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Characterization and Stability Studies of Egusi Melon Seed Oil (*Citrullus colocynthis* L.)

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Abstract
The physical value of oil depends upon its chemical composition, even today these values play a vital role while using different oil for industrial products and also, despite the vast nutritional and medicinal significance of egusi melon, there are little details on the shell life and stability of its oil over time. Therefore, the influence of time and temperature on melon seed oil was investigated at temperatures of 0^oC and 30^oC at different weeks to ascertain its physicochemical value and storage stability. For week zero, at 0^oC and ambient temperature (30^oC), the result revealed iodine value 124.09, Acid value 3.64 mgNaOH/g, Free Fatty Acid value 1.84 mgNaOH/g, Saponification 217.35 mgKOH/g, Peroxide value 1.25 mg/g oil, pH 5.89 and thiobarbituric acid value 0.1383 respectively. In the 5^{th} week, at 30^oC, the result revealed iodine value 91.1543, acid value 12.8921 mgNaOH/g, free fatty acid value 6.4988 mgNaOH/g, Saponification 346.42 mgKOH/g, Peroxide value 9.5mg/g oil, pH 3.2 and thiobarbituric acid value 0.413 respectively. Also at 0^oC in the 5^{th} week, the results were observed as follow: Iodine value 102.53, Acid value 7.96 mgNaOH/g, Free Fatty Acid value 4.01 mgNaOH/g, saponification 287.51 mgKOH/g, Peroxide value 6.1 mg/g oil, pH 5.05, and thiobarbituric acid value 0.2658 respectively. Refrigeration (0^oC) of oil reduced the rate of most of the oxidative deterioration that produces rancidity. These values are within recommended range for edible oils. These results indicate that egusi melon oil could be a good source of table oil. The statistical results show that there was a significant difference between the melon seed oil stored at 0^oC and 30^oC ($P < 0.001$).
Keyword: Thiobarbituric acid, stability studies, saponification value, rancidity, seed oil, egusi.

Introduction

Many plant proteins usually in the form of protein extracts or seed flours are being investigated and tested for new products such as low cost fabricated foods which are nutritious, attractive and acceptable to consumers just like conventional foods from meat, fish and dairy products [1]. Seeds have nutritive and calorific values, which make them necessary in diets. Research attentions that are geared towards increasing utilization of plant protein sources for food use include pumpkin [2, 3, 4, 5, 6]. The ultimate success of utilizing plants proteins as ingredients depends largely upon the beneficial qualities they impact to foods, which in turn depend largely on their nutritional and functional properties [7].

Citrullus lanatus (egusi melon) is the biological ancestor of the watermelon now found all over the world, but originated from West Africa. Egusi melon is a member of the Cucurbitaceae family. Unlike the common watermelon, whose flesh is sweet and red, the egusi melon's juicy flesh is pale yellow or green, and also tastes bitter. A creeping annual herb, the egusi melon has hairy stems, forked tendrils and lobed hairy leaves.

Melon seeds popularly called 'Egusi' (yoruba), ′ogili′ (ibo), 'iguana agushi' (hausa) and 'dende'(Fulani), contains about 53% oil, 28% protein and some other important mineral nutrients [8]. The ''Egusi'' melon (*Colocynthis citrullus L.*) is a widely cultivated and consumed oil seed crop in West Africa. It is a creeping annual plant and an intercropping plant use in traditional farming practice; it thrives well on rich light soil in the hot climate regions of Africa [9].

Comprising 50% oil and 35% protein [10], the seeds have both nutritional and cosmetic importance. The seeds contain vitamin C and B2, minerals, ribflavin, fat, carbohydrates and protein [11]. Despite the vast nutritional and medicinal significance of egusi melon, little details on its shell life and stability of its oil over time.

Egusi seed oil is very rich in essential fatty acids (linoleic) but poor in linolenic acid, this therefore shows that the lower the linolenic acid content of oil, the more suitable is the oil for frying oil, making it good for the fight against

cardiovascular diseases [12]. The physical value of oil depends upon its chemical composition; even today these values play a vital role while using different oil for industrial product [13].

