EVALUATION OF PHYSICOCHEMICAL PROPERTIES AND TOTAL PHENOL CONTENTS OF WATERMELON (ROTHMAS AND SUGAR BABY) SEED OILS

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ABSTRACT

This study investigated the physiochemical properties of watermelon (Rothmas and Sugar baby) seed oils and the effects of temperature were evaluated and compared with bitter melon (conventional) oil. This was with a view to utilizing the oils for both domestic and industrial purposes. The iodine values were 183.2, 193.0, and 197.1 g iodine/100 g oil for bitter melon, rothmas and sugar baby oils respectively. The total phenol contents were 454.2, 538.6, and 590.3 mg/kg for rothmas, bitter melon and sugar baby oils respectively. The refractive indices of watermelon oils were higher than that of bitter melon oil while the surface tension, specific gravity, smoke point and viscosity of bitter melon oil were higher than that of watermelon seed oils. The properties of watermelon oils revealed that the oils could be useful as drying oil in paint industry, releasing agent in baking processes, production of biodiesel, as lubricant and in deep-frying purposes. They could also be dietary sources of natural antioxidants.

Keywords: Melon Oil, Nonconventional, Physicochemical Properties, Smoke Point, Total Phenol

INTRODUCTION

There has been an increase in the demand for vegetable oils for both domestic and industrial purposes which has led to increase in world prices of these important commodities. The world vegetable oil consumption as at 2010 was put at 150.8 million metric tons (USDA , 2011) and the commodity prices for 2005 released by the World Bank (World Bank, 2005) were for groundnut (1) palm kernel (2) soybean (3) and palm oils (4) \$1101.3 (1) \$655.6 (2) \$548.0 (3) and \$421.7 (4)/metric ton respectively. When these prices were compared with earlier ones, it was obvious that the prices have increased astronomically and the vegetable oils are vast getting out of the reach of the poor. Biodiesel, which is now generally believed to be suitable replacement for fossil fuel because of its advantages over the latter will put more pressure on the demand for conventional vegetable oils hence further increase in prices of these vegetable oils, is expected. As a result, it has become imperative to source for other vegetable oils from some of the underutilized oil seeds available around . If this is done, it will help reverse the upward trend in the movement of the prices of these essential commodities.

Nutritionally, vegetable oils have been reported to be sources of natural antioxidants majority of which are polyphenolic in nature (Akanni *et al.,* 2005; Andrikopoulos *et al.,* 2002). Antioxidant compounds in vegetable oil protect or defend it against lipid oxidation (known to cause rancidity) and also of immense benefit to humans and animals if ingested through plant foods (Andrikopoulos *et al.,* 2002; Tapiero *et al.,* 2002). Some diseases such as cancer, coronary heart disease and inflammation have been linked to oxidative stress (Tapiero *et al.,* 2002; Sharma and Sultana, 2004).

Polyphenols are natural antioxidants that have been reported to play important role in human nutrition as protective agents against several oxidative stress related diseases such as coronary heart disease (Rababah *et al.,* 2004) and some cancer (Hu *et al.,* 2004).

Watermelon (*Citrullus lanatus*) is cultivated mainly for its juice, nectars and fruit while its seeds are regarded as waste. The seed that could have been used as human food to compensate for its high cost is regarded as waste. It has been reported that watermelon seeds can be utilized successfully as a source of good quality edible oil and protein for human consumption (Akoh and Nwosu, 1992). The seeds are utilized for human consumption in India, some African and Arabian countries (Mello *et al.,* 2000). The seeds have been reported to be rich in protein and lipids (Mabaleha *et al.,* 2007, Wani *et al.,* 2008). Bitter melon (*Citrullus colocynthis L.*) known as *Egusi* in Western Nigeria on the other hand is cultivated for its seeds and oil. Its fruit is not edible because of its bitter taste, but the seeds are employed both as condiment and thickener in Nigerian local soup. The seed is very rich in oil (53 %) and protein (28 %) (Ntui *et al.,* 2009). The seed oil has been investigated for its potential as a raw material for biodiesel production (Giwa *at al.,* 2010). Although, investigations have been carried out on the chemical composition and physical properties of watermelon seed oils, the information is scanty or inadequate. The present study was designed to investigate the characteristics of watermelon seed oils with a view to comparing their physicochemical properties with that of bitter melon oil (conventional oil). The results of this study will provide information on the possibility of utilizing watermelon seeds, regarded as waste, for the production of valuable vegetable oils. This will eventually help to solve the problem of high prices of conventional vegetable oils.

