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USE OF SKIN AND SEED EXTRACT FROM *LYCOPERSICON ESCULENTUM* FOR INCREASING BIODIESEL OXIDATION STABILITY

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Abstract

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This study aims to use carotenoids substances extracted from the skin and seeds of *Lycopersicon esculentum* at protection of biodiesel from fish oil against oxidative degradation. Oxidation stability is an important parameter widely known as material control and evaluation of different types of oil and the effectiveness of antioxidants. There were formed two sets of samples of biodiesel. A sample without additives and other with additives with 0.1% mixture of 0.1% and 0.1% extract extract seed and skin of *Lycopersicon esculentum*. Samples were incubated for 4 weeks at 50°C. Weekly, the samples of biodiesel have been determined following parameters: Oxidation Stability, Peroxide value and acidity. Additives samples showed values higher than those without additives oxidation stability range 187-515 min. Toward the samples without additives for 90-450 min. Biodiesel samples without additives show an increase in Peroxide value during storage between 1.5-62.2 mEqO_2/kg to the additives of 1.0-39.4 m EqO_2/kg. Variation in acidity during storage is between 0.4-1.4 mg KOH/g for samples without additives and 0.36-1.0 mg KOH/g for samples additives.

Key words: carotene, *Lycopersicon esculentum*, antioxidants, biodiesel

Introduction

Vegetable oils and animal fats are an important source of energy, possessing, after processing, similar characteristics and the type of diesel fuel held. It turned out that the product of fatty acid esterification namely ester (biodiesel) obtained by processing fats has characteristics closely resembling those of classical fuel. Chemical reaction and flow technology of biodiesel (Ilgen et al.2007; Dumitru and Grecu, 2010; Bița et al., 2012; Chemical Bussines and www.chemstations.com rev) are shown in Figures 1 and 2.

An important disadvantage of biodiesel is the relatively small period of this fuel conservation due to the action of oxygen on unsaturated carbon atoms (Chul et al., 2004 and Divya and Tyagi, 2006). Degradation chemistry of biodiesel is the same as that oils it comes from.

The unsaturated fatty acid chains in biodiesel are susceptible to oxidation, and the mechanism is well known. Autooxidation is a chain reaction including three basic steps: initiation, propagation, and termination (Topallar et al., 1997; Schaich, 2005; Wyrick, 2005 and Dumitru et al., 2009).

Initially, the allylic hydrogen of unsaturated fatty acid chains is easily removed to form a carbon-based radical (R^*), which then reacts with oxygen in a propagation step to form a peroxy radical (ROO^*) and a hydroperoxide (ROOH). Two free radicals can react with one another in a termination step. The ROOH concentration is very low during the initial period; however, in the propagation period, the ROOH level increases rapidly, indicating the onset of the overall oxidation process. The hydroperoxide species can form acids, aldehydes, and dimers, finally resulting in polymer formation even at ambient temperatures. These polymers may potentially plug fuel filters and injection systems.

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Initiation

$$L_1H \xrightarrow{k_i} L_1^*$$

Propagation

Free radical chain reaction established

$$L_1^* + O_2 \underset{k_\beta}{\overset{k_o}{\rightleftharpoons}} L_1OO^*$$

$$L_1OO^* + L_2H \xrightarrow{k_{p1}} L_1OOH + L_2^*$$

$$L_2OO^* + L_3H \xrightarrow{k_{p1}} L_2OOH + L_3^* \text{ etc.} \dashrightarrow L_nOOH$$

Free radical chain branching (initiation of new chains)

$$L_nOOH \xrightarrow{k_{d1}} L_nO^* + OH^- \quad \text{(reducing metals)}$$

$$L_nOOH \xrightarrow{k_{d2}} L_nOO^* + H^+ \quad \text{(oxidizing metals)}$$

$$L_nOOH \xrightarrow{k_{d3}} L_nO^* + {}^*OH \quad \text{(heat and uv)}$$

$$\left.\begin{matrix} L_nO^* \\ L_nOO^* \\ HO^* \end{matrix}\right] + L_4H \xrightarrow[k_{p3}]{\overset{k_{p2}}{k_{p1}}} \left[\begin{matrix} L_nOH \\ L_nOOH \\ HOH \end{matrix}\right] + L_4^*$$

$$L_1OO^* + L_nOOH \xrightarrow{k_{p4}} L_1OOH + L_nOO^*$$

$$L_1O^* + L_nOOH \xrightarrow{k_{p5}} L_1OH + L_nOO^*$$

Termination (formation of non-radical product)