The aim of the present study therefore is to determine some physical and chemical properties as well as stability (which could be used in determining the quality of the oil) of egusi melon oil obtained locally from a South-south region of Nigeria.

Materials and Methods

Chemicals

All chemicals used were of analytical grade and were products of BDH Chemicals Ltd., Poole, England unless otherwise stated.

Procurement and Preparation

Fresh Seeds of watermelon (*Citrullus colocynthis L.*) of the Cucurbitaceae family were purchased from the local market in Ekosodin, Benin city, Nigeria and were identified as *Citrullus lanatus* by the taxanomist in the department of Crop Science, Faculty of Agriculture, University of Benin, Benin City. The melon seeds was purchased with the husks and then screened to remove bad ones, shelled manually and further screened. The melon seeds were oven dried to a constant weight at 70^{o}C, this is necessary prior to solvent extraction, because many organic solvents cannot easily penetrate into foods containing water, and therefore extraction would be inefficient. Some of the seeds were subsequently deposited at the herbarium of the faculty.

Particle size reduction

The Dried melon seed are finely ground using a mechanical blender, put in an air-tight container and stored in the dessicator prior to solvent extraction to produce a more homogeneous sample and to increase the surface area of lipid exposed to the solvent, followed by the extraction of oil using soxhlet extraction with chloroform and methanol in the ratio of (2:1) as solvent for 8 h (60-80°C boiling range) according to the method described by AOAC [14]. At the end of the extraction, the extraction solvent was evaporated in a rotary evaporator (Cehmglass). The extracted oil was used for feed formulation and the remaining stored in light proof, airtight and moisture proof container at -4°C for further analysis.

Physico-chemical characteristics of seed oil samples.

The thiobarbituric acid value was determined using the International Union of Pure and Applied Chemistry (IUPAC), applied chemistry division commission on Oils, Fats and derivatives [15]. The physico-chemical properties like acid value, saponification value, iodine value and peroxide value of melon seed oil, used in this study were determined by American Oil Chemists' Society (AOCS) Recommeded Practices (Official Methods of Analysis of AOAC [16]. The values reported in this study are average of five (5) replicate measurements. The results were compared with those of common vegetable oils / conventional oils in Nigeria.

pH of the Oil

A clean dried test tube was used to measure out 2.0 g of the oil and 13 mL of hot distilled water was added to the oil in the tube. The resulting mixture was stirred slowly and cooled in a water bath at 25^{o}C. A standardized pH electrode was immersed into the sample and pH value was recorded. The procedure was repeated with same weight of oil samples.

Statistical Analysis

Statistical analysis were performed using SPSS 16 statistical software (SPSS inc, Chicago, 1L), the data were subjected to one way analysis of variance (ANOVA) and the differences between samples were determined by Scheffe and Linene's test for equality of variance. P values < 0.01 were regarded as significant.

Results and Discussion

The extraction yield of melon seed oil 60.08% obtained by soxhlet extraction method using chloroform and methanol in a ratio of 2:1 showed that melon seed has a high oil content that can be exploited.

