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Physiochemical Properties and Fatty Acids Compositions of Three Species of Cucurbitaceae

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ABSTRACT

Increasing world population which directly increases the demand and consumption of vegetable oil puts pressure on soybean, groundnut, and other leading plant crops used in the production of vegetable oil in the world. On this bases the seed of *Cucumeropsis manni, Trianchosanthes angunia* and *Cucumis sativa* were analyzed to establish their physicochemical and fatty acids properties using standard procedures of Association of Official Analytical Chemists (AOAC) and GC-MS respectively. The specific gravity values showed no significant differences among the species. Meanwhile, there were significant differences in peroxide value, acidic, saponification, free fatty acid pH and viscosity and iodine values. The seed oil of the three species contained high level of unsaturated fatty acid (linoiec acid) with the value of 43.55%, 65.41%, 53.62% for *C. sativa, T. anguina* and *C. manni* respectively. Oleic acid was found in *C. manni*. The results of the physicochemical and fatty acids of the three Cucurbitaceae have proved their great importance in nutrition and therefore, will reduce the pressure on well-known oil producing plants.

Keyword: Vegetable oil, Cucurbitaceae, physiochemical properties, fatty acids

INTRODUCTION

Oil and fat are important nutritional components with varieties of functions in our body as an energy source, membrane structures, regulating body temperature, and insulate organs (Endo, 2018). Vegetable oils are consumed in all parts of the world. It accounts for 80% of the world's natural oils and fat supply (FAO, 2007). Its increasing importance in nutrition and commerce is due to its dietary energy, antioxidant, bio-fuels, and raw materials potentials (Fasina and Colley, 2008). Increasing world population which directly increases the demand and consumption of vegetable oil puts pressure on soybean, groundnut, and other leading plant crops used in the production of vegetable oil in the world (Mielke, 2018). These strains on edible vegetable oil are compounded as more of these oil resources are diverted towards the production of biodiesel (Albishri *et al.*, 2013).

Cucumeropsis mannii Naudin (melon) (known as "*egusi*" in most countries in West Africa (Essien, *et al.*, 2012) is a plant that grows in temperate and tropical regions of south-west Nigeria area. It has a fibrous and shallow root system, a characteristic of the family Cucurbitaceae. It is an annual crop that crawls and it's grown mostly as a subsidiary crop that can be planted with yam and maize in some savanna areas in Nigeria (Fokou and Achu, 2004). The melon seed is

very rich in oil and has a higher percentage level of protein and amino acids that are essential except for lysine (Fokou *et al*. (2004); El-Adawy and Taha (2001); Badiru *et al.* (1991); Sharma *et al.*, (1986); Nwokolo and Sim (1987) and Martin, (1998). The oils of the seeds are of great importance in industries, drug production (medicinal), and nutrition (Oderinde *et al.*, 2009).

Trichosanthes anguina L. (snake gourd or serpent gourd) is cucurbit with unusual long cucurbit with white spackled fruits. Morphologically, it resembles snake, hence the name snake gourd. It is widely grown as a vegetable. Snake gourd is a variety of *T. cucumerina*. The fruits of *T. anguina* are good sources of nutrients necessary for human and animal health thus contributing its nutritional value (Rahman *et al*., 2008). The roots and seeds are used as treatment to expel worms and also to treat diarrhea and syphilis (Kirtikar and Basu, 1987; Ali and Pandey, 2007).

Cucumis sativus L. (Cucumber) is another plant that is produced worldwide commercially and is known as a seasonal vegetable crop. Cucumber is one of the popular vegetables in many countries. Water is the main component (96%) of the crop and there are a lot of vitamins, minerals, and organic acids in this plant which makes it a nutritious product. It is extremely eaten fresh in cooked vegetables or as a salad (Sotiroudis *et al.*, 2010). Different pharmacological activities which include antioxidant, antimicrobial, antidiabetic, anti-wrinkle and the potentials of lipid-lowering agents of this plant have been reported. Little bioactive compounds belonging to different chemical groups have been discovered from this plant. The cucumber also has some polyphenol contents which have also been reported (Melo, *et al.*, 2006). Curing of burning sensation, constipation, tonic, and intermittent fevers are the usefulness of the seed (Warrier, 1994). Significant ulcer potential of methanolic extract of *C. sativus* seeds is due to the antioxidant activity (Gill *et al.*, 2009).