Radical recombinations

$$\left.\begin{matrix} L_n^* \\ L_nO^* \\ L_nOO^* \end{matrix}\right] + \left[\begin{matrix} L_n^* \\ L_nO^* \\ L_nOO^* \end{matrix}\right] \xrightarrow[k_{t3}]{\overset{k_{t1}}{k_{t2}}} \text{polymers, non-radical monomer products (ketone, ethers, alkanes, aldehydes, etc.)}$$

Radical scissions

$$\left.\begin{matrix} LOO^* \\ LO^* \end{matrix}\right] \xrightarrow[k_{ts2}]{k_{ts1}} \text{non-radical produscts (aldehydes, ketones, alcohols, alkanes, etc.)}$$

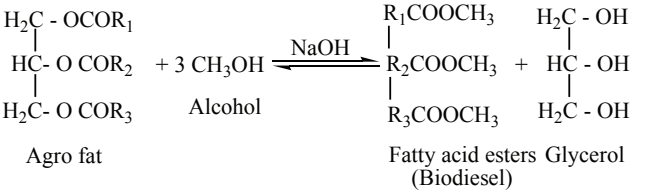

Fig. 1. The reaction for obtaining biodiesel from fatty acids

Factors which influence the oxidative stability of biodiesel include fatty acid composition, natural antioxidant content, the level of total glycerin, and the conditions of fuel storage such as temperature, exposure to light and air, and tank material of construction (Schober and Mittellbach, 2005 and Sendzikiene et al., 2005). In Figure 3 shows the mechanism of autooxidation of oleate, linoleate at Linolenate (Frankel, 2005).

In this study, a fish oil biodiesel was used from which the content of fatty acids, in particular unsaturated fatty acids with more than three double bonds (Table 1), has a significant degradation during storage Cherng-Yuan and Rong, 2009).

To reduce oxidation reactions that occur during storage use antioxidants.

In a system, an antioxidant can be defined as any substance present in low concentrations compared to that of a substrate oxidable and delays or prevents oxidation of the substrate (Halliwell et al., 1995). Antioxidants are natural or synthetic, which reacts with atmospheric oxygen or free radicals in the environment, protecting against self-oxidation compounds.

After origin, antioxidants are classified into natural and synthetic, and after structure: phenolic, amino, endiolic (re-