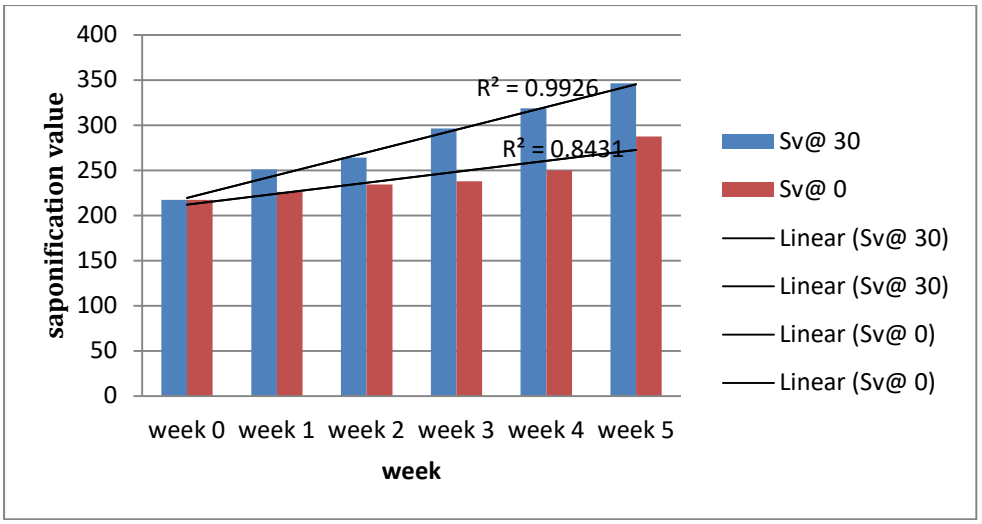

Figure 1: Saponification Value (SV)

The result in figure 1 revealed that saponification value increase significantly with increase in storage time. The saponification value increases from (217.3525 – 346.4175) mgKOH/g in 5 weeks at room temperature 30^{o}C and an increase from (217.3525 – 287.5125) mgKOH/g in 5 weeks at a refrigerated temperature 0^{o}C. The analysis of variance result in figure showed existence of highly significant differences in saponification value due to the effect of storage at different temperature due to the increase in the rate at which rancidity occur at the room temperature compared with the rate of rancidity in the refrigerated temperature. The saponification value was higher compared to that of egusi melon oil 192.0±43.7 mg KOH g^{-1} obtained by Pearson [17]. However, The saponification value of egusi melon oil agrees with the values obtained for some vegetable oils such as coconut oil (253.0 mg KOH g^{-1}), palm kernel oil (247.0 mg KOH g^{-1}) and butter fat (225.0 mg KOH g^{-1}) [18]. As reported by Pearson [17] oil with higher saponification values contain high proportion of lower fatty acids. Therefore, the values obtained for egusi melon oil in this study show that it contains high amounts of higher fatty acids.

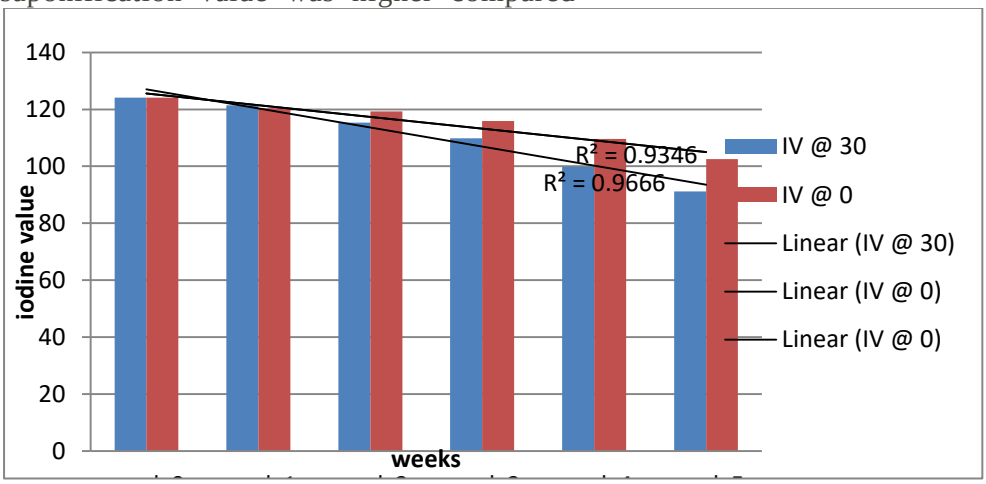

Figure 2: Iodine Value (IV)