MATERIALS AND METHODS

Materials

Two species (Sugar baby and Rothmas) of Watermelon (*Citrulus lanatus*) and the dried seeds of bitter melon (*Citrullus colocynthis* L.) were purchased from fruits market in Ile-Ife, Nigeria. These samples were identified in the herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Processing Techniques

Watermelon was cut open with knife and the seeds were extracted with hand and washed with tap water. The seeds were air dried at room temperature for a period of two weeks. The dried seeds of watermelon and bitter melon were shelled manually. The kernels were removed with hand and ground into fine powder using National, MX-795N, blender Matsushita Selangor, Malaysia. The fine seed powder was stored in a plastic container inside a refrigerator at between 4 - 5°C until used.

Reagents and Chemicals

All reagents used in the study were of analytical grade (Sigma Chemical Co. St. Louis, MQ, USA and BDH Chemicals Ltd, Poole England).

Methods

The oils were extracted with petroleum spirit (60-80°C boiling range, Sigma) using AOAC method (AOAC, 1990). The chemical parameters were determined as reported by the Association of Official Analytical Chemists' methods (AOAC 920.158; AOAC 936.15; AOAC 936.16; AOAC 933.08 and AOAC 965.33) for iodine, saponification, acid, peroxide values and unsaponifiable matter, respectively (AOAC, 1990).

The total phenol (TP) was extracted from oil samples as previously described (Morello *et al.,* 2004) and then estimated spectrophotometrically using Folin – Ciocalteu's phenol reagent assay reaction and garlic acid as standard (Falade *et al.,* 2008). The TP content was expressed as mg/kg gallic acid equivalent (GAE) and the linearity range for the standard was between 0 – 40 mg/L GAE (R^2 = 0.9928).

The Spiking method was used for quality control of the total phenol analysis. Bitter melon oil (3.00 g) was weighed in triplicate and each was spiked with 20 mg/L gallic acid and phenol extracted as described earlier (Morello *et al.,* 2004) and then determined spectrophotometrically (Falade *et al.,* 2008).

Viscosity measurement [in centistokes (cSt)] was determined using an Oswald Kinematics viscometer (Schott Instrument GmbH, Germany) with an attached water bath and a thermometer within a temperature range of 30 - 80°C. Specific gravity has also determined at a temperature range of 30 – 80°C using a specific gravity bottle (BS 733 Jaytec, UK). The refractive index and the surface tension of the oils (at room temperature) were determined with an Abbe refractometer (Carl Zeiss 114484, Germany) and a "drop number" method, respectively (AOAC, 1990). Smoke point was determined by placing 20 mL of oil sample into a stainless steel crucible. A thermometer was inserted in the oil sample and the crucible with the oil was heated under strict temperature regulation using a thermostat-equipped hot plate. Smoke point was taken as the temperature at which the sample gave off a thin, continuous steam of smoke (Hoffmann, 1986).

Statistical Analysis

Results were expressed as mean ± SD, n = 3. Data were subjected to one way analysis of variance (ANOVA) to determine the levels of significant difference by performing a multiple comparison post test (Tukey) $p \leq 0.05$ was considered significant. Correlation coefficient (Pearson r), which assumed a Gaussian distribution, was also determined between refractive index and iodine value and also between free fatty acid (FFA) and smoke point by using GraphPad InStat version 3.06 for Windows 2003 and considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The chemical properties of the oil samples are presented in Table 1. The ether extract which provides information on the oil content ranged between 55.7 ± 1.1 and 58.8 ± 1.7 % for sugar baby and bitter melon respectively. The results showed that there was no significant difference ($p < 0.05$) in the oil contents of the samples which implies that the watermelon seeds compared favourably well with this conventional oil seed as sources of vegetable oil. The oil content of the watermelon seeds was higher than 49.95 ± 0.5 % reported earlier for a species of watermelon (Das *et al.,* 2002). The iodine value (IV) which is a measure of the degree of unsaturation of vegetable oil was observed to vary

Table 1. Chemical Properties of the Melon Seed Oils

Characteristics	Bitter Melon	Rothmas	Sugar baby
Ether extract †	58.8 ± 1.7 [a]	57.5 ± 2.2 [a]	55.7 ± 1.1 [a]
Iodine value ††	183.2 ± 2.9 [a]	193.0 ± 2.9 [a]	197.1 ± 5.4 [b]
Saponification value †††	173.9 ± 0.3 [b]	166.3 ± 1.2 [a]	171.6 ± 1.4 [b]
Unsaponifiable matter †	0.8 ± 0.3 [a]	1.0 ± 0.2 [a]	1.2 ± 0.1 [a]
Acid value †††	1.4 ± 0.4 [a]	1.6 ± 0.2 [a]	3.5 ± 0.3 [b]
FFA (as oleic acid) †	0.7 ± 0.1 [a]	0.8 ± 0.1 [a]	1.8 ± 0.2 [b]
Peroxide value ††††	13.4 ± 2.8 [b]	5.9 ± 1.5 [a]	8.4 ± 1.4 [b]
Total phenols †††††	538.6 ± 13.8 [b]	454.2 ± 19.7 [a]	590.3 ± 22.3 [c]

