Improvement of the oxidation stability of biodiesel using natural antioxidants

by

Emmanuel Kongolo

Bachelor of Technology: Chemical Engineering

A thesis submitted in fulfilment of the requirements for the degree of Master of Engineering: Chemical Engineering in the Faculty of Engineering and the Built Environment at Cape Peninsula University of Technology

Supervisor: Prof Oluwaseun Oyekola

Co-Supervisor: Dr Debbie De Jager

Bellville campus, 26 August 2023

ABSTRACT

Efforts to meet the constantly increasing global energy demand without adverse environmental impacts have led to the development of alternative energy sources. Biodiesel, a biomass alternative, has been identified as a source with a potential substitute for fossil fuel-derived diesel for transportation purposes. Oxidation degradation is one of the main drawbacks to large-scale commercialisation of biodiesel. This study investigated the effect of three natural antioxidants, vitamins A, C and E (OA, OC and OE, respectively), on the oxidation stability of biodiesel derived from waste cooking oil (WCO). The antioxidants were first investigated individually and then in pairs. The effect of antioxidant concentration on oxidation stability was studied using the Rancimat method, which measures the biodiesel's induction period. When employed individually, it was shown that all of the antioxidants, with OC being the most effective, enhanced the oxidative stability of WCO biodiesel. At a concentration of 1000 ppm, OC lengthened the Rancimat induction duration of the WCO-biodiesel from 0.79 to 7 hours. Moreover, using OC as an antioxidant resulted in biodiesel meeting the EN14112/IS-15607 standard. This was explained by OC's low bond dissociation energy (BDE, 318.5 kJ/mol), low molecular weight (Mw, 176.16 g/mol), and acetyl palmitate production, which is known to exhibit potent antioxidant behaviour in oils. Following OC were OA and OE, which improved the oxidation stability of biodiesel. However, biodiesel prepared with added OA or OE did not meet the ASTM 6751 standard of 3 hours.

The combined effects of antioxidants in a 1:1 ratio demonstrated a synergistic influence of a mixture of OA and OC, OC and OE, and OA and OE with induction periods of 12, 7, and 0.33 hours, respectively. It can be concluded that combining natural antioxidants may have more excellent beneficial effects on the oxidation stability of biodiesel prepared from waste cooking oil than using them individually. This study has revealed the potential benefits associated with the use of selected natural antioxidants. Moreover, it offers valuable insight into using natural antioxidants in the biodiesel industry faced with the oxidation stability challenge when considering waste cooking

oil as feedstock. Further studies should consider investigating the mechanistic phenomena leading to the observed antioxidant activity at the molecular level to understand and improve antioxidant activity.

DECLARATION

I, Emmanuel Kongolo, declare that the contents of this dissertation/thesis represent my own unaided work and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed -- **Date** 26 August 2023 --------------------------------

ACKNOWLEDGEMENTS

I wish to thank and pass my sincere gratitude to:

- The Lord Jesus Christ, my Lord and saviour, for holding me together and seeing me through till the end of the master's degree.
- Prof. Oluwaseun Oyekola, my supervisor and mentor, for his guidance, mentorship, and patience with me throughout the journey, And to Doctor Shabani Malonda and Doctor Emmanuel Alechine Ameh for their assistance by reviewing this thesis.
- My church family of JTL Ministries and my Pastors (Rev. Kiluba wa Kiluba, Pastor Ebenezer, and Doctor Joshua) for their prayers and support.
- My brother and companion, Pastor Jonathan, and his wife, Sipiwe Katende, for their consistent love, support and encouragement from the beginning of this journey till the end. Zezethu Siqungathi for being a supportive colleague in this journey.
- My amazing family and friends, Job Kongolo for cooking and taking care of me, Thierry Kongolo, Rose Kongolo and Jacob Kongolo, Olivier Kasikala, Fabrice

  Kapiamba for their love and consistent support.

DEDICATION

This thesis is dedicated to my dear parents, Joseph Nkongolo Kabasele and Leonie Muyombe Kaosha, for their commitment to seeing me succeed in life by achieving great goals. Their love, moral, spiritual and financial support; for always being there for me at all times. I hope this achievement brings joy to your hearts and crowns all the sacrifices you made for me throughout this journey. Thank you very much for everything, I love you dearly, and I am serious about it.

ABBREVIATIONS

ASTM	American Society for Testing and Materials
AV	Acid value
BDE	Bond dissociation energy
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CNSL	cashew nutshell liquid
DG	Dodecyl gallate
EN	European norm
EQ	Ethoxyquin
GC-MS	Gas chromatography mass spectroscopy
IP	Induction period
OG	octyl gallate
OSI	Oil stability index
PG	Propyl gallate
PY	Pyrogallol
TBHQ	Tertiary-butylhydroquinone
TPSA	Topological polar surface area
WCO	Waste cooking oil

TABLE OF CONTENTS

TABLE OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

1.1 Background and motivation

The environmental concerns associated with the widespread use of fossil fuels (pollutants generation, global warming, diseases, etc.) and their depletion in a century characterised by high energy demand have resulted in a global energy crisis. Intense research efforts are invested in developing alternative energy sources (Edeh, 2020; Aransiola et al., 2014). Biofuels derived from biomass have been identified as potential alternatives or substitutes for fossil fuel energy sources (Khan et al., 2021). Biodiesel is a biofuel commonly produced via the transesterification of vegetable oils or animal fats with an alcohol in the presence of a catalyst, resulting in fatty acid alkyl esters (commercially known as biodiesel) and glycerol as a by-product (de Sousa et al., 2014). Due to its very low toxicity and high biodegradability, biodiesel is considered environmentally friendly. Its high flash point makes it safe to store and handle (Živković & Veljković, 2018).

Despite the numerous advantages biodiesel offers over conventional petroleum diesel, biodiesel production has been faced with several challenges, such as the high cost of conventional feedstock vegetable/edible oils, which poses debate about its demand between the biodiesel and food industry; high product separation and purification costs, including the challenge of concern regarding the oxidative degradation (oxidation stability) of biodiesel product upon storage over time (Alsultan et al., 2021; Romola et al., 2021). When exposed to light, heat, and the atmosphere over a period of time, biodiesel tends to undergo oxidative degradation (de Sousa et al., 2014). The oxidation degradation of biodiesel is one of the most significant technical drawbacks in considering its production and hinders its commercial use in automobiles (Kumar, 2017).

The oxidation stability (OS) of biodiesel depends on the feedstock oil or fat used, the concentration of naturally occurring antioxidants in the feedstock and the storage conditions (Alsultan et al., 2021). OS has been reported to be affected by the degree of unsaturation of alkyl esters in biodiesel, of which higher levels would result in increased susceptibility to oxidation (Botella et al., 2014). Thus, adding antioxidants to biodiesel is a suitable approach to retard or mitigate biodiesel oxidation degradation. Several studies have reported on the use of biodiesel antioxidants, aiming to improve the oxidation stability of the product (Mittelbach & Schober, 2003; Agarwal et al., 2015; Varatharajan & Pushparani, 2018; Pantoja et al., 2013; Ramalingam et al., 2016; Liang et al., 2006; Buosi et al., 2016). The antioxidants prevent free radicals' propagation and minimize the formation of compounds that lead to fuel degradation (Kivevele, 2020). Further, researchers have classified biodiesel antioxidants into synthetic and natural oxidants (Romola et al., 2021).

Most studies to date have focused on synthetic antioxidants, which are the most commonly employed and have proven successful in retarding the oxidation degradation of biodiesel obtained from various feedstocks (Mittelbach & Schober, 2003; Agarwal et al., 2015; Varatharajan & Pushparani, 2018; Pantoja et al., 2013). However, due to consumers' concerns regarding the biosafety of synthetic antioxidants, several researchers are shifting their focus towards natural antioxidants. Waste cooking oil (WCO), proven as a potential feedstock for biodiesel in previous studies (Math et al., 2010; Knothe & Steidley, 2009) is the feedstock of choice in this study. The feedstock is low-cost and readily available (Gaur et al., 2021). Its use for biodiesel production would serve both as a waste management strategy and environmental alleviation associated with minimizing waste disposal (Vidigal et al., 2021; Tshizanga et al., 2017).

1.2 Problem statement

The oxidation stability of biodiesel has come to the forefront of studies mainly because of the presence of unsaturated fatty acids derivatives in biodiesel that are readily oxidised, thus resulting in the formation of oxidation products such as peroxides and hydro-peroxides (Joshi et al., 2010). These products increase biodiesel's acidity, thus forming corrosive acids that can cause engine and fuel pump wear (Kumar, 2017). Furthermore, the process generates undesirable product deposits, such as polymers responsible for obstructing fuel filters and injection systems (Jain & Sharma, 2010b). These drawbacks have hindered the wide use of biodiesel in engine and fuel injection equipment, thus impeding the biodiesel market expansion. This study investigates the effect of natural antioxidants OA, OC and OE, their concentration and blending on the oxidative stability of biodiesel derived from WCO (WCO-biodiesel). Studies reported in the literature on using natural antioxidants to improve the oxidation stability of WCO are relatively scarce. Hence this study will investigate the ability of natural antioxidants to increase the oxidation stability of WCO.

1.3 Research aim and objectives

This study aims to investigate the capacity of selected natural antioxidants to improve biodiesel’s oxidation stability derived from waste cooking oil (WCO) feedstock. The following objectives have been defined in order to achieve the overall aim of the study:

- To select suitable and readily available natural antioxidants for stabilizing WCO-biodiesel.
- To investigate the effect of the various natural antioxidants on the oxidative stability of biodiesel
- To investigate the effect of antioxidant(s) concentration on biodiesel’s oxidative stability.

- To investigate the effect of blending the various antioxidants on biodiesel oxidative stability

1.4 Research significance

Study on the effect of selected natural antioxidants on biodiesel derived from WCO is limited. This study will provide industry and academia with knowledge and insight into the use of readily available antioxidants for biodiesel obtained from a waste by-product (WCO). This work could also amplify the pilot-scale studies and application of WCO for biodiesel production.

1.5 Research design and methodology

The following approaches were employed to meet the set objectives of the study:

1.5.1 Selection of natural antioxidants

Antioxidants that are natural and readily available were considered for this study. The idea was to use substances that can easily be obtained, are cost-effective, and can improve biodiesel oxidation stability. The study conducted was theoretical and based on information available in the literature.

1.5.2 Preparation and characterization of waste cooking oil-biodiesel

In a world with significant waste handling challenges, using WCO to produce a valuable product plays a role in addressing the challenge. Biodiesel was produced from WCO and characterised to ensure it complies with the specification standards.

1.5.3 Studies on effect of antioxidant addition on prepared biodiesel

Selected antioxidants were applied individually and also combined onto the WCO biodiesel. The Rancimat apparatus was used to determine the induction period, an indicator of biodiesel's oxidative stability. The effect of the concentration of the selected oxidants on biodiesel oxidative stability was investigated.

1.6 Research delineation

The investigations carried out were limited to biodiesel derived from WCO. This study did not investigate transesterification parameters, such as catalyst type, dosage, or alcohol-to-oil ratio.

1.7 Thesis outline

The outcome of this study is a comparative analysis of selected natural antioxidants and their synergistic effects in retarding the oxidative degradation of biodiesel. The thesis outline is as follows:

- **Chapter 1: Introduction**

The first chapter presents a general background on the topic under consideration. The problem to be solved is formulated, the objectives of this study are defined, and a general overview of how the problem will be resolved is presented.

- **Chapter 2: Literature Review**

The second chapter reviews the literature relevant to the oxidative degradation of biodiesel that occurs during the biofuel’s handling and storage. Various studies on the chemistry of oxidative degradation and the factors that enhance it are presented. In addition, different techniques used to retard the oxidation degradation of biodiesel are reported with a view to showing the existing literature gaps.

- **Chapter 3: Materials and Methods**

The third chapter describes the methodology used to solve the problem formulated in Chapter 1. The different materials and instruments used for the current study are presented. The experimental procedures involved are also presented.

- **Chapter 4: Results and Discussion**

The fourth chapter reports detailed outcomes of experiments conducted. The performances of selected natural antioxidants are compared. The results from antioxidants combined effects are also presented. The findings are discussed in line with existing literature.

- **Chapter 5: Conclusion and Recommendation**

Finally, the fifth chapter presents the conclusion drawn from the experimental results and recommendations are made for further studies.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Biodiesel has been identified as an attractive, suitable, and sustainable alternative to replace conventional petroleum diesel (Bashir et al., 2022). Biodiesel is biodegradable and has many technical advantages over petroleum diesel, such as higher cetane number and lubricity, reduced life cycle emissions of greenhouse gases, lower flammability with high flash point (up to 160°C), and less environmental damage in the event of spillage (Demirbas, 2009; Khounani et al., 2018; Dunn, 2008; Aransiola et al., 2014). Because of biodiesel's various advantages, it has been successfully used to substitute petroleum diesel in diesel engines (Kumar, 2017). Different techniques are used to produce biodiesel, including the supercritical fluid (Ayoola et al., 2021). Biodiesel production by transesterification is the most commonly used method due to its ease of operation (Aransiola et al., 2014; Ayoola et al., 2021). The process involves a reaction between the triglycerides contained in lipids feedstock with alcohols in the presence of acidic or basic catalysts to produce fatty acids alkyl esters (commercially known as biodiesel) and glycerol (Sivaprakasam & Saravanan, 2007) (Figure 2.1).

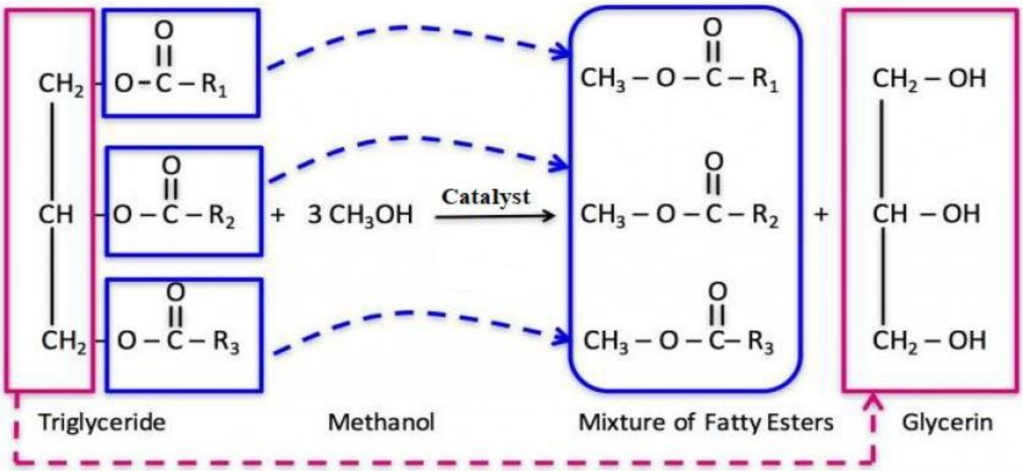

Figure 2.1: Transesterification reaction (with methanol) for biodiesel production (Almeida et al., 2018)

Several reported research studies have highlighted and elaborated on the oxidation degradation and the oxidative stability of biodiesel (Pullen & Saeed, 2012; Jain & Sharma, 2010a; Fattah et al., 2014; Romola et al., 2021). The parameters that influence the oxidation stability of biodiesel have been extensively reviewed, and different methods that can be used to improve oxidation stability and retard the oxidation degradation of biodiesel have been discussed (Jakeria et al., 2014; Bukkarapu & Krishnasamy, 2022). Fattah et al. (2014) reviewed different studies on the efficiency of antioxidants used individually and as blends on the oxidation stability of biodiesel derived from different feedstocks. Sorate and Bhale (2015) reviewed the effects of various antioxidants on the oxidation stability of biodiesel. They investigated the use of biodiesel containing antioxidants on the performance and emission of an engine.

This chapter reviews the literature relevant to the oxidative degradation of biodiesel that occurs during biodiesel handling and storage in the long term. Studies on the oxidation mechanism, the factors that enhance it, and its effects on biodiesel quality are presented. In addition, different techniques used to retard the oxidation degradation of biodiesel are presented to highlight gaps in the literature that further research should consider bridging.

2.2 Oxidative stability of biodiesel

Oxidation stability refers to the resistance to oxidation responsible for chemical changes during the handling or long-term storage of biodiesel (Yesilyurt et al., 2020). Oxidation stability indicates biodiesel’s shelf-life before complete oxidation by atmospheric oxygen, leading to the generation of undesired products that are harmful to automobile engines (Anwar & Garforth, 2016). The oxidative stability of biodiesel and its blends is generally much lower than that of petroleum diesel under the same storage and handling conditions (Santos et al., 2012). Light, heat, moisture, and contaminants make biodiesel prone to degradation (Saluja et al., 2016; Verma et al., 2016; Serrano et al., 2013). Products such as peroxides and hydroperoxides are generated when biodiesel is oxidised. These products further react to produce compounds such as ketones, aldehydes, alcohols, and low molecular weight acids (Joshi et al., 2010). A thorough understanding of the causes of oxidative degradation of biodiesel is required in order to be able to retard or even mitigate the degradation process.

2.2.1 Causes of oxidative degradation of biodiesel

Biodiesel oxidation results from the unsaturation of free fatty acid molecules in biodiesel. The unsaturation is due to the presence and position of double bonds, which make compounds susceptible to attacks by oxygen (Kumar, 2017; Jain & Sharma, 2010a). The reactivity of free fatty acids is greatly enhanced by the presence of double bonds in the acids' structure, especially when the molecules are exposed to water or air (Christensen & McCormick, 2014). The extent of unsaturation determines biodiesel's stability; the degradation rate is higher at high degrees of fatty acids unsaturation. There is a correlation between the number and position of bis-allylic sites on free fatty acid molecules and the oxidative degradation of biodiesel. Monounsaturated fatty acids are less susceptible to oxidation than polyunsaturated fatty acids due to allylic methylene structure (Yaakob et al., 2014).

Naturally occurring fatty acids are generally polyunsaturated with the methylene interrupted configuration; their structure comprises multiple cis-double bonds with a single methylene unit in-between (Saluja et al., 2016). The degree of stability in fatty acids with methylene interrupted structure is much lower than that of its isomers or conjugated structures; in this sense, the delocalization of pi-electrons results in partial stabilization of the structure in the latter. Polyunsaturated fatty acids contain bis-allylic sites, which are sensitive when exposed to ambient conditions and are easily attacked by oxygen (Kumar, 2017). A specific example is creating a bis-allylic site at 11th carbon, which is created in linoleic acid and methyl linoleate with carbon-carbon double bonds at the 9th and 12th carbon positions (Sia et al., 2020) (Figure 2.2).

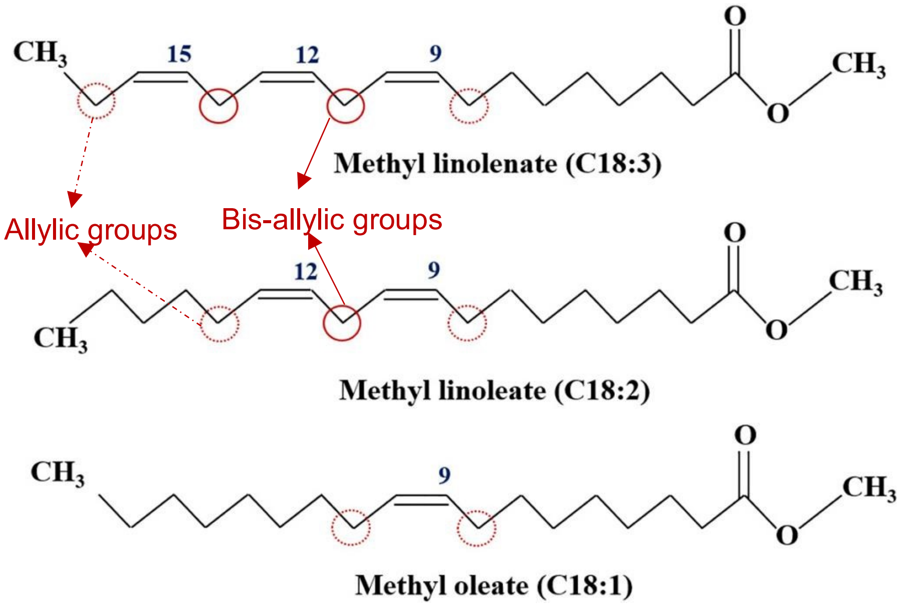

Figure 2.2: Allylic and bis-allylic groups on methyl linolenate, methyl linoleate and methyl oleate (Kongprawes et al., 2021)

Since hydrogen radicals on the bis-allylic site can be easily removed at the initiation step of oxidation, the bis-allylic site becomes weaker and, therefore more sensitive to oxidation attack than allylic sites (Saluja et al., 2016). Linolenic acid and methyl linolenate contain three carbon-carbon double bonds at the 9th, 12th and 15th positions, giving it two bis-allylic sites at the 11th and 14th carbons, making it more unstable than linoleic acid and methyl linoleate.

2.2.2 Chemistry of oxidation degradation of biodiesel

The process of oxidation degradation of fatty oils involves multiple steps: conjugated diene and hydroperoxides, referred to as primary degradation products, which decompose and then react with each other. The reactions are known to generate several secondary products such as formic acid, formate esters, aliphatic alcohols, aldehydes, fatty acids (short chains) and various high molecular weights compounds

(Sharma et al., 2008). The multiple processes involved in oxidation degradation can be subdivided into two main stages, which explain the chemistry of oxidative degradation: primary and secondary oxidation stages. The primary steps involve the following stages (**Figure 2.3**): initiation, propagation, and termination (Mofijur et al., 2013).