The present study analyzed the phytochemical and fatty acid properties of *C. manni, T. angunia* and *C. sativa* with aim of determining their nutritional values.

MATERIALS AND METHODS

Sample collection

The seeds were collected from various locations in Ile-Ife, Osun State and Ilorin, Kwara State, Nigeria (Table 1) and were taken to Forestry Herbarium Ibadan (FHI), and University of Ibadan Herbarium and University of Ilorin Herbarium for proper identification.

Species	Shape of Fruit	English name	Nigerian local names	Place of collection	Global Positioning System	Voucher number
Cucumis sativa Linn.	Oblanceolate	Cucumber	Cucunba (Y), Uniokirihio (I), Kokumba (H)	Ilorin, Kwara State	8º30'0"N 4º33'0"E	UIL/002/525/2021
Trichosanthes anguina Linn.	Spherical	Snake gourd	Tomato elejo (Y), Ogiri (I), Guna (H)	Ile-Ife, Osun State	7º28'0"N 4º34'0"E	UIL/003/1425
Cucumeropsis manni Naud.	Round	Melon	Itoro (Y), Ahuelu (I)	Ilorin, Kwara State	8º30'0"N 4º33'0"E	UIL/004/1426

Keys: Yoruba (Y), Hausa (H), Igbo (I).

Extraction of seeds oils

Two grams (2g) of seeds were weighed and dehusked through the removal of testa. They were cleaned and sun-dried. After drying, the seeds were crushed by pounding with laboratory mortar and pestle. The oil was extracted with soxhlet apparatus using petroleum ether 60-80^{o}C (Cocks and Van Rede, 1996).

Determination of physical properties

Specific gravity

A measuring cylinder containing 1cm^3 of oil was weighed. The volume of oil was determined by subtracting the weight of the empty container from the weight of the container containing the oil. Specific gravity was determined by using the following formula.

$$\text{Specific gravity} = \frac{\text{Weight of oil in (g)}}{\text{Volume of oil } (cm^3)}$$

(Temple, 1989).

Viscosity

This is the ratio of the shear stress to the rate of shear of a fluid. The viscosity was determined by placing a suspended level viscometer (type bs/IP/SL) in a constant temperature bath while maintaining the capillary in a vertical position. In capillary viscometer flow times are proportional to kinematic viscosity (Lakshminarayana *et al.*, 1983).

Refractive index

The refractive index of oil was taken as the ratio of the sine of the angle of incidence to the sine of the angle of refraction when a ray of light of wavelength 589.3 mμ passes from air into the oil (Biakales, 1978). The oil samples were dried and filtered before refractive index determinations. A drop of oil was placed in between the glass prisms provided on the refractometer. An ample time of about 5 min was allowed for the oil and prisms of the instrument to attain a steady temperature. The knob was well adjusted after which the readings were taken.

Determination of Chemical Properties

Peroxide Value

Three grams (3 g) of oil was weighed into a 250 ml conical flask. 10 ml chloroform was added to dissolve the oil. Fifteen-milliliter acetic acid, 1.0 ml KI (Potassium iodide) solutions were mixed and left for 5 min in the dark. Thirty milliliter of distilled water was added and 1 ml starch indicator was titrated with sodium thiosulphate. The process was repeated for blank (without oil). Peroxide Value was determined using the following formula:

$$\text{Peroxide Value} = B - A \times 0.1 \times 1000$$

Where: B = blank, A = sample W = weight

Free Fatty Acid

One gram (1g) of seed oil was weighed and dropped into 250 mL conical flask containing 50ml Chloroform. After 5 drops of m-cresol indicator had been added the solution was titrated to purple as end point with aqueous 0.05M NaOH.

