THERMAL DEGRADATION AND BIODIESEL PRODUCTION USING *CAMELLIA OLEIFERA* SEED OIL

by

CHARLES B. ALLEN, JR

(Under the Direction of John M. Ruter)

ABSTRACT

Oil derived from the seed of *Camellia oleifera* has been used across China and Southeast Asia for centuries for a range of purposes. Certain physiochemical properties of Camellia oil provide nutritional benefits while contributing to its heat stability and potential for use as biodiesel. Smoke point testing was conducted on 17 different cooking oils. Camellia oil was shown to be a high heat cooking oil with various possible cooking applications including deep fat frying. Biodiesel was then produced from the waste oils and compared to virgin oil biodiesel product. Overall, peanut oil showed the most thermal stability. Its fatty acid profile most resembled its virgin oil control, peroxide and acid values showed the least variation over time, and total polymeric materials increased only 8 units. Camellia produced a biodiesel comparable to soybean. Camellia shows great potential as a cooking oil and biodiesel feedstock.

INDEX WORDS: Camellia, *oleifera*, cooking oil, smoke point, fatty acid profile, peroxide value, acid value, total polymeric materials, biodiesel, flash point temperature, cloud point temperature

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B.S.A., The University of Georgia, 2012

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2015

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August 2015

DEDICATION

I dedicate this thesis to my mother and father. Thank you for making me who I am.

ACKNOWLEDGEMENTS

I would like to thank Dr. John Ruter for all of his guidance throughout this process. My advisory committee, Dr. Ron Pegg and Dan Geller, for all of their patience and revisions as well as access to their lab spaces and personnel. I would like to thank Vickie Waters, Susan Hawkins, and Betty Schroder for their help, advice, and most importantly their encouragement throughout this graduate process. I would like to thank Daniel Jackson of the University of Georgia Agricultural and Environmental Services Lab as well as Down to Earth Energy™ for their assistance in my process of learning new analytical methods. Lastly, I would like to thank Arette Organics© for their donation of the commercial Camellia oil used in this project.

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CHAPTER 1

Introduction and Literature Review

Introduction

Camellia is a genus of evergreen flowering trees and shrubs native to China, Japan, and other regions of Southeast Asia. *Camellia* is a member of the family Theaceae and botanical tribe Gordonieae which is characterized by the formation of a seed within a capsule [22]. Its taxonomy is as follows [1]:

- Kingdom-Plantae
    - Division- Magnoliophyta
        - Class- Magnoliopsida
            - Order- Ericales
                - Family- Theaceae
                    - Genus- Camellia
                        - Species- *C. oleifera*

The genus was named by Carolus Linnaeus in honor of Georg Josef Kamel, famous Jesuit naturalist and apothecary, where he first identified *C. japonica and C. sinensis* [22]. *C. sinensis* has become most familiar internationally with its dried leaves being used to produce green, black, jasmine, and oolong tea beverages. The English word for "tea" is actually derivative of the Chinese "tê" in the Min nan dialect [22]. High demand of the tea beverage eventually led to the introduction of ornamental species, such as *C. japonica*. Today, there are many cultivated species which can be identified by their floral and leaf characteristics. These species include, *Camellia oleifera, C. semiserrata, C. meiocarpa, C. vietnamensis, C. yuhsienensis, C. chekiangoleosa, C. gigantocarpa, C. reticulata, and C. octopetala* [22]. The cultivation area of Camellia in China covers

roughly 3.5 million hectares in 17 different provinces with 98% of this land being devoted specifically to *C. oleifera* production [25].

Morphologically, *Camellia oleifera* are large shrubs ranging from 10-25 feet tall at maturity [5]. The crop normally takes two to three years to mature and can produce fruit for fifteen to sixty years [41] with one year from flowering to harvest fruit. They grow naturally in the southern region of China between 18º and 34ºN latitude in acidic soils where average January temperatures don't drop below 2°C and annual precipitation ranges from 40 in – 80 in a year [5]. This allows for some cultivars to be well suited for growing in the southeastern region of the United States. *C. oleifera* hybrids have been widely used in the United States as hardy ornamentals since the late 1970's [25] but no studies have focused on developing them as an oilseed crop. Flowering requires full sun but shade is preferred on non-flowering plants in production with greatest growth recorded at 30% light exclusion [24]. Cross pollination of Camellia primarily takes place through insects with some wind dispersion giving the genetic background great potential for wide variation in pest and environmental resistances as well as productivity [40]. It was originally thought that Camellia was non-self-fertile but many superior lines recently developed in China show a high rate of self-pollination [6]. Introduction of *Camellia* oil outside of China and the region was delayed until the recognition of its many health benefits [22].

Camellia oleifera has been shown to be propagated by both seed and cuttings. For quickest germination, seed must first be cold stratified at low temperatures, below 2 °C, for a period of 15 – 30 days [25]. Germination rates exceeding 96% have been observed within 10 – 30 days after sowing [24]. Non- stratified seed have been shown to germinate

non-uniformly over a longer period of time when compared to stratified seed [25]. One to two node cuttings are ideal when vegetatively propagating. When misted frequently and given a controlled rate of fertilizer, rooting percentages average 93% for some selections [25]. In China, hypocotyl grafting has grown in popularity with success rates exceeding 95% [40].

The most common disease effecting the propagation success and subsequent yield of *Camellia* is the pathogen *Collectotrichum gleosporioides*, commonly known as Anthracnose. Disease progression starts with small spots on the fruits and leaves that expand to form large lesions [17]. These symptoms eventually lead to fruit and bud drop, leaf loss, death of branches and even death of the plant [17]. Conventional breeding techniques [24], as well as genetic technologies [17] are being employed to incorporate more Anthracnose resistance into the genome. Camellia are also highly susceptible to dieback and canker caused by the fungus *Glomerella cingulata*. Initial symptoms of infection include leaf loss and death of branch tips progressing to cankers causing loss of vigor in the infected branch and eventually death [8]. To treat and help prevent the spread of *G. cingulata*, plant crops in well drained acidic soils, provide adequate fertilization, and remove any dead branches by pruning below the cankered area [8]. The most common pest effecting Camellia production is the tea scale (*Fiorinia theae)*. They feed by attaching to the underside of leaves with heavy infestations causing leaf drop, fewer and smaller blossoms, leaf chlorosis, twig dieback, and even death [8].

All C*amellia* species produce oil but *C. oleifera* has been cultivated in China for thousands of years for this purpose primarily. *Camellia oleifera* is considered to be one of the four main oil-bearing trees along with olive, palm, and coconut [22]. Oil constitutes

roughly 40-50% of the seed weight making it a high oil content crop [24]. In 2007, *C. oleifera* production in China yielded an estimated 150 thousand tons of oils at a value of $1 billion USD giving great value to this product [22]. The two primary means of extracting and processing Camellia oil is mechanic expression and solvent extraction [42]. Mechanical expression tends to be free of solvents and inexpensive while solvent extraction is more efficient [5]. Seed extract from *C. oleifera* is also known as camellia oil, oil-tea camellia, tea oil, tea oil camellia, tea seed extract, and tea seed oil. Camellia oil should not be confused with tea tree oil, an essential aromatic oil extracted from the leaves of *Melaleuca alternifolia*.

Recent efforts have been made to characterize the main genes that control the biosynthesis of fatty acids within *C. oleifera*. Much of the research on optimizing *Camellia* production focuses on breeding and planting strategies with little emphasis on biological and genetic manipulation. Bioinformatic and homological analysis reveal 15 genes central to controlling biosynthesis of fatty acids [39]. One acyl carrier protein (ACP) was found to control long chain saturated fatty acid (SFA) synthesis by catalyzing palmitic acid to form mainly stearic acid [39]. Stearic acid is dehydrogenated to oleic acid through enzymes coded on three stearoyl-ACP desaturase (SAD) genes [35]. Oleic acid is then further catalyzed by fatty acid desaturase (FAD), represented by three genes, to polyunsaturated fatty acids (PUFAs) such as linoleic and alpha-linolenic acid [39]. This data can be applied to improve lipid yields genetically.

Even though Camellia production is focused on tea and cooking oil in southeastern Asia, the major value of *Camellia* globally lies in its aesthetic contribution as an ornamental plant. About 2.5 million *Camellia* plants, mostly *Camellia japonica*, are

produced per year in Spain and exported throughout Europe as ornamentals. *C.japonica* has the highest economic value of the *Camellias* due to its ornamental beauty [27]. *Camellia oleifera* also hosts a number of industrial uses. Throughout Southeast Asia, Camellia is used in the production of cosmetics and lotions. Essential oils from the leaf shoots of *C. oleifera* contain alpha-terpineol (13.179%), linalool (12.959%) trans-geraniol (6.172%) and other components that increase penetration of the skin [36]. They facilitate absorption by temporarily increasing the permeability of the skin to allow for materials to pass to lower levels of the dermis[36]. The seed cake remaining after oil extraction can be applied to lawns and crops as a biopesticide to deter larval development [24] of pests including cutworms, cotton aphids, long horded beetles and leeches [29]. The seed cake has also been shown to control rice blast, sheath and culm blight of rice, wheat rust, and rice hopper [29]. Other non-food applications include fertilizers, paints, soaps, hair oil, and lipstick [22].

Cooking Oil Applications

Edible oils are composed mostly of triacylglycerides (TAG) which up make 95%-99% of the oil composition. This TAG profile determines physiochemical and nutritional properties of the oil as well as its quality [27]. To maximize health benefits, a cooking oil should be dominated by monounsaturated fatty acids (MUFA) with minimal levels of saturated fatty acids (SFA) [22]. Generally, the fatty acid profile of *C. oleifera* is comprised of 65 % - 75 % MUFA, 5 % - 10 % SFA, and 10 % -20 % polyunsaturated fatty acids (PUFA) [13]. The most notable fatty acids within the profile are oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) which have been reported at 56%, 22%, and 0.3% respectively [26]. Studies on the variants with the highest oleic

content show oleic acid, linoleic acid, and linolenic acid at 82%, 6.8%, and .4% respectively in refined oil [9]. Oleic acid, a common plant derived MUFA, has been known to help reduce low density lipoprotein (LDL) levels and serum triglycerides while increasing high density lipoprotein (HDL) levels in the blood [4]. Linoleic and alpha-linolenic acids are both "essential" to human health in that they cannot be produced *de novo* and must be obtained from food sources.

There are other physiochemical properties of the oil apart from its fatty acid profile that contribute to its health properties. Camellia oil has been reported to have a relatively high smoke point when compared to commercial oils at around 252 °C [41]. When an oil reaches its smoke point it degrades and starts to increase free radical production. Oxygen derived free radicals have been found to be related to the formation of cancer, inflammation, atherosclerosis, ischemia-reperfusion injuries, aging, Alzheimer's disease, shock, diabetes, cataracts, hypertension, cardiovascular disease, exercise related muscle damage and infertility [10]. The oil also contains a class of chemical compounds called saponins which have been shown to have the same beneficial effects as oleic acid while also reducing liver reactive oxygen species in rats [38]. High levels of vitamins A, D, E, and K have also been found in Camellia oil [20].

Methanol extraction has been reported to produce the highest yield and strongest antioxidant activity [15]. The total phenolic content of Camellia oil has been reported at 1.63mg gallic acid equivalents/g oil, higher than values for olive oil (0.55mg GAE/g oil) [5]. Phenolics and flavanoids are non-nutritive compounds that have antioxidant, anti-carcinomic, anti-mutagenic, and anti-inflammatory activities while also reducing atherosclerosis [30]. Trolox is a commonly used antioxidant standard [21]. Equivalence

assays show Camellia oil to average 0.70µM Trolox equivalent/g sample, higher than both olive oil (0.63 µM Trolox equivalent/g) and peanut oil (0.61µM Trolox equivalent/g) [5].

Both the meal and oil were also shown to contain Sesamin and one of its structural variants, compound B, that have been shown to have antioxidant effect [15]. Sesamin was successfully identified through MS, infrared (IR) spectroscopy, hydrogen nuclear magnetic resonance (H NMR), carbon nuclear magnetic resonance (C NMR) data, and high performance liquid chromatography (HPLC) [15]. Sesamin and compound B were both found to suppress oxidative injury to red blood cells by 49% and 48% respectively. During liver metabolism, sesamin increases the frequency of hydroxyl groups on phenyl structures thereby increasing their antioxidant activity [12]. Sesamin in unbaked sesame was even shown to suppress growth in lymphoid leukemia Molt 4b cells and induce apoptosis [19].

Well known epidemiological analysis of cancer show that 35% of cancer in the west can be attributed diet and since the 1950's, natural products have been recognized as anticancer agent by the United States National Cancer Institute (NCI)[7]. Camellia oil and its meal have been shown to be anti-proliferative activities against three lines of human cancer: SiHa uterus, MCF-7 breast, and HT-29 colon[5]. Many synthetic agents have shown to have antioxidant and anticancer activities but these drugs are expensive and could possibly contribute to long term health issues[5]. Natural antioxidant and anticancer agents found in oils that prevent lipid peroxidation are preferred to synthetic butylated hydroxyanisole (BHA) and butylated hydroxytolulene (BHT) [31].

Bio-fuel Applications

Biodiesel is defined as a fuel composed of monoalkyl esters of long chain fatty acids derived from vegetable oil or animal fat [2]. Production of biodiesel is important globally. Countries without native concentrations of petroleum oil face a crisis to get the energy that they need and due to the large number of vehicles that require liquid fossil fuels, it is unlikely that there will be a shift in engine design to support a different type of fuel [14]. In accordance with the biodiesel standards of the United States, China, and Germany a criterion for testing Camellia biodiesel has been developed as follows [41]:

- Oil content greater than 30% in seeds
- Acid value less than 1mg/g KOH in oleic acid equivalents (OAE)
- Iodine number less than 120mg Iodine/100g oil
- Cetane number greater than 49 units
- Viscosity between 1.9 and 6.1mm^2/s
- Cold Filter Plugging Point (CFPP) not above O °C

Straying from these guidelines can cause potential problems with fuel quality or economic loss. Certain properties of Camellia oil make it an ideal candidate for biodiesel production.

Oil yield from the seed has been reported at between 30-32% [26] but is known to reach levels of 50% [41]. Increased commercial use of biodiesel has led to American standard ASTM D6751 and European standard EN 14214 including acid number as an important quality[3]. Acid values for various Camellia species rage from 0.41 to 4.71mg/g KOH clearly eliminating some species from contention based on biodiesel

standards [41]. Too high of an acid value can cause easy saponification of oil. The iodine value of TSO has been reported at between 85 and 91mg iodine/ 100g oil [26]. The iodine number should be kept low because too much iodine can cause polymerization during combustion leaving sediment in the engine [41]. Cetane number values were found to be between 55.7 and 56.4. Low cetane values can cause accelerated wear of crucial engine parts [41]. Viscosity is a measure of how well a liquid flows. Values in the camellia family range from 3.31 and 3.58mm^2/s [41]. Increases in viscosity can be detrimental to engine performance due to the decrease ability of the fuel to be atomized during the combustion process [33]. A major limitation of biodiesel across the board is its cold weather properties. At low temperatures crystallization can occur and plug the engine filter. CFPP in the Camellia family range from -9.8 to -12.2 °C [41].

Plant components other than the oil have also been shown potential for use in alternative energy production. The seed kernel hull is a byproduct of the Camellia seed processing that is treated as a waste and is left to eventually degrade[18]. Properties of the hull were analyzed through gas chromatography-mass spectrometry (GC/MS). With the kernel hull contributing only 6 - 8 % of the seed weight, 32.37% of the hull contained 2-furancarboxaldehyde, 5-(hydroxymethyl)-furfural[18] which can be converted into biofuel. Pyrolyzate extractions also show 21.95% ethane, and fluoro [37], which can also be converted into bio-fuels [23].

The emissions profile of biodiesel makes it an attractive alternative energy source. In general, biodiesel greatly reduces hydrocarbons (HC), carbon monoxide (CO) and particulate matter (PM) emissions with CO being lowered 30%-50% [11] depending on the percentage of biodiesel in the blend. Neat, or 100%, biodiesel has the lowest CO

emissions of all blends tested [32]. Emission temperatures were decreased, however, due to the high oleic content of *C. oleifera*, nitrous oxide (NO_x) emissions were increased [32]. Vegetable oil fuels are beneficial due to their high calorific value [14] and increased thermal efficiency which causes more complete combustion of the fuel [32]. Oxidation stability becomes an issue when storing fatty acid methyl esters (FAMEs) due to the auto-oxidation process [28]. Studies indicate that stability of the oil significantly decreases when storage temperature is increased [34]. Daylight and fluorescent lighting greatly accelerate oil oxidation, while ultraviolet radiation has less of an effect [34]. Natural and synthetic antioxidants can be added to biodiesel to increase its oxidation stability [16] while also increasing production costs.