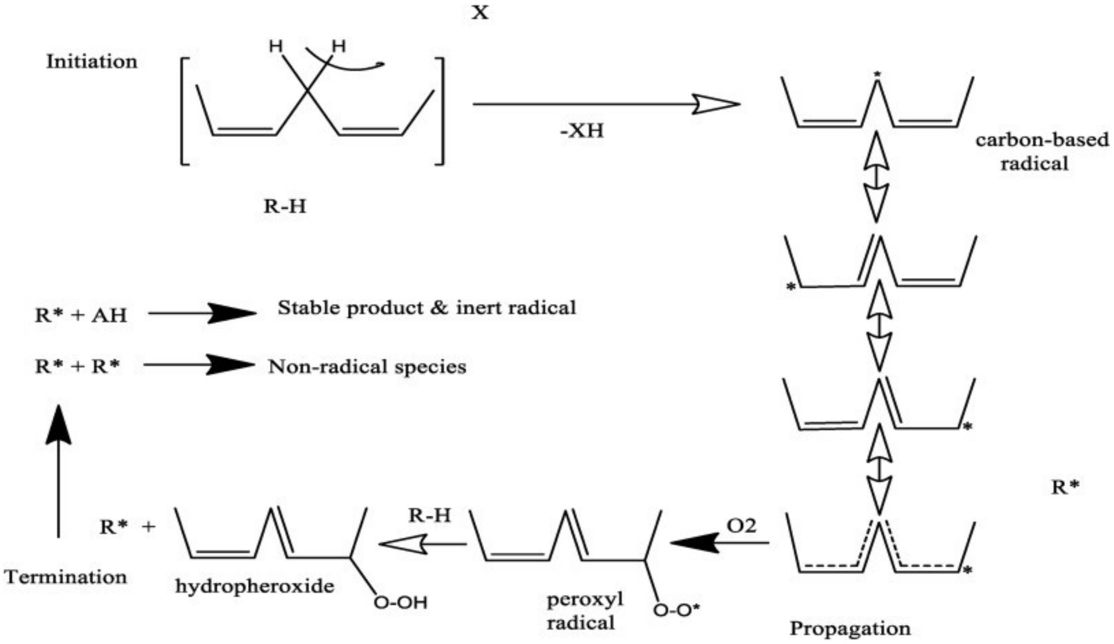

Figure 2.3: Stages of primary phases of biodiesel oxidation reaction (Amran et al., 2022)

The initiation step results in the production of carbon-free radicals (R•); this occurs through the removal of hydrogen from carbon atoms of free fatty acids alkyl esters (RH) by an initiator radical (I). Hydrogen atoms removal from polyunsaturated fatty acids leading to the formation of carbon-based radicals (R•) is the main characteristic of the initiation step. The radical hydrocarbon groups formation occurs on the bis-allylic carbon. As the process proceeds, there is a chemical reaction between free radicals generated in the initiation phase and antioxidants present the biodiesel this retarding biodiesel oxidation. The process depletes the antioxidants compounds present in the biodiesel without altering the fuel quality. The duration of the initiation phase is determined by the concentration of reactive species and antioxidants, and environmental factors such as light, heat and moisture (Schaich et al., 2012).

The propagation phase follows the initiation stage, also referred to as peroxidation. At this stage, peroxide formation occurs as oxygen is consumed at high rates. Hydroperoxides are generated as radicals of reaction with oxygen. Alkyl radicals formation also occurs during the oxidation propagation stage (Saluja et al., 2016). The presence of hydroperoxide radicals further enhances the propagation stage. The rate of peroxy radical production is significantly increased by the presence of diatomic oxygen in polyunsaturated fatty acids, thus hindering the substantial production of carbon-based radicals (Jain & Sharma, 2010a). Peroxy radicals are less reactive than carbon free radicals. However, they are reactive enough to attract hydrogen atoms, forming carbon-free radicals and hydroperoxides (ROOH) (Saluja et al., 2016; Jain & Sharma, 2010a). The diatomic oxygen present reacts with carbon-free radicals, resulting in increased propagation.

Before the propagation oxidation reaction, the ROOH concentration is relatively low. At the end of the initiation process and the onset of the propagation reaction, there is a spike in ROOH concentration; this makes it possible to determine the oxidative stability of fatty acids or biodiesel under stress conditions (Rashedul et al., 2014). There is a direct or indirect alteration of fatty acids or biodiesel properties at stressed conditions. A rapid increase in ROOH concentration characterizes the end of the induction period (Verma et al., 2016). As the propagation reaction occurs, carbon-free radicals from the initiation stage, which are highly reactive, immediately react with available bi-radical oxygen, leading to the formation of peroxide radicals (ROO•) (Saluja et al., 2016). Hydrogen atoms from the fatty acids alkyl esters (FAAE) molecules are removed by peroxide radicals resulting in the formation of form fatty acid hydroperoxides (ROOH) and carbon free radicals (Rashed et al., 2015).

Formation of non-radical products due to the reaction between the accumulated peroxides occurs as the termination stage begins. Chain reactions stop as radicals (R• or ROO•) react to form more stable products. Stable products are the result of the reaction between two alkyl radicals or two peroxide radicals (Figure 2.4) (Strömberg et al., 2013):

$$R\bullet + R\bullet \rightarrow R-R$$
$$ROO\bullet + ROO\bullet \rightarrow \textit{stable products}$$

Figure 2.4: Termination/secondary stage of oxidative degradation

The secondary oxidation phase, referred to as vinyl polymerization process, is characterized by hydroperoxide decomposition, leading to the formation of aldehydes (propanals, hexanals and heptanals), aliphatic alcohols, formic acid, and formate esters. These compounds alter the properties of biodiesel (Yaakob et al., 2014). Biodiesel acidity increases due to the presence of short-chain free fatty acids formed during the secondary oxidation stage. In both primary and secondary oxidation phases, the oxidation reactions will proceed with complete exhaustion of reactive sites of the fatty acid molecules. In the secondary phase, the peroxide formation rate is lower than the peroxide degradation rate. Hydroperoxide residues remaining after peroxidation and other degradation products are either polymerised to sludge or transformed into short-chain acids and aldehydes. Rapid generation of acids and other degradation products heavily alters biodiesel properties. When used in automobiles, degraded biodiesel leads to the clogging of filters and injectors due to the presence of the sludge (Strömberg et al., 2013; Christensen & McCormick, 2014).

The primary products formed during oxidation are allylic hydroperoxides species, where double bonds initially present in the fatty acids shift or undergo cis/trans isomerisation. Various products are generated in the secondary oxidation stage due to the rearrangement, fission, and dimerization of unstable hydroperoxides. Different products such as monomeric, oligomeric, and short chains are formed during the secondary oxidation stage. At the end of the secondary oxidation stage, different products are formed as reactions such as dehydration, cyclization, rearrangement, radical substitution, chain cleavage, and dimerization occur simultaneously. The number of products from the secondary oxidation stage varies; and is determined mainly by the nature of the substrate (Knothe, 2007). Secondary oxidation products comprise saturated and unsaturated aldehydes, acids, and dimers, etc (Ukaew et al., 2014). An increase in acidity occurs during oxidation, owing to hydrolysis reactions caused by the presence of water and oxidation by oxygen (Bondioli et al., 1995). The presence of unstable compounds in biodiesel leads to the formation of free radicals upon oxidation (Mantovani et al., 2018). The radicals react with oxygen, resulting into the formation of a larger number of free radicals. The reaction between the free radicals formed with olefinic species produces gums (Kumar, 2017). Secondary oxidation reactions occurring at high rates and forming large amounts of free radicals and peroxide compounds must be eliminated to preserve biodiesel quality during handling and storage (Kivevele & Huan, 2015).

2.2.3 Consequences of oxidative degradation

Unsaturation of fatty acids associated with the presence and position of double bonds makes it prone to polymerization reactions, resulting in adverse effects on biodiesel properties (Saluja et al., 2016). Polymerisation reactions, in turn, produce gums in biodiesel which clog engine filters and injection systems. Hydroperoxides and acids generated in large quantities during oxidation degradation have been found to lead to corrosion in the engine system, adversely affecting engine efficiency and performance (Kumar, 2017). Kreivaitis et al. (2011) observed a significant increase in engine wear off when biodiesel stored for 70 days was used. They attributed their observations to the possible formation of deep scratch marks in the engine, resulting from impaired absorption lubrication film, consequently leading to direct contact between metal and oxidation products. Peroxides formed during the propagation phase and acids produced during the initiation stage tend to deteriorate the absorption film and the lubrication properties of biodiesel (Fox et al., 2004). At the end of the induction period, highly unstable hydroperoxide molecules promote the breaking down of fatty acids, further degrading biodiesel lubrication properties (Kumar, 2017). Engine wear is also enhanced due to the interaction of hydroperoxide molecules with the engine surface (Kreivaitis et al., 2013). Engine corrosion and wear in the overall engine surface are due to the formation of acids during the secondary oxidation stage. Engine operation and emissions are significantly affected by the oxidative degradation of biodiesel. Monyem and Van Gerpen (2001) have reported a 15.1 increase of brake specific fuel consumption (BSFCs) of a diesel engine when using oxidized biodiesel. The observation was attributed to possible variation in combustion timing because of biodiesel’s higher cetane number, injection time and lower heating value. Further, oxidized biodiesel showed a lower carbon footprint as compared to unoxidized biodiesel. The former had no significant effect on the brake thermal efficiency (BTE) and showed high NOx emissions at standard injection timing.

The composition of biodiesel and physicochemical properties (such as density, acid value (AV), flash point, viscosity, induction period (IP) and peroxide value (PV)) are altered during storage and handling due to oxidation degradation (Jain & Sharma, 2010b; Bondioli et al., 2003; Bouaid et al., 2007; A. Sarin et al., 2009; Sharma et al., 2009). These properties are used to monitor the extent of biodiesel oxidation. The most common properties indicators of biodiesel quality are AV and viscosity (Kumar, 2017). Oxidation degradation results into a tremendous increase in biodiesel AV, which can be explained by the formation of different products during the secondary oxidation stage, which oxidise hyperoxide molecules to produce acids (Pattamaprom et al., 2012). Thus, biodiesel's physical properties are altered mainly due to oxidation products: hyperoxides, acids, aldehydes, ketones, etc.

2.2.4 Measurement of oxidative stability

European standards EN14213 and EN14214 and US standards ASTM D6751 have set standard specifications for biodiesel oxidative stability (Knothe & Razon, 2017; Anwar & Garforth, 2016; Saluja et al., 2016; Jain & Sharma, 2010a). The Rancimat method (EN14112) and the Oxidative Stability Index (OSI) are among the techniques used to measure biodiesel's oxidative stability (Saluja et al., 2016). Both these methods involve heating biodiesel samples to temperatures around 110 °C in a sealed tube (Monirul et al., 2015). Oxidation is simulated by passing air through a biodiesel sample. A gas containing primary oxidation products is released from the oxidation sample, and passed through a tube containing distilled water, equipped with a conductivity meter (Jain & Sharma, 2010a). The conductivity, an indicator of oxidation degradation, is monitored over time until a sharp increase occurs. This increase indicates the generation of water-soluble products such as short-chain carboxylic acids (formic acid and acetic acid) (Rashed et al., 2015). The time corresponding to the sharp increase in conductivity is the induction period (Saluja et al., 2016).

2.3 Factors affecting oxidation

The following have been identified as factors associated with biodiesel degradation: the presence of bacteria or fungi resulting in microbial degradation; elevated temperatures causing thermal oxidation; the presence of moisture leading to hydrolysis; and oxidation due to the presence of oxygen (Dunn, 2005; du Plessis et al., 1985; Thompson et al., 1998). The factors associated with the oxidation process include fatty acid structure, trace metals, heat, various impurities, peroxides, light, and the contact surface area between biodiesel and air (Knothe, 2007). These factors catalyse the initiation process, removing the hydrogen atoms from fatty acids alkyl esters.

2.3.1 Structure of fatty acid

Biodiesel comprises fatty acid mono-alkyl esters (FAAE) and long-chain unsaturated species and long chain saturated species which are monosaturated and/or polyunsaturated in different proportions. There are minimal differences between the chemistry of biodiesel degradation and that of the fatty oils it derives from. The feedstock type is one of the main determining factors of fuel properties (Altun, 2014). The presence of unsaturated fatty acid chains makes biodiesel prone to oxidation. The greater the degree of unsaturation of fatty acids the more biodiesel is likely to undergo oxidation degradation. Unsaturated fatty acids are easily attacked by oxygen (Thompson et al., 1998; Dunn, 2002). For example, studies have shown that Crambe oil biodiesel was more stable than soybean oil biodiesel due to the difference in fatty acid profile (i.e., degree of saturation) (Laghetti et al., 1995; Wazilewski et al., 2013). Crambe oil is mainly made of erucic acid (56%), a 22 carbon atoms chain with one double bond. On the other hand, soybean oil contains the 18 carbon atoms chains linoleic acid (54%) and oleic acid (24 %); the former acid contains 2 double bonds and the later 1.

Moser (2009) conducted studies of the oxidative stability of fatty acid alkyl esters (FAEE) by comparing the Rancimat technique and the pressurized differential scanning calorimetry (PDSC) method. The paper reported a decrease in the oxidation stability of FAEE with an increased number of double bonds, a reduction in the distance between the unsaturation and ester group, and a decline in the number of hydroxyl groups. Moreover, it was reported that there was higher sensitivity to cis-isomers' oxidation than trans-unsaturated sites. However, conjugated trans-unsaturated compounds were more susceptible to oxidation than cis-unsaturated compounds.

The higher number of reactive bis-allylic sites in polyunsaturated fatty acids than in monounsaturated acids makes them more prone to oxidation (Karavalakis et al., 2010). Radicals easily attack the bis-allylic protons; thus, the molecules containing them easily undergo oxidation and lead to the generation of polar oxy compounds. The high reactivity is caused by the attraction of electrons near the double bonds at each side of the methylene group. The hydrogens in the methylene group become more acidic, translating into easy removal of atoms in the presence of a radical initiator (Karavalakis et al., 2010). Hence, biodiesel derived from oils rich in unsaturated acids such as soybean, sunflower and grape seed oils which contain large concentrations of linoleic and linolenic acids has low oxidation stability.

On the other hand, biodiesel derived from oils with very low unsaturated fatty acids (such as palm oil, olive oil, and almond oil) tend to exhibit high resistance to oxidation (Ramos et al., 2009). Moreover, the rate of oxidation of fatty compounds is determined by the degree of unsaturation (number and position of double bonds). The oxidative stability has been reported to decrease with increasing ester side chain length (Jain & Sharma, 2010a). It is suggested that longer side chains contain sites more easily accessible to oxidation, making them prone to cleavage than shorter chains. Knothe and Dunn (2003) have observed that when two molecules have the same number of double bonds, the one with higher molecular weight exhibits more excellent oxidation stability as measured by the oil stability index (OSI).

A study conducted on biodiesel oxidation observed that the induction period of biodiesel derived from soybean (2.68 h) was much longer than that of biodiesel derived from poultry fats (0.52 h). The concentration of linolenic and linoleic acids in soybean oil (63%) was, however, much higher than in poultry fats (28.4%) (Kivevele & Huan, 2015). This is because soybean oil-biodiesel contained a higher concentration of natural antioxidants than biodiesel derived from poultry fats. These observations indicate that biodiesel obtained from vegetable oils tends to be more resistant to oxidation degradation than animal fat-derived biodiesels.

2.3.2 Effect of external factors on biodiesel degradation

Biodiesel can be oxidized without any external initiation; however, external factors affect the oxidation reaction rate. Studies have shown that factors such as temperature, aging, exposure to open atmosphere (air, sunlight, etc.) greatly influence the rate of biodiesel oxidation (Sia et al., 2020; Aquino et al., 2012). Biodiesel is known for its tendency to absorb moisture, which is a factor that accelerates biodiesel oxidative degradation. It has been however observed that compared to other external factors, moisture's effect is generally minimal (Yang et al., 2016). Bouaid (2007) conducted studies on the oxidation degradation of biodiesels prepared using high oleic sunflower oil (HOSO), high and low Erucic Brassica Carinata oil (HEBO and LEBO) and used frying oil (UFO). He subjected the biodiesels prepared from selected oils to a set of storage conditions for a duration 30 months. The findings showed an acceleration of the oxidation process with increased storage time for all the biodiesel samples under observation. Sunlight only had significant effects after 12 months of observation. Storage temperature had a significant impact on oxidation degradation. Higher temperatures resulted in higher degradation rates and increased sample loss due to evaporation associated with highly volatile products formed from the cleavage of fatty acid chains. An analysis of the volatile products revealed the presence of peroxides, alcohols, aldehydes, ketones, and various low molecular weight species.

Thompson et al. (1998) studied the oxidation degradation of rapeseed methyl and ethyl esters over two years of storage. They found that indoor storage resulted in higher oxidation degradation than outdoor storage, probably due to higher mean temperature indoors. Lin and Chiu (2010) investigated the oxidation degradation of palm oil-derived biodiesel at 20 °C and 60 °C, with and without antioxidant addition. The biodiesel without antioxidants and stored at a higher temperature, degraded faster than the biodiesel sample containing antioxidants at the same temperature. Furthermore, it was reported that the degradation rate accelerated over time. Leung et al. (2006) studied the effect of storage temperature on biodiesel degradation. They reported that high temperature storage increased the degradation rate much more than lower temperature storage. Likely, Bondioli (2003) investigated the oxidation degradation of biodiesel derived from rapeseed oil for extended storage periods under various conditions. It was observed that the storage container's characteristics and temperature affected the path of oxidation. Berrios et al. (2012) also investigated the effect of temperature on the oxidation degradation of biodiesel. Both studies reported that temperature significantly influences the degradation process of biodiesel. Some studies even recommended underground tank biodiesel storage (Kumar, 2017).

A study was conducted by (Agarwal et al., 2015) on the effect of sunlight and air exposure on the oxidation stability of biodiesel. The authors observed that the two factors significantly affected the degradation of biodiesel. The induction period of the biodiesel exposed to sunlight while stored in a metal container open to the atmosphere decreased from 21.12 to 2.25 hours. Another biodiesel sample stored in a non-metal container and exposed to the same atmospheric air and sunlight resulted in an induction period of 21.12 to 3.42 hours. Thus, the process of biodiesel degradation was accelerated when stored in a metal container (Das et al., 2009). Aquino et al. (2012) observed a significant acceleration in biodiesel degradation when the sample was exposed to light and stored at high temperatures. The study showed that the best storage conditions for biodiesel are the absence of light and room temperature. Thus, it has been concluded by most investigations that the external (storage) conditions have a significant effect on biodiesel degradation (Bondioli et al., 2003; Leung et al., 2006; Berrios et al., 2012; Thompson et al., 1998; Sharma et al., 2009), calling for a need to investigate such conditions (in this work) to enhance the shelf life of biodiesel and ascertain its suitability for large scale commercialisation (Christensen & McCormick, 2014).

2.3.3 Effect of impurities and storage vessel

The quality and stability of biodiesel are affected by possible trace substances remaining in the product after preparation (A. Sarin et al., 2009; Jakeria et al., 2014).

The presence of trace metals can catalyse reactions leading to the formation of free radicals, and this could result in degradation of biodiesel properties (Lepri et al., 2011). Moreover, during storage in various kinds of vessel or whilst used in engine, biodiesel is exposed to various metals (copper, zinc, lead, tin, and bronze) with the potential to accelerate the rate of oxidative degradation. According to Fazal et al. (2022), the presence of metallic ions tends to increase the rate of peroxide generation (Figure 2.5).

$$RH + I = R' + IH$$

$$ROO + M^{2+} = RO' + OH + M^{3+}$$

$$ROOH + M^{3+} = ROO' + H' + M^{2+}$$

Figure 2.5: Formation of initiator radical (Fazal et al., 2022)

Sarin et al. (2009) investigated the effects of metal contaminants' concentration on the oxidation degradation of biodiesel derived from jatropha oil. It was found that metal content had a detrimental effect on biodiesel's oxidation stability. The concentration of the metals had minimal effect such that both low and high metal concentrations resulted in nearly the same degree of degradation extent. Copper (Cu) resulted in highest detrimental effect based on the catalytic effect observed. Further, it was also shown that the antioxidant concentration used depends on the type of metal present in biodiesel. Observations made by Sarin et al. (2009), showed that antioxidant concentrations of 500, 700, 900 and 1000 ppm was needed to inhibit the oxidation degradation of biodiesel containing iron, nickel, cobalt and copper, respectively; with the view to obtain biodiesel sample that could comply with the EN-14112 specification. Knothe and Dunn's (2003) studies also showed that copper (Cu), among Fe and Ni metals, showed the highest adverse effect on the oxidation stability of biodiesel; with Fe being the least.

The affinity of monounsaturated methyl oleate in biodiesel is greater towards copper than steel and other metals. Contact between biodiesel and copper increases the linoleate formation rate, which adversely affects biodiesel's oxidation stability. Exposure to metal surfaces also increases biodiesel’s hygroscopic properties (Cursaru et al., 2014). Hence based on given literature, the presence of metals in biodiesel catalyses the initiation stage of biodiesel oxidation, increasing the rate at which free radicals are produced and leading to poor oxidation stability of biodiesel (Aquino et al., 2012).

2.4 Effect of oxidation of biodiesel on fuel properties

When biodiesel is oxidised, the fuel chemistry and physicochemical characteristics are affected. The effect of oxidation on biodiesel properties is reviewed in the following subsection.

2.4.1 Lubricity and wear capacity

When biodiesel is oxidised, free radicals react with atmospheric oxygen and substrates, yielding peroxides, hydroperoxides, carboxylic acids, and other complex compounds (Fu et al., 2017). Polymerization reactions, being part of the biodiesel oxidation process, adversely alter the lubricity of biodiesel. Consequently, gums are produced, making biodiesel applications prone to clogging filters and injection systems of automobiles. Corrosion in automobile fuel systems may also occur due to the action of hydroperoxides and high acids concentrations produced during oxidation. As mentioned earlier, the factors would result in engine performance deterioration (Jain & Sharma, 2010b).

Kreivaitis et al. (2011) reported a significant deterioration of biodiesel’s lubrication properties due to oxidation. The study involved four balls tested in biodiesel samples stored over time. A greater extent of biodiesel oxidation was associated with more significant wear off the balls and deeper scratch marks. This was due to the deterioration of the adsorption layer in the balls, which resulted in direct metal contact with biodiesel. The stability of the lubrication adsorption film was destroyed by peroxides formed during the early stages of oxidation. These, in return, enhanced the decomposition of Fatty acids chains, resulting in significant deterioration of biodiesel lubrication properties (Christensen & McCormick, 2014). Compounds such as formic, acetic, propionic, caproic acids, etc., resulting from biodiesel oxidation, are responsible for the corrosive and overall wear capacity of biodiesel (Goto et al., 2009). Biodiesel's lubricity has been reported to improve during long-term storage, whereas friction and wear characteristics are negatively affected due to oxidation (Milano et al., 2022). The utilisation of biodiesel leads to increased engine oil dilution, resulting in increased complexity of the lubrication of the engine's moving parts. Hydroperoxides produced during biodiesel oxidative degradation, further react to generate hydrocarbon and proxy radicals, and the compounds further enhance engine wear upon contact with the metal surface (Baena & Calderón, 2020).

