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African *Cucurbita pepo* L.: properties of seed and variability in fatty acid composition of seed oil

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Abstract

Pumpkin (*Cucurbita pepo*) seeds are used locally in Eritrea to treat tapeworm. Seeds were found to be rich in oil (~35%), protein (38%), α-tocoferols (3 mg/100 g) and carbohydrate content (~37%). The physico-chemical properties and fatty acid composition of the seed oil were examined. The four dominant fatty acids found are: palmitic C16:0 (13.3%), stearic C18:0 (8.0%), oleic C18:1 (29.0%) and linoleic C18:2 (47.0%). The oil contains an appreciable amount of unsaturated fatty acids (78.0%) and found to be a rich source of linoleic acid (47.0%). Within the three localities of the study, variations exist in seed properties and the fatty acid composition of the oil. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Pumpkin seed oil; *Cucurbita pepo*; Cucurbitaceae; Fatty acid composition

1. Introduction

Pumpkin, *Cucurbita pepo* L., (English name; summer pumpkin, locally known in Eritrea as Dubba) is a herbaceous, monoecious, annual plant of the Cucurbitaceae family (Grosch and Belitz, 1987; Trease and Vans, 1983; Bombardelli and Morzzoni, 1997). Seeds have long been used as a remedy for various ailments, particularly, as a treatment against worms (Schiebel-Schlosser and Friederich, 1998; Lewis et al., 1997). In Eritrea, Sudan and Ethiopia, pumpkin seeds are used to treat tapeworm, where the dried seeds are eaten in an empty stomach. For many years, particularly in Europe, extracts from pumpkin seeds, *C. pepo*, have been used in folk medicine as a remedy for micturition caused by Benign Prostatic Hyperplasia (BPH) (Madaus, 1979; Mandressi et al., 1987; Carbin et al., 1990; Silverio et al., 1993). In a recent study, the therapeutic use and safety of a pumpkin seed extract were tested in a multicentric clinical trial with 2245 patients suffering from BPH. Urinary symptoms were recorded by the International-Prostate-Symptom-Score (I-PSS) according to the American Urological Association, the influence on quality of life has been recorded by a quality of life questionnaire (LQ-Index). Patients were treated with capsules containing 500 mg of a pumpkin seed extract. The I-PSS decreased by 41.4%, life quality improved by 46.1% during therapy. More than 96% of the patients had no side effects under this treatment (Schiebel-Schlosser and Friederich, 1998). Neither the constitution and the properties of the seeds, nor the physico-chemical properties and fatty acid composition of the seed oil of the African variety of *C. pepo* L. have been reported so far (Younis and Ghirmay, 1998). Earlier analysis of the European and the American varieties and the allied species of *C. pepo* have been made (Franklin and James, 1996; Murkovic et al., 1996a; Murkovic et al., 1996b; vas Concellos et al., 1980; Kusmenolglu, 1996; Murkovic, 1996).

The dominant fatty acids that are found in pumpkin

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seed oil are: palmitic C16:0, stearic C18:0, oleic C18:1 and linoleic C18:2. Previous studies have shown that in different varieties, which are used for oil production, palmitic occurs in the range of 10.3–11.7%, stearic 4.1–5.4%, oleic 30.5–40.8% and linoleic 42.1–51.5% (Wenzel, 1987).

The aim of the present investigation is to study the characteristics and fatty acid composition of the seed oil as well as the properties of the seed of *C. pepo* L. (African variety), grown in three different ecological zones in Eritrea (Africa); namely, the high lands (2100–2400 m), the Red Sea hills (900–1800 m) and the low lands (600–700m). This is a continuation to our earlier reported studies (Younis and Ghirmay, 1998).