Cooking Oil Research Objectives

The fatty acid profile of Chinese grown *C. oleifera* extract has been studied but there has been little research on its smoke point and no information on the frying performance of the oil. The overall goal of this project in reference to cooking oil was to determine the frying performance of *C. oleifera* oil in comparison to peanut oil and soybean oil. Various tests were conducted on samples to get a depiction of the physiochemical breakdown of the oil over time. This data along with smoke point temperatures was used to determine the health benefits associated with the consumption of tea seed oil in comparison to peanut oil and soybean oil. Waste oil from this project was converted into biodiesel for further testing.

Biodiesel Research Objectives

There has been only one publication on the production of *C. oleifera* biodiesel using a supercritical methanol transesterification process requiring high temperature and

pressure to carry out the reaction (Lin & Fan, 2011). There has been to our knowledge no literature published on production of biodiesel using the standard and more cost-effective catalyzed method. Due to this fact, there is a need for further characterization of *C. oleifera* biodiesel to fully evaluate how well it will perform in accordance to international standards. The overall goal of the biodiesel project was to successfully synthesize and test *C. oleifera* biodiesel for various properties to determine how well it compares to the standards of the industry. Product from fresh oil was compared to biodiesel prepared from the oil produced during frying performance testing.

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CHAPTER 2

Smoke Point Testing of *Camellia oleifera* and Selected Commercial Cooking Oils

[1]Allen, C. and J. Ruter. To be submitted to the *European Journal of Lipid Science and Technology*

Abstract

Camellia oleifera is an oilseed crop native to the southern provinces of China and Southeast Asia where it has been used for thousands of years as a cooking oil. In the region it is commonly referred to as the "eastern olive oil" and has been found to exhibit similar health benefits as this oil when consumed. *Camellia oleifera* is currently being developed as a new oilseed crop for the southeastern United States. The purpose of this experiment was to evaluate the smoke point temperature of *Camellia* oil relative to other common cooking oils. The smoke point of cooking oil is defined as the temperature in which a continuous stream of smoke is emitted from a heated sample. It is thought that at this temperature the oil degrades and starts to produce free radicals. Seventeen oils were tested using American Oil Chemists Society (AOCS) official method Cc 9a-48 with slight modifications. Of the oils tested, Hollywood® Enriched Gold Peanut Oil and Hollywood® Enriched Expeller Pressed Safflower Oil had the highest smoke points with mean values of 244 °C and 243 °C, respectively. Crude *Camellia* oil had the lowest smoke point of 158 °C due to the natural impurities in the sample. Commercial Camellia oil had a smoke point of 210 °C.

Introduction

Camellia is a genus of evergreen flowering trees and shrubs native to China, Japan, and other regions of Southeast Asia. It is a member of the family Theaceae and botanical tribe Gordonieae which is characterized by the formation of a seed within a capsule [29]. All Camellia species produce oil but *C. oleifera* has been cultivated in Southeast Asia for thousands of years for this purpose [30]. Morphologically, Camellia

are large shrubs ranging from 10-25 feet tall at maturity in some species [15]. The crop normally takes two to three years to mature and can produce fruit for a period of fifteen to sixty years [14]. Camellias grow naturally in the southern region of China between 18 °N and 34 °N latitude where average January temperatures don't drop below 2 °C [31].This allows for some cultivars to be well suited for growing in the southeastern region of the United States. Historically, the United States National Arboretum has released 12 cultivars of Camellia but they are currently only used for ornamental purposes, although, previous studies show great potential for *C. oleifera* as food oil in the United States [7,22,30]. Due to this likeness, the University of Georgia has developed a program to introduce and further develop *C. oleifera* as an oilseed crop in the Southeast [31]. Camellia is considered to be one of the four main oil-bearing trees along with olive, palm, and coconut [29]. It has been found to be an important source of edible oil with health benefits said to compare to olive oil [35], with the heat stability of peanut oil. Camellia oil has also been found to possess significant antioxidant activity. The most notable result is the presence of Sesamin and a structural variant deemed "Compound B" found at levels of 33.8 mg and 18.4 mg, respectively, in 100 g of oil. Both compounds were found to suppress oxidative injury to red blood cells by ~49% while also increasing hydroxyl groups on phenyl structures in the liver to improve their antioxidant activity [20]. Oil extracted from Camellia seed is also known as camellia oil, oil-tea camellia, tea oil, tea oil camellia, tea seed extract, and tea seed oil. Camellia oil, however, should not be confused with tea tree oil, an essential oil extract derived from the *Melaleuca alternifolia.* Tea oil and the meal remaining after extraction have also been found to be versatile with many other non-food applications. Internationally they are used in the

production of paint, fertilizer, soap, hair oil, sun protection creams, animal feed, bio-pesticides, and lubricants [15,30].

Edible oils are made of mostly triacylglycerol's (TAGs), which make 95%-99% of the total lipid content and determine the physiochemical and nutritional properties of the oil as well as its quality [32]. The desired characteristics of any cooking oil for maximum health benefit include a fatty acid profile dominated with monounsaturated fat (MUFA) and minimal levels of saturated fat (SFA) [29]. Profiles for commercially refined Camellia oil typically contain 5 % - 10 % SFA, 65 % - 75 % MUFA and 10 % – 20 % polyunsaturated fatty acids (PUFAs) [23]. These ratios of saturated to unsaturated fat also play a role in the heat stability of the oil. Saturated fats contribute greatly to this characteristic. The linear structure and chemically inert nature of SFAs allow them to withstand high heat relative to other fatty acids [5]. Excess SFA consumption can be detrimental to human health raising total cholesterol and low density lipoprotein (LDL) levels directly contributing to the development of heart disease [12]. Metabolically, the best fats to consume are MUFAs. They have been shown to lower LDL levels while raising HDL levels [12]. MUFAs contain a single double bond granting them chemical reactivity and lessened heat stability relative to SFAs [5]. Oleic acid, a common plant derived MUFA, has been reported at over 75% of the fatty acid profile in commercially refined tea oils [36]. As saturation increases, reactivity increases while heat stability of that fatty acid decreases. Tea oil also been shown to contain linoleic and alpha-linolenic acids at 22.4%, and 0.25%, respectively [15]. Linoleic acid is an omega 6 PUFA while alpha-linolenic is an omega 3 PUFA. Both fatty acids are classified as "essential" in that

they cannot be produced *de novo* in humans and must be obtained from food sources. Also contributing to associated health benefits.

The indicator of food oil stability that was examined in this study the smoke point. The AOCS official method Cc 9a-48 defines the smoke point as "the temperature indicated by the thermometer when the test portion gives off a thin, continuous stream of bluish smoke" [34]. These temperatures can vary based on refinement and origin of production materials. As the oil is heated, oxidation is caused by the transfer of oxygen from the air into the fat and is responsible for its aging [5]. TAGs are actively being broken apart into their individual glycerol and free fatty acid (FFA) structures creating free radicals within the oil or in the body when consumed [11]. Glycerol can be further degraded into acrolein which, when volatilized, can irritate the eyes and throat [25]. Free radicals have been shown to react with cyclooxygenase in the body to produce PGs1 and PGs2 prostaglandins with the latter being linked to pro-inflammatory and pro-carcinogenic responses [16]. FFAs have been linked to the growing obesity issue seen in the United States. Research suggests elevated serum FFA levels are a key component of insulin resistance in patients with type 2 diabetes [9]. They affect glucose metabolism by directly inhibiting glycolysis, glycogenesis, and glycogen uptake leading to hepatic insulin resistance [9]. While it is accepted that reactive oxygen species (ROS) are necessary in the fine tuning of metabolic processes, unbalanced and prolonged presence of these species can lead to oxidative stress, apoptosis and necrotic cell death [27]. Oils with higher smoke points reduce the chance of exposure to free radicals especially when cooking with high heat. An extensive literature search shows very little experimentation conducted on *Camellia oleifera* oil in the United States with limited research on smoke

point temperature. There is also very little statistically validated data for many of the common commercial cooking oils used in households today. Having a reference for more accurate smoke point data can be greatly beneficial to the health of the consumer. Each oil was placed into specific categories that pair the heat stability of the oil with recommended cooking uses.

Materials and Methods

Several oils were used for the comparison of smoke points. They are as follows: Earth Fare® Expeller Pressed Grape Seed Oil (Fletcher, North Carolina, USA), International Collection® Almond Oil (Hull, England), Kroger® Pure Olive Oil (Cincinnati, Ohio, USA), Kroger® Pure Vegetable Oil, Spectrum® Expeller Pressed Walnut Oil (Petaluma, California, USA), Kroger® Pure Sunflower Oil, Hollywood® Enriched Gold Peanut Oil (Boulder, Colorado, USA), Kroger® Pure Canola Oil, Georgia Olive Farms ™ Extra Virgin Olive Oil (Lakeland, Georgia, USA), Hollywood® Enriched Expeller Pressed Safflower Oil, Kinloch© Virgin Pecan Oil (Winnsboro, Louisiana, USA), Arette© Organic Extra Virgin Tea Seed Oil (Sunnyvale, California, USA), Kroger® Value Shortening, Kroger® Corn Oil, and Spectrum® High Oleic Peanut Oil. Two extracts from Georgia grown Camellia seed, one crude and one centrifuged, were also tested. With the exception of the crude Camellia that was tested, all oils were commercially refined, bleached and deodorized.

The apparatus used to conduct the smoke point test was engineered by the University of Georgia Design and Instrument Fabrication Shop (Athens, GA) based on dimensions specified in AOCS official method Cc 9a-48 [34]. It can be seen in Figure

2.1.The apparatus was decreased by 10cm in depth and 5cm in height to accommodate the size of the fume hood. Florida Method of Test for Smoke Point Designation FM 5-519 was also considered when designing the protocol for this experiment [8]. The oil was heated with the standard 12.7cm Bunsen burner with 1.11cm diameter. The temperatures were measured with a Maveric® Dual Sensor ET-85 Thermometer (Edison, New Jersey, USA). Pieces of metal wool were used to clean heavy debris from cup. Low odor mineral spirits was used to dissolve residual oil from the cup. The smoke points were found using the Cleveland open cup smoke point method, Cc 9a-48, and Florida State Test Method FM 5-519 [8,34]. Sixty-five mL of oil was measured, volumetrically, into the testing cup. Special care was taken not to drip any oil on the rim of the cup or the tripod holding the cup. Stray oil can cause premature smoking and an inaccurate smoke point reading. The thermometer tip was submerged approximately 2mm below the surface of the oil and was secured in place throughout the duration of the test. The oil was heated in the apparatus fitted in the fume hood with the door of the fume hood shut to prevent any draft. Once the oil reached a temperature ~24°C below the suspected smoke point, the heating was slowed to 2-5°C until the smoke point was reached. This temperature was recorded and 5 repetitions were taken of each oil. To ensure accurate testing, all parts coming in constant contact with heat (cup, tripod, and thermometer) were allowed to return to room temperature between each repetition.

Data Analysis

Data was analyzed using the "R" statistical programming console (R-Project, Vienna, Austria). An analysis of variance (ANOVA) was conducted to determine differences in smoke point for each oil tested (n=17). Multiple comparisons tests were

conducted on analysis results measured with a 95% confidence interval using Tukey's Honest Significant Difference Test. Table and graphs were produced using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

Results

The study found that oil type had an influence on the smoke point of a cooking oil ($p \leq 0.001$). Due to lack of smoke point temperature labeling by oil producers, mean values observed in this study were compared to values obtained from consumer reports as well some scientific literature. Expected values were also of oils with similar refinement to those tested in this study. As can been seen in Table 2.1, Hollywood® Enriched Gold Peanut Oil and Hollywood® Enriched Expeller Pressed Safflower Oil had the highest smoke point values. Observed peanut oil temperatures slightly exceeded the reported range of 229 - 232 °C [3,13] with a temperature of 244 ± 0.8 °C. Safflower oil, having a mean smoke point of 243 ± 1.0 °C, fell comfortably within the range of values reported from 212 – 265 ˚C [3,19]. Crude and centrifuged Camellia oils had the lowest smoke points. No other values could be found for Camellia oils of these classes however crude peanut oil was found to have a similar smoke point temperature at ~160 °C [3]. Arette© Organic Extra Virgin Tea Seed Oil had a much higher smoke point than the other unrefined Camellia oils, but it was not different from Spectrum® Kroger® Brand Corn Oil. Consumers and oil producers have often reported tea seed oil temperatures at ~252 °C [2,6]. Experimental values were found to be closer to 210 ± 1.7 °C. This result was consistent with 210 °C – 215 °C reported in the few other academic publications that could be found testing the smoke point of *C. oleifera* [17,24]. Corn oil was found to have a smoke point temperature of 212 ± 1.6 °C when reports show temperatures around 232

°C [3,6]. Vegetable shortening smoke point values are typically seen around 180 °C [6,33]. Testing showed a mean temperature of 215 ± 0.9 °C. Mean experimental values for grapeseed oil were found to be 202 ± 3.5 °C. These values were on the lower end of the rage of 195 °C – 229 °C. Both crude and centrifuged Camellia samples had lower estimated smoke point temperatures than Kroger® Brand Pure Olive Oil and Georgia Olive Farms ™ Extra Virgin Olive Oil. Experimental values for pure olive oil were lower than expected at 187 ± 1.8 °C. Smoke point temperatures have been reported closer to 225 °C [6]. Experimental extra virgin olive oil smoke point temperatures were between reported values of 165 – 207 °C. Commercially refined Camellia oil did have a significantly higher smoke point than both of the olive oils ($p \leq 0.001$) however peanut oil had a significantly higher smoke point temperature than Camellia. No smoke point values could be found specifically for high oleic peanut oil and none were provided by the manufacturer.

Discussion

The mean smoke point was used to place each oil into a heat recommendation category as seen in Table 2.2. These categories were constructed are based on information provided by the cooking oil industry, specifically Spectrum Organics®, and the United States Department of Agriculture Food Safety and Inspection Service [4]. Each recommendation represents a range of heating temperatures an oil can be safely used within to best minimize potential free radical exposure. Based on the expected smoke point values presented in Table 2.1, some oils could potentially fall into multiple heat recommendation categories. Extra virgin olive oil smoke points have been reported at a wide range from a medium heat oil at 165 °C to almost a high heat oil at 207 °C

[6,10]. Grapeseed oil temperatures span from a medium high heat oil at 195 °C to well within the high heat category at 229 °C. Although they don't span heating recommendation categories, some reported smoke point temperatures still present much variation. Safflower oil has a reported range of 33 °C with temperatures well exceeding what was found in this study. These differing reports could be attributed to many things. With much of the smoke point data being produced by commercial oil companies, it can be comfortably assumed that various methods were used to obtain these temperatures. This fact would allow for much variation in testing results. There could have also been smoke points included within a reported range that were of a different refining than the majority of the oils. Such great variation presents a need for more accurate labeling by cooking oil manufacturers of smoke points to reflect the natural change that may be seen in their feedstock.

Oils with low smoke points, below 121 °C, are best when used with no heat such as in salad dressings or poured onto a finished dish [1]. Due to random selection of oils, none from this study that fell into the lowest category. Unrefined canola and safflower oils have been reported in this category with a smoke point temperature of 107 °C [3]. Medium heat oils have smoke points ranging from 121 – 176 °C [4]. Both of the non-refined Camellia oils fell into this category. Even though they don't have the highest smoke point, these oils can contain varying levels of certain physiochemical constituents relative to commercially refined oils. The degumming process was found to be beneficial in removing excess phospholipids to stabilize the oil [28]. However the excess bleaching can lead to degradation of low molecular weight products in the oil, causing an increase in harmful volatile production during cooking [28]. These oils are best used for light

sautéing and sauces. Oils with smoke point temperatures 176 – 210 °C are ideal for medium-high heat. They should be used for higher heat sautéing and baking [4]. High heat oils, 210 °C and above, can be used as all-purpose cooking oils and are ideal for usage in deep fat frying [1]. The commercially refined Camellia oil can be found in this category giving it a wide range of cooking uses while presumably preserving key nutritional qualities under high heat stress.

