MULBERRY SILKWORM (*Bombyx mori*) PUPAE EXTRACT AS A SUBSTITUTE FOR SYNTHETIC ANTIOXIDANTS IN VEGETABLE COOKING OILS

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A Research Thesis Submitted in Partial Fulfillment of the Requirements for the Award of a Degree of Master of Science in Food Security and Sustainable Agriculture

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DECLARATION

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This thesis is my original work and has not been presented for an award of a degree or diploma in any other university or institution.

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ACKNOWLEDGEMENT

My gratitude goes to Jaramogi Oginga Odinga University of Science and Technology (JOOUST) through the Africa Center of Excellence in Sustainable Use of Insects as Food and Feeds (INSEFOODS) for granting me the opportunity to further my studies and also funding this project.

I would also like to sincerely appreciate my supervisors; Dr. Solomon Omwoma and Dr. Alice Nakhumicha for their tremendous and tireless support during the period of my studies.

My appreciation also goes to Dr. John Kinyuru, Department of Food Science and Technology for the facilitation on lab space, equipment and an enabling environment to carry out this study and Mr. David Abuga, Food Biochemistry Laboratory, for his invaluable support with laboratory work. Both of Jomo Kenyatta University of Agriculture and Technology.

To Dr. Boniface Ngoka of Commercial Insects Programme, ICIPE and Silk Origin Limited, I am so grateful for the provision of pupae required for the project. Finally, I would like to thank my friends and colleagues who in various capacities supported me during the course of my studies, I am so much grateful.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluol
EDTA	Ethylenediaminetetraacetic acid
GC-MS	Gas chromatography–mass spectrometry
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
RBD	Refined bleached and deodorized
SFA	Saturated fatty acids
TBHQ	Tert-butylhydroquinone
TBA	Thiobarbituric acid
IV	Iodine Value

DEFINITION OF TERMS

Antioxidant. These are compounds that deter degradation of fats and oil by allowing their hydrogen to react with free radicals that are generated in the initial and propagation phases of autoxidation.

Autoxidation. A free radical chain reaction which occurs in the presence of oxygen in fats and oils.

Vegetable oils. This is used to refer to oils derived from plant parts such as leaves, fruits or seeds. Most of them are liquid at room temperature.

Shelf life. The period of time after processing and packaging during which a food product remains fit for consumption.

Extract. A preparation containing the active ingredient of a substance in concentrated form.

Pupae. An insect in the non-feeding stage of development between the larvae and the final form of the adult.

Fortification. The process of adding micronutrients (essential trace elements and vitamins) to food.

Oxidative stability. The measure of resistance of a fat or oil to oxidation.

Refining. The process of removing unwanted elements or impurities in edible oils processing.

Photo-oxidation. A reaction involving carbon and oxygen atoms of oils in the presence of sunlight that leads to the formation of new products.

Functional food. A food with the ability to positively affect human health beyond general nutrition.

Radical scavenging activity. The ability to remove or deactivate unstable molecules formed from oxidation of fatty acids in fats and oils.

Peroxide value. A measure of the extent of primary oxidation in oils. It is the reactive oxygen content expressed in terms of milliequivalents (meq) of free iodine per kilogram of oil.

Carcinogenic. Tendency to cause cancer.

ABSTRACT

Shelf stability of vegetable cooking oils is affected by their oxidation process (rancidity). Rancidity is a chemical process that involves oxidation or hydrolysis of oils/fats on exposure to moisture, air, light or bacteria giving rise to products such as ketones, aldehydes or free fatty acids. Rancid oils have a bad smell which affects vegetable oil quality. Synthetic antioxidants such as butylated hydroxytoluene (BHT) have the ability to slow down the oxidation process. However, their usage is discouraged due to toxicity after prolonged intake. Natural antioxidants may hold the solution to the rancidity problem. Silkworm pupae extract has been tested and found to be a potent natural antioxidant. This work evaluates the efficiency of the silkworm pupae oil extract as an antioxidant in vegetable cooking oils**.** Silkworm pupae extract was obtained by chloroform/methanol/water extraction. The extract was then added to sunflower and corn oils and their fatty acid profiles determined by GC-MS analysis. Thermal stability of the treated vegetable cooking oils and efficiency of the extract against autoxidation was also evaluated. The data obtained was analyzed using Stata version 12 package. Variations in studied parameters were compared using analysis of variance at a confidence level of 95% ($p < 0.05$). The oxidative stability of mulberry pupae extracts enriched-vegetable oils under typical cooking and shelf storage conditions was also statistically determined. The addition of silkworm extract into corn and sunflower oil as well as storage period had a significant effect on the physical and chemical characteristics of the oils. The silkworm extract contributed to more than half of the SFA in all the corn oil samples, specifically the corn oil treated with 200ppm of silkworm extract had significantly high levels of myristic acid (9.23%) while lauric acid was notably high in corn oil samples (7.8% & 2.13%) with both 100 ppm and 200 ppm silkworm extract respectively. Oil samples preserved using 100 ppm BHT had significantly high viscosity (25.41 & 32.03 Pa. s for corn and sunflower respectively) and density (0.91 & 0.92 Kg/m^3 corn and sunflower respectively) as compared to samples preserved using 100ppm silkworm extract (21.83 &
28.25 Pa. s for corn and sunflower respectively) and (0.89 & 0.88Kg/m^3 for corn and sunflower respectively); 200ppm silkworm extract (24.43 & 26.85 Pa. s for corn and sunflower respectively) and (0.88Kg/m^3 for both corn and sunflower oils respectively. This therefore means that the silkworm extract had better influence on the flowability of the oils compared to BHT. The oils also had improved fatty acid profiles, corn and sunflower oils preserved using 200ppm silkworm extract had the highest MUFA content (36% & 35.5% for corn and sunflower oils respectively) with the predominant MUFA being oleic acid. Oil samples preserved using 100ppm and 200 ppm silkworm extract PUFAs accounted for 49% and 36% of the total fatty acid content; the major PUFA was linoleic acid. Oleic and linoleic acid are unsaturated fatty acids important for cardiovascular health, immunity and brain function. This study concludes that the incorporation of silkworm pupae oil extract improves vegetable cooking oils quality and supports the use of edible insects as a sustainable source of nutrition.

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Vegetable oils are an integral to the human diet, serving as a primary source of energy and essential fatty acids. They contribute significantly to overall health by providing vital nutrients that support cardiovascular function, brain health, and immune system performance (Orsavova et al., 2015). The consumption of vegetable oils, such as corn and sunflower oil, is widespread due to their versatility in cooking and food preparation. However, these oils are susceptible to oxidative degradation, which can lead to the formation of harmful compounds and a reduction in nutritional quality (Omwamba et al., 2011).

Antioxidants are substances that inhibit oxidation, a chemical reaction that can produce free radicals, leading to cellular damage and various health issues (Bansal et al., 2013; Dimitrios, 2006). In the context of vegetable oils, antioxidants are crucial for prolonging shelf life, maintaining quality, and preventing rancidity. They work by neutralizing free radicals and interrupting the oxidative process, thereby preserving the integrity of the oils.

Oxidative deterioration when edible oils are stored is the main reason why they become unfit for human use (Esfarjani et al., 2019). Their vulnerability to oxidation is strongly dependent on the saturation state of lipids. In the last few decades, there has also been a shift from the use of oils and fats that contain high amount of saturated fatty acids, to vegetable oils comprising of additional healthy mono and polyunsaturated lipids (Matthäus, 2010; Mattson & Grundy, 1985). While oils containing unsaturated fatty acids offer better health benefits compared to their saturated counterparts, they are notably susceptible to oxidation due to weaker hydrogen -methylene bond strength in the fatty acid molecule (Choe & Min, 2006).

The byproducts of oxidation can react with other ingredients in the food such as amino acids or proteins resulting in loss of essential nutrients. Consequently, changes in color, viscosity and solubility occur. Most importantly, loss of essential fatty acids could also take place (Omwamba et al., 2011). The by-products of oxidation have also been linked with health concerns such as cancer, malformations during pregnancy and rise in blood pressure (Grootveld et al., 2006; Perumalla & Subramanyam, 2016; Vieira et al., 2017). Oxidation therefore is very significant in terms of the nutritional value, palatability and toxicity of edible oils.

The oxidation process cannot be completely stopped. Once it has begun, it can only be retarded or minimized (Kamal Eldin, 2010). Recent tendencies that are aimed at enhancing the storage stability

of edible oils by altering the composition of fatty acid from polyunsaturated to greater levels of monounsaturated fatty acids are linked to some disadvantages, mainly based on a nutritional perspective (Matthäus, 2010). Preventing contact with factors such as temperature, oxygen and metal traces has proved inefficient and uneconomical (Greyt, 2013). Therefore, the most effective and easy to delay oxidation is by use of antioxidants. In the past two decades, a lot of research using natural extracts in edible oils have been carried out so as to minimize or avoid the use of synthetic food additives, (Taghvaei & Jafari, 2015) due to the complexity associated with their toxicological studies and controversy over their safety.

Currently, antioxidants can be classified into two main categories: synthetic and natural. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are commonly used due to their effectiveness in preventing oxidation (Bansal et al., 2013; Dimitrios, 2006). Currently, the vegetable oil industry in Kenya uses butylated hydroxytoluol(BHT) and tert-butyl hydroquinone (TBHQ), (Lourenco et al., 2019). However, concerns regarding their safety andpotential health risks have prompted a shift towards natural alternatives. Natural antioxidants, including tocopherols (vitamin E), ascorbic acid (vitamin C), and phenolic compounds, are derived from plant sources and are generally regarded as safer options (Choe & Min, 2006). While these natural antioxidants are effective, they may have limitations in terms of stability and efficacy, particularly under high-temperature cooking conditions.

The efficiency of an antioxidant is dependent on the presence of on-going oxidative stress. However, the primary requirement is a high antioxidant content in the food matrix for screening of antioxidant potential in novel foods (Ioannone et al., 2017; Lettieri-Barbato et al., 2013). The stability during storage and quality of edible oils is significantly determined by fatty acid composition and tocopherol content apart from external factors such as temperature (Choe and Min., 2006). Thermal decomposition decreases with increase in saturation; unsaturated fatty acids decomposition begins at(200ºC to 380ºC) while for saturated fatty acids begin at (480ºC - 600ºC) (Santos et al.,2004). The oxidation process may get delayed or slowed down in case the oils contain higher amounts of naturalor added antioxidants (Abdel-razek et al., 2010).

Alternative sources of antioxidants are gaining attention for their potential benefits in food preservation. One such source is mulberry silkworm pupae extract, which is rich in bioactive compounds, including polyphenols and flavonoids (Arabshahi-Delouee & Urooj, 2007; Ramesh et al., 2014). These compounds exhibit strong antioxidant properties, making silkworm pupae a promising candidate for enhancing the oxidative stability of vegetable oils. Research has shown that

silkworm pupae can effectively scavenge free radicals and improve the overall quality of oils (Di Mattia et al., 2019).

Despite the known benefits of natural antioxidants, there are still significant knowledge gaps regarding their application in vegetable oils. For instance, no studies have evaluated the impact of silkworm pupae extract on the fatty acid profiles of commonly used vegetable oils like corn and sunflower oil. Additionally, the antioxidant efficiency of silkworm pupae extract has not been comprehensively tested across a range of physical and chemical parameters to assess oxidative stability. Furthermore, the thermal stability of silkworm pupae extract-fortified oils under typical cooking conditions remains unexplored.

In summary, the incorporation of natural antioxidants, particularly from alternative sources like mulberry silkworm pupae, offers a promising approach to improving the stability and quality of vegetable oils. As such, there is a need for continued research on various natural antioxidants so as to find ones that are as effective as artificial antioxidants. This study aims to fill the existing knowledge gaps by investigating the effects of silkworm pupae extract on the fatty acid profiles, antioxidant efficiency, and thermal stability of vegetable oils, thereby contributing to the development of safer and more effective food preservation methods.

1.2 Statement of the Problem

Shelf stability and safety of edible oils are crucial factors for both consumers and the industry. Oxidation in stored oil is detrimental to its quality. Recently, researchers have tried to improve oil storage stability by altering the composition of fatty acids from polyunsaturated to a greater quantity of monounsaturated fatty acids. Such interventions result in nutritional loss. The most effective and economical way to delay oxidation is to use antioxidants. While the refining process naturally results in loss of essential fatty acids and phenolic compounds, oxidation worsens it.

The increasing prevalence of oxidative deterioration in vegetable oils poses significant challenges to food quality, safety, and nutritional value. Traditional synthetic antioxidants, such as BHT, have been widely used to mitigate these issues; however, concerns regarding their long-term health effects and regulatory restrictions have prompted a search for safer, natural alternatives. This study aims to address the gap in knowledge regarding the efficacy of natural antioxidants in enhancing the stability and nutritional quality of vegetable oils.

Silkworm pupae extract presents a promising alternative due to its rich composition of unsaturated fatty acids, functional pigments, and antioxidant compounds, which can enhance the nutritional

profile of oils while providing effective protection against oxidation. By utilizing silkworm pupae extract, this study seeks to demonstrate its potential as a sustainable and health-promoting ingredient in cooking oils, thereby contributing to improved food quality and safety. The findings will not only support the use of edible insects in food products but also align with global efforts to promote sustainable food sources and enhance food security.

1.3 Objectives

1.3.1 General objective

To assess the effect of mulberry silkworm pupae oil extract on nutrition, oxidation and shelf stability of vegetable oils.

1.3.2 Specific objectives

i. To determine the effect of mulberry silkworm pupae extract on chemical characteristics of vegetable cooking oils (corn and sunflower oils) including refractive index, purity, fatty acid profile, colour, peroxide value, iodine value, saponification value, moisture value, acid value and viscosity.
ii. To asses shelf stability of vegetable cooking oils (corn and sunflower oils) after addition of mulberry silkworm pupae extract and commercial antioxidant (BHT)
iii. To asses oxidative stability at cooking temperatures of vegetable cooking oils (corn and sunflower oils) after addition of mulberry silkworm pupae extract and commercial antioxidant (BHT)

1.4 Hypotheses

$H0_1$: Addition of mulberry silkworm pupae extract in vegetable cooking oils does not affect their nutritional content

$H0_2$: Addition of mulberry silkworm pupae extract in vegetable cooking oils does not affect their shelf stability.

$H0_3$: Addition of mulberry silkworm pupae extract in vegetable cooking oils does not affect nutritional value of the cooking oils at high temperatures.

1.5 Justification

The use of synthetic antioxidants is under strict regulation due to their potential hazards thus the trend towards the use of natural antioxidants (Taghvaei & Jafari, 2015). Moreover, more energy is also required to metabolize synthetic antioxidants compared to natural antioxidants which are readily absorbed into the body. Therefore, the use of silkworm pupae extract will go a long way in improving the safety and functionality of vegetable oils which is a basic necessity in most

households.

The processing of edible oils has a significant effect on their nutritional composition (Matthäus, 2010). Crude oils consist of triacylglycerides, free fatty acids, phospholipids, phytosterols, waxes, colour and aroma components. Some of these components impair the stability of the oil since some ofthem undergo oxidative deterioration during processing therefore they have to be removed by refining. The refining process eliminates even the vital constituents such as free fatty acids, tocopherol, phenolic compounds and carotenoids (Matthäus, 2010). These compounds are not only important for shelf stability but also nutritionally hence they have to be added back through synthetic fortification which has sometimes proven inefficient since some of the fortificants are lost during storage (Diosady & Krishnaswamy, 2018). Silkworm pupae are a good source of high-quality unsaturated fatty acids, as well as functional pigments such as lutein, neoxanthin, carotenoids, and phenolic compounds (Longvah et al., 2011; Tomotake et al., 2010). Therefore, the functional and nutrient profile of the oils can be enhanced without the need for additional fortification, making the process more sustainable and economical.

Furthermore, the incorporation of silkworm pupae extract significantly increases the levels of beneficial unsaturated fatty acids, such as oleic and linoleic acids, in the vegetable oils (Longvah et al., 2011; Tomotake et al., 2010). This enhancement not only improves the nutritional quality of the oils but also supports cardiovascular health and overall well-being. Using silkworm pupae extract can transform conventional cooking oils into functional foods, thereby promoting healthier dietary choices among consumers.

Silkworm pupae extract can also effectively prolong the shelf life of vegetable oils while maintaining their quality. It not only reduces the reliance on synthetic additives, which may pose health risks, but also enhances the safety and longevity of food products. This aligns with consumer preferences for natural ingredients and can lead to increased market acceptance of products containing insect-derived components.

The use of silkworm pupae extracts as a natural antioxidant in cooking oils is relevant for the health-conscious Kenyan consumer. It offers a safer alternative to synthetic antioxidants which have been linked to toxicity concerns. Additionally, promoting the use of natural antioxidants from locally available sources like silkworm pupae aligns with the Kenyan government's focus on value addition and agro-processing of agricultural products. It can support the growth of small and medium enterprises in the food processing sector.