2. Results and discussion

The *C. pepo* L. seeds upon Soxhlet extraction with hexane for 16 h have yielded 35, 24 and 22% of oil for seeds grown in the high lands, the Red Sea hills and the low lands, respectively. The recovered oil has a nut-like taste and a brownish yellow color. The physico-chemical properties of the oil are given in Table 1. The observed variability of the seed oil content found in the present investigation, Table 2, could only be attributed to climatic factors, more probably to the differences in: temperature, humidity, soil nature and other climatic factors prevailing in the locality in which the pumpkin seeds are cultivated. The oil content value found in this investigation ranges between 22 and 35%, which is much lower than that reported (60%) for the European variety of *C. pepo* L. (Murkovic et al., 1996a, 1996b). It was claimed that such differences in yield could be attributed to genetic diversity (Idourain et al., 1996). Moreover, the oil content of the seeds tends to be higher (35 and 24%) for seeds grown at high altitudes where the average temperature is low, and tends to be lower (22%) for seeds grown at low altitudes where the average temperature is high,

Table 1
Physico-chemical properties of *Cucurbita pepo* L. seed oil

S. No.	Determinations	Value (mean ± SD)
1	Specific gravity[a]	0.915 ± 0.00
2	Refractive index[a]	1.4695 ± 0.0007
3	Optical rotation[a]	(+) 0.35 ± 0.07
4	Acid value (%)	0.66 ± 0.20
5	Free fatty acid (%)	0.33 ± 0.66
6	Saponification value	132.33 ± 0.02
7	Iodine value	123.00 ± 0.10
8	Energy (kcal/100 g)[b]	480.30, 505.60, 410.40

[a] Determined at 23°.
[b] High lands, low lands and Red Sea hills seeds, respectively.

Table 2
Chemical composition of *Cucurbita pepo* L. seeds grown in different localities

Locality	Altitude ($m \times 10^3$)	Temperature (°C)	Oil content (%)	Moisture content (g/100 g)	Protein content (g/100 g)	Carbohydrate content (g/100 g)	Ash content (g/100 g)	Cholesterol content (mg/100 g)	Dl-α-tocoferol content (mg/100g)	Fatty acid composition					Reference
										C16:0	C18:0	C18:1	C18:2	C18:3	%[a]	
High land	2.4–2.1	27–10[b]	35	6.2	38.0	37.9	3.3	< 0.2	3.0	11.2	8.2	28.3	50.3	0.2	79.3	Exp.[c]
Low land	0.7–0.6	42–17[d]	21.9	4.0	32.0	36.5	3.0	3.0	3.0	14.0	8.0	34.0	43.0	0.17	7.6	Exp.
Red Sea hills	1.8–0.9	40.25[d]	23.9	6.6	28.0	37.0	3.3	< 0.2	3.0	11.6	8.0	30.6	47.6	0.2	78.9	Exp.
Germany I	n.s.[e]	n.s.	n.s.	–	–	–	–	–	–	11.1	4.8	35.5	46.5	0.3	82.7	(Wenzel, 1987)
Germany II	n.s.	n.s.	n.s.	–	–	–	–	–	–	11.8	5.8	28.9	51.9	0.3	81.6	(Cerney et al., 1971)

[a] Total percentage of unsaturated fatty acids.
[b] Maximum and minimum average temperature. Lowest average temperature prevails.
[c] Exp.: experimental data from this study.
[d] Maximum and minimum average temperature. Highest average temperature prevails.
[e] n.s.: not stated.

Table 2. In contrast, the protein, carbohydrate, ash, cholesterol, with the exception of α-tocoferol values, are also comparable to those found for seeds of European variety (Murkovic et al., 1996a, 1996b). Values for free fatty acid content (0.33%), acid value (0.66%), iodine value (123), saponification value (132.3), specific gravity (0.9150) and refractive index (1.4695) are similar to those values reported for soybean, cotton seed, corn and safflower (Murkovic et al., 1996a, 1996b; vas Concellos et al., 1980). Gas chromatography (GC) analysis have shown that the dominant fatty acids found in the oil of seeds cultivated in the three localities are: palmitic C 16:0 (11.2–14%); stearic C18:0 (8.0–8.2%), oleic C18:1(28.2–34.0%) and linoleic C18:2 (43.0–53.0%). The content of these four main fatty acids ranges from 97.7 to 99.0% of the total fatty acid composition of the oil. The fatty acid composition of oil samples from seeds cultivated in the three regions is given in Table 2.