$$\text{FFA} = \frac{1000\ x\ 0.5\ x\ V}{W}$$ (Cocksand Van Rede, 1996)

Where V = volume of NaOH, W = weight of oil

Acid value

The following solutions employed for the acid value determination was prepared as prescribed by Cocks and Van Rede (1996). 1g of oil was dissolved in the neutral solvent (0.1 m NaOH solution) and titrated with phenolphthalein indicator. The end point was shown by a persistent pale-pink colouration.The acid value was determined using the following formula:

$$\text{Acid value} = \frac{5.61\ x\ N\ x\ V}{W}$$

Where: N = Normality of NaOH,V = Volume (ml) of NaOH, W = Weight of sample

Saponification Value

Ethanolic KOH solution (10cm^3) was measured into a volume metric flask after which the flask was heated gently. 1g of seed oil and 2 drops of phenolpthalein indicator was then titrated against 0.5m HCl to a colourless end point.The blank titration were carried out under the same conditions. The saponification value was calculated from the formula below

Saponification value $=5.61\ N\ (B\ A)$ (Cocks and Van Rede, 1996) where B = volume of HCl used for blank titration, A = volume of

HCl used for sample titration, N = normality of HCl, W = sample weight in g.

Iodine Value

One gram of seed oil was weighed into a conical flask and 15cm^3 CCl_4 (tetrachloromethane) was added to dissolve the oil. 25cm^3 of Wig's solution was added and the mixture stoppered. This was kept in the dark for 2h. At the end of this period, 20cm^3 of 10% KI (Potassium iodide) solution and 150cm^3 of distilled water was added to the solution. The solution was then titrated against the sodium thiosulphate solution to a faint yellow colouration after which 1cm^3 of starch solution was added. Titration was continued until colouration disappeared. A blank titration was also carried out under the same condition. The iodine value was calculated thus:

Iodine Value = $\frac{12.69N\ (B-A)}{W}$ (Cocks and Van Rede, 1996)

Where: B = titre value of blank titration, A = titre value of test solution, W = weight of oil, N = normality of thiosulphate solution

Acidity/Alkalinity (pH)

The pH of the oil sample was measured using a pre-calibrated pH meter. One gram of the oil sample was taken and dissolved in 100ml of H_2O to determine the pH value after two hour with digital pH meter.

Determination of fatty acid composition:

Fatty acids were converted to their methyl esters (FAME) following the method of He and Xia (2007) with a slight modification. Gas chromatography technique was employed for determining the composition of the oils. The Agilent Technologies GC/MS Instrument Agilent 5975 Series MSD version was used to analyse the fatty acid composition of the seed oils under the following condition: Column type 19091S-433HP-5MS, 325^{0}C (30 m x 250 µm; 0.25 µm film thickness), automatic injector with injection volume as 1 µL.Injection temperature 250^{o}C, interface temperature 300^{o}C Helium carrier gas flow rate was 79.5. 5 mL/ min, split ratio 50:1, split flow 75 mL/min.ms zones set at MS Source 230^{0}C and MS Quad 150^{o}C .The oven temperature programme was 35^{o}C for 5mins, then 4^{o}C/min to 150^{o}C for 2 mins, then 20^{o}C/mins to 250^{o}C for 5mins. Data processing was performed using the NIST library to identify the resulting peaks.

Statistical analyses

Data collected were analyzed using Univariate analysis of variation under general linear model of Statistical Package for Social Science (SPSS) software version 17. Results were expressed as means ± Standard Deviation (SD). A probability value at $p < 0.05$ was considered to denote the statistically significant differences while cluster and principal component analysis (PCA) for the percentage fatty acid composition was done using UPGMA algorithm on Paleontological statistics software (PAST).