1.6 Limitations and Conceptual Framework

1.6.1 Limitations of the Study

The study was conducted using a limited number of vegetable oil types (corn and sunflower oils). The findings may not be generalizable to other types of vegetable oils or to oils with different processing methods. Future research should include a broader range of oils to validate the results. The study also focused on specific concentrations of silkworm pupae extract (100 ppm and 200 ppm). The effects of varying concentrations beyond these limits were not explored, which may limit the understanding of the optimal dosage for antioxidant efficacy.

Additionally, the oxidative stability tests were conducted over a relatively short period (5 days). Long-term storage studies are necessary to assess the sustained effectiveness of silkworm pupae extract in preventing oxidation over extended periods. This study also did not account for environmental factors such as light exposure, humidity, and temperature fluctuations during storage, which can significantly influence the oxidative stability of oils. Future studies should consider these variables to provide a more comprehensive understanding of the extract's effectiveness. Lastly, this study primarily focused on chemical analyses and did not include sensory evaluations (taste, aroma, etc.) of the treated oils. Sensory properties are crucial for consumer acceptance and should be assessed in future research.

1.6.2 Description of the Conceptual Framework

This framework provides a structured approach to understanding the mechanisms by which silkworm pupae extract influences the properties of vegetable oils. It emphasizes the potential of natural antioxidants in food applications and supports the study's objectives. The framework begins with the input of silkworm pupae extract into vegetable oils (input variables), highlighting the concentrations used in the study. It then outlines the processes of lipid oxidation and the role of antioxidants in mitigating these effects (process variables). Finally, the framework connects these processes to the output variables, which include improved oxidative stability and enhanced fatty acid profiles, ultimately leading to better nutritional quality and safety of the oils.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview and nutritional composition of Mulberry Silkworm

Mulberry Silkworm (**Figure. 2.1**) is an insect under the order Lepidoptera, commercially reared for silk production. Although, the other silkworm varieties *tasar, eri and muga* are also used for silk production, most of the world's silk is produced from the Mulberry Silkworm (Sheikh et al., 2018).They account for 90% of commercial silk production in the world (Patil et al., 2019). At the pupal stage of its lifecycle, silkworms build a shielding cocoon made up of raw silk. After pupation, the cocoons are chemically or heat treated to release an enzyme that breaks open the cocoon to release the pupae (**Figure. 2.1**). The spent pupae from yarn reeling are not discarded as waste; instead, theyare utilized as food and feed in various applications, and some are processed into compost for use as manure. The Mulberry Silkworm pupae is widely utilized as food and feed in Asian countries andother parts of the world due to its high-quality nutrient profile; they have a high protein content andfat rich in monounsaturated and polyunsaturated fatty acids, (Zhou & Han, 2006).

Mulberry Silkworm pupae is rich in omega-3 and omega-6 fatty acids, averagely 40% and 5% (of total fatty acids by weight) respectively (Kwon et al.,2012). Omega-3 (docosahexaenoic acid) which is the most dominant is an essential fatty acid also found naturally in some sea foods and maternal breast milk. It is responsible for organ development in premature babies. The pupae therefore have a great potential in human diets. In China for instance, the silkworm pupae have been approved as a novel food source by Ministry of Health, (Patil et al., 2019).

The composite nutritional quality of Mulberry silkworms is as a result of their plant-based feeding (Di Mattia et al., 2019). Mulberry Silkworms solely feed on Mulberry leaves. The table below (**Table 2.1**) shows the nutritional composition of the leaves.

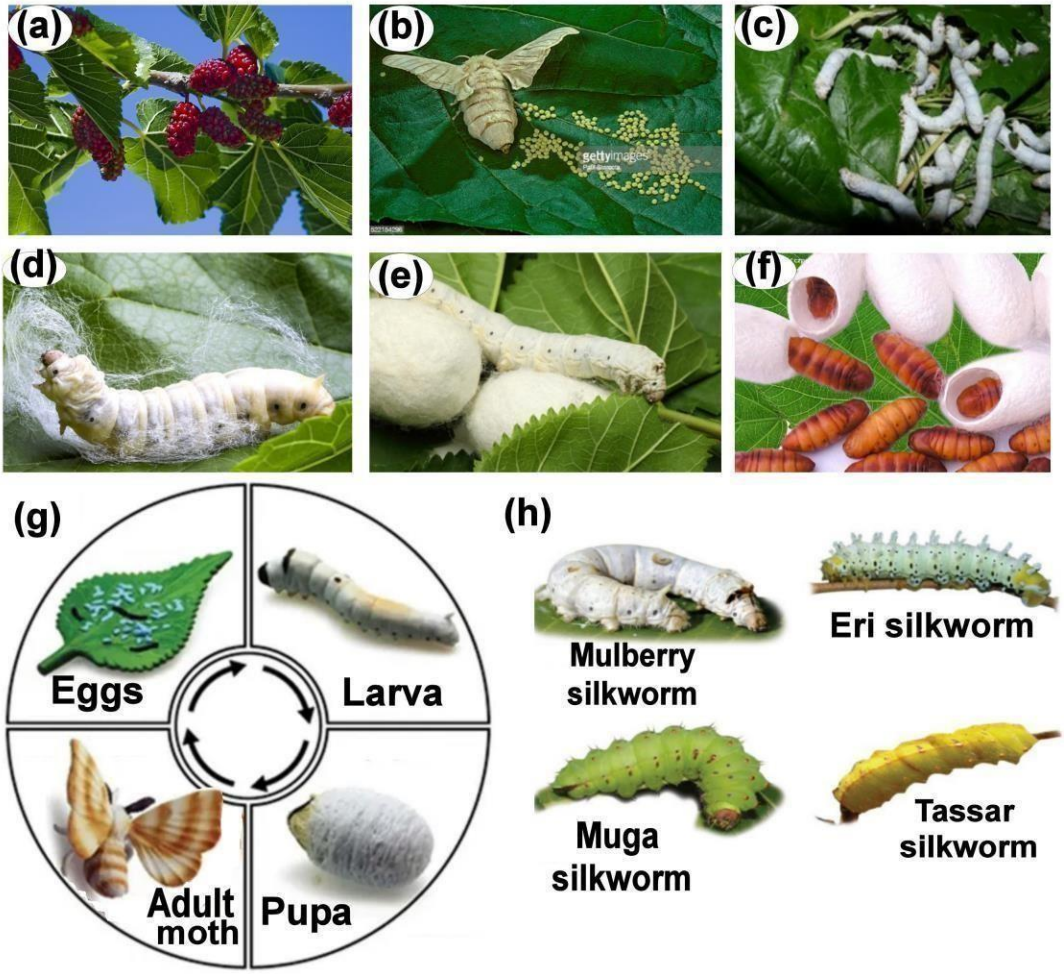

Figure 2.1: a) Mulberry plant, b) Adult mulberry moth eggs, c) Mulberry larva, d) Mulberry larva making silk, e) Mulberry silkworm cocoon with pupae, f) Mulberry pupae, g) Mulberry silkworm lifecycle and h) Various types of silkworms.

Source: *(International Sericultural Commission, 2013).*

Table 2.1: Nutritional composition of Mulberry leaves

Constituent	Amount (Per 100g of Mulberry Leaves)
Crude protein (%)	12.4
Crude fat (%)	4.0
Moisture (%)	67.8
Total free sugar(mg/g)	10.4
C16:0 (%)	20.1
C16:1 (%)	1.4
C18:0 (%)	4.3
C18:1 (%)	9.5
C18:2 (n-6) (%	27.5
C18:3 (n-3) (%)	36.5
Saturated Fatty acids (%)	24.4
Monounsaturated fatty acids (%)	10.9
Polyunsaturated fatty acids (%)	64.0
Neoxanthin (ug/g)	38.8
Violaxanthin (ug/g)	51.9
Antheraxanthin (ug/g)	26.4
Lutein (ug/g)	197
β-Carotene (ug/g)	148
Tot. carotenoids (ug/g)	462

Source: (Chieco et al., 2019)

Silkworm pupae is fed directly to livestock, as well as used a protein supplement in swine, poultry and cattle feeds (Sheikh et al., 2018). While the silkworm pupae have been explored as food and other uses, the functionality of their biologically active compounds remain under-explored (Di Mattia et al., 2019; Kotake-Nara et al., 2002).

Mulberry leaves have been reported to contain many antioxidative flavonoid and phenolic compounds with free radical scavenging activity that are expressed in the silkworms as a result of their feeding (Arabshahi-Delouee & Urooj, 2007; Ramesh et al., 2014). The chemical component of Mulberry Silkworms is primarily determined by their diets (Chieco et al., 2019). This therefore suggests their role as a source of potent antioxidants. Additionally, a study to determine the antioxidant potential of edible insects has proven that silkworm pupae have an antioxidant capacity three times higher than that of olive oil, a functional food that is important in balancing the antioxidant network in humans (Di Mattia et al., 2019).

Table 2.2: Artificial antioxidants in oils and their effect on the fatty acid content of selected edible oils

Vegetable oils	Fatty acids			Artificial antioxidants
	monounsaturated	polyunsatured	saturated	
Olive	71.3	12.7	16.0	–
Rapeseed (A)	65.2	29.3	5.5	citric acid/E vitamin
Rapeseed	65.0	29.0	5.0	–
Sunflower (A)	22.8	65.2	12.0	citric acid/E vitamin
Sunflower	23.0	65.0	12.0	–
Corn (A)	33.5	51.0	15.5	citric acid/TBHQ
Corn	34.0	50.0	16.0	–
Soybean	24.3	60.0	15.7	citric acid/TBHQ
Rice	40.8	40.1	19.1	–

(A) presence of artificial antioxidants

Source: *(Santos et al., 2004).*

Most antioxidants function by reacting with radicals from the free radical chain mechanism to form more stable compounds (Taghvaei & Jafari, 2015). Others function by destroying the hydroperoxides formed, scavenging of oxygen or synergism. Synthetic antioxidants such as BHT, TBHQ, citric acid and vitamin E **(Table 2.2)** have been used over time mainly because of their high stability, performance and low costs; however, their safety has always been controversial (Lourenço et al., 2019). Prolonged consumption of synthetic antioxidants is associated with health problems, including skin allergies, gastro intestinal tract issues, and in some cases increased risk of cancers (Botterweck et al., 2000; Choe & Min, 2006; Engin et al., 2011; Randhawa & Bahna, 2009). Hence the trend towards natural antioxidants.

2.2 Shelf life of vegetable oils

Vegetable oils play an important role in the routine diets of individuals globally. Based on the US Department of Agriculture, about 189.11 million metric tons of vegetable oils are produced across the world (Madhujith & Sivakanthan, 2019). In the recent past, the production of vegetable oils across the globe has been consistently increasing particularly palm, soybean and sunflower. This is because vegetable oils provide a healthier alternative to animal fats due to their cholesterol free nature and fatty acid profile (Orsavova et al., 2015). Hence, the oil processing industry ought to maintain the high quality of the product after processing until the point of use by the consumer. Vegetable oils differ in their features majorly in accordance with their composition. As a result, oils are utilized as an ingredient in different foods and for cooking. The oils go through a number of processing techniques, majorly heat treatment.

The main quality deteriorative reaction taking place in edible oils is oxidation (Choe & Min, 2006). The oxidative stability of edible oils depends on the raw material used, the processing steps involved and the conditions during storage. Vegetable oils consist of about 96% triacylglycerides, composed of various fatty acids (Orsavova et al., 2015). Fatty acids, either bound to glycerol or free are susceptible to oxidation resulting in a wide range of degradation products (Omwamba et al., 2011). The most obvious characteristic change is the emergence of bad taste and smell. Other transformations include change in viscosity and colour. Consequently, there is destruction of vitamins and their precursors, loss of essential fatty acids and formation odor-intensive compounds. These changes in the long run influence the sensory and nutritional value of the vegetable oils (Matthäus, 2010).

2.3 Mechanism of Lipid Oxidation

The vulnerability of edible oils to oxidation is dependent on the saturation levels of fatty acids, the content and form of antioxidants, temperature, oxygen available, and the amount of light and trace metals (Choe & Min, 2006). Therefore, the most critical role in their production and commercialization is to ensure that these determinants remain at the lowest level to a point where no unfavorable alterations are expected beyond a given time (Greyt, 2013).

Oxidation of edible oils starts with the double bonds of fatty acids in the triacylglycerol molecule (Omwamba et al., 2011). The first products of lipid oxidation are hydroperoxides, which are generally odorless and tasteless. Since hydroperoxides are very unstable, they undergo a series of reactions to form products such as aldehydes, ketones, hydrocarbons, alcohol and lactones (Kerrihard et al., 2015). These products have a very low threshold for taste and smell and hence become very obvious in the oil once formed. The major channel for the development of hydroperoxides is autoxidation yet still irradiation, enzymatic oxidation and photo-oxidation are also possible (Matthäus, 2010). The mechanisms of these pathways are technically similar although they differ in radical formation.

2.3.1 Autoxidation

The major constituents in this reaction are oxygen and unsaturated fatty acids, which can be free or bound in the triacylglycerol molecules. Normally, atmospheric oxygen exists as triplet oxygen. A direct reaction between the fatty acid molecule and the triplet oxygen is not possible due to a spin-forbidden reaction although triplet oxygen can react with free fatty acid radicals. This reaction is known as autoxidation (Choe & Min, 2006).

It is a free radical chain reaction that occurs in four phases; initiation phase, propagation, chain branching, and termination (Figure 2.2).

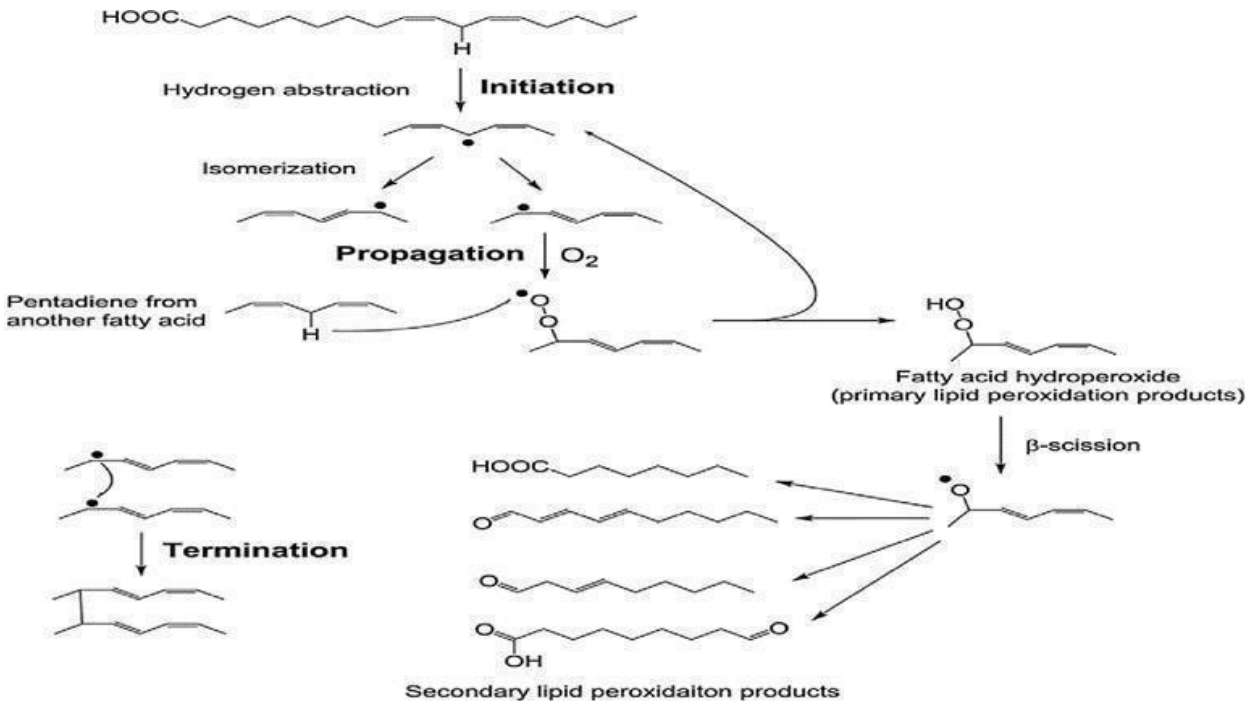

Figure 2.2: Mechanism of lipid oxidation in edible oils

Source: *(Wang et al., 2017)*

The initiation phase which begins with hydrogen atom elimination from an intact fatty acid molecule in order to form a radical is necessary for the chain reaction to begin. This forms a radical that can react with triplet oxygen. The reaction between a fatty acid radical and triplet oxygen forms a peroxy radical (Kerrihard et al., 2015). The reaction occurs within a very short time since the fatty acid radical is very unstable and no activation energy is required for the reaction to occur. The reaction is also strongly dependent on the oxygen available for the reaction and the ability of oxygen to dissolve in the lipid. An increase in temperature results in a decrease in solubility of oxygen in the lipid. The decrease in solubility of oxygen in lipids, results in slowdown or termination of the formation of peroxy radicals. Without the availability of oxygen, other reactions like polymerization take over (Ahmed et al., 2016; Kamal Eldin, 2010).