The fatty acid profile should also be considered within each category to pair the maximum heat stability for the cooking application with the greatest nutritional benefit. All fatty acid profiling data was obtained from previous literature examining non-heated oils of similar refining as to what was tested in this study. Peanut oil was found to have the highest smoke point temperature with a mean of 244 °C. This smoke point has made it a popular frying oil in the southeastern United States. Fatty acid profiling shows that peanut oil has a saturated fat content of ~24% [26] while commercial Camellia oil averages 9.5% [15]. Safflower oil had virtually the same smoke point as peanut oil with a saturated fat content averaging 13%. This increase in saturated fat in peanut and safflower oil can potentially account for the increased smoke point relative to commercial Camellia oil [5]. In contrast, an increase in saturated fat has shown to be a detriment to human health by increasing total cholesterol (TC) and LDL levels contributing to the development of heart disease [25]. Unrefined Camellia oil was found to have a saturated fat content comparable to peanut oil at ~20 % [15] but other physiochemical properties not examined in this study prevented an elevated smoke point. Commercially refined tea-seed oil also contains up to 80% oleic acid [15], considerably higher than peanut at 52% [26]. Safflower oil had the lowest oleic acid content at only 17 % of the profile [25].

Oleic acid is less stable thermally than saturated fats but can still contribute greatly to the smoke point of an oil due to its ability to resist oxidation [13]. It is also more beneficial for human health to consume higher amount of monounsaturated fats, like oleic acid, relative to saturated fats due to their ability to lower LDL cholesterol while raising HDL cholesterol levels [25]. While contributing greatly to its health advantages, polyunsaturated fats can lower the heat stability of an oil. Tea-seed oil was found to average 15 % [15] PUFA with peanut oil averaging 25 % [25]. Safflower oil PUFA levels average 70 % of the profile [18]. Moderate levels of SFAs and MUFAs may account for the increase smoke point in safflower oil. For all three oils, the main form of polyunsaturated fatty acid found in the profile is linoleic acid. Linoleic acid is an omega 6 fatty acid, essential to human health, which can lower LDL levels but also potentially lower HDL cholesterols levels [25]. Due do the increase in unsaturated bonds, PUFAs are the most susceptible to oxidation [21]. In high temperature situations, research has shown that PUFAs easily degrade producing toxic compounds in the oil even before the smoke point is reached. For high heat cooking, having lower levels of theses fatty acids may actually be beneficial to oil stability and human health.

Conclusions

The results of this study allow for the consumer to make a more informed decision of which cooking oil to use based on the heat required for cooking, minimizing exposure to dangerous free radicals. With a smoke point of 210 ± 1.7 °C, commercially refined Tea-seed oil can be classified as a cooking oil with high heat tolerance. This high smoke point allows the oil to be used in various applications while resisting degradation and maintaining oxidative stability. Although peanut oil was the most stable against

degradation at high temperatures, oils within the same heat recommendation category offer the same flexibility in cooking application with physiochemical properties more favorable for proper nutrition as seen in Table 2.3. *Camellia* oil shares a heat recommendation category with peanut oil but offers increased oleic acid levels with decreased SFA levels. Safflower oil also offers lowered SFA levels with an increase in PUFAs relative to peanut oil. However, safflower oil may not be as suitable for high heat usage due to the dominant nature of PUFAs in the profile and the toxic nature of their degradation products. Further research should be conducted to get more accurate values of other popular commercial cooking oils with a wider range of smoke points. There is also a need of more accurate labeling by major commercial cooking oil producers to aid the consumer in making the most health conscious decision. Assays should also be performed on heated samples to quantify the change in antioxidant activity of the oil to draw conclusions about its free radical content.

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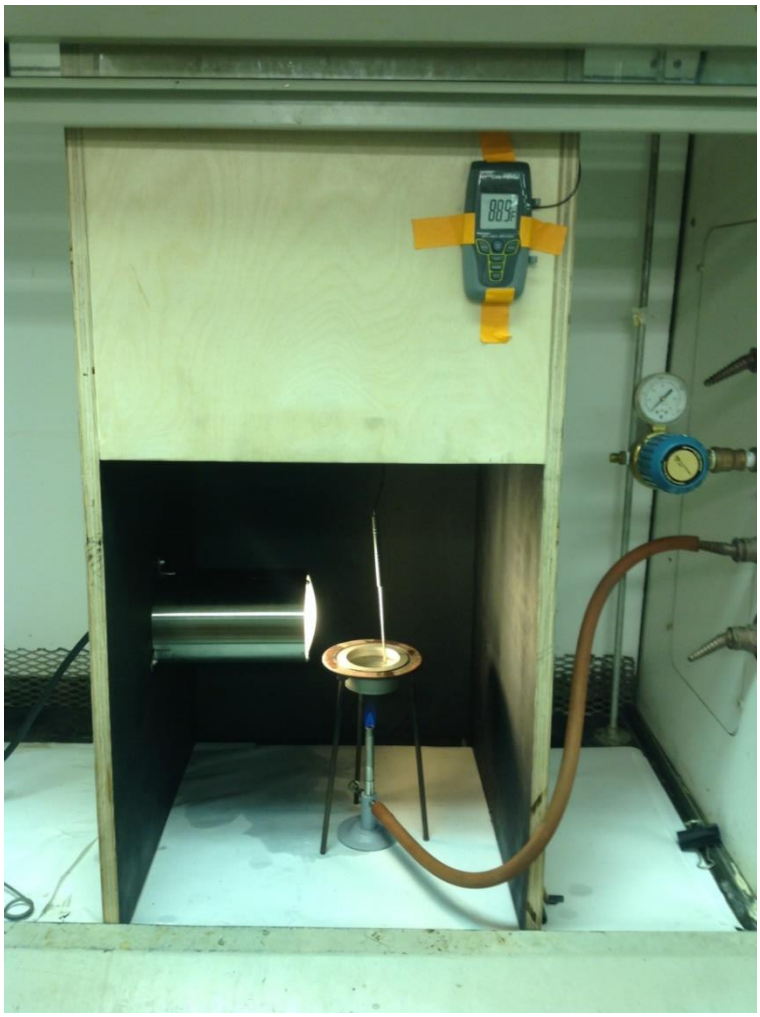

Figure 2.1: Smoke point testing apparatus

Table 2.1: Mean smoke point temperature of cooking oils with Tukey grouping

Oil	Mean (°C)	reported
Peanut	244 ± 0.8 $_{a}$	232
Safflower	243 ± 1.0 $_{a}$	232-265
Sunflower	236 ± 0.4 $_{b}$	226
Pecan	234 ± 2.0 $_{b}$	243
Walnut	228 ± 1.2 $_{c}$	204
Vegetable	227 ± 0.8 $_{c}$	205-232
Almond	223 ± 0.6 $_{d}$	221
Canola	223 ± 1.3 $_{d}$	204
High Oleic Peanut	218 ± 1.3 $_{e}$	
Shortening	215 ± 0.9 $_{e,f}$	187
Corn	212 ± 1.6 $_{f,g}$	232
Commercial Camellia	210 ± 1.7 $_{g}$	252
Grape Seed	202 ± 3.5 $_{h}$	195-229
Pure Olive	187 ± 1.8 $_{i}$	225
Extra Virgin Olive Oil	182 ± 3.0 $_{j}$	165-207
Centrifuged Camellia	164 ± 1.1 $_{k}$	
Crude Camellia	158 ± 1.9 $_{l}$	

Shown are the observed mean smoke points (°C) of each cooking oil tested and their reported values. Observed temperatures with the same letter were not significantly different based on Tukey grouping.

Table 2.2: Recommended heating range of cooking oils to minimize free radical exposure

Heat Recommendation	**Oil**	
No Heat: below 121°C	N/A	
Medium Heat: 121 - 176°C	Crude Camellia Centrifuged Camellia	
Med-High Heat: 176 - 210°C	Extra Virgin Olive Pure Olive Grapeseed	
High Heat: 210°C and above	Commercial Camellia Corn Shortening High-Oleic Peanut Canola Almond	Vegetable Walnut Pecan Sunflower Safflower Peanut

Each oil is placed into a heat recommendation category based on their observed smoke point temperatures. These categories determine the cooking applications of the oil.

Table 2.3: Comparison of selected oils

Oil	Smoke Point (°C)	Saturated Fatty Acid (% SFA)	Oleic Acid (% MUFA)	Polyunsaturated Fatty Acid (% PUFA)
Peanut	244	24	52	25
Safflower	243	13	17	70
Commercial Camellia	210	9.5	80	15

Camellia oil is compared to peanut and safflower oil on basis of smoke point and fatty acid profile.

CHAPTER 3

Thermal Degradation of Camellia and Selected Cooking Oils

[2]Allen, C., R. Pegg and J. Ruter. To be submitted to the *European Journal of Lipid Science and Technology*

Abstract

The oil extracted from the seed of *Camellia oleifera* has been used in China and Southeast Asia for thousands of years as a cooking oil. Camellia oil has certain physiochemical properties that are beneficial to human health and these properties also contribute to the heat stability of the oil. This study examined how Camellia oil and oils with similar chemical characteristics, peanut and soybean, respond to high heat stress during frying and what degradation products were produced. Over five consecutive days, each oil was subjected to high temperatures through the frying of potatoes. A series of analysis were conducted on an oil sample taken from each day to determine the fatty acid profile, peroxide value, acid value and total polymeric materials in the waste oil. These tests are important to determine how the beneficial and potentially negative nutritional aspects of the oil change during heating as this could have effects on human health. Overall, peanut oil was most stable under high heat and frying conditions followed by Camellia and soybean oils, respectively. The fatty acid profile for peanut oil changed the least during frying, peroxide values peaked at 0.19 mg peroxide/kg oil, acid values at 0.45 mg KOH/g oil, and total polymeric material values topping 10.8% Camellia oil also exhibited a small change in fatty acid profile, peroxide values peaked at 1.79 mg peroxide/kg oil, acid values at 1.1 mg KOH/g oil, and total polymeric material values topping 19.2%

Introduction

Camellia oleifera, an oilseed crop native to Southeast Asia, has recently been adapted for production in the Southeastern United States. A member of the family *Theaceae*, Camellia are a genus of flowering evergreen trees ranging from 10-25 feet tall

at maturity [7]. A plant becomes fully mature in two to three years and can flower for at least 60 years [22]. The oil, derived from the seed, is a popular cooking oil in its native regions and is known to have a relatively high smoke point with refined oils averaging 215 °C [15]. Camellia, with its seeds containing up to 40% oil w/w [17], is considered as one of the four main oil bearing trees, along with olive, palm, and coconut [15]. China is currently the largest producer of Camellia oil in the world with production centered in the southern provinces. Production can be optimized in this region due to the milder climates [7]. Annually, China produces approximately 150 thousand tons of oil with an estimated value of over $1 billion [21]. Camellia possess a number of industrial uses. It can be used to manufacture paint, fertilizer, soaps, and anti-wrinkle creams [15] although roughly one seventh of the Chinese population uses the oil for cooking purposes [17].

Commercial Camellia producers describe the oil as having a smoky or nutty flavor and being quite mild in comparison to olive oil [15]. Traditionally, Camellia oil has been used for medicinal purposes to treat a range of ailments from stomachache to mild burns [12]. In addition to the oil, the seed meal remaining after extraction has also been found to be anti-proliferative against human uterus, breast, and colon cancer lines [7] while containing Sesamin compounds that can reduce oxidative injury of red blood cells by up to 49 % [10]. Introduction of Camellia oil into the United States was delayed until the recognition if its potential health benefits but popularity for the oil has yet to grow. Although two cultivars of *oleifera* have been released by the United States Arboretum, they are currently only being used for ornamental purposed despite their potential as a food oil crop[2,18].

Deep-fat frying is a common food preparation technique used in kitchens around the world. It is estimated that in the United States the food frying sector is worth upwards of $83 billion with that only representing half of the global market [13]. Frying is a rapid and energetic process that requires submerging the desired material in hot oil or fat heated to 150 – 190 °C with temperature dependent on the food product. During the process, a simultaneous transfer of heat and mass from the oil, food, and air occur contributing to the development of flavors and textures [5]. Over time, absorption of oil by the food requires the medium to be partially replenished to maintain initial frying conditions, however repeated use of an oil can lead to the accumulation of degradation products [8]. High oil temperatures combined with the moisture and chemical makeup of the food product along with incorporation of oxygen lead to a series of reactions including dimer and polymer formation, fatty acid oxidation, fat hydrolysis, and Maillard reactions [5]. As the products oxidation increase, the quality of the fried product produced from this oil decreases and the health risks associated with its consumption increase.

Cooking oils are composed mostly of triacylglycerol (TAG) which have a strong influence in determining nutritional qualities as well as the oils resistance to thermal degradation at frying temperatures [19]. To achieve the greatest health benefits, an oils fatty acid profile should be composed mostly of monounsaturated fatty acids (MUFAs), moderate levels of polyunsaturated fatty acids (PUFAs) and minimal levels of saturated fatty acids (SFAs) [15]. The fatty acid profile of Camellia oil typically contains 65% - 75% (MUFA), 10% - 20% PUFA, and 5% - 10% SFA [11]. Other physiochemical characteristics of the oil also contribute to the behavior of the oil under stress and *in vivo*.

The sum of compounds not representing triglycerides are defined as the oils total polar materials (TPM). These materials include free fatty acids (FFAs), sterols, antioxidants, antifoamers, carotenoids, crystal inhibitors, soap residues, bleaching earth, or any other materials that could have been emulsified or solubilized into the oil [16]. Initial TPM levels have been found to impact taste, creating off-flavor compounds, while effecting primary oxidation rates [14]. While under heat stress, changes in TPM can be contributed to in part by the oils acid value and peroxide value (PV) [14]. The acid value of an oil strictly measures the number of carboxylic acid groups present in a given sample. An elevated acid value can lead to increased hydrolysis and oxidation within the oil, decreasing its shelf life [3]. An oils PV is a measure of the primary lipid oxidation products, or hydroperoxides, within the oil. Rancidity can be palated once PV levels reach 30 – 40meq/kg oil [11]. Oils with high initial PV levels are also more likely to contribute to a higher TPC over time [14]. An ideal frying oil would be mild in flavor, contain very low moisture and peroxide levels, have a smoke point above 170 °C, high oleic acid values, low linoleic/linoleic acid levels, and a low FFA content [20].

The purpose of this study was to observe how well Camellia oil withstands thermal degradation in comparison to peanut and soybean oils. Changes in fatty acid profile, TPC, AN, and PV will be observed. Peanut and Vegetable (soybean) oils were selected for comparison because of their ability to withstand degradation under high heat stress and their consumer popularity, especially for frying purposes, in the United States.

Materials and Methods

Frying Performance Testing

Frying oil performance tests were conducted on Arette© Organic Extra Virgin Tea Seed Oil (Sunnyvale, California, USA), Bakers and Chefs Peanut Oil (Geylang, Singapore), and Admiration® Pure Vegetable Salad Oil (Brundidge, Alabama, USA). All three oils tested were commercially refined, deodorized, and bleached. 3.5 liters of each oil was randomly added to one of three Rival® CZF725 professional style deep-fryers and heated to a temperature of approximately 180 °C. Potatoes were washed and cut into 9mm x 9mm pieces of varying length using a Nemco® N55450 potato press. 200g of potatoes were added to each fryer and cooked for seven minutes per batch averaging one batch every 10-12 minutes. Each oil was subjected to five hours of frying each day for a period of five consecutive days. After each day, 100mL of oil was taken from each fryer and stored at 0 °C for future analysis. Sufficient fresh oil was added to each fryer at the beginning of each frying day to return the oil to its original volume. TPM values were recorded using a Testo® 265 TPM probe at the end of each day of frying. The TPM probe was previously calibrated according to the instructions listed in the manual.