RESULTS AND DISCUSSION

The physicochemical properties of the three plant species (*C. sativa*, *T. anguina* and *C. manni*) were analyzed and showed in Table 1. The specific gravity for all the oils ranges from 0.90 ± 0.01 to 0.91± 0.01 g/cm^3 with *T. anguina* having the least value while *C. manni* and *C. sativa* have the highest values. Earlier, Ali *et al.* (2011), Oyeleke *et al.* (2012) and FAO/WHO (2009) reported similar values from *Trichosanthes anguina* (0.92g/cm^3), *S mahagoni* oil (0.91) and watermelon seed oil (0.91g/cm^3), and cucurbits respectively.

The viscosity of the oil samples ranges from 24.38± 2.53 to 36.50±3.02 with *T. anguina* having the highest value while *C. sativa* is has the lowest viscosity. This showed that *T. anguina* is thicker compared to the other oils. The viscosity of the oil is the main factor that governs oil absorption and drainage. The higher the oil viscosity, the slower is the oil drainage (Ziaiifar *et al.*, 2008). The viscosity of oil depends on oil type, frying temperature, and oil quality.

The refractive index (RI) is the ratio of the speed of light in a vacuum to the speed of light through a given material (Mohammed and Ali, 2015; Jack *et al.*, 2013). The RI of oil is related to the degree of saturation and distinctive for each type of oil and is

indicative of oil purity. The results of the RI are same for all the oils of the different plant species with a value of 1.48±0.01 which is similar to the research conducted by Ali *et al.*, 2010 on *T. anguina* (1.48±0.01) but are different from those reported by Lazos (1986) for pumpkin (1.46), Oluba *et al.* (2008) for egusi melon seed oil (1.45) and Ardabili *et al.* (2011) for pumpkin seeds (1.47). The values obtained were slightly above the level (1.466-1.470) recommended by FAO/WHO. This reveals that these oils are thicker, which can be compared to most drying oils (Duel, 1951). The difference in unsaturation as the fat or oil is hydrogenated can be measured using a RI. Molecular weight, fatty acid chain length, and the degree of unsaturation are factors that the RI depends on (Nichols and Sanderson 2003). The high RI of these oils is attributable to the high number of carbon atoms in their fatty acid composition (Falade *et al.*, 2008).

Peroxide value is a useful indicator of rancidity occurring under a mild condition and it is a measure of the primary lipid oxidation products which value gives better information about the deterioration of oil, and thus gives a measure of the primary oxidative products (peroxides and hydroperoxide) of fat and oil (Kapoor *et al.*, 2009). The peroxide value of the studied cucurbits ranges from 2.20±0.1Meq/kg (*T. anguina*) to 9.01±0.08 Meq/kg (*C. manni*). The peroxide value of *C. manni* is higher in this analysis than those reported in the research of Achu *et al.* (2006) (8.48±3.85 Meq/kg) and Arinola and Ogunbusola (2013) (0.14 ± 0.01 Meq/kg). The heating of oil during extraction gives rise to the oxidation of fatty acid which produces more peroxides, thus brings about a higher peroxide value in the oil (Cheftel and Cheftel, 1992). Peroxide value is dependent on factors such as fatty acid present, the quantity of oxygen consumed (state of oxidation), and method of extraction (particularly heat applied). The peroxide values (between 2.20±0.1 and 9.01±0.08) of the selected curbits are within the range of good and edible oil for consumption (Aremu *et al.*, 2016). Studied cucurbits oil can be stored for a long time without deteriorating since rancidity occurs when peroxide value falls between 20.0 – 40.0 mg/kg (Onyeike and Acheru, 2002) as low values indicate resistance to oxidation and translates to oil having higher saturation and possibly contains high antioxidants increasing its shelf life (Decker *et al.* 2010 and Commission, 2001).

