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Oxidation Rancidity Test of Biodiesel Formulation from Mixtures of [Oil + Animal Fats and Poultry Fats] of Three Species of Cucurbitaceae Family Seeds Oil. (Ash, Bottle and Ornamental Gourds)

[1] Abdulhameed MA, [2] Owoeye Gbenga, [3] Esan

[1, 3] Department of Chemistry, School of Pure and Applied Sciences, LAUTECH, Ogbomoso, Oyo State, Nigeria
[2] Department of Chemistry, Federal University of Technology Akure, Ondo State, Nigeria

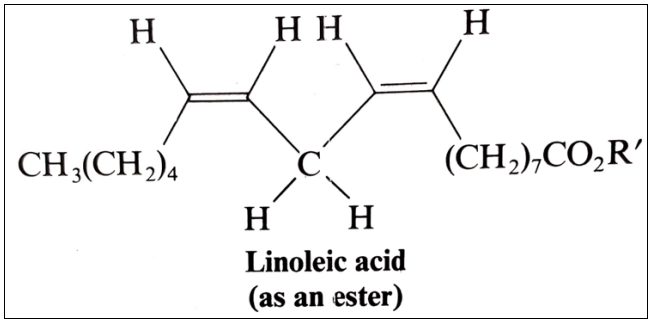
Corresponding Author: **Owoeye Gbenga**

Abstract
Oxidation stability test are one of the criteria for biodiesel wide spread use, because the user must be satisfy during uses and has the confident on the producer. Therefore, this paper is investigating the oxidation rancidity test on biodiesel produce from three species of gourd seed optimized oils using standard method. (ASTM N5304 - 03). The results observed indicated that, the biodiesel produced from the gourd samples has some significant traces of oxidation reaction when investigated and this problem can easily be amended by the use of improvers and by using appropriate container for storage. In conclusion, oxidation stability test has shows that oxidation used to occur and it becomes real when the fatty acid profile indicate the presence of unsaturated fatty acid present in the produced biodiesel, and the storage problems when antioxidant is used.

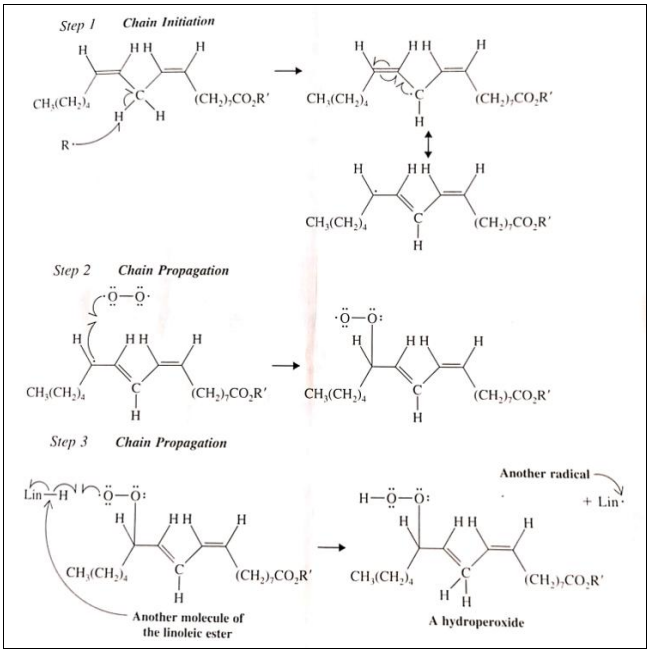
Keywords: Oxidation Stability, Biodiesel Formulation and Analysis