Materials used to build diesel engines include elastomers, ferrous (cast irons and steel) and non-ferrous alloys (aluminium and copper alloys) (Yamagata, 2005). According to the aforementioned literature, oxidised biodiesel is detrimental to metals (such as aluminium, zinc, brass, bronze, and copper) (Bhardwaja et al., 2014), such that biodiesel has been deemed more corrosive than diesel (Cursaru et al., 2014). Geller et al. (2008) studied the compatibility of Carbon Steel, 316 Stainless Steel, copper, and brass with poultry fat-biodiesel and diesel fuel mixtures. It was observed that neither weight loss nor corrosion occurred for both carbon steel and 316 stainless steel for all biodiesel concentrations investigated over ten months, except for copper, followed by brass.

2.4.2 Efficiency and greenhouse gases emission

Alteration of the chemistry of biodiesel due to oxidation greatly influences its effects on engine efficiency and gases emissions. Studies done by Monyem and Van Gerpen

(2001) to assess the influence of oxidized biodiesel on engine performance and emissions using a turbocharged direct-injection diesel engine. They selected oxidized, non-oxidized biodiesel and diesel fuel for their investigations at different loads and injection times. According to their finding, oxidised biodiesel gave the highest brake specific fuel consumption, followed by diesel and non-oxidized biodiesel. It was suggested that this observation was due to the lower heating value and higher cetane number of non-oxidised biodiesel, which relate to combustion and injection time changes. Oxidised biodiesel, however, did not affect the brake thermal efficiency and was further found to emit the least amount of carbon monoxide and the most NOx gases. High NOx and low CO emissions reported for oxidised biodiesel probably resulted from high oxygenated product contents. Thus, high cetane number and high oxygen content in oxidised biodiesel, make it prone to total combustion and increased engine power, resulting in high fuel consumption than diesel and unoxidized biodiesel (Pattamaprom et al., 2012).

2.4.3 Effect on fuel quality

The wide commercialisation of biodiesel is impeded by the oxidation degradation effects on the fuel quality (Kumar, 2017). Physicochemical properties of biodiesel; density, viscosity, acid value (AV), peroxide value (PV), flash point and composition; tend to alter during the product storage (Kumar, 2017). Again, the changes in biodiesel properties mainly owe to formed oxidation products such as hydroperoxides, aldehydes, ketones, and acids. The physicochemical properties of biodiesel and its composition indicate the extent of biodiesel degradation. According to Pullen & Saeed (2012), acid value and viscosity are the most common properties used to monitor biodiesel oxidation.

2.4.3.1 Acid value

The acid value, also called acid number, indicates the acidity of biodiesel. The acid number corresponds to the quantity in milligrams of potassium hydroxide per gram of biodiesel sample required for the titration of the sample in the solvent and whose volume corresponds to a non-aqueous buffer solution (Dijkstra, 2016). The acid value can be affected by several factors, such as the water content of the feedstock and the degree of oxidation of biodiesel (Haijing et al., 2017). The oxidative degradation of biodiesel significantly increases the acid value (Zuleta et al., 2012). A study was conducted on the effect of storage temperature on the oxidation stability of biodiesel over a year (Moser, 2009). An increase in acid value over time was reported, and this was proportional to decreased oxidation stability.

2.4.3.2 Oil feedstock type

Yang et al. (2013) investigated the effect of oil feedstock type on biodiesel using three biodiesel samples (soybean, canola, and animal fat) to determine the change in acid value. It was found that soybean-derived biodiesel showed the most significant increase in acid value compared to the other biodiesels used due to the polyunsaturated nature of soybean.

2.4.3.3 Viscosity

Viscosity significantly affects the fuel injection characteristics, making it an essential characteristic of a direct injection fuel. Biodiesel becomes more viscous upon oxidation (Zuleta et al., 2012). An increase in viscosity is favoured by polymerisation reactions occurring during biodiesel oxidation. This process produces insoluble, long-chain saturated compounds, sediments, and gums that plug engine filters and injection systems (Komariah et al., 2018). Biodiesel oxidation produces biodiesel with long chain molecules and high molecular weight free fatty acids (FFA) with cis or trans double bond isomerization (Knothe, 2007). Biodiesel oxidation leads to an increased degree of saturation of the biofuel, which correlates to increased viscosity. Geller et al. (2008)

conducted a study on the influence of long-term storage on different physical properties of poultry fat-derived biodiesel and blends. It was observed for all samples that the viscosity either slightly dropped or remained relatively constant over time. The study also showed sediment accumulation at the bottom of storage containers, with the most sediments in pure biodiesel. Moser (2011) investigated the effect of long-term storage on the kinematic viscosity of biodiesel over 12 months. His findings showed a slight increase in the kinematic viscosity at the end of the observation period for biodiesel stored at low temperatures. On the other hand, the viscosity increase was substantial over the observation period for biodiesel stored at high temperatures.

2.4.3.4 Density

Biodiesel density is a function of the average molecular weight. Studies were conducted on the oxidation stability of palm oil, jatropha oil, coconut oil-derived biodiesel and some of their blends over three months (Shahabuddin et al., 2012). A density increase over the period was reported. The change in density was explained by the formation of insoluble sediments and generation of oxidation products. Biodiesels with shorter chain-hydrocarbons and highly saturated fatty acids exhibited the highest density increase due to oxidation products formed and condensing into the resultant density. Another study found that storage time also affected biodiesel density (Khalid et al., 2013). An increase in density was associated with longer storage times, possibly due to increased molecular interactions upon forming peroxides during oxidation.

2.5 Prevention of oxidation

Researchers have been developing different methods to improve biodiesel's oxidation stability (Karavalakis et al., 2011; Hazrat et al., 2021; Amit Sarin et al., 2009). The most promising methods are associated with the structural modification of species contained in biodiesel and using substances with antioxidant properties.

2.5.1 Structural modification

It has been established that biodiesel's oxidation degradation depends on its composition and the structure of the molecules it comprises. The ability to vary biodiesel composition can influence its oxidation stability (Kumar, 2017). The main compounds that make up biodiesel are triglycerides. Triglycerides are either saturated, monounsaturated, or polyunsaturated (Fukuda et al., 2001). Fatty acids in biodiesel contain saturated and unsaturated triglycerides and are long chain-hydrocarbons of 16 - 18 carbons. The most common are palmitic, stearic, oleic, linoleic, and linolenic acid. Other fatty acids occur in small concentrations in most oils and fats used to prepare biodiesel (Jafarihaghighi et al., 2020; Ramos et al., 2009). High number of saturated fatty acids (SFAs) and a relatively low of unsaturated fatty acids (UFA), would result in biodiesel having high viscosity, poor cold flow properties, high cetane number, high calorific value, and resistance to oxidation degradation (Kumar, 2017). To obtain biodiesel with high oxidative stability and good cold flow properties, it will require that the oil feedstock used consists of a higher concentration of SFAs as compared to unsaturated ones (Kumar, 2017). Thus, enhancing SFA concentration of SFAs in biodiesel will improve its oxidation stability, at the expense of cold flow properties (Sia et al., 2020). Nonetheless, using monounsaturated fatty acid (MUFA) oils offers the possibility of establishing a good balance between oxidative stability and cold flow properties of biodiesel (Li et al., 2013; Kumar, 2017). Oxidation stability of biodiesel could be improved by increasing the concentration of monosaturated fatty acids (MUFAs) (e.g., oleic acid) and decreasing the concentration of polyunsaturated fatty acids (PUFAs) (e.g., linoleic and linolenic acids); the latter being the factor to oxidation degradation (Serrano et al., 2013). Some researchers recommend the use of oil feedstocks that comprise shorter-chain fatty acids owing to good oxidation stability. Such oil feedstocks do not require transesterification; and could be of an advantage to lowering the biodiesel production cost (Brahma et al., 2022).

Chemical modifications of oil feedstocks through processes such as hydrogenation and ozonation, offer the possibility of improving the oxidation stability of biodiesel (Kivevele & Huan, 2015). The hydrogenation of vegetable oils is a common practice used improve its oxidation stability and texture to the required standard (Dijkstra & van Duijn, 2016). Studies on the hydrogenation of polyunsaturated fatty acids methyl esters (PUFA) and monoesters in the presence of a suitable catalyst, have shown significant improvements in the biodiesel's oxidation stability and its storage properties (Lu et al., 2018). Ozonation has also been used to modify vegetable oils to improve their oxidation stability (Sadowska et al., 2008).

Vegetable oils have mostly been genetically modified in the food industry. Such modifications show great potential for the fuels sector. The use of chemical modification to improve oxidation stability of vegetable oils, is currently limited in industrial applications: lubricants, precursors, or diesel oil substitutes (Salimon et al., 2012). Genetic modification offers greater potential than chemical modification when it comes to improvement of oxidation stability of biodiesel (Chellamuthu et al., 2022).

2.5.2 Use of antioxidants

One way of improving the oxidative stability of biodiesels is by adding substances capable of hindering the oxidation process, antioxidants. Antioxidants are chemical compounds that can delay, inhibit, and control autoxidation of substrates; thus, minimising the generation of undesired chemical species (Varatharajan & Pushparani, 2018). Antioxidants slow down the oxidation process by hindering polymerization reactions that occur due to free radicals and subsequent oxidizing reactions (Dunn, 2008). Antioxidants are classified as either primary or secondary antioxidants (hydroperoxide decomposers) (Pauquet, 1999). The general mechanism by which chain breaking antioxidants occur, illustrated using phenolic and amine antioxidants, is, shown in (Figure 2.6):

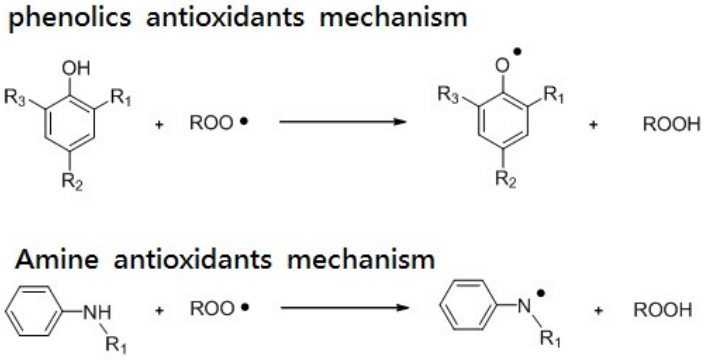

Figure 2.6: Chain breaking antioxidant mechanism (Easchem Co., n.d.)

Primary antioxidants (AH) donate a hydrogen atom to a free radical (especially peroxyl radical ROO*), so the propagation phase of the oxidation is slowed down or inhibited. Removing the hydrogen atom from the antioxidant molecule is easier than removing a fatty oil or ester (Yamane et al., 2007). Consequently, the oxidation chain reaction is interrupted while the antioxidant is consumed. The hydrogen atom, removed from the active OH or NH groups of primary antioxidants, reacts with free radicals (Yamane et al., 2007). Further, primary antioxidants cannot eliminate a light-induced photo oxidation process.

Secondary antioxidants react with hydroperoxides, forming alcohols, and an innocuous oxidized form of the antioxidant is shown in Figure 2.7. Secondary antioxidants are often combined with primary antioxidants to enhance their effectiveness (Wypych, 2018).

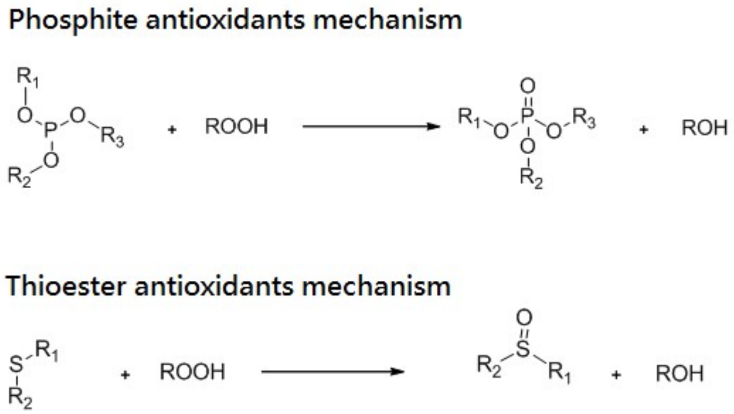

Figure 2.7: Secondary antioxidant mechanism (Easchem Co., n.d.)

Oxidation stability is an essential parameter for biodiesel quality control. In addition to fatty acid and the nature of oil feedstock mentioned previously, the oxidative degradation process of biodiesel depends on the presence of moisture (moisture content), the temperature to which it is subjected and exposure to light (Jain & Sharma, 2010a). Biodiesel is subject to oxidative and thermal degradation. Besides the nature of feedstock or type, the susceptibility to oxidation is determined by the presence of naturally occurring antioxidants in biodiesel. Autoxidation is biodiesel's most common degradation process; atmospheric oxygen is introduced in a C–R bond to form a hydroperoxide. Understanding the oxidation propagation stage is required to establish a suitable antioxidant mechanism. During the transesterification process, the fatty acid chains remain unchanged, implying that the chemical steps involved in degradation mechanism of biodiesel are similar to the steps undergone by the feedstock oil (Waynick, 2005).

2.5.2.1 Antioxidants' action on initiation and propagation reactions

Removal of hydrogen atoms from unsaturated fatty acids (UFAs) is influenced by factors such as heat, light, high-energy radiation, metal ion catalysts and moisture (Amran et al., 2022). The initiation reaction results in the formation of free radicals, as in equation 2.1.

$$RH + O_2 \rightarrow R\bullet + HO_2\bullet \qquad (2.1)$$

Where: RH is the organic substrate being oxidized (FAME), R• is a carbon-centred alkyl radical derived from RH and $HO_2\bullet$ is hydro-peroxyl radical.

The use of primary antioxidants cannot inhibit the initiation reaction. The- latter will, however, react with free radicals to produce thermodynamic-stable products (Amran et al., 2022). Thus, the initiation reaction can be activated by the action of secondary antioxidants and slowed down or deactivated using metal ion catalysts (Kumar, 2017).

In the propagation stage, unstable and highly reactive carbon radicals (R•) generated during the initiation phase, reacts with oxygen to produce peroxy radical (ROO•), as shown in equation 2.2 (Varatharajan & Pushparani, 2018).

$$R\bullet + O_2 \rightarrow ROO\bullet \qquad (2.2)$$

The peroxy (ROO•) radical formed is more stable and less reactive than the carbon radical (R•). However, reactive enough to rapidly detach another hydrogen atom with low bond dissociation energy (BDE). It does so by attacking the weak part of the FAME structure, thus producing a hydroperoxide (ROOH•) and an additional free radical R• (Equation 2.3) (Amran et al., 2022).

$$ROO\bullet + RH \rightarrow ROOH\bullet + R\bullet \qquad (2.3)$$

The low bond enthalpy bis-allylic hydrogen is highly susceptible to attacks by peroxy radicals, resulting in the collapse of the FAME structure (Varatharajan & Pushparani, 2018). The additional free radical (R•) generated reacts with diatomic oxygen to produce more peroxy radicals and propagates as a chain reaction (Amran et al., 2022). The addition of primary antioxidants interrupts the autoxidation chain reaction by producing antioxidant radicals (AO•), owing to the deactivation of peroxy radical

(ROO•) and termination of the carbon-centred free radical chain (R•) (Fattah et al., 2014). The antioxidant radical formed is stable and inert such that it can neither initiate nor propagate the oxidation reaction. The process ceases the propagation chain as the products formed are inactive and do not initiate further oxidation as shown below.

$$ROO\bullet + AH \rightarrow ROOH + A\bullet \qquad (2.4)$$

$$RO\bullet + AH \rightarrow ROH + A\bullet \qquad (2.5)$$

$$ROOH + A\bullet \rightarrow ROOA \qquad (2.6)$$

$$ROH + A\bullet \rightarrow ROA \qquad (2.7)$$

Antioxidant effect on propagation reaction (Fattah et al., 2014)

The reactions shown in equation 2.4 - 2.7 are exothermic, heat is released to the surrounding with increasing BDE, leading to increased activation energy (AE) of compounds formed and higher oxidant efficiency. Compounds with low activation and dissociation energy can easily donate their hydrogen atoms and form stable radical intermediates, making them suitable antioxidants (Amran et al., 2022).

2.5.3 Selection of antioxidants for biodiesel

The oxidation stability is one of the key aspects in biodiesel production regarding compliance with quality specification standards (EN 14214 in Europe and ASTM 6751 in USA). To produce biodiesel that complies with the standards and for commercialisation, state-of-the-art technologies require the addition of antioxidants. No existing technology can completely eliminate the undesired oxidation degradation of biodiesel; however, using antioxidants can significantly delay the degradation process and make biodiesel suitable for commercialisation. To maximise antioxidant benefits, they should be added to the biodiesel immediately after preparation. When biodiesel has become rancid, the addition of antioxidants only has minimal effect. Ideal antioxidants for biodiesel should possess the following qualities (Amran et al., 2022):

- Non-toxicity, high solubility in biodiesel and effective at low concentrations
- Low volatility, high thermal stability, and high stability in the presence of light, availability, and cost-effective.
- Low bond dissociation energy (BDE) makes the transfer of hydrogen atoms to free radicals easy and rapid, forming more stable products.
- High molar mass (molecular weight) for long-term storage of biodiesel due to the high number of hydrogen atoms available to be transferred, causing a large number of initiation sites to be reached and thus enhancing the formation of free radicals.

A suitable antioxidant should possess strong radical-scavenging properties whilst suppressing any side reactions. In addition to the aforementioned qualities, the effectiveness of an antioxidant to improve the oxidative stability of biodiesel also depends on factors such as number of hydroxyl groups, topological surface area (TPSA), and melting point (Romola et al., 2021; Amran et al., 2022).

Low molecular weight antioxidants tend to be more effective than those with high molecular weight. However, these are volatile and susceptible to evaporation (Romola et al., 2021). and hence are not suitable for long term storage and stability of biodiesel (Varatharajan & Pushparani, 2018). Antioxidants with multiple hydroxyl groups exhibit greater effectiveness than those with a single hydroxyl group. Hydroxyl groups provide hydrogen atoms which react with free radicals to terminate the oxidation degradation process (Romola et al., 2021). Topological polar surface area (TPSA) is the total surface area of polar atoms and depends on structural conformation (Prasanna & Doerksen, 2009).

Researchers have investigated the effect of various antioxidants on the oxidation stability of biodiesel (Mittelbach & Schober, 2003; Agarwal et al., 2015; Varatharajan & Pushparani, 2018; Pantoja et al., 2013; Ramalingam et al., 2016; Liang et al., 2006)

And these have been classified into two categories: synthetic and natural antioxidants.

2.5.3.1 Synthetic antioxidants

The use of synthetic antioxidants to improve the oxidation stability of biodiesel and other fuels has been reported in the literature (Focke et al., 2012; van der Westhuizen & Focke, 2018). Their wide use owes to their availability, cost, and efficiency (Buosi et al., 2016). Many studies conducted so far have focused on synthetic antioxidants. The most used synthetic antioxidants for biodiesel are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary-butylhydroquinone (TBHQ), propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG), pyrogallol (PY) and ethoxyquin (EQ) (Jakeria et al., 2014).

Yang et al. (2013) reported on the use of TBHQ, a soluble fat-synthetic antioxidant that easily blends with biodiesel. According to his finding, it was observed that when TBHQ was applied at a concentration of 3000 ppm, the oxidative stability of soybean biodiesel was best improved, and TBHQ also acted as a corrosion inhibitor. Saluja et al. (2016) reported using BHA, a blend of 2-tert-butyl-4hydroxyanisole and 3-tert-butyl-4-hydroxyanole. Their results showed that the antioxidant in question (BHA) applied at concentrations of 200 ppm and 1000 ppm in biodiesel (vegetable, animal fat, grease based), optimally improved the oxidative stability of biodiesel. Scherer et al. (Scherer et al., 2011) and Saluja et al. (2016) in their studies, found that BHT at different concentrations in different types of biodiesels optimally improved the oxidative stability of soybean-based biodiesel at 1300 ppm, that of poultry-based biodiesel at 500 ppm, and that of recycled soybean-based biodiesel at 3000 ppm: whilst also leading to compliance of the various biodiesels with the EN standards. Likely, PG has also proven to be a successful antioxidant for different types of biodiesels at different concentration and EN compliance standards (Ramalingam et al., 2018; Díaz-Ballote et al., 2018). In a study by Rashedul et al. (2015), PY demonstrated the best effect on oxidative stability of jatropha oil-biodiesel compared to TBHQ, BHA, BHT and PG (Rashedul et al., 2015).

Synthetic phenolic antioxidants can effectively inhibit oxidation by scavenging free radicals. When used as additives in refined-petroleum distillates like gasoline and low level biodiesel blends, these antioxidants exhibited excellent performance. Still, their efficiency is reportedly low in high-level biodiesel blends. Although widely used, synthetic antioxidants have been reported to be more effective in animal fat-based biodiesel than in biodiesel from vegetable oils (Varatharajan & Pushparani, 2018). The Consumers' concerns about biosafety regarding synthetic antioxidants have hindered the consideration of their use in commercial/industrial levels, triggering researchers to seek natural antioxidant alternatives (Focke et al., 2012).

2.5.3.2 Natural antioxidants

Using natural antioxidants in fuels has been considered for health and environmental reasons (Romola et al., 2021; Amran et al., 2022). Nature offers a variety of plant materials with high phenolic contents, which have shown antioxidant activity in oils and fats. Vegetable oils contain natural antioxidants such as tocopherols, polyphenols, carotenoids, and many others that can alleviate oxidation degradation (Şahin et al., 2020). The challenge, however, associated with the majority of naturally occurring antioxidants in oils/fats is that these tend to become ineffective during the transesterification and/or post-refining processes of biodiesel (Tang et al., 2008). Nonetheless, it has been demonstrated that biodiesels from unrefined vegetable oils contain more natural antioxidants and exhibit better stability (Moser, 2009).

Other sources of natural antioxidants are plants that contain phenolic compounds (Romola et al., 2021). Yang et al. (2013) reported that cashew-nutshell liquid (CNSL) contains cardanol, which causes it to exhibit antioxidant activity. It was found that adding the liquid (CNSL) to biodiesel obtained from beef tallow improved the oxidation stability, leading to a 50 % increased induction period. In a study by Uğuz et al. (2019), the use of potato peels extracts as a natural antioxidant led to a similar effect as the synthetic antioxidant TBHQ at the same concentration. Same with ethanolic potato peel extract (solanum tuberosum) in another study, the antioxidant resulted in an improved oxidation stability of biodiesel from Mesua ferrea oil (increased induction period from 5.63 to 6.21 hours and biodiesel compliant with the EN standards). Moreover, Devi et al. (2019) reported using ethanolic leaf extract of Thuja oreantalis on biodiesel from WCO (WCO-biodiesel). They found an increase in the biodiesel's induction period from 4.55 h to 9.62 h, including achieving quality compliance with the EN standards.