Fatty Acid Profiling

To obtain the fatty acid profile for each oil on each day, samples were first transmethylated to create fatty acid methyl esters. Approximately 80mg oil was loaded into 5mL glass vials along with a C17:0 internal standard and transmethylation reagent comprised of sulfuric acid in methanol with hydroquinone as an antioxidant. The samples were then incubated at 65 ºC to 70 ºC for at least 14 hours. Next, samples were subjected to several water and hexane wash steps. Remaining hexane was evaporated from the sample under nitrogen gas using an N-EVAP system. Preparation was complete after

samples have been re-suspended to equal volume and transferred to 2mL glass gas chromatography vials. Profiles were obtained using an Agilent© 7683 gas chromatograph with flame ionized detection (GC-FID) and 7683B auto-sampler. A 10µL syringe was used to inject 1µL samples through an 11mm inlet septum. Both the injector and detector temperatures should be set to 250 °C. Samples were loaded on a Supelco© SP-2560 column having a length of 100m, inner diameter of 0.25mm, and a film thickness of 2µm. Standard retention times were obtained for the column using a 37 FAME standard mix to determine the relative response factors (RRF) for each fatty acid identified. The GC was set to a split ratio of 50:1 under constant flow with helium as the carrier gas generating a head pressure of ~40psi. The flame produced by the FID component was generated from a mixture of compressed air flowing at 450mL/min, helium at 40mL/min, and helium makeup gas at 23.9mL/min. The initial oven temperature was set to 140 °C and held for 5 minutes. The temperatures was then increased at a rate of 5 °C/min to a final temperature of 240 °C. This temperature was held for 15 minutes resulting in a total run time of 45 minutes.

Peroxide Value

Peroxide values were tested according to AOCS method Cd 8b-90. Approximately 2g of each sample was weighed into a 250mL Erlenmeyer flask. The oil was combined with 50mL 3:2 acetic acid isooctane solution (v/v) and 0.5mL saturated potassium iodide solution creating a golden-orange coloration. After being agitated for one minute, 30mL distilled water was added to neutralize the reaction. The mixture was then titrated with 0.1M sodium thiosulfate pentahydrate until a pale yellow color was observed. The titrant was previously standardized using potassium dichromate. A blue coloration was caused in the sample by adding 0.5mL 10% sodium dodecyl sulfate (SDS)

and 0.5mL starch indicator. The titration was completed right as the starch-iodine complex disappears. Units for this analysis were measured in meq. peroxide O_2/kg oil. The volume of titrant needed for the reaction was used to calculate the peroxide value with the following equation:

Peroxide Value = ((S-B)×M×1000)/W, where:
B = volume titrant for blank (mL)
S = volume titrant for sample (mL)
M = molarity of sodium thiosulfate solution
W = sample weight (g)

Acid Value

Acid values were calculated based on ASTM D974. Approximately 2 g of each sample were added to a 250mL flask along with a 100:1:99 (v/v/v) mixture of toluene, water, and 2-propanol respectively that served as the titration solvent. 0.5mL *p*-naptholbenzein indicator solution was added to the sampled and vigorously agitated. The sample was then immediately titrated with 0.1M potassium hydroxide solution until a green colored chromophore was held in the mixture for at least 15 seconds. Potassium hydroxide standardization was performed on the titration solution. Acid values were calculated using the following equation:

Acid Value = ((A-B)×M×56.1)/ W, where:
A = vol titration for sample (mL)
B = vol titrant for blank (mL)
M = molarity of the KOH solution
W = sample weight (g)

Data Analysis

A univariate repeated measures design was used to determine differences in each fatty acid over time. Analysis of variance was conducted using the "R" statistical

programming console (R-Project, Vienna, Austria). This was performed separately for each oil (n=3). Each specific fatty acid examine served as the whole unit treatment with time (n=6) as the within-unit factor. A repeated measures design was also used to determine differences in TPM, PV, and acid value between each oil over time. Each separate oil served as the whole unit treatment for this model with time again as the within-unit factor. Coefficients of determination (R^2) were determined using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Regression analysis was conducted between TPM, PV, acid values, and oleic/linoleic acid ratios (O/L) using linear and quadratic functions. Daily aliquots taken for testing represent the experimental unit and the subunit was represented by the individual replications taken from each aliquot for each analysis. Control measurements were obtained from non-thermally degraded samples of each oil are a represented in each analysis at hour zero. Differences means were considered significantly different at $p \leq 0.05$.

Results and Discussion

The composition of major FAs present in fresh Camellia oil are as follows: palmitic acid (C16:0), 9.05g/100g oil; stearic acid (C18:0), 2.26g/100g oil, oleic acid (C18:1) 78.4g/100g oil; linoleic acid (C18:2), 7.88g/100g oil; gadoleic acid (C20:1), 0.69g/100g oil, α-linolenic acid (C18:3), 0.23g/100g oil, eicosadienoic acid (C20:2), 0.55g/100g oil, behenic acid (C22:0), 0.41g/100g oil. These findings are generally consistent with data produced by Arette© on the major fatty acid content of the Camellia oil they distribute with the exception of behenic acid. Levels reported by Arette© average 0.02g/100g oil, roughly 5% of what was observed in this study. Fresh Camellia oil also contained small amounts of α-linolenic acid that could not be detected after first day of

frying. The fatty acid profile of Peanut oil saw the least deviation in fatty acids overall but even its α-linolenic acid levels decreased by 18 % on the first day of testing. Due to the instability of polyunsatured fats, it was likely that the low concentrations of this particular fatty acid were degraded.

The profile of Camellia oil showed differences for every fatty acid with the exception of behenic acid ($p \leq 0.052$). The high heat stability of behenic acid can be attributed to it being both long chained, with a backbone of 22 carbon atoms, and completely saturated. There was a general decrease in % PUFA after 25 hours of frying. The difference in %PUFA at 25 hours compared to the control decreased 4.99%. %MUFA exhibited a general increase after 25 hours of frying. Compared to the control, after 25 hours of frying the %MUFA in Camellia oil increased 0.92%. There was a decrease in %SFA after five hours of frying with a general rise in concentration up to 25 hours. The difference in %SFA at five hours compared to control decreased 7.99% with the final value after 25 hours being 0.98% below the control. These general trends are expected during thermal degradation of oil as PUFA's degrade to MUFA's. MUFA then degrade to form SFA's accounting for its increase. As can be seen in Table 3.2, Soybean oil also exhibited differences in over time for every fatty acid with the exception of behenic acid ($p \leq 0.459$). There was a general decrease in %PUFA after 25 hours, as expected. The difference in %PUFA at 25 hours relative to the control decreased 1.5%. There were general increases in both %MUFA and %SFA, also as expected. %MUFA increased 4.63% at 25 hours relative to the control while %SFA increased 13.2% relative to the control.

Table 3.3 shows the changes in the fatty acid profile of peanut frying oil over time. Stearic ($p \leq 0.001$), linoleic ($p \leq 0.001$), gadoleic ($p \leq 0.001$), α – Linolenic ($p \leq 0.001$), eicosadienioic ($p \leq 0.001$), and lignoceric acid ($p \leq 0.012$) all showed significant differences in peanut oil. There were no significant changes seen in oleic ($p \leq 0.277$) and behenic ($p \leq 0.059$) acids. As observed in the other oils tested in this study, %PUFA of peanut oil generally increased after 25 hours of frying. The difference in %PUFA at 25 hours decreased 2.68% relative to the control. %MUFA and %SFA both show decreases after five hours of frying followed by a general rise in concentration up to 25 hours. The difference in %MUFA at five hours compared to the control decreased 0.17% with the final value after 25 hours being 0.12% below the control. Differences in %SFA at five hours decreased 1.42% relative to the control with the final value after 25 hours of frying being 0.67% below the control. At the conclusion of frying, peanut oil possessed the fewest fatty acids affected over time. %SFA, %MUFA, and %PUFA values for peanut oil were also closest to their control at the conclusion of frying relative to the other oils tested in this study.

The ratio of oleic acid to linoleic acid (O/L) can be used as a measure of an oils oxidative stability at frying temperatures and resistance to rancidity at ambient storage temperatures (Das, 2013). Higher ratios are favored for this characteristic. Fresh Camellia oil was found to have the highest O/L ratio with a mean of 9.96. These values were much higher than those observed in fresh peanut and soybean oils at 2.81 and 0.41 respectively. A regression analysis of the O/L ratio and TPM values showed a significant quadratic relationship in Camellia oil ($R^2=0.864$) as can be seen in Figure 3.6. Significant relationships were observed between O/L ratio and TPM in both peanut ($p \leq 0.002$) and

soybean oils ($p \leq 0.001$) but coefficients of determination were weak for both linear and quadratic models. Such high ratios would indicate an increased shelf life relative to peanut due to a slower rate of oxidative rancidity development (Das, 2013). This would also indicate a higher level of stability against thermal degradation but other physiochemical characteristics must be considered.

The acid value is a measure of the free fatty acid content in lipids. A high acid value implies an elevated level of FFAs which leads to decreased thermal and oxidative stability. This could also lead to rapid hydroperoxide development (Lin, 2011). Table 3.4 shows the mean acid values and standard deviations obtained in this experiment. Differences were observed between each oil over time ($p \leq 0.001$). Both Camellia and soybean oils possessed the highest acid values at 1.06 ± 0.04mg KOH/g oil and 1.18 ± 0.10 mg KOH/ g oil respectively. Increases in acidity can be attributed to the oxidation and hydrolysis occurring within the oil under frying conditions. Although Camellia acid value levels were higher than peanut oil, they were still comparable to soybean oil. Figure 3.1 graphically represents the change of acid value over time for each oil. Addition of oil to each fryer to return it to its initial volume could have an effect on the trends of fatty acid breakdown. Significant responses could be observed between TPM and AV for Camellia ($p \leq 0.001$), peanut ($p \leq 0.001$), and soybean oils ($p \leq 0.001$). TPM can be seen here as an overall measure of the extent of thermal degradation in each tested oil. The relationship between TPM and AV for Camellia, peanut, and soybean oils can be seen in Figure 3.4. Camellia oil exhibited a positive quadratic response ($R^2=0.913$) while soybean oil possessed a negative quadratic response ($R^2=0.901$). No further trends could be observed between TPM and AV for peanut oil.

Results for peroxide value testing can be seen in Table 3.5. The PV can be seen as an indicator of the level of primary oxidation occurring within the oil during both the initiation and propagation of phases of degradation. Oxidative stability of an oil decreases with increased PV levels. At frying temperatures, hydroperoxides are constantly being created and decomposed into volatile and nonvolatile compounds causing the PV levels to rise and fall [16]. Differences were observed between each oil over time ($p \leq 0.001$). Initial PV and acid values are two quality characteristic that have been shown to directly influence the rate of TPM change in an oil at frying temperatures (R., 2013). Figure 3.5 shows regression analysis between PV and acid value for Camellia, peanut, and soybean frying oils. Regression analysis between PV and acid value for Camellia oil showed a significant relationship ($p \leq 0.001$) with a positive quadratic model associated with the data ($R^2=0.913$). Soybean ($p \leq 0.001$) and peanut ($p \leq 0.004$) oils both exhibited significant relationships between PV and acid value however coefficients of determination were weak for both linear and quadratic models. Peanut oil also had the lowest acid values which directly contributes to the rate of peroxide formation at frying temperatures (Lin, 2011). All values observed for PV testing for each oil were lower than 2 meq peroxide/kg oil, the point at which an oil becomes rancid. PV's for Camellia oil were highest with values peaking at 1.76 ± 0.02meq peroxide/kg oil on the fourth day of frying. Differences could be observed between every interval with the exception of intervals four and five. High PV concentration in Camellia oil can be attributed to its higher acid value relative to the other oils tested.

It has been long accepted that an analysis of TPM is one of the most reliable oil deterioration indicators [9]. The presence of these polar compounds indicate triglyceride

degradation resulting in the formation of monoglycerides and triglyceride dimers [16]. TPM testing results can be seen in Table 3.6. All three oils exhibited a change in TPM over time with Figure 3.3 showing an increase of TPM for each oil. Due to the accuracy of the instrument used, standard deviations for this portion of the experiment are mostly uniform. Testing with TPM probes have however been proven to be as accurate as other column chromatography techniques used to find these values [16]. Due to health concerns, many have stated that oil should be discarded when polar materials equal or exceed 27% [4,9]. This has caused many countries to set 25% as the legal limit for consumption [6]. Soybean was the only oil to exceed legal limits for this analysis exhibiting the highest peak value of 28.33 ± 0.29g/100g oil after 25 hours of frying.

These high TPM values can be linked to the high initial PV and acid number values relative to the other oils tested which directly affect the rate of polymeric material formation. The TPM of peanut oil showed the lowest initial values and least deviation over time with an increase of only 7.66 ± 0.29g/100g oil. Peanut oil contained the lowest initial PV and acid values, directly contributing to its oxidative stability. Rapid increases in these values can be attributed to the elevated initial PV's observed in this study relative to other oils. At the conclusion of the study, Camellia oil TPM levels were still 150% lower than soybean oil. Regression analysis between the ratio of PV to acid value against TPM for Camellia, peanut, and soybean oils can be seen in Figure 3.7. Camellia ($p \leq 0.001$), peanut ($p \leq 0.017$), and soybean oils ($p \leq 0.001$) all exhibited significant relationships. PV/AV and TPM for both Camellia ($R^2=0.945$) and soybean oils ($R^2=0.933$) produced strong quadratic responses with a decrease in PV/AV ratio leading to an increase in TPM. No strong linear or quadratic models were observed for peanut oil.

Conclusion

Overall, peanut oil was the most successful at withstanding thermal degradation. Its fatty acid profile, PV, acid value, and TPM exhibited the least change over time. The results of this study, however, do favor Camellia oil for potential use in the frying industry. Although Camellia's fatty acid profile reacted more to frying temperatures than peanut, it still possesses a lower SFA and higher unsaturated FA content than peanut giving it greater nutritional value with the same functionality. Camellia TPM value also never exceeded legal limits as could be seen in soybean oil, another leading cooking oil in United States markets.

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Table 3.1: Change in fatty acid profile of Camellia oil over time

Time (h)	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Gadoleic (C20:1)	Eicosadienoic (C20:2)	Behenic (C22:0)	%SFA	%MUFA	%PUFA
0	9.05 ± 0.01	2.26 ± 0.00	78.4 ± 0.01	7.88 ± 0.01	0.69 ± 0.00	0.55 ± 0.00	0.41 ± 0.00	11.7	79.1	8.43
5	7.48 ± 0.02	2.93 ± 0.01	79.4 ± 0.33	7.77 ± 0.03	0.53 ± 0.00	0.50 ± 0.01	0.36 ± 0.01	10.8	79.5	8.39
10	7.74 ± 0.01	2.88 ± 0.01	79.3 ± 0.15	7.36 ± 0.03	0.55 ± 0.01	0.50 ± 0.03	0.34 ± 0.02	11.0	79.7	8.32
15	8.15 ± 0.00	2.88 ± 0.02	79.3 ± 0.20	6.69 ± 0.03	0.55 ± 0.01	0.56 ± 0.01	0.29 ± 0.01	11.3	80.0	8.27
20	8.47 ± 0.01	2.85 ± 0.01	79.6 ± 0.16	5.90 ± 0.00	0.56 ± 0.00	0.60 ± 0.01	0.28 ± 0.01	11.6	79.9	8.14
25	8.59 ± 0.01	2.78 ± 0.00	79.3 ± 0.15	6.30 ± 0.01	0.57 ± 0.00	0.57 ± 0.05	0.23 ± 0.02	11.6	79.9	8.01
Significance	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.052$			

This figure shows the change in each fatty acid in the profile of Camellia over time with standard deviation. Significance values are listed for each fatty acid individually. Change in %SFA, %MUFA, and %PUFA are also presented here and calculated from relative values observed at each day. Control data is represented by hour zero. The unit of measure for this analysis is g/100 g oil.