It can be seen that the total percentage of the unsaturated fatty acids ranges from 78 to 79%. This value is relatively lower than that reported for the seed oil of *C. pepo*, German variety (81.6 and 82.7%), (Cerny et al., 1971; Wenzel, 1987), but relatively higher than that reported for soybean 75% (Cerny et al., 1971); peanut 78% (Sanders, 1980) seed oils. Moreover, the composition of linoleic acid was found to be in the range of 43–50%, which is comparable to that found for the European variety, 36.6–60.8% (Murkovic et al., 1996a, 1996b). This indicates that the *C. pepo* L. seed oil is a rich source of linoleic acid, and that the unsaturated nature of this oil qualifies it to be a promising edible oil. It is worth mentioning that epidemiological studies show that the probability of coronary artery disease decreases linearly with the increase of quantities of the unsaturated fatty acids in the food stuff (Key, 1970). Moreover, studies on human subjects using diets rich in linoleic acid showed that in the groups provided with higher amounts of soybean oil (50% linoleic acid content), the mortality rate due to coronary artery disease decreases significantly. It is observed that the percentage composition of linoleic acid is always (1) higher in localities where lower average temperature (10 and 25°C, respectively) prevails; 50.3% for the high lands, 47.5% for the Red Sea hills and (2) lower in localities where higher average temperature (42°C) prevails; 43.0% for the low lands.

Furthermore, for all oil samples of seeds cultivated in the three different localities, whenever the composition of linoleic acid C18:2 is high, the composition of oleic acid C18:1 is low and vice versa. The composition of stearic acid C18:0 remains constant. The trace amounts of the other higher unsaturated fatty acids, e.g. linolenic acid C18:3 (0.2%) and nervonic acid C24:1 (0.3%), found in the oil of seeds cultivated in localities where the average low temperature prevails are also higher than those found in the oil of seeds cultivated in localities where the average high temperature prevails, 0.1 and 0.2%, respectively (Table 2). This conclusion is based on the assumption that experimental error encountered in the measurement of these trace amounts is negligible. These observations are in accordance with the reported findings that due to the temperature dependence of the microsomal oleoyl phosphatidylcholine desaturase (ODS) in sunflower seeds, more linoleic acid is produced at lower temperature (Mancha et al., 1995). von Marquard (1990) has also shown that several plants including *C. pepo* L. produce more linoleic acid when grown in areas where colder climate predominates. Accordingly, lipids tend to be more highly unsaturated, and thus remain fluid, at lower temperature (Mead et al., 1986). The fatty acids: myristolic acid C14:1; 11,14-eicosadienoic acid C20:2; homogamma linoleic acid C20:3 and arachidonic acid C20:4 were not present in the seed oil samples studied. It is worth mentioning that a trace amount of nervonic acid C24:1 (0.2–0.3%) has been found in all the seed oil samples analyzed in this study. This has not been reported before.

Cucurbita pepo L. seeds that have been researched so far, in reported studies, are based on crossed varieties. This can result in a much better genotopic and phenotopic variations. Accordingly, the European varieties of *C. pepo* L. have acquired upgraded seeds with improved properties and, accordingly, high oil content. In comparison, seeds studied in this work could be considered as inbreed of pumpkin, though cultivated in that region of Africa for several years. Given the mass of a typical pumpkin seed and the yield of oil, protein, etc., it can be concluded that these seeds may be useful as a food source.

3. Experimental

3.1. General information

Seeds of *C. pepo* L. were obtained from fruit samples provided by the Agricultural Research Center in Asmara, Eritrea. The fruits were collected and sampled out from the following ecological zones: the high lands (2100–2400 m), the Red Sea hills (900–1800 m) and the low lands (600–700 m). The highest and the lowest average temperatures are 10–27, 25–40, and 17–42°C, respectively. Within each locality, fruit samples were collected from farms spreading over an area ranging from 100 to 500 km^2. The seeds were spread out and shade dried.

3.2. Determination of the oil content

The oil content of the seeds was determined by

treating the weighed powdered seeds with hexane and refluxed for 16 h in a Soxhlet extractor. The solvent was removed by rotary evaporator. The oil sample was then placed in a vacuum oven kept at 60°C for 30 min, and then accurately weighed and the percentage yield calculated. The physico-chemical properties of the oil were determined, Table 1. The oil content values for seed samples collected from the high lands, the low lands and the Red Sea hills are given in Table 2. The values shown are mean of three determinations.

3.3. Determination of the moisture content

The moisture content was determined using the standard procedure described in Ref. (Nordic Committee for Food Analysis, 1991). Values for moisture content for seed samples collected from the high lands, the low lands and the Red Sea hills are given in Table 2. They are mean of three determinations.