In this study, three natural antioxidants, namely oxidant A, oxidant C and oxidant E (OA, OC and OE respectively) known to possess antioxidant properties (Palace et al., 1999; Traber & Atkinson, 2007; Traber & Stevens, 2011) have been selected for use as antioxidants for biodiesel to be prepared from WCO. OA is a group of fat-soluble unsaturated hydrocarbons (retinoids) that exhibit both antioxidant and pro-oxidant properties (Dao et al., 2017). OC is a strong antioxidant and free radical scavenger (Njus et al., 2020). Its antioxidant behaviour has been demonstrated in its ability to donate a hydrogen atom to form an ascorbyl-free and stable radical (Pehlivan, 2017). OE is an oil-soluble antioxidant known for its role in inhibiting lipids peroxidation.

In the open literature, there is limited investigations on the use of OA and OC to improve the oxidation stability of biodiesel, compared to the wide use of OE (Chakraborty & Baruah, 2012; Liang et al., 2006; Varatharajan & Pushparani, 2018; Serrano et al., 2013; Díaz-Ballote et al., 2018). For example, in a study by Liang et al. (2006), it was found that pure OE at a concentration of only 1000 ppm, was sufficient to improve the oxidation stability of palm oil-biodiesel (increased induction period from 3.52 h to 6.42 h). In another study by Serrano et al. (2013), OE at same concentration (1000 ppm), improved on the oxidation stability of rapeseed oil-biodiesel and sunflower oil-biodiesel with an increase in induction period from 8 to 14 hours and 15 to 33 hours respectively.

Based on the above reports, the use of OE as antioxidant has been limited to biodiesel produced from sunflower oil, rapeseed oil, palm oil, soybean oil, poultry fat and yellow grease. According to the author's knowledge, this antioxidant use owes to the exception of biodiesel produced from waste cooking oil (WCO), being of interest in this work. Thus, the present study investigates the application of natural antioxidant A (OA), C (OC) and E (OE) to improve the oxidation stability of biodiesel obtained from WCO (WCO-biodiesel). WCO is a low cost, readily available and promising feedstock for biodiesel production (Vidigal et al., 2021). More to it, using WCO as feedstock is a good strategy for waste management that will alleviate the environmental pollution and economic demand associated with its disposal challenge.

Synthetic and natural antioxidants differ in performance, in effectiveness based on number of peroxides formed in lipids over time and differ in ability to provide stability under different processing conditions (Sarin et al., 2010). The focus for using natural antioxidants has been due the effectiveness of plant phenolics or phenolic-containing extracts (Ramos et al., 2018). A growing body of literature has pointed out the importance of natural antioxidants from many plants, which could be used to reduce oxidative degradation. These are not only limited to foods or lipid derivatives but also in the human body (Xu et al., 2017). The preference for natural antioxidants is also emphasised on the fact that these can be harvested from nature or directly from any organic source (such as herbs, fruits, vegetables, and organic waste). In contrast, synthetic antioxidants are produced artificially to prevent rancidity. Natural antioxidants, therefore, are presented as alternatives to synthetic antioxidants due to the growing concern among consumers about synthetic antioxidants and their toxicological effects (Rodrigues et al., 2020). Moreover, from the review, it should be noted that there is limited application of natural antioxidants in commercial industries, except for their emergence to be considered alternatives to synthetic antioxidants. Lastly, the open literature does not report the use of OA, OC, and OE for WCO biodiesel. Based on the success of the current study, the use of natural antioxidants could achieve commercial consideration.

CHAPTER 3: MATERIALS AND METHODS

This chapter outlines the methods and procedures for the experiments to improve biodiesel's oxidative stability using natural antioxidants. The experimental stages include set-up, design, and procedures. The materials and reagents used are presented in section 3.2.1

3.1 Basis for selection of natural antioxidants

A theoretical study was conducted to find readily available natural antioxidants with the potential to retard biodiesel oxidation based on their structural properties.

3.2 Materials and method

3.2.1 Feedstock and chemical reagents

Methyl alcohol (CH_3OH, 99.8%) and potassium hydroxide pellets (KOH, 99.99%) used for biodiesel preparation were obtained from Sigma-Aldrich. Antioxidant A ($C_{20}H_{30}O$, 95%), antioxidant E ($C_{29}H_{50}O_2$, 95.5%) and antioxidant C ($C_6H_8O_6$, 99%) tablets used as antioxidants were purchased from Merck. All chemicals were used as received without further purification. The antioxidants A, C and E will be referred to as OA, OC and OE, respectively. The waste cooking oil (WCO) used to prepare biodiesel was purchased from Super Oil South Africa. The oil herein was derived from restaurant cooking operations, mainly fish, chicken frying, etc.

Feedstock preparation

3.2.2 Feedstock preparation

The WCO received from super oils contained debris of solid particles. The oil was transferred from the storage container into a 1000 mL beaker, where large solid particles were let to settle. This was allowed to set until it was visually observed that the solids particles had settled. The oil sample, containing the remaining finer particles, was transferred into another beaker, from which the particles were filtered through a Buchner flask (Figure 3.1) until visible solid particles in the oil were no longer observed. The filtered oil was heated to 105 °C to evaporate the water content in the WCO.

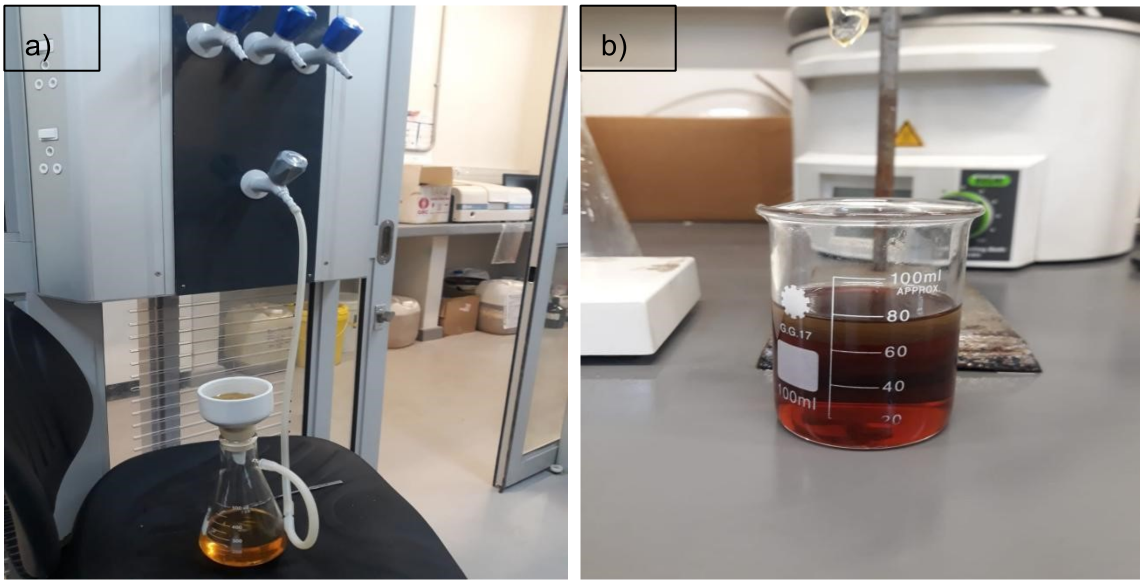

Figure 3.1: (a) Waste cooking oil filtration using Buchner flask. (b) Filtered waste cooking oil.

3.2.3 Biodiesel preparation by transesterification of WCO

Biodiesel from WCO was produced in a water-bath apparatus preheated to a transesterification temperature of 65 °C. Twenty ml methanol was mixed with 100 ml oil in a 250 ml beaker. The mixture was then transferred to a two-necked round bottom flask, and 1.5 g KOH catalyst was added. A mechanical stirrer was submerged in the reaction mixture through the central neck of the flask, and a thermometer was inserted into the flask to account for and regulate the reaction temperature. A reflux system was put in place to prevent pressure build-up. The transesterification reaction was run for 3 hours at 65 °C with continuous stirring at 300 rpm. The product mixture was transferred into a separating funnel, which settled overnight. The phases in the mixture were then separated using a centrifuge vessel, and the biodiesel was collected as the top phase and glycerol as the bottom phase.

The process diagram showing oil pre-treatment (as per section 3.1.2) and the set-up for the transesterification procedure of WCO for biodiesel production are presented in Figure 3.*2*.

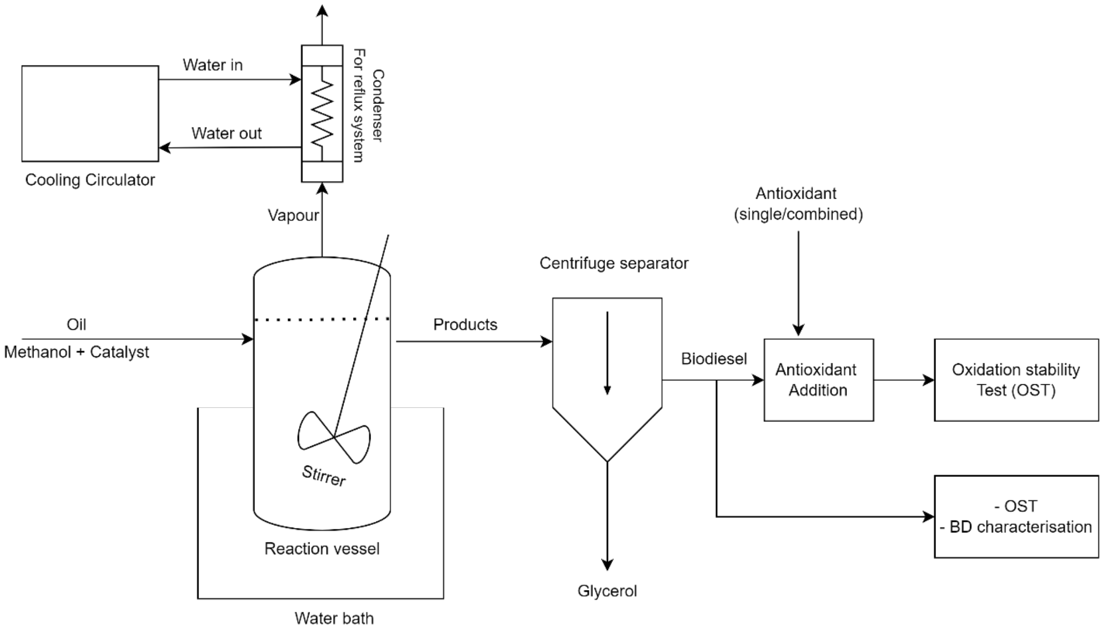

Figure 3.2: Process diagram illustrating the biodiesel production from WCO *and antioxidant addition.*

The yield of biodiesel was determined using equation 3.1.

$$Biodiesel\,yield\,(\%) = \frac{Volume\ of\ biodiesel}{Volume\ of\ WCO} \times 100 \qquad 3.1$$

3.2.4 Antioxidant addition and WCO/biodiesel characterisation

After the transesterification process, the biodiesel sample was collected in a beaker after separation the reaction products from the vessel. The selected antioxidants (OA, OC and OE) were ground in crucibles. Varying amounts of each antioxidant were added to the biodiesel at different concentrations. The antioxidants were used individually in the first set of experiments and a combination of the two to study the synergistic effects of antioxidants blends. The matrix of samples prepared is summarized in Table 3.1.

Table 3.1: Biodiesel samples and codes for oxidation stability test using various antioxidants at different concentrations.

Biodiesel samples + Antioxidants		
Biodiesel sample	**Sample name**	**Antioxidant concentration (ppm)**
B100	Biodiesel	0
B-OA250	Biodiesel + OA	250
B-OA500		500
B-OA1000		1000
B-OA2000		2000
B-OA3000		3000
B-OC250	Biodiesel + OC	250
B-OC500		500
B-OC1000		1000
B-OC2000		2000
B-OC3000		3000
B-OE250	Biodiesel + OE	250
B-OE500		500
B-OE1000		1000
B-OE2000		2000
B-OE3000		3000
B-OAC	Biodiesel + OA + OC	2000*
B-OAE	Biodiesel + OA + OE	2000*
B-OCE	Biodiesel + OC + OE	2000*

*The concentration of each antioxidant was 1000 ppm. Since they were blended, the total antioxidant concentration amounted to 2000 ppm.

3.2.4.1 Physical properties

WCO and biodiesel were characterised at Oil watch laboratories in Cape Town, South Africa, to determine the physical properties such as density, flash point, viscosity, and water content. The following instrument was used for each of the above properties mentioned:

- Water content: A Metrohm 808 Titrando + 801 Stirrer was used to measure the water content present in the fuel.
- Viscosity: A Stabinger Viscometer SVM 300 was used to measure the viscosity of the fuel. The kinematic viscosity was measured at 40 °C, and the dynamic viscosity was measured in cSt.
- Flashpoint: To measure the flash point of the fuel, a Flash point tester: Herzog; Pensky Martens Closed Cup HFP 339 was used.
- Density: A density meter SVM 300 was used to measure the density of the fuel.
- The FFA measurements were conducted according to the procedure presented in (Win & Trabold, 2018).

3.2.4.2 Gas Chromatography Mass Spectrometry (GC-MS)

Identification of products was performed on a gas chromatograph (6890N, Agilent Technologies network) coupled to Agilent Technologies inert XL EI/CI Mass Selective Detector (MSD) (5975, Agilent Technologies Inc., Palo Alto, CA). The GC-MS system was coupled to a CTC Analytics PAL autosampler. The fatty acids were separated on a ZB-FFAP (30 m, 0.25 mm ID, 0.25 μm film thickness) (Restek, USA) capillary column. Helium was used as the carrier gas at a 1 ml/min flow rate. The GC-MS analysis of WCO and biodiesel was conducted at the University of Stellenbosch's central analytical facilities in South Africa. Samples of WCO and biodiesel for GC analysis were prepared by diluting each sample in 1 mL hexane separately. Each sample was injected into the column in a split ratio of 40:1 at 320 °C injection port and interface line temperature.

The calibration curve was constructed for each sample based on the detection/identification and concentrations of components present therein.

3.2.4.3 Oxidation stability test and equipment operation

The oxidation stability tests of all samples (Table 3.1) were carried out at the Willowton group laboratories in Pietermaritzburg, South Africa. The tests were conducted using Metrohm 743 Rancimat equipment to determine the induction period of each biodiesel sample according to the ASTMD6751 and EN14214 standards. The biodiesel samples containing different concentrations of antioxidants (Table 3.1) were placed in the Rancimat equipment. The Rancimat technique is accelerated oxidation that naturally occurs slowly when biodiesel reacts with air after a certain period. A computer controlled the Rancimat equipment through an interface (RS-232) and an evaluating computer program to automatically generate the conductivity measurements as a function of time to determine the induction period of each biodiesel sample. The Rancimat technique allows the biodiesel sample to be exposed to an airflow of 10 L/h at a constant temperature ranging from 50 to 200 °C. For the test in this study, the temperature was kept at 120 °C. The biodiesel sample was placed in a glass reaction vessel and a heating block. Then the Rancimat internal pump supplied the airflow of 10 L/h at 120 °C through a fluorinated ethylene-propylene tube into the reaction vessel. The formed volatile compounds flew into the measuring vessel via a silicone tube. An electrode in the measuring vessel detected the conductivity when the volatile compounds (mainly formic acid) were absorbed in the distilled water in the measuring cell (Figure 3.6). The induction period was measured as the time required for a rapid increase in conductivity detected by the electrode in the measuring vessel. Figure 3.3 presents the operation of the Rancimat technique.

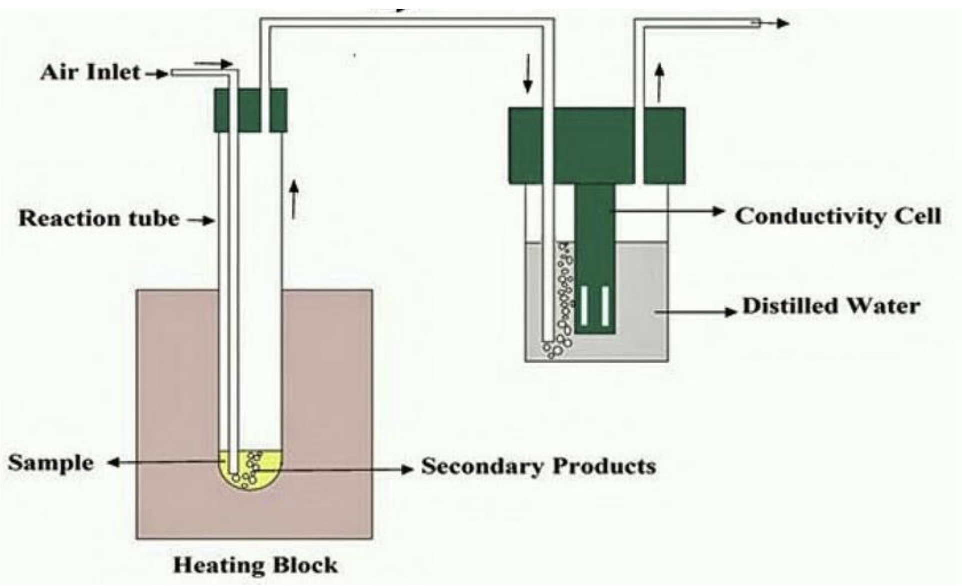

Figure 3.3: Diagram of Rancimat apparatus (Sakthivel et al., 2019)

CHAPTER 4: RESULTS AND DISCUSSION

This chapter analyses and discusses the experimental results obtained from various WCO and biodiesel sample analyses. The results provide insight into the oxidation stability of biodiesel from WCO using natural antioxidants. The chapter is divided into the following sections:

- Characterization of biodiesel from WCO
- The effect of natural antioxidants on the oxidative stability of biodiesel
- The effect of antioxidants concentration on the oxidative stability of biodiesel •

The effect of antioxidants blending on biodiesel oxidative stability

4.1 Characterization of WCO and biodiesel

The physicochemical properties of WCO, characterized by methods discussed in section 3.1.4 (3.1.4.1 -3.4.1.2), are presented in Table 4.1.

Table 4.1: Physicochemical Properties of WCO

Property	Value
Kinematic Viscosity at 40 °C, cSt	50.13
Density at 20 °C, g/ml	0.92
Water content, %	0.03
FFA content (wt% of oil)	1.00
Colour	Brownish dark
Molecular weight, g/mol	812.00
Acid value, mg KOH/ g oil	2.50

The above properties are typical for WCO obtained from restaurants' frying oil (Erchamo et al., 2021; Degfie et al., 2019). It is essential to characterise waste cooking oil, especially its acid content, before biodiesel preparation. The fatty acid profile of

WCO, characterised via GC-MS (see appendix - Figure A.*0.**2*** for the chromatograms) as described in section 3.1.4.2. are reported in Table 4.2.

Table 4.2: Fatty acid composition of WCO from GC-MS analysis

Fatty acid type	Carbon chain	wt. %
Caprylic	C8:0	0.30
Palmitic	C16:0	8.40
Stearic	C18:0	8.80
Oleic	C18:1	14.30
Linoleic	C18:2	68.10

The physicochemical properties of WCO, as shown in Table 4.1, are critical in determining the quality of biodiesel produced from the waste cooking oil. The water content and FFA content of WCO, for instance, can affect the biodiesel production process and the quality of the final product (Erchamo et al., 2021). A high-water content in WCO can lead to the formation of emulsions during the transesterification process, which can hinder the separation of the glycerol and biodiesel phases (Degfie et al., 2019). This can lead to the formation of soap and other impurities that can affect the quality of the biodiesel produced. Similarly, a high FFA content in WCO can lead to the formation of free fatty acids during the transesterification process, which can react with the catalyst used and reduce its effectiveness (Erchamo et al., 2021). This can lead to a lower yield of biodiesel and higher amounts of impurities in the final product.

The fatty acid profile of WCO, as shown in Table 4.2, is also important in determining the quality of biodiesel produced from the waste cooking oil. The high content of linoleic acid in WCO, for instance, can result in a lower oxidative stability of the biodiesel produced. This is because linoleic acid is highly unsaturated and prone to oxidation, which can lead to the formation of harmful compounds that can negatively affect the engine's performance. Therefore, it is important to consider the physicochemical properties and fatty acid profile of WCO before producing biodiesel from the waste cooking oil. This can help to optimize the biodiesel production process and improve the quality of the final product.

The presence of free fatty acids (FFA) in WCO is due to repeated heating or thermal stress from the use of cooking oil (Susilowati et al., 2019). The WCO contained a high percentage of linoleic acid (68 wt. %), a polyunsaturated fatty acid. High linoleic acid content is a characteristic of vegetable oils from soy, corn, or canola (Fritsche, 2014). Linoleic acids are also known to make up the highest percentage of free fatty acids in most consumed meats (beef, chicken, pork) prepared in restaurants, usually contributing up to 85% (Whelan & Fritsche, 2013). The linoleic acid was followed by another unsaturated fatty acid, oleic acid, a mono-unsaturated fatty acid, which comprised 14.30% of the free fatty acid content. Biodiesel prepared from WCO with high concentrations of FFAs exhibits low oxidation stability (Erchamo et al., 2021; Degfie et al., 2019); thus, the need to add antioxidants. The biodiesel prepared by transesterification of WCO, under prescribed conditions and procedure described in section 3.1.3, was characterized to determine its physicochemical properties. The biodiesel was characterised following the South African National Standard (SANS 342:2016 requirements). The result of the physicochemical properties of WCO, viscosity, density, flashpoint, and water content, are presented in Table 4.3.