Table 3.2: Change in fatty acid profile of peanut frying oil over time

Time (h)	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Arachidic (C20:0)	Gadoleic (C20:1)	α – Linolenic (C18:3 n3)	Eicosadienoic (C20:2)	Behenic (C22:0)	Lignoceric (C24:0)	%SFA	%MUFA	%PUFA
0	7.75 ± 0.05	2.67 ± 0.03	59.0 ± 0.06	21.0 ± 0.02	1.19 ± 0.01	1.89 ± 0.00	2.86 ± 0.02	0.41 ± 0.00	2.53 ± 0.00	1.40 ± 0.00	14.1	60.9	24.2
5	7.77 ± 0.02	2.53 ± 0.01	58.9 ± 0.11	20.9 ± 0.01	1.14 ± 0.00	1.82 ± 0.00	2.35 ± 0.03	0.40 ± 0.02	2.50 ± 0.01	1.37 ± 0.00	13.9	60.8	24.1
10	7.79 ± 0.04	2.54 ± 0.02	59.0 ± 0.15	20.8 ± 0.06	1.14 ± 0.01	1.82 ± 0.01	2.30 ± 0.03	0.46 ± 0.04	2.51 ± 0.00	1.38 ± 0.01	14.0	60.7	23.9
15	7.73 ± 0.12	2.54 ± 0.03	58.5 ± 0.61	20.4 ± 0.17	1.13 ± 0.02	1.82 ± 0.02	2.24 ± 0.06	0.45 ± 0.05	2.51 ± 0.03	1.37 ± 0.02	13.9	60.7	23.7
20	7.76 ± 0.03	2.55 ± 0.00	58.8 ± 0.04	20.3 ± 0.00	1.14 ± 0.00	1.83 ± 0.00	2.20 ± 0.02	0.51 ± 0.02	2.52 ± 0.01	1.37 ± 0.00	1.9	60.7	23.6
25	7.83 ± 0.03	2.55 ± 0.00	58.8 ± 0.05	20.3 ± 0.09	1.13 ± 0.01	1.84 ± 0.00	2.17 ± 0.05	0.55 ± 0.05	2.54 ± 0.01	1.38 ± 0.00	14.0	60.8	23.6
Significance (p≤)	0.421	0.001	0.277	0.001	0.001	0.001	0.001	0.001	0.059	0.012			

This figure shows the each fatty acid in the profile of peanut oil over time with standard deviation. Significance values are listed for each fatty acid individually. Change in %SFA, %MUFA, and %PUFA are also presented here and calculated from relative values observed at each day. Control data is represented by hour zero. The unit of measure for this analysis is g/100 g oil.

Table 3.3: Change in fatty acid profile of soybean frying oil over time

Time (h)	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	γ – Linolenic (C18:3 n6)	Gadoleic (C20:1)	α – Linolenic (C18:3 n3)	Eicosadienoic (C20:2)	Behenic (C22:0)	%SFA	%MUFA	%PUFA
0	10.1 ± 0.00	4.50 ± 0.00	21.1 ± 0.00	51.0 ± 0	0.14 ± 0.00	0.37 ± 0.00	6.02 ± 0.00	0.20 ± 0.00	0.40 ± 0.00	15.0	21.5	58.2
5	10.9 ± 0.05	4.94 ± 0.02	21.9 ± 0.06	51.5 ± 0.05	0.17 ± 0.00	0.39 ± 0.01	6.76 ± 0.02	0.22 ± 0.00	0.42 ± 0.00	16.3	21.8	58.4
10	11.2 ± 0.02	5.03 ± 0.01	22.4 ± 0.17	51.1 ± 0.01	0.18 ± 0.00	0.40 ± 0.01	6.53 ± 0.00	0.22 ± 0.01	0.44 ± 0.00	16.6	22.1	58.6
15	11.4 ± 0.02	5.12 ± 0.01	22.5 ± 0.04	50.5 ± 0.10	0.19 ± 0.00	0.41 ± 0.01	6.31 ± 0.02	0.22 ± 0.01	0.44 ± 0.02	16.9	22.3	58.2
20	11.6 ± 0.01	5.23 ± 0.00	22.8 ± 0.03	49.8 ± 0.08	0.20 ± 0.00	0.44 ± 0.00	6.05 ± 0.02	0.25 ± 0.01	0.44 ± 0.00	17.3	22.4	57.8
25	11.6 ± 0.01	5.23 ± 0.01	22.8 ± 0.03	49.6 ± 0.07	0.22 ± 0.00	0.44 ± 0.02	5.92 ± 0.14	0.23 ± 0.03	0.44 ± 0.00	17.3	22.5	57.4
Significance (p≤)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.019	0.459			

This figure shows the each fatty acid in the profile of soybean oil over time with standard deviation. Significance values are listed for each fatty acid individually. Change in %SFA, %MUFA, and %PUFA are also presented here and calculated from relative values observed at each day. Control data is represented by hour zero. The unit of measure for this analysis is g/100 g oil.

Table 3.4: Change in acid value (AV) for Camellia, peanut, and soybean frying oils over time ($p \leq 0.001$)

Time (h)	Camellia	Peanut	Soybean
0	0.05 ± 0.00	0.10 ± 0.02	0.16 ± 0.00
5	0.25 ± 0.08	0.26 ± 0.01	0.79 ± 0.03
10	0.39 ± 0.13	0.60 ± 0.01	1.07 ± 0.08
15	0.85 ± 0.02	0.64 ± 0.02	1.08 ± 0.05
20	1.10 ± 0.12	0.44 ± 0.15	1.15 ± 0.05
25	1.06 ± 0.04	0.45 ± 0.03	1.18 ± 0.10

This figure shows the mean AV for Camellia, peanut, and soybean oils at each time interval along with its standard deviation. Differences in AV were observed for each oil ($p \leq 0.001$). Control data is represented by hour zero. Acid values are reported in mg KOH/g oil.

Table 3.5: Change in peroxide value (PV) of Camellia, peanut, and soybean frying oils over time ($p \leq 0.001$)

Time (h)	Camellia	Peanut	Soybean
0	0.56 ± 0.05	0.06 ± 0.02	0.08 ± 0.03
5	0.73 ± 0.07	0.17 ± 0.03	0.09 ± 0.04
10	1.02 ± 0.03	0.19 ± 0.01	0.10 ± 0.08
15	1.68 ± 0.09	0.15 ± 0.01	0.89 ± 0.03
20	1.76 ± 0.2	0.14 ± 0.00	0.95 ± 0.10
25	1.29 ± 0.08	0.19 ± 0.02	1.01 ± 0.02

This table shows the mean peroxide value for Camellia, peanut, and soybean oils at each time interval along with its standard deviation. Differences in peroxide value were observed for each oil ($p \leq 0.001$). Control data is represented by hour zero. The unit of measure for this analysis is meq peroxide/kg oil.

Table 3.6: Change in total polymeric materials (TPM) of Camellia, peanut, and soybean frying oils over time (p≤0.001)

Time (h)	Camellia	Peanut	Soybean
0	1.33 ± 0.29	3.17 ± 0.29	6.33± 0.29
5	8.67 ± 0.29	7.67 ± 0.29	14.7 ± 0.29
10	12.2 ± 0.29	9.83 ± 0.29	19.7 ± 0.29
15	14.8 ± 0.29	8.83 ± 0.29	23.2 ± 0.29
20	17.5 ± 0.29	9.83 ± 0.29	23.8 ± 0.29
25	19.2 ± 0.29	10.8 ± 0.29	28.3 ± 0.29

This table shows the mean TPM value and standard deviation for Camellia, peanut, and soybean oils at each time interval. Differences in TPM were observed for each oil ($p \leq 0.001$). Control data is represented by hour zero. Similarities in standard deviation are due to the limited resolution of the instrument used. The unit of measure for TPM is g/100g oil.

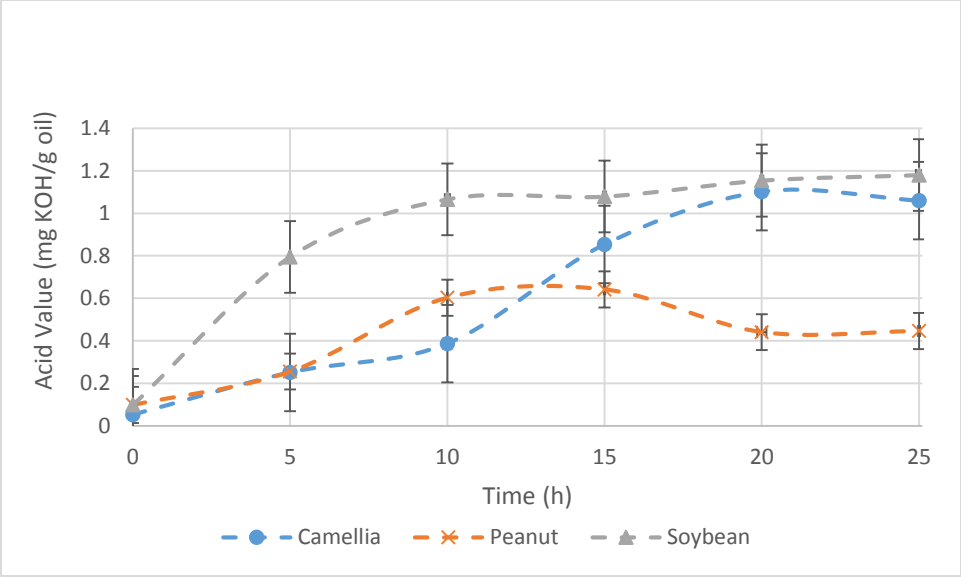

Figure 3.1: Change in acid value (AV) of Camellia, peanut, and soybean frying oils over time ($p \leq 0.001$)

This figure graphically represents the change in AV over time for Camellia, peanut, and soybean oils. Differences in AV were observed between each oil ($p \leq 0.001$). Each point represents a mean along with its standard deviation represented by the error bars. Control data is represented by hour zero. Error bars are presented in the graphic but, due to such small standard deviations some points in the data set they could not be visualized.

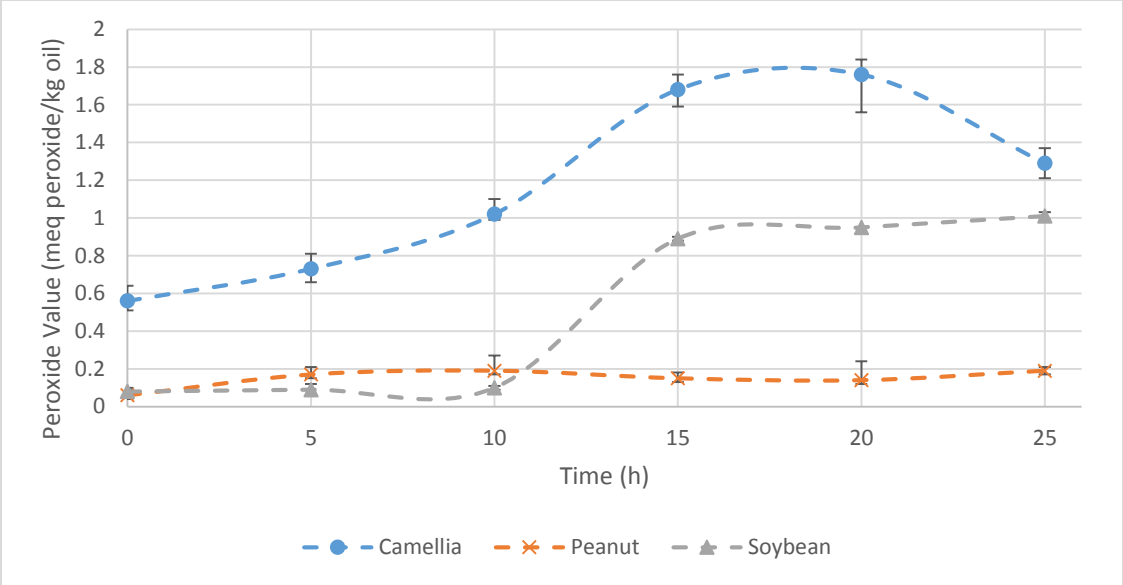

Figure 3.2: Change in peroxide value (PV) of Camellia, peanut, and soybean frying oil over time ($p \leq 0.001$)

This figure graphically represents the change in PV over time for Camellia, peanut, and soybean oils. Differences in PV were observed between each oil ($p \leq 0.001$). Each point represents a mean along with its standard deviation represented by the error bars. Control data is represented by hour zero. Error bars are presented in the graphic but, due to such small standard deviations some points in the data set they could not be visualized.

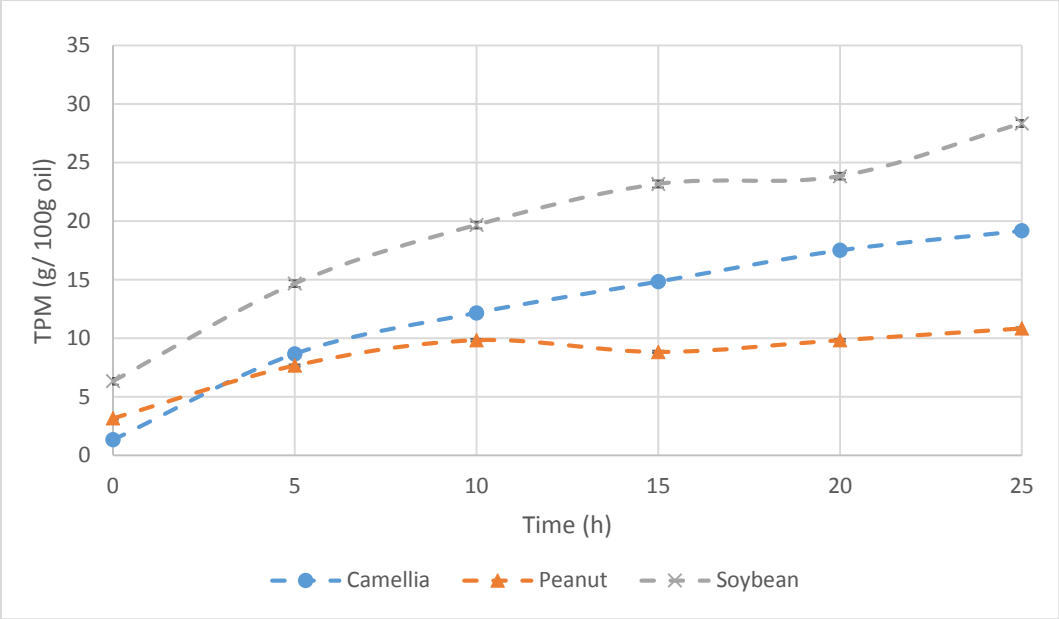

Figure 3.3: Change in total polymeric materials (TPM) of Camellia, peanut, and soybean frying oil over time ($p \leq 0.001$)

This figure graphically represents change in TPM over time for Camellia, peanut, and soybean oils. Differences were observed between each oil ($p \leq 0.001$). Each point represents a mean along with its standard deviation represented by error bars. Control data is represented by hour zero. Error bars are presented in the graphic but, due to such small standard deviations some points in the data set they could not be visualized.

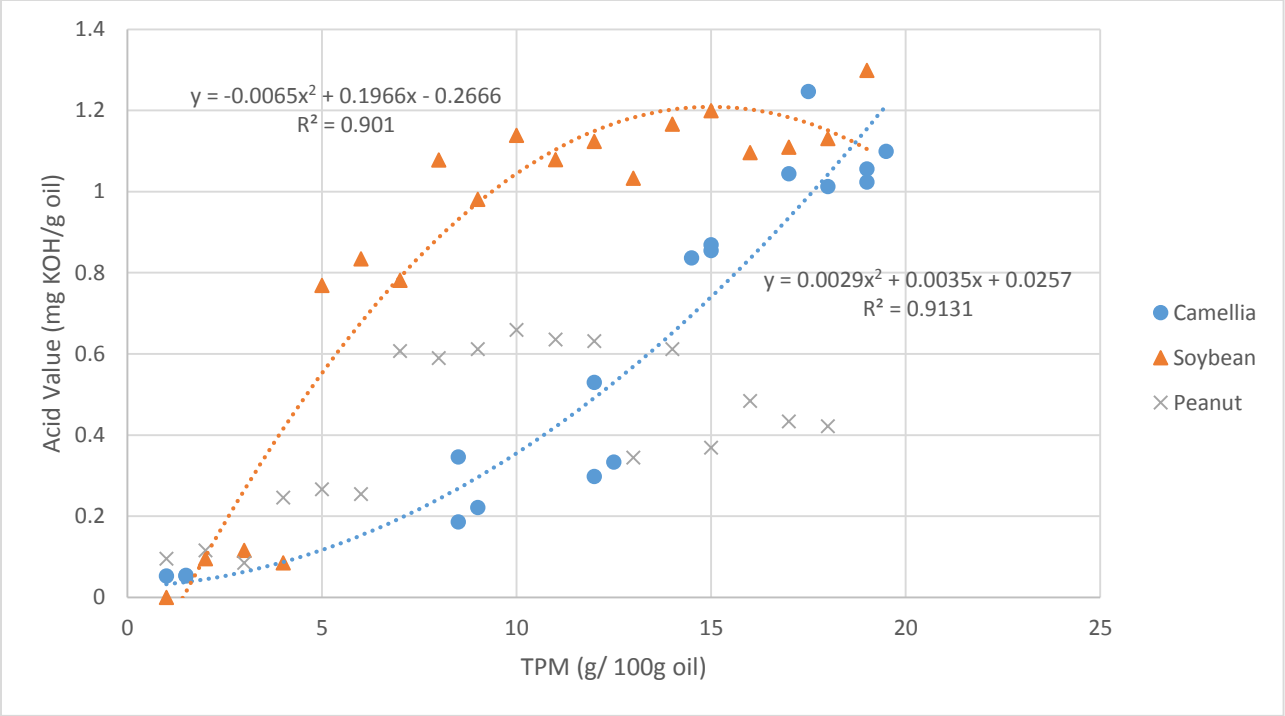

Figure 3.4: Regression analysis between TPM and AV for Camellia, peanut, and soybean frying oils

This figure shows regression analysis between TPM and acid value for Camellia ($p \leq 0.001$), peanut ($p \leq 0.001$), and soybean oils ($p \leq 0.001$). Camellia ($R^2=0.913$) and soybean ($R^2=0.901$) oils both exhibited a quadratic response with the formula for their models shown above. No strong model could be observed between TPM and acid value for peanut oil. Control data is represented by hour zero.

