UNIVERSITY OF CAPE COAST

ANALYSIS OF DEODORIZED PALM KERNEL OIL USING OPTICAL AND CONVENTIONAL TECHNIQUES

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BY

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Thesis submitted to the Department of Physics of the School of Physical Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Masters of Philosophy Degree in Physics

JUNE 2021

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of this has been presented for another degree in this university or elsewhere

Candidate's Signature……..... Date……

Georgina Esinam Fianoo

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Supervisor's Signature……..... Date……..

Dr Samuel Sonko Sackey

Co-Supervisor's Signature……..... Date……..

Mr. Patrick Mensah- Amoah

ABSTRACT

Palm kernel oil has been deodorized using medicinal plants (fresh and dry cinnamon leaves, fresh lemongrass) and the changes monitored using optical (absorbance (ABS), surface reflectance (SR), laser induced fluorescence (LIF)) and conventional (phosphor molybdate method using ascorbic acid) techniques. Changes were again monitored when the oil was exposed to sunlight with samples protected and not protected from ultraviolet radiation (UV). The ABS measurements were conducted using a spectrophotometer while for the SR measurements, white light source was focused into a two-way optical fibre probe and the reflected light channelled into a spectrometer on to the sample. The LIF was measured at longer wavelengths using a spectrometer at 90^{o} to a 445 nm laser source. Principal component analysis was computed using the fluorescence data in discriminating between the refined deodorized and refined non-deodorized oils. The ABS increased with increasing deodorant mass while the SR decreased. The conventional technique established an increase in antioxidant capacity (AC) as deodorant mass increased. This corresponded to an increase in vitamin E and chlorophyll contents, associated with longer wavelengths in the fluorescence emission spectrum. Correspondingly, increase in deodorant mass resulted in increase in florescence intensity (FI) at longer wavelengths. The fresh cinnamon and the lemongrass deodorant had 169 % and 78 % in AC respectively. There was reduction 91 % in AC after exposure to sunlight protected and not protected from UV, with the reduction of 91.4 % being greater for samples not protected. FI peaks at longer wavelength of the samples decreased after exposure to sunlight.

KEY WORDS

Antioxidants

Deodorization

Multivariate data analysis

Palm kernel oil

Spectroscopic techniques

Ultra violet (UV)

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DEDICATION

To my late mother Beatrice N. Amedzake and my brother Darlington Ahuble.

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LIST OF ACRONYMS

ABS	Absorbance
AC	Antioxidant Capacity
CD	Cinnamon Dry leaves
CDNP	Cinnamon Dry leaves Not Protected
CDP	Cinnamon Dry Protected
CF	Cinnamon Fresh leaves
CFP	Cinnamon Fresh Protected
CFNP	Cinnamon Fresh Not Protected
DRS	Diffuse Reflectance Spectroscopy
FDA	Food and Drugs Authority
FI	Florescence Intensity
FS	Fluorescence Spectroscopy
L	Lemongrass leaves
LGNP	Lemongrass Not Protected
LGP	Lemongrass Protected
LIF	Laser Induced Fluorescence
PCA	Principal Component Analysis
PIR	Peak Intensity Ratios
PKO	Palm Kernel Oil
PKOR	Palm Kernel Oil Residue
RBDPKO	Refined Bleached Deodorized Palm Kernel Oil
SR	Surface Reflectance
UV	Ultraviolet

CHAPTER ONE

INTRODUCTION

Background to the Study

Palm kernel Oil (PKO) is mainly semi-solid oil at room temperature and obtained from the core of palm (*Elaeisguineensis*) fruits. It is extracted from the kernel of the palm fruit through a process of milling, draining, degumming and refining (Reeve & Weihrauch, 1979). The palm kernel oil obtained after this process is very useful due to its benefits such as its nutritional values, application in the cosmetic industry and its uses on the industrial and domestic fronts.

Nutritional Value of Palm Kernel Oil

Analysis of PKO by Atasie and Akinhanmi (2009) showed that it contains 42% fat/oil, 7.01% crude protein, 6.5% moisture, 11.09% crude fibre and 33.40% carbohydrate (by difference) while its elemental composition (mg/100 g) includes: Na (37.00±0.40), K (39.51±0.22), Mg (3.60±0.10), Ca (19.0±0.42), Fe (20.04±0.28), Zn (2.82±0.30) and P (3.4±0.00). Its physicochemical properties include: saponification value (232.82 mg KOH/g), refractive index (1.453 at room temperature), iodine value (41.24 g/100 g), acid value (11.60 mg KOH/g) and peroxide value (1.70 meq/kg) (Atasie and Akinhanmi, 2009). Furthermore, PKO is completely free of cholesterol, unlike animal-based fats such as butter. It is also known to contain high levels of saturated fatty acids like lauric acid (45-55%) and myristic acid (14-20%), with low degree of unsaturation (11-28 %) similar to coconut oil (Atasie and Akinhami,2009).

PKO contains Vitamin A which is essential for proper vision, and also prevents the occurrence of different eye disorders such as night blindness. It contains vitamin K, an essential fat- soluble vitamin required for bone-health, and also a blood coagulation factor (Reeves &Weihrauch, 1979). Palm kernel oil is necessary for the prevention of wrinkles and fine lines, and also protects against harmful Ultra Violet (UV) rays and other toxins so as to keep the skin healthy and youthful. It promotes cuticle and skin health. It prevents ragged cuticles and itchy skin. For this reason, palm kernel oil is an essential component in various soaps and skin creams. In addition to its anti-aging benefits, palm kernel oil makes the skin naturally soft and lustrous averting a greasy look (Saudagar & Sisodiya, 2018).

PKO promotes hair growth. It conditions, strengthens and thickens hair thereby reducing hair-fall. When used as a hot-oil-treatment, it gives a soothing effect and provides the nourishment it contains. According to (Hartwell 1971), palm kernel oil is used as a liniment for indolent tumours. Palm kernel oil has been used to replace milk-fats in the production of ice cream. In some settings it is distilled into biofuels, with the Palm Kernel Oil Residue (PKOR) being potentially useful as energy and/or protein source for feeding most species of livestock. Its use is therefore widespread.

Antioxidant Capability of PKO

A key reason for the widespread application of PKO, especially for cooking and other purposes, is its antioxidant capability.

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. They are used for the stabilization of polymeric products,

petrochemicals, foodstuffs, cosmetics and pharmaceuticals and involved in the defence mechanism of the organism against pathologies associated to the attack of free radicals. Antioxidants have these capabilities due to the presence of beta-carotene and vitamin E, namely tocopherols and tocotrienols.

Under high cooking temperatures and upon storage, PKO has been reported to be capable of resisting oxidation (Engelsen, 1997). These factors make PKO a better alternative than other vegetable oils, especially for cooking purposes. It is therefore essential to continually study and understand the antioxidant behaviour of PKO since its activity is akin to its usefulness.