Introduction

Oxidation Stability of biodiesel is one of the major issues limiting the biodiesel uses or consumption at various constituents. This is because fatty acid methyl ester are more prone to oxidation which changes the physicochemical properties and biological properties of the fatty acid methyl ester than the conventional diesel. Dun (2002) [1]. The unsaturated fatty acid chains are more susceptible to free radical attack which then react with other fatty acid chain. Oxidation stability is also a physical properties that is used to describe the ageing behavior of fuels during transport and storage and during biodiesel blends with diesel which can results in the formation acids and polymer solids which affect plug filters and leaves problematic deposit throughout fuel supply system. The concern of oxidation stability is the tendency of a fuel to react with oxygen, which is highly depend especially on the composition of unsaturated fatty acid methyl ester in biodiesel especially component with bis-allylic, methylene group adjacent to the double bond present on the unsaturated fatty acid methyl esters. The peroxides or higher oxides are the end product of a series of reaction via radical routes, the reaction ends with the formation of alcohol, aldehyde, ketone or carboxylic acids. Frankel (1993) [6], Gartner (1983) and Dun (2005) [4].



Linoleic acid (as an ester)

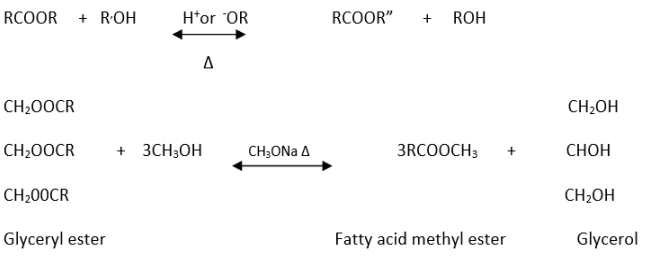


The occurrence of oxidation stability in biodiesel takes three main stages as shown above Fig 1 (Auto-oxidation reaction): a induction stage in which free radicals react with antioxidant compound preferably rather than free fatty acid methyl esters. b The experimental stage is the period in which 80 - 90% antioxidant have being reacted or consumed and free fatty acids methyl esters rapidly reacted with oxygen. c The last stage is the termination stage in which the rate of peroxide formation/degradation exceeds the rate of peroxide formation and the fuel quality is significantly change. Furthermore, the oxygen molecules that react with the unsaturated fatty acid chain exist in two forms: the triplet state ($^3O_2$), its ground state and the excited singlet state ($^1O_2$) that is more reactive than the triplet state by 22.5kcal/mol because of the chemistry, two oxidation mechanism usually occurs a thermal oxidation (autoxidation) and photo-oxidation. The photo oxidation is faster than auto-oxidation by several order of magnitude due to the reactant $^1O_2$ that is being highly energetic and is more reactive than the di-oxygen in the ground state. Dun (2005) [4], and Chen *et al*. (2011) [5]. The mechanism can be more, explanatory as follows: step 1 the reaction is initiated by the attack of a radical on one of the hydrogen atom of the ---CH_2---- group between the two double bond, this abstraction produce a radical that is resonance hybrid, step 2 this radical react with oxygen in the first two propagation step to produce oxygen containing radicals, of which in step 3 can abstract a hydrogen from another molecule of the linoleic acid ester (Lin—H). The result of the second chain propagating step is the formation of hydrogen peroxide and a radical (Lin) that can bring about the repletion of step 2. Lin ---O -----O* + In---H ⟶ Lin ---O----OH + In*. Solomon (2005) and FA0/WH0.

The impact/effect of oxidation on biodiesel fuel characteristics are on: density, peroxide value, acid value, compositions, extent of gumming and sulfur contents. The sensitivity to oxidation varies and depend on the raw materials and the presence of natural antioxidant in the finished biodiesel and oxidation due to exposure to atmospheric air and high temperature Dinko *et al* (2009), lapuetas *et al* (2012). The autoxidation reaction can be prevented by the use of antioxidant, which break the chain reactions and react to form a stable products. [*hydroperoxide decomposer (antioxidant) convert hydroperoxide into, non – radical, non reactive and thermally stable products].* Some factors to be considered in stabilizing lipids or biodiesel especially if the fuel compose of much unsaturated glycerides in the biodiesel formulation. Such factor include: temperature, fatty acid composition of biodiesel, light, metal catalyst, inhibiting compound and availability of oxygen. One of such factors is use determine the stability of the prepared samples, used in this research and investigation, that is Oxygen using standard method. The following are some of the reliable technique use in the determination of oxidation stability of biodiesel fuel. ASTM D 2274 for the ageing behavior of diesel fuel, ASTM N 5304-03, ASTM D02.14 and RSSOT method.