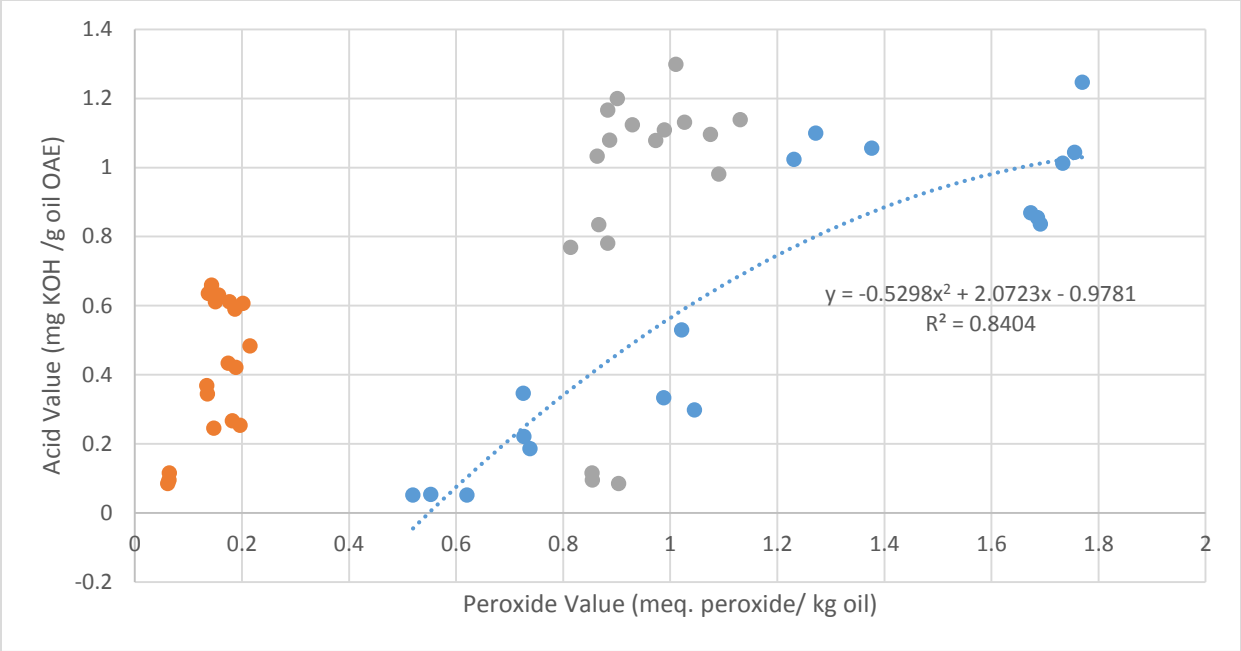

Figure 3.5: Regression analysis between PV and AV for Camellia, peanut, and soybean frying oils

This figure shows regression analysis between peroxide and acid value for each oil. Soybean ($p \leq 0.001$) and peanut ($p \leq 0.004$) oils both exhibited a response however further trends could not be observed for peanut. Regression analysis between PV and acid value for Camellia oil ($R^2=0.913$) produced a quadratic response with the formula for the model shown above. Control data is represented by hour zero.

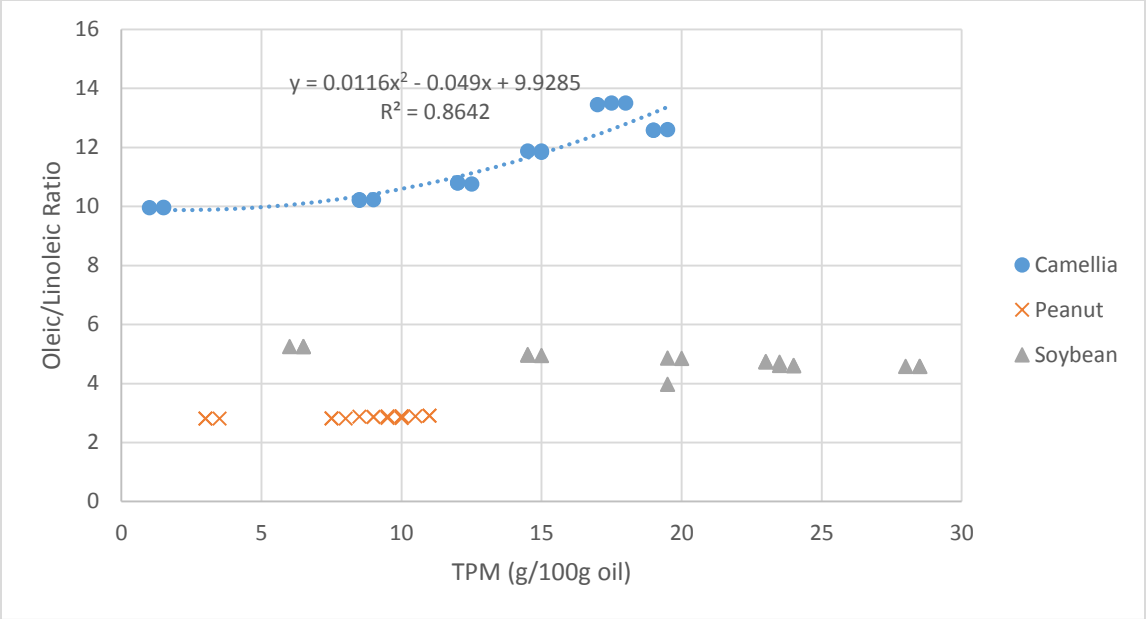

Figure 3.6: Regression analysis between the ratio of oleic acid and linoleic acid (O/L) against total polymeric materials (TPM) for Camellia, peanut, and soybean frying oils

This figure shows regression analysis between the oleic acid/linoleic acid (O/L) ratio and TPM for each oil. Camellia ($p \leq 0.001$), peanut ($p \leq 0.002$), and soybean oils ($p \leq 0.001$) all exhibited a response however further trends could not be observed for peanut and soybean. O/L and TPM for Camellia oil ($R^2=0.864$) produced a quadratic response with the formula for the model shown above. Control data is represented by hour zero.

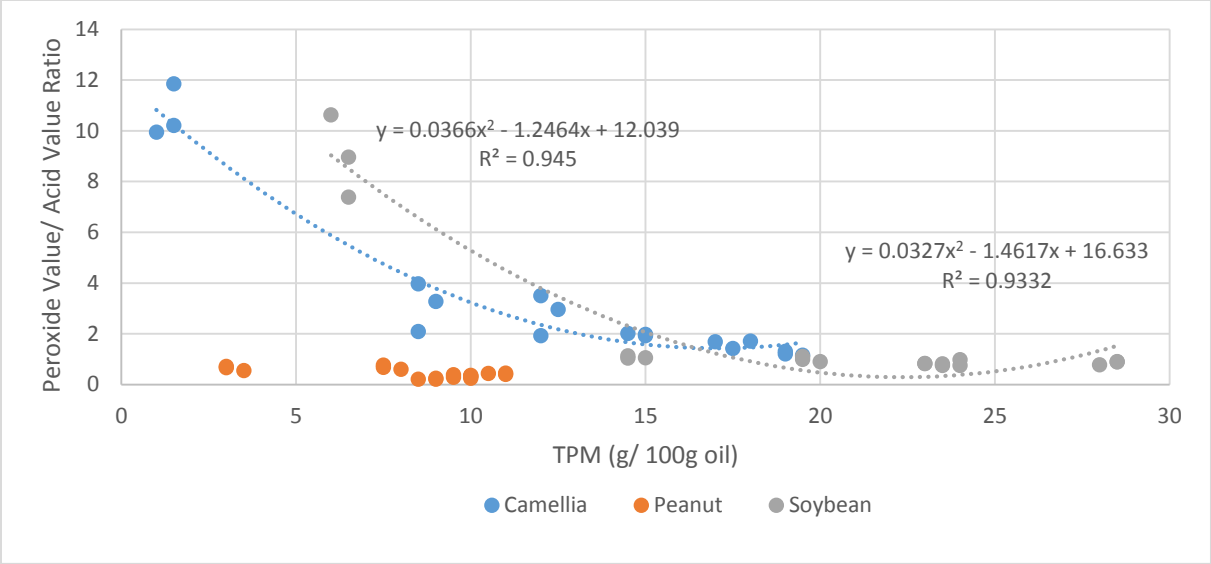

Figure 3.7: Regression analysis between the ratio of peroxide value (PV) and acid value (AV) against total polymeric materials (TPM) for Camellia, peanut, and soybean frying oils

This figure shows regression analysis between the ratio of PV and AV against TPM for each oil. Camellia ($p \leq 0.001$), peanut ($p \leq 0.017$), and soybean oils ($p \leq 0.001$) all exhibited a response however further trends could not be observed for peanut. PV/AV and TPM for both Camellia ($R^2=0.945$) and soybean oils ($R^2=0.933$) produced a quadratic response with the formula for their models shown above. Control data is represented by hour zero.

CHAPTER 4

Thermal Degradation Analysis of Biodiesel Made from Camellia, Peanut, and Soybean oils

[3]Allen, C., D. Geller, R. Pegg, and J. Ruter. To be submitted to *Biofuel Research Journal*

Abstract

Camellia oleifera is an oilseed crop native to the southern provinces of China, where it has been used traditionally as a cooking oil for thousands of years. Certain physiochemical properties of Camellia oil provide nutritional benefits while contributing to its heat stability and potential for use as biodiesel. Biodiesels are fuels composed of monoalkyl esters of long chain fatty acids which have been converted from animal fats and vegetable oils. This study examined the physiochemical properties of biodiesel produced from *C. oleifera,* peanut, and soybean oils before and after thermal degradation has occurred. Waste cooking oil from the previous study was converted to biodiesel and compared to samples produced from fresh cooking oil. Camellia produced a biodiesel product comparable to soybean oil, a leading biodiesel feedstock in the United States, while meeting the fuel standards of both the U.S. and European Union. Changes in fatty acid profile were minimal for all three oils tested. Peroxide values for Camellia increased by 0.2meq peroxide/kg oil, acid values increased by 0.04mg KOH/g oil, flash point temperature increased by 1 ˚C and cloud point temperature decreased by 12 ˚C.

Introduction

Limitations of petroleum fuel reserves and growing concern over society's contribution to global warming has made biodiesel an attractive alternative energy source. The most widely adapted technique currently used in the industry to produce biodiesel is transesterification due to the small amount of catalyst required, short reaction time, and high conversion rates [6]. The chemical composition of biodiesel allows for it to be blended with diesel fuel or used in its pure form known as "neat" biodiesel. The United States currently consumes approximately 188 million tons of diesel per year with

global consumption approaching 1 billion tons [15]. Soybean oil, the leading feedstock for biodiesel production in the U.S., can meet only 0.3 % of the biodiesel demand due to oil supply [3]. This presents the need for another reliable source to contribute to biodiesel production globally and in the United States.

Camellia oleifera is an oilseed crop native to Southeast Asia where it supplies over one seventh of the Chinese population with cooking oil [12]. *Camellia* is a genus of flowering evergreen trees and shrubs ranging from 15 – 25 feet tall taking an average of 2 – 3 years to mature [1]. Once mature, a plant can produce seed for 15 – 60 years with one year from flowing to fruit [17]. Camellia is considered one of the four main oilseed crops, along with coconut, palm and olive, with oil constituting approximately 40 – 50 % of the seed weight [13]. China is currently the world's leader in Camellia oil production. Roughly 3.5 million hectares of Camellia are planted across 17 provinces with 98 % of the cultivated space being dedicated to *C. oleifera [16]*. Each year 150 thousand tons of oil are produced worth an estimated $1 billion [11]. The defatted cake remaining from processing also has potential uses. Protein levels have been found at upwards of 20% making it an ideal candidate for animal feed [18]. Triterpenoid saponins extracted from the cake have been found to have strong antimicrobial effect against *E. coli, A. niger, P. citrinum,* and *C. uitilis* while also deterring larval development in insects making Camellia a natural biological pesticide [11].

The purpose of this study is to examine the biodiesel properties of *C. oleifera* before and after thermal degradation. There are various nutritional and medicinal benefits associated with consuming Camellia oil allowing it to demand a higher price in consumer markets. These same physiochemical properties make Camellia a potential biodiesel

feedstock. It is possible that the oil can be used for cooking purposes and then biodiesel production while creating a fuel comparable to what would obtained from its virgin oil. To assess fuel quality, fatty acid profiling, acid value testing, peroxide value analysis, and cloud and flash point temperature determination will be performed.

Materials and Methods

Frying Performance Testing

Frying oil performance tests were conducted on Camellia, peanut, and soybean oils. *C. oleifera* oil to be tested has been donated by commercial tea seed oil producer Arette at a total of fifteen liters of oil, ten liters of refined oil and five liters of their "natural" less refined oil. 3.5L of each oil was randomly added to one of three Rival® CZF725 professional style deep-fryers and heated to a temperature of approximately 180 ºC. Potatoes were washed and cut into 9mm x 9mm pieces of varying length using a Nemco® N55450 potato press. 200g of potatoes were added to each fryer and cooked for seven minutes per batch averaging one batch ever 10-12 minutes. Each oil was subjected to five hours of frying each day for a period of five consecutive days. After each day, 100mL of oil was taken from each fryer and stored at 0 ºC for future analysis. Sufficient oil was added to each fryer at the beginning of each frying day to return the oil to its original volume.

Biodiesel Production

Waste oil recovered from the frying performance testing was used to produce biodiesel. 500mL oil was first filtered through cheese cloth to remove any heavy debris. To remove any remaining water the oil was slowly heated to 100°C and held until any boiling stopped. The temperature was then raised to 130°C and held for ten minutes. Acid

values were obtained from each oil sample to determine the amount of potassium hydroxide catalyst needed to complete the reaction. Once acid values are obtained, catalyst needed can be calculated using the following equation:

$$Potassium\ Hydroxide\ (mg) = (A * V) + (3.5 * V)$$ where,
A = acid value (mg)
V = volume biodiesel to be made

The appropriate amount of catalyst was then combined with methanol equaling twenty percent of the weight of the oil sample. The vegetable oil was then lowered and held within a range of 48 – 54 °C. The methanolic potassium hydroxide solution can now be added to the oil. The mixture was kept at temperature under constant agitation for 1 hour and then transferred to a separatory funnel. After settling for at least 8 hours, a distinct glycerol layer forms below the biodiesel and was removed. To wash the biodiesel of any contaminants, water equaling forty percent of the biodiesels volume was added to the funnel. The funnel was then vigorously agitated for five minutes and then allowed to separate. A new biodiesel sample was prepared if a distinct separation did not occur within two hours. The sample was allowed to separate for 12-24 hours upon which the water was removed from the bottom portion of the sample. The water wash step was repeated a minimum of three times or until the wash water remained clear after separation. Processed biodiesel samples were then heated to 55°C to remove any residual water and triple filtered. Cloud point values were obtained using a Phase Technology © CPA-T30 portable cloud point analyzer. Flash temperatures were produced using a Koehler K16500 closed cup rapid flash tester.