Table 1: Physicochemical properties of *Cucumis sativa, Trichosanthes anguina and Cucumeropsis manni*

Taxa	*Cucumis sativa*	*Trichosanthes anguina*	*Cucumeropsis manni*
Specific gravity (g/cm^3)	0.91±0.01	0.90±0.01	0.91±0.01
Viscosity (cSt)	24.38±2.33	36.50±3.02	31.00±1.41
Refractive index at 25°c	1.48±0.01	1.48±0.01	1.48±0.01
Peroxide value (mg/g)	7.01±0.01	2.20±0.10	9.01±0.08
Free fatty Acid	2.51±0.01	4.30±0.01	2.72±0.01
Acid Value (mgKOH/g)	5.01±0.01	8.40±0.01	5.48±0.01
Saponification Value (mgKOH/g)	186.01±0.01	185.01±0.01	204.18±0.01
Iodine value (mgKOH/g)	123.02±0.02	126.40±0.01	112.77±0.01
pH value	6.80±0.01	6.55±0.01	6.30±0.01

The free fatty acid (FFA) percentage in oil indicates its quality and level of degeneration. Storage condition of seeds and duration of storage are factors that also influence the value of FFA (Fokou *et al.*, 2009). The FFA content is more in *T. anguina* (4.40±0.01) compared to *C. sativa* which has the least content (2.51±0.01). These results of FFA is similar to what Ali *et al.* (2011) reported for *T. anguina* (4.2± 0.31), and Akubugwo *et al.* (2008) for *Treculia africana* (4.22±0.01) and *Cocos nucifera* (4.80±0.06). The FFA value indicates the transesterification effect of glycerides. The rottenness of the fat leads to the release of FFAs from triacylglycerol (Ekwenye, 2006). FFA is produced by the hydrolysis of oils and fats and it is not desired in the oil meant for consumption although the method of processing the seeds can be attributed to the high value of FFA.

The acid value is a measure of the FAAs in the oil. The higher the acid value found, the higher the level of FFAs which translates into decreased oil quality. The oil's acid value in this research ranges from 5.01±0.01 to 8.40±0.01 mgKOH/g. *Trichosanthes anguina* has the highest value while *C. sativa* has the least value. The acid value result of *T. sativa* oil (9.01±08 mgKOH/g) was reported by Ali *et al.* (2010) was a little different. Ouattara *et al.* (2015) also reported on Sheanut (11.17 ± 1.62 mgKOH/g), *Sesamum indicum* (1.16 ± 0.06 mgKOH/g), *Cucurbita pepo* (1.29 ± 0.05), and *Cucumis melodrama* (2.51 ± 0.13 mgKOH/g) with most of the results having a lower acid value. The use of oil for human consumption or industrial usage can be influenced by its acid value (Akinyeyea *et al.*, 2011). Acid value for edible oil should not exceed 0.6mg KOH/g as reported by Amoo (2004) and recommended by WHO/FAO indicating that the acid values of the study samples fall outside the nutritional limit. Consuming rancid edible oil is unlikely to cause immediate health impact, but can reduce significantly the nutritional value of foods by degrading the essential fatty acids and nutrients (De Souza *et al*. 2015).

The saponification value (SV) is high in *C. manni* (204.18±0.01 mgKOH/g) than others with *T. anguina* having the least (185.01±0.01 mgKOH/g) and *C. sativa* (186.01±0.01 mgKOH/g). The saponification values of *T. anguina* (187.7±1.0 mgKOH/g), and crude castor oil (185.83 mgKOH/g) and refined castor oil (181.55 mgKOH/g) in the works of Ali *et al.* (2010) and Akpan *et al.* (2006) respectively were similar to the value obtained in this present work. SV is an estimation of oxidation during storage and indicating the level of oil decomposition (Neagu *et al.*, 2013). SV is an important parameter for the industrial use of oil, specifically for soap production (Olanrewaju and Moriyike, 2013). As reported by Akbar *et al.* (2009), high saponification value indicates that oils are normal triglycerides and very useful in production of liquid soap and shampoo industries. Oils with low SV value can be used for the production of soap, candle, and raw materials for lubricants (Agatemor, 2006). SV value of all the studied cucurbits can be used in soap production and other industrial oil uses.