Table 4.3: Physicochemical properties of biodiesel obtained from WCO SANS 342:2016 Requirements

Property	Value	SANS 342:2016	ASTM D6751	Pass/ Fail
Viscosity @ 40°C, cSt	4.10	2.00 - 5.30	1.9 - 6.0	Pass
Density, g/ml	0.88	0.80 (min)	0.86 - 0.90 (EN 14214:2012)	Pass
Flashpoint, °C	65.00	55.00 (min)	52.00 (min)	Pass
Water content, %	0.03	0.04 (max)	0.05 (max)	Pass
Oxidation stability, hrs	0.79	3.00 (ASTM-6751)	3.00 (min)	Fail

The low oxidation stability of the WCO-biodiesel, as shown in Table 4.3, could be attributed to the high content of unsaturated fatty acids in the WCO used to produce the biodiesel. As shown in Table 4.2, the WCO used in this study had a high content of linoleic acid, which is highly unsaturated and prone to oxidation. The low oxidative stability of the WCO-biodiesel could also be due to the presence of bis-allylic methylene groups close to the double bond within the WCO-biodiesel (Yaakob et al., 2014). The high FFA content and water content in the WCO used to produce the biodiesel, as shown in Table 4.1, could also contribute to the lower oxidative stability of the WCO-biodiesel. Several strategies can be employed to improve the oxidative stability of biodiesel produced from WCO. One strategy is to use antioxidants during the production process to reduce the rate of oxidation. As shown in previous sections of this report, natural antioxidants such as OC, OA, and OE can improve the oxidative stability of WCO-biodiesel. Another strategy is to use different feedstocks with a lower content of unsaturated fatty acids. For instance, biodiesel produced from algae or animal fats has been found to have higher oxidative stability than biodiesel produced from vegetable oils (Yaakob et al., 2014).

Table 4.4: FAMEs composition of WCO-biodiesel

FAME	Carbon chain	wt %
Myristic	C14	0.08
Pentadecylic	C15	0.02
Palmitic	C16	6.67
Palmitoleic	C16:1	0.07
Margaric	C17	0.01
Stearic	C18	6.39
Oleic	C18:1cis	18.70
Linoleic	C18:2cis	66.35
Arachidic	C20	0.41
Behenic	C22	0.89
arachidonic	C20:4n6	0.01
Tricosylic	C23	0.03
Tetracosanic	C24	0.22
Nervonic	C24:1cis	0.01
Others	n/a	0.15

It is well known that polyunsaturated fatty acids such as linoleic acid contain bis-allylic sites, which are sensitive to ambient conditions and are easily attacked by oxygen. This lower oxidation capacity may be of concern as it reveals a high possibility of further oxidation upon extended oil storage.

The result of the fatty acid profile (fatty acids methyl esters, FAMEs) of the obtained WCO-biodiesel, as also characterised via GC-MS as described in section 3.1.4.2, is presented in Table 4.4. In Table 4.4, the GC-analysis results of WCO-biodiesel indicated the dominance of fatty acid content with 66.35% linoleic acid which is a polyunsaturated fatty acid (PUFA), followed by 18.70% oleic acid which is a monounsaturated fatty acid (MUFA), 6.67 % palmitic acid, 6.39% stearic acid and other fatty acids which are saturated made up the balance. Thus, the finding suggests that WCO biodiesel has low oxidative stability (OS), which can be attributed to the high degree of unsaturated fatty acids content (PUFA + MUFA ≈87 wt %) in the biodiesel product (Rashed et al., 2015). The above agrees with results obtained by Bondioli et al. (1995) and Lin et al. (2006) regarding the degree of unsaturation of soybean and distilled sunflower oil, respectively, vis-à-vis the oxidation stability. The fatty acid content significantly affects the different biodiesel properties, including its oxidative stability (Chhetri et al., 2008). The auto-oxidation of fatty acids is one of the mechanisms to decrease oxidative biodiesel stability. The susceptibility to oxidation is a function of the level of unsaturation and the number of double bonds present in the free fatty acid. A high number of double bonds enhances oxidation degradation due to the pi bond's weak and low energy, making the unsaturated compound highly reactive (Romola et al., 2021).

4.2 Selection of natural antioxidants

The structure of active molecules determines the performance of antioxidants, their operation mechanism, the system in which they are present, and the storage and processing conditions (Shahidi, 2015). In the current study, the structural properties of readily available molecules have been used as the main selection criteria. Therefore, three antioxidants, i.e., OA, OC and OE, known to display antioxidant properties (Palace et al., 1999; Traber & Atkinson, 2007; Traber & Stevens, 2011) were selected for the study. The structures of the antioxidant used are shown in Figure 4.1.

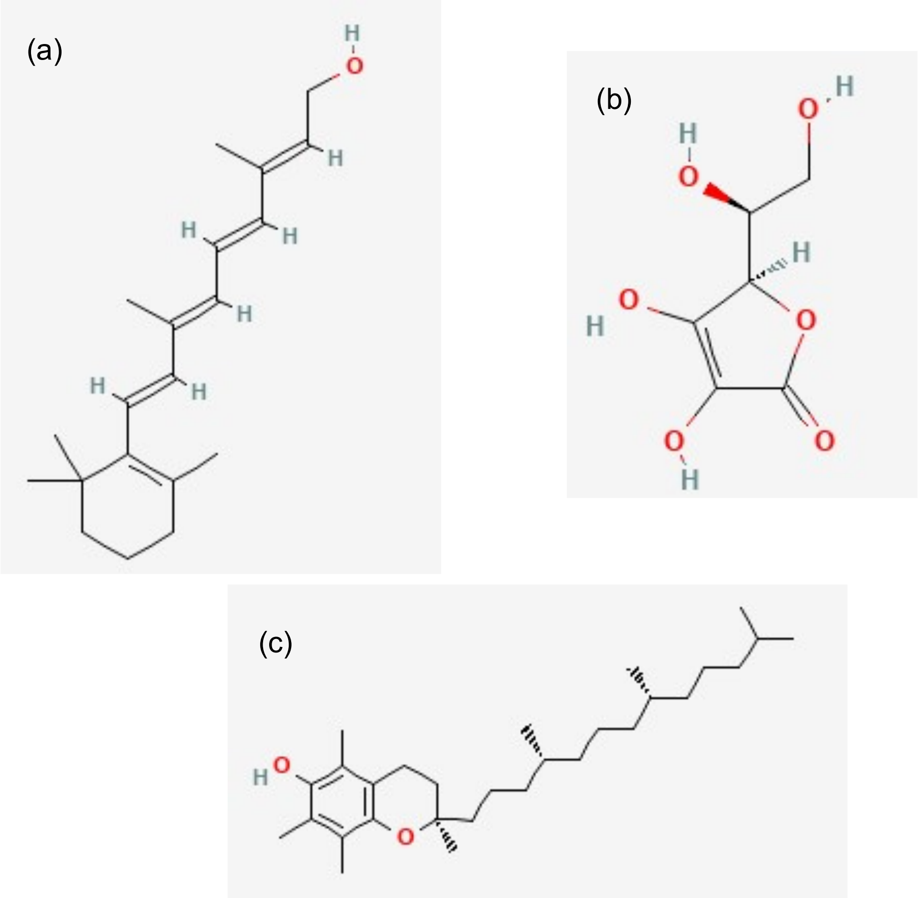

Figure 4.1: Selected antioxidants chemical structures: (a): OA (b) OC (c) OE

It can be noticed from the structures of the molecules under consideration that OC has four hydroxyls while OA and OE only have one hydroxyl group each. The hydroxyl group is one of the factors responsible for the vitamins' antioxidant properties (Traber & Atkinson, 2007). Therefore, OC having a more significant number of hydroxyl radicals than OA and OE implies that it has the potential to donate a more significant number of hydrogen-free radicals or electrons to eliminate oxidative free radicals. The performance of the antioxidants is also determined by the strength of the most reactive O-H bond. Hence, the O-H bond dissociation energy (BDE) is a good predictor of antioxidant activity (Romola et al., 2021). Previous studies have estimated the BDE of OA, OC and OE in water (Lucarini et al., 1994; Pandithavidana & Jayawardana, 2019; Evgeny & Taisa, 2010). The results are summarized in Table 4.5, with the molecular masses of each selected component included.

Table 4.5: BDE and molecular mass of selected antioxidants

Antioxidant	Bond dissociation energy (kJ/mol)	Molecular mass (g/mol)
OC	318.50	176.12
OE	330.10	430.71
OA	356.00	286.40

The antioxidants' molecular mass can be used to indicate antioxidant activity (Romola, 2021). OC has the lowest molecular mass of the three selected antioxidants, followed by antioxidant OA and antioxidant OE.

OC was found to have the lowest BDE of the three selected antioxidants, followed by OE. OA showed the highest BDE of the antioxidants selected for this study. The antioxidant capacity is generally significant in molecules with lower BDE (Romola et al., 2021). This observation can also relate to the larger molecules offering a high ability to donate electrons, and hydrogen may dictate their effectiveness as antioxidants. Another advantage of larger molecules is the potential for multiple electron donations, the metal's chelating capabilities, and the potential for complex formation with the radical that needs to be scavenged (Tyrakowska et al., 1999).

4.3 The effect of natural antioxidant types on oxidative biodiesel stability

The effect of three antioxidants, OA, OC and OE, on the oxidative stability of WCO biodiesel was investigated. The initially selected concentration of each antioxidant in WCO biodiesel samples was 1000 ppm (section 3.1.4, Table 3.1). The induction time determined using a Rancimat device indicates the oxidative stability of the respective biodiesel samples. Figure 4.2 present the Rancimat analysis curve obtained for biodiesel without any antioxidant addition and biodiesel with added antioxidants (1000 ppm).

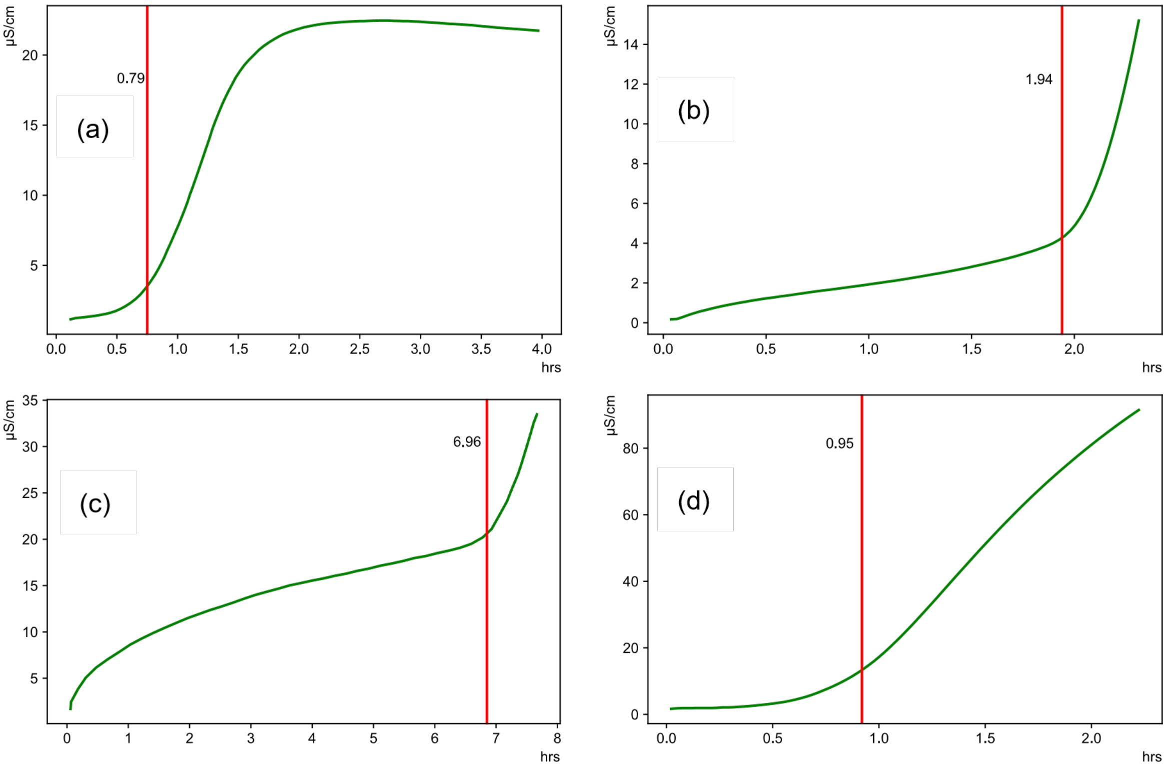

Figure 4.2: Rancimat curve for induction period estimation for WCO-biodiesel with (a) No antioxidant addition (b) 1000 ppm OA (c) 1000 ppm OC (d) 1000 ppm OE

Initially, a gradual increase in conductivity is due to the dissociation of volatile organic acids generated as a result of biodiesel oxidation (Figure 4.2). The point of inflection shows that accelerated biodiesel oxidation is occurring, marking the induction period of biodiesel. The addition of antioxidants to WCO-biodiesel samples improved their oxidative stability, as evidenced by the longer induction periods obtained. The induction period of the WCO-biodiesel sample without any added antioxidant was only 0.79 hours, while the addition of OA, OC, and OE increased the induction period to 1.94, 6.96, and 0.95 hours, respectively. This suggests that OC is the most potent antioxidant, followed by OA and OE. The differences in the effectiveness of the antioxidants can be related to their chemical structures and properties (Oyetunji et al., 2019). For instance, OC has a large number of hydroxyl groups, which can scavenge free radicals and inhibit the oxidation process. OA and OE also have hydroxyl groups, but their effectiveness may be lower due to differences in their molecular weight and branching. Additionally, the antioxidant activity of these compounds may also be influenced by their solubility and reactivity with the free radicals generated during the oxidation process.

Summarily, the results suggest that the addition of antioxidants to WCO-biodiesel samples can improve their oxidative stability and increase their shelf life. OC, OA, and OE are potential candidates for use as antioxidants in biodiesel production, and further research is needed to optimize their concentration and application in industrial-scale biodiesel production. Figure 4.3 presents the results of Rancimat analysis of induction time upon adding antioxidants to the various biodiesel samples.

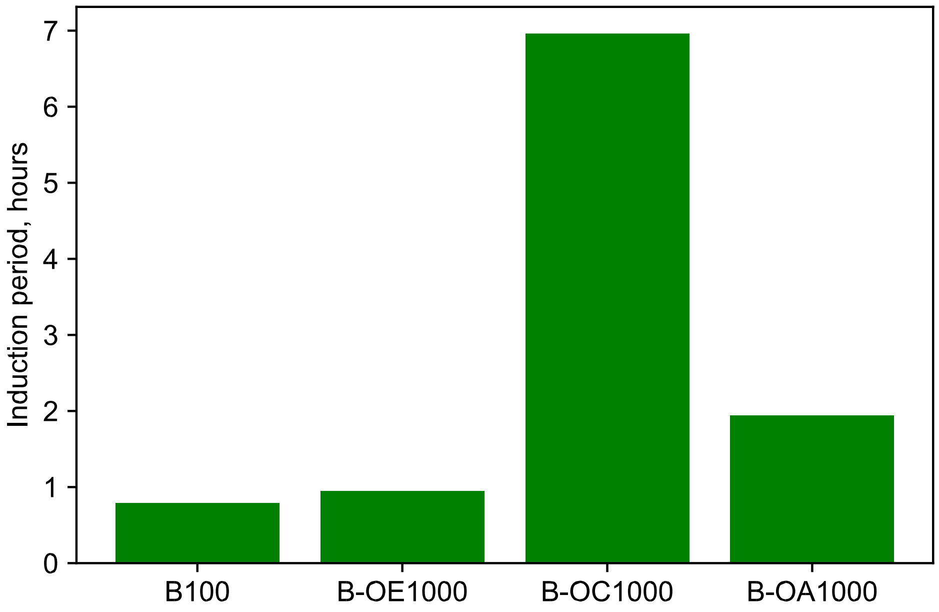

Figure 4.3: Rancimat induction periods for WCO-biodiesel with natural antioxidants (antioxidant concentration = 1000 ppm). B100: biodiesel without antioxidant, and B-O: biodiesel with added antioxidants of OE, OC and OA.

It is observed in Figure 4.3 that the WCO-biodiesel (B100), prior to antioxidant addition, did not comply with EN-14112/IS-15607's standard induction period of 6 hours, neither with the ASTM-6751 limit of 3 hours. According to Nagarajan & Narayanasamy (2020) and (Bharti & Singh, 2019), the induction period of biodiesel produced from WCO feedstocks usually does not comply with these minimum standards. This can also be explained by the high poly-unsaturated fatty acid methyl esters (PUFA) content (66 %) in WCO-based biodiesel, as reported earlier in Table 4.4. Moreover, the WCO used to prepare the biodiesel was unsaturated, and this resulted in biodiesel prepared from it having low oxidation stability.

Nonetheless, the results in Figure 4.3 reveals that all the natural antioxidants employed improved the oxidation stability of WCO-biodiesel. OC showed the most significant effects based on a more extended induction period of 6.96 hours. OC is insoluble in oils and partially soluble in glycerol (PubChem, 2021; Othmer, 1969; O'Neil, 2013). The study showed that OC appeared to dissolve in waste cooking oil biodiesel. Two possible explanations exist: firstly, OC can dissolve in the water contained in biodiesel (cite references & indicate how OC is water-soluble). The oil used for this study is the waste product of restaurant oils used for frying purposes, which contains water, as shown in the physicochemical characterization of the waste cooking oil biodiesel (see Table 4.3). Secondly, OC could react with methyl palmitate to form ascorbyl palmitate, the oil-soluble form of OC (Lewis, 2007). Interestingly, the gas chromatography analysis of the waste cooking biodiesel prepared for this study revealed the presence of methyl palmitate (6.67 wt%) (Table 4.4). This observation explains the reaction between methyl palmitate and OC to produce ascorbyl palmitate.

The findings from this study are consistent with those of (Park et al., 2019), who reported that the oxidation stability of biodiesel produced from waste cooking oil feedstocks did not comply with minimum standards, possibly due to high PUFA content. They also found that methyl palmitate was present in significant quantities in the biodiesel, which could react with OC to form ascorbyl palmitate, the oil-soluble form of OC. Park et al. (2019) identified methyl oleate, methyl linoleate, methyl palmitate, methyl linolenate, and methyl stearate as the significant components of fatty acid in biodiesel produced from restaurant waste cooking oils. In the current study, the induction period of biodiesel was increased from 0.79 to 6.96 hours with 1000 ppm of OC as an antioxidant. The performance of OC can be attributed to the presence of its four hydroxyl groups. Many OH groups mean more sites allow complexes between free radicals and antioxidant radicals, thus, stabilizing the biodiesel (Liang et al., 2006). Another possible explanation is the formation of ascorbyl palmitate, which exhibits strong antioxidant properties. The structure of the ascorbyl palmitate is shown in Figure 4.4. Multiple OH groups greatly enhance the ascorbyl palmitate's antioxidant behaviour.

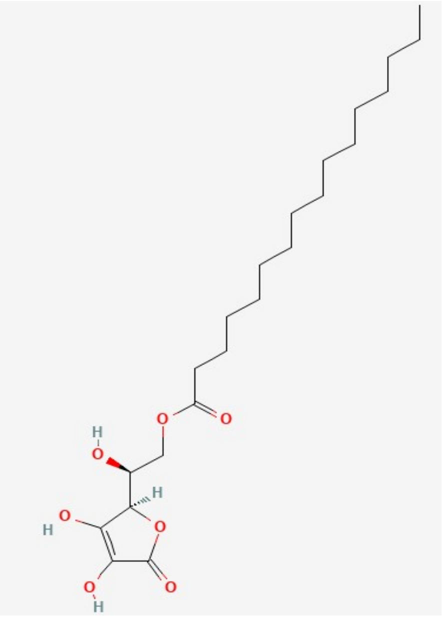

Figure 4.4: Ascorbyl Palmitate structure

It can be seen that OA performed better than OE. The induction times of waste cooking oil-based biodiesel prepared with 1000 ppm of OA and OE antioxidants were 1.94 and 0.95 hours, respectively (Figure 4.3). The molecular mass of OE is approximately 1.5 times bigger than that of OA (Table 4.5), while the value of the BDE of the OA is greater than OE's (Table 4.5). The lower molecular mass of OA compared to OE provides a possible explanation for its better performance. Studies conducted on OE have shown that the antioxidant activity of OE is limited, which is more effective in hydrocarbon than in vegetable oils (Waynick, 2005). The waste cooking oil used for the present study is vegetable oil. This explains the low performance of OEs in improving biodiesel oxidation stability. A study by Ramos et al. (2018) on the evaluation of natural antioxidant action in the oxidative stability of commercial animal-vegetable oil-based biodiesel showed that using OE had little effect on the oxidation stability of biodiesel. To the best of our knowledge, there is no report in the literature on using OA as a natural antioxidant to reduce oxidation degradation of waste cooking oil-based biodiesel. The observations above show that OA will perform better as an antioxidant for waste cooking oil biodiesel than OE.

4.4 The effect of antioxidants concentration on oxidative biodiesel stability

Following the application of the three antioxidants (OA, OC, and OE) over WCO biodiesel at a fixed concentration of 1000 ppm, the oxidation stability (OS) tests using the Rancimat method were further investigated at varying concentrations between 250 to 3000 ppm as prescribed in section 3.1.4 (Table 3.1).

4.4.1 Influence of OA concentration on oxidation stability

Figure 4.5 presents the effect of the varying concentrations of antioxidants on biodiesel oxidation stability (in terms of rancimat’s induction period).

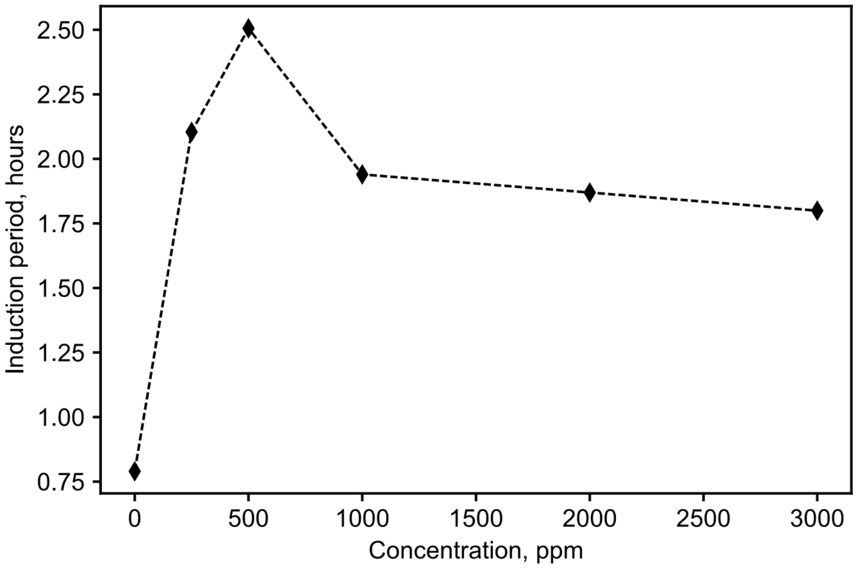

Figure 4.5: Effect of the concentration of antioxidant A (OA) on the induction period of WCO-biodiesel

An increase in the induction period was observed for OA concentrations up to 500 ppm (Figure 4.5). Despite the beneficial effect of OA addition, the oxidation stability did not meet the ASTM-6751 limit of 3 hours for waste cooking oil-based biodiesel. One possible explanation is the high content of unsaturated fatty acids in WCO, which are highly susceptible to oxidation. Previous studies have shown that biodiesel produced from WCO has lower oxidative stability than biodiesel produced from other feedstocks, such as animal fats or algae (Mohammad et al., 2013). The high content of free fatty acids and water in WCO may also contribute to the lower oxidative stability of the resulting biodiesel. At an OA concentration of 500 ppm, the antioxidant activity has reached its peak value, as beyond 500 ppm OA, the induction period was observed to decrease. Some studies have shown the existence of an antioxidant saturation point beyond which no significant or even pro-oxidant effect is observed (Varatharajan & Pushparani, 2018). It has been reported that natural antioxidants show high sensitivity to concentration and exhibit pro-oxidant behaviour when used in large quantities (Prankl, 2005). At OA concentrations of 2000 ppm and above, the induction period reached a plateau value of about 2 hours.

