DETERMINATION OF FATTY ACID AND TOCOPHEROL CONTENTS OF THE OILS OF VARIOUS PUMPKIN CULTIVARS (*CUCURBITA* SPP.) SEEDS

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Abstract
The pumpkin is a rich source of nutrients and has medicinal properties. However, owing to the perception that it is a traditional food and majority of the Kenyan populace being unaware of its high nutritional value it still remains unexploited. The main aim and objective of this work was to determine the composition of fatty acids and vitamin E in seed oils of pumpkins (Cucurbita spp) collected from selected regions in Kenya. The pumpkin samples were graded on the basis of colour, nine varieties were identified. The seeds were removed from the fruits and dried under shade and grounded into fine flour prior to analysis. Data analysis was done using Genstat statistical package. The oil yields were obtained by solvent extraction and analysed for fatty acids by Gas Chromatography (GC). The oil yields of the seeds were found to be ranging between 31.9 to 39.44%. Both saturated and unsaturated fatty acids were identified in all the seed oils with the latter being the predominant with linoleic acid ranging between 43.4% to 58.29% and oleic acid ranging between 20.27% to 40.51%. Vitamin E ranged between 9.42 and 122.65 µg/g. There was significant decrease in percentage saturated FA, increase in percentage unsaturated FA with increase in vitamins E concentration in all the seed oils. The seed oils have many FA such as oleic acid, and α-linoleic acids which are of biological and industrial significance to humans thus could be potentially exploited domestic use.

Keywords: Pumpkin, seed oil, gas chromatography, α-tocopherol

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1. INTRODUCTION

Considerable proportions of oil and fats, whether for domestic consumption or for industrial use is currently derived from plant sources mainly vegetables (Ramadan *et al*., 2006). Despite the wide range of vegetable oils sources, global consumption is largely dominated by soybean, palm, rapeseed, and sunflower oils accounting for 31.6, 30.5, 15.5, and 8.6 million tons consumed per year, respectively (Soy, 2005). Oilseed crops are generally grown for the purpose of exploiting oil in their seeds and they vary considerably in their content, quality, and composition. (Hablila *et al*., 2012).

Vegetable oils possess different compositions, such as fatty acids and tocopherols and sterols, enabling improved performance in food and industrial applications, as well as nutritional benefits (Stevenson *et al*., 2007). A good number of vegetable oils are either consumed directly or indirectly mostly as ingredients in food and the oils serve a number of purposes in this role (Dubois *et al*., 2007). A high consumption of vegetable oils has been reported (Rezanka and Singler, 2009; Ixtaina *et al*., 2011) which has substantially increased the demand for alternative plant based oils which are lower in saturated fats, higher in mono-unsaturates.

Currently, pumpkin seed oil is not either used commercially or domestically even though it has characteristics that are well suited for industrial applications and can contribute to healthy human diets (Karanja *et al*., 2013).

Pumpkin seed are either brown or white the seed oil varies from dark green to brown in color. It is a highly unsaturated oil, with predominantly oleic and linoleic acids present (Karanja *et al.,* 2013). Very low levels of linolenic acid or other highly unsaturated fatty acids are present, providing pumpkin seed oil with high oxidative stability for storage or industrial purposes and low free radical

production in human diets (Stevenson *et al*., 2007).
The highly unsaturated fatty acid composition of pumpkin seed oil makes it well-suited for improving nutritional benefits from foods (Stevenson *et al*., 2007). A number of biological and medicinal properties such as reducing the prostate size and preventing its growth (Tsai *et al*., 2006; Gossell-Williams *et al*., 2006), retarding the progression of hypertension (Zuhair *et al*., 2000), arresting hypercholesterolemia effects Zuhair *et al*., 1997) anti-inflammatory (Fahim *et al*., 1995) and anti-diabetic (Fu *et al*., 2006) have been ascribed to pumpkins seed oil.
In this study we investigate the oil content, fatty acid composition, and α-tocopherol content of 9 pumpkin cultivars, cultivated in Kenya. To date, nothing is known about the oil characteristics of any of the pumpkin cultivars selected in this study. Knowledge gained from this study will help to determine the potential for seed oil from these pumpkin cultivars to be commercially exploited for industrial applications and incorporation into food formulations to improve human health.

2. MATERIALS AND METHODS

2.1. Collection and preparation of samples

The pumpkins were purchased from farmers and traders in East and Central between July and September 2011. The samples were then grouped into 9 categories based on the colour as shown in Table 1. The seeds were separated from the fruits manually after cutting with a sharp steel knife. The seeds were cleaned and dried under a shade for a period of four weeks. The seeds were ground using an electric grinder the material with uniform size, passing through 80-mesh sieve was used for extraction purposes. Three replicates were assayed for each pumpkin cultivar.