The iodine value of the seed oils of the studied plants ranges from 112.77±0.01 to 126.40±0.01 mgKOH/g. *Cucumeropsis manni* has the least value while *T. anguina* has the highest value. This high value is an indication of high degree of unsaturation. The iodine value for *T. anguina* is close to the result of Ali *et al.* (2011) (127.6±1.7 mgKOH/g) and Younis *et al.* (2000) (123.0 mgKOH/g). The results are higher than those reported by Akubugwo *et al.* (2008) for the iodine value of the seed oils of *Pentaclethram acrophylla* (20.50 mgKOH/g), *Treculia africana* (27.50 mgKOH/g), *Persea gratesima* (52.40 mgKOH/g) and *Telferia occidentalis* (49.4 mgKOH/g). The relatively high iodine value is indicative of the presence of higher C = C double bonds i.e. the higher the iodine value, the greater the number of unsaturated bonds number. The oils with unsaturated fatty acid (monounsaturated or polyunsaturated) are merely absorbed and comparably easier to

decompose into calories than a saturated fatty acid. Aremu *et al.* (2006) reported that the lower the iodine value the lesser the number of unsaturated bonds; thus the lower the susceptibility of such oil to oxidative rancidity. The cucurbits oil studied iodine value is high which is proportional to greater unsaturation and therefore is recommended for consumption. Studies have also recommended switching from saturated to unsaturated fats because of the risk of cardiovascular disease associated with high consumption of saturated fatty acids (De Souza *et al*., 2015; Li *et al*., 2015; German and Dillard, 2004 and Nettleton, 1995).

The pH value for all the species are very close with *C. manni* value being little acidic (6.33±0.01) than the others. The other oil sample of *C. sativa* (6.80±0.01) and *T. augunia* (6.55±0.01) are close to neutral.

The seed oils were also analyzed for their fatty acid contents (Tables 2). Both saturated and unsaturated acids are found in the oil samples. Palmitic acid, linoleic acid, oleic acid, lauric acid, and stearic acid were all present in different seed oils, at different proportions. The presence of higher amount of unsaturated fatty acid of linoleic acid was discovered in *T. anguina* (65.41%), followed by palmitic acid (20.02%) and stearic acid (14.57%) in order of increasing fatty acid content. This is different from Ali *et al.*, (2011), where linoleic acid (20.1%) and palmitic acid (6.4%) are lower, while that of stearic (11.6%) is a little similar. *Cucumeropsi manni* contains a higher percentage of linoleic acid (53.62%) with palmitic acid (22.2%) more than oleic acid (13.05%) and stearic acid (11.13%). *Cucumis sativa* has 43.55% linoleic acid 35.86% stearic acid, 9.01% palmitic acid, and 6.57% lauric acid. From the result, it was observed that unsaturated fatty acid of linoleic acid is present in all the plant species and has the highest content of fatty acid. This result indicated that the GC-FID with conditions and parameters used was stable in detecting fatty acids.

Table 2: Fatty acids component identified in the *Cucumis sativa, Trichosanthes anguina and Cucumeropsis manni*

S/N	Common names	Indentity of fatty acids	Symbol	*Cucumis sativa*	*Trichosanthes augina*	*Cucumeropsis manni*
1	Palmitic acid	Hexadecanoic acid	16:0	9.01	20.02	22.2
2	Stearic acid	Methyl Stearate	18:0	35.86	14.57	11.13
3	Lauric acid	Dodecanoic acid	12.0	6.51	-	-
4	Linoleic acid	9,12-Octadecanoic acid (Z,Z)	18:2	43.55	65.41	53.62
5	Oleic	7-octadecanoic acid	18:1	-	-	13.05

