Short note

Assessment on seed oil percentage and physicochemical properties of watermelon (*Citrullus lanatus*)

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Recent studies have shown that seeds of some Cucurbitaceae family plants contain a substantial amount of oil that can be exploited and for this reason, two watermelon accessions W1-1 and PI-186490 with 14.75% and 22.25% seed oil were used, respectively, and their physicochemical properties analysis in this study. Five main fatty acids (FAs) were detected through Gas Chromatography-Mass Spectrometry. W1-1, fatty acids percentage increased from α-Linolenic acid (1.12%) to Stearic acid (10.23%) to Palmitic acid (19.63%) to Linoleic acid (26.37%) to Oleic acid (26.82%), whiles in PI-186490 it increased from α-Linolenic acid (0.31%) to Stearic acid (10.81%) to Palmitic acid (13.67%) to Oleic acid (17.21%) to Linoleic acid (40.78%). Correlation between the various fatty acids and seed oil percentages (SOP) were examined to understand possible relationship that may exist. Significant difference was observed in SOP and concentration of fatty acids. Other properties that were examined were acid value (mg KOH/g), free FAs (%), oxidizability value (Cox), saponification value (mg KOH/g), unsaponifiable matter (%), specific gravity (25°C). The results of watermelon seed oil properties compared with standards set for consumable oils, confirmed the domestic and industrial economic potentials for watermelon seed oil.

Keywords: Watermelon, seed oil percentage, fatty acid composition, physicochemical properties.

INTRODUCTION

Cucurbitaceae family produce many fruits which supplies significant nutrients needed for effective growth in humans [1]. Diploid watermelon (2n = 2x = 22) is of great influence in the cucurbitaceae family not just for the fruit value but also for the promising seed-oil extraction potential [2]. The global market for vegetable oils is mainly dominated by soybean, sunflower, palm and recently rapeseed oil [3]. For the past ten years (2004 to 2013) under review, the global vegetable oil import and export grew by 65.4% and 67.1% respectively [4]. This estimated sharp increase of 6.5% in the year-by-year consumption of vegetable oil for domestic and industrial use by these major sources cannot meet the increasing demand. Therefore, the urgent need and responsibility to explore other lesser-known sources to increase global supplies. Proximate analysis revealed watermelon seed contains major elements such as Zinc, Calcium and Magnesium, Protein, Crude fibre, Fat, Carbohydrates [5, 6]. Vegetable oils are known to be fats that are extracted from some seeds, nuts or fruits possessing liquid state at room temperature. Vegetable oils are either used for domestic (cooking oil, margarine, non-dairy creamers, ice cream, make-up products and cosmetics) or industrial purposes in various forms. Chromatographic analysis is needed for the characterisation of vegetable oil through analysing the TAG and fatty acid

compositions [7]. The main essential fatty acid source for nourishment is through oils from various oil seeds [8]. The greatest enemy of essential fatty acids is light that causes the destruction of vital biological properties of these acids [9]. Omega-3 and 6 fatty acids are precursors for several substances in the body, which are involved in a blood pressure regulation and anti-inflammatory process. The α-Linolenic acid which is part of omega-3 acids are necessary for prostaglandins production. Omega-3 plays important functions in our bodies by having preventive effect on many modern lifestyles diseases by reducing the risk of cardiovascular disease, modifying of the blood pressure, improving the elasticity of blood vessels and reducing the level of cholesterol and blood coagulation [9].

Fatty acids play a key role in human physiology as energy source and membrane constituent. Fatty acid (FA) composition of vegetables is usually categorised as saturated, monounsaturated and polyunsaturated [10]. Saturated fatty acids (SFAs) controls signalling in many cells and covalent modification of proteins [11]. Consumption of it in high quantities is linked to increase in total and low-density lipoprotein cholesterol leading to the high risk of cardiovascular disease (CVD) [11-14].

This study is to explore, through established protocols, the seed oil percentages of watermelon seeds and whether they agree with the recommended standards set for vegetable oil for food or for other industrial uses.

MATERIALS AND METHODS

W1-1 and PI-186490 as shown in Figure 1 are the watermelon accessions utilised for this study. W1-1 seeds were solicited from the Laboratory of Molecular Genetic Breeding in Watermelon and Melon, NEAU, China. Angela R. Davis of South-Central Agricultural Research Laboratory (USDA) and currently working with Sakata Seeds Company in the United States donated the PI-186490 seeds for this study. The seedlings were raised in the nursery under recommended growth conditions and transplanted at three weeks after sowing.