4.4.2 Influence of OE concentration on oxidation stability

Figure 4.6 shows the influence of OE, also known as tocopherol, as an antioxidant in waste cooking oil biodiesel.

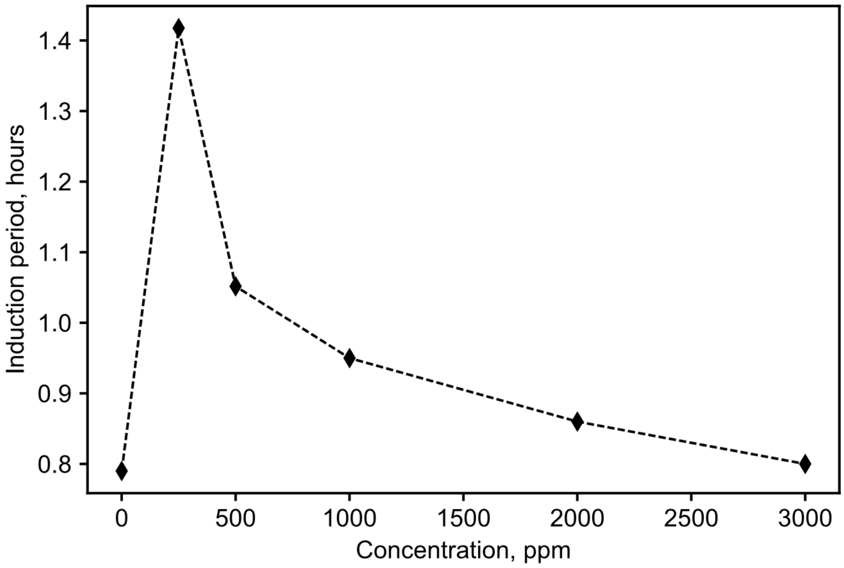

Figure 4.6: Effect of the concentration of OE on the induction period of biodiesel

The effect of the concentration of OE follows similar trends as OA shown above (Figure 4.5), such that there is an optimum induction period of 1.4 hours at a concentration of 500 ppm and a sharp decrease at higher concentrations. It is to be specified that OE is a well-recognized natural antioxidant used to improve the oxidation stability of biodiesel (Serrano et al., 2013; Liang et al., 2006; Varatharajan & Pushparani, 2018). Petroleum diesel containing about 600 ppm of OE was characterized by an optimum induction period of 25 hours. At the same time, refined palm oil methyl esters showed an optimum induction period of 3.5 hours for an OE antioxidant concentration of 600 ppm (Liang et al., 2006). Our value of 1.4 hours for an OE concentration of 500 ppm is slightly below the 3.5 hours observed in the previously mentioned study. In another study, OE has been found to have little or even slight negative impact on the oxidation stability of yellow grease and poultry fat-based biodiesels (Tang et al., 2008). OE, although capable of improving the oxidation stability of biodiesel, has been reported to exhibit pro-oxidant behaviour in the presence of metal impurities (Romola et al., 2021).

The pro-oxidant behaviour observed in this study at OE concentrations above 250 ppm could be due to enhanced degradation of OE at high concentrations due to the presence of metal impurities in the waste cooking oil used to prepare the biodiesel.

4.4.3 Influence of OC concentration on oxidation stability

OC provided the best antioxidant activity, as indicated in section 4.3. Figure 4.7 shows the effect of OC, also known as ascorbic acid, on the induction period.

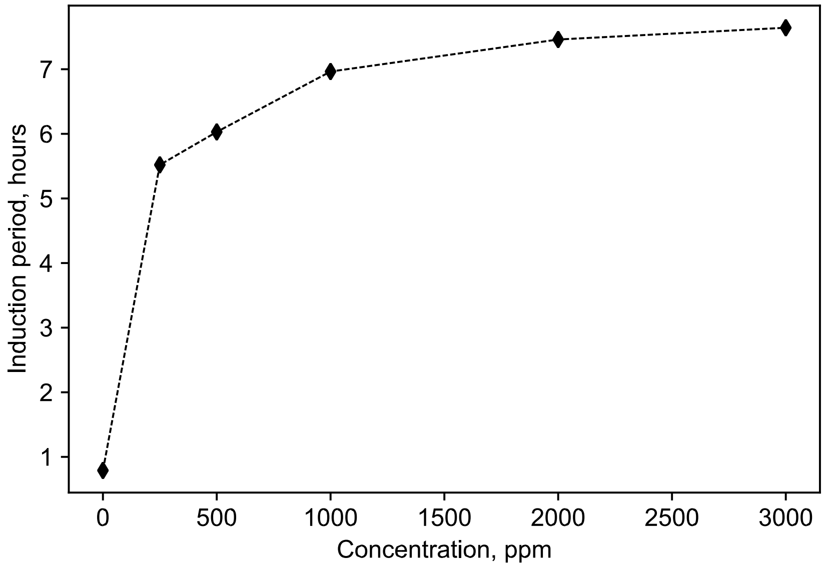

Figure 4.7: Effect of the concentration of OC on the induction period of biodiesel

Upon the addition of 250 ppm OC, a sharp increase in the induction period is observed. The increase peaks at 2000 ppm, where the induction period observed is around 7 hours. At OC concentrations higher than 2000 ppm, no significant increase in the induction period is observed. Similar trends were observed with synthetic antioxidants PY and PG to improve the oxidation stability of frying oil-based biodiesel (Mittelbach & Schober, 2003). A saturation antioxidant concentration exists beyond which antioxidant activity improvement should not be expected. Further addition of antioxidants might promote biodiesel degradation (Varatharajan & Pushparani, 2018).

The results obtained from the individual use of antioxidants in this study suggest that using natural antioxidants, including OC, OA, and OE, can improve the oxidative stability of WCO-biodiesel. The increase in the induction period observed at different concentrations of each antioxidant is consistent with previous studies. For instance, a study by Meher (2006) found that the induction period of WCO-biodiesel can be increased by using synthetic antioxidants such as BHT, TBHQ, and tert-butyl hydroxyanisole (TBHA). Similarly, a study by Vatandoost et al. (2014) found that the induction period of WCO-biodiesel can be increased by using natural antioxidants such as tocopherol and carotenoids. However, the optimal concentration of each antioxidant reported in this study differs from previous reports. For instance, the optimal concentration of OC in this study was above 1000 ppm, while a previous study by Kumar et al. (2018) found that the optimal concentration of OC in WCO-biodiesel was 500 ppm. Similarly, the optimal concentration of OA and OE reported in this study differs from those reported in previous studies. The differences in the optimal concentration of each antioxidant reported in this study could be attributed to differences in the experimental conditions, such as the type of biodiesel used, the source of the waste cooking oil, and the analytical methods used to determine the induction period.

4.4.4 Synergistic effects of antioxidants on biodiesel oxidative stability

The antioxidant blending and their effect on the oxidation stability of biodiesel were conducted as described in section 3.1.4. Figure 4.8 presents the result of the antioxidants’ combined effect on the oxidation stability of WCO-biodiesel.

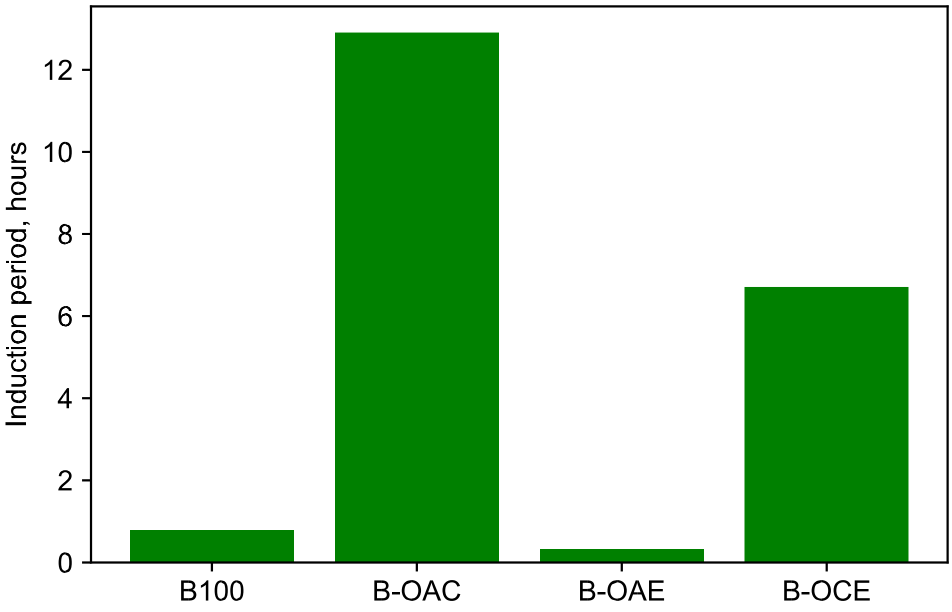

Figure 4.8: Effect of antioxidants blends on the induction period of biodiesel.

As shown in Figure 4.8, combining OA and OC (OAC) into the biodiesel led to an induction period of 12.9 hours for the antioxidant ratio of 1 to 1 (combined antioxidant concentration 2000 ppm). This proves to be almost 6 times and 2 times better than the individual antioxidant (OA and OC) performance, respectively, demonstrating maximum activity at 2 and 7 hours, respectively (Figures 4.5 and 4.6). On the other hand, combining antioxidants OA and OE (OAE) did not improve the oxidation stability of biodiesel but rather had an adverse effect, leading to an induction period of 0.33 hour. This can be attributed to the fact that OA acts as a free radical scavenger and a metal chelator. In contrast, OE acts as a singlet oxygen quencher and a chain-breaking antioxidant. When combined, the different mechanisms of action of OA and OE might interfere with each other, leading to a decrease in their effectiveness and a consequent decline in the oxidation stability of biodiesel. The chemical interaction between the two antioxidants could also contribute to the adverse effect observed. Further studies could be conducted to investigate the exact mechanism of this adverse effect. It was, therefore, unnecessary to have the two (OA and OE) antioxidants blended for use in biodiesel’s antioxidation application, compared to their performance individually for 2 hours and 0.9 hours, respectively.

4.5 Summary

Natural antioxidants like OA, OC and OE showed good antioxidant activity by improving waste cooking oil biodiesel induction periods. Unlike the OA and OE, OC showed a minimum increased induction period of 5.5 hours at 500 ppm concentration. This was further increased to an optimum induction period of over 7 hours upon varying concentration to 1000 ppm. Therefore, WCO-biodiesel treated with OC at an optimised concentration in WCO-biodiesel was the only biodiesel sample that complied with the ASTM-6751 limit of 3 hours. OC has resulted in being the best antioxidant in improving the oxidation stability of biodiesel. Its large number or hydroxyl groups explain this, lower BDE and molecular weight as compared to OA and OE (See Table 4.5).

These findings have significant implications for the storage and commercialization of biodiesel. Biodiesel is susceptible to oxidative degradation during storage, forming harmful compounds that can negatively affect the engine's performance. Using natural antioxidants like OC can help increase biodiesel's shelf life and reduce the formation of harmful compounds during storage. This can reduce the cost of biodiesel storage and transportation, making biodiesel a more economically viable alternative to traditional fossil fuels. In addition, the study's findings also have implications for the commercialization of biodiesel. Natural antioxidants can improve the biodiesel's quality and make it more attractive to potential buyers. In addition, using natural antioxidants can help address concerns about the environmental impact of biodiesel production, as these antioxidants are derived from renewable sources and are biodegradable.

In conclusion, the study's findings suggest that using natural antioxidants like OC can significantly improve the oxidative stability of WCO-biodiesel. This has important implications for the storage and commercialization of biodiesel, as it can increase biodiesel's shelf life and make it more attractive to potential buyers. Further research is needed to investigate the long-term effects of natural antioxidants and to identify other potential benefits of their use in biodiesel production.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

Biodiesel has been identified as an attractive, suitable, and sustainable alternative to conventional petroleum diesel. Biodiesel is biodegradable and offers more significant technical advantages than petroleum diesel, such as higher combustion efficiency, higher cetane number, low emission of aromatic and sulphurous compounds, low flammability with a high flash point, and high lubricity. Because of biodiesel's various advantages, it has successfully been used to replace petroleum diesel in diesel engines partially. Biodiesel is prepared using lipid or oil feedstock via transesterification in the presence of appropriate catalysts.

One of the major drawbacks associated with the commercialisation of biodiesel is oxidation degradation. Biodiesel is prone to degradation upon radical chemical reactions. Biodiesel oxidation occurs due to the presence and position of double bonds (unsaturation) in fatty acid molecules. The double bonds of the unsaturated fatty acids increase their reactivity with oxygen, especially when the molecules are exposed to water or air. To tackle the oxidation stability challenge, antioxidants are added to biodiesel. Antioxidants have the potential to retard the biodiesel oxidation process. Furthermore, these antioxidants prevent free radicals' propagation and minimize the formation of compounds that promote biodiesel degradation.

Several investigations on the improvement of biodiesel's oxidative stability have been conducted. Various antioxidants have been proposed; these can be classified as synthetic and natural. Most studies have focused on synthetic antioxidants, which are the most commonly used. However, consumers’ concerns regarding the biosafety of synthetic antioxidants have shifted research focus toward natural antioxidants, which are the subject of this project. Waste cooking oil (WCO) which has been identified as a promising oil feedstock for biodiesel production, has been used for the present study. This is due to its low cost and the fact that it is readily available. Using WCO as feedstock for biodiesel means putting to good use a waste product often discarded inappropriately with a negative environmental impact.

Biodiesel was successfully produced from WCO via transesterification using a potassium hydroxide catalyst. Physicochemical analysis of WCO-biodiesel via GC showed that the product dominantly consisted of unsaturated fatty acids (85 %) (66.35% Linoleic-PUFA acid and 19% monounsaturated fatty acids). Consequently, the derived biodiesel was associated with low oxidation stability with an induction period of 0.79 hours. The produced biodiesel complied with the South African National Standard biodiesel specification (SANS 342:2016) regarding the measured physicochemical properties (viscosity, density, flash point and water content) but not oxidation stability.

To tackle the oxidation stability challenge, antioxidants are added to biodiesel. Antioxidants have the potential to retard the biodiesel oxidation process. This study selected three natural biodiesel antioxidants: OA, OC and OE. The antioxidants were first investigated individually and then in pairs. The effect of antioxidant concentration on oxidation stability was studied using the Rancimat method. The technique is used to determine the biodiesel's induction period, which indicates oxidation stability. Antioxidant OC performed best for biodiesel oxidation stability with the lowest Bond Dissociation Energy (BDE, 318.5 kJ/mol) and molecular weight (176.16 g/mol). Addition of 1000 ppm OC led to an increase in the Rancimat induction period from 0.79 to 7 hours. A study on the effect of the concentration of OC (250 – 3000 ppm) on oxidation stability showed an improvement in the induction period for all concentrations studied. Using 3000 ppm OC led to an induction period of 7.64 hours. Biodiesel prepared with OC addition with concentrations higher than 500 ppm complied with EN-14112/IS-15607's standard induction period of 6 hours and the ASTM-6751 limit of 3 hours. Using OA and OE at different concentrations improved the oxidation stability of biodiesel. However, biodiesel prepared with either OA or OC did not comply with EN-

14112/IS-15607’s standard or with the ASTM-6751 standard.

The combined effects of antioxidants selected for this study were assessed in pairs on a 1 to 1 ratio. Antioxidant OA combined with OC (OAC) resulted in WCO biodiesel with an induction period of 12.9 hours from 0.79 hours of biodiesel without antioxidants. An induction period of 7 hours was obtained by combining OC and OE (OCE). The effect of combined OA and OE (OAE) was detrimental and resulted in an induction period of 0.33 hours. Antioxidants appeared to have synergistic effects when used in pairs; they exhibit better antioxidant activity than when used individually, except for the OAE combination.

This study has shown the potential benefits natural antioxidants offer to improve the oxidation stability of waste cooking oil biodiesel. Such a study will provide great insight for industries interested in harnessing the waste cooking oil potential in biodiesel manufacture.

5.1 Recommendations for further studies

The mechanistic phenomena leading to the observed antioxidant activity are worth investigating on the molecular level to understand and improve antioxidant activity. Furthermore, a comparative study between synthetic and commercial antioxidant on WCO biodiesel and natural antioxidants whose performance have been exalted in this study will be justifiable. Lastly, owing to the knowledge gained in this study, it will be useful to assess the economic impact of using the applied natural antioxidants OA, OC, and OE compared to synthetic antioxidants.

REFERENCES

Agarwal, A.K., Khurana, D. & Dhar, A. 2015. Improving oxidation stability of biodiesels derived from Karanja, Neem and Jatropha: Step forward in the direction of commercialisation. *Journal of Cleaner Production*, 107: 646–652.

Almeida, E.L., Andrade, C.M.G. & dos Santos, O.A. 2018. Production of Biodiesel Via Catalytic Processes: A Brief Review. *International Journal of Chemical Reactor Engineering*, 16(5).

Alsultan, A.G., Asikin-Mijan, N., Ibrahim, Z., Yunus, R., Razali, S.Z., Mansir, N., Islam, A., Seenivasagam, S. & Taufiq-Yap, Y.H. 2021. A Short Review on Catalyst, Feedstock, Modernised Process, Current State and Challenges on Biodiesel Production. *Catalysts*, 11(11): 1261.

Altun, Ş. 2014. Effect of the degree of unsaturation of biodiesel fuels on the exhaust emissions of a diesel power generator. *Fuel*, 117(Part A): 450–457.

Amran, N.A., Bello, U. & Hazwan Ruslan, M.S. 2022. The role of antioxidants in improving biodiesel's oxidative stability, poor cold flow properties, and the effects of the duo on engine performance: A review. *Heliyon*, 8(7): e09846.

Anwar, A. & Garforth, A. 2016. Challenges and opportunities of enhancing cold flow properties of biodiesel via heterogeneous catalysis. *Fuel*, 173: 189–208.

Aquino, I.P., Hernandez, R.P.B., Chicoma, D.L., Pinto, H.P.F. & Aoki, I. v. 2012. Influence of light, temperature and metallic ions on biodiesel degradation and corrosiveness to copper and brass. *Fuel*, 102: 795–807.

Aransiola, E.F., Ojumu, T. v., Oyekola, O.O., Madzimbamuto, T.F. & Ikhu-Omoregbe, D.I.O. 2014. A review of current technology for biodiesel production: State of the art. *Biomass and Bioenergy*, 61: 276–297.

Ayoola, A.A., Fayomi, O.S.I., Adegbite, O.A. & Raji, O. 2021. Biodiesel Fuel Production Processes: A Short Review. *IOP Conference Series: Materials Science and Engineering*, 1107(1): 012151.

Baena, L.M. & Calderón, J.A. 2020. Effects of palm biodiesel and blends of biodiesel with organic acids on metals. *Heliyon*, 6(5): e03735.

Bashir, M.A., Wu, S., Zhu, J., Krosuri, A., Khan, M.U. & Ndeddy Aka, R.J. 2022. The recent development of advanced processing technologies for biodiesel production: A critical review. *Fuel Processing Technology*, 227: 107120.

Berrios, M., Martín, M.A., Chica, A.F. & Martín, A. 2012. Storage effect in the quality of different methyl esters and blends with diesel. *Fuel*, 91(1): 119–125.

Bhardwaja, M., Guptab, P. & Kumarc, N. 2014. Compatibility of Metals and Elastomers in Biodiesel: A review. *International Journal of Research (IJR)*, 1(7).

Bharti, R. & Singh, B. 2019. Green tea (Camellia assamica) extract as an antioxidant additive to enhance the oxidation stability of biodiesel synthesized from waste cooking oil. *Fuel*, 262: 116658.

Bondioli, P., Gasparoli, A., della Bella, L., Tagliabue, S. & Toso, G. 2003. Biodiesel stability under commercial storage conditions over one year. *European Journal of Lipid Science and Technology*, 105(12): 735–741.

Bondioli, P., Gasparoli, A., Lanzani, A., Fedeli, E., Veronese, S. & Sala, M. 1995. Storage stability of biodiesel. *Journal of the American Oil Chemists' Society*, 72(6): 699–702.

Botella, L., Bimbela, F., Martín, L., Arauzo, J. & Sánchez, J.L. 2014. Oxidation stability of biodiesel fuels and blends using the Rancimat and PetroOXY methods. Effect of 4allyl-2,6-dimethoxyphenol and catechol as biodiesel additives on oxidation stability. *Frontiers in Chemistry*, 2(43): 1–9.

Bouaid, A., Martinez, M. & Aracil, J. 2007. Long storage stability of biodiesel from vegetable and used frying oils. *Fuel*, 86(16): 2596–2602.

Brahma, S., Nath, B., Basumatary, B., Das, B., Saikia, P., Patir, K. & Basumatary, S. 2022. Biodiesel production from mixed oils: A sustainable approach towards industrial biofuel production. *Chemical Engineering Journal Advances*, 10: 100284.

Bukkarapu, K.R. & Krishnasamy, A. 2022. A critical review on available models to predict engine fuel properties of biodiesel. *Renewable and Sustainable Energy Reviews*, 155: 111925.