Vegetable as a whole are considered as natural sources of nutrient gifted by God almighty to human being, their fresh and edible parts of the herbaceous plants in which traditional African, has select and exploited to provide adequate nutrition, food security and income generation Akubor (2008) [11]. They are also inexpensive wild plant which comes in the forms of edible, roots, stems, leaves, fruits or seeds and valuable food ingredients which can be successfully utilized by human being and for industrial uses. Cucurbitaceae family are an example of such plant in which the seeds extracted oil can be use in the production of biodiesel. such include; Ash, Bottle and Ornamental gourds.

Transeterification Reactions: When a glyceride ester refluxed with alcohol (other than the one use in its preparation) preferably in the presence of small amount of acid or sodium alkoxide as a catalyst, the original alcohol residue in the ester gets replaced by new alcohol. The ester interchange is called alcoholysis. Therefore, the splitting of ester by alcohol is called transesterification. The reaction is useful for the preparation of ester derived from insoluble acid.



The mechanism of transesterification is most analogus to the hydrolysis of ester. In the case of hydrolysis water act as a nucleophile and displace alcohol, whereas in transesterification, the alcohol act as nucleophile and displace another alcohol. It is an equilibrium reaction. To make the reaction to completion, it becomes necessary either to use large excess of the added alcohol or to remove one of the product from the reaction mixture. When the alcohol use is having a higher boiling point than the alcohol produced then, the removal of the latter by distillation offers a useful method for increasing the ester yield. Kuevele *et al*. (2011) and Dun (2002) [1].

Justification

Several reports has been known about oxidation rancidity /stability on biodiesel produce from various vegetable seed oil but few was reported on oxidation stability/rancidity of biodiesel produce from cucurbitaeae family, and the use of

antioxidant as an inhibitor of oxidation rancidity need special attention because during storage antioxidant is consumed and because of this and the integrity and purpose to be prevented there is need to carry out oxidation stability of biodiesel. Therefore this paper seek to study oxidation rancidity analysis of biodiesel produce from three species of cucurbitaceae, (Ash, bottle and ornamental gourd) seed oil. with the aim of formulating lasting solution to the detrimental effect of oxidation, such measures as implementing provision of promoters, improvers, doubling the antioxidant concentration, use of two antioxidant and other positive measures.

Materials and Merthods

Experimental

Sample Preparation: Samples of ash, bottle and ornamental gourd seeds were processed from the main gourd fruits by plucking from the main stem and allowed to dried, break into two or three and gently remove the seeds from the dried fruits and separated to the individual gourd species and latter dried very well inside a regulated oven at 50oC for a period of 2—4hours. After the oven dried period, It was allowed to cool and stored in three different PVC plastic container from where the epicarp / curticle was removed living the main useful seeds that the oil inside can be extracted usually with soxhlet extractor. In the analytical Laboratory Federal University of Technology Akure. The de-husked seeds was later store back in there different label PVC plastic containers.

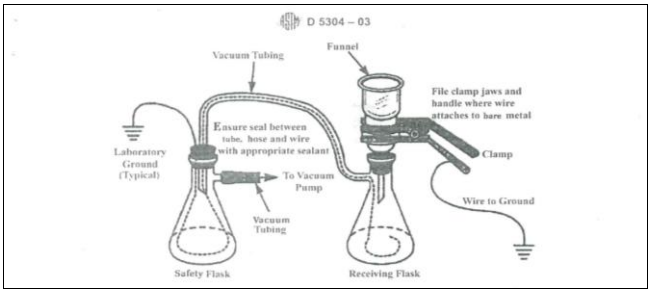
Methods: Oils Extraction; This was carried out by blending each of the seeds inside each container differently. The blended seeds was packed inside the thimble of the sohxlet extractor and later position at the right place of the equipment. The extraction took place for a period of 2—4hours usually when the solution at the thimble is cleared. Each of the saw treated the same. [Ash, bottle and ornamental].