The peroxy radical being very reactive, it attracts a hydrogen atom from another stable unsaturated fatty acid, leading to the formation of a hydroperoxide, which is the main product of the degradation process of edible oils (Omwamba et al., 2011). The chain reaction continues after the first phase as the newly formed radical merges with triplet oxygen and abstracts hydrogen from another unsaturated fatty acid, creating other hydroperoxides (Ned, 2013). The autoxidation process then goes on exponentially. Initially the formation of hydroperoxides is slow then later accelerates to a level which is detectable, a phase known as the induction period (Šimon et al., 2000). The oxidation process is accelerated by the presence of metal ions such as copper and iron (Omwamba et al., 2011).

The vulnerability of fatty acids to hydrogen removal significantly relies on the bond strength of the hydrogen -methylene group in the fatty acid molecule (Juan & Maria, 2003). The peroxy radical always abstracts the weakest bound hydrogen in the fatty acid molecule. Although the bond strength of hydrogen in saturated fatty acids is nearly 99kcal/mol, only about 80kcal/mol is required to abstract a hydrogen atom from a methylene group in oleic acid and the abstraction of hydrogen from the double allylic methylene group of linoleic acid only needs 69kcal/mol (Matthäus, 2010; Ying et al., 2018). For linolenic acid that has two doubly allylic methylene groups it is 40kcal/mol. Various fatty acid molecules have various hydrogen bond strengths, and hence they go through lipid oxidation at different rates (Hongyan et al., 2013; Martín-Polvillo et al., 2004). Hence, this means that edible oils with great quantity of unsaturated fatty acids are subject to faster autoxidation during storage compared to oils with monosaturated and saturated fatty acids.

2.3.2 Photo-oxidation

Another alternative means of activation of the fatty acid molecule is photo-oxidation and it occurs in two types (Porter, 2013); Type I: light activates the sensitizer, which conveys energy to the fatty acid resulting in the development of a radical that is able to react with triplet oxygen.

Type II: the sensitizer reacts with triplet oxygen after activation by light to form reactive singlet oxygen, which also reacts with the fatty acid molecule.

Reaction of fatty acids with singlet oxygen is faster about 1500 times than with triplet oxygen (Choe & Min, 2006). Singlet oxygen can react directly with the double bond of the fatty acid without further activation (Hongyan et al., 2013; Juan & Maria, 2003). This therefore means that oxidative deterioration takes place very fast since it occurs without an induction period.

There is no possibility of the oxidation process being terminated by use of antioxidants since in type II reactions there is no radical formation however, the reaction can be inhibited by quenchers whichtake up the activation energy of light without formation of any reactive species (Choe & Min, 2006).

2.3.3 Irradiation

This occurs in two different ways (Ahmed et al., 2016; Madhujith & Sivakanthan, 2019; Matthäus, 2010); Direct formation of radicals from lipids by abstraction of hydrogen from the allylic methylene group of an unsaturated fatty acid as ionization energy is way higher, 105 times greater than the energy required for abstraction.

Development of other radicals such as hydroxyl radicals through radiolysis of water which then abstracts hydrogen from the allylic methylene group. The resultant radical of the fatty acid then reacts according to the free radical chain mechanism of a hydroperoxide.

2.3.4 Enzymatic oxidation

Hydroperoxides are also formed through enzymatic oxidation by enzyme lipoxygenase belonging to the oxido-reductases group. This enzyme is found in nearly all living cells, they enhance the reaction between oxygen and cis, cis-unsaturated fatty acids, resulting in hydroperoxides. Their main reactants are free fatty acids. Some of them utilize triacylglycerides as a reactant, but with a reduced specificity. A number of lipoxygenases are very specific for the cis, cis-9, 12-dienesystem of fatty acids. For instance, based on enzyme product specificity, linoleic acid is oxidized in position 9 or in position 13 (Wang & Hammond, 2010).

2.4 Formation of secondary reaction products

Hydroperoxides cannot be detected by consumers since they are tasteless and odorless. Changes in quality only become clear after the secondary reaction products are formed with many aroma- active compounds (Matthäus, 2010; Porter, 2013).

The amount and type of compound formed depends on the fatty acid composition. Although hexanal is the major product of the decomposition of linoleic acid, significant amounts of trans, trans-2, 4-heptadienal are formed from linolenic acid (Greyt, 2013). Therefore, the rapid deterioration of edible oils with greater amounts of linolenic acid is not only due to the high vulnerability of this fatty acid to oxidation but also the very low threshold values of the aroma- compounds formed (Madhujith & Sivakanthan, 2019; Matthäus, 2010). The oxidation of hydroperoxides can occur spontaneously or in the presence of metal ions.

2.5 Effects of oxidation on the nutritional and sensory quality of edible oils

2.5.1 A decline in sensory quality during storage

This is the major and the most common or expected effect of oxidative deterioration. Generally, the negative characteristic of the oil is described as rancid which means ‘unfavorable’, ‘stinky’ or ‘nasty’ (Khan et al., 2011). The rancid sensation perception is varied and it depends on the composition fatty acids of the oil and the resultant degradation products.

A typical example of the off-flavor formation is the green, beany and grassy perception of soybean oil at an onset stage of storage which shortly changes to painty or fishy. Crude soybean oil already has this green-beany flavor, but it is eliminated during refining, leading to in a tasteless and odorless product (Greyt, 2013; Vieira et al., 2017). However, the flavor re-emerges during the early stage of storage. A number of other oils also develop specific off-flavor during storage depending on their source, such as `animal flavor' for lard and tallow, `fishy' for rapeseed,
`grassy/painty' for rapeseed and linseed, and `painty/rancid' for palm oil (Villarino et al., 2007).

2.5.2 Effect on nutritional quality

Edible oils are a significant source of fatty acids like linoleic and linolenic acid, and Vitamin-E-active compounds like tocopherol or tocotrienols (Aladedunye & Przybylski, 2009). Oxidation leads to degradation of these compounds and if they were the only source of dietary lipids and vitamin E, then there will be a deficiency (Aladedunye & Przybylski, 2009; Vieira et al., 2017). Moreover, there is a reduction in amino acid availability in a protein-rich food prepared using oxidized oil as a result of reactions involving lipid degradation products (Madhujith & Sivakanthan, 2019; Omwamba et al., 2011).

A surge in the uptake of oxidized oils also lead to an accelerated rate of turnover of Vitamin-E-active compounds to maintain the immune system of the body, which causes a greater need for Vitamin-E-active compounds, otherwise the balance between oxidized species from oxidized oil and the antioxidant defense system becomes disturbed (Burton & Traber, 1990; John et al., 2001).

2.6 Protection of edible oils against oxidation

2.6.1 Modification of the fatty acid composition

This is done through genetic modification or natural plant breeding to improve the oxidative stability of vegetable oils (Richard, 2012). Refined vegetable oils such as peanut, soybean, rapeseed or sunflower oils have high levels of unsaturated fatty acids linolenic and linoleic acids; hence they arenot recommended for repeated deep frying. Despite some oils like palm kernel and being stable, theirhigh levels of saturated fatty acids hinder their application (Sakurai et al., 2011) and therefore geneticand breeding methods are used to modify the saturates composition (Lee et al., 2018).

In the recent past, mid and high-oleic acid oil crops have been developed through breeding techniques; examples include Nexera™ (Omega-9 canola and Omega-9 sunflower oils) and Vistive-Gold™(low-saturated high-oleic soybeans)by Monsanto Co.(St. Louis, USA)(Bellaloui et al., 2015;Kaushik & Grewal, 2017; Richard, 2012) .Oil crops produced through genetic approaches have higher levels of oleic acid than normal include soybean (from 24% to 84%), palm(from 36% to 59%),canola (from 57% to 89%), sunflower (from 29% to 84%), peanut (from
55% to 76%), cottonseed (from 13% to 78%), and safflower (from 10% to 81%) (Richard, 2012). Mutation has also enabled scientists to develop soybean phenotypes with linolenic acid less than 4%, a level described as low linolenic and less than 2% which is ultra-low linolenic (Clemente & Cahoon, 2009); since a greater composition of linolenic acid is the major responsible determinant of the poor oxidative stability of a number of oils like soybean oil. Incomplete hydrogenation of highly unsaturated oils also improves the oxidative stability substantially. However, it is discouraged due to the formation of trans fats; hence fatty acid composition modification by genetic and breeding methods remains effective (Richard, 2012). This not only improves the oxidative stability of the oils but also the nutritional value (O'Keefe et al., 1993).

2.6.2 Modification of oil processing

The heat applied during the conventional oil processing techniques accounts for the major loss of antioxidants naturally present in edible oils. Cold-pressing technique enables oils retain higher levels of antioxidants and have an acceptable shelf life with no added antioxidants (Hassanein & Abdel-razek, 2012). Edible oils acquired through cold-pressing are referred to as virgin oils and are popularly known because of their distinct taste, colour and flavor. Due to the absence of heat treatment, most of the oil's natural constituents are left intact.

According to (Abdel-razek et al., 2010), cold-pressed olive oil has a stronger antioxidant activity, which is linked to the availability of natural phenolic compounds. Additionally, flushing oil with nitrogen during processing is also effective in reducing oxidative changes in sunflower and rapeseed oil (Wroniak et al., 2016).

2.6.3 Blending

Blending is achieved by mixing of different oils together. This combines the desirable qualities of two different oils to achieve a compounded effect on the quality of the resultant oil. It results in oils with modified fatty acid composition, functional and physicochemical properties with no alteration of the chemical composition (Abdel-razek et al., 2010).

A study by (Okogeri, 2015), investigating the stability of frying peanut oil blended with palm kernel oil at various ratios (90:10, 80:20, 70:30 and 60:40) revealed that all blends contained less polar compounds than control after used for frying. Considering the advantages and disadvantages of a single oil as cooking medium, blended oils seem to be more suitable than single oil for culinary purposes while at the same time economical.

2.6.4 Types of antioxidants and their use

Antioxidants are substances that significantly inhibit oxidation of the substrate by inhibiting formation of free radicals or by interrupting propagation of the free radical when introduced at a low concentration compared to that of an oxidizable substrate (Bansal et al., 2013; Bera et al., 2006).

Antioxidant addition during processing of oils is one of the most efficient and practical technique to slow down oxidation in edible fats and oils. There are two types of antioxidants in accordance with their techniques of action: primary and secondary antioxidants. They can additionally be categorized into natural and synthetic.

2.6.4.1 Primary antioxidants

Primary antioxidants also known as chain-breaking antioxidants, have the ability to of neutralize lipid free radicals by halting their aggressive state through donation of hydrogen. Examples of primary antioxidants are; butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butyl hydroquinone (TBHQ), tocopherol, and flavonoids (Bansal et al., 2013; Dimitrios, 2006).

2.6.4.2 Secondary antioxidants

Secondary antioxidants slow down the rate of oxidation through the removal of the substrate or singlet oxygen quenching mechanism (Ana et al., 2015; Bente et al., 2002). Their mechanism of action, including metal chelation, singlet oxygen quenching and inactivation of photosensitizers and lipoxygenase (Okogeri, 2015). Metals act as pro-oxidants by lowering the activation energy of the oxidation process, particularly in the first phase, to impede oil oxidation. The metals form metal complexes that are not soluble or provide steric hindrance between the components of metals and foodand examples include citric acid, EDTA, polyphenols, lignans and ascorbic acid (Hussainet al., 2015;Jiang & Youling, 2016).

The mechanism of action of singlet oxygen quenchers entails deactivation of singlet oxygen to the ground-state triplet oxygen or getting oxidized themselves by singlet oxygen (Rather et al., 2016). Tocopherol, carotenoids, phenolics, and ascorbic acid slow down oxidation of lipids through quenching singlet oxygen (Jie et al., 2012).

Photosensitized compounds such as riboflavin and chlorophyll transfer energy to triplet oxygen to form singlet oxygen or a superoxide anion radical which reacts with lipids to form free radicals (Orsavova, 2015). Energy of the photosensitizers is transferred to the singlet state of antioxidant, leading to triplet state of antioxidant, which is altered to singlet state by transferring the energy to the surrounding or releasing phosphorescence (Fernandez et al., 2019).

Combining primary and secondary antioxidants has proven more effective than the effect of a single antioxidant due to their synergistic effect which increases the length of the induction period (Bente et al., 2002; Fernandes et al., 2019).

2.6.4.3 Synthetic and natural antioxidants

BHT, BHA, and TBHQ are the most largely utilized antioxidants in the edible oils industry due to their effectiveness. A number of have tried to examine the effect of addition of synthetic antioxidants on the edible plant oils. Bente et al. (2002) assessed the impact of the direct incorporation of TBHQ, BHT, and blend of (TBHQ and BHT) on the oxidative stability of palm olein, soybean oil and linseed oil at room temperature and 70^{o}C for 168 hrs. They found that TBHQ had substantial impact on the oxidative stability of palm olein at 70^{o}C, and the combination of TBHQ and BHT had synergetic effect on stability of soybean oil at room temperature and Linseed oil at 70^{o}C.According to Xiu-qin et al. (2009), α-tocopherol, tocopherol esters and BHA show lower antioxidant effect at frying temperatures while ascorbic acid-6 palmitate and phytosterols fractions contain greater antioxidant activity.

However, recently they are being discouraged mainly based on the results linked to possible toxicity and carcinogenicity of these synthetic antioxidants as revealed by studies focusing on animals (Botterweck et al., 2000; Jeong et al., 2005; Pokorný, 2007). For instance, BHA and BHT have been revealed to contain carcinogenic activity in rodents. This has resulted in a significant shift towards the utilization of natural antioxidants as an alternative to synthetic antioxidants for enhancing the oxidative stability of oils (Amalia et al., 2017; Ke-Zheng et al., 2016).

Natural antioxidants are majorly from plant sources like grains, seeds, cereals, nuts, fruits, vegetables and spices (Rather et al., 2016). The compounds responsible for the antioxidative effect include flavonoids (quercetin, kaempferol, myricetin), catechins or phenols (carnosol, rosmanol, rosamaridiphenol) and phenolic acids (carnosic acid, rosmarinic acid) (Amalia et al., 2017; Embuscado, 2015).

Tocols are the natural antioxidants found in plant-based oils. The α-tocopherol is the most active biological isomer while γ-tocopherol is taken to be the best antioxidant (Taghvaei &Jafari, 2015). They offer protection against peroxidation of polyunsaturated fatty acids (George et al., 2004). Of all forms of tocopherol, α-tocopherol has the least stability, and therefore it is easily destroyed at high temperatures (Giovanna & Arnoldi, 2011). (Sandrine et al., 2016) reported that α- tocopherol are significantly lost during frying compared to at room temperature.

Edible oils with high amounts of unsaturated fatty acids like peanut, corn, sesame, sunflower and soybean oils are easily degraded during continuous frying as a result of their high polyunsaturated

fatty acid content (Xiu-qin et al., 2009). The presence of natural substances such as tocopherols, oryzanol, sterol fraction and squalene however enhance their stability at higher temperatures.

In the previous few decades, studies have largely focused on the use of natural plant extracts as sources of antioxidants as an alternative to the synthetic antioxidants (Rather et al., 2016); with the tendency towards the use of agro-industrial by-products increasing (Amalia et al., 2017; Casagrande et al., 2018; Lourenço et al., 2019). Extracts from natural plant have been shown to contain higher antioxidant activity and thermal stability, which are critical criteria for an antioxidant to be utilized for fats and oils (Bansal et al., 2013; Lourenço et al., 2019).

Examples of natural sources of antioxidants that have been used include pomegranate peels, green tea, olive waste, sesame cake, sesame seed, rosemary, Eucalyptus leaf, celery, oregano (Origanum vulgarem) and cinnamon(Bouaziz et al., 2008; Fernandes et al., 2019; Orsavova et al., 2015; Zhang et al., 2020).Extensive studies have been done on the antioxidative effect of α- tocopherol (vitamin E),which is a fat soluble carotenoid, rosemary extract, citric acid and rosemary extract(Embuscado, 2015).

Sesame cake extract possesses more antioxidant activity compared to BHA and BHT although less than that of TBHQ (Carvalho et al., 2012). Sesame seeds antioxidant activity is a result of its natural antioxidant components sesamolin, sesamin and sesaminol (Ruslan et al., 2018). Apart from enhancing the oxidative stability of sunflower oil, it greatly improved its fatty acid profile. Green tea, a powerful antioxidant exhibits excellent antioxidant activity at a concentration of 200ppm and above in both sunflower and soybean oils; its antioxidant activity is higher than that of BHA and BHT but still lower than that of TBHQ. (Casarotti & Jorge, 2014) studied the thermoxidative stability of soybean oil with rosemary extract at 3000 ppm and TBHQ at 50 ppm, from this study, it is evident that natural antioxidants can only be more effective than TBHQ when used in higher concentrations. Henceforth, based on circular economy, the trend of use of natural sources of antioxidants has increased the interest of researchers for new raw materials with antioxidant power (such as by- products from the agricultural food industry), without affecting the consumers' perceptions and the quality of the final products and at the same time producing a functional food with added value (Lourenço et al., 2019).