2.2. Oil extraction

Approximately 2 g of the ground seed sample were subjected to oil extraction using a Soxhlet apparatus (Gerhardt) according to AOCS Official methods Am 2-93 (Anonymous, 2003).

Table 1: External Fruit physical characteristics of different pumpkins

Variety	*Fruit skin Colour*	*Sample(n)*
A	Cream plain	7
B	Cream greenish	4
C	Yellow orange plain	2
D	Yellow orange cream spots	3
E	Yellow orange green spots	1
F	Dark green	5
G	Light green	5
H	Green with cream spots	9
I	Green with orange spots	9

2.3. Fatty acid analysis

The methyl esters of the fatty acids were prepared according to IUPAC (Anonymous, 1987) and analyzed using a Shimadzu GC-2010 gas chromatograph equipped with a DB-23 column (60m x 0.25mm i.d. and 0.25 µm film thickness) (J&W). Injector, column and detector temperatures were 230, 190 and 240 °C, respectively. Split ratio was 80:1. Carrier gas was helium at 1.0 ml/min ratio.

2.4. Tocopherol analysis

The sample was prepared by dissolving 1g of oil in 9ml of hexane and injected into a normal phase HPLC to analyze tocopherols (Anonymous, 2003), using a Shimadzu SCL-10A HPLC system. The chromatographic separation was done with a Lichrosorb Si60-5 (250 x 4.6 mm id, particle size S-5µm) (Hichrom, Reading, UK). The column temperature was maintained at 25^{o}C. Separation of the tocopherols was based on isocratic elution with n-hexane (99%) and iso-propanol (1%) at 1 mL/min. The eluate was monitored at 295 nm. α-tocopherol was quantified based on peak areas compared with an external standard α tocopherol.

2.6. Statistical analysis

Data for each of the 9 varieties were recorded as means±standard deviation and analyzed by Genstat statistical package. One-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out to test any significant differences between cultivars.

3. RESULTS AND DISCUSSION

The oil yields of the 9 seed oils are presented in Table 2. The yield showed that variety E had the highest oil content of 39.44 ± 1.60 percent. It was followed by variety B (39.18 ± 1.20%), variety D had the lowest oleic content (31.9 ± 1.50%). The Vitamin E (VE) concentration was found to be highest in variety E (122.65 ± 2.42 µg/g) and lowest in variety A (9.42 ± 2.10 µg/g). Both the oil yield and vitamin E content exhibited significant differences at $p<0.05$. The fatty acid composition in the seed oils studied showed presence of various components which may be of nutritive value since they contain appreciable amounts of essential fatty acids (EFA) which humans cannot synthesise (Mc-Murray *et al*., 2010) and vitamin E (VE) that play a critical role in human life.

Table 2. Percentage oil yield and Vitamin E concentration of the pumpkin cultivars

VARIETY	Oil yield %	Vitamin E µg/g
A	34.77±1.1^{abcd}	9.42±0.9^{a}
B	39.18±1.9^{bd}	23.5±1.9^{a}
C	34.24±2.0^{abcd}	24.42±1.0^{a}
D	31.90.6±^{a}	36.29±5.1^{a}
E	39.44±0.7^{bcd}	122.65±2.0^{b}
F	35.23±1.8^{abcd}	35.42±3.1^{a}
G	33.33±4.0^{ab}	31.02±1.2^{a}
H	33.98±0.7^{a0bc}	13.08±0.7^{a}
I	36.28±2.6^{abcd}	23.98±0.5^{a}

The crude fat in the seeds varied from 31.9% to 39.44%, These values were higher compared to *Citrullus lanatus, Cucumis prophetarum, Cucumis sativus*, *Luffaechinata,* and *Cucumis* seeds of *Cucurbit* species cultivated in Sudan ranging from 10.9-27.1 reported by Mariod*et al*. (2009) but relatively lower than those of *Cucumeropsis mannii*, *Cucurbita maxima*, *Cucurbita moschata*, *Lagenaria siceraria* and *Cucumis sativus*, cultivated in Cameroon which had oil content ranging from 40-56% (Fokou *et al*., 2009).

In addition to the fatty acid composition, the composition of tocopherol is an important characteristic feature to describe the identity of vegetable oils. Although tocopherols represent an important class of antioxidants, the pumpkin seed oil contained α-tocopherols content of between 9.42-122.65 µg/g with no significant variation ($p<0.05$) among the cultivars. α-tocopherol act as an natural antioxidants which aid in protecting the polyunsaturated fatty acids against peroxidation (Kamal-Eldin and Andersson, 1997). Presence of considerable amount of α-tocopherol, especially in cultivar E might be linked with the good oxidative stability of these oils.