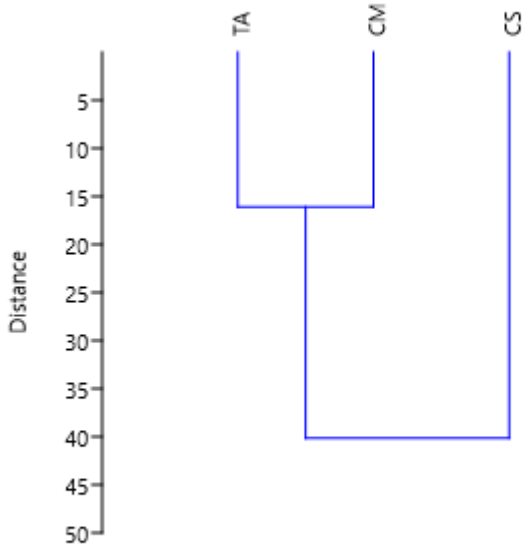

Fig. 1. Cluster analysis showing the relationship among *Cucumis sativa, Trichosanthes anguina and Cucumeropsis manni* based on fatty acids composition (Using UPGMA)

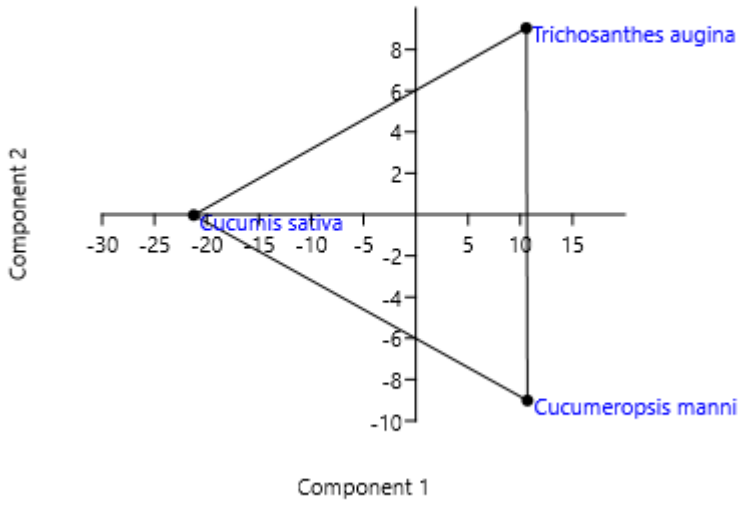

Fig. 2: Principal component analysis performed on fatty acids of *Cucumis sativa, Trichosanthes anguina and Cucumeropsis mannii.*

The dendrogram showing the relationships established among the studied species (Fig. 1), based on the fatty acid composition of the three species underlined a great affinity that exists between *Trichosanthes augina and Cucumeropsis manni* while *Cucumis sativa* is a bit distantly related to the other two. The similarity between *Trichosanthes augina and Cucumeropsis manni and Cucumis sativa* suggesting a relatively evolutionary division explained by Hutchintson and Dalziel, (1954).

The two components of PCA explained 100% of the total variance (89.46 and 10.5% respectively Fig. 2). This would imply that the data on elemental composition of the examined elements can be explained by the two variables without loss of the total variance. The first component which account almost total variance, (89.46%) explain a lot on the similarity of the three species and this is in consistent with criteria established by Sneath and Sokal (1973), who affirm that the PC used for interpretation must explain at least 70% of the total variance.

CONCLUSION

The current finding demonstrated that these cucurbits seeds have great potential to be utilized as a source of new vegetable oil for human consumption and their full potential should be exploited in the search for alternative oil sources, particularly those rich in unsaturated fatty acids as fatty acids content of these cucurbits has more of unsaturated fatty acids.

Conflict of interest

The authors declare that they have no conflict of interests.

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