Biodiesel Formulation: Biodiesel was produced from each of the extracted oils by a process called transesterification reaction. Each was processed as follows:

Mixtures of ash gourd seeds oil + animal fat + poultry fats in the ratio: 50%: 30%: 20% respectively was mixed with sodium alkoxide solution and were pour inside a separating funnel, stirred for two hour and latter transfer to a regulated water bath at 60^{o}C and was stirred intermittently for 2hour. Then the funnel was hanged on a stand for separation and latter removed the lower layer which is the glycerine living only the biodiesel (fatty acid methyl ester). Bottle and ornamental gourd seed oil was treated the same. The resulted biodiesel solution was analysed for oxidation stability.

Oxidation Stability test: The was carried out by using ASTM N 5304 - 03. aparatus. It was operated by filtring the biodiesel solution through a device connected to the vacuum pump and air for specific time as; 24 hours, 48hours, 72hours and 96hours. Respectively. After each experiment the samples were analysed for, peroxide value, extent of gum, and antioxidant. The test was carried out in triplicate.

Statistical analysis: The results obtained were subjected to analysis of variance (ANOVA). In other to ascertain the source of variance. Least Significant Different between the mean. The significant was at P< 0.05.



ASTM Method N 5304 -03

Results, Discussion and Conclusion

Table 1: Results of Ash gourd methanol biodiesel

Test times	24hrs	48hrs	72hrs	96hrs
Peroxide value Meg/kg	0.02±0.1	0.02±0.1	0.02±0.1	0.04±0.10
Extent of gum mg/100ml	0.02±0.01	0.02±0.01	0.03±0.01	0.04±0.01
Antioxidant ppm	12.10±0.02	10.20±0.02	9,60±0.03	8.05±0.02

± = standard deviation. Results of three analysis.

Table 2: Results of Ash gourd ethanol biodiesel

Test time	24hrs	48hrs	72hrs	96hrs
Peroxide value Meg/kg.	0.00±0.1	0.00±0.1	0.00±0.1	0.00±0.1
Extent of gum mg.100ml	0.02±0.01	0.002±0.02	0.007±0.02	0.007±0.07
Antioxidant ppm	10.20±0.1	9.80±0.1	8.40±0.1	6.50±0.1

± = Standard deviation. Results of three analysis.

Table 3: Results of Bottle gourd methanol biodiesel

Test time	24hrs	48hrs	73hrs	96hrs
Peroxide value Mg/kg	0.00±0-1	0.00±0.1	0.00±0.1	0.00±0.1
Extent of gum mg/100ml	0.04±0.1	0.04±0.1	0.04±0.1	0.04±0.1
Antioxidant ppm	12.40±0.01	12.00±0.02	10.40±0.02	8.50±0.01

± = standard deviation. Results of three analysis.

Table 4: Results of Bottle gourd Ethanol biodiesel

Test time	24hrs	48hrs	72hrs	96hrs
Peroxide value Mg/kg	0.00±0.1	0.00±0.1	0.00±0.1	0.00±0.1
Extent of gum mg/100ml	0.008±0.01	0.008±0.01	0.008±0.01	0.008±0.01
Antioxidant ppm	14.50±0.1	13.85±0.1	10.40±0.1	8.30±0.1

± = Standard deviation. Results of three analysis.

Table 5: Results of Ornamental gourd methanol biodiesel

Test time	24hrs	48hrs	72hrs	96hrs
Peroxide value Mg/kg	0.00±0.1	0.00±0.1	0.00±0.1	0.00±0.1
Extent gum mg/100ml	0.02±0.1	0.02±0.1	0.022±0.1	0.024±0.01
Antioxidant ppm	15.20±0.01	13.50±0.1	12.10±0.1	10.20±0.1

± = Standard deviation. Results of three analysis.