Buosi, G.M., da Silva, E.T., Spacino, K., Silva, L.R.C., Ferreira, B.A.D. & Borsato, D. 2016. Oxidative stability of biodiesel from soybean oil: Comparison between synthetic and natural antioxidants. *Fuel*, 181: 759–764.

Chakraborty, M. & Baruah, D.C. 2012. Investigation of oxidation stability of Terminalia belerica biodiesel and its blends with petrodiesel. *Fuel Processing Technology*, 98: 51–58.

Chellamuthu, M., Eswaran, K. & Subramanian, S. 2022. *Genetic Engineering for Oil Modification*. I. S. Niang, ed. London: IntechOpen.

Chhetri, A.B., Watts, K.C. & Islam, M.R. 2008. Waste Cooking Oil as an Alternate Feedstock for Biodiesel Production. *Energies 2008, Vol. 1, Pages 3-18*, 1(1): 3–18.

Christensen, E. & McCormick, R.L. 2014. Long-term storage stability of biodiesel and biodiesel blends. *Fuel Processing Technology*, 128: 339–348.

Cursaru, D.L., Brănoiu, G., Ramadan, I. & Miculescu, F. 2014. Degradation of automotive materials upon exposure to sunflower biodiesel. *Industrial Crops and Products*, 54: 149–158.

Dao, D.Q., Ngo, T.C., Thong, N.M. & Nam, P.C. 2017. Is Vitamin A an Antioxidant or a Prooxidant? *Journal of Physical Chemistry B*, 121(40): 9348–9357.

Das, L.M., Bora, D.K., Pradhan, S., Naik, M.K. & Naik, S.N. 2009. Long-term storage stability of biodiesel produced from Karanja oil. *Fuel*, 88(11): 2315–2318.

Degfie, T.A., Mamo, T.T. & Mekonnen, Y.S. 2019. Optimized Biodiesel Production from Waste Cooking Oil (WCO) using Calcium Oxide (CaO) Nano-catalyst. *Scientific Reports 2019 9:1*, 9(1): 1–8.

Demirbas, A. 2009. Political, economic and environmental impacts of biofuels: A review. *Applied Energy*, 86(Suppl. 1): S108–S117.

Devi, A., Das, V.K. & Deka, D. 2019. A green approach for enhancing oxidation stability including long storage periods of biodiesel via Thuja oreantalis L. as an antioxidant additive. *Fuel*, 253: 1264–1273.

Díaz-Ballote, L., Gómez-Hernández, K., Vega-Lizama, E.T., Ruiz-Gómez, M.A., Maldonado, L. & Hernández, E. 2018. Thermogravimetric Approach for Assessing the Oxidation Level of a Biodiesel Sample. *Química Nova*, 41(5): 492–496.

Dijkstra, A.J. 2016. Vegetable Oils: Composition and Analysis. *Encyclopedia of Food and Health*: 357–364.

Dijkstra, A.J. & van Duijn, G. 2016. Vegetable Oils: Oil Production and Processing. *Encyclopedia of Food and Health*: 373–380.

Dunn, R.O. 2008. Antioxidants for improving storage stability of biodiesel. *Biofuels, Bioproducts and Biorefining*, 2(4): 304–318.

Dunn, R.O. 2005. Effect of antioxidants on the oxidative stability of methyl soyate (biodiesel). *Fuel Processing Technology*, 86(10): 1071–1085.

Dunn, R.O. 2002. Effect of oxidation under accelerated conditions on fuel properties of methyl soyate (biodiesel). *Journal of the American Oil Chemists' Society*, 79(9): 915–920.

Easchem Co. Antioxidant effect and their mechanism. *Easchem Co.* http://www.easchem.com.tw/en/product-info.php?id=167 20 October 2022.

Edeh, I. 2020. Biodiesel Production as a Renewable Resource for the Potential Displacement of the Petroleum Diesel. *Biorefinery Concepts, Energy and Products*.

Erchamo, Y.S., Mamo, T.T., Workneh, G.A. & Mekonnen, Y.S. 2021. Improved biodiesel production from waste cooking oil with mixed methanol-ethanol using enhanced eggshell-derived CaO nano-catalyst. *Scientific Reports* |, 11: 6708.

Evgeny, D. & Taisa, D. 2010. Dissociation Energies of O − H Bonds of Phenols and Hydroperoxides. , (2): 1858–1866.

Fattah, R.I.M., Masjuki, H.H., Kalam, M.A., Hazrat, M.A., Masum, B.M., Imtenan, S. & Ashraful, A.M. 2014. Effect of antioxidants on oxidation stability of biodiesel derived from vegetable and animal based feedstocks. *Renewable and Sustainable Energy Reviews*, 30: 356–370.

Fazal, M.A., Rubaiee, S., Al-Zahrani, A. & Ghazali, S. 2022. Biodiesel degradation mechanism upon exposure of metal surfaces: A study on biodiesel sustainability. *Fuel*, 310: 122341.

Focke, W.W., Westhuizen, I. van der, Grobler, A.B.L., Nshoane, K.T., Reddy, J.K. & Luyt, A.S. 2012. The effect of synthetic antioxidants on the oxidative stability of biodiesel. *Fuel*, 94: 227–233.

Fox, N.J., Tyrer, B. & Stachowiak, G.W. 2004. Boundary Lubrication Performance of Free Fatty Acids in Sunflower Oil. *Tribology Letters 2004 16:4*, 16(4): 275–281.

Fritsche, K.L. 2014. Linoleic Acid, Vegetable Oils & Inflammation. *Missouri Medicine*, 111(1): 41–43.

Fu, J., Hue, B.T.B. & Turn, S.Q. 2017. Oxidation stability of biodiesel derived from waste catfish oil. *Fuel*, 202: 455–463.

Fukuda, H., Kondo, A. & Noda, H. 2001. Biodiesel fuel production by transesterification of oils. *Journal of Bioscience and Bioengineering*, 92(5): 405–416.

Gaur, A., Mishra, S., Chowdhury, S., Baredar, P. & Verma, P. 2021. A review on factor affecting biodiesel production from waste cooking oil: An Indian perspective. *Materials Today: Proceedings*, 46: 5594–5600.

Geller, D.P., Adams, T.T., Goodrum, J.W. & Pendergrass, J. 2008. Storage stability of poultry fat and diesel fuel mixtures: Specific gravity and viscosity. *Fuel*, 87(1): 92–102.

Goto, S., Oguma, M. & Chollacoop, N. 2009. *Benchmarking of Biodiesel Fuel Standardization in East Asia*. Shinichi Goto, ed. Jakarta: EAS-ERIA.

Haijing, W., ZeXue, D. & GuoQiang, G. 2017. Influence factors of acid value of biodiesel and method of reducing acid value. *China Oils and Fats*, 42(6): 121–124.

Hazrat, M.A., Rasul, M.G., Khan, M.M.K., Mofijur, M., Ahmed, S.F., Ong, H.C., Vo, D.V.N. &

Show, P.L. 2021. Techniques to improve the stability of biodiesel: a review. , 19(3): 2209–2236.

Jafarihaghighi, F., Ardjmand, M., Salar Hassani, M., Mirzajanzadeh, M. & Bahrami, H. 2020. Effect of Fatty Acid Profiles and Molecular Structures of Nine New Source of Biodiesel on Combustion and Emission. *ACS Omega*, 5(26).

Jain, S. & Sharma, M.P. 2010a. Review of different test methods for the evaluation of stability of biodiesel. *Renewable and Sustainable Energy Reviews*, 14(7): 1937–1947.

Jain, S. & Sharma, M.P. 2010b. Stability of biodiesel and its blends: A review. *Renewable and Sustainable Energy Reviews*, 14(2): 667–678.

Jakeria, M.R., Fazal, M.A. & Haseeb, A.S.M.A. 2014. Influence of different factors on the stability of biodiesel: A review. *Renewable and Sustainable Energy Reviews*, 30: 154–163.

Joshi, H., Moser, B.R., Toler, J. & Walker, T. 2010. Preparation and fuel properties of mixtures of soybean oil methyl and ethyl esters. *Biomass and Bioenergy*, 34(1): 14–20.

Karavalakis, G., Hilari, D., Givalou, L., Karonis, D. & Stournas, S. 2011. Storage stability and ageing effect of biodiesel blends treated with different antioxidants. *Energy*, 36(1): 369–374.

Karavalakis, G., Stournas, S. & Karonis, D. 2010. Evaluation of the oxidation stability of diesel/biodiesel blends. *Fuel*, 89(9): 2483–2489.

Khalid, A., Tamaldin, N., Jaat, M., Ali, M.F.M., Manshoor, B. & Zaman, I. 2013. Impacts of biodiesel storage duration on fuel properties and emissions. *Procedia Engineering*, 68: 225–230.

Khan, M.A.H., Bonifacio, S., Clowes, J., Foulds, A., Holland, R., Matthews, J.C., Percival, C.J. & Shallcross, D.E. 2021. Investigation of Biofuel as a Potential Renewable Energy Source. *Atmosphere 2021, Vol. 12, Page 1289*, 12(10): 1289.

Khounani, Z., Hosseinzadeh-Bandbafha, H., Nizami, A.S., Sulaiman, A., Goli, S.A.H., Tavassoli-Kafrani, E., Ghaffari, A., Rajaeifar, M.A., Kim, K.H., Talebi, A.F., Aghbashlo,

M. & Tabatabaei, M. 2018. Unlocking the potential of walnut husk extract in the production of waste cooking oil-based biodiesel. *Renewable and Sustainable Energy Reviews*, 119: 109588.

Kivevele, T. 2020. Storage and thermal stability of biodiesel produced from manketti nut oil of Southern Africa origin with the influence of metal contaminants and antioxidants. *SN Applied Sciences*, 2(5): 1–10.

Kivevele, T. & Huan, Z. 2015. Review of the stability of biodiesel produced from less common vegetable oils of African origin. *South African Journal of Science*, 111(9–10): 01–07.

Knothe, G. 2007. Some aspects of biodiesel oxidative stability. *Fuel Processing Technology*, 88(7): 669–677.

Knothe, G. & Dunn, R.O. 2003. Dependence of Oil Stability Index of Fatty Compounds on Their Structure and Concentration and Presence of Metals. *JAOCS, Journal of the American Oil Chemists' Society*, 80(10): 1021–1026.

Knothe, G. & Razon, L.F. 2017. Biodiesel fuels. *Progress in Energy and Combustion Science*, 58: 36–59.

Knothe, G. & Steidley, K.R. 2009. A comparison of used cooking oils: A very heterogeneous feedstock for biodiesel. *Bioresource Technology*, 100(23): 5796–5801.

Komariah, L.N., Hadiah, F., Aprianjaya, F. & Nevriadi, F. 2018. Biodiesel effects on fuel filter; Assessment of clogging characteristics. In *Journal of Physics: Conference Series*. Institute of Physics Publishing.

Kongprawes, G., Wongsawaeng, D., Hosemann, P., Ngaosuwan, K., Kiatkittipong, W. & Assabumrungrat, S. 2021. Improvement of oxidation stability of fatty acid methyl esters derived from soybean oil via partial hydrogenation using dielectric barrier discharge plasma. *International Journal of Energy Research*, 45(3): 4519–4533.

Kreivaitis, R., Gumbyte, M., Kazancev, K., Padgurskas, J. & Makarevičiene, V. 2013. A comparison of pure and natural antioxidant modified rapeseed oil storage properties. *Industrial Crops and Products*, 43(1): 511–516.

Kreivaitis, R., Padgurskas, J., Gumbyte, M., Makarevičiene, V. & Spruogis, B. 2011. The influence of Oxidation on Tribological Properties of Rapeseed Oil. *Vilnius Gediminas Technical University*, 26(2): 121–127.

Kumar, N. 2017. Oxidative stability of biodiesel: Causes, effects and prevention. *Fuel*, 190: 328–350.

Kumar, R., Sharma, S.K. & Srivastava, R.K. 2018. Production and characterization of biodiesel from waste cooking oil using a heterogeneous catalyst. *Fuel*, (233): 412–418.

Laghetti, G., Piergiovanni, A.R. & Perrino, P. 1995. Yield and oil quality in selected lines of Crambe abyssinica Hochst. ex R.E. Fries and C. hispanica L. grown in Italy. *Industrial Crops and Products*, 4(3): 203–212.

Lepri, F.G., Chaves, E.S., Vieira, M.A., Ribeiro, A.S., Curtius, A.J., de Oliveira, L.C.C. & de Campos, R.C. 2011. Determination of Trace Elements in Vegetable Oils and Biodiesel by Atomic Spectrometric Techniques—A Review. *Applied Spectroscopy Reviews*, 46(3): 175–206.

Leung, D.Y.C., Koo, B.C.P. & Guo, Y. 2006. Degradation of biodiesel under different storage conditions. *Bioresource Technology*, 97(2): 250–256.

Lewis, R.J. 2007. *Hawley's Condensed Chemical Dictionary*. 15th ed. American Chemical Society.

Li, H., Fan, Y.W., Li, J., Tang, L., Hu, J.N. & Deng, Z.Y. 2013. Evaluating and predicting the oxidative stability of vegetable oils with different fatty acid compositions. *Journal of food science*, 78(4).

Liang, Y.C., May, C.Y., Foon, C.S., Ngan, M.A., Hock, C.C. & Basiron, Y. 2006. The effect of natural and synthetic antioxidants on the oxidative stability of palm diesel. *Fuel*, 85(5–6): 867–870.

Lin, C.Y. & Chiu, C.C. 2010. Burning characteristics of palm-oil biodiesel under long-term storage conditions. *Energy Conversion and Management*, 51(7): 1464–1467.

Lu, Z., Cherepakhin, V., Kapenstein, T. & Williams, T.J. 2018. Upgrading Biodiesel from Vegetable Oils by Hydrogen Transfer to Its Fatty Esters. *ACS Sustainable Chemistry and Engineering*, 6(5): 5749–5753.

Lucarini, M., Pedulli, G.F. & Cipollone, M. 1994. Bond Dissociation Enthalpy of α-Tocopherol and Other Phenolic Antioxidants. *Journal of Organic Chemistry*, 59(17): 5063–5070.

Mantovani, A.C.G., Chendynski, L.T., Salviato, A., Borsato, D., Santana, V.T. & di Mauro, E. 2018. Monitoring free radicals formation in the biodiesel oxidation reaction via electronic paramagnetic resonance. *Fuel*, 224: 255–260.

Math, M.C., Kumar, S.P. & Chetty, S. v. 2010. Technologies for biodiesel production from used cooking oil — A review. *Energy for Sustainable Development*, 14(4): 339–345.

Meher, L.C., Vidya Sagar, D. & Naik, S.N. 2006. Technical aspects of biodiesel production by transesterification—a review. *Renewable and Sustainable Energy Reviews*, 10(3): 248–268.

Milano, J., Shamsuddin, A.H., Silitonga, A.S., Sebayang, A.H., Siregar, M.A., Masjuki, H.H., Pulungan, M.A., Chia, S.R. & Zamri, M.F.M.A. 2022. Tribological study on the biodiesel produced from waste cooking oil, waste cooking oil blend with Calophyllum inophyllum and its diesel blends on lubricant oil. *Energy Reports*, 8: 1578–1590.

Mittelbach, M. & Schober, S. 2003. The influence of antioxidants on the oxidation stability of biodiesel. *JAOCS, Journal of the American Oil Chemists' Society*, 80(8): 817–823.

Mofijur, M., Masjuki, H.H., Kalam, M.A. & Atabani, A.E. 2013. Evaluation of biodiesel blending, engine performance and emissions characteristics of Jatropha curcas methyl ester: Malaysian perspective. *Energy*, 55: 879–887.

Mohammad, M., Kandaramath Hari, T., Yaakob, Z., Chandra Sharma, Y. & Sopian, K. 2013. Overview on the production of paraffin based-biofuels via catalytic hydrodeoxygenation. *Renewable and Sustainable Energy Reviews*, 22: 121–132.

Monirul, I.M., Masjuki, H.H., Kalam, M.A., Zulkifli, N.W.M., Rashedul, H.K., Rashed, M.M., Imdadul, H.K. & Mosarof, M.H. 2015. A comprehensive review on biodiesel cold flow properties and oxidation stability along with their improvement processes. *RSC Advances*, 5(105): 86631–86655.

Monyem, A. & van Gerpen, J.H. 2001. The effect of biodiesel oxidation on engine performance and emissions. *Biomass and Bioenergy*, 20(4): 317–325.

Moser, B.R. 2009. Comparative oxidative stability of fatty acid alkyl esters by accelerated methods. *JAOCS, Journal of the American Oil Chemists' Society*, 86(7): 699–706.

Moser, B.R. 2011. Influence of extended storage on fuel properties of methyl esters prepared from canola, palm, soybean and sunflower oils. *Renewable Energy*, 36: 1221–1226.

Nagarajan, J. & Narayanasamy, B. 2020. Effects of natural antioxidants on the oxidative stability of waste cooking oil biodiesel. *Biofuels*, 12(5): 485–494.

Njus, D., Kelley, P.M., Tu, Y.J. & Schlegel, H.B. 2020. Ascorbic acid: The chemistry underlying its antioxidant properties. *Free Radical Biology and Medicine*, 159: 37–43.

O'Neil, M.J. 2013. *The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals*. 13th ed. New Jersey: Merck and Co. Inc.

Othmer, K. 1969. *Encyclopedia of Chemical Technology*. 2nd ed. New York: John Wiley & Sons.

Oyetunji, O.J., Olutoye, M.A. & Ajayi, O.O. 2019. Comparative evaluation of antioxidants on the oxidative stability of waste cooking oil biodiesel. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 41(18): 2211–2223.

Palace, V.P., Khaper, N., Qin, Q. & Singal, P.K. 1999. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. *Free radical biology & medicine*, 26(5–6): 746–761.

Pandithavidana, D.R. & Jayawardana, S.B. 2019. Comparative study of antioxidant potential of selected dietary vitamins; Computational insights. *Molecules*, 24(9): 1–9.

Pantoja, S.S., da Conceição, L.R. v., da Costa, C.E.F., Zamian, J.R. & da Rocha Filho, G.N. 2013. Oxidative stability of biodiesels produced from vegetable oils having different degrees of unsaturation. *Energy Conversion and Management*, 74: 293–298.

Park, S.H., Khan, N., Lee, S., Zimmermann, K., Derosa, M., Hamilton, L., Hudson, W., Hyder, S., Serratos, M., Sheffield, E., Veludhandi, A. & Pursell, D.P. 2019. Biodiesel Production from Locally Sourced Restaurant Waste Cooking Oil and Grease: Synthesis, Characterization, and Performance Evaluation. *ACS Omega*, 4(4): 7775–7784.

Pattamaprom, C., Pakdee, W. & Ngamjaroen, S. 2012. Storage degradation of palm-derived biodiesels: Its effects on chemical properties and engine performance. *Renewable Energy*, 37(1): 412–418.

Pauquet, J.R. 1999. Breakthrough Chemistry for Processing Stabilisation of Polypropylene. *Journal of Macromolecular Science, Part A* , 36 A(11): 1717–1730.

Pehlivan, F.E. 2017. Vitamin C: An Antioxidant Agent. In A. H. Hamza, ed. *Vitamin C*. IntechOpen.

du Plessis, L.M., de Villiers, J.B.M. & van der Walt, W.H. 1985. Stability studies on methyl and ethyl fatty acid esters of sunflowerseed oil. *Journal of the American Oil Chemists' Society*, 62(4): 748–752.

Prankl, H. 2005. Stability of Biodiesel. In Gerhard. Knothe, J. Harlan. Van Gerpen, & J. Krahl, eds. *The Biodiesel Handbook*. AOCS Publishing: 134–143.

Prasanna, S. & Doerksen, R. 2009. Topological Polar Surface Area: A Useful Descriptor in 2D-QSAR. *Current Medicinal Chemistry*, 16(1): 21–41.

PubChem. 2021. Ascorbic acid. *National Center for Biotechnology Information*. https://pubchem.ncbi.nlm.nih.gov/compound/Ascorbic-acid 29 December 2021.

Pullen, J. & Saeed, K. 2012. An overview of biodiesel oxidation stability. *Renewable and Sustainable Energy Reviews*, 16(8): 5924–5950.

Ramalingam, S., Govindasamy, M., Ezhumalai, M. & Kaliyaperumal, A. 2016. Effect of leaf extract from Pongamia pinnata on the oxidation stability, performance and emission characteristics of calophyllum biodiesel. *Fuel*, 180: 263–269.

Ramos, M.J., Fernández, C.M., Casas, A., Rodríguez, L. & Pérez, Á. 2009. Influence of fatty acid composition of raw materials on biodiesel properties. *Bioresource Technology*, 100(1): 261–268.

Ramos, T.C.P.M., de Souza, E.F., Pina, C.C., Cavalheiro, A.A., Fiorucci, A.R. & da Silva, M.S. 2018. Evaluation of Natural Antioxidants Action in Oxidative Stability of Commercial Biodiesel. *Orbital: The Electronic Journal of Chemistry*, 10(1): 26–30.

Rashed, M.M., Kalam, M.A., Masjuki, H.H., Rashedul, H.K., Ashraful, A.M., Shancita, I. & Ruhul, A.M. 2015. Stability of biodiesel, its improvement and the effect of antioxidant treated blends on engine performance and emission. *RSC Advances*, 5(46): 36240–36261.

Rashedul, H.K., Masjuki, H.H., Kalam, M.A., Ashraful, A.M., Rashed, M.M., Sanchita, I. & Shaon, T. 2014. Performance and emission characteristics of a compression ignition engine running with linseed biodiesel. *RSC Advances*, 4(110): 64791–64797.

Rashedul, H.K., Masjuki, H.H., Kalam, M.A., Teoh, Y.H., How, H.G. & Rizwanul Fattah, I.M. 2015. Effect of antioxidant on the oxidation stability and combustion–performance–emission characteristics of a diesel engine fueled with diesel–biodiesel blend. *Energy Conversion and Management*, 106: 849–858.

Rodrigues, J.S., do Valle, C.P., Uchoa, A.F.J., Ramos, D.M., da Ponte, F.A.F., de Sousa Rios, M.A., de Queiroz Malveira, J. & Ricardo, N.M.P.S. 2020. Comparative study of synthetic and natural antioxidants on the oxidative stability of biodiesel from Tilapia oil. *Renewable Energy*, 156: 1100–1106.

Romola, C.V.J., Meganaharshini, M., Rigby, S.P., Moorthy, I.G., Kumar, R.S. & Karthikumar, S. 2021. A comprehensive review of the selection of natural and synthetic antioxidants to enhance the oxidative stability of biodiesel. *Renewable and Sustainable Energy Reviews*, 145: 111109.