Antioxidants can also be found in various other insects, and there is potential for formulating these antioxidants for use in food preservation and other applications. Many insects are known to possess antioxidant properties due to their biochemical composition. For example, insects like crickets,

mealworms, and grasshoppers have been studied for their nutritional profiles, which include antioxidants such as phenolic compounds, flavonoids, and vitamin E (Aiello et al, 2023).

2.7 Importance of antioxidants in human health

Antioxidants play a crucial role in human health by protecting cells from oxidative stress linked to various chronic diseases. Antioxidants are compounds that inhibit oxidation, a chemical reaction that can produce free radicals i.e. unstable molecules that damage cells (Taghvaei & Jafari, 2015). This oxidative stress is associated with several health issues. For instance, oxidative stress can lead to DNA damage, contributing to the development of cancer. Free radicals can also oxidize low-density lipoprotein (LDL) cholesterol, leading to atherosclerosis. Additionally, conditions like Alzheimer's and Parkinson's are linked to oxidative damage in neurons.

Some of the health benefits of antioxidants include reducing inflammation, which is a precursor to many chronic diseases. Furthermore, by neutralizing free radicals, antioxidants support the immune system and enhance its ability to fight infections. Antioxidants can also protect the skin from damage caused by UV rays and pollution, potentially reducing the signs of aging. Certain antioxidants, like lutein and zeaxanthin, are known to support eye health and may reduce the risk of age-related macular degeneration (Bansal et al., 2013; Bera et al., 2006).

2.8 Antioxidant activity of silkworm

A lot of silkworm pupae is produced from yarn reeling yet little has been done to find its utilization. Apart from some of it being used as livestock and poultry feed, most of it end up in dumping sites. Despite the chemical composition of desilked silkworm pupae having attracted great attention worldwide and as a good source of a large number of bioactive substances, little research has been done with regard to the same.

Mulberry Silkworms are endowed with a unique pattern of redox ingredients such as phenolic compounds (Butkhup et al., 2012). This chemical species is able to counteract oxidative stress from lipophilic and water environments. According to (Kotake-Nara et al., 2002 and Ravinder et al., 2016), the silkworm pupae contain antioxidant, tocopherol; about 180micrograms per gram of extract. Carotenoids, lutein, violaxanthin and neoxanthin approximately 5.12, 0.28 and 0.88 (ug/g) pupae (wet basis) respectively, are also present in Mulberry Silkworms (Tabunoki et al., 2004). These compounds are known for their radical scavenging effect and other varied functions, such as antiallergy, antityrosinase and anti-inflammatory activities (Park et al., 2008; Yokohira et al., 2008).

A study by (Pachiappan et al., 2016) to investigate the antioxidant potential of silkworm pupal products; pupal powder, chitosan and pupal oil by measuring their radical scavenging activity reported that there was no significant difference between the radical scavenging activity of these products and that of ascorbic acid, which is known to be a great antioxidant. The by-products exhibited a 60% to 76% radical scavenging ability at 10 µg/ml against 64% of ascorbic acid at the same concentration. Winitchai et al., (2011) also reports free radical scavenging activity to be attributed to by phospholipids and tocopherol, which play an important role in protecting the lipids against oxidation. Investigation of the antioxidant activity of silkworm by determination of the phenolic and flavonoid content revealed that they also have an average of 15.5 mg catechin/g and 5.4 catechin/g phenolic and flavonoid contents respectively (Deori et al., 2014).

The two major types of pigments that account for cocoon color in silkworms are ether-soluble yellowish carotenoids and water-soluble green flavonoids (Kurioka & Yamazaki, 2002; Tabunoki et al., 2004; Tamura et al., 2002). The coloring content is dependent on the Mulberry silkworm strain. Flavonoid compounds, in addition to fibroin and sericin proteins in mulberry silkworm are also responsible for the antioxidant properties (Kato et al., 1998; Kurioka & Yamazaki, 2002).

Conclusively, silkworm pupae are good in antioxidant activity and they could be used as a source of antioxidants in food, pharmaceutical and cosmetic industries. Despite most researchers citing antioxidant potential of silkworms and it's by products including the possibility of their use as natural antioxidants in food products, little research has been done to test its effect in foods.

As the existing literature on antioxidants in vegetable oils is reviewed, several knowledge gaps emerge that this study aims to address. First, no studies have evaluated the effect of silkworm pupae extract on the fatty acid profiles of commonly used vegetable oils, such as corn and sunflower oil. Understanding this impact is crucial for assessing the nutritional implications of using silkworm extract as an antioxidant. Second, the antioxidant efficiency of silkworm pupae extract has not been comprehensively tested across a range of physical and chemical parameters to assess its oxidative stability. This study will fill this gap by evaluating how effectively the extract preserves oil quality under various conditions. Lastly, the thermal stability of silkworm pupae extract-fortified oils under typical cooking conditions has not been investigated. This research will explore how the extract performs when subjected to high temperatures, which is essential for practical applications in cooking. By addressing these gaps, this study will contribute valuable insights into the potential of silkworm pupae extract as a natural antioxidant in vegetable oils, enhancing their stability.

CHAPTER THREE
METHODOLOGY

3.1 Raw materials

3.1.1 Acquisition of Mulberry Silkworm pupae and vegetable oils

The raw silkworm pupae that were used were obtained from Silk Origin Limited, (1.2646° S, 36.8047° E) through ICIPE, Commercial Insects Programme, Sericulture Unit, (1.2219° S, 36.8966° E). Both in Nairobi. Freshly-prepared, refined, bleached and deodorized (RBD) corn and sunflower oils devoid of any additives was sourced from Bidco Africa Limited, Thika. The vegetable oils were stored at -4^{o}C until use. Corn and sunflower oils are widely used cooking oils in many households, making them relevant choices for evaluating the effectiveness of natural antioxidants.

3.1.2 Silkworm pupae collection and preparation

Mulberry silkworm cocoons were collected by simple random sampling. The pupae were taken out of the cocoon balls by cutting; this was done by carefully cutting around the outside of the cocoon along the long axis. Stopping periodically to peek into the cocoon to make sure that one is not cutting into the pupae that's inside, they were cut until there were two halves of the cocoon and the pupae removed.

The pupae were then screened for defective ones by separating the diseased and damaged ones from the intact ones. The intact ones were then washed thoroughly using distilled water and 95% alcohol remove foreign materials then oven dried at 55 °C for 24 hours, (Pachiappan et al., 2016).

3.2 Research design

The experimental design employed in this study was a randomized complete block design (RCBD). This design was chosen to minimize the effects of variability among the experimental units (corn and sunflower oils) and to ensure that the treatments (different concentrations of silkworm pupae extract and BHT) were applied uniformly. Each type of oil served as a block, and within each block, the treatments were randomly assigned to the experimental units. The design included a control group (antioxidant-free oil) and three treatment groups: oils with 100 ppm BHT, 100 ppm silkworm extract added to the oil, and 200 ppm silkworm extract added to the oil. Each treatment was replicated twice to ensure statistical reliability. This design allows for the comparison of the effects

of different antioxidants on the physical and chemical properties of the oils, (Hasan *et al.* (2019). Each of the treatments was assigned to the experimental units within the blocks randomly and replicated twice. Each of the vegetable oil samples were then mixed for 5 minutes in a water bath to be used for subsequent analyses. After the incorporation of antioxidants, 750mls of the vegetable oils; both corn and sunflower treated with the various antioxidants were heated in a beaker at about 170˚C for 0, 10, 20,30,40 and 60 minutes (a thermometer was used to check the temperature) for 5 days. Samples were then drawn each day for 5 days to analyze the physical and chemical parameters to determine the efficiency of the antioxidants in the vegetable oils. The fatty acid profiles of the oils were also determined after the 5 days of heat treatment, (Pachiappan et al., 2016). All the determinations were carried out in duplicate.

3.3 Determination of the fatty acid profiles of vegetable oils incorporated with silkworm pupae extract.

The fatty acid profile was determined by gas chromatography. The extraction of the lipids was done by a modification of the Bligh and Dyer method (1959). Samples of each 1000g of silkworm pupae, were crushed and placed in a glass stoppered five-liter Conical flask and then 2000ml of methanol-chloroform-water (2:1.01) v/v) was added and the mixture shaken overnight at room temperature. The samples were centrifuged and the supernatant to decanted and the residue suspended in 1000ml of methanol-chloroform-water (2:1:0.1), the homogenate was centrifuged. Then 500ml each of chloroform and water were added to the supernatant and the mixture centrifuged. The chloroform phase was extracted and dried in a vacuum rotary evaporator at 40^{O}C. The residue was completely dried in oven at 60 ^{O}C for one hour then to a desiccator for weight difference. The samples were centrifuged and the supernatant decanted and the residue suspended in 9.5ml of methanol-chloroform-water (2:1:0.8), the homogenate was centrifuged. Then 7.5ml each of chloroform and water were added to the supernatant and the mixture centrifuged. The chloroform phase was extracted and dried in a vacuum rotary evaporator at 40 °C. The residue was completely dried in a desiccator over KOH pellets. Then the lipid was methylated by placing 2mg of the sample in a flask and refluxing with 2ml of 95%methanol-HCl for 1 hour. The methyl esters were extracted with 3 portions of hexane (1ml) and then washed with distilled water (3ml). The hexane layer was dried in vacuum rotary evaporator and the residue dissolved in a small drop of hexane. Then 0.2ul was

injected into the GC (Shimadzu GC-2010) with a capillary column, supelcowax (30m x 0.53mm); injection temperature of 240 and detection temperature, 260^{O}C under a flame ionization detector and mass spectrophotometer. Identification of the fatty acid methyl esters was by comparison of retentiontimes with standards and expressed as percentages of total methyl esters.

3.4 Establishing the efficiency of silkworm pupae extract against oxidation in vegetable oils

3.4.1 Physical quality parameters

The physical changes in the vegetable oils were assessed by colour, viscosity, refractive index and density as described by (AOAC,1995). All the determinations were carried out in duplicates.

3.4.1.1 Colour

The colour of the vegetable oils was measured using a Minolta colorimeter (Model CR-200, Osaka, Japan). Prior to measurement, the colorimeter was calibrated with standard white and black tiles to ensure accuracy. The oil samples were placed in a sample holder, and the colour was assessed using the CIE L*a*b* color space, which provides a comprehensive representation of colour. The L* value indicates lightness, while the a* and b* values represent the green-red and blue-yellow colour axes, respectively. The hue angle (H) was calculated from the a* and b* values according to (AOCS,1998).

Hue angle (H°) = arctan (b/a)

This method allows for a precise quantification of colour differences, which is essential for evaluating the quality and stability of the oils. The colour measurements were taken in triplicate to ensure reliability, and the average values were reported for each sample.

3.4.1.2 Viscosity

The viscosity of the vegetable oils was determined using a HAAKE Viscometer VT-02 (Ontario, Canada), which is a rotating viscometer designed for precise viscosity measurements. Approximately 25 mL of each oil sample was placed in the outer cylinder of the viscometer, and a coaxial bob (Bob No. 2) was inserted. The radius of the tube was 16.25 mm and the radius of the bob was 15.5 mm while the length of the bob is 54 mm. The viscometer was set to the appropriate measuring mode (MS 19), and the measurement time was fixed at 60 seconds to ensure consistency across samples.

To maintain a constant temperature during the viscosity measurement, a circulatory water bath was set at 30°C. This temperature is critical, as viscosity can vary significantly with temperature. The viscometer provided readings in Pascal-seconds (Pa. s), which were recorded for each sample. Each measurement was conducted in duplicate to ensure accuracy, and the average viscosity values were calculated for each treatment group. This detailed approach to measuring viscosity is important for understanding the flow properties of the oils, which can impact their usability in cooking and storage.

3.4.1.3 Refractive Index

This was done by use of Abbe Refractometer. The refractometer was first charged by opening the

double prism using a screw head and placing few drops of the oil sample on the prism. The prisms were then firmly closed by tightening using the screw head. The refractometer was left to stand a few minutes before reading, such that the temperature of the test sample and instrument were the same. Measurements were based upon observation of position of border line of total reflection in relation to faces of flint glass prism. The border line was brought into the field of vision of telescope by rotating the double prism by means of alidade in the following manner; the sector was held firmly and the alidade moved backward or forward until field of vision is divided into light and dark portion. The Line dividing these portions is the "border line," and, as a rule, is not supposed to be a sharp line but a band of color. Colors were eliminated by rotating the screw head of the compensatoruntil sharp and a colorless line obtained. The border line was then adjusted so that it falls on point of intersection of cross hairs. *n* was read directly on scale of sector and estimated to 4th decimal place. Readings were taken 3, approaching intersection alternately from one field to other, and averaged. The range of readings should be 0.0002. The prisms were cleaned in between readings by wiping off the oil with soft cloth, then with cotton pad moistened with solvent (e.g., trichloroethylene,toluene, or petroleum ether), and let dry.

3.4.1.4 Density

This was done using a pycnometer of 50 mL capacity, equipped with a cap and thermometer graduated in 0.1°C (Kimble Glass Inc. No. 15). The pycnometer was cleaned by filling with chromic acid cleaning solution and letting it stand several hours prior to use and emptied then rinsed thoroughly with distilled water. A water bath at a constant temperature of (±5°C) at which determination (*T*) was made was also used.

To calibrate the pycnometer, it was filled with recently boiled water cooled to approximately 5°C below constant temperature bath. The seal thermometer was then carefully inserted avoiding any airbubbles and placed in the constant temperature bath. After 1 hour, the water level was adjusted to a proper point on the pycnometer and stoppered then the temperature reading(*T*) taken to 0. 1°C.The pycnometer was then removed from the bath, wiped dry with a clean cloth, left to cool and weighedto 0.1 mg. The pycnometer was then emptied, rinsed several times with alcohol and then ether, left todry completely, ether vapor removed, thermometer replaced then capped and weighed to 0.1 mg. The following measurements were then recorded;

Volume (mL) of pycnometer at temperature $T = V_T V_T = [(W - W)/ (, _T)] [1 + a (T - T)]$

Where; W = weight (g) of pycnometer empty and filled with H_2O, respectively; $_T$ =density (g/mL) of H_2O at temperature (T) and a = mean coefficient of cubic expansion of pycnometer (a = 0.000010 for borosilicate glass, 0.000025 for soda lime glass).

For determination, a clean empty pycnometer with cap and thermometer was weighed (to 0.1 mg) then filled with the oil sample at a temperature below that of constant temperature bath, and the procedureas calibration repeated.

Finally, the density (D_T) of the oil sample at temperature T in g/mL was then calculated asfollows:

D_T (g/mL) = $\frac{W - W'}{V_T}$

where W and W = weight (g) of pycnometer empty and filled with test sample; V_T = volume of pycnometer (mL) at temperature T.

For volumes of oil measured at temperature $T°$ close to T:

D_T (g/mL) = D_T (g/mL) + 0.00068($T - T$)

where 0.00068 is the correction coefficient.

3.4.2 Chemical quality parameters

The chemical changes in the vegetable oils were assessed by free fatty acid value, peroxide value, thepercentage of insoluble impurities, iodine value, saponification value, moisture content and acid value as described by (AOAC,1995). All the parameters were also assessed in duplicates.

3.4.2.1 Free fatty acids

This represents the extent to which glycosides in the oil have been decomposed by lipase.