3.4. Determination of the ash content

The ash content was determined using the standard procedure described in Ref. (Nordic Committee for Food Analysis, 1987). Values for ash content for the seed samples collected from the high lands, the low lands and the Red Sea hills are given in Table 2. They are mean of three determinations.

3.5. Determination of the protein content

The protein content of the defated seeds was determined using the AOAC Official Method 968.06 (4.2.04) (Official Methods of Analysis, 1995). Perkin–Elmer, PE3410 N-Nitrogen Analyzer, Model 29 A was used. The percentage nitrogen determined is used to calculate the percentage protein content. The protein content for seed samples collected from the high lands, the low lands and the Red Sea hills are given in Table 2. The values are mean of three determinations.

3.6. Determination of the α-tocoferol (vitamin E) content

The α-tocoferols in the seeds were extracted and the content determined with the aid of High Performance Liquid Chromatography (HPLC) analysis, described in Ref. (Manz and Philipp, 1981). The HPLC modules and conditions are as follows: Module: Injection valve type 7125, Rheodyne, Catati, CA 94928, USA; double piston pulse-free pump, Kontron, Zurich, Switzerland. Florescence detector, 650Lc Perkin–Elmer, Norwalk, CT 06856, USA. Integrator, Mod. 7270, Spectra-Physics, San Jose, CA 95134, USA. Recorder, Mod. W + W 312, Kontron, Zurich, Switzerland.

3.6.1. Conditions

Column: Stainless steel, length 12.5 cm, inner diameter 4 mm, stationary phase: silica gel-HIBAR prepared column filled with LiChrosorb Si 60.5 m, Darm stadt, FRG. Mobile phase: *n*-hexane containing 3% dioxane, isocratic. Flow rate: 1 ml/min. Pressure: 33 bar. Temperature: ambient. Injection volume: 50 μl (loop). Detection: Florescence detection, excitation at approximately 293 nm. And emission at approximately 326 nm. Retention times 5–5.5 min. Run time: 30 min. Standard solution for external calibration: 10 μg α-tocoferol/ml. The values for dl-α-tocoferol content of the seed samples collected from the high lands, the low lands and the Red Sea hills are given in Table 2. Values are mean of three determinations.

3.7. Determination of cholesterol content by gas chromatography (GC)

The cholesterol in the oil was extracted and content determined with the aid of GC analysis, described in Ref. (Franz and Hermine, 1992). Varian Vista 3400 CX Chromatograph with Quadrex OV 351 was used. The column used is a fused silica capillary column (30 × 0.25 mm i.d.) coated with DB-5, film thickness 0.25 m. (J&W Scientific, Folsom, CA, USA). The instrument is equipped with split/spills injector (temperature programmable). The initial injector temperature was 40°C and the initial auxiliary temperature was 270°C. A flame ionization detector, ADCB (10 V) type was used. The detector temperature was 260°C. Chromatograms were recorded with SP 4270 computing integrator. Helium was the carrier gas and delivered to the column at a head pressure of 0.5 bar. The split vet flow was 47 ml/min. The temperature of the column was held for a minute at 110°C, and programmed at a rate of 4–300°C/min. Cholesterol was quantified by means of internal standard, cholestane. For quantification purpose, cholesterol/cholestane mixtures in hexane were prepared and chromatographed. Test mixtures consisted of cholestane (50 μg) and a varying amount of cholesterol (10–250 μg) made up to 0.25 ml with hexane. The injection volume was 10 μl/s. Results are shown in Table 2.

3.8. Determination of fatty acid composition of the seed oil by gas chromatography

Fatty acid methyl esters (Aldrich, Dorset-SP8 4JL, England) standard solution mixture was used. The extracted seed oil was methylated in the usual manner by refluxing with methanolic sodium hydroxide solution, boron trifluoride reagent and heptane (Official Methods of Analysis, 1997). The fatty acid composition of the esterified oil was characterized and quantified using Varian Vista 3400 CX Chromatograph

described above. Quadrex OV 351 (25 m × 0.32 mm) column was used. The initial column temperature was 110°C and the final temperature was 220°C. The initial injector temperature was 40°C, and the auxiliary temperature was 270°C. The detector temperature was 260°C. An auto-sampler type 8200 was used; and the injection volume was 10 μl. Results are shown in Table 2.

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