According to a review conducted by (Pisoschi and Negulescu 2011), the methods of antioxidant capacity evaluation include spectrometry, chromatography and electrochemical techniques. Whereas chromatography techniques are laborious and technical, the electrochemical methods also require large samples and the use of reagents which could be relatively expensive. The use of spectrometry methods, especially Fluorescence Spectroscopy (FS) and Diffuse Reflectance Spectroscopy (DRS), are more favourable as these methods require little or no sample preparation, are non-destructive, and relatively less expensive.

Various Spectroscopy methods have been used to study antioxidant activity in other oils but not in PKO. In this work spectroscopic methods (FS and DRS) will be used in tandem with conventional methods to measure anti-oxidant levels in PKO. Because of the less expensive nature and easy-to-set-up capability of the optical spectroscopy technique, this study will explore the use of FS and DRS as alternatives for evaluating the antioxidant levels in PKO and deodorized PKO.

Off-flavours and Deodorization of PKO

Off-flavours are taints in food products caused by the presence of undesirable compounds that affect the sensory impression of the product. In PKO this is caused by the oxidation reaction and decomposition of oxidative products and results in the unpleasant acrid and rancid odour normally experienced (Pokorny, J., Yanishlieva, N., & Gordon, M., 2001). Even though PKO is appreciated for its comparatively cheap cost (among other cooking oils), easy accessibility and several applications, this unpleasant odour makes it unattractive and inconvenient for use. This therefore calls for procedures to eliminate the offensive smell so as to make it more acceptable.

In an attempt to reduce the pungent smell, industry or large-scale commercial producers of PKO usually go through a final step of refining which includes deodorizing. Deodorizing removes the volatile materials causing odour in the oil and also incorporates other aroma of preference. Industrially, however, deodorizing is usually done with synthetic chemicals which end up removing nutritionally valuable carotenoids to yield oils of low colour and affecting the nutritional potency, most especially its antioxidant capacity (FAO & WHO Report, 1994). An alternative approach adopted for deodorizing in local production is blending the oils with plants extracts having medicinal values.

Such plants extracts are not only aimed at enhancing the smell but also enriching the nutritional value, especially the antioxidant levels of the oil. The actual impact on the antioxidant enhancing capacity of the PKOs deodorized with medicinal plants has not been fully explored and this study seeks to investigate that.

PKO Exposure to Sunlight

The sun's radiation has a continual energy spectrum over a wavelength range of about 0.7 nm to 3000 nm, with the portion reaching the earth's surface stretching from 290 nm to 3000 nm (Reinert, Fuso, Hilfiker & Schmidt, 1997). Between 290 nm and 400 nm is the UV region (UVA and UVB) – a region that poses a threat due to its potential for biological damage. It has a destructive effect on the skin as it causes sunburns and premature ageing accompanied by lines and wrinkles, ocular damage, and skin cancer (Almahroos & Kurban 2004; Cummings, Palacin, 1997; Sliney, 2001; Tripp & Herrmann, 1997; Young, 1992, 2003).

It is also known to have a detrimental effect on our foods (Kolakowska, 2003). Figure 1 is a picture showing some retailers having packaged PKO in plastic and glass bottles exposed to sunlight. A previous study has confirmed that some of these bottles are transparent to UV radiation (Sackey et al., 2015). The concern therefore is what happens to the antioxidant capability of the oil after such exposure. This study seeks to investigate that as well.

Figure 1: Package Palm Kernel Oil in Plastic and Glass Bottles Exposed to Sunlight

Problem Statement

PKO is often deodorized with various plant extracts during its local production. The question now is; what are the changes in flavour and nutritional content after deodorization? Antioxidants are abundant in PKOs, and even improve after deodorization. What happens to the antioxidant level when the oil is stored in a vessel transparent to UV radiation and exposed to sunlight?

Research Objectives

- To deodorize edible PKO using some selected medicinal plants (cinnamon leaves and lemon grass) and monitor changes that take place.
- To determine the changes in properties of deodorized palm kernel oil after exposure to UV radiation, using optical techniques.

Specific Objectives

The specific objectives of the study were as follows:

1. Determine the changes in flavor of PKO after Deodorization with local plant extracts
2. Determine changes in Antioxidant Capacity, using Optical and Conventional Techniques, after Deodorization with plant extracts
3. Establish relation between Deodorant mass and Antioxidant capacity using optical techniques
4. Distinguish between Deodorized and Non-Deodorized PKO
5. Determine the effect of sunlight on Antioxidant Capability of Deodorized PKO

Relevance of the Study

This work will help provide a simple and rapid approach for determining antioxidant levels in PKOs. It will also become a basis to explore the impact of several other medicinal plants that can be used for deodorizing PKO? It will establish the effect of UV radiation on deodorized PKO.

Organization of the Thesis

Chapter One provides a general prologue to palm kernel oil, its nutritional value, deodorization and radiation from the sun. Chapter Two is

devoted to the review of related literature and provides support for the study. In this section the theoretical explanations of the concepts used in the study are reviewed. Chapter Three details the materials, methods and procedures used. In this chapter, the system characteristics and method used for data collection and analysis will be explained. In Chapter Four the results obtained from all the measurements that were carried out are discussed. Chapter Five gives a summary of key findings, the conclusion and recommendations for future work.

Chapter Summary

This chapter gave an overview of the different optical techniques and their biological and chemical applications in biological or medicinal plants. The scope of the work and the organization of the study were included in this chapter

CHAPTER TWO

LITERATURE REVIEW

Introduction

This chapter reviews the literature on spectroscopy with emphasis on fluorescence, absorbance and UV. The antioxidants found in medicinal plants are outlined and finally the theories involved in the multivariate data analysis discussed.

Antioxidants and Free Radicals

An antioxidant is a molecule capable of slowing or preventing oxidation. In a biological system they are needed to maintain optimal cellular, body health and protect cells from damage caused by unstable molecules known as free radicals (Sies, 1997). In recent years significant attention has been directed toward exploring plant-based natural antioxidants, especially the phenolic and tocophenols (Chaovanalikit & Wrolstad, 2004, Katalinic et al., 2006). These are natural antioxidants are not only reported to have anticarcinogenic potential that protects the foods from oxidative deterioration, but are also associated with other health beneficial effects such as lowering the incidence of aging, inflammation, cardiovascular diseases and certain cancers (Frankel et. al, 1995; Liu &Yao, 2007).

In biochemistry and medicine, antioxidants are enzymes or organic substances such as vitamin E or β-carotene that are capable of counteracting the damaging effects of oxidation in tissues (Buyukokuroglu et. al., 2001; Huang et al., 2005). In the chemical industry, antioxidants often refer to compounds that retard autoxidation of a chemical product such as rubber and plastics (Huang et al., 2005).

In food science, antioxidants prevent fats in food from becoming rancid. A dietary antioxidant significantly decreases the adverse effects of reactive species such as reactive oxygen and nitrogen species in executing their normal physiological function in humans (Chu et al., 2000). Oxidation reactions generate free radicals that start-off chain reactions. This spontaneous process of oxidation causes rancidity and food spoilage. Moreover, oxidative stress leads to many lethal diseases such as cancer in humans (Varsha et al., 2010). Synthetic antioxidants are currently being used in the food and pharmaceutical industries to extend product shelf-life (Gende et al., 2008), but there is increasing interest to explore natural sources of antioxidants to minimize the damage caused by synthetic ones. Phytonutrients are increasingly known for antioxidant activity (Karau et al., 2012).

Types and Benefits of Antioxidants

Two main types of antioxidants can be identified; the synthetic and natural kinds. The synthetic types are made from chemical synthesis, with the most ones being butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ). In most countries, the usage level of such synthetic antioxidants is regulated and the safety of the compounds involved tested based on long-term toxicity studies. Natural antioxidants are obtained from medicinal plant extracts and are known to increase the antioxidant capacity of the plasma and reduce the risk of certain diseases (Kumar, Yadav, Ahmad, Narsaiah, & Safety, 2015).

Recommendations based on epidemiological studies have confirmed that fruits, vegetables and less-processed staple foods ensure the best protection against the development of diseases caused by oxidative stress such

as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract (Carlsen et al., 2010). This is due to health benefits brought about by the presence of antioxidants in fruits and vegetables (Carlsen et al., 2010). There are numerous antioxidants such as carotenoids, phenolic compounds, benzoic acid derivatives, flavonoids, proanthocyanins, stilbenes, coumarins, lignans, and lignins in dietary plants (Lindsay & Astley, 2002). Antioxidants are known to play a key role in the protective influence exerted by plant foods and fight inflammatory processes. Such processes are the causes of many chronic diseases in the body (de Quirós, Costa, & Analysis, 2006).

Determination of Antioxidant Levels

Methods used to determine antioxidant levels in substances fall under well-defined groupings such as electrochemical, chromatographic, and spectrometric techniques. With electrochemical techniques, cyclic voltammetry and biamperometry are the most broadly used. Chromatographic methods are often applied to antioxidant separation and detection, and used before spectrophotometric or electrochemical assessment of the total antioxidant capacity. Spectrometric techniques rely on the reaction of a radical cation or complex with an antioxidant molecule capable of donating a hydrogen atom (Pisoschi & Negulescu, 2012).

While chromatography techniques are laborious and technical, electrochemical methods require large samples and the use of reagents which can be expensive. The use of spectrometry methods, such as FS or fluorimetry, are therefore more favourable as these methods require little or no sampling, non-destructive, and relatively less expensive. Fluorimetry technique was used in this study.

Fluorimetry

Fluorimetry is the quantitative study of the fluorescence of fluorescent molecules. Fluorescence is the phenomenon where a molecule absorbs light within its absorption band and emits this light at longer wavelengths within its emission band. In this an orbital electron of the molecule relaxes to its ground state by emitting a photon of light after being excited to a higher quantum state by the absorbed light. In most cases, the emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation.

Fluorescence is unique from other spectroscopic techniques with its multidimensional character (Christensen et al., 2006). When molecules absorb radiation energy, it causes a difference in energy levels between the ground and excited states for a particular fluorophore. Consequently, the radiation of a lower energy level is specific for a particular molecule during its deactivation. Excitation and emission are two fluorescence properties of every compound. It is not all absorbing molecules that fluoresce and this contributes to fluorescence selectivity as opposed to absorption spectra. Higher sensitivity of fluorescence is a unique advantage as its sensitivity is 100 to1000 times higher than absorption techniques, allowing concentrations measurements up to parts per billion levels (Guilbault, 1990).

The fluorescent investigation of refined bleached deodorized palm kernel oils takes advantage of the presence of natural fluorescent components, including phenolic compounds, tocopherols and pheophytins, and their oxidation products. There has been an increase in the use of fluorescence for food analysis and its analytical applications to oils include discrimination between the different quality grades, adulteration detection, authentication,

quantification of fluorescent components, monitoring thermal and photo-oxidation and quality changes during storage (Christensen et al., 2006; Karoui & Blecker, 2011; Sadecka & Tothova, 2007). This fluorescence food analysis has led to the determination of their antioxidant content and phenolic compounds (Chong, Venegas, & Olsher, 2007; de Quirós et al., 2006).

Fluorescence Spectroscopy

Fluorescence is the emission of light subsequent to absorption of ultraviolet or visible light by a fluorophore (Lakowicz, 2013). The general principle of the phenomenon is illustrated by Jablonski diagram as shown in Figure 2. The singlet ground, first and second electronic states are depicted by S_0, S_1 and S_2, respectively. The first step of fluorescence is the excitation of the molecule from the ground state (S_0) to an excited state (S_1) by the absorption of light

This is followed by a vibrational relaxation or internal conversion, where the molecule undergoes a transition from an upper excited state (S_1;3) to a lower one (S_1;0), without any radiation.

Finally, the fluorescence occurs typically around 10^{-8} s after excitation, when the electron returns to the ground state (S_0). The emitted light has energy equal to the difference between energies of the ground and excited states.

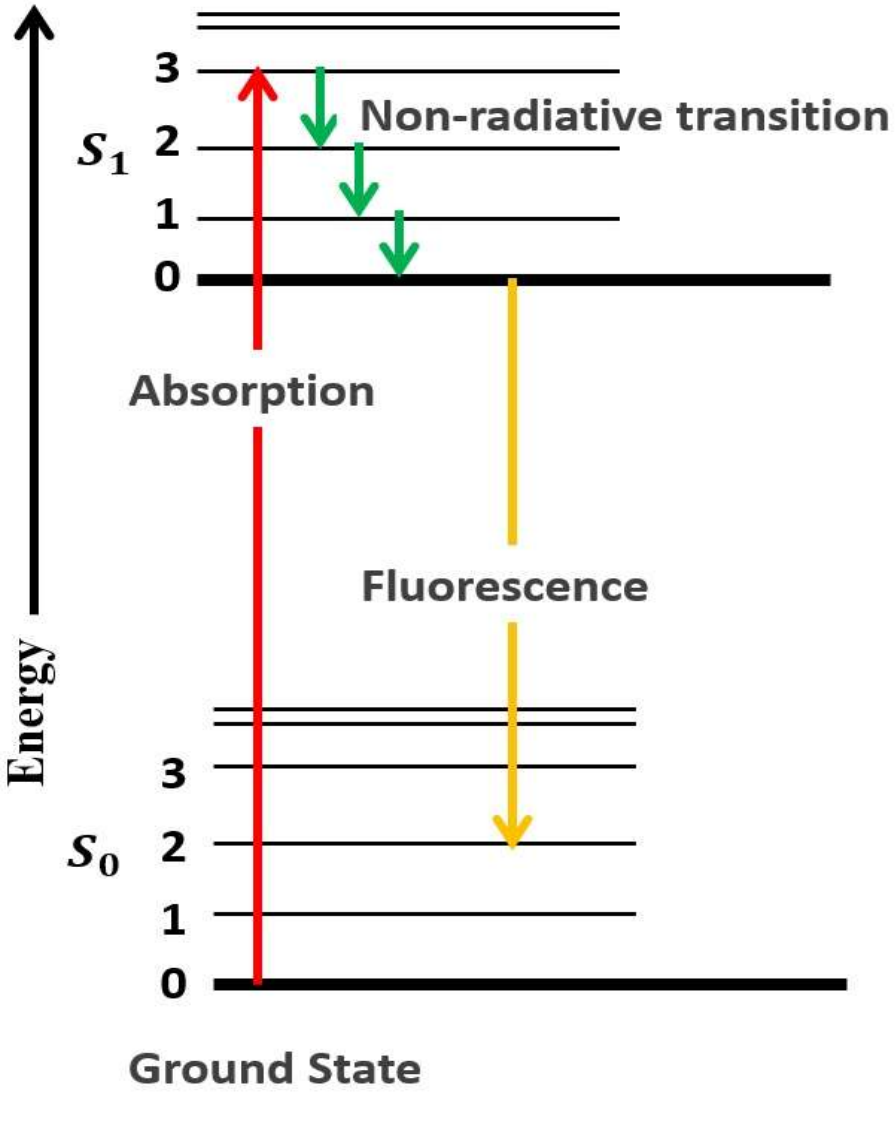


Figure 2: Jablonski Diagram Describing the Electronic Energy Levels of Compounds.

Fluorescence Measurement

Fluorescence measurement can be broadly classified into two types: steady-state and time-resolved. Steady-state measurements are those performed with constant illumination and observation, and the intensity or emission spectrum recorded.

The time-resolved technique is used to measure intensity decays or anisotropy decays. To take the measurements, the sample is exposed to a pulse

of light, where the pulse width is normally shorter than its decay time. The decay intensity is recorded with a high-speed detection system that allows the measurement of its intensity or anisotropy on a timescale. The periodic measurement requires complex and expensive instrumentation as compared to the time-resolved. Also, most molecular information available from fluorescence are lost during the time averaging process in steady-state measurement.

Fluorescence measurements are done using a spectrofluorometer which uses an excitation monochromator (device which includes a wavelength dispersing component as opposed to a filter). Resolution is obtained with changeable fixed slits. The advantage of a spectrofluorometer is that it allows for varying wavelength selection where the operator can scan a substance over a range of wavelengths (Guilbault, 1990; Lakowicz, 2013; Lakowicz & Gryczynski, 1992).

The fluorescence spectral data are generally presented as emission spectra. A fluorescence emission spectrum is a plot of the fluorescence intensity versus wavelength (nanometer) or wavenumber (cm^{-1}). The quantum efficiency of most complex molecules is independent of the wavelength of extinction coefficient of the compound; in other words, the corrected excitation spectrum of a substance will be the same as its absorption spectrum. A fluorophore is characterized by its fluorescence lifetime and quantum yield (Lakowicz et al., 2008; Mycek & Pogue, 2003).

If are the radiative (Γ) and non-radiative (K_{NR}) decay rates, are measured fluorescence lifetime τ (which is the average time spent by a

molecule in the excited state prior to decay to the ground state) can be calculated using equation (1) as:

$$\tau = \frac{1}{\mathrm{T}+\mathrm{K}_{NR}} \quad (1)$$

The quantum yield (Φ), which is defined as the number of emitted photons relative to the number of absorbed photons, can be calculated using equation (2) as:

$$\Phi = \frac{\tau}{\mathrm{T}+\mathrm{K}_{NR}} \quad (2)$$

The value for Φ ranges between 0 and 1, with a higher value implying brighter fluorescence.

Excitation and emission spectra are two basic fluorescence properties. The shape and location of the excitation and emission spectra are not influenced by particular emission and excitation wavelengths for any system containing a single fluorophore. For systems comprising several fluorophores, multidimensional measurement methods are required as single wavelength spectra is inadequate for detailed description of fluorescent properties. The first to introduce a multicomponent fluorescent system was (Weber, 1961) and its detailed characterization is achieved by determining the excitation-emission matrix which is also called total luminescence spectrum or fluorescence landscape. This technique was generally used to explore oil fluorescence after its successful application in edible oils. Total luminescence spectra are commonly attained when emission spectra are measured at several excitation wavelengths which is depicted as a three-dimensional plot of fluorescence intensity as a function of the excitation and the emission wavelengths (Guilbault, 1999; Ndou & Warner, 1991). Total luminescence can also be represented by using two-dimensional contour maps of emission

against excitation wavelength. The outlines are plotted by joining points of equal fluorescence intensity. The total luminescence spectrum offers a detailed description of the mixture fluorescent components as this may be used as a distinctive fingerprint to identify and characterize the sample investigated. The acquisition of contour maps at sufficient resolution (determined by the individual emission spectra numbered and recorded) on orthodox spectrofluorometers is laborious and time wasting, and requires the use of large numerous scans for each sample (Guilbault, 1999). (Lloyd 1971) suggests that multicomponent fluorescent systems can be studied by the synchronous fluorescence methods.

Absorption Measurement

Absorption is a total transfer of energy from a photon to a molecule, after which the photon ceases to exist. A photon with inherent energy can be expressed in equation (3) as

$$E = hv \tag{3}$$

But $v = \frac{c}{\lambda}$

Therefore $E = \frac{hc}{\lambda}$

The photon can only be absorbed by a molecule if the photon energy corresponds to a difference in energy, ΔE, between two allowed states in the molecule's energy levels, E1 and E2. This relationship is defined by Bohr's frequency condition (Ball, Gary, Ye, & Sanford, 2011) and expressed in equation (4) as:

$$\Delta E = E_1 - E_2 \tag{4}$$

$$\Delta E = \frac{hc}{\lambda}$$

The absorption of spectral energy excites the molecule to a higher state and excess energy is lost over time. The loss of energy mostly occurs as heat dissipation, but can also result in the emission of a new photon, perceived as fluorescence or phosphorescence, or as photochemical reactions (Holick, MacLaughlin, Parrish, & Anderson, 1982). The allowable energy levels depend on the molecular structure and vary between different molecules. The absorption varies for each wavelength, resulting in the fingerprint by which the molecules can be identified (Stratis-Cullum, et al., 2003)

Diffuse Reflectance Spectroscopy

Diffuse reflection is the reflection of light or other waves or particles from a surface such that a beam incident on the surface is scattered at many angles rather than at just one angle as in the case of specular reflection. The most general mechanism by which a surface gives diffuse reflection does not involve exactly the surface as most of the light is contributed by scattering centres beneath the surface. Only the part of the beam that is scattered within a sample and returned to the surface is considered to be diffused reflection. Figure 3 gives an illustration of the diffuse reflection phenomenon.

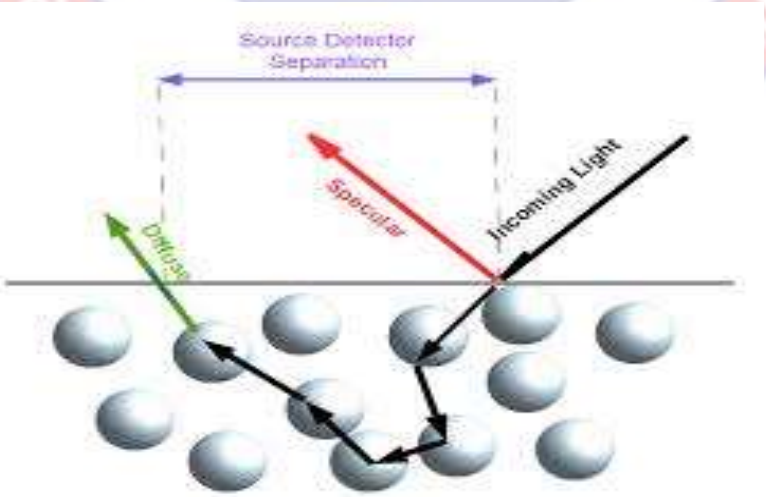


Figure 3: Diffuse Reflectance on a Surface of a Liquid (Frisk, 2016)

Diffuse reflectance spectroscopy (DRS) is the type of spectroscopy where the diffuse reflection of radiation by a sample is measured. It allows the accurate measurement of the flux per wavelength of light reflected in a scattered manner from the sample. DRS rely on the incidence of the beam into the sample where it is reflected, scattered and transmitted through the sample material. The back reflected and diffusely scattered light are then collected into the sensor. Such spectroscopic technique makes the analysis of a wide range of samples easier, faster and more efficient. Advanced options for diffuse reflectance provide the ability to heat the sample and monitor a reaction process. DRS has applications in the food, pharmaceutical and textile industries, building materials, paper and pulp materials as well as in adsorption studies and other basic investigations in physical, inorganic and organic chemistry (Frei, 1976).

Deodorization

A deodorant is a substance that removes or masks an unpleasant or offensive smell. PKO, the presence of off-flavours makes it objectionable and undesirable to consumers. It has therefore become important for it to be refined to make it more appealing. The process of deodorization therefore eliminates the offensive odour to make it more acceptable, and also improves its nutritional value (Pokorny et al., 2001). Deodorants used are either synthetic chemicals or plants extracts. The application of synthetic antioxidants has been recently restricted due to their toxicological effects and the suspicion that they may be carcinogenic. Consumers therefore have shown preference to the use of natural antioxidants (Byrd, 2001; Johnston et al., 2005). Plants can inhibit oxidative rancidity, delay the development of off-

flavour and improve the nutritional value of the oil by increasing its antioxidant potency.

Some of the plant extracts used includes cinnamon leaves and lemon grass.

Cinnamon

Cinnamon (Cinnamomum verum) spice is a source of antioxidants which augments the efficiency of other vital antioxidants. It contains a number of potent antioxidants and serves as a vital spice for kerbing oxidative stress. The antioxidant potential of cinnamon contains a constituent of flavonoid compounds. The essential oils in cinnamon such as cinnamaldehyde, eugenol, and linalool, have been investigated using peroxynitrite induced nitration and lipid peroxidation (Aqil et al., 2006). Cinnamon extract is effective in the reduction of oxidation of lipid in palm oil and this has proved to be effective as synthetic antioxidants in food preparations (Ghobadi, Akhlaghi, Shams, & Mazloomi, 2018). Besides being an antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering and cardiovascular disease-lowering compound, cinnamon is known to be effective against neurological disorders, such as Parkinson's and Alzheimer's disease (Rao, P. V., & Gan, S. H. 2014).

Lemongrass Leaves

Lemongrass (Cymbopogon citratus) is a tall perennial grass which grows in almost all tropical and subtropical countries (Cheel et al., 2005). It is an aromatic herb known to be effective in scavenging free radicals and has the potential as a powerful antioxidant. Studies have also demonstrated antibacterial, antifungal and insecticide efficacy, as well as potential anticarcinogenic activity (Onawunmi, Yisak, & Ogunlana, 1984; Tian et al.,

2011). Both the freshly cut or partially dried leaves can be used medicinally, with their scent resembling that of lemons (cirtruslimon).

Effect of Solar Exposure on Oils and Antioxidant Activity

A previous study on effects of UV radiation on the chemical and sensory characteristics of virgin olive oils concluded that even small doses of UV radiation induced oxidation of the oil samples. The total phenols and fatty acids contents and the fruity sensory attributes decreased in the process while intensity of the rancid sensory attribute, on the other hand, increased (Luna et al., 2006).

Oils stowed in the dark essentially contained primary oxidation products, while those kept in light have secondary oxidation products as established by the K270 values and this exceeded the legal limits of purification by means of alumina (Caponio et al., 2005).

The shelf life of oils exposed to light have been confirmed to be shorter than those kept in the dark (Caponio et al., 2005).

Chapter Summary

This chapter reviewed the literature on spectroscopy with emphasis on fluorescence, absorbance and UV. The antioxidants found in medicinal plants were outlined.

CHAPTER THREE

METHODOLOGY

Introduction

This chapter firstly describes the procedure for deodorizing the PKO used for the study. Secondly, details are given about the various experiment setups and techniques used to study the antioxidant behaviour of the palm kernel oil, and the effect after exposure to sunlight.

Sample Preparation

The PKO used for the study was obtained directly from the producers at Abura in the Central Region of Ghana. The PKO was stored in white plastic gallons warped with aluminium foil and placed in an ice chest before transporting to the laboratory.

The cinnamon leaves and lemongrass leaves were fetched from the University of Cape Coast botanical garden. They were carefully washed and some of the cinnamon leaves was air-dried at room temperature. It took five (5) days to dry them. Figure 4 shows a picture of dry (left) and fresh (right) cinnamon leaves, while Figure 5 shows a picture of fresh lemongrass being cultivated.

Figure 4: Cinnamon dry (left) and Cinnamon Fresh (right) leaves

Figure 5: Fresh Lemongrass

The flow chart in Figure 6 describes the sample preparation process for the PKO prior to any of the analytical measurements conducted.

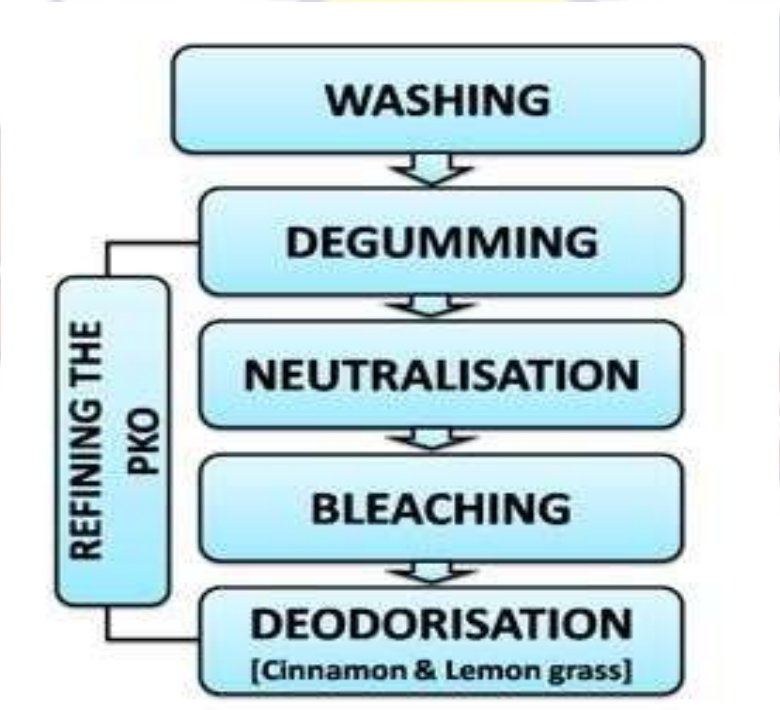


Figure 6: The Preparation process for PKO before Analytical Measurements

Details of Sample Preparation Washing

About 50 ml of the crude palm kernel oil was measured into a clean conical flask. 50 ml distilled water was added to the crude oil sample in the conical flask and heated for about 25 minutes within the temperature range of 45 -80 ^{o}C on a hot plate. The content in the conical flask was allowed to cool after which it was separated *using* a separatory funnel. This procedure was repeated for two more times to clear many impurities from the crude palm kernel oil. Figure 7 is a composite picture in which 7a shows a mixture of the crude palm kernel and distilled water being heated on the hot plate, and Figure 7b shows the mixture being separated after cooling using a separating funnel.

Figure 7: Sample Preparation (A) – the crude PKO and Distilled Water being heated on a Hot-Plate. (B)– The mixture being separated using a Separating Funnel

Refining

The refining process involved degumming, neutralization, bleaching and deodorization processes.

Degumming Process

This involved two procedures; treatment with phosphoric acid (H_3PO_4) and with sodium hydroxide (NaHO).

Treatment with Phosphoric Acid

A 0.017 M concentration of phosphoric acid (H_3PO_4) was prepared by dissolving a 0.115 ml of the phosphoric acid in 50 ml of distilled water and stirred. About 50 ml of 0.017 M phosphoric acid was measured and transferred into a conical flask containing 50 ml of the washed oil. The mixture was heated for about 15 minutes after which the gum or phosphatide formed was removed or separated from the oil using a separatory funnel.

Treatment with Sodium Hydroxide

About 20 ml of prepared sodium hydroxide (NaOH) was added to 50 ml of the acid-treated oil. The mixture was then shaken till a clear solution or mixture was observed, after which it was centrifuged. The soap precipitate was then removed from the top layer leaving the oil at the bottom. Figure 8 is a composite picture in which (8a) and (8b) show the oil treatment with phosphoric acid, while (8c) and (8d) show the treatment with sodium hydroxide and the mixture being centrifuged respectively.

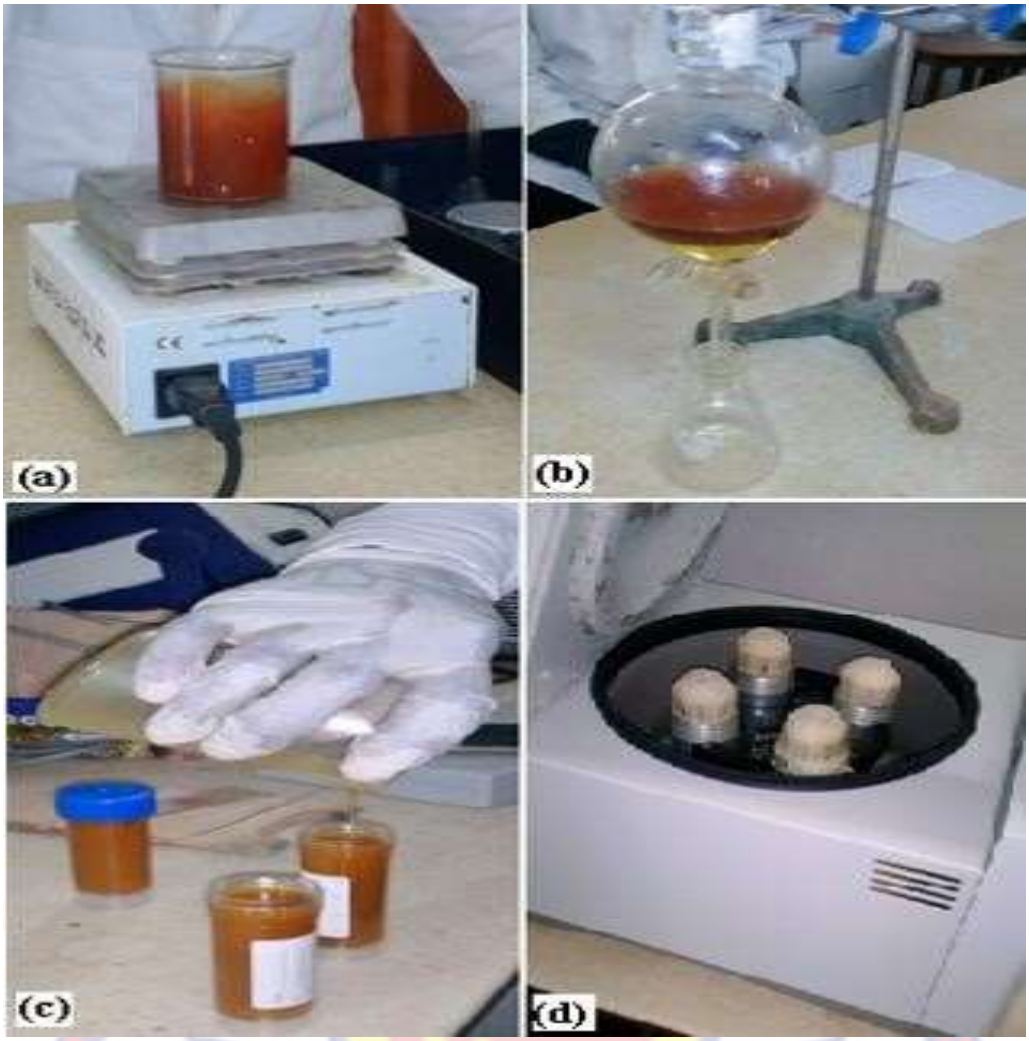


Figure 8: (a) Sample preparation, (b) Oil treatment with Phosphoric acid. (c) Treatment with Sodium Hydroxide and (d) the mixture centrifuged

Neutralization Process

About 50 ml of degummed oil and 100 ml of distilled water were measured into a conical flask and heated for about 10 minutes to allow the water boil and remove any traces of soap in the oil. The solution was allowed to cool and the content centrifuged. The oil was separated from the water with the aid of a separatory funnel. The process was repeated to ensure efficient removal of the traces of soap and other impurities.

Bleaching

50 ml of the degummed crude PKO was measured into a conical flask after which 1.0 g of activated carbon was mixed with the oil. PKO was then allowed to stand for 24 hours. The benefit of this bleaching stage was the removal of soap and other organic contaminants as the activated carbon simply absorbs these compounds. The activated carbon was separated from the oil via vacuum filtration. Figure 9 is a composite picture in which (9a) illustrates the activated charcoal mixed with the RDPKO that had undergone neutralization, (9b) illustrates the vacuum filtration process employed in separating the activated charcoal from the oil and (9c) illustrates the final 'bleached' PKO.

Figure 9: Sample preparation: (a) - activated charcoal mixed with the RBPKO (b) - the vacuum filtration process that separated the activated charcoal from the oil (c) the final 'bleached' RBPKO.

Deodorization Process

3300 ml of the degummed and bleached PKO was measured and poured into twenty-two (22) different beakers containing 150 ml of oil each. One of the beakers was used as reference and labelled REFF. The reference had no deodorant in it. The deodorants used for the deodorization were fresh

cinnamon leaves, dry cinnamon leaves and fresh lemon grass. Each beaker was filled with different leaves with specific masses 0.5, 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g. The leaves were chopped into very small pieces to reduce the surface area and hence enabled the quick absorption of nutrient into the oil when heated. After heating the oil with the various deodorants at a temperature of 85 ^{0}C, the deodorized oil was allowed to cool and sieved to remove the leaves residue. The refined deodorized oils were stored in plastic containers, well-sealed and covered in aluminium foil to prevent any interaction with light, to prevent bleaching. They were all stored in a dark room. Figure 10 shows some stages of the deodorization process of the bleached PKO using cinnamon fresh leaves (10a) and cinnamon dry leaves (10b).

Figure 10: Deodorization of the bleached PKO using (a) cinnamon fresh leaves and (b) cinnamon dry leaves.

Experimental Measurements

The flow chart in Figure 11 describes the experimental measurements carried out on the oil samples.

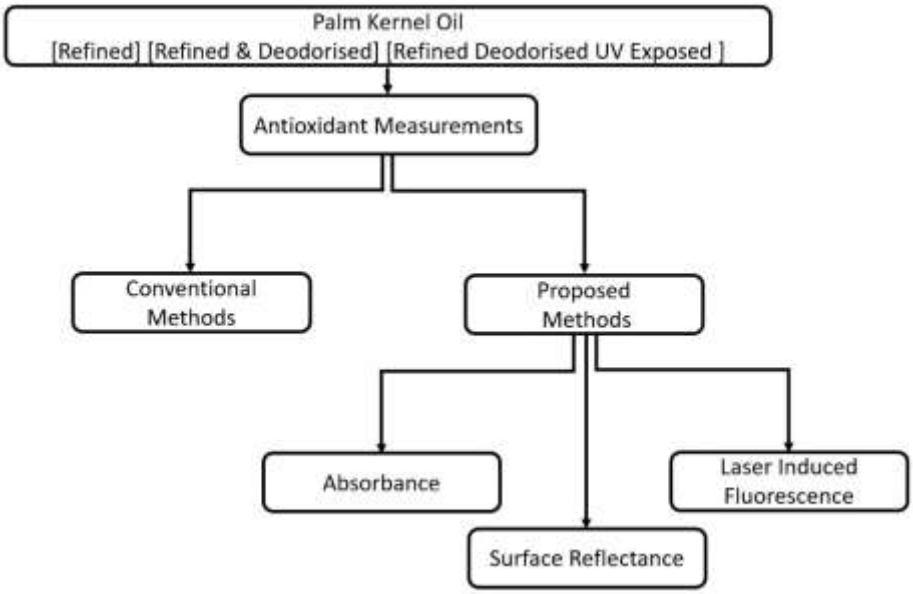


Figure 11: Schematic diagram of the experimental measurements carried out

Experimental Research Design

The various samples were placed in separate plastic bottles and labelled as shown in Figure 12. The labels assigned to the samples (deodorised and non-deodorised) are given in Table 1.

Figure 12: Samples of refined bleached deodorized palm kernel oil

Table 1: Labels Assigned to the Various Samples Investigated.

Mass (g)	Deodorant		
	Dry Cinnamon leaves	Fresh Cinnamon leaves	Fresh Lemon grass
0.5	CD1	CF1	L1
1.0	CD2	CF2	L2
2.5	CD3	CF3	L3
5.0	CD4	CF4	L4
10.0	CD5	CF5	L4
15.0	CD6	CF6	L6
20.0	CD7	CF7	L7

The non-deodorized palm kernel oil was named REFF.

Conventional Approach for Determination of Total Antioxidant Capacity

The total antioxidant capacity of the fraction was determined by phosphor molybdate method using (2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one (ascorbic acid) as a standard. An aliquot of 0.1ml of each of the oil samples was mixed with 1.0 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM Ammonium molydate added) as shown in Figure 13(b). The tubes were caped and incubated in a water bath at 95 ^{o}C for 90 minutes as shown in Figure 13(c). After the sample had cooled to room temperature, the absorbance of the mixture was determined at 765 nm against a blank. A typical blank contained 1.0 ml of the reagent solution and the appropriate volume of solvent was incubated under the same conditions when the ascorbic acid was used as the standard. The total antioxidant capacity was estimated as mg/kg ascorbic acid equivalent (AAE). The assay was based on the reduction of phosphomolybdate acid (Mo^{6+}) to phosphate/Mo^{5+} complex which was green in colour at acidic Ph, and whose

absorbance was measured with a spectrophotometer at 695 nm (Prieto et al., 1999) as shown in Figure 13 (a).

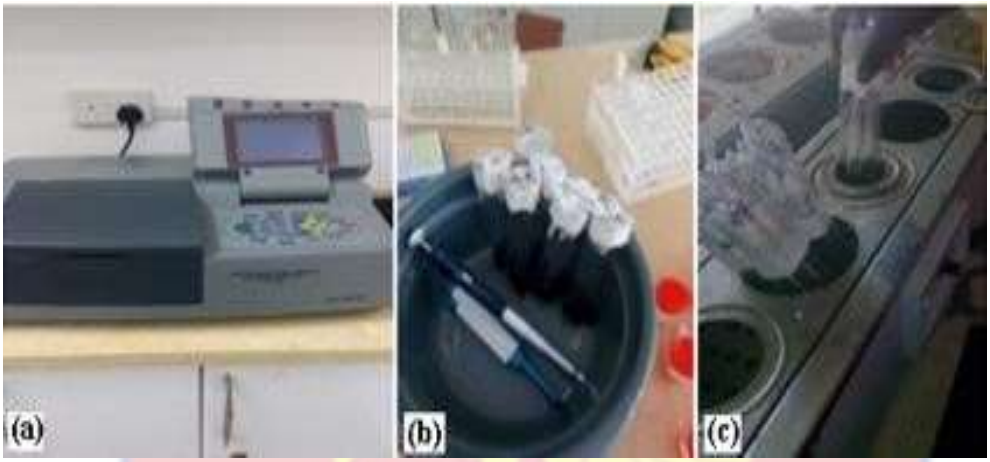


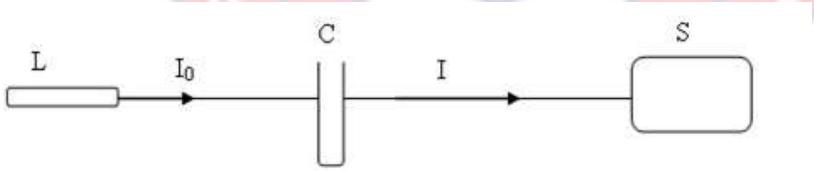
Figure 13: Sample preparation (a) determination of total antioxidant ability with spectrophotometer (b) Samples in reagent solution and (c) samples in water heater

Proposed Measurements

Three unique optical measurements were conducted on the samples. These were absorbance, Surface reflectance and Laser Induced Fluorescence (LIF) measurements.

Absorbance Measurements

This was carried out using a spectrophotometer to determine the capacity of the samples to absorb light at different wavelengths. The schematic diagram for the spectrophotometer is given in Figure 14.



L – White light source

I_O – Intensity of light entering the sample

I – Intensity of light coming out sample

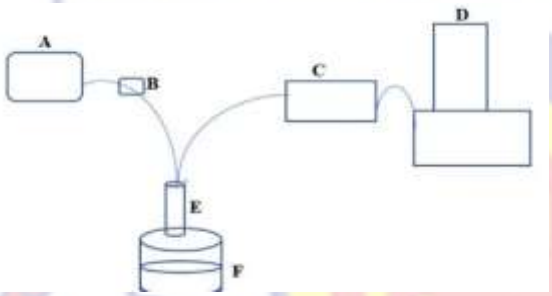
C – Cuvette containing the sample

S - Spectrometer

Figure 14: Schematic diagram for spectrophotometer used for absorbance

Surface Reflectance Measurement

This was conducted to determine the fraction of incoming radiation that was reflected after incident on the sample. The experimental setup used is shown in Figure 15. A white light source (fibre optic illuminator model 77501) was focused into a microscope objective into a two-way optical fibre probe (delivers light on to the sample via a single fibre and collects the reflected light using six other surrounding fibres) and the reflected light channelled into a spectrometer (USB 2000). The spectrometer was connected to a computer from which the reflectance spectrum from the sample could be visualized.



A – White light source
B – Microscope objective
C – Spectrometer
D – Computer
E – Bifurcated fibre probe
F - Sample

Figure 15: Experimental setup for surface reflectance measurement

Laser Induced Fluorescence (LIF) Measurement

The schematic diagram for this measurement is shown in Figure 16. During measurements, the sample was put in a cuvette using a disposable pipette and inserted into a sample holder. Each sample was excited by the laser source and the spectrometer used to detect the various emission spectra.

The laser source of power 100 mW was emitted at 445 nm. It is equipped with a laser diode driver requiring an input rating of 12 V and 2 A respectively, with TTL modulation of 0 to 20 KHz. A Neutral density (ND) filter (2.0) was used to reduce the intensity of the laser to prevent photobleaching of the various samples and saturation in the detector while the data was taken. The emission spectra were recorded, using OOIB32 software which was done under an integral time of 307 ms, an average of 2 and a boxcar of 10. To ensure validity of comparison, each spectrum represented the average of 580 scans recorded after 360 s of excitation. All measurement were done under the same conditions. The data was extracted and plotted graphs for the various samples. The data analysis was done using the different peak intensities and the ratios at different wavelengths.

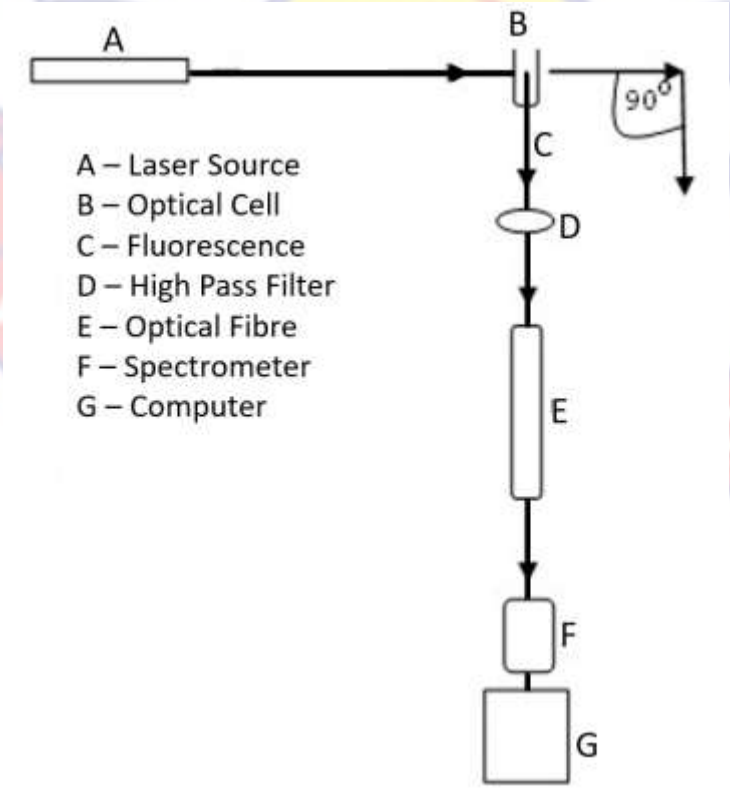


Figure 16: Experimental setup for LIF measurement

Measurement of UV Effect on Total Antioxidant Ability

Part 1: Measurement of UV Transparency of Materials

The UV transparency of specific materials known to be used as containers to store oil, especially by market women, were, determined. Figure 17 is a typical market scene in Ghana showing some RDPKO stored in bottles made from different materials and exposed to the sun.

Figure 17: A typical market scene in Ghana showing some RDPKO stored in bottles made from different materials and exposed to the sun

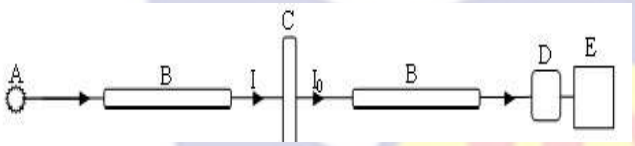
Some of these materials, and the form in which they are used, are presented in Table 2.

Table 2: Some of the commonly used Materials for the Storage of Refined Deodorized Palm Kernel Oil RDPKO

Material	