10g of the oil was taken, mixed with 11mls of neutralized alcohol spirit and 11mls of diethyl either.1 ml of phenolphthalein indicator was then added to the mixture and titrated with 0.01N NAOH, shaking constantly until pink color persist for 15 seconds. The percentage of free fatty acids were then calculated as follows;

FFA% = $\frac{V1 * M_1 * N}{10 * W_1}$

Where;

V_1 = volume of 0.01 NAOH

M_1 = molecular weight of the acid in oilN = normality

W1 = weight of the sample

3.4.2.2 Peroxide value

This was based on the measurement of iodine produced from potassium iodide by peroxides present in the sample using iodometric titration. 2.5 grams of oil sample was weighed into a glass stoppered flask. 25 ml of acetic acid: chloroform mixture was then added to all the flasks containing the oil samples and a blank.1 ml of saturated potassium iodide was added and the sample gently stirred for one minute. The sample was then placed in the dark for 30 minutes after which 30ml ofwater was added and shaken well. About 1ml of starch solution was then added and titration donewith 0.01N-sodium thiosulphate until the blue colour disappeared. The peroxide value was calculated as follows;

PV= ((A-B) × F × 10)/Weight of oil used Where;

A=Volume of 0.01N sodium thiosulphate required for the sample (ml)B=Volume of 0.01N-sodium thiosulphate required for the blank (ml) F=Factor of 0.01N- sodium thiosulphate

3.4.2.3 Insoluble impurities

This measures the number of insoluble particles present in the finished product. Oil containing insoluble impurities higher than 0.07% is considered unfit for consumption. 5gms of the oil was weighed(w_o) into the flask and dissolved in 200mls of petroleum ether left to stand for 1 hour. Afilter paper was dried in the oven for 10 minutes, then used for filtration of the mixture. The weight of the dry filter paper was taken as (w_1). After filtration the excess petroleum ether was washed off until no more oil stains remained on the filter paper and dried in the oven for 15minutes then the weight taken as (w2). The percentage of insoluble impurities was calculated as follows:

$$\text{Insoluble Impurities (\%)} = \frac{(w_2 - w_1)}{10W} \times 100$$

3.4.2.4 Iodine Value

2g of oil sample was weighed into a glass stoppered flask,10 ml of carbon tetrachloride added and a blank test done at the same time. 25ml of Wijs solution was then pipetted into all the flasks which were then placed in the dark for one hour. 20ml of 10% potassium iodide and a 100 ml of

distilled water were then added and mixed well. The solution was titrated with 0.1N-sodium thiosulphate solution until the colour changed to yellow and then a few drops of starch solution were added and the titration continued until the blue colour disappeared. Iodine Value was calculated as follows;

IV= ((B-A) × F × 126.9 × 10^{-3})/ weight of oil used) × 100Where;

B= volume of 0.1N-Sodium thiosulphate required for the blank (ml)A=volume of 0.1N-Sodium thiosulphate required for the sample (ml)F=Factor of 0.1N-sodium thiosulphate

3.4.2.5 Saponification value

2g of the oil samples were weighed into flasks.25ml of 0.5N-alchoholic potassium hydroxide was pipetted into each of the flasks and a blank test done at the same time. The samples were then boiled under a reflux condenser for 30 minutes. A few drops of phenolphthalein were then added and the solution titrated with 0.5N-hydrochloric acid (HCL) until the pink colour disappeared. The SV was calculated as follows;

SV= ((B-A) × F × 28.05)/ weight of oil used)

3.4.2.6 Moisture content

The moisture dishes were washed and placed in a cabinet drier at 105℃ for one hour. They were then placed in a desiccator to cool and the initial weight of the dish recorded afterwards (W1). Two grams of sample were taken and placed in the moisture dish and weight recorded (W2). The dishes were then placed in a cabinet drier for 3 hours. After the drying time the dishes were removed from the drier, cooled in a desiccator and the final weight recorded(W3). The moisture content of the samples was calculated as below;

Moisture content (%) = (W3 – W1)/ (W2 – W1) × 100

3.4.2.7 Acid value

The acid value was determined by titration of the oil samples with 0.1N NaOH using phenolphthalein as an indicator.5mls of the oil was diluted with 25ml of distilled water, then10mlof the diluted oil was used for titration with 0.1N Sodium Hydroxide using phenolphthalein as an indicator. The acid value was expressed as % c acid using the formula;

% Acid Value = Sample reading (ml)*Dilution factor/sample weight (ml)* acid factor (0.278) *100

3.5 Evaluating oxidative stability of silkworm pupae extract-incorporated vegetable cookingoils

3.5.1 Determination of Thiobarbituric acid reactive substances (TBARS)Reagents preparation

Thiobarbituric acid (TBA) stock solution (0.04 M) was prepared by dissolving 2.88 g of 2-Thiobarbituric acid (TBA) in50 ml distilled water in a 500 ml volumetric flask and diluted to volume with glacial acetic acid. The solution was then put on a magnetic stirrer overnight.0.3 M Na2SO3 solution was as a result of dissolving 18.91 g of sodium sulphite in 500 ml distilled water and 0.28 M TCA solution by dissolving 22.87 g of trichloroacetic acid (TCA) in 500 ml distilled Water.0.22 g TEP (1,1,3,3, tetraethoxypropane) in 100 ml distilled water on a magnetic stirrer to make 0.01 M TEP stock solution; the TEP stock solution was diluted to 0.0001 M TEP work solution. For TBA work solution which must be prepared a maximum 30 minutes before analysis, 180 ml TBA stock solution, 120 ml chloroform and 15 ml sodium sulphite solution were mixed.

3.5.2 Preparation of oil samples (Effect of high temperature and prolonged heating)

60mls of oil was heated in a beaker at about 170˚C for 0, 10, 20, 30, 40, and 60 min (a thermometer was used to check the temperature) then 10 ml removed at every heating stage and put in a separate test tube. This was done for both the corn and sunflower oils treated with the various antioxidants.

3.5.3 Sample analysis

This was done in triplicate. Ten mls of oil was transferred into a centrifuge glass tube using a dropper,5 ml TBA work solution added and the tube closed tightly with a fitting cap. The emulsion was then mixed for 15 seconds on a vortex to dissolve the oil and the tubes incubated in a water bath with almost boiling water (95°C) for 45 min then cooled down under running cold water. 2.5 ml TCAsolution was added to the resultant solution and mixed by inverting the tube afew times then centrifuged for 10 min at 2500 rpm to separate the pink water phase from the chloroform phase (bottom layer). Finally, the absorbance of the water phase was measured at 538nm in 10 mm QS glass cuvettes against the blank that was prepared as above, using reagents only, without the oil.

If the absorbance of the sample was too high (> 900), the water phase was diluted with 55% acetic acid glacial then the absorbance calculated by the formula;

E532 x 12.9, where: E is the extinction value at 532 and 12.9 is a conversion factor.

3.6 Data analysis

Data analysis was performed using Stata version 12 software. The analysis included descriptive statistics to summarize the data and inferential statistics to determine the significance of differences between treatment groups. A two-way ANOVA was conducted to assess the effects of different treatments on the viscosity, density, and fatty acid profiles of the oils. Post-hoc tests (Bonferroni method) were applied to identify specific differences between treatment means. The significance level was set at $p < 0.05$. This detailed approach to data analysis ensures that the results are statistically valid and can be confidently interpreted in the context of the study's objectives. The results were then used to draw inferences on the oxidative behavior of the selected vegetable oils comparing the antioxidative capacity of BHT, and Mulberry Silkworm pupae extract.

CHAPTER FOUR

RESULTS

4.1 Fatty acid profile

The fatty acid profile of corn oil preserved using BHT and silkworm oil is shown below. The major SFA in the corn oil samples was preserved using 100 ppm BHT, 100 ppm silkworm and 200ppm silkworm, and palmitic acid. The palmitic acid content of corn oil preserved using 100 ppm and 200 ppm silkworm was similar to the control. Furthermore, the palmitic acid content of corn oil preserved using 100 BHT was significantly lower than the control. The present study results show that palmitic acid contributes more than half of the SFA in all the corn oil samples (Table 4.1). Interestingly corn oil preserved using 200ppm silkworm oil showed significantly high levels of myristic acid (Table 4.1). Statistical analysis further shows that corn oil preserved using 100 ppm and 200 ppm silkworm oil had significant amounts of lauric acid as compared to the control.

The corn oil preserved using 200ppm silkworm had the highest MUFA content followed by corn oil preserved using 100ppm BHT while the control was the least (Table 4.1). The predominant MUFA in all the corn oil samples was oleic acid. Low amounts of Cis-10 heptadecanoic acid were detected in corn oil samples preserved using 100 ppm BHT, 100 ppm and 200 ppm silkworm oil (Table 4.1). Furthermore, low amounts of palmitoleic acid were detected in corn oil preserved using 100 ppm silkworm oil. Myristoleic and elaidic acids were not detected in all the corn oil samples (Table 4.1). PUFAs contributed to over half of all the fatty acid content in the control and the corn oil preserved using 100 ppm BHT. In corn oil samples preserved using 100 ppm and 200 ppm silkworm oil PUFAs accounted for 49% and 36% of the total fatty acid content. The predominant PUFA in all the corn oil samples was linoleic acid while trace amounts of linolenic acid were also detected.

Table 0.1: Fatty acid profile (%) of corn oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

	Chemical structure	Control	BHT 100ppm	Silkworm 100ppm	Silkworm 200ppm
Butyric acid	C4:0	Nd	Nd	Nd	Nd
Caprylic acid	C8:0	Nd	0.09 ± 0.01a	Nd	Nd
Capric acid	C10:0	Nd	Nd	Nd	Nd
Lauric acid	C12:0	Nd	Nd	7.80 ± 0.02^{a}	2.13 ± 0.03^{b}
Tridecanoic acid	C13:0	Nd	Nd	Nd	Nd
Myristic acid	C14:0	Nd	Nd	Nd	9.23 ± 0.21^{a}
Pentadecanoic acid	C15:0	Nd	0.14 ± 0.003^{c}	0.10 ± 0.02^{b}	0.07 ± 0.008^{a}
Palmitic acid	C16:0	13.42 ± 1.33^{a}	7.02 ± 0.32b	13.48 ± 0.19^{a}	14.53 ± 0.40^{a}
Heptadecanoic acid	C17:0	Nd	Nd	Nd	Nd
Stearic acid	C18:0	2.35 ± 0.11^{c}	0.31 ± 0.03^{a}	0.19 ± 0.03^{a}	1.82 ± 0.07b
Arachidic acid	C20:0	0.20 ± 0.09^{a}	0.17 ± 0.003^{a}	0.24 ± 0.01^{a}	0.26 ± 0.008^{a}
Behenic acid	C22:0	0.08 ± 0.001^{c}	0.07 ± 0.003b	Nd	0.05 ± 0.001^{a}
Lignoceric acid	C24:0	Nd	Nd	Nd	Nd
SFA		16.1	7.8	21.8	28.1
Myristoleic acid	C14:1	Nd	Nd	Nd	Nd
Palmitoleic acid	C16:1	Nd	Nd	0.07 ± 0.006^{a}	Nd
Cis-10 heptadecanoic acid	C17:1	nd	1.77 ± 0.08^{c}	0.86 ± 0.02^{b}	0.29 ± 0.05^{a}
Oleic acid	C18:1	27.52 ± 6.01^{a}	32.77 ± 1.02^{a}	28.26 ± 0.66^{a}	35.58 ± 0.51^{a}
Elaidic acid	C18:1	nd	Nd	Nd	Nd
Nervonic acid	C24:1	0.05 ± 0.003^{a}	0.05 ± 0.002^{a}	0.06 ± 0.005^{a}	0.05 ± 0.007^{a}
MUFA		27.1	34.7	29.4	36
Linoleic acid	C18:2	55.15 ± 4.91^{a}	57.18 ± 0.82^{a}	45.05 ± 0.60^{b}	28.20 ± 0.26^{c}
Linoelaidic acid	C18:2	nd	Nd	Nd	Nd
Linolenic acid	C18:3	1.24 ± 0.003^{b}	0.44 ± 0.02^{a}	3.90 ± 0.05^{c}	7.81 ± 0.18^{d}
PUFA		56.4	57.6	49	36

Values are means ± SD. Means with different superscript letters across the rows are significantly different at $p<0.05$. *nd-not detected.*

The predominant SFA in the sunflower oil samples was palmitic acid with sunflower oil preserved using 200ppm silkworm having the highest value followed by the control while the sunflower oil preserved using 100ppm BHT and 100ppm silkworm oil were the least (Table 4.2). Significant amounts of arachidic acid were detected in all the sunflower oil samples with the samples preserved using 200 ppm silkworm oil having the highest amounts while all the other samples had the least amounts (Table 4.2). Behenic and lignoceric acids were also detected in the control and sunflower oil preserved using 100ppm BHT and 100ppm silkworm oil.

Oleic acid was the predominant MUFA in the sunflower oil samples with sunflower oil samples preserved using 100ppm and 200ppm silkworm oil having the highest values as compared to the control while sunflower oil preserved using 100ppm BHT had the least amounts (Table 4.2). Cis-10 heptadecanoic acid was also detected in all the sunflower oil samples with the control having the highest amounts and sunflower preserved using 100ppm silkworm oil the least amounts. Palmitoleic acid was detected in the sunflower oil preserved using 200ppm silkworm oil while myristoleic and elaidic acids were not detected in any of the sunflower oil samples.

PUFAs accounted for over half of all the fatty acid content in the control and in sunflower oil preserved using 100ppm BHT and 100ppm silkworm oil. Linoleic acid was the major PUFA while trace amounts of linolenic acid were also detected. Linoelaidic acid was not detected in any of the sunflower oil samples (Table 4.2).

Table 0.2: Fatty acid profile (%) of sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

	Chemical structure	Control	BHT 100	Silkworm100	Silkworm200
Butyric acid	C4:0	nd	Nd	nd	Nd
Caprylic acid	C8:0	nd	Nd	nd	Nd
Capric acid	C10:0	nd	Nd	nd	Nd
Lauric acid	C12:0	nd	3.75 ± 0.21^{b}	0.06 ± 0.001^{a}	Nd
Tridecanoic acid	C13:0	nd	Nd	nd	Nd
Myristic acid	C14:0	nd	3.03 ± 0.11^{b}	0.28 ± 0.02^{a}	Nd
Pentadecanoic acid	C15:0	nd	0.56 ± 0.09^{b}	0.16 ± 0.004^{a}	1.57 ± 0.13^{c}
Palmitic acid	C16:0	12.86 ± 0.52^{b}	8.80 ± 2.87^{a}	8.65 ± 0.63^{a}	23.94 ± 2.54^{c}
Heptadecanoic acid	C17:0	0.16 ± 0.005^{a}	0.058 ± 0.003^{a}	0.12 ± 0.024^{a}	3.85 ± 0.37^{b}
Stearic acid	C18:0	nd	0.94 ± 0.10^{a}	0.53 ± 0.04^{a}	2.93 ± 0.37^{b}
Arachidic acid	C20:0	0.38 ± 0.01^{a}	0.47 ± 0.02^{a}	0.59 ± 0.04a	2.48 ± 1.24^{b}
Behenic acid	C22:0	0.30 ± 0.007^{b}	0.29 ± 0.02^{b}	0.01 ± 0.001^{a}	Nd
Lignoceric acid	C24:0	0.46 ± 0.02^{b}	0.16 ± 0.02^{a}	0.12 ± 0.01^{a}	Nd
SFA		14.3	18.2	10.6	34.9
Myristoleic acid	C14:1	nd	Nd	nd	Nd
Palmitoleic acid	C16:1	nd	Nd	nd	0.38 ± 0.14^{a}
Cis-10 heptadecanoic acid	C17:1	2.54 ± 0.01^{d}	0.85 ± 0.07^{c}	0.11 ± 0.01^{a}	0.59 ± 0.05^{b}
Oleic acid	C18:1	22.42 ± 0.55^{b}	14.45 ± 0.69^{a}	35.38 ± 0.76^{c}	34.51 ± 1.40^{c}
Elaidic acid	C18:1	nd	Nd	nd	Nd
Nervonic acid	C24:1	nd	Nd	0.02 ± 0.001	Nd
MUFA		24.9	15.4	35.4	35.5
Linoleic acid	C18:2	59.61 ± 0.76^{c}	65.99 ± 1.84^{d}	50.02 ± 0.69^{b}	22.19 ± 2.00^{a}
Linoelaidic acid	C18:2	nd	Nd	nd	Nd
Linolenic acid	C18:3	1.30 ± 0.07^{a}	0.74 ± 0.02^{a}	3.95 ± 0.24^{b}	7.56 ± 0.72^{c}
PUFA		60.9	66.7	54	29.8

Values are means ± SD. Means with different superscript letters across the rows are significantly different at $p<0.05$. *nd-not detected.*

4.2 Physical parameters

4.2.1 Colour

The lightness of the corn oil samples was significantly affected by the preservative used and the storage period ($p<0.001$). At day 0 the corn oil preserved using 100ppm BHT and 100ppm silkworm oil was significantly lighter as compared to the control (Table 4.3). In all the corn oil samples the

lightness significantly reduced with increase in storage time with samples in day 5 having the least values for lightness.

The preservative and storage time had a significant influence on the lightness of the sunflower oil samples ($p<0.001$). In the sunflower oil samples only the sample preserved using 100ppm BHT was similar in lightness as the control while sunflower oil samples preserved using 100ppm and 200ppm silkworm oil had least lightness values as compared to the control (Table 4.3). The lightness in all the sunflower oil samples decreased with storage time with samples in day 5 having the least values. Interestingly sunflower oil samples preserved using 100ppm and 200ppm silkworm oil at day 5 were significantly lighter as compared to the samples preserved using BHT (Table 4.3).