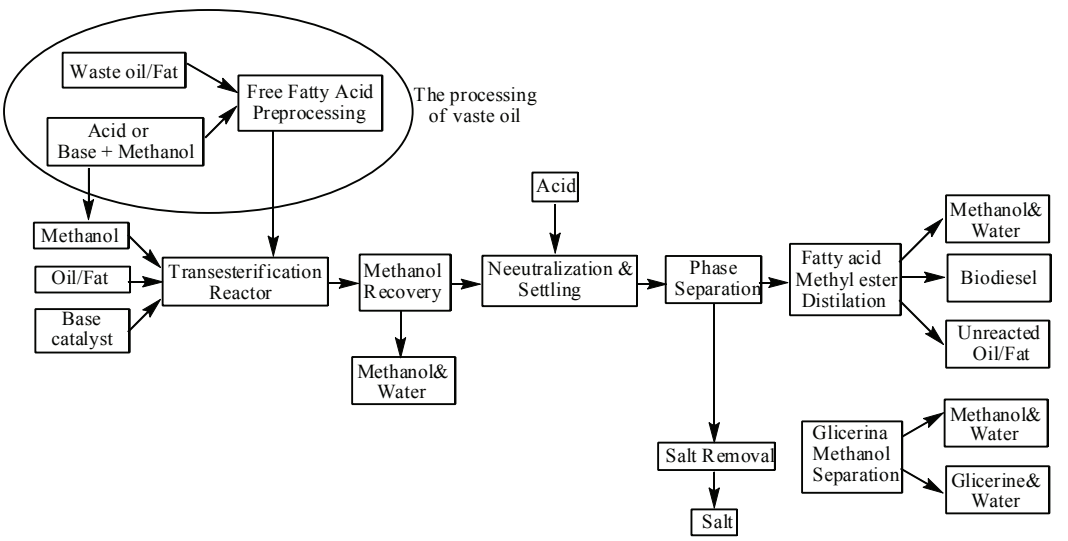

Fig. 2. Technological flow of obtaining biodiesel

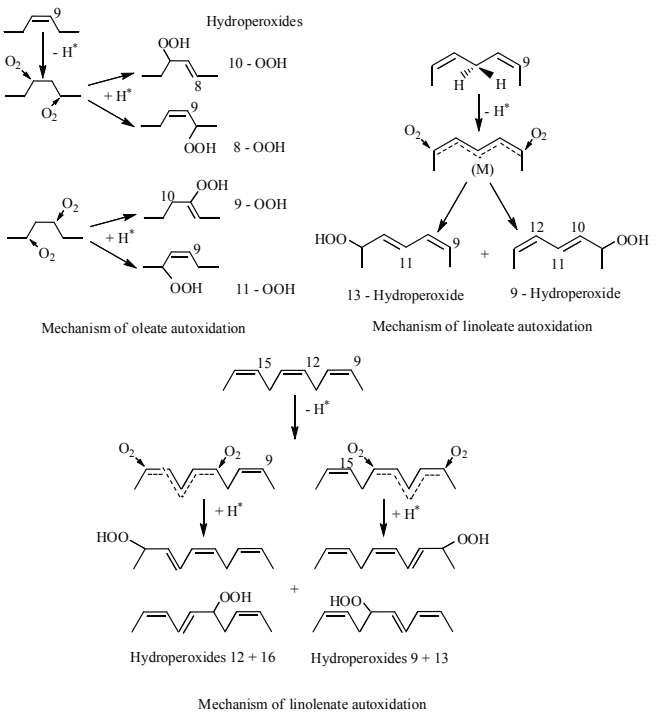

Fig. 3. Mechanism of oleate, linoleate at linolenate autoxidation

ductones), heterocyclic and izoprenoidic. By mode of action, they are classified into primary or discontinue the kinetic chain by direct reaction with free radicals and secondary, acting through other mechanisms of reaction. Antioxidants are the primary hydrogen donor (tocopherols, ascorbic acid, gallic acid and its esters, flavones etc.) to stabilize free radicals thereby blocking its kinetic chain.

In the study a natural antioxidant from residues resulting from the industrialization process of tomato (*Lycopersicon esculentum*) was used which is an effective way to provide carotenoids especially β-carotene and lycopene. These residues consist mainly of skins and seeds, husks containing 5 times more lycopene than the pulp (Delia et al., 1975; Sharma, 1996 and Knoblich et al., 2005).

The Experiment

To achieve experiment the following three steps were necessary:

Extraction of carotenoid substances

Carotenoid substances were extracted from the skins and seeds of *Lycopersicon esculentum* by solvent extraction (hexane) using Soxhlet (Bița and Preda, 2004 and Temime et al., 2004). Extracts obtained were evaporated under reduced pressure in an evaporator skin type RV 05-Below Basic type and stored at -25°C until use.

Table 1
Fatty acid composition of fish oil, %

Types of fatty acids	Chemical structure	Biodisel from marine fish oil
Myristic acid	C14:0	3.16
Palmitic acid	C16:0	19.61
Palmitoleic acid	C16:1	5.16
Heptadecanoic acid	C17:0	1.82
Stearic acid	C18:0	5.24
Oleic acid	C18:1	20.94
Linoleic acid	C18:2	2.69
Linolenic acid	C18:3	0.90
Arachidic acid	C20:0	4.75
Eicosadienoic acid	C20:2	0.81
Eicosatetraenoic acid	C20:4	2.54
Eicosapentaenoic acid	C20:5	3.70
Behenic acid	C22:0	1.55
Docosaenoic acid	C22:1	0.98
Docosatetraenoic acid	C22:4	3.86
Docosahexaenoic acid	C22:6	15.91
Saturated fatty acids	-	37.06
Mono-unsaturated	-	26.35
Long carbon-chain fatty acid	C20-C22	37.30

Determination of antioxidant activity of the extract mixture of 0.1% skin of Lycopersicon esculentum and 0.1% of Lycopersicon esculentum seed extract

For the determination of the lipophilic fraction antioxidant activity, the reaction mixture contained 56 mM ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 24.5 mM $K_2S_2O_8$ in ethanol (dilution 1:100) in a total volume of 1 mL. In this case, 5 µL of the lipophilic fraction (organic phase) were added to the reaction mixture and the decrease in absorbance at 734 nm was determined after 5 min. The total time needed to carry out each assay was approximately 6 min. The absorbance decrease was determined from the difference between the A_{734} values before and after addition of sample. The antioxidant activity may be expressed as % inhibition of radicalic cation (Tommonaro, 2008).