Generally oils from Cucurbit seeds are highly valued as potential sources of tocopherols Nyam *et al*. (2009). The concentration of α-tocopherol from cultivar E 122 µg/g was higher than those reported for soy bean (99) and palm oil (89) oils but lower than ground nut oil (178), cotton seed (338) and rape seed (202) oils (Rossell, 1991). In a study by Nyam *et al*. (2009) reported that Malaysian Cucurbits had high amounts of α-tocopherol ranging from 151-442.2mg/kg. Nakić e*t al*. (2006) reported that *Cucurbita pepo* seed oil had 454-709mg/kg of total tocopherols with contribution of α-tocopherol being (6.9-14.46 mg/kg).

The values of tocopherols reported by Murkovic *et al*. (1996) in oil extracted from 100 breeding lines of *C. pepo* varied from 0 to 91 mg/kg and from 41 to 620 mg/kg for α- and γ-tocopherol, respectively. It is also reported that the content of α- and γ-tocopherol in the *C. pepo* oil was 37.5 and 383 mg/kg, respectively (Murkovic *et al*., 2004). François *et al*. (2006) reported concentration of α- and γ-tocopherol in raw *C. pepo* seed oil of 76.9 and 964 mg/kg, respectively. In the seed of African *C. pepo*, however, the α-tocopherol content was reported to be 30 mg/kg (Younis *et al*., 2000) while Murkovic *et al*. (2000) reported content of α-tocopherol as ranging from 19.9 to 78.7 mg/kg, and γ-tocopherol content from 52.3 to 644 mg/kg.

The results showed that the seeds have good oil yield which could be exploited for domestic and commercial use.

The fatty acid analyses of the seed oils are presented in Table 3. The fatty acids found and determined as FAMEs were oleic (40.51-20.27%), linoleic (58.29-43.4%), palmitic (8.66-18.2%) and stearic (0.52-3.56%) acids. Of the four fatty acid dominant, only oleic acid showed significant differences at $p<0.05$.

Table 3. Fatty acid composition ofthe pumpkin seed oil

Variety	Stearic	Palmitic	Linoleic	Oleic
A	2.01±0.1[a]	8.66±0.15[a]	52.23±1.0[a]	23.64±0.3[a]
B	0.577±0.5[a]	12.52±0.35[a]	56.35±0.4[a]	28.49±0.6[ab]
C	0.16±0.5[a]	18.2±1.4[a]	50.49±0.1[a]	30.5±0.1[ab]
D	5.555±0.0[a]	11.75±0.4[a]	52.21±1.4[a]	28.44±0.0[ab]
E	0.52±0.3[a]	13.45±0.2[a]	43.4±6.1[a]	40.51±0.0[b]
F	3.013±0.2[a]	13.39±0.1[a]	47.84±0.1[a]	24.05±0.4[a]
G	1.015±[a]	17.92±0.8[a]	47.84±2.2[a]	30.46±0.4[ab]
H	3.562±0.1[a]	17.6±1.2[a]	49.13±0.7[a]	25.02±0.9[a]
I	2.296±0.1[a]	12.16±0.7[a]	58.29±0.5[a]	20.27±0.45[a]

The most abundant fatty acid detected in the tested seed oils was linoleic acid, followed by oleic acid, palmitic and stearic acid respectively (Table 3) variety I (58.29%) had the highest amount of linoleic acid, while cultivar E (43.4%) had the lowest. Sew *et al*., (2010) also reported that winter melon seed oil had linoleic acid (67.37%) as the principal component, followed by palmitic acid (17.11%), oleic acid (10.21%) and stearic acid (4.83%), respectively.

As for the fatty acid content, our data are in agreement those reported by Applequist *et al*. (2006) that linoleic acid is 40.4%–57.2 %, Alfawaz *et al*., (2005) reported 18.14 % oleic acid, 53 % linoleic acid and 1.27 % linolenic acid. The fatty acid composition may vary depending on the climatic conditions. It is documented that when the temperature are lower during the last weeks of seed filling, there is normally a shift in fatty acid content from oleic to linoleic acid. Linoleic acid content is normally high in areas where lower average temperature prevails (Younis *et al*., 2000; Murkovic *et al*., 1996). Younis *et al*. (2000) found out that *C. pepo* oil content of oleic and palmitic acid decreased as the average growing temperature decreased. Also, it is confirmed that decreased oleic acid content is followed increased linoleic acid content (Younis *et al*., 2000; Murkovic *et al*., 1996).

The high content of linoleic acid makes the pumpkin seed oil suitable for development as a high linoleic acid crop. As reported by (Green, 1986; Haris *et al.*, 1980; Singh *et al.*, 1998) the proportion of oleic and linoleic acid determines the quality of oil and its use. Seed crops richly endowed in linoleic acid have considerable agricultural importance in the market for edible vegetable oil which is expanding rapidly (Singh *et al*., 1998).

4. CONCLUSIONS

The oil content of the seeds was imperatively high and had a higher proportion of unsaturated fatty acids compared to saturated ones, this is very important in human food because of its prevention of cardiovascular disease. The overall results of this work may offer a scientific basis for use of the seeds and oils in human diet.

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