The result in figure 2 revealed that the iodine value showed significant decrease with the increase in time. The iodine value decreases from (124.0983 - 91.1543) in 5 weeks at room temperature 30^{o}C and a decrease from (122.0983 – 102.5278) in 5 weeks at refrigerated temperature 0^{o}C. The analysis of variance result in figure showed existence of highly significant differences in iodine value due to the effect of storage at different temperature. The result further substantiate that iodine value at room temperature increase more progressively with time due to the increase in the rate at which rancidity occur is more in at the room temperature compared with the rate of rancidity in the refrigerated temperature. The iodine

value of the egusi melon seed oil, was similar to the egusi melon seed oil 110.0±8.2 mg iodine g^{-1} obtained by Aremu [18] and to those of unsaturated fatty acid-rich oils such as peanut (86.0-107.0), cottonseed (100.0-123.0), sesame (104.0-120.0), sunflower (118.0-141.0) but lower than that of soybean oil (124.0-139.0) [18]. Egusi melon seed oil however has iodine value higher than those of saturated fatty acid-rich oils such as *Theobroma cacao*, cocoa butter (32.0-42.0) [19], coconut (6.0-10.0), palm oil (50.0-55.0), palm kernel (14.0-1.0) [18]. The iodine value is indicative of high unsaturation fatty acid-rich oils showing that the oil is rich in unsaturated fatty acids.

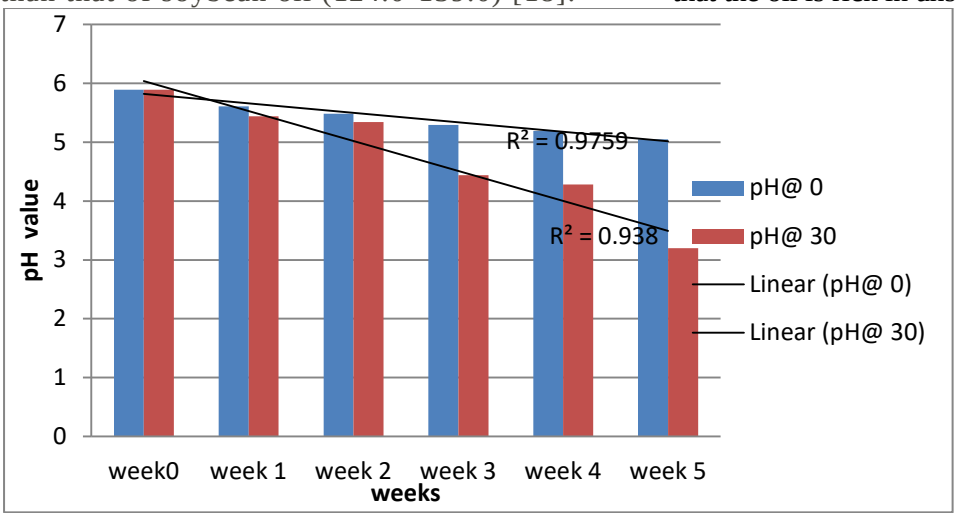

Figure 3: pH Value

The result in figure 3 revealed that the pH value at refrigerated temperature 0°C (5.89 – 4.62) decreases more than at 30°C (5.89 – 5.05) in 5 weeks, this would correspond with higher oxidation at 30°C than at 0°C and progressive oxidation during storage at both temperatures. The correlation coefficients between pH and storage time were high and negative at both storage temperature. That is, as the storage time increased, the pH also decreased indicative of lower acidity during storage that can be ascribe to accumulative of acidic entities during storage.