Table 0.3: Colour (lightness) of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn oil			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	25.43 ± 0.11^{a}	25.43 ± 0.11^{a}	25.43 ± 0.11^{a}
Day 0	27.47 ± 0.11^{e}	28.33 ± 0.11^{f}	25.83 ± 0.11^{a}
Day 1	23.43 ± 0.11^{g}	27.33 ± 0.11^{e}	24.63 ± 0.11^{h}
Day 2	20.46 ± 0.11^{i}	26.7 ± 0.11^{j}	22.7 ± 0.11^{k}
Day 3	16.37 ± 0.11^{b}	19.37 ± 0.11^{l}	17.43 ± 0.11^{d}
Day 4	16.3 ± 0.11^{b}	17.53 ± 0.11^{d}	14.43 ± 0.11^{m}
Day 5	15.63 ± 0.11^{c}	15.77 ± 0.11^{bc}	12.6 ± 0.11^{n}
Sunflower oil			
Control	29.63 ± 0.11^{a}	29.63 ± 0.11^{a}	29.63 ± 0.11^{a}
Day 0	29.47 ± 0.11^{a}	23.4 ± 0.11^{i}	22.67 ± 0.11^{g}
Day 1	20.33 ± 0.11^{f}	22.73 ± 0.11^{g}	26.87 ± 0.11^{h}
Day 2	18.63 ± 0.11^{b}	18.4 ± 0.11^{b}	20.5 ± 0.11^{f}
Day 3	18.2 ± 0.11^{b}	16.4 ± 0.11^{e}	17.23 ± 0.11^{k}
Day 4	15.67 ± 0.11^{d}	16.33 ± 0.11^{e}	15.7 ± 0.11^{d}
Day 5	12.67 ± 0.11^{j}	14.53 ± 0.11^{c}	14.3 ± 0.11^{c}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

The hue angle of the corn oil samples was significantly affected by the concentration of preservative used as well as storage period. Corn oil preserved using 100ppm BHT had significant higher hue angle values as compared to the control and sunflower oil preserved using 100ppm and 200ppm silkworm oil at day zero (Table 4.4). In corn oil preserved using 100ppm BHT the hue angle significantly decreased up to day 3 followed by a significant increase up to day 5. In corn oil preserved using 100ppm silkworm oil the hue angle significantly decreased in day 2 followed by a significant increase up to day 5 (Table 4.4). In corn oil preserved using 200 ppm silkworm oil the

hue angle significantly decreased in day 2 of storage followed by an increase and a decrease in day 3 and 4 respectively and a final increase in day 5.

The preservative used and storage period had a significant influence on the hue angle of the sunflower oil samples ($p<0.001$). The sunflower oil preserved using 100ppm BHT had significantly lower hue angle value as compared to the control in all storage days apart from day 5. Interestingly in sunflower oil samples preserved using 100ppm silkworm oil the samples at day 1 had similar hue angle with the control while samples in other storage days differed significantly with the control. For sunflower oil samples preserved using 200 ppm silkworm oil hue angles of samples in day 2, 3 and 5 were similar to the control (Table 4.4).

Table 0.4: Colour (hue angle) of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn oil			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	105.76 ± 1.66^{bd}	105.76 ± 1.66^{bd}	105.76 ± 1.66^{bd}
Day 0	126.69 ± 1.66^{h}	76.91 ± 1.66^{a}	100.99 ± 1.66^{bdf}
Day 1	108.01 ± 1.66^{b}	79.35 ± 1.66^{ac}	106.86 ± 1.66^{b}
Day 2	81.89 ± 1.66^{ac}	58.18 ± 1.66^{g}	79.56 ± 1.66^{ac}
Day 3	49.25 ± 1.66^{g}	83.65 ± 1.66^{ac}	95.88 ± 1.66^{ef}
Day 4	87.75 ± 1.66^{ce}	79.30 ± 1.66^{ac}	75.75 ± 1.66^{a}
Day 5	95.18 ± 1.66^{ef}	106.22 ± 1.66^{b}	96.40 ± 1.66^{def}
Sunflower oil			
Control	104.34 ± 1.83^{abc}	104.34 ± 1.83^{abc}	104.34 ± 1.83^{abc}
Day 0	32.32 ± 1.83^{i}	86.14 ± 1.83^{de}	144.69 ± 1.83^{k}
Day 1	51.17 ± 1.83^{f}	103.65 ± 1.83^{abc}	126.98 ± 1.83^{l}
Day 2	87.84 ± 1.83^{deg}	42.78 ± 1.83^{f}	109.57 ± 1.83^{c}
Day 3	80.72 ± 1.83^{dh}	15.89 ± 1.83^{k}	107.52 ± 1.83^{bc}
Day 4	84.88 ± 1.83^{de}	70.80 ± 1.83^{h}	48.57 ± 1.83^{f}
Day 5	97.27 ± 1.83^{abg}	85.70 ± 1.83^{de}	95.18 ± 1.83^{aeg}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

The chroma of the corn oil samples was significantly affected by the preservative used and the duration of storage ($p<0.001$). The corn oil preserved using 100ppm BHT and 200ppm silkworm oil at day 5 had the highest chroma values while control and corn oil preserved using 100ppm BHT at day 1 were the least (Table 4.5). In corn oil preserved using 100ppm BHT the chroma values increased significantly with increase in storage time. Similarly, in corn oil preserved using 100ppm silkworm chroma values increased with increase in storage time up to day 3 followed by a significant decline (Table 4.5). In corn oil samples preserved using 200ppm silkworm chroma values declined

significantly with increase in storage time up to day 2 followed by a significant increase with further increase in storage time.

The preservative used and the storage period had a significant influence on the chroma value of the sunflower oil samples ($p<0.001$) with the highest value being observed in the 5th day storage in sunflower oil preserved using 100ppm BHT and 200ppm silkworm oil and the least values in the 1st day of storage in sunflower oil preserved using 100ppm BHT (Table 4.5).

Table 0.5: Colour (chroma) of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn oil			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	1.59 ± 0.09^{a}	1.59 ± 0.09^{a}	1.59 ± 0.09^{a}
Day 0	0.84 ± 0.09^{b}	0.44 ± 0.09^{b}	2.79 ± 0.09^{d}
Day 1	1.61 ± 0.09^{a}	0.54 ± 0.09^{b}	1.95 ± 0.09^{ac}
Day 2	1.89 ± 0.09^{ac}	0.71 ± 0.09^{b}	2.20 ± 0.09^{ce}
Day 3	1.85 ± 0.09^{ac}	3.89 ± 0.09^{h}	2.92 ± 0.09^{df}
Day 4	2.50 ± 0.09^{de}	3.39 ± 0.09^{fg}	2.44 ± 0.09^{de}
Day 5	4.42 ± 0.09^{i}	3.57 ± 0.09^{gh}	4.46 ± 0.09^{i}
Sunflower oil			
Control	2.82 ± 0.09^{af}	2.82 ± 0.09^{af}	2.82 ± 0.09^{af}
Day 0	1.18 ± 0.09^{bg}	1.50 ± 0.09^{bd}	0.86 ± 0.09^{gi}
Day 1	0.17 ± 0.09^{h}	0.45 ± 0.09^{hi}	2.00 ± 0.09^{de}
Day 2	2.60 ± 0.09^{ac}	1.23 ± 0.09^{bg}	1.59 ± 0.09^{bd}
Day 3	4.32 ± 0.09^{k}	2.19 ± 0.09^{ce}	3.22 ± 0.09^{f}
Day 4	2.24 ± 0.09^{ce}	2.44 ± 0.09^{ace}	1.51 ± 0.09^{bd}
Day 5	5.51 ± 0.09^{j}	2.64 ± 0.09^{ac}	5.86 ± 0.09^{j}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.2.2 Viscosity

The viscosity of the corn oil samples was significantly affected by the storage period ($p<0.001$) while the preservative had no significant influence on viscosity ($p= 0.9979$). The control had the highest viscosity while the least value was observed in all the corn oil samples stored for 5 days. In corn oil samples preserved using 100ppm BHT there was a significant decline in viscosity in the 5th day storage. In corn oil samples preserved using 100ppm and 200ppm silkworm oil a significant decline in viscosity was noted in the 4th day of storage followed by a constant decline in the 5th day of storage.

The viscosity of the sunflower oil samples was significantly affected by the preservative used and storage period ($p<0.001$). The control had the highest viscosity while the least values were observed

in sunflower oil preserved using 100ppm and 200ppm silkworm stored for 5 days (Table 4.6). In sunflower oil samples preserved using 100ppm BHT and 100ppm silkworm oil there was a significant decrease in viscosity in day 2 followed by a further decrease in viscosity with increase in storage time. For sunflower oil preserved using 200ppm silkworm oil viscosity significantly decreased with storage time (Table 4.6).

Table 0.6: Viscosity of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	39.24 ± 2.38^{c}	39.24 ± 2.38^{c}	39.24 ± 2.38^{c}
Day 0	33.15 ± 2.38^{ac}	33.76 ± 2.38^{ac}	34.15 ± 2.38^{ac}
Day 1	30.41 ± 2.38^{acde}	30.51 ± 2.38^{acde}	30.75 ± 2.38^{ace}
Day 2	25.74 ± 2.38^{abcde}	26.37 ± 2.38^{abcde}	26.34 ± 2.38^{abcde}
Day 3	24.74 ± 2.38^{abde}	22.80 ± 2.38^{abde}	22.78 ± 2.38^{abde}
Day 4	21.21 ± 2.38^{abde}	17.55 ± 2.38^{bde}	17.12 ± 2.38^{bd}
Day 5	15.96 ± 2.38^{b}	13.55 ± 2.38^{b}	15.45 ± 2.38^{b}
Sunflower			
Control	37.49 ± 0.38^{a}	37.49 ± 0.38^{a}	37.49 ± 0.38^{a}
Day 0	37.38 ± 0.38^{ai}	35.00 ± 0.38^{bc}	35.31 ± 0.38^{bci}
Day 1	36.17 ± 0.38^{aci}	33.51 ± 0.38^{bm}	31.71 ± 0.38^{hm}
Day 2	34.40 ± 0.38^{bc}	29.96 ± 0.38^{gh}	27.64 ± 0.38^{kl}
Day 3	30.54 ± 0.38^{gh}	26.54 ± 0.38^{fk}	24.94 ± 0.38^{ef}
Day 4	29.10 ± 0.38^{gl}	24.32 ± 0.38^{de}	22.58 ± 0.38^{d}
Day 5	24.60 ± 0.38^{def}	20.18 ± 0.38^{j}	18.93 ± 0.38^{j}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.2.3 Refractive index

The refractive index of the corn oil and sunflower oil samples was not significantly affected by the preservative used and storage period (p= 0.1416 and p=0.2515) (Table 4.7).

Table 0.7: Refractive index of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	1.48 ± 0.001^{a}	1.48 ± 0.001^{a}	1.48 ± 0.001^{a}
Day 0	1.47 ± 0.001^{a}	1.48 ± 0.001^{a}	1.47 ± 0.001^{a}
Day 1	1.47 ± 0.001^{a}	1.47 ± 0.001^{a}	1.47 ± 0.001^{a}
Day 2	1.48 ± 0.001^{a}	1.47 ± 0.001^{a}	1.47 ± 0.001^{a}
Day 3	1.47 ± 0.001^{a}	1.47 ± 0.001^{a}	1.47 ± 0.001^{a}
Day 4	1.47 ± 0.001^{a}	1.47 ± 0.001^{a}	1.47 ± 0.001^{a}
Day 5	1.47 ± 0.001^{a}	1.47 ± 0.001^{a}	1.47 ± 0.001^{a}
Sunflower			
Control	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}
Day 0	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}
Day 1	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}
Day 2	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}
Day 3	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}
Day 4	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}
Day 5	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}	1.47 ± 0.0004^{a}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.2.4 Density

The preservative used and storage period had a significant effect on the density of the corn oil samples ($p<0.001$) whereby corn oil preserved using 100ppm BHT at day 0 had the highest density while corn oil preserved using 200ppm silkworm oil at day 2, 3,4 and 5 had the least density (Table 4.8).

The density of the sunflower oil was significantly affected by the preservative used and storage period whereby the control and sunflower oil preserved using 100ppm BHT and all stages of storage had the highest density while sunflower oil preserved using 200ppm silkworm oil stored for 3, 4 and 5 days and the least density (Table 4.8).

Table 0.8: Density of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	0.92 ± 0.001^{ab}	0.92 ± 0.001^{ab}	0.92 ± 0.001^{ab}
Day 0	0.93 ± 04.001^{b}	0.91 ± 0.001^{fg}	0.90 ± 0.001^{dh}
Day 1	0.92 ± 0.001^{abg}	0.91 ± 0.001^{hi}	0.90 ± 0.001^{d}
Day 2	0.91 ± 0.001^{fi}	0.90 ± 0.001^{d}	0.89 ± 0.001^{c}
Day 3	0.92 ± 0.001^{afg}	0.90 ± 0.001^{de}	0.89 ± 0.001^{c}
Day 4	0.92 ± 0.001^{ab}	0.89 ± 0.001^{ce}	0.89 ± 0.001^{c}
Day 5	0.92 ± 0.001^{ab}	0.89 ± 0.001^{ce}	0.89 ± 0.001^{c}
Sunflower			
Control	0.91 ± 0.01^{a}	0.91 ± 0.01^{a}	0.91 ± 0.01^{a}
Day 0	0.91 ± 0.01^{a}	0.88 ± 0.01^{ab}	0.89 ± 0.01^{ab}
Day 1	0.91 ± 0.01^{a}	0.90 ± 0.01^{ab}	0.89 ± 0.01^{ab}
Day 2	0.91 ± 0.01^{a}	0.90 ± 0.01^{ab}	0.89 ± 0.01^{ab}
Day 3	0.91 ± 0.01^{a}	0.88 ± 0.01^{ab}	0.87 ± 0.01^{b}
Day 4	0.91 ± 0.01^{a}	0.88 ± 0.01^{ab}	0.87 ± 0.01^{b}
Day 5	0.91 ± 0.01^{a}	0.88 ± 0.01^{ab}	0.87 ± 0.01^{b}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.3 Chemical parameters

4.3.1 Free fatty acids

The free fatty acid content of the corn oil samples was significantly affected by the preservative used as well as duration of storage ($p<0.001$). Corn oil preserved using 200ppm silkworm oil and stored for 4 and 5 days had the highest amounts of free fatty acids while the control had the least amounts (Table 4.9).

In sunflower oil samples free fatty acid content was significantly affected by preservative used and storage time ($p<0.001$). Sunflower oil preserved using 200ppm silkworm oil at day 3, 4 and 5 and sunflower oil preserved using 100ppm silkworm oil at day 5 of storage had the highest amounts of free fatty acids while the control had the least amounts (Table 4.9).

Table 0.9: Free fatty acids content of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	0.059 ± 0.001^{b}	0.059 ± 0.001^{b}	0.059 ± 0.001^{b}
Day 0	0.066 ± 0.001^{bc}	0.11 ± 0.001^{h}	0.12 ± 0.001^{d}
Day 1	0.073 ± 0.001^{c}	0.12 ± 0.001^{d}	0.12 ± 0.001^{de}
Day 2	0.071 ± 0.001^{c}	0.12 ± 0.001^{de}	0.13 ± 0.001^{a}
Day 3	0.073 ± 0.001^{c}	0.13 ± 0.001^{a}	0.13 ± 0.001^{a}
Day 4	0.099 ± 0.001^{f}	0.13 ± 0.001^{ae}	0.14 ± 0.001^{g}
Day 5	0.098 ± 0.001^{f}	0.13 ± 0.001^{ae}	0.15 ± 0.001^{g}
Sunflower			
Control	0.041 ± 0.001^{a}	0.041 ± 0.001^{a}	0.041 ± 0.001^{a}
Day 0	0.054 ± 0.001^{m}	0.074 ± 0.001^{bc}	0.087 ± 0.001^{ei}
Day 1	0.061 ± 0.001^{n}	0.077 ± 0.001^{cd}	0.093 ± 0.001^{jk}
Day 2	0.067 ± 0.001^{h}	0.082 ± 0.001^{de}	0.10 ± 0.001^{l}
Day 3	0.069 ± 0.001^{bh}	0.089 ± 0.001^{ij}	0.11 ± 0.001^{f}
Day 4	0.073 ± 0.001^{bc}	0.097 ± 0.001^{kl}	0.11 ± 0.001^{g}
Day 5	0.082 ± 0.001^{de}	0.11 ± 0.001^{fg}	0.11 ± 0.001^{fg}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.3.2 Peroxide value

The peroxide value of the corn oil samples was significantly affected by the preservative used and the storage time ($p<0.001$). The control had the least peroxide value while corn oil preserved using 100ppm silkworm oil had the highest peroxide value (Table 4.10). In corn oil preserved using 100ppm BHT the peroxide value increased with storage time up to day 2 followed by a significant decline in day 3 and a further increase with increase in storage period. In corn oil samples preserved using 100ppm silkworm oil an increase in peroxide value with increase in storage period was observed up to day 3 followed by a significant decrease in day 4 and a significant increase in day 5 (Table 4.10). In corn oil preserved using 200ppm silkworm oil there was a significant increase in peroxide value in day 1 of storage while no change in peroxide value was observed in day 2, 3 and 4.