EXTRACTION OF SEED OILS

Oil from watermelon seeds were extracted using the Soxhlet extraction method with n-Hexane as the extraction solvent according to AOAC1995 Official Method 963.15 [15]. Initial 15 g seeds of 5% moisture content from each accession were separately pulverised using a regular kitchen blender into fine powder. Four (4 g) of powder was immediately weighed and transferred into filter paper and folded tightly with the help of staple-pins. The extraction duration was set at 4 hours due to the small sample size. Total extracted oil was expressed as percentage of 4 g seed powder. All reagents used for analysis were of the analytical grade.

FATTY ACID METHYL ESTER PREPARATION

Due to the unrefined (crude) nature of the extracted oils with expected free fatty acids (FFAs) to be above 0.5%, Fatty Acid Methyl Ester (FAMEs) was prepared according to EN ISO 12966-2:2011 general transmethylation/methylation method [16]. 50 mg of oil was transferred into 10 ml ground-glass necked flask. 2 ml 0.2 mol/l sodium methoxide in methanol and boiling chips were added. The mixture was refluxed under heat using reflux condenser for 7 min to be clear. The flask was removed from the heat source, waited for the reflux to end and separated from the condenser. Two drops of phenolphthalein solution were added.

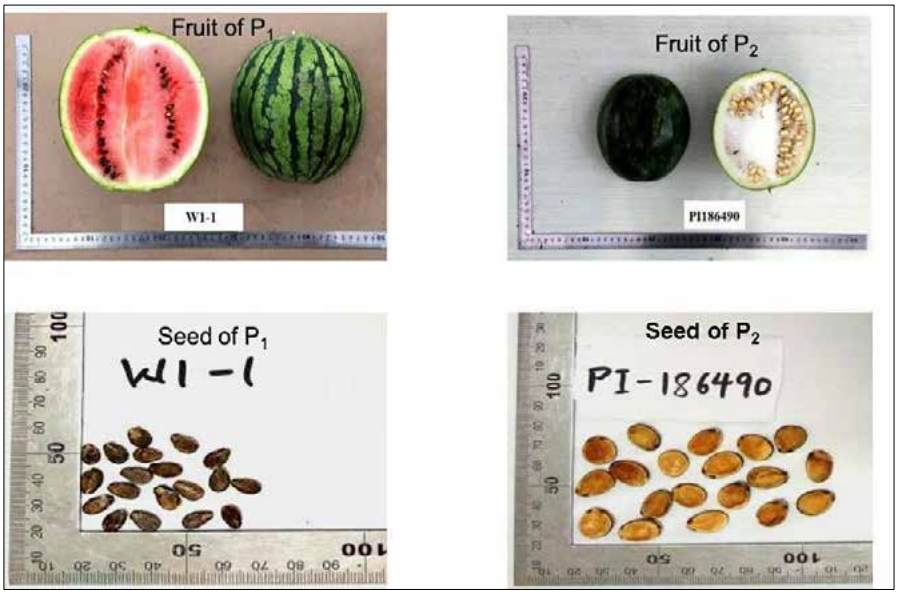

Figure 1 - Fruit and seed of the two accessions (W1-1 and PI-186490)

Sufficient 1 mol/l sulfuric acid in methanol solution was added until the solution becomes colourless. The flask was fitted to the condenser and heated again for 7 min. Mixture was removed from the heat source and allowed to cool under running water. 4 ml sodium chloride solution was added and shaken briefly. 1 ml isooctane added, and flask plugged, the flask was shaken vigorously for 15 sec and allowed to settle for two phases separation. Sodium chloride solution was added again until the aqueous layer reached the lower end of the flask neck while the upper layer containing the methyl esters filled the flask neck. The upper isooctane layer was transferred into a GC sample vial for analysis.

CHROMATOGRAPHY ANALYSIS

A robust Agilent GC 6890-MS 5973N Gas Chromatography-Mass Spectrometer furnished with Column: DB-5 (60m × 0.25mm) and 0.25 µm film thickness. Chromatographic conditions: inlet temperature 250°C, carrier gas He flowing at 1.0mL/min. It was temperature-programmed to raise from room temperature to 150°C for 2 minutes, then raised to 250°C at 5°C per minute for 10 minutes at this temperature. Splitless injection of 1 µl of sample was applied. The solvent then delayed for 10 minutes. Mass spectrometry ion source was scanned at 225°C and Ionisation mode: electron energy 70 eV; 50 ~ 500 amu scanning mass range.

CHEMICAL PROPERTIES

All chemical properties were analysed according to their respective official protocols stated below.
Modified official AOCS Method Ca 5a-40 [17] by titration and indicator as phenolphthalein was used in determining the acid value. The equations: Acid value = [Titre (mL) × 5.61] / Sample weight, Free Fatty Acid = Acid value × 0.503 previously reported by Egbuonu et al [18] were used for the calculation. Oxidizability value (Cox) was worked out using the equation reported previously as COX = [1 (Oleic acid%) + 10.3 (Linoleic acid%) + 21.6 (α-Linolenic acid %)]/100 [19]. Saponification value was measured according to the AOAC Official Method (2005) 920.160. Using 2 g of oil dissolved in 0.7 N alcoholic KOH solution. The mixture was refluxed for complete saponification. After 2 h, the solution was titrated with a 1 N sulfuric acid solution using a phenolphthalein solution 1% in ethanol solution as an indicator.