Fatty Acid Profiling

Profiles were obtained using an Agilent© 7683 gas chromatograph with flame ionized detection (GC-FID) and 7683B auto-sampler. A 10µL syringe was used to inject 1µL samples through an 11mm inlet septum. Both the injector and detector temperatures should be set to 250 °C. Samples were loaded on a Supelco© SP-2560 column having a length of 100m, inner diameter of 0.25mm, and a film thickness of 2µm. The GC was set to a split ratio of 50:1 under constant flow with helium as the carrier gas generating a head pressure of ~40psi. The flame produced by the FID component was generated from a mixture of compressed air flowing at 450mL/min, helium at 40mL/min, and helium makeup gas at 23.9mL/min. The initial oven temperature was set to 140 °C and held for 5 minutes. The temperatures was then increased at a rate of 5 °C/min to a final temperature of 240 °C. This temperature was held for 15 minutes resulting in a total run time of 45 minutes.

Data Analysis

Data was analyzed using the "R" statistical programming console (R-Project, Vienna, Austria). An analysis of variance (ANOVA) was conducted to determine differences in fatty acid profile between control and waste biodiesel products for each oil separately (n=3). Oils subject to frying performance testing, represented by waste biodiesel values for each analysis, serve as the whole-unit treatment group (n=2). ANOVA was also conducted to determine differences between control and waste biodiesel samples for PV, acid value, cloud point, and flash point separately. Frying performance testing again served as the treatment group for each analysis. Comparisons for these analyses were conducted between all three oils. Differences in means were

measured with a 95% confidence interval using Tukey's Honest Significant Difference Test. Table and graphs were produced using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

Peroxide Value

Peroxide values were tested according to AOCS method Cd 8b-90. Approximately 2g of each sample was weighed into a 250mL Erlenmeyer flask. The oil was combined with 50mL 3:2 acetic acid isooctane solution (v/v) and 0.5mL saturated potassium iodide solution creating a golden-orange coloration. After being agitated for one minute, 30mL distilled water was added to neutralize the reaction. The mixture was then titrated with 0.1M sodium thiosulfate pentahydrate until a pale yellow color was observed. The titrant was previously standardized using potassium dichromate. A blue coloration was caused in the sample by adding 0.5mL 10% sodium dodecyl sulfate (SDS) and 0.5mL starch indicator. The titration was completed right as the starch-iodine complex disappears. Units for this analysis were measured in meq. peroxide /kg oil. The volume of titrant needed for the reaction was used to calculate the peroxide value with the following equation:

Peroxide Value = ((S-B)×M×1000)/W, where:
B = volume titrant for blank (mL)
S = volume titrant for sample (mL)
M = molarity of sodium thiosulfate solution
W = sample weight (g)

Acid Value

Acid values were calculated based on ASTM D974. Approximately 2g of each sample were added to a 250mL flask along with a 100:1:99 (v/v/v) mixture of toluene, water, and 2-propanol respectively that served as the titration solvent. 0.5mL *p*-

naptholbenzein indicator solution was added to the sampled and vigorously agitated. The sample was then immediately titrated with 0.1M potassium hydroxide solution until a green colored chromophore was held in the mixture for at least 15 seconds. Potassium hydroxide standardization was performed on the titration solution. Acid values were calculated using the following equation:

Acid Value = ((A-B)×M×56.1)/ W, where:
A = vol titration for sample (mL)
B = vol titrant for blank (mL)
M = molarity of the KOH solution
W = sample weight (g)

Results and Discussion

Control and waste Camellia biodiesel fatty acid profiles can be found in Table 4.1. Each biodiesel shows a significant change in fatty acids when compared to its control. There were 5 fatty acids observed in the profile of Camellia biodiesel with significant differences seen between control and waste samples for each fatty acid. Camellia biodiesel was mostly composed of oleic acid. Levels of this fatty acid specifically were found at 79.4 ± 0.02% and 80.0 ± 0.10% for Camellia control and waste oil sample respectively. High oleic content can be a direct contributor to increased thermal stability. Elevated levels of this fatty acid in addition to high linoleic acid content produce high levels of nitric oxide and nitrogen dioxide when combusted [14]. Camellia exhibited the least change between control and waste biodiesel for total SFA, MUFA, and PUFA compared to peanut and soybean oils. There were increases in both total SFA ($p \leq 0.001$) and MUFA ($p \leq 0.001$) relative to the control for Camellia oil. The high thermal stability of oleic acid slows the rate of total MUFA degradation leading to its increase.

The difference in total SFA waste biodiesel increased 2.65% relative to the control with total MUFA increasing 0.74%. Total PUFA decreased in wasted samples 10.5% below the control ($p \leq 0.001$). Decreases in PUFA can be attributed to the instability of these structures at high temperatures.

Fatty acid profiles for control and waste soybean biodiesel samples can be found in Table 4.3. Significant differences were observed between control and waste values for each fatty acid in the profile. There was a decrease in both total PUFA ($p \leq 0.001$) and MUFA ($p \leq 0.001$) in soybean oil compared to the control. The difference in total PUFA decreased 16.1% relative to the control while MUFA decreased 13.2%. An increase was observed in total SFA values relative to the control ($p \leq 0.001$) with a difference of 33.5%.

Table 4.2 shows the fatty acid profiles of both waste and control peanut biodiesel. There were 9 fatty acids observed in both profiles with significant differences observed between control and waste values for each sample. These samples had the highest initial concentrations of PUFA's, 35.9 ± 0.04 %, negatively effecting the thermal and oxidative stability of the fuel [7]. There was a decrease in total PUFA observed between control and waste peanut biodiesel, consistent with expected results ($p \leq 0.001$). The difference in total PUFA decreased 35.9% below the control, greater than both Camellia and soybean biodiesel. Decreasing PUFA values have a direct effect on total MUFA. There was an increase in total MUFA in waste biodiesel relative to the control ($p \leq 0.001$). Peanut also saw the greatest change in this property compared to Camellia and soybean biodiesel with a 24.3% decrease relative to the control. A decrease of 16.1% relative to the control was also observed for total SFA values ($p \leq 0.001$).

Results for PV, acid number, cloud point, and flash temperature testing can also be found in the first three tables with their respective oil types. A graphic representation of peroxide values for each oil can be found in Figure 4.3. An oils PV is a measure of primary oxidation products, or hydroperoxides. There are currently no limits for PV set by the United States or 0European Union. There was a significant relationship between peroxide value and oil type ($p \leq 0.002$). Control peanut oil samples were found to have the highest values at 1.27 ± 0.02meq peroxide/100g oil. These values were significantly different from biodiesel produced from control Camellia oil which had the lowest PV's at 0.136 ± 0.01meq peroxide/100g oil. Such low values suggest a high degree of oxidative stability and a slower rate of lipid deterioration in reference to the other oils tested. There was also no difference between control and waste Camellia PV's implying similar levels of primary oxidation [5]. These values are drastically lower than the 7.31meq peroxide/100g oil produced in other studies with Camellia using supercritical-methanol transesterification [7]. No other values could be found for biodiesel produced from *C. oleifera* using the standard methanol transesterification employed in this study.

There was no significant difference in PV observed between soybean control and waste biodiesel ($p \leq 0.073$). No significant difference was also observed between soybean control and peanut waste biodiesel ($p \leq 0.424$). Vegetable oil was also found to have a much lower peroxide value than was expected. Studies show biodiesel produced from pure soybean oil to have a PV of 5meq peroxide/100g oil [10] when this study found control sample values at 0.97 ± 0.16meq peroxide/100g oil. Such discrepancy between the PV's observed in this study and what was expected can be attributed to differences in production method that resulting in slightly altered products.

Figure 4.4 graphically represents the acid values observed in both waste and control biodiesel produced from Camellia, peanut, and soybean oils. The acid value of an oil is a physical measure of the carboxylic acid content of a sample. There was a significant relationship observed between acid value and oil type ($p \leq 0.001$). Control and waste peanut oil had the highest acid values with no difference between them ($p \leq 1.000$). There was also no significant difference between waste Camellia and both waste ($p \leq 0.854$) and control ($p \leq 0.824$) peanut biodiesel. A high acid value implies an elevated free fatty acid content which leads to decreased thermal and oxidative stability. This could also lead to a rapid hydroperoxide development (Lin, 2011). Mean values for each oil, control and waste, were below the limits set by the United States and European Union of a maximum 0.50mg KOH/g oil [2].

Acid value analysis show no difference between control and waste concentrations in Camellia biodiesel ($p \leq 0.241$). Similarities in acid value between control and waste Camellia suggest this oil is able withstand oxidative stress. With Camellia demanding a higher price as a cooking oil due to its nutritive properties, it is important that a comparable fuel can be produced even after prolonged exposure to high heat. There was also no significant difference observed between waste Camellia and waste soybean biodiesel products ($p \leq 0.131$). The difference in acid value between waste Camellia and both control ($p \leq 0.021$) and waste soybean ($p \leq 0.029$) biodiesel were significant. These results show that acid values in both control and waste Camellia are comparable to soybean biodiesel. Control vegetable biodiesel levels were much lower than expected. This study observed an acid value of 0.06 ± 0.02mg KOH/g oil [10] when previous studies show levels closer to 0.90mg KOH/g oil. Biodiesel produced from waste Camellia

oil also had slightly lower values than expected. Previous research show acid values for Camellia to average 0.22mg KOH/g oil [17] when a value of 0.14 ± 0.03mg KOH/g oil was observed in this study. Differences between observed and expected values can be attributed to differences in feedstock and production method.

The cloud point is defined as the temperature in which the first stages of crystallization occur in a biodiesel product [15]. A graphical representation of the cloud point temperature of both control and waste biodiesel product for each oil is shown in Figure 4.2. There was a significant relationship observed between cloud point temperature and oil type ($p \leq 0.001$). Waste Camellia biodiesel had the lowest cloud point temperature at -5.52 ± 0.03 °C. These values are much lower than 7.52 ± 0.77 °C, the cloud point observed for control Camellia samples. Degradation of fatty acids, specifically SFA, during the frying performance testing phase could account for the lowered temperatures in the waste oil biodiesel. No other loud point values could be found for *C. oleifera* biodiesel. Cloud point temperatures for waste Camellia biodiesel were also found to be significantly different than both control ($p \leq 0.001$) and waste soybean biodiesel ($p \leq 0.001$). The difference in cloud point temperature of waste Camellia biodiesel compared to control and waste soybean products decreased by 75.5% and 86.2% respectively. These results show that in addition to improving cold weather properties compared to the control, thermally degraded Camellia oil produces cloud point temperatures well below soybean oil, the leading biodiesel feedstock used in American markets.

There was no significant difference cloud point between control and waste peanut oil samples ($p \leq 0.371$). Their temperatures were 15.8 ± 0.33 °C and 16.4 ± 0.31 °C

respectively. Similarities between control and waste samples can be attributed to the increase stability of peanut oil during frying performance testing relative to Camellia and soybean oils. Such high cloud points would limit the uses of this biodiesel in cold environments. Efforts could be made to decrease the cloud point through winterization but this would in effect increase the cost associated with production using this feedstock. The expected cloud point for soybean biodiesel was -4.08 ± 0.12 °C [15]. This study found a slightly higher temperature for control samples at -1.35 ± 0.08 °C. It is possible that biodiesel samples used in this study contained higher levels of SFA resulting in a higher cloud point. Differences in storage time could also play a role in elevated cloud points of all samples. Virgin and waste oils were stored for several months between thermal degradation testing and biodiesel production possibly leading to the development of residues causing premature crystallization [2].

Figure 4.1 shows the flash point temperatures of control and waste biodiesel for Camellia, peanut, and soybean oils. The flash point is the temperature at which volatile production from the sample has saturated the testing environment, causing an ignition of the fuel but not supporting combustion [4]. There was a significant relationship observed between flash point temperature and oil type ($p \leq 0.002$) although there is little variation between values. Both control and waste samples for Camellia, peanut, and soybean oil exceed minimum requirements set by the United States at 130 °C [2]. Waste Camellia biodiesel was found to have the highest flash point temperature in the study at 147 ± 0.03 °C. These values are consistent with a flash point temperature of 150 °C found in the literature [7]. Although waste Camellia exhibited the highest mean value, no significant difference could be observed when compared to control Camellia ($p \leq 0.998$), control

soybean ($p \leq 0.998$), control ($p \leq 0.214$) and waste peanut biodiesel ($p \leq 0.998$). Similar flash points observed in these products would imply comparable stability and storage properties in addition to diminished fire risk from elevated environmental temperatures [8]. Flash points for vegetable oil was also lower than expected. Waste soybean exhibited the lowest flash point with a mean of 143 ± 0.02 ˚C however there was no significant difference from control peanut biodiesel ($p \leq 0.214$). Previous studies show soybean oil producing a flash point of 165 °C. Differences in observed and expected flash points can also be attributed storage time due constant rate of fatty acid and carboxylic acid hydrolysis occurring within the oil [9].

Conclusions

This study has shown that a viable biodiesel product can be produced from *C. oleifera* oil. Fuel standards of the United States and European Union were still met when the oil was subjected to thermal degradation. Some parameters improved in biodiesel produced from waste Camellia when compared to fresh oil. With the high market price of Camellia oil it is important that a quality product can still be derive from used oil.

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Table 4.1: Fuel properties of control and waste Camellia biodiesel

Property	Unit	Camellia – Control	Camellia – Waste	Significance (p≤)
Palmitic acid	mass %	9.02 ± 0.00	8.54 ± 0.00	0.001
Stearic acid	mass %	2.25 ± 0.00	2.50 ± 0.00	0.001
Oleic acid	mass %	79.4 ± 0.00	80.0 ± 0.10	0.001
Linoleic acid	mass %	7.98 ± 0.00	7.14 ± 0.02	0.001
γ-linolenic acid	mass %	0.67 ± 0.00	0.63 ± 0.00	0.001
Total SFA	mass %	11.0 ± 0.00	11.3 ± 0.00	0.001
Total MUFA	mass %	80.0 ± 0.02	80.6 ± 0.10	0.001
Total PUFA	mass %	7.98 ± 0.00	7.14 ± 0.02	0.001
Peroxide Value	meq/100g oil	0.14 ± 0.01	0.26 ± 0.03	0.002
Acid Value	mg KOH/100g oil	0.10 ± 0.02	0.14 ± 0.03	0.021
Cloud Point	**°C**	7.52 ± 0.77	-5.52 ± 0.03	0.001
Flash Point	**°C**	146 ± 0.02	147 ± 0.03	0.998

This table shows the fuel properties of both control and waste camellia oil. Means including standard deviation and unit of measure are listed for each property. Significance values represent the relationship between control and waste samples for each property. Multiple comparisons were conducted using Tukey's honestly significant difference (HSD) test with a 95% confidence interval.

Table 4.2: Fuel properties of control and waste peanut biodiesel

Property	Unit	Peanut – Control	Peanut – Waste	Significance (p≤)
Palmitic acid	mass %	10.1 ± 0.00	7.92 ± 0.06	0.001
Stearic acid	mass %	3.36 ± 0.01	2.60 ± 0.01	0.001
Oleic acid	mass %	44.6 ± 0.02	58.4 ± 1.54	0.001
Linoleic acid	mass %	33.0 ± 0.02	20.9 ± 0.15	0.001
Arachidic acid	mass %	1.08 ± 0.00	1.13 ± 0.00	0.001
γ-linolenic acid	mass %	1.06 ± 0.01	1.79 ± 0.02	0.001
α-linolenic acid	mass %	2.82 ± 0.01	2.07 ± 0.04	0.001
Behenic acid	mass %	2.31 ± 0.00	2.46 ± 0.00	0.001
Lignoceric Acid	mass %	1.14 ± 0.00	1.35 ± 0.00	0.001
Total SFA	mass %	16.8 ± 0.01	14.1 ± 0.07	0.001
Total MUFA	mass %	45.6 ± 0.03	60.2 ± 1.56	0.001
Total PUFA	mass %	35.9 ± 0.04	23.0 ± 0.19	0.001
Peroxide Value	meq/100g oil	1.24 ± 0.02	1.00 ± 0.14	0.001
Acid Value	mg KOH/100g oil	0.16 ± 0.03	0.16 ± 0.03	0.186
Cloud Point	°C	15.8 ± 0.33	16.4 ± 0.31	0.37
Flash Point	°C	145 ± 0.03	146 ± 0.03	0.376

This table shows the fuel properties of both control and waste peanut oil. Means including standard deviation and unit of measure are listed for each property. Significance values represent the relationship between control and waste samples for each property. Multiple comparisons were conducted using Tukey's honestly significant difference (HSD) test with a 95% confidence interval.