Values are means of triplicate determination ± standard deviation of mean.
Values in the same row with the same superscripts are not significantly different at the 5 % probability level.
† Values are expressed in %
†† Values are expressed in g iodine / 100 g oil
††† Values are expressed in mg KOH / g oil
†††† Values are expressed in meq Peroxide / kg
††††† Values are expressed in mg / kg
% Recovery for the total phenols is: 105

between 183 ± 2.9 and 197.1 ± 5.4 g iodine / 100 g oil for bitter melon and sugar baby, respectively. There was no significant difference ($p < 0.05$) between the iodine value of bitter melon and rothmas but sugar baby was significantly higher than both. This showed that sugar baby contained more unsaturated fatty acids than the other two samples making its oil more preferable from the nutritional view point. The findings were in agreement with that of Nyam *et al.* (2009) who observed that the polyunsaturated fatty acid in watermelon was higher than that of bitter melon. Oils rich in unsaturated fatty acids have been reported to reduce heart diseases associated with cholesterol (Yuan *et al.,* 2004). The high iodine value also implies that the watermelon oils have high drying property and hence could have good application in the coating industries (FAO/WHO, 1993). The negative effect of oil with high iodine value is that it has short shelf-life because it will be susceptible to lipid oxidation. Hence, watermelon oils must be protected with an anti-oxidant compound if it must be stored for long. As a result of their high iodine values, the watermelon oils are not suitable for use as biodiesel fuels. This is because high iodine value vegetable oils become viscous due to polymerization (Purdy, 1986), and have a low degree of atomization if use for biodiesel (Snell, 1971). Moreover high iodine value oils could easily polymerize during deep frying of foods (Abramovic and Abram, 2005).

Saponification value which provides information on the suitability or otherwise of vegetable oil for the production of soap was observed to range between 166.3 ± 1.2 and 173.9 ± 0.3 mg KOH/g oil for rothmas and bitter melons respectively. The results showed that the saponification values of watermelon oils compared well with that of conventional oil (bitter melon oil). In fact, the values obtained for sugar baby oil was not significantly different ($p < 0.05$) from that of bitter melon. The value obtained in this study for sugar baby agreed favourably with 173.2 ± 2.1 mg KOH / g reported for Kalahari melon (Nyam *et al*., 2009). High saponification values also indicated that the fatty acids content of these watermelon oils are of high number of carbon atoms (Snell, 1971) which implies that watermelon oils, after hydrogenation, could complement or even substitute some conventional oils in soap making.

Unsaponifiable matter which includes plant sterols, tocopherols, some hydrocarbons, as well as small amounts of pigment and minerals ranged between 0.8 and 1.2 % for bitter melon and sugar baby respectively. There was no significant difference ($p < 0.05$) in the unsaponifiable matter of the samples. This parameter is believed not to have any effect on the quality of biodiesel obtained from vegetable oils (De Bussy, 1975), but it will negatively affect the quantity of soap produced.

The acid value and percentage of free fatty acids (FFA) are indicators of the status of the vegetable oil. It is a general knowledge that free fatty acids are prone to lipid peroxidation leading to reduced smoke point, increase in rancidity and production of offensive odour compared to fatty acids in esterified forms (triacylglycerol) (Nikogosyan, 1997). These parameters ranged between 1.4 ± 0.4 and 3.5 0.3 mg KOH/g oil (acid value) for bitter melon and sugar baby, respectively and between 0.7 ± 0.1 and 1.8 ± 0.2 % (FFA) for bitter melon and sugar baby oils respectively. The values of these two parameters were not significantly different ($p < 0.05$) in bitter melon and rothmas, but were significantly higher ($p < 0.05$) in sugar baby oils. The implication of the acid value and percentage FFA results is that sugar baby oil is prone to lipid oxidation faster than bitter melon oil. Apart from the negative effect of FFA on the stability of vegetable oil, its presence in large amount in vegetable oil also affects its conversion to biodiesel due to the neutralization of alkaline solution used in transesterification reaction leading to the formation of soap (De Bussy, 1975). The soap formed can prevent separation of the biodiesel from the glycerin fraction. This shows that Sugar baby oil can only be suitable for biodiesel production if its FFA content is removed or at least reduced significantly.