Table 6: Results of Ornamental gourd ethanol biodiesel

Test time	24hrs	48hrs	72hrs	96hrs
Peroxide value Mg/kg	0.00±0.1	0.00±0.1	0.00±0.1	0.00±0.1
Extent of gum mg/100ml	0.008±0.01	0.007±0.01	0.007±0.01	0.007±0.01
Antioxidant ppm	15,40±0.1	14.30±0.1	12.40±0.1	10.50±0.1

± = Standard deviation. Results of three analysis.

The above results indicate the observed values for all the experiment conducted, Table 1 indicated values for methanol ash gourd biodiesel, 0.02±0.1 Meg/kg for peroxide value, after the first twenty four hours and it does not change until the 96hours was completed, the value reveal was: 0.04±0.1 Meg/kg for ash gourd methanol biodiesel. The extend of gum represent the gumming layer left after evaporating a certain volume of the biodiesel 0.02±0.01mg/100ml for 24hours, 0.02±0.01mg/100ml and it increases significantly after 72hours and 96hours respectively to: 0.03±0.01mg/100ml and 0.03±0.01mg/100ml for ash gourd methanol biodiesel sample respectively. The antioxidant value observed fall gradually as the number of hours increases significantly indicating that some reaction has occurs and needs to prevent and preserve the quality of the biodiesel sample. The values were: 12.00±0.2ppm at 24hours, 10.20±0.02ppm, 9.60±0.02ppm and 8.05±0.02ppm at 96hours. Table 2 indicated the results of oxidation stability test on biodiesel produce using ethanol. The peroxide value observed were: (0.00±0.01) nil for all the testing period and the extent of gum indicated 0.02±0.01mg/100ml for 24hours test, and 0.02±0.01mg/100ml for 48 hours while 0.007±0.01mg/100ml value observed for 72 hours and 96hours respectively, it also indicated some change of reaction. The antioxidant values observed at 24hours was 10.20±0.1ppm, 9.80±0.1ppm for 48 hours. 8.40±0.1ppm for 72hours and was observed to decrease to 6.50±0.1ppm at 96hours which shows that the antioxidant improver are necessary for the quality. Table 3 shows the observed value for rancidity test on bottle gourd seed oil methanol biodiesel, the peroxide value was 0.00±0.01Meg/kg (nil) for all the test, while the extent of gum indicated 0.04±0.1mg/100ml for all the tested hours, and the antioxidants were: 12.40±0.01ppm, 12.00±0.01ppm, 10.40±0.02ppm and 8.50±0.01ppm for: 24hours, 48hours, 72hours and 96hours respectively for the test. Indicating the values decreases as the number of hours used increases, and the effect of the antioxidant which is to inhibit the radical reactions found decreases and there is need to for the supply of improver. Table 4 shows the results for oxidation rancidity test for bottle gourd seed oil ethanol biodiesel indicating values: 0.00±0.1Mg/kg for peroxide value for all the tested hours, and the extent of gum were 0.008±0.1ppm for all the tested hours while the antioxidant were 14.50±0.1ppm,for 24 hours, 13.85±0.1ppm for 48hours, 10.40±0.1ppm for 72hours and 8.30±ppm for 96hours indicating the same observation for, methanol biodiesel in the, way the value decreases showing the effect of antioxidant on produce biodiesel, and the need for addition of an improver. Table 5 indicated the observed values for ornamental gourd seed oils methanol biodiesel as: 0.00±0.1Mg/kg for peroxide in all the test period, and the extent of gum as 0.02±0.1g/100ml for 24hours, 0.02±0.1g/100ml for 48hours, 0.022±0.1g/100ml for 72hours and 0.024±0.1g/100ml for 96hours while the antioxidant values were 15.20±0.01ppm, 13.50±0.01ppm, 12.10±0.01ppm and 10.20±0.01ppm for 24hours, 48hours, 72hours and 96hours respectively. Table 6 shows the observed results for ornamental seed oil ethanol biodiesel with the value for peroxide as: 0.00±0.1Mg/kg (nil) for all the tested period. The extent of gum indicated observed value as 0.008±0.01mg/100ml at 24hours and 0.007±0.01mg/100ml value for 48hours, 72hours and 96hours respectively while the antioxidant indicated values as: 15.40±0.1ppm for 24hours, 14.30±0.1ppm for 48hours, 12.40±0.1ppm for 72hours and 10.50±0.1ppm for 96hours. The observed value for ornamental gourd seed oil biodiesel both for methanol and ethanol has similar effect as it has a decreasing value for antioxidant and need for improver. There is general observation of significant differences in the values observed for methanol biodiesel and ethanol biodiesel especially on the extent of gumming and mainly on the antioxidant values observed. The zero value for peroxide value indicated that the biodiesel is good. The autoxidation reaction does not occur easily except through the antioxidant when left for a long time during storage, because the are consume in the process. Chen *et al*. (2011) [5], and (Frankel 1993) [6].