Table 4.3: Fuel properties of control and waste soybean biodiesel

Property	Unit	Soybean – Control	Soybean – Waste	Significance (p≤)
Palmitic acid	mass %	4.11 ± 0.01	6.34 ± 0.00	0.001
Stearic acid	mass %	2.00 ± 0.00	2.92 ± 0.00	0.001
Oleic acid	mass %	58.4 ± 0.00	50.7 ± 0.00	0.001
Linoleic acid	mass %	19.0 ± 0.01	26.0 ± 0.03	0.001
γ-linolenic acid	mass %	1.67 ± 0.00	1.40 ± 0.00	0.001
α-linolenic acid	mass %	8.12 ± 0.01	6.40 ± 0.01	0.001
Behenic acid	mass %	0.36 ± 0.00	0.46 ± 0.00	0.001
Total SFA	mass %	6.47 ± 0.01	9.73 ± 0.00	
Total MUFA	mass %	60.0 ± 0.00	52.1 ± 0.00	
Total PUFA	mass %	32.3 ± 0.04	27.1 ± 0.02	
Peroxide Value	meq/100g oil	0.97 ± 0.17	0.80 ± 0.02	0.073
Acid Value	mg KOH/100g oil	0.06 ± 0.02	0.09 ± 0.02	0.058
Cloud Point	°C	-1.35 ± 0.08	0.76 ± 0.06	0.001
Flash Point	°C	146 ± 0.02	143 ± 0.02	0.007

This table shows the fuel properties of both control and waste soybean oil. Means including standard deviation and unit of measure are listed for each property. Significance values represent the relationship between control and waste samples for each property. Multiple comparisons were conducted using Tukey's honestly significant difference (HSD) test with a 95% confidence interval.

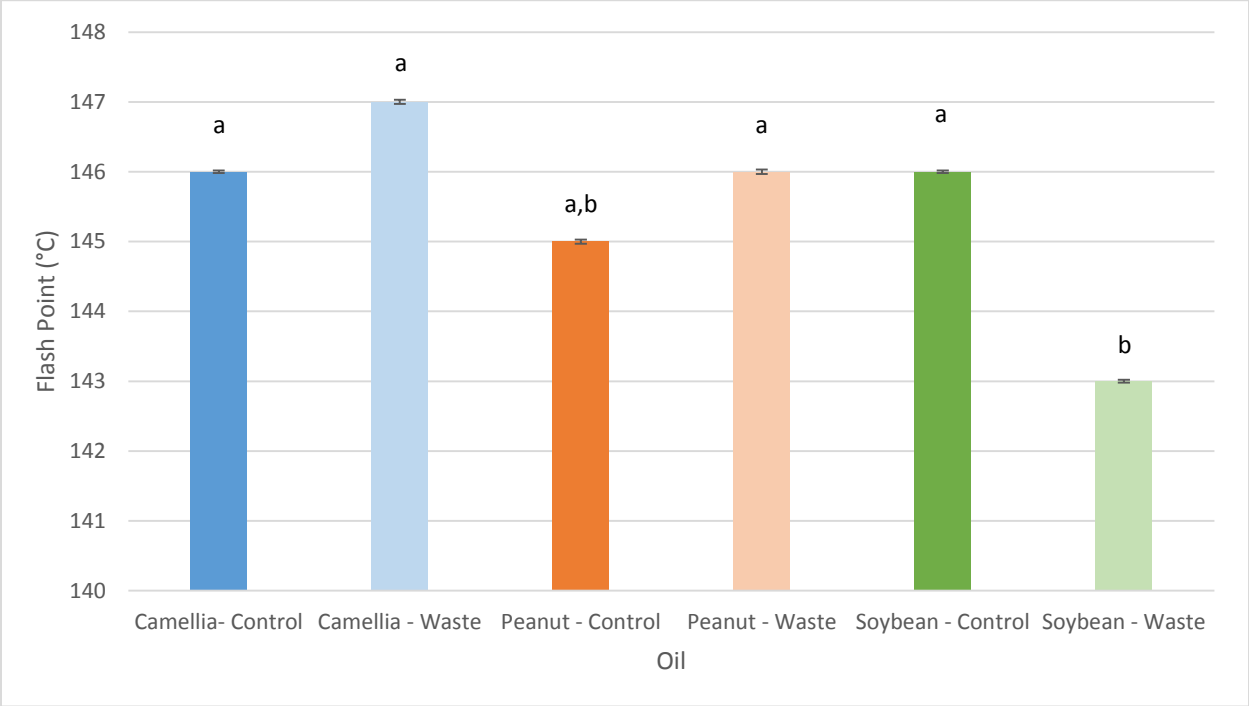

Figure 4.1: Flash point temperature of control and waste biodiesel for Camellia, peanut and soybean oils ($p \leq 0.002$)

This figure shows the mean flash point temperatures of both control and waste biodiesel for Camellia, peanut, and soybean oils. Standard deviation for each oil is represented by error bars. Temperatures are reported in °C. There was a significant relationship between flash temperature and oil type ($p \leq 0.002$). Multiple comparisons were conducted using Tukey's honestly significant difference (HSD) test with a 95% confidence interval, represented by the letter above each data point.

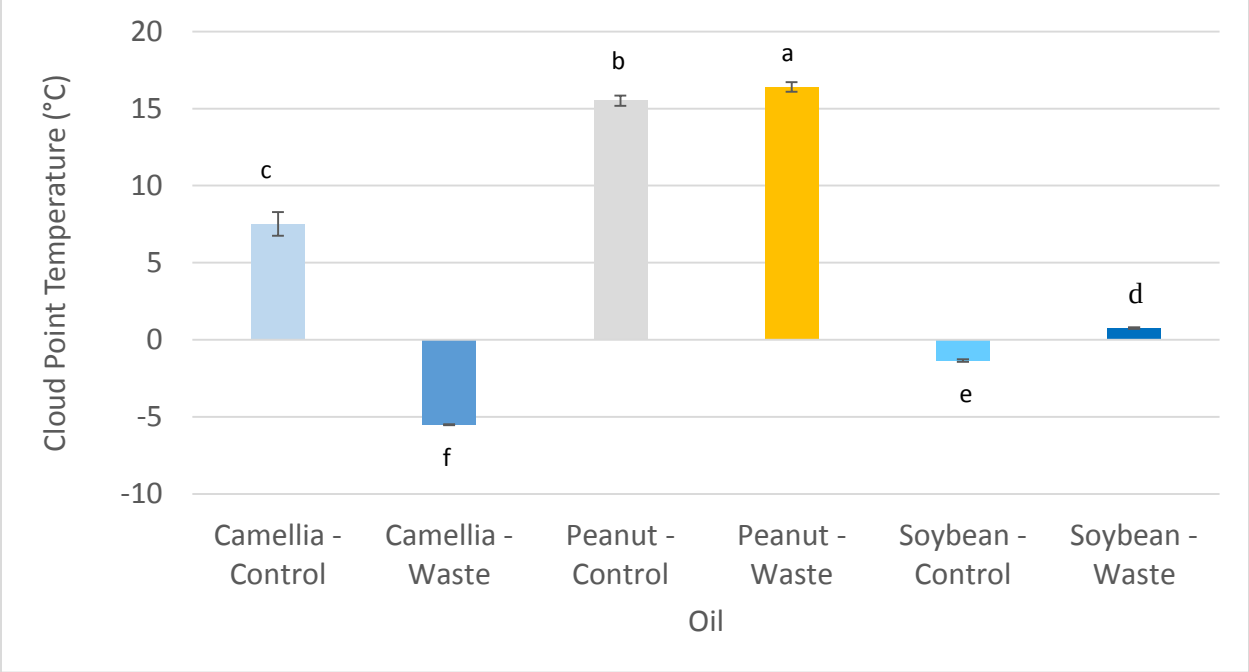

Figure 4.2: Cloud point temperature of control and waste biodiesel products for Camellia, peanut, and soybean oils ($p \leq 0.001$)

This figure shows the mean cloud point temperatures of both control and waste biodiesel for Camellia, peanut, and soybean oils. Standard deviation for each oil is represented by error bars. Temperatures are reported in °C. There was a significant relationship between cloud temperature and oil type ($p \leq 0.001$). Multiple comparisons were conducted using Tukey's honestly significant difference (HSD) test with a 95% confidence interval, represented by the letter above each data point.

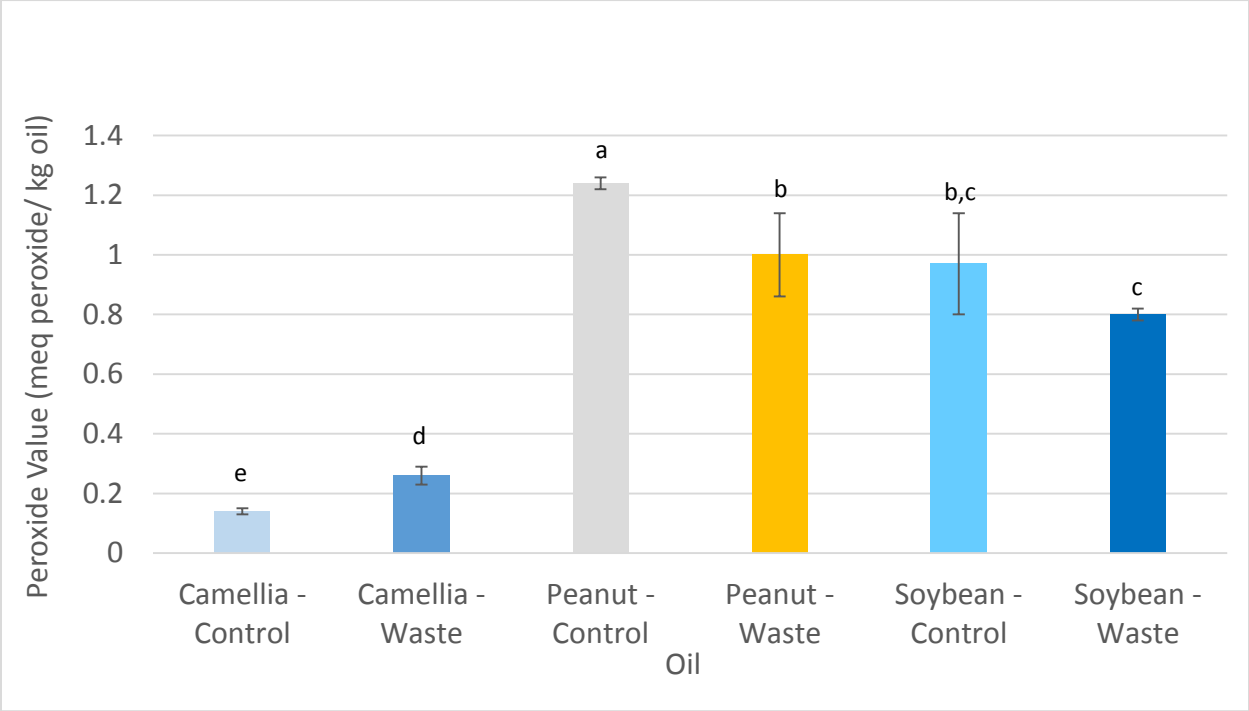

Figure 4.3: Peroxide values (PV) of control and waste biodiesel products for Camellia, peanut, and soybean oils ($p \leq 0.001$)

This figure shows the mean peroxide value (PV) of both control and waste biodiesel for Camellia, peanut, and soybean oils. Standard deviation for each oil is represented by error bars. PV's are reported in meq peroxide/kg oil. There was a significant relationship between PV and oil type ($p \leq 0.001$). Multiple comparisons were conducted using Tukey's honestly significant difference (HSD) test with a 95% confidence interval, represented by the letter above each data point.

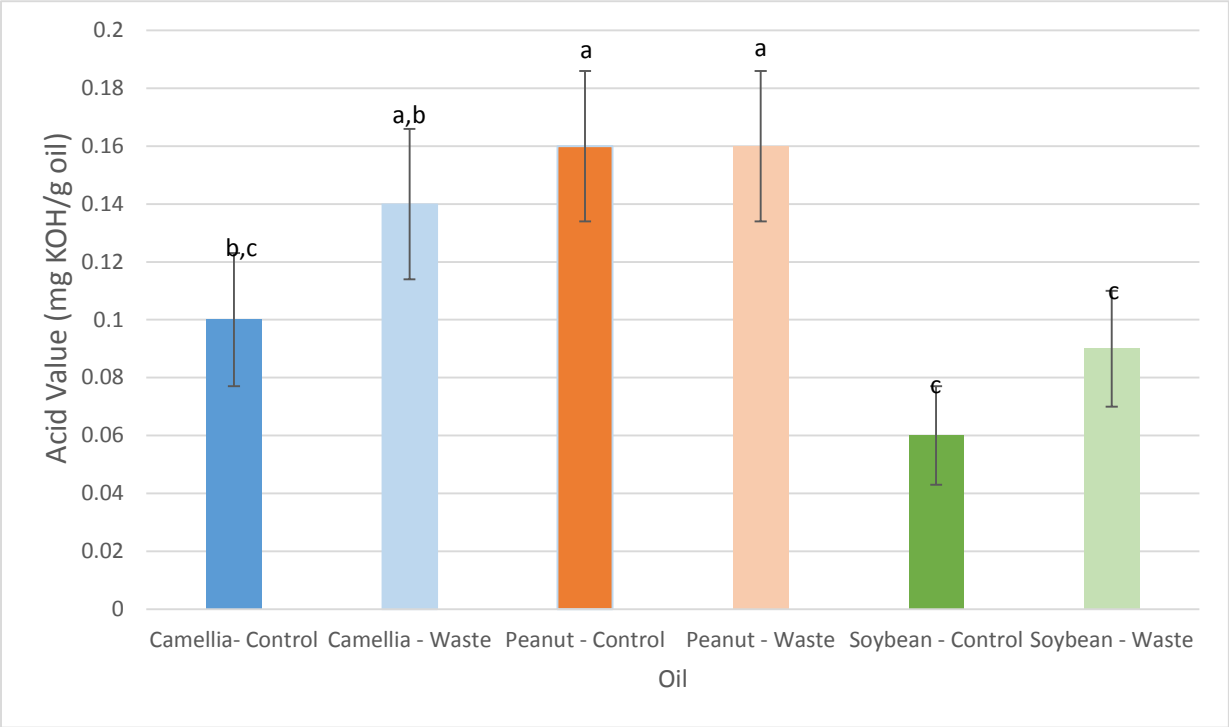

Figure 4.4: Acid values of control and waste biodiesel products for Camellia, peanut, and soybean oils ($p \leq 0.001$)

This figure shows the mean acid value of both control and waste biodiesel for Camellia, peanut, and soybean oils. Standard deviation for each oil is represented by error bars. Acid values are reported in mg KOH/g oil. There was a significant relationship between acid value and oil type ($p \leq 0.001$). Multiple comparisons were conducted using Tukey's honestly significant difference (HSD) test with a 95% confidence interval, represented by the letter above each data point.

CHAPTER 5

Conclusions and Future Directions

Physiochemical analysis show *Camellia oleifera* possessing great potential as a cooking oil and biodiesel feedstock before and after thermal degradation. Smoke point testing has placed Camellia among cooking oils with high heat recommendation giving them a wide variety of cooking used including deep fat frying. During frying performance testing and analysis, Camellia oil exhibited a high degree of heat stability relative to peanut and soybean oils. Pre and post thermal degradation biodiesel production also show Camellia oil to retain its value after use.

The Camellia oil project has been proven to be very promising and research should be continued. Due to limitations in yield from this program, most Camellia oil used during this project was commercially produced. Efforts could be made to characterize the various *C. oleifera* cultivars produced in the southeast and compare those to their Asian relatives. More in depth study could also be conducted on the seed hulls as well as the meal remaining from oil extraction. Both have been proven to have various industrial, agricultural, an even pharmaceutical properties. With previous efforts already being made to adapt the crop to the southeast, these finding may present an opportunity for great economic gain for the region.