Peroxide value (PV) (a primary oxidation product), like acid value, is a quality evaluation parameter. The peroxide value varied between 5.9 ± 1.5 and 13.4 ± 2.8 meq Peroxide/kg oil for rothmas and bitter melon, respectively. There was no significant difference ($p < 0.05$) in the peroxide value of bitter melon and sugar baby oils. These showed that rothmas oil was more stable towards lipid oxidation than the other two samples. It has been reported that for oil to have acceptable storage stability, its PV should be less than five (Snell, 1971). Based on this, it might be safe to conclude that none of the oil samples should be kept for too long except they are well processed or protected with antioxidant compound.

The summary of the total phenol (TP) content is also presented in Table 1. The TP content of the oil samples varied between 454.2 ± 19.7 and 590.3 ± 22.3 mg/kg for rothmas and sugar baby, respectively. The TP content of sugar baby oil was significantly higher ($p < 0.05$) than the other two samples; hence it is expected to have a better shelf life than the two oils but was not so when the peroxide values of the samples were compared. For example, sugar baby oil with the highest TP recorded PV value of 8.4 meq Peroxide/kg as against 5.9 meq Peroxide/kg in rothmas with the least TP. When correlation coefficient was calculated between PV and TP, a non significant negative correlation ($r = 0.50$ at $p < 0.05$) was obtained. This showed that there are other compounds with antioxidant property such as tocopherols in the oil apart from the phenolic compounds that are protecting the oils against lipid oxidation. It has been reported earlier that phenolic compounds contributed about 30 % to oil stability (Ray *et al.,* 1984). The TP content of all the oil samples was higher than 30.3 mg/kg reported for groundnut oil (Falade *et al.,* 2008) which implied that these oils are better sources of anti-oxidants than groundnut oil.

The summary of physical properties of the oil samples are presented in Table 2. The refractive index values for watermelon oils were significantly higher ($P < 0.05$) than that of bitter melon oil. These values are within the range reported for conventional oil such as soybean (1.466 – 1.470) (De Bussy, 1975). High refractive index is an indication of high number of carbon atoms (Rudan-Tasic and Klofutar, 1999) which means that the watermelon oils contain high number of carbon atoms. A linear relationship has been established between refractive index and IV (Rudan-Tasic and Klofutar, 1999) which is in agreement with a high correlation coefficient ($r = 0.70$ at $p < 0.05$) obtained in this study for refractive index versus iodine values. Surface tension of the oil samples varied between 0.0292 ± 0.0002 and 0.0322 ± 0.0001 for rothmas and bitter melon, respectively. The value obtained for bitter melon was significantly higher ($p < 0.05$) than those obtained for watermelon oils. Since surface tension is a measure of the forces of cohesion among the particles of a given liquid, the lower value of surface tension in watermelon oils can be an advantage in their application in making shortenings, salad dressings and as a releasing agent in baking processes.

Table 2. Physical Properties of the Melon Seed Oils

Characteristics	Bitter Melon	Rothmas	Sugar baby
Refractive index	1.4531 ± 0.0001^{a}	1.4653 ± 0.0001^{c}	1.4612 ± 0.0002^{b}
Surface tension [Nm^{-1}]	0.0322 ± 0.0001^{c}	0.0292 ± 0.0002^{a}	0.0300 ± 0.0002^{b}
Specific gravity	0.8942 ± 0.0001^{c}	0.8867 ± 0.0001^{a}	0.8914 ± 0.0001^{b}
Smoke point [^{o}C]	228.3 ± 1.5^{c}	221.3 ± 1.5^{b}	210.0 ± 1.0^{a}
Viscosity [cSt]	14.79 ± 1.32^{a}	12.71 ± 1.10^{a}	13.90 ± 1.14^{a}

Values are means of triplicate determination ± standard deviation of mean.
Values in the same row with the same superscripts are not significantly different at the 5% probability level.

The viscosity of watermelon oils compared favourably with that of bitter melon oil because there was no significant difference ($p < 0.05$) in the values of this parameter in all the oil samples analyzed. These values were also lower than the values reported for some other conventional oils such as soybean (31 cSt), cottonseed (36 cSt), sunflower (43 cSt) at 30 ^{o}C (Kammann and Philips, 1985) and Groundnut oil (63.8 cSt). Low viscosity is a distinct advantage for the use of these melon oils for biodiesel production because low viscosity will make the biodiesel obtained from such oil to have a high degree of atomization resulting in a short ignition delay (Ray *et al.,* 1984). The only problem that can prevent their use in biodiesel production as mentioned earlier is their high iodine values which can be reduced by a controlled hydrogenation of the oils.