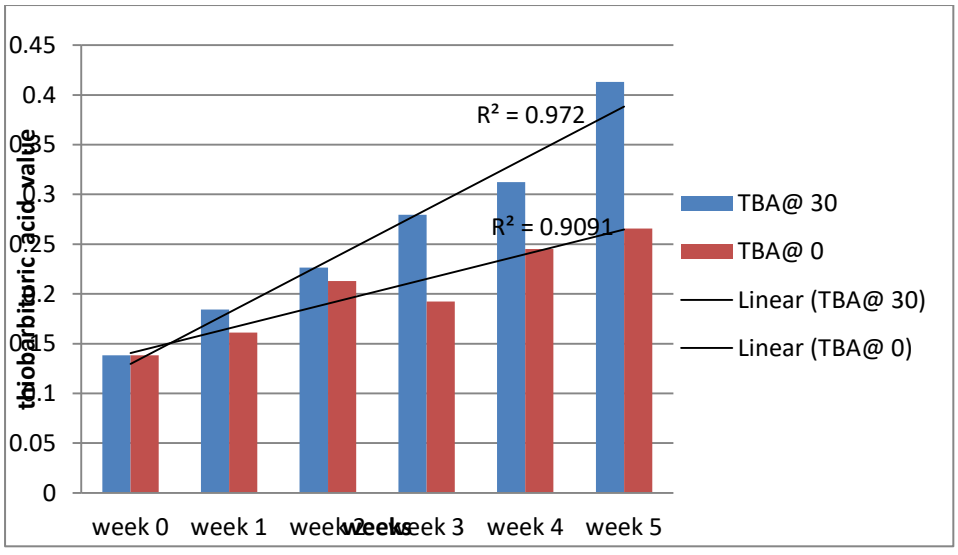

Figure 4: Thiobarbituric Acid Value (TBA)

The graphical presentation of the results in figure 4 indicates that there was also a high correlation coefficient between TBA values and Storage time. Additionally the correlation was positive, this means that as the storage time was increasing, TBA value were also increasing, in agreement with earlier observation, TBA is one way of assessing oxidation in oil as in fats, therefore increase in TBA during storage meant that the oil sample were undergoing oxidation. Figure 4 also revealed, that oxidation was more at 30°C than at 0°C. The analysis of variance

results in figure 4, showed existence of highly significant differences in thiobarbituric acid value due to the effect of storage at different temperature. The thiobarbituric acid value is also used as an indicator for the degree of lipid oxidation. It shows the amount of polar secondary reaction products. The leaf oil contained higher thiobarbituric acid values, but lower oil yield than the seed oil. Lukaszewicz *et al.* [20] observed that the presence of thiobarbituric acid in oil indicates that oxidation has already occurred and can be quantified from the amounts present. Thiobarbituric acid values can also serve as a yardstick for sensory testing to detect food rancidity [21].

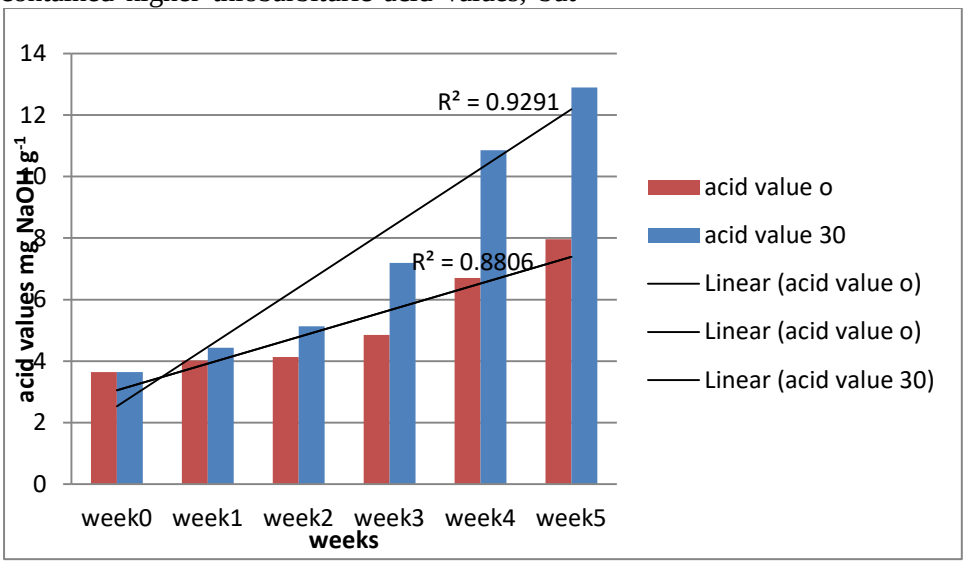

Figure 5: Acid Value (AV)