Biodiesel sample preparation

Two sets of samples were formed:

- a set of biodiesel made from fish oil without additives
- a set of biodiesel made from fish oil mixture admixed with 0.1% skin of *Lycopersicon esculentum* and 0.1% of *Lycopersicon esculentum* seed extract. Samples were placed in dark glass and incubated at 50°C for 4 weeks. Analysis of the main indicators of quality biodiesel samples (Oxidation stability, Peroxide value and Acid value) was determined at the beginning of storage, 2 weeks, 3 weeks and 4 weeks of storage.

Oxidation stability by Zürcher-Hadorn method (Rancimat)

This method consists in the biodiesel oxidation in accelerate conditions. The method permits the establishment of the induction period, which corresponds, with the initiation step of the biodiesel auto-oxidation.

To determine the stability in oxidation it was used an installation, which used oxidated Biodiesel samples (10 g) at a temperature of 110°C (Sensidoni et al., 1974; Schwarz et al., 2001; Kiritsakis et al., 2002; Dumitru and Grecu, 2010; Bița et al., 2012 and David and Tremblay). Through the biodiesel samples, it was barboted air with a debit of 8 liters per hour. Because of the oxidation reactions, which take place in a reactor, the formed volatile acids are trained by the air current and absorbed in the measurement cell where there is bidistilled water. The measurement of the solution conductibility is done with a conductometer of Radelkis type. In the beginning, we notice a slow increasing of the solution conductibility, after that it appears a sudden increasing of this because of the formation of volatile acids. The induction period is considered the interval until the moment of the suddenly curve's change (Figure 4).

Determination of peroxide value level by Hara-Totani method

Peroxide of biodiesel samples was determined at the beginning and after determining oxidation stability by Hara-Totani (Hara and Totani, 1988; Bița and Preda, 2004 and Dumitru and Grecu, 2010).

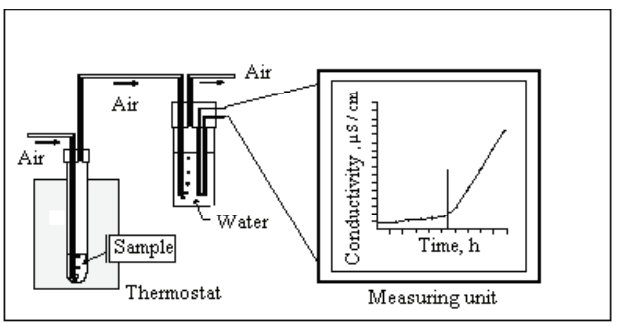

Fig. 4. Installation for the determination of stability in the biodiesel oxidation

In a 250 mL conical bottle was weighed on analytical balance a fixed quantity of biodiesel (20 mg) and were dissolved in 10 mL chloroform. Add 15 mL glacial acetic acid and mix. After replacing air with nitrogen or CO_2, add 0.3 mL saturated KI solution and shake for 1 minute, followed by cooling in ice water bath in the dark. Add 100 mL cold distilled water and shake and then titrated potentiometrically with sodium thiosulfate solution 0.001N keeping the vial in ice bath. During titration, there is a change in potential that in the equivalence moment shows a sudden drop. Parallel running a blank solution without biodiesel where volume 0.001N sodium thiosulphate used in titration until the equivalence point should not exceed 0.15 mL. If this amount is exceeded then another will prepare a saturated solution of KI. Index value is calculated in milliequivalents peroxide oxygen/kg lipid using formula:

$$IP = \frac{(V - V_m) \cdot F \cdot N \cdot 1000}{m} \quad mE\,O_2/Kg$$

where: V - number ml 0.001N sodium thiosulphate solution used for titration of sample to be analyzed
Vm - number ml 0.001N sodium thiosulphate solution used in blank titration
m - mass of biodiesel sample (g)
F - factor solution 0.001N sodium thiosulphate
N - normality of 0.001N sodium thiosulphate

