 (0.01)	0.06 (0.02)
14:0	0.18 (0.05)	0.46 (0.01)
14:1	0.13 (0.01)	0.19 (0.03)
15:0	0.03 (0)	0.07 (0.01)
16:0	22.5 (0.7)	38.6 (2.1)
16:1n-7	0.52 (0.01)	0.48 (0)
18:0	8.7 (0.3)	23.2 (1.2)
18:1n-9	101 (3.3)	135 (7.2)
18:1n-7	2.1 (0.1)	2.9 (0.2)
18:2n-6	18.3 (0.6)	92.0 (5.4)
18:3n-6	0.03 (0.01)	0.25 (0.2)
18:3n-3	0.32 (0.01)	0.57 (0.04)
20:0	1.08 (0.02)	1.75 (0.01)
20:1	0.31 (0.01)	0.39 (0.02)
22:0	0.23 (0.02)	0.47 (0.02)
22:1	0.12 (0.03)	0.22 (0.03)
24:0	0.38 (0)	0.43 (0.04)
24:1	ND	0.6 (0.14)
Total fatty acid (mg/g dry wt)	155	298

*Number in parentheses is the difference between the means; ND, not detected (<0.005 mg/g dry weight).

contain reports about the content of various nutrients in the seeds of cultivated pumpkin and the seeds of the spontaneous plant *C. esculentus*, since the amounts of particular nutrients can differ between regions because of environmental conditions and genetic factors, we wanted to determine the amounts of essential fatty acids, amino acids and minerals and trace elements that the seeds of these two plants might provide to human diets.

In general, the results contained in this report parallel findings from other studies. For example, in the case of pumpkin seed, our finding that protein contributes greater than 50% to the dry weight of the seed (Table 1) agrees with data reported by Mansour and colleagues (3), El-Adawy and Taha [22] and Bosch and coworkers [2]. Our results underscore the observation of El-Adawy and Taha [22] that lysine is the limiting amino acid in unfractionated pumpkin seed protein. On the other hand, we did not find, as claimed by Mansour and colleagues [3], that isoleucine and valine are the nutritionally limiting amino acids in pumpkin seed protein. The high tryptophan content of pumpkin seed reported in our study (15.3 mg/g) is in agreement with that of 1.54 g/16 g N reported by Zdunczyk and coworkers in their study comparing pumpkin seed cake to soybean meal and casen [5].

With regard to the issue of fatty acids, our data are in accord with those of Murkovic and associates [6] and Tsaknis and coworkers [7] which show that four fatty acids (palmitic, stearic, oleic and linoleic) account for >97% of the fatty acid total in pumpkin seeds, and that this plant food is a good source of the essential fatty acid linoleic acid. As for minerals and trace elements, the data in Table 3 are in good agreement with the findings of Juranovic and colleagues [13] who, as we did in the present study, used atomic emission spectrometry to quantify these same elements.

Compared to pumpkin seed, the literature contains far fewer reports of the nutrient composition of *C. esculentus* seeds. Bosche and coworkers [2] subjected the proteins in *C. esculentus* nuts from plants grown in Africa to acid hydrolysis, and separated and quantified the resultant amino acids. Their major findings and ours are in good agreement: 1) arginine was one of the most abundant amino acids; 2) the proportions of histidine and tyrosine were low; and 3) the percentage composition of each of the essential amino acids met or exceeded the standards set by the WHO/FAO for human diets.

It is useful to interpret the amino acid, mineral and fatty acid data in Tables 1, 3 and 4, respectively, in terms of what fraction of an adult's daily nutritional requirement might be satisfied by consumption of a reasonable portion of pumpkin seeds or the seeds of *C. esculentus*. We therefore selected key essential nutrients and asked the question What percentage of an adult's daily requirement could be satisfied by the consumption of 10 grams (dry weight) of either seed. As shown in Table 6, for most of the nutrients listed, pumpkin seeds appear to represent a more useful source of critical nutrients than the seeds of *C. esculentus*. For example, setting aside for the moment any consideration of the matter of bioavailability, 10 grams of pumpkin seed alone would provide 17% of an adult's protein requirement, 17% of their requirement for potassium, and 7.5–14% of their requirements for the critical elements magnesium, zinc, selenium, copper, chromium and molybdenum. Pumpkin seeds also contain useful amounts of linoleic acid; in particular, 10 grams (dry weight) of these seeds would provide a 1 to 8-year-old child with 15.4% of their daily requirement for this essential n-6 fatty acid (22) (Table 6). It is noteworthy that neither *C. esculentus* nor pumpkin seeds contain very substantial amounts of iron or calcium that are required for, among other things, red cell synthesis and bone formation, respectively

Table 6. Comparison of the content of selected nutrients in 10 grams of two plant foods from Niger versus the USDA recommended daily values (RDV)

		Percent of RDV	
Nutrient	RDV*	*Cyperus esculentus*	Pumpkin seed
Fat (g)	65	2.4	4.5
Protein (g)	50	1.2	12
Sodium (g)	2400	3.4	0.3
Potassium (mg)	3500	16	17
Calcium (mg)	1000	0.2	0.4
Iron (mg)	18	3.0	5.9
Magnesium (mg)	400	1.9	14
Zinc (mg)	15	0.8	7.5
Selenium (ug)	70	4.0	18.4
Copper (mg)	2	1.2	7.7
Chromium (ug)	120	24	24
Molybdenum (ug)	75	0.7	11

*Relative daily value; from the Food and Nutrition Board (22).

In terms of their nutrient content, the seeds of *C. esculentus* compare poorly with those of pumpkin; except for potassium and chromium, *C. esculentus* seeds seem capable of satisfying less than 5% of an adult's requirement for the various nutrients listed in Table 5.

Of course, neither of the plant foods we describe herein are consumed in isolation; they are commonly part of the larger diet. Thus, in a practical sense, *a priori* concern about the low lysine content of pumpkin seeds, would be mitigated by the likelihood that these seeds would be eaten together with other protein sources that contain a much higher proportion of lysine. For example, in certain parts of Africa, pumpkin seed meal is added to wheat flour in bread making [4].

An inherent limitation in any study based on the chemical analysis of nutrients in foods is that it does not take into consideration the important issue of bioavailability. The

usefulness of the foods we have analyzed in the present report in fulfilling a particular nutrient need will depend on the extent to which these foods are digested and that nutrient in question is absorbed from the intestine. For example, pumpkin seed contains phytates that can bind calcium and other divalent cations and protease (e.g., trypsin) inhibitors that can reduce protein digestion [4]. Nevertheless, the data we provide in this report should be of interest to nutritionists and public health officials in West Africa where pumpkin seeds and the seeds of *C. esculentus* are part of the diets local populations.

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