CHEMICAL REAGENTS

All chemical reagents used were of analytical grade and sourced from reputable companies. Potassium hydroxide (KOH), n-hexane (GC/MS grade), Phenolphthalein, Isooctane (2,2,4-Trimethylpentane), Ethanol, Methanol. Sodium methoxide (CH_3NaO), Sulfuric acid (H_2SO_4), Sodium chloride (NaCl)

STATISTICAL ANALYSIS

For results reliability, analysis had to be replicated 3 times. Microsoft Excel 2013 and SPSS version 23 were used for data analysis.

RESULT AND DISCUSSION

SEED OIL PERCENTAGE

The seed oil percentage of 4 g seed powder were 14.75 and 22.25 for W1-1 and PI-186490 respectively manifested significant differences as displayed Table I. The results were in line with the earlier findings of *Citrullus lanatus* var. *Citroides* 19% [21], *Citrullus Vulgaris* 26.52% [22] and *Citrullus lanatus* 28.25 to 35.65% [23] watermelon seed. This suggests that watermelon seed can be considered as an economically viable seed oil source.

FATTY ACID COMPOSITION

The concentration of unsaturation fatty acids in oil is said to be directly proportional to the oxidative deterioration [24]. The key indicators tested for to guarantee the wholesomeness are possible adulteration, stability and nutritive value of the oil [25]. Five major fatty acids were identified in oils from the two watermelon accessions through Gas Chromatography-Mass Spectrometry analysis as demonstrated in Table II expressed as % of total lipid. Significant statistical difference was recorded in the fatty acids of the

Table I - Seed oil Percentage (means, standard deviation) of two watermelon lines

Trait	W1-1 Mean±SD	PI-186490 Mean±SD
Seed Oil Percentage (%)	14.75 ± 1.11**	22.25 ± 0.45**

Values followed by ** implies significant different ($p<0.05$)

Table II - Fatty acid profile of seed oil from two watermelon (Citrullus lanatus) accessions W1-1 and PI-186490

	Palmitic acid		Stearic acid		Oleic acid		Linoleic acid		α-Linolenic acid	
	W1-1	PI-186490	W1-1	PI-186490	W1-1	PI-186490	W1-1	PI-186490	W1-1	PI-186490
Mean	19.63**	13.67**	10.23**	10.81**	26.82**	17.21**	26.37**	40.78**	1.12**	0.31**
SD	0.13	0.35	0.11	0.06	0.08	0.20	0.39	0.07	0.07	0.01
Min	19.50	13.27	10.13	10.75	26.73	17.00	25.92	40.70	1.06	0.30
Max	19.75	13.93	10.35	10.85	26.88	17.40	26.64	40.84	1.20	0.32

Within each fatty acid type, values ** were significantly different ($p<0.05$)

oils from the two study materials. In W1-1, the fatty acids percentage increased from α-Linolenic to Stearic to Palmitic to Linoleic to Oleic, whiles in PI-186490 it increased from α-Linolenic to Stearic to Palmitic to Oleic to Linoleic. The two oils contained encouraging amount of essential fatty acids (α-linolenic and linoleic) signifying our findings [26]. Consumption of food lacking the daily requirements of these five major fatty acids can lead to serious health problems [27]. In W1-1, values of 29.86%, 26.82% and 27.49% were recorded for (SFA, MUFA and PUFA) respectively. That of PI-186490 were 24.48%, 17.21% and 41.09% for (SFA, MUFA and PUFA) respectively as noted in Table III. The recorded total saturated fatty acid in the two examined oils were higher in earlier report [9]. The α-linolenic acid value of 1.12% and 0.31% for W1-1 and PI-186490 was within reported range [28]. Identification of palmitic and stearic acids as major saturated fatty acid agreed to similar findings presented before [24, 29]. However, other researches recorded indicate that watermelon seed oil is constituted of high unsaturated fatty acids [2].

PUFA value of (27.49% and 41.09%) for the two accessions conformed to the range of values by previous investigations [24, 29]. PUFA/SFA score above 1 is considered to have nutritional value [25]. A low PUFA/SFA value of 0.92 was recorded for W1-1 seed oil and high PUFA/SFA value of 1.68 for PI-186490.