The preservative used and the storage period significantly affected the peroxide value of the sunflower oil samples ($p<0.001$) whereby the control had the least peroxide value and sunflower oil preserved using 200ppm silkworm oil at the 5^{th} day of storage having the highest peroxide value (Table 4.10).

Table 0.10: Peroxide value (meq/kg) of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	0.35 ± 0.02^{f}	0.35 ± 0.02^{f}	0.35 ± 0.02^{f}
Day 0	0.69 ± 0.02^{j}	0.94 ± 0.02^{abc}	0.84 ± 0.02^{c}
Day 1	0.69 ± 0.02^{j}	1.02 ± 0.02^{abd}	0.97 ± 0.02^{abc}
Day 2	1.06 ± 0.02^{bdg}	1.29 ± 0.02^{ei}	0.98 ± 0.02^{ab}
Day 3	0.92 ± 0.02^{ac}	1.41 ± 0.02^{ik}	0.95 ± 0.02^{abc}
Day 4	1.17 ± 0.02^{egh}	1.29 ± 0.02^{ei}	1.04 ± 0.02^{abd}
Day 5	1.25 ± 0.02^{eh}	1.48 ± 0.02^{k}	1.14 ± 0.02^{dgh}
Sunflower			
Control	0.25 ± 0.02^{b}	0.25 ± 0.02^{b}	0.25 ± 0.02^{b}
Day 0	0.27 ± 0.02^{be}	0.66 ± 0.02^{af}	0.66 ± 0.02^{af}
Day 1	0.32 ± 0.02^{be}	0.62 ± 0.02^{acf}	0.64 ± 0.02^{acf}
Day 2	0.29 ± 0.02^{be}	0.56 ± 0.02^{cd}	0.58 ± 0.02^{acd}
Day 3	0.33 ± 0.02^{be}	0.59 ± 0.02^{acd}	0.69 ± 0.02^{fg}
Day 4	0.34 ± 0.02^{e}	0.58 ± 0.02^{acd}	0.76 ± 0.02^{gh}
Day 5	0.52 ± 0.02^{d}	0.58 ± 0.02^{acd}	0.83 ± 0.02^{h}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.3.3 Impurities

The levels of impurities in the corn oil samples was significantly influenced by the preservative used and the storage period ($p<0.001$). The least levels of impurities were observed in the control, the corn oil preserved using 100ppm BHT at all stages of storage and corn oil preserved using 100ppm silkworm oil at 2nd day of storage while the highest levels of impurities were in corn oil preserved using 100ppm silkworm at the 3rd day of storage (Table 4.11).

The preservative used and storage duration had a significant effect on the levels of impurities in the sunflower oil samples whereby the control had the least levels of impurities and sunflower oil preserved using 100ppm silkworm oil at the 5th day storage and sunflower oil preserved using 200ppm silkworm oil at the third day of storage had the highest levels of impurities (Table 4.11).

Table 0.11: Impurities (%) in corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	1.65 ± 0.23^{a}	1.65 ± 0.23^{a}	1.65 ± 0.23^{a}
Day 0	1.66 ± 0.23^{a}	3.41 ± 0.23^{fgh}	5.31 ± 0.23^{cde}
Day 1	1.98 ± 0.23^{a}	2.78 ± 0.23^{afh}	5.92 ± 0.23^{de}
Day 2	1.95 ± 0.23^{a}	1.80 ± 0.23^{a}	5.25 ± 0.23^{bcde}
Day 3	1.64 ± 0.23^{a}	6.11 ± 0.23^{e}	4.53 ± 0.23^{bcg}
Day 4	2.21 ± 0.23^{af}	3.94 ± 0.23^{bgh}	5.06 ± 0.23^{bcde}
Day 5	2.59 ± 0.23^{af}	4.59 ± 0.23^{bcdg}	5.04 ± 0.23^{bcde}
Sunflower			
Control	0.67 ± 0.4^{a}	0.67 ± 0.4^{a}	0.67 ± 0.4^{a}
Day 0	1.35 ± 0.4^{abc}	5.18 ± 0.4^{de}	1.43 ± 0.4^{abc}
Day 1	1.26 ± 0.4^{abc}	6.39 ± 0.4^{d}	3.44 ± 0.4^{bce}
Day 2	1.24 ± 0.4^{abc}	5.98 ± 0.4^{d}	3.51 ± 0.4^{ce}
Day 3	1.21 ± 0.4^{ab}	4.12 ± 0.4^{de}	6.08 ± 0.4^{d}
Day 4	1.34 ± 0.4^{abc}	4.81 ± 0.4^{de}	1.88 ± 0.4^{abc}
Day 5	2.05 ± 0.4^{abc}	6.02 ± 0.4^{d}	4.46 ± 0.4^{de}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.3.4 Iodine value

The iodine value of the corn oil samples was significantly affected by the preservative used and the storage time ($p= 0.0002$). Corn oil preserved using 200ppm silkworm oil stored for 4 and 5 days had the highest iodine value while corn oil samples preserved using 100ppm BHT stored for 2 and 3 days had the least iodine value (Table 4.12). The iodine value of corn oil samples preserved by 100ppm BHT at all stages of storage were similar to the control. In corn oil samples preserved using 100ppm silkworm oil the highest vale was observed in the sample stored for 1 day while the control had the least value (Table 4.12).

The iodine value in the sunflower oil samples was not significantly affected by the preservative used and the storage time ($p= 0.3379$).

Table 0.12: Iodine value (IV per 100g) of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	107.14 ± 1.44^{ab}	107.14 ± 1.44^{ab}	107.14 ± 1.44^{ab}
Day 0	107.74 ± 1.44^{abd}	113.48 ± 1.44^{abcde}	112.34 ± 1.44^{abcde}
Day 1	109.31 ± 1.44^{abde}	118.47 ± 1.44^{c}	113.03 ± 1.44^{abcde}
Day 2	105.62 ± 1.44^{a}	114.13 ± 1.44^{bcde}	115.67 ± 1.44^{cde}
Day 3	105.81 ± 1.44^{a}	112.45 ± 1.44^{abcde}	116.24 ± 1.44^{ce}
Day 4	108.67 ± 1.44^{abde}	112.48 ± 1.44^{abcde}	117.79 ± 1.44^{c}
Day 5	106.04 ± 1.44^{ab}	112.26 ± 1.44^{abcde}	120.38 ± 1.44^{c}
Sunflower			
Control	121.63 ± 2.24^{a}	121.63 ± 2.24^{a}	121.63 ± 2.24^{a}
Day 0	127.06 ± 2.24^{ab}	129.10 ± 2.24^{abc}	130.24 ± 2.24^{abc}
Day 1	127.15 ± 2.24^{ab}	130.33 ± 2.24^{abc}	132.10 ± 2.24^{abcd}
Day 2	131.16 ± 2.24^{abc}	132.81 ± 2.24^{abcd}	137.75 ± 2.24^{bcd}
Day 3	129.14 ± 2.24^{abc}	132.12 ± 2.24^{abcd}	140.35 ± 2.24^{cd}
Day 4	131.87 ± 2.24^{abcd}	133.86 ± 2.24^{abcd}	144.30 ± 2.24^{d}
Day 5	134.47 ± 2.24^{bcd}	135.64 ± 2.24^{bcd}	141.53 ± 2.24^{cd}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.3.5 Saponification value

The preservative used and the storage period had a significant effect on the saponification value of the corn oil samples whereby the least value was reported in corn oil preserved using 200ppm silkworm and stored for 3 days while, the highest saponification values were in the control, corn oil samples preserved using 100ppm BHT stored for 0, 1, 2, 3 and 4 days and corn oil preserved using 100ppm silkworm oil at day 0 of storage (Table 4.13).

The saponification value of the sunflower oil samples was significantly affected by the preservative used and the storage period ($p<0.0001$) whereby the control had the highest value while sunflower oil sample preserved using 100ppm BHT and stored for 4 days had the least value (Table 4.13).

Table 0.13: Saponification value corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT 100ppm	Silkworm 100ppm	Silkworm 200ppm
Control	191.19 ± 1.84^{a}	191.19 ± 1.84^{a}	191.19 ± 1.84^{a}
Day 0	193.87 ± 1.84^{a}	193.90 ± 1.84^{a}	190.13 ± 1.84^{ac}
Day 1	194.05 ± 1.84^{a}	189.09 ± 1.84^{acd}	180.56 ± 1.84^{bcde}
Day 2	192.11 ± 1.84^{a}	189.92 ± 1.84^{ac}	177.09 ± 1.84^{be}
Day 3	191.54 ± 1.84^{a}	186.41 ± 1.84^{abcd}	174.78 ± 1.84^{e}
Day 4	195.08 ± 1.84^{a}	185.34 ± 1.84^{abcd}	186.54 ± 1.84^{abcd}
Day 5	184.99 ± 1.84^{abcde}	179.14 ± 1.84^{bde}	179.32 ± 1.84^{be}
Sunflower			
Control	191.15 ± 1.61^{d}	191.15 ± 1.61^{d}	191.15 ± 1.61^{d}
Day 0	185.33 ± 1.61^{abd}	182.57 ± 1.61^{abcd}	183.84 ± 1.61^{abcd}
Day 1	185.03 ± 1.61^{abd}	183.39 ± 1.61^{abcd}	180.29 ± 1.61^{abce}
Day 2	178.29 ± 1.61^{abcef}	181.84 ± 1.61^{abc}	180.20 ± 1.61^{abce}
Day 3	175.24 ± 1.61^{cef}	180.92 ± 1.61^{abc}	183.30 ± 1.61^{abcd}
Day 4	170.96 ± 1.61^{f}	181.99 ± 1.61^{abc}	186.31 ± 1.61^{bd}
Day 5	171.51 ± 1.61^{ef}	183.00 ± 1.61^{abcd}	177.15 ± 1.61^{acef}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.3.6 Moisture content

The preservative used and the period of storage had no significant influence on the moisture content of all the corn samples as well as the control ($p= 0.4685$).

The storage period had a significant influence on the moisture content of sunflower oil samples preserved using 100ppm silkworm oil ($p= 0.0014$). Samples stored for 2 days had a significantly higher moisture content as compared to the control and all the other sunflower oil samples preserved using 100ppm silkworm oil (Table 4.14).

Table 0.14: Moisture content (%) of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	0.009 ± 0.63^{a}	0.009 ± 0.63^{a}	0.009 ± 0.63^{a}
Day 0	0.006 ± 0.63^{a}	0.007 ± 0.63^{a}	0.003 ± 0.63^{a}
Day 1	0.004 ± 0.63^{a}	0.005 ± 0.63^{a}	0.005 ± 0.63^{a}
Day 2	0.021 ± 0.63^{a}	2.90 ± 0.63^{a}	0.005 ± 0.63^{a}
Day 3	0.006 ± 0.63^{a}	0.004 ± 0.63^{a}	0.007 ± 0.63^{a}
Day 4	0.007 ± 0.63^{a}	0.007 ± 0.63^{a}	0.007 ± 0.63^{a}
Day 5	0.027 ± 0.63^{a}	0.006 ± 0.63^{a}	0.008 ± 0.63^{a}
Sunflower			
Control	0.008 ± 0.001^{ac}	0.008 ± 0.001^{ab}	0.008 ± 0.001^{ab}
Day 0	0.005 ± 0.001^{abc}	0.004 ± 0.001^{ac}	0.007 ± 0.001^{ab}
Day 1	0.004 ± 0.001^{ab}	0.007 ± 0.001^{abc}	0.006 ± 0.001^{abc}
Day 2	0.004 ± 0.001^{abc}	0.01 ± 0.001^{b}	0.003 ± 0.001^{ac}
Day 3	0.007 ± 0.001^{abc}	0.004 ± 0.001^{abc}	0.007 ± 0.001^{ab}
Day 4	0.003 ± 0.001^{ac}	0.007 ± 0.001^{ab}	0.005 ± 0.001^{abc}
Day 5	0.003 ± 0.001^{ac}	0.007 ± 0.001^{c}	0.006 ± 0.001^{abc}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.3.7 Acid value

The acid value of the corn oil samples was significantly affected by the preservative used and the storage period with the highest acid value being recorded in corn oil preserved using 200ppm silkworm oil and stored for 5 days while the least acid value was recorded in corn oil preserved using 100ppm BHT at day o of storage (Table 4.15). In corn oil preserved using 100ppm BHT, 100ppm silkworm oil and 200ppm silkworm oil there was a significant increase in acid value with increase in storage period (Table 4.15).

The acid value of the sunflower oil samples was significantly affected by the preservative used and the storage period ($p<0.0001$) with the highest acid value being recorded in sunflower oil preserved using silkworm oil stored for 5 days and the least value in sunflower oil preserved using 100ppm BHT at day 0 of storage. The acid value of sunflower oil preserved using 200ppm silkworm oil had significant higher values as compared to sunflower oil preserved using 100ppm silkworm at all stages of storage (Table 4.15). In sunflower oil samples preserved using 100ppm BHT there was a significant increase in acid value at day 5 of storage. In sunflower oil samples preserved using 100ppm silkworm oil there was a significant increase in acid value at day 2 followed by a further increase in acid value with increase in storage time (Table 4.15). In sunflower oil samples preserved using 200ppm silkworm oil there was a significant increase in day 1 followed by a constant increase

up to day 3 and a further significant increase in day 4 which remained constant up to the last day of storage (Table 4.15).

Table 0.15: Acid value (mgKOH/g) of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	5.2 ± 0.05^{b}	5.2 ± 0.05^{b}	5.2 ± 0.05^{b}
Day 0	4.9 ± 0.05^{h}	7.5 ± 0.05^{k}	7.9 ± 0.05^{c}
Day 1	5.5 ± 0.05^{i}	7.9 ± 0.05^{c}	8.0 ± 0.05^{cd}
Day 2	5.9 ± 0.05^{j}	8.2 ± 0.05^{ad}	8.2 ± 0.05^{ad}
Day 3	6.3 ± 0.05^{f}	8.4 ± 0.05^{a}	8.5 ± 0.05^{ae}
Day 4	6.5 ± 0.05^{fg}	8.7 ± 0.05^{e}	8.7 ± 0.05e
Day 5	6.7 ± 0.05^{g}	9.1 ± 0.05^{l}	10.1 ± 0.05m
Sunflower			
Control	3.8 ± 0.08^{ab}	3.8 ± 0.08^{ab}	3.8 ± 0.08^{ab}
Day 0	3.7 ± 0.08^{a}	5.4 ± 0.08^{f}	6.5 ± 0.08^{g}
Day 1	3.9 ± 0.08^{abc}	5.8 ± 0.08^{f}	7.1 ± 0.08^{h}
Day 2	4.0 ± 0.08^{abc}	6.6 ± 0.08^{g}	7.7 ± 0.08^{d}
Day 3	4.3 ± 0.08^{c}	7.2 ± 0.08^{h}	8.1 ± 0.08^{de}
Day 4	4.2 ± 0.08^{bc}	7.7 ± 0.08^{d}	8.6 ± 0.08^{ei}
Day 5	4.8 ± 0.08^{j}	8.2 ± 0.08^{de}	8.7 ± 0.08^{i}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

4.4 Oxidative stability

4.4.1 Thiobarbituric acid reactive substances TBRAS value

Oxidative stability of the corn oil samples was significantly affected by the preservative used and the storage period ($p= 0.0051$). The control and the corn oil preserved using 100ppm BHT at day o of storage showed the best oxidative stability while corn oil preserved using 100ppm silkworm oil at the 5th day of storage had the least oxidative stability (Table 4.16). In the corn oil sample preserved using 100ppm BHT, 100ppm silkworm oil and 200ppm silkworm oil oxidative stability significantly decreased with increase in storage time.

The preservative used and storage period significantly affected the oxidative stability of the sunflower oil samples with the control having the best oxidative stability and sunflower oil preserved using 100ppm silkworm stored for 5 days having the least oxidative stability (Table 4.16). In sunflower oil sample preserved using 100ppm BHT, 100ppm silkworm oil and 200ppm silkworm oil oxidative stability significantly decreased with increase in storage time (Table 4.16).