$$RH \rightarrow R^* + H^*$$

$$R^* + O_2 \rightarrow ROO^*$$

$$ROO^* + RH \longrightarrow ROOH + R^*$$

$$R^* + R^* \rightarrow RR$$

$$ROO + ROO \longrightarrow \text{stable product.}$$

Autoxudation reaction is multistep process where primary oxidation product such as hydroperoxide and epoxide décompose, react with each other and form a stable secondary oxidation products. This product can be aldehydes, aliphatic alcohol, formic acid, formate, ester, shorter chain fatty acids, and oligomers with molecular weight of up to 1600g/mol. (high molecular weight oligomers). The product of oxidation can lead to higher emissions, a higher corrosiveness, fuel filter blockage, clogging of fuel injections and formation of deposit in the entire fuel system. Kechar (2011), Charlce 1911 and Frankel (1991). The preventive measures for this harmful oxidation products calls for the use of an antioxidant that is needed to be added to diesel fuel, also several high quality effective synthetic antioxidant has been develop. However, antioxidants are been consumed during storage especially for a long period, there is partial loss to there efficiency against oxidative stress. During the period of storage, especially, if the induction period is exceeded, all antioxidant will be consumed and the harmful oxidation product would be form again at this period and before, the oxidation stability test needs to be conducted or analysed for. But, the use of two antioxidant and double concentration will amend some of the returned back oxidation and would rather prolong the storage period. Example: phenolic and amionic antioxidant break the chain reactions by abstracting hydrogen radicals. This hydrogen radicals react with radicals from autoxidation and form a stable products. The resulting antioxidant radicals is either stable or further engage in a reaction to form a stable molecules. Kivevele, *et al*. (2011) [12] and Bosato *et al*. (2010).

Furthermore, Improvers are solvent that could be added to biodiesel in other to increase the cold flow process and the quality of the fuel. Such improver include; alcohol, acetone, diethyl ether, ethyl acetate, polymer surfactant (surfactant act by displaying hydrophobic and hydrophilic properties possess) etc. The cold flow properties problem can also be addressed by adding nickel and magnesium as an improver.

Some metals speed up oxidation therefore, there is need to avoid them, metals like stainless still, aluminium or carbon still are recommended for storage of biodiesel fuel in other to improve stability. Moreover pipelines and vessels containing impurities that affect biodiesel stability could be prevented by using two antioxidant and doubling the concentration.

Conclusion

The oxidation rancidity is not optional in the production of biodiesel but it can be prevented and taken care when occur in the process of use. The above experiment has showed that when it happens it can easily be detected and amended and cured and other method can also be use as confirmatory test to the above, therefore, oxidation rancidity or stability has being confirmed as one of the quality that determined the biodiesel stability and widespread use.

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