Specific gravity is presented in Table 2. The specific gravity of watermelon oils was significantly lower ($p < 0.05$) than that of bitter melon oil. The specific gravity of these oils followed the same trend as observed under viscosity.

The viscosity and specific gravity of the oils decreased with increase in temperature (Tables 3 and 4). The rates with which the oils lost their viscosity with increase in temperature are 64.9, 62.1 and 54.7% at 80^{o}C for bitter melon, sugar baby and rothmas, respectively. These rates were lower than 75% and 77% losses reported earlier for groundnut and *A. colei* oils, respectively (Falade *et al.,* 2008) which implied that these oils will be better as lubricating oil than groundnut and *A. colei* oils.

Table 3. Effect of Temperature on Viscosity of the Melon Seed Oils

	Temperature, [^{o} C]					
Oils	30	40	50	60	70	80
Bitter Melon	14.79 ± 1.32 ^{a}	11.77 ± 1.26 ^{a}	9.51 ± 1.34 ^{b}	8.80 ± 1.57 ^{a}	7.11 ± 0.16 ^{a}	5.19 ± 0.07 ^{a}
Rothmas	12.71 ± 1.10 ^{a}	10.17 ± 1.12 ^{a}	8.04 ± 1.18 ^{a}	7.88 ± 1.40 ^{a}	7.30 ± 0.14 ^{a}	5.76 ± 0.07 ^{b}
Sugar baby	13.90 ± 1.14 ^{a}	10.65 ± 1.06 ^{a}	8.47 ± 1.17 ^{ab}	7.75 ± 1.38 ^{a}	6.79 ± 0.14 ^{ab}	5.27 ± 0.07 ^{a}

Values are means of triplicate determination ± standard deviation of mean in cSt.
Values in the same column with the same superscripts are not significantly different at the 5% probability level.

Table 4. Effect of Temperature on Specific Gravity of the Melon Seed Oils

	Temperature, [^{O} C]					
Oils	30	40	50	60	70	80
Bitter Melon	0.8942 ± 0.0001 ^{a}	0.8904 ± 0.001 ^{a}	0.8881 ± 0.0000 ^{a}	0.8878 ± 0.0001 ^{a}	0.8869 ± 0.0001 ^{a}	0.8800 ± 0.0002 ^{a}
Rothmas	0.8867 ± 0.0001 ^{b}	0.8846 ± 0.0000 ^{b}	0.8828 ± 0.0000 ^{b}	0.8817 ± 0.0001 ^{b}	0.8822 ± 0.0000 ^{b}	0.8775 ± 0.0001 ^{b}
Sugar baby	0.8914 ± 0.0001 ^{c}	0.8893 ± 0.0000 ^{a}	0.8863 ± 0.0001 ^{c}	0.8855 ± 0.0001 ^{c}	0.8848 ± 0.0000 ^{c}	0.8793 ± 0.0001 ^{c}

Values are means of triplicate determination ± standard deviation of mean.
Values in the same column with the same superscripts are not significantly different at the 5% probability level.

Smoke point is the point at which a cooking oil or fat breaks down, when heated. At this point, oil smokes or burns, and imparts unpleasant taste on food. The smoke points of 221.3 and 210.0^{o}C for rothmas and sugar baby oils, were lower than that of bitter melon oil (228.31.5^{o}C) but compared favourably with groundnut oil (213.5^{o}C) a conventional oil (Falade *et al.,* 2008). A strongly significant negative correlation ($r = -0.90$ at $p < 0.001$) was obtained between FFA and smoke point. These results agreed favourable with the joint report of FAO and WHO (1993) that the higher the free fatty acid content, the lower the smoke point. The smoke point revealed that the melon oils could be used in deep-frying purposes. The quality of oils for frying purposes can be further improved by processing the oils to reduce their FFA content.

CONCLUSION

In conclusion, the oil content of the two species of watermelon seeds compared favourably with bitter melon seed. The high iodine value of the watermelon oils implied that the oils will be better as drying oil than bitter melon oil hence could be used to replace conventional oils used as vehicle in the paint industry. The higher iodine value also showed that the Watermelon oils will have higher levels of unsaturated fatty acids, hence better than bitter melon oil from nutritional view point. The saponification value and the total phenol content of the watermelon oils revealed that the oils could be sources of raw material for soap industry and good sources of antioxidant compounds respectively. The physical properties of watermelon oils revealed that the oils could be used in the production of shortenings, salad dressings and as a releasing agent in baking processes. The oils could be used in the production of biodiesel if it is processed to reduce their iodine values. The oils could also be used as lubricant and in deep-frying purposes.

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