Figure 5 revealed that the acid value significantly increase with storage time. The acid value increases from (3.6445 – 12.8921) $mgNaOHg^{-1}$ in 5 weeks at room temperature 30^{o}C. At 0^{o}C, the value increased from (3.6445 – 7.9615) $mgNaOHg^{-1}$ in 5 weeks. The result further substantiate that acid value at room temperature increase more progressively with time due to the increase in the rate at which rancidity occur is more in at the room temperature compared with the rate of rancidity in the refrigerated temperature. The acid value obtained is very similar to the 3.5±0.3 mg KOH g^{-1} obtained for egusi melon seed oil and fluted pumpkin (3.5 mg KOH g^{-1}) and relatively low compared to that reported for tropical almond (7.6 mg KOH g^{-1}) [18]. The low acid value of the oil indicates that it is good as edible oil.

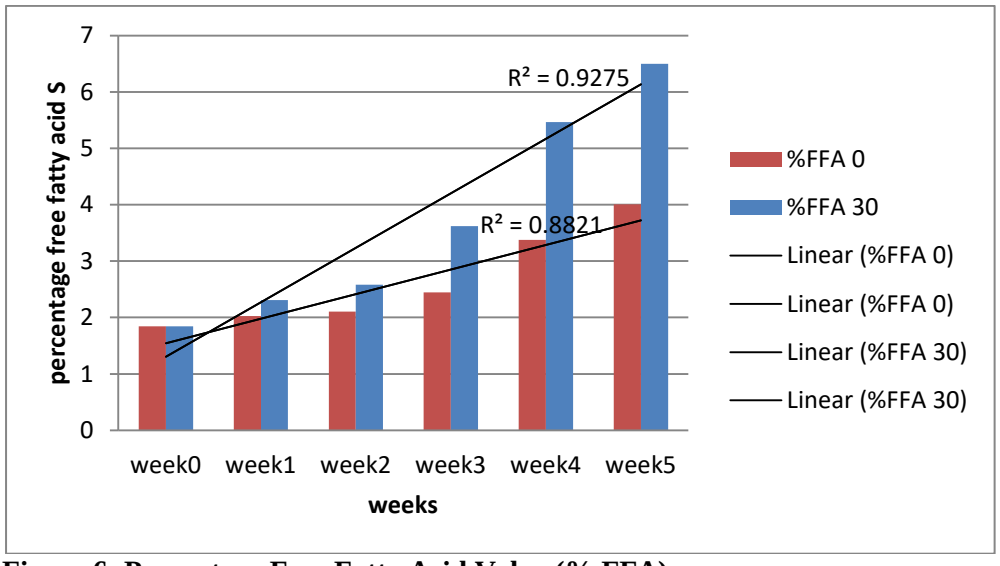

Figure 6: Percentage Free Fatty Acid Value (% FFA)

It can be observed that as in the previous discussion, there was a strong correlation (positive) between storage time and % FFA content of the stored melon seed oil. This implies that as the storage time was increasing the % FFA content (as Oleic acids) was increasing. % FFA is one of the important parameter used in grading oils. High contents are undesirable because it means the oil has been the subject of extensive lypolysis either by purely hydrolytic processes or by enzyme-mediated breakdown which occur in microbe-infested samples. High% FFA may also be the result of auto-oxidation predicted deterioration of oils and fats. Therefore, it is obvious that a combination of these factors could be used to explain the increasing % FFA. During the storage of the melon seed oil as depicted in figure 6, the lypolysis apparently occurred at a higher rate during storage at 30^{o}C and 0^{o}C, which again illustrate the importance of temperature on the kinetics in natural products.