Sadowska, J., Johansson, B., Johannessen, E., Friman, R., Broniarz-Press, L. & Rosenholm, J.B. 2008. Characterization of ozonated vegetable oils by spectroscopic and chromatographic methods. *Chemistry and Physics of Lipids*, 151: 85–91. www.sciencedirect.com 20 August 2022.

Şahin, S., Elhussein, E., Gülmez, Ö., Kurtulbaş, E. & Yazar, S. 2020. Improving the quality of vegetable oils treated with phytochemicals: a comparative study. *Journal of Food Science and Technology*, 57(11).

Sakthivel, R., Ramesh, K., Mohamed Shameer, P. & Purnachandran, R. 2019. Experimental investigation on improvement of storage stability of bio-oil derived from intermediate pyrolysis of Calophyllum inophyllum seed cake. *Journal of the Energy Institute*, 92(3): 768–782.

Salimon, J., Salih, N. & Yousif, E. 2012. Industrial development and applications of plant oils and their biobased oleochemicals. *Arabian Journal of Chemistry*, 5(2): 135–145.

Saluja, R.K., Kumar, V. & Sham, R. 2016. Stability of biodiesel – A review. *Renewable and Sustainable Energy Reviews*, 62: 866–881.

Santos, N.A., Cordeiro, A.M.T.M., Damasceno, S.S., Aguiar, R.T., Rosenhaim, R., Carvalho Filho, J.R., Santos, I.M.G., Maia, A.S. & Souza, A.G. 2012. Commercial antioxidants and thermal stability evaluations. *Fuel*, 97: 638–643.

Sarin, Amit, Arora, R., Singh, N.P., Sarin, R., Malhotra, R.K. & Kundu, K. 2009. Effect of blends of Palm-Jatropha-Pongamia biodiesels on cloud point and pour point. *Energy*, 34(11).

Sarin, A., Arora, R., Singh, N.P., Sharma, M. & Malhotra, R.K. 2009. Influence of metal contaminants on oxidation stability of Jatropha biodiesel. *Energy*, 34(9): 1271–1275.

Sarin, A., Singh, N.P., Sarin, R. & Malhotra, R.K. 2010. Natural and synthetic antioxidants: Influence on the oxidative stability of biodiesel synthesized from non-edible oil. *Energy*, 35(12): 4645–4648.

Sazzad, B.S., Fazal, M.A., Haseeb, A.S.M.A. & Masjuki, H.H. 2016. Retardation of oxidation and material degradation in biodiesel: a review. *RSC Advances*, 6. www.rsc.org/advances 7 October 2022.

Schaich, K.M., Shahidi, F., Zhong, Y. & Eskin, N.A.M. 2012. Lipid Oxidation. *Biochemistry of Foods*: 419–478.

Scherer, M.D., Oliveira, S.L., Lima, S.M., Andrade, L.H.C. & Caires, A.R.L. 2011. Determination of the biodiesel content in diesel/biodiesel blends: A method based on fluorescence spectroscopy. *Journal of Fluorescence*, 21(3): 1027–1031.

Serrano, M., Martínez, M. & Aracil, J. 2013. Long term storage stability of biodiesel: Influence of feedstock, commercial additives and purification step. *Fuel Processing Technology*, 116: 135–141.

Shahabuddin, M., Kalam, M.A., Masjuki, H.H., Bhuiya, M.M.K. & Mofijur, M. 2012. An experimental investigation into biodiesel stability by means of oxidation and property determination. *Energy*, 44(1): 616–622.

Shahidi, F. 2015. Antioxidants: Principles and applications. *Handbook of Antioxidants for Food Preservation*: 1–14.

Sharma, B.K., Suarez, P.A.Z., Perez, J.M. & Erhan, S.Z. 2009. Oxidation and low temperature properties of biofuels obtained from pyrolysis and alcoholysis of soybean oil and their blends with petroleum diesel. *Fuel Processing Technology*, 90(10): 1265–1271.

Sharma, Y.C., Singh, B. & Upadhyay, S.N. 2008. Advancements in development and characterization of biodiesel: A review. *Fuel*, 87(12): 2355–2373.

Sia, C.B., Kansedo, J., Tan, Y.H. & Lee, K.T. 2020. Evaluation on biodiesel cold flow properties, oxidative stability and enhancement strategies: A review. *Biocatalysis and Agricultural Biotechnology*, 24: 101514.

Sivaprakasam, S. & Saravanan, C.G. 2007. Optimization of the transesterification process for biodiesel production and use of biodiesel in a compression ignition engine. *Energy and Fuels*, 21(5): 2998–3003.

Sorate, K.A. & Bhale, P. v. 2015. Biodiesel properties and automotive system compatibility issues. *Renewable and Sustainable Energy Reviews*, 41: 777–798.

de Sousa, L.S., de Moura, C.V.R., de Oliveira, J.E. & de Moura, E.M. 2014. Use of natural antioxidants in soybean biodiesel. *Fuel*, 134: 420–428.

Strömberg, N., Saramat, A. & Eriksson, H. 2013. Biodiesel degradation rate after refueling. *Fuel*, 105: 301–305.

Susilowati, E., Hasan, A. & Syarif, A. 2019. Free Fatty Acid Reduction in a Waste Cooking Oil as a Raw Material for Biodiesel with Activated Coal Ash Adsorbent. *Journal of Physics: Conference Series*, 1167(1): 012035.

Tang, H., Wang, A., Salley, S.O. & Ng, K.Y.S. 2008. The effect of natural and synthetic antioxidants on the oxidative stability of biodiesel. *JAOCS, Journal of the American Oil Chemists' Society*, 85(4): 373–382.

Thompson, J.C., Peterson, C.L., Reece, D.L. & Beck, S.M. 1998. Two-year storage study with methyl and ethyl esters of rapeseed. *American Society of Agricultural and Biological Engineers*, 41(4): 931–939.

Traber, M.G. & Atkinson, J. 2007. Vitamin E, Antioxidant and Nothing More. *Free radical biology & medicine*, 43(1): 4–15.

Traber, M.G. & Stevens, J.F. 2011. Vitamins C and E: Beneficial effects from a mechanistic perspective. *Free radical biology & medicine*, 51(5): 1000–1013.

Tshizanga, N., Funmilayo Aransiola, E. & Oyekola, O. 2017. Optimisation of biodiesel production from waste vegetable oil and eggshell ash. *South African Journal of Chemical Engineering*, 23(2017): 145–156.

Tyrakowska, B., Soffers, A.E.M.F., Szymusiak, H., Boeren, S., Boersma, M.G., Lemańska, K., Vervoort, J. & Rietjens, I.M.C.M. 1999. TEAC antioxidant activity of 4hydroxybenzoates. *Free Radical Biology and Medicine*, 27(11–12): 1427–1436.

Uğuz, G., Atabani, A.E., Mohammed, M.N., Shobana, S., Uğuz, S., Kumar, G. & AlMuhtaseb, A.H. 2019. Fuel stability of biodiesel from waste cooking oil: A comparative evaluation with various antioxidants using FT-IR and DSC techniques. *Biocatalysis and Agricultural Biotechnology*, 21: 101283.

Ukaew, S., Beck, E., Meki, M.N. & Shonnard, D.R. 2014. Application of the roundtable on sustainable biofuels method to regional differences in nitrous oxide emissions for the rapeseed hydrotreated renewable jet life cycle. *Journal of Cleaner Production*, 83: 220–227.

Varatharajan, K. & Pushparani, D.S. 2018. Screening of antioxidant additives for biodiesel fuels. *Renewable and Sustainable Energy Reviews*, 82: 2017–2028.

Vatandoost, M., Shariati, A. & Shariati, M. 2014. A review on the performance and emission of biodiesel in diesel engine. *Renewable and Sustainable Energy Reviews*, (31): 113–127.

Verma, P., Sharma, M.P. & Dwivedi, G. 2016. Impact of alcohol on biodiesel production and properties. *Renewable and Sustainable Energy Reviews*, 56: 319–333.

Vidigal, I.G., Siqueira, A.F., Melo, M.P., Giordani, D.S., da Silva, M.L.C.P., Cavalcanti, E.H.S. & Ferreira, A.L.G. 2021. Applications of an electronic nose in the prediction of oxidative stability of stored biodiesel derived from soybean and waste cooking oil. *Fuel*, 284: 119024.

Waynick, J.A. 2005. *Characterization of biodiesel oxidation and oxidation products: technical literature review.* Lakewood: National Renewable Energy Laboratory.

Wazilewski, W.T., Bariccatti, R.A., Martins, G.I., Secco, D., Souza, S.N.M. de, Rosa, H.A. & Chaves, L.I. 2013. Study of the methyl crambe (Crambe abyssinica Hochst) and soybean biodiesel oxidative stability. *Industrial Crops and Products*, 43(1): 207–212.

van der Westhuizen, I. & Focke, W.W. 2018. Stabilizing sunflower biodiesel with synthetic antioxidant blends. *Fuel*, 219: 126–131.

Whelan, J. & Fritsche, K. 2013. Linoleic Acid. *Advances in Nutrition*, 4(3): 311.

Win, S.S. & Trabold, T.A. 2018. Sustainable waste-to-energy technologies:

Transesterification. In *Sustainable Food Waste-to-Energy Systems*.

Wypych, G. 2018. Effect of Additives on Weathering. In *Handbook of Material Weathering*. ChemTec Publishing: 591–624.

Xu, D.P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.J. & Li, H. bin. 2017. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *International Journal of Molecular Sciences*, 18(1).

Yaakob, Z., Narayanan, B.N., Padikkaparambil, S., Unni K., S. & Akbar P., M. 2014. A review on the oxidation stability of biodiesel. *Renewable and Sustainable Energy Reviews*, 35: 136–153.

Yamagata, H. 2005. *The science and technology of materials in automotive engines*. Cambridge: Woodhead Publishing Limited.

Yamane, K., Kawasaki, K., Sone, K., Hara, T. & Prakoso, T. 2007. Oxidation stability of biodiesel and its effects on diesel combustion and emission characteristics. *International Journal of Engine Research*, 8(3): 307–319.

Yang, J., Caldwell, C., Corscadden, K., He, Q.S. & Li, J. 2016. An evaluation of biodiesel production from Camelina sativa grown in Nova Scotia. *Industrial Crops and Products*, 81: 162–168.

Yang, Z., Hollebone, B.P., Wang, Z., Yang, C. & Landriault, M. 2013. Factors affecting oxidation stability of commercially available biodiesel products. *Fuel Processing Technology*, 106: 366–375.

Yesilyurt, M.K., Cesur, C., Aslan, V. & Yilbasi, Z. 2020. The production of biodiesel from safflower (Carthamus tinctorius L.) oil as a potential feedstock and its usage in compression ignition engine: A comprehensive review. *Renewable and Sustainable Energy Reviews*, 119.

Zhang, X., Peterson, C., Reece, D., Haws, R. & Möller, G. 1998. Biodegradability of biodiesel in the aquatic environment. *Transactions of the ASAE*, 41(5): 1423–1430.

Živković, S. & Veljković, M. 2018. Environmental impacts the of production and use of biodiesel. *Environmental Science and Pollution Research*, 25(1): 191–199.

Zuleta, E.C., Baena, L., Rios, L.A. & Calderón, J.A. 2012. The oxidative stability of biodiesel and its impact on the deterioration of metallic and polymeric materials: a review. *Journal of the Brazilian Chemical Society*, 23(12): 2159–2175.

APPENDIX: CHROMATOGRAPHS OF FATTY ACIDS

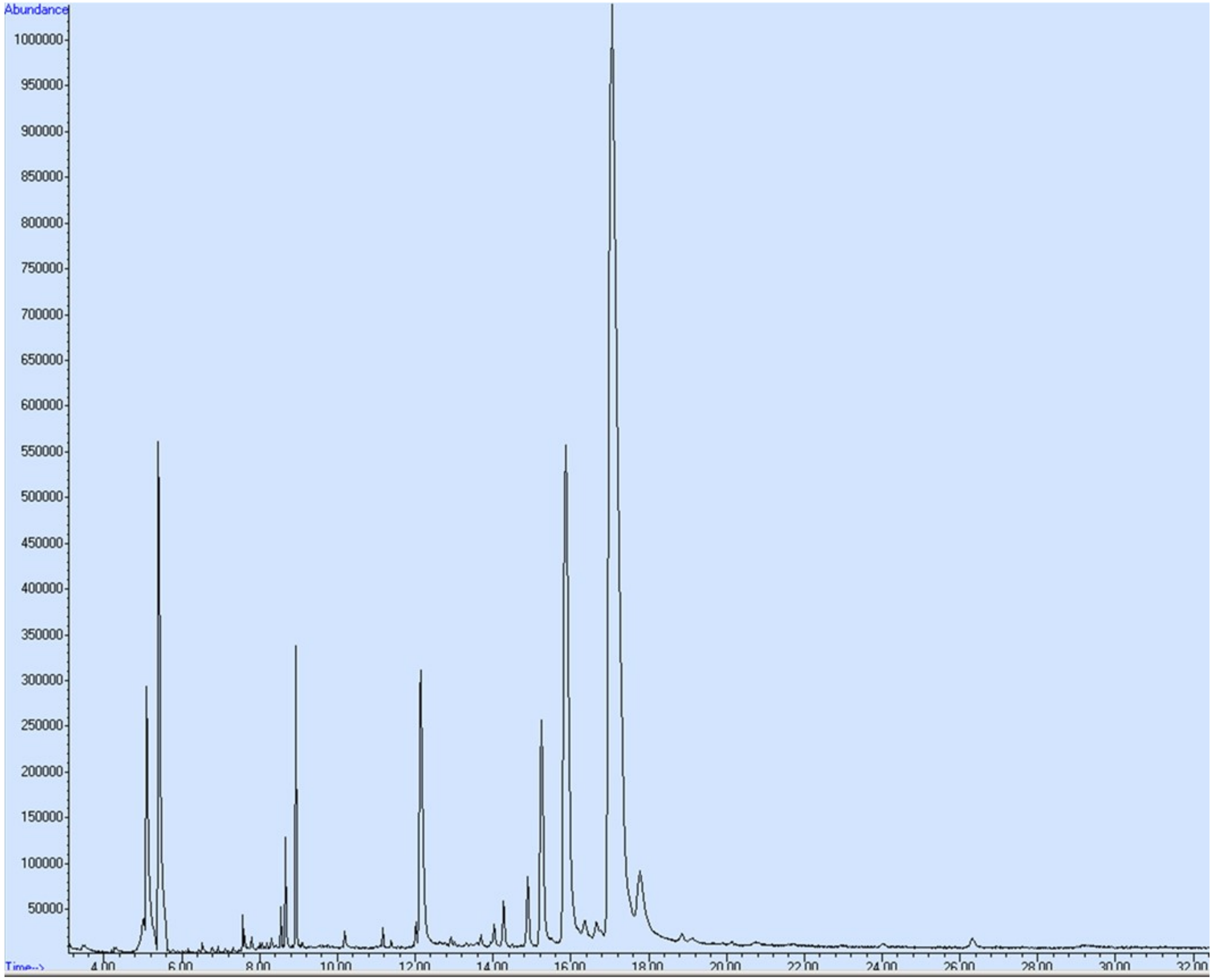

Figure A.0.1: Waste cooking oil (concentrated) chromatogram

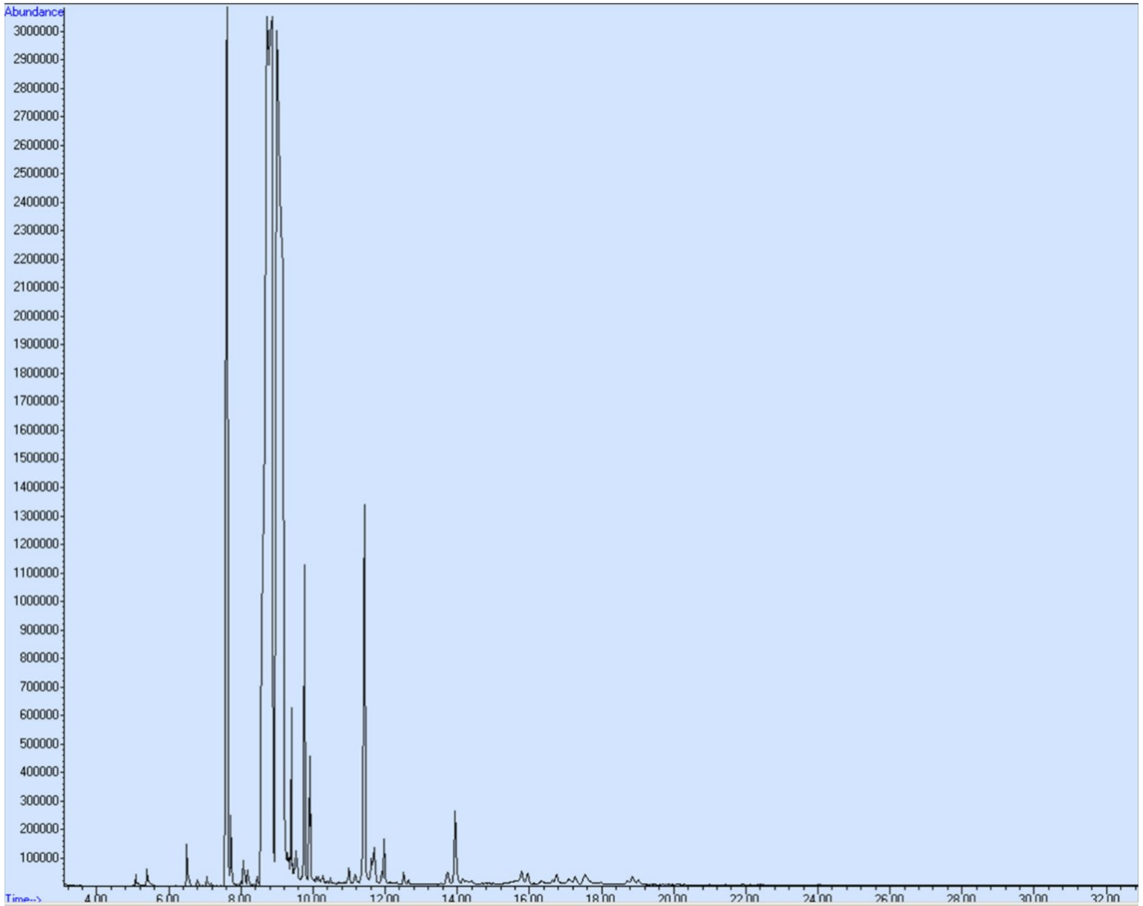

Figure A.0.2: Biodiesel oil (10x diluted) chromatogram.

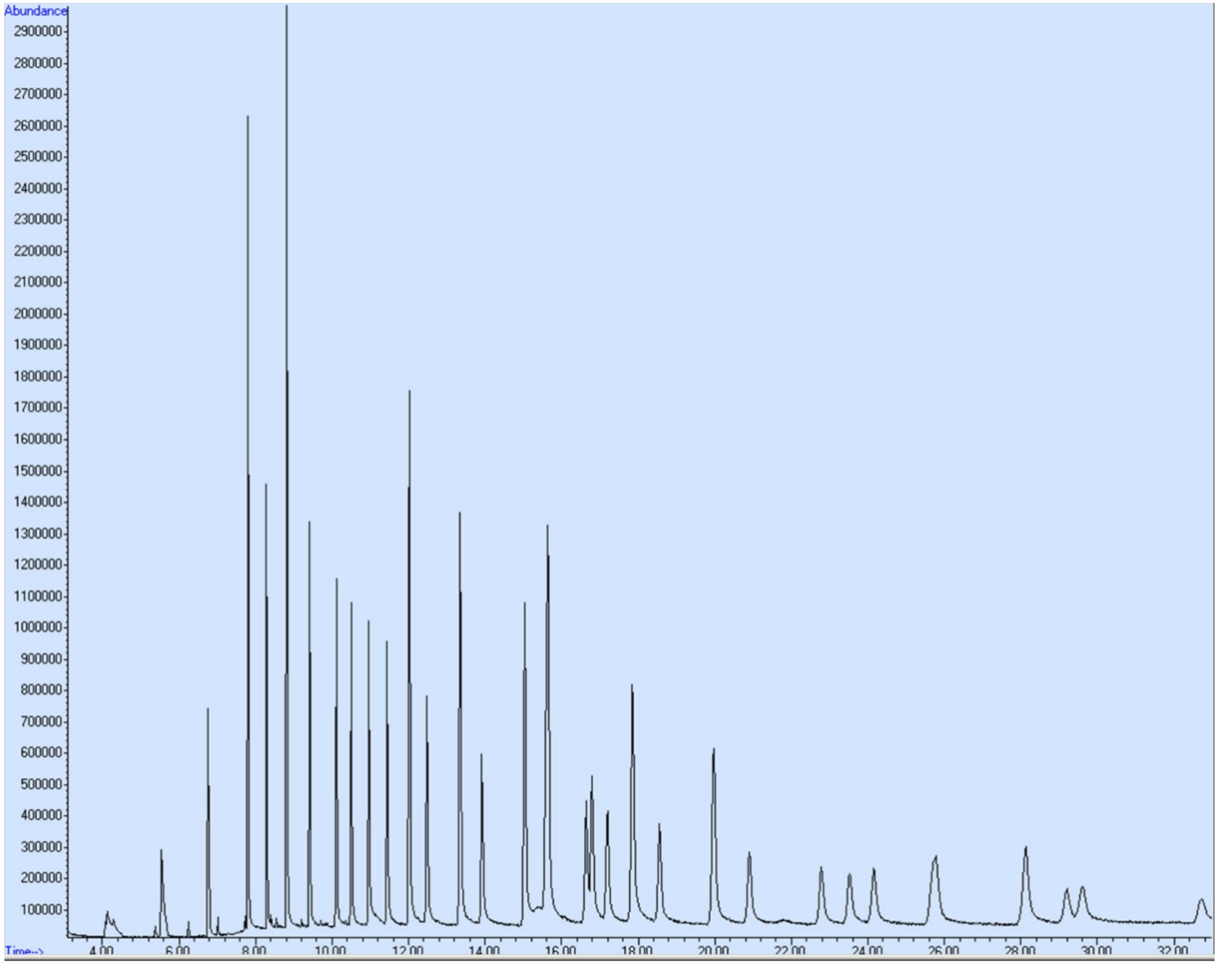

Figure A.0.3: 500 ppm standard mixture chromatogram.