Determination of acidity of biodiesel samples

The acidity value, Ia represents the number of milligrams of potassium hydroxide necessary to neutralize the free fatty acids into a gram of oil. This index modifies it self according to the length and to the conditions of storage of the oil. The fresh oils have a very small acidity. The acidity value grows with the age and grade of rancidity of the oil and indicates the grade of hydrolyses of the fat (Boz et al., 2009; Tyson, 2001; Bița et al., 2012 and Chemical Bussines). The acidity was established with the formula:

$$\text{Acidity value} = \frac{K \cdot V}{M}$$

where: K - titre of the solution of KOH 0.1N
V - volume of the hydroxide used at titration, mL
M - mass of the biodiesel used at titration, g

Results and Discussions

After determining the antioxidant activity of lipophilic extracts from skin and seeds of *Lycopersicon esculentum* it was obtained a value of 89.7%, which demonstrates increased

antioxidant capacity of the mixture of extracts from skins and seeds.

Oxidation stability by Zürcher-Hadorn method (Rancimat)

After determination of the oxidation stability of biodiesel samples during storage were obtained results that are consistent with the EN 14112 and other authors, (Wynick, 2005; Lin and Li, 2009 and Dunn, 2008) (Figure 5).

According to the European Standard (EN 14214), the induction period with a minimum of 6 hours at 110°C.

The high content of compounds with double bonds (unsaturated, Table), fish oil leads to a more pronounced degradation with formation of secondary compounds in higher concentrations (Knothe and Dunn, 2003; Frankel, 2005; Reyes and Sepulveda, 2006; Knothe, 2008 and Lin and Li, 2009). The presence of antioxidants from skins and seeds of *Lycopersicon esculentum* into biodiesel contributed to reducing the degradation process of the operation of radicals formed. Peroxyl radical ROO* attack-taking place according to the following scheme:

The mechanism of antioxidant action of carotenoids without OH groups differs therefore from the mechanism of the antioxidant phenolic structure, the radical ROO* inactivation is achieved due to OH group.

Determination of parameters by Hardorn - Zürcher method (Figure 6) shows a variation throughout the test.

There is a correlation between induction period and quality degradation. Thus, biodiesel additives samples have high oxidation stability between 187 min and 515 min, values that are higher than without additives (90 min - 450 min).

Percentage decrease in oxidation stability is between 13% and 63% for samples additives and 15% to 80% for those without additives. There is a significant increase in degradation between the second and third weeks of storage (63.3%) in samples without additives to the additive showing the increase between the third and fourth weeks (63%). These figures highlight the role of skins and seeds of *Lycopersicon esculentum* extract the antioxidant activity of biodiesel.

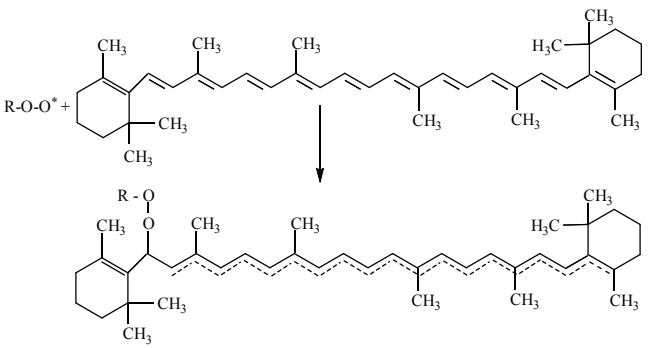

Fig. 5. Peroxyl radical attack by β-carotene

Determination of acidity of biodiesel samples

Acidity is one of the essential properties for biodiesel quality. It arises from the process of formation of peroxides which decompose and interacts as formation of numerous oxidation products including aldehydes, which are oxidized to acids (Knothe and Dunn, 2003; Frankel, 2005 and Cherng-Yuan and Rong, 2009).

Following the determinations made in samples of biodiesel results in a value on acidity is shown in Figure 7.

It is noted that biodiesel additives extract samples from skins and seeds of *Lycopersicon esculentum* have a variation of pH during storage between 0.4-1.4 mgKOH/g for samples without additives and 0.36-1.0 mg KOH/g for samples additives. Small amounts of acid additives samples are available on the influence antioxidant, which causes a decrease in the number of double bonds, which oxidizes reduced leading to an accumulation of oxidation products. This reduced accumulation of oxidation products leads to decrease acidity (Biţa and Preda, 2008).