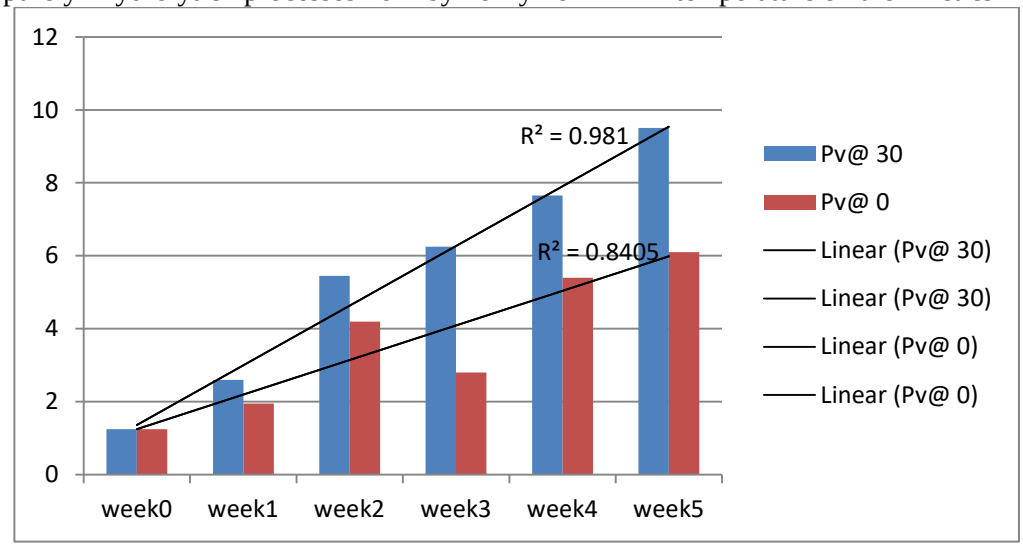

Figure 7: Peroxide Value (PV)

The result presented in figure 7 revealed that the peroxide acid value increased with storage time. The peroxide value increase from 1.25 to 9.5 mg/g oil in 5th week at room temperature 30^{o}C and 1.25 to 6.10 mg/g at 0^{o}C in the 5th week. In accordance with figure 7, it is observe that the correlation between PV and storage time was positive and high. Increase PV during storage is tantamount to increased oxidation during storage. Peroxide value depends on a number of factors such as the state of oxidation (quantity of oxygen consumed), the method of extraction used and the type of fatty acids present in the oil. The high peroxide value recorded for egusi melon oil in this study may be due to too much exposure of the seeds to sun during drying, causing lipid oxidation resulting from the absorption of oxygen, which increases the formation of peroxides. Secondly, it may be attributable to heating of the oil during its extraction process. Heat favours oxidation of fatty acids increasing the formation of peroxides [22]. Thirdly, the oil contains mostly polyunsaturated fatty acids which easily undergoes oxidation, raising peroxide value of the oil. The peroxide value obtained in this study is however lower than 15 mg equiv. O_2 kg^{-1} of oil (the maximum level for cold pressed and virgin oils) [18], showing that egusi melon oil is good for consumption.

Conclusion

Melon seeds contain appreciable golden yellow colored oil that can be explored. The oil can be used in Food, soap, paint and Pharmaceutical industries.

Refrigeration will reduce the rate of the oxidative reactions that produces rancidity. Peroxide value, acid value, saponification value, thiobarbituric acid values at room temperature increase more with storage time, than at 0^{0}C, indicating the accentuating role of elevated temperature in spoilage of melon seed oil. The present study indicates that egusi seed oil has acid, iodine, peroxide and saponification values within recommended limits. The iodine value is close to those of unsaturated fatty acid-rich oils (corn, cottonseed, sesame, sunflower and peanut oils) showing that the oil is rich in unsaturated fatty acids. The seeds could be extracted for oil and furthermore used for edible

purposes, and the meal could be used as a meat substitute, and also for animal and poultry feed or protein production. These oils owe their industrial value to their ability to dry into hard, solid films after being applied as thin layers.

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