OIL PERCENTAGE AND FATTY ACIDS CORRELATION

Pearson correlation assessment was necessary to identify the relationship between oil percentage and constituent fatty acids as shown in Table IV. Seed oil percentage (SOP) had no significant correlation at (p<0.05) but positive significant correlations was observed at (p<0.01) with stearic, linoleic but negatively correlated with palmitic, oleic and α-Linolenic acids. Palmitic and α-Linolenic acids correlated negatively with stearic and linoleic acids. Stearic acid correlated positively only with linoleic acid.

PHYSICOCHEMICAL PROPERTIES

Physicochemical properties information plays integral role in the quality assessment of the oils. Reports attested of values for physicochemical properties of vegetable oils showing considerable variations as a result of their diverse sources [30, 31]. This study analysed six physicochemical properties of oils from two parents and their corresponding result values as in Table V below.

Acid value of the oil which is the KOH amount in mg required in neutralising organic acids in 1g of oil helps in the determination of the wholesomeness of oils since high acid value indicates increased susceptibility of oils to rancidity [32]. The acid values of 2.78 and 2.88 mg KOH/g for W1-1 and PI-186490 respectively conformed to the global recommended level of 4 mg KOH/g [33]. An increase in the amount of FFA is an element in the testing of degradation in oil [34].

The acid and free fatty acids (as percentage of oleic acid) values recorded for W1-1 was significantly higher than that of PI-186490. 2.78 mg KOH/g oil and 2.88 mg KOH/g oil, 1.39% and 1.44% for W1-1

Table III - Summary of Fatty acid types of W1-1 and PI-186490 watermelon seed oil samples (% of total lipid).

Fatty Acid Types	Means	
	W1-1	PI-186490
Total SFA	29.86%	24.48%
Total USFA	54.31%	58.30%
MUFA	26.82%	17.21%
PUFA	27.49%	41.09%
MUFA/PUFA	0.98	0.42
PUFA/SFA	0.92	1.68
Total SFA/ Total USFA	0.54	0.42

SFA: saturated fatty acids, USFA: unsaturated fatty acids, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid.

Table V - Means and Standard deviation of seed oil physicochemical properties.

Physicochemical properties	Means±SD	
	W1-1	PI-186490
Acid value (mg KOH/g)	2.78±0.02**	2.88±0.01**
Free fatty acid (%)	1.39±0.01**	1.44±0.05**
Oxidizability value (Cox)	3.23 ±0.02**	4.44 ±0.04**
Saponification value (mg KOH/g)	210.42±1.33	211.33±1.30
Unsaponifiable matter (%)	0.90±0.02	0.91±0.03
Specific gravity (25°C)	0.83±0.01	0.86±0.02

Values with ** are significantly different (p<0.05)

Table IV - Seed oil percentage and fatty acids Pearson correlation coefficients

	SOP	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	α-Linolenic acid
SOP	1					
Palmitic acid	-.996**	1				
Stearic acid	.968**	-.965**	1			
Oleic acid	-.999**	.999**	-.971**	1		
Linoleic acid	.998**	-.996**	.975**	-.999**	1	
α-Linolenic acid	-.991**	.994**	-.964**	.995**	-.992**	1

** Significant at the 0.01 level.

and PI-186490 seed oils, respectively. The detected free fatty acid agreed with the recommended below 5.0% [30]. Oils with low free fatty acid content tends to have enhanced acceptability and nutraceutical value.

The oxidizability value (COX) results for W1-1 and PI-186490 oils were 3.23 and 4.44 as shown in the Table V. Significant difference was recorded at $p<0.05$ between values of the two oils. The watermelon oil had higher oxidative stability that is higher than reported for palm oil (1.615) and camellia oil (1.772) Xu et al [19].

Saponification value in the two accessions demonstrated high significance. Observed saponification values (210.42 mg KOH/g - 211.33 mg KOH/g) were higher than existing 205.3 mg KOH/g [18] but lower than 220 mg KOH/g [30] both reported in watermelon seed oils, suggesting watermelon oils possess lighter average molecular weight of triglycerides and may be ideal for domestic and industrial production of cosmetic products like soap and shampoo.

Specific gravity of oil which is the heaviness of the oil compared to that of water. Bottle pycnometer was used for measuring the specific gravity in accordance with AOAC (2005). The experiment's result was 0.83 and 0.86 for W1-1 and PI-186490 respectively. These results were in close agreement to 0.899-0.920 range set for vegetable oils [32, 35]. Specific gravity of 0.87 is an ideal indicator of suitability for cosmetic use [18]. This attests that both oils examined in this study can be suitable for domestic and industrial purposes as stated earlier above.

CONCLUSION

Results on the various watermelon seed oil properties agreed with the recommended standards set for vegetable oil. The results presented by this study will provide useful information for future breeding and a key to unlock the great potential of watermelon seed oil as novel source to help alleviate the high demand on the global vegetable oil market.

Conflicts of interest

The authors hereby openly declare the absence of any conflicts of interest.

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