Determination of peroxide value

Peroxide is a parameter used to determine the oxidation of biodiesel. It measures only primary oxidation products, hydroperoxides (Wynick, 2005).

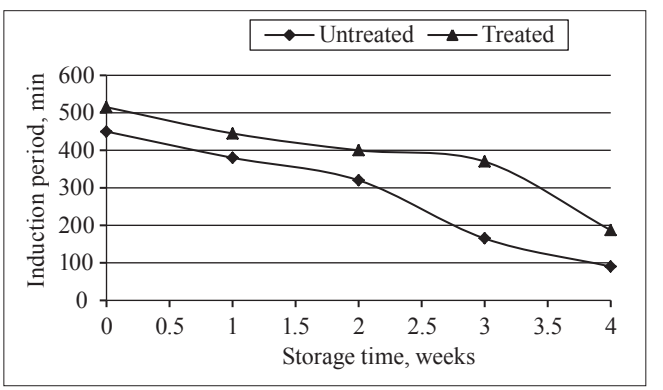

Fig. 6. Oxidation stability of biodiesel samples

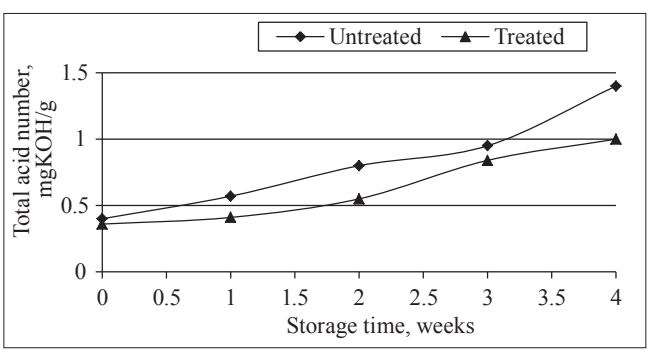

Fig. 7. Biodiesel samples acidity

Although the peroxide value is not specified in the standards that characterize biodiesel, it is a parameter that influences cetane number; parameter is specified in the standards. An increase in the peroxide involves an increase in cetane number and therefore may reduce ignition (Monyem and Gerpen, 2000 and Boulifi et al., 2010). From literature (Berner, 1989) it results in a value less than 10 mEq/g.

Experimental data obtained (Figure 8) show an increase in the peroxide value during storage between 1.5-62.2 $mEqO_2$/kg the samples without additives and 1.0-39.4 $mEqO_2$/kg the evidence additives.

Small values of the samples of peroxide additives are determined by the used extract and reduced accumulation of oxidation products (Monyem, 2000) due to inactivation of peroxide radicals by β-carotene.

Conclusion

Effect of natural antioxidant from the skin and seeds of *Lycopersicon esculentum* into biodiesel stability was identified from the results as a viable means to improve its stability to oxidation.

Small amounts of peroxide, low acidity and stability of biodiesel samples treated with the extract clearly shows their potential as an alternative source of natural antioxidants. The results were generally in agreement with studies available in the literature. Further study will be needed to establish some general conclusions on the use of natural antioxidant extracted from the skins and seeds of *Lycopersicon esculentum*. Results obtained in this research are available to reduce the effects of oxidative degradation produces biodiesel during storage.

Abundance of raw materials and high content of antioxidant substances character skins and seeds of *Lycopersicon esculentum* recommends its use in the protection of the products of oxidative degradation effect.

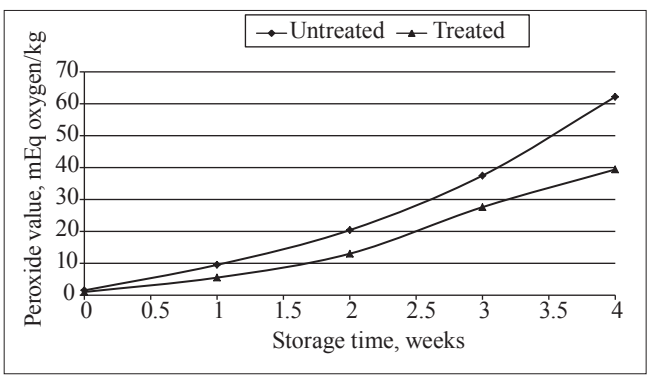

Fig. 8. Peroxide values of the samples of biodiesel

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