Table 0.16: Thiobarbituric acid reactive substances TBRAS value (mg MDA/kg) of corn and sunflower oil preserved for 5 days using 100ppm BHT, 100ppm and 200ppm silkworm oil

Corn			
	BHT100ppm	Silkworm100ppm	Silkworm200ppm
Control	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}
Day 0	0.13 ± 0.01^{a}	0.21 ± 0.01^{e}	0.15 ± 0.01^{a}
Day 1	0.22 ± 0.01^{e}	0.29 ± 0.01^{f}	0.23 ± 0.01^{e}
Day 2	0.32 ± 0.01^{f}	0.38 ± 0.01^{cg}	0.34 ± 0.01^{fg}
Day 3	0.38 ± 0.01^{cg}	0.45 ± 0.01^{bd}	0.42 ± 0.01^{bc}
Day 4	0.41 ± 0.01^{bc}	0.44 ± 0.01^{bd}	0.45 ± 0.01^{bd}
Day 5	0.49 ± 0.01^{dh}	0.55 ± 0.01^{i}	0.53 ± 0.01^{hi}
Sunflower			
Control	0.12 ± 0.004^{a}	0.12 ± 0.004^{a}	0.12 ± 0.004^{a}
Day 0	0.13 ± 0.004^{a}	0.13 ± 0.004^{a}	0.13 ± 0.004^{a}
Day 1	0.20 ± 0.004^{c}	0.23 ± 0.004^{b}	0.20 ± 0.004^{c}
Day 2	0.24 ± 0.004^{b}	0.29 ± 0.004^{d}	0.25 ± 0.004^{b}
Day 3	0.30 ± 0.004^{d}	0.38 ± 0.004^{e}	0.34 ± 0.004^{h}
Day 4	0.39 ± 0.004^{ef}	0.46 ± 0.004^{g}	0.41 ± 0.004^{f}
Day 5	0.46 ± 0.004^{g}	0.55 ± 0.004^{j}	0.49 ± 0.004^{i}

Values are means ± SE. Means with different superscript letters along the columns and across the rows are significantly different at $p<0.05$.

CHAPTER FIVE
DISCUSSION

5.1 Fatty acid profile

In corn and sunflower oil linoleic acid (PUFA) was the predominant fatty acid accounting for more than half of the total fatty acid content in the control and the oil preserved using 100ppm BHT while in corn oil preserved using 200ppm silkworm oil had the least amount of linoleic acid. Furthermore, it is worth noting that corn oil samples preserved using 100ppm silkworm oil had trace amounts of palmitoleic acid which is a trans-fat. Similarly, in sunflower oil preserved using 200ppm silkworm oil small amounts of palmitoleic acid were detected. Studies have documented that silkworm oil has palmitoleic acid and thus the trace amounts of palmitoleic acid in the oil samples could have been as a result of addition of silkworm oil (Ji *et al.*, 2016). Furthermore, in corn and sunflower oil samples oleic acid was the major MUFA. The oleic acid content in sunflower oil varied significantly with oils preserved using 100ppm and 200ppm silkworm oil having highest values than the control BHT. Palmitic acid was the major SFA and its content in corn oil preserved using 100ppm, 200ppm silkworm oil and the control were similar. In sunflower oil samples preserved using 200ppm silkworm oil had the highest palmitic acid content followed by the control while the rest had the least content. Thus, addition of silkworm oil into corn and sunflower oil had some effect on the fatty acid content.

5.2 Physical parameters

The colour of corn and sunflower oil was significantly affected by addition of BHT and silkworm oil as a preservative as well as storage period. Evidence suggests that silkworm excreta are used predominantly as natural colorant sin the food and pharmaceutical industry (Kumar and Srivastava, 2020). In both the corn and sunflower oil, the lightness of the oil was observed to decrease with storage time. The changes in lightness of corn and sunflower oil could be linked to oxidation of the oil which consequently results to degradation of natural pigments such as carotenoids and pheophytins which are responsible for oil colour (Morelló *et al.*, 2004). Furthermore, oxidation products adsorbing in the visible spectra range may as well explain the change in lightness of corn and sunflower oil (Sikorska *et al.*, 2007). The hue angle and chroma of the corn oil and sunflower oil samples was as well affected by addition of silkworm oil and the duration of storage. The hue angle of corn oil samples preserved using 100ppm BHT, 100ppmsilkowm oil and 200ppm silkworm oil ranged between 49.25 to 126 °, 58.18 to 106 ° and 75 to 105° respectively which was indicative of yellow to green colour. Furthermore, the intensity of the oil colours differed significantly as indicated by the chroma values. Colour in oils is usually due to presence of carotenoids and

chlorophyll with carotenoids being responsible for yellow coloration and chlorophyll the green coloration (Ramos-Escudero *et al.*, 2019). Studies have documented that carotenoids and chlorophyll are degraded due to oxidation, polymerization and other chemical changes (Karoui *et al.*, 2011). Similarly, evidence indicates that sunflower oil rapidly changes in colour from light yellowto brown/orange color as a result of heating (Maskan, 2003). Similarly, in the present study the colour of sunflower oil and intensity of the color changed with increase in storage time.

Viscosity in the corn and sunflower oil samples was affected by storage time. In the present study oil samples were heated and then stored thus storage temperature impacted on the viscosity. Evidence suggests that an increase in storage temperature results to an increase in kinetic energy which consequently enhances movement of molecules and reduces the intermolecular forces. Thus layers of the liquid easily pass over one another and thus results to a reduction in viscosity (Okparanta, 2018). Studies have also reported that viscosity of oil is also dependent on molecular structure and a degree of unsaturation whereby the viscosity decreases with a decrease in saturation (Kim *et al.*, 2010). Ideally oil degrades with storage time and temperature where by oxidation reactions results to decrease in saturation of oils. The viscosity of sunflower oil samples was significantly affected by the preservative used whereby samples preserved using 100ppm BHT had higher viscosity as compared to samples preserved using 100ppm and 200ppm silkworm oil. This therefore shows that BHT was more effective in preventing oxidation of the oil as compared to silkworm oil.

The refractive index of the corn oil and sunflower oil samples was not affected by type of preservative used as well as storage time. The refractive index of corn oil and sunflower oil in the present study were similar to the values of 1.47 reported by Mudawi, Elhassan and Sulieman, (2014). Density of corn oil samples was significantly affected by storage time as well as type of preservative use. Corn oil samples preserved using 100ppm BHT had higher densities as compared to samples preserved using 100ppm and 200ppm silkworm oil. Evidence indicates that density of oil increases with increase in degree of unsaturation and vice versa (Abramovič and Abram, 2005). Thus, it is clear that BHT was significantly better in preventing oxidation of corn oil as compared to silkworm oil. Interestingly for the sunflower oil samples 100ppm BHT and 100ppm silkworm oil had similar capabilities in preventing sunflower oil oxidation. A decline in density was observed in sunflower oil preserved using 200ppm silkworm which was indicative of oxidation of sunflower oil. Thus 200ppm silkworm oil was less effective in preventing sunflower oil oxidation as compared to 100ppm BHT and 100ppm silkworm oil.

5.3 Chemical parameters

The release of free fatty acids in oils is an indication of hydrolytic or oxidative rancidity a phenomenon that results to unpleasant odors and taste of oils (Medeiros Vicentini-Polette *et al.*, 2021). In the present study corn oil samples preserved using 200ppm silkworm stored for 4 and 5 days had the highest amounts of free fatty acids. Similarly, sunflower oil preserved using 200ppm silkworm oil started showing high free fatty acid content from day 3 of storage up to day 5. This basically implies that 200ppm silkworm oil is less effective in preventing oxidative degradation of corn oil as compared to 100ppm BHT and 100ppm silkworm oil. Furthermore, the study findings show that the amount of free fatty acids increased with an increase in storage period. Evidence indicates that storage temperature results to thermal degradation of triacylglycerol molecules into free fatty acids (Choe and Min, 2006). Furthermore, studies have shown that increase in temperatureresults to an increase in agitation of atoms which makes the triacylglycerol bonds more unstable thus favoring their breakage (Sanibal and Mancini Filho, 2002).

In the present study corn oil preserved using 100ppm silkworm oil had high peroxide values across the storage days as compared to 100ppm BHT and 200ppm silkworm oil. In sunflower oil samples preserved using 200ppm silkworm oil and stored for 5 days had the highest peroxide value. It is worth noting that the highest peroxide values in all samples was observed at the 5^{th} day of storage. Thus, silkworm oil was less effective in preventing lipid oxidation as compared to 100ppm BHT. Peroxide value indicates the formation of hydroperoxides of unsaturated fatty acids as a result of lipid oxidation. The peroxide values in corn and sunflower oil samples were very unstable, at first the values seemed to increase followed by a decline and a further increase with increase in storage time. The observed fluctuations in peroxide value could be attributed to instability of hydroperoxides of unsaturated fatty acids which breakdown into a wide variety of volatile flavor compounds and nonvolatile compounds (Kaleem *et al.*, 2015). Evidence further indicates that the decrease in PV is due to formation of secondary oxidation products such as hydrocarbons, alcohols, ketones and aldehydes (Karakaya and Şimşek, 2011).

In the present study corn oil samples preserved using 100ppm BHT as well as the control had the least levels of impurities. Evidently addition of silkworm oil resulted to an increase in levels of impurities in the oil. The percentage level of impurities in the corn oil and sunflower oil samples was significantly high than the permissible level of 0.05% given by the codex standard (CODEX-STAN210-1999). The levels of impurities in edible oils may further cause oxidation and rancidity in the oils. Furthermore, the impurities may affect other physicochemical parameters and reduce the nutritional values of edible oils. The presence of impurities in edible oils is an indication of in efficiency of clarification during oil extraction process (Hasan *et al.*, 2019).

Iodine value represents the degree of unsaturation of edible oils or fat (Onyeike and Oguike, 2016). Furthermore, the IV indicates the stability of oils to oxidation. Corn oil samples preserved using 100ppm silkworm and 200ppm silkworm oil had considerably higher iodine values as compared to corn oil samples preserved using 100ppm BHT. It is worth noting that storage period had no significant effect on the IV of corn oil. Evidence shows that all the corn oil samples were within the range of 103- 108 reported in other studies (Sanders, 2003). Interestingly in sunflower oil samples preservative used and storage time had no effect on the IV of the oil samples. The IV of sunflower oil samples were comparable with 133 reported in other studies. However small deviations may exist due to differences in climate, soil and variety (Subila, 2016).

Saponification value is usually an index of average molecular mass of fatty acids in oils (Endo, 2018). The saponification value of corn oil was significantly affected by the preservative added as well as storage period since the least saponification value was observed in corn oil preserved using 200ppm silkworm oil stored for 3 days. It is worth noting that the saponification value of a majority of the corn oil samples were comparable to the expected range of 187- 198 mg KOH/g (Endo, 2018). The significantly low saponification value observed in corn oil preserved using 200ppm silkworm oil stored for 3 days suggests that the mean molecular weight of fatty acids is lower or the number of ester bonds is less (Zahir *et al.,* 2017). Furthermore, more this could imply that the fat molecules were not intact with each other (Denniston, Topping and Caret, 2004). The saponification value of sunflower oil was comparable to 177.06 mg KOH/g reported by (Neagu *et al.,* 2014).

The maximum allowable moisture content in edible oils is 0.2% (Australian Oilseeds Federation, 2011). The corn oil and sunflower oil samples in the present study had a moisture content significantly lower than 0.2%. Furthermore, the moisture content of corn oil is comparable to 0% as reported by Barrera-Arellano, Badan-Ribeiro and Serna-Saldivar, (2018). The low moisture content of the corn and sunflower oil samples is an indication of utilization of high technology in oil production (Orji and Mbata, 2008). Therefore, due to the low moisture content the oil samples in thecurrent study were less likely to undergo rancidity (Negash *et al.*, 2019).

Acid value is a measure of free fatty acids in fats and oils (Neagu *et al.*, 2014). Furthermore, AV is an index of purification of fats and oils and usually an acid value of ≤ 0.6 mg KOH/g is desirable for refined edible fats and oils (Codex Alimentarius, 2021). In the present study the acid value was significantly higher than 0.6 mg KOH/g. Furthermore, corn oil and sunflower oil samples preserved using 100ppm and 200ppm silkworm oil had significantly higher acid value as compared to samples preserved using 100ppm BHT. Additionally, corn and sunflower oil samples stored for five days showed had the highest acid value which implies that storage period had a significant impact on acid

value of the oil samples. Evidently the acid value of the sunflower oil samples was also higher than 0.22mg KOH/g and 1.7mg KOH/g as reported by Neagu *et al.*, (2014) and Negash *et al.*, (2019). The acid value is an important parameter of evaluating the quality of oils as well as the degree of refining. Ideally the low the acid value the better the quality in terms of degree of freshness and thebetter the degree of refining. High acid value in edible oils is undesirable since it may cause human gastrointestinal discomfort, diarrhea and liver damage. The high acid value in corn and sunflower oil may be an indication of adulteration of the oil as evidence indicates that unscrupulous traders and manufacturers may add low quality oils to vegetable oils in order to maximize on profits (Zhang*et al.*, 2015).

5.4 Oxidative stability

Thiobarbituric acid reactive substances (TBRAS) test helps shows levels of secondary oxidative products in polyunsaturated fatty acids such as malonaldehyde. Usually the primary product hydroperoxides reacts with oxygen to form MDA which contributes to off flavor of the oil (Zhang *et al.*, 2010). TBRAS have been used widely as an indicator of oxidative stress (Yim *et al.*, 2013). The TBRAS of the corn oil and sunflower oil samples preserved using 100ppm and 200ppm silkworm were significantly higher than those of oil samples preserved using 100ppm BHT. Therefore, 100ppm BHT was more effective in preventing oxidation of corn and sunflower oil as compared to silkworm oil. Interestingly in sunflower oil samples 200ppm silkworm oil was significantly more effective in preventing oxidation of oil as compared to 100ppm silkworm oil. Furthermore, the TBRAS values increased with an increase in storage days. Similarly, other studies have reported an increase in TBRAS values in edible oils with increase in storage time (Goli, Barzegar and Sahari, 2005).

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study aimed to investigate the stability and quality of vegetable cooking oils enhanced with Mulberry silkworm pupae extract as an antioxidant. The following conclusions were drawn based on the specific objectives; first, the analysis of the fatty acid profiles of vegetable oils treated with silkworm pupae extract revealed no significant differences compared to the control and BHT-treated oils. This indicates that the incorporation of silkworm extract does not adversely affect the fatty acid composition of the oils. Second, the oxidative stability of the vegetable oils was significantly enhanced by the addition of silkworm pupae extract. The oils treated with silkworm extract demonstrated improved resistance to oxidation when exposed to typical cooking temperatures, comparable to those treated with synthetic antioxidants like BHT. Lastly, the addition of silkworm extract influenced the physical properties of the oils, particularly viscosity and density. Oils preserved with silkworm extract exhibited lower viscosity and density compared to those treated with BHT, suggesting that silkworm extract may enhance the flowability of the oils. In summary, the study concludes that Mulberry silkworm pupae extract can serve as an effective natural antioxidant in vegetable oils, improving their oxidative stability and maintaining their fatty acid profiles without compromising their physical properties.

This research contributes to knowledge in many ways. First, this study evaluates the efficiency of silkworm pupae extract as a natural antioxidant in vegetable cooking oils like corn and sunflower oil. This provides evidence for using silkworm pupae as a potential alternative to synthetic antioxidants. The research also determines the effect of silkworm pupae extract on the fatty acid profiles of the vegetable oils. It finds that oils preserved with silkworm extract have improved fatty acid compositions, with higher levels of beneficial unsaturated fatty acids like oleic and linoleic acid. Additionally, the study statistically analyzes the oxidative stability of silkworm pupae extract-enriched vegetable oils under typical cooking and storage conditions. This helps understand the antioxidant efficacy of the extract in preserving oil quality.

6.2 Recommendations

Based on the results of this study, several recommendations can be made regarding the use of silkworm pupae extract as a natural antioxidant in vegetable oils. First, the findings indicate that silkworm pupae extract has a significant effect on the physical properties of corn and sunflower oils, particularly in terms of viscosity and oxidative stability. Therefore, it is recommended that food

manufacturers consider incorporating silkworm extract as a natural antioxidant in their oil products. This could provide a healthier alternative to synthetic antioxidants like BHT, appealing to health-conscious consumers. Future research should explore the effects of varying concentrations of silkworm extract beyond the 200 ppm used in this study. This could help identify the optimal concentration for maximizing antioxidant efficacy while maintaining desirable oil characteristics.

It is also recommended that further studies be done to investigate the long-term stability of vegetable oils preserved with silkworm extract under different storage conditions (e.g., temperature, light exposure). Understanding how these factors influence the effectiveness of the extract over time will be crucial for practical applications in food. Further studies should also be done to explore the effect of feeding mulberry on other insects.

Given the potential for silkworm pupae extract to cause allergic reactions or toxicity in some individuals, it is essential to conduct comprehensive safety assessments. Future research should include toxicity studies and allergenicity testing to evaluate the safety of silkworm extract for human consumption. This is particularly important for individuals with shellfish allergies, as silkworms are arthropods and may pose similar risks.

Furthermore, it is also recommended to conduct consumer acceptance studies to gauge public perception of silkworm extract as a food additive. Understanding consumer attitudes towards this ingredient will be vital for its successful integration into food products. Additionally, further research should be conducted to explore the long-term effects of using silkworm pupae extract in various food applications, as well as its potential benefits in other sectors such as cosmetics and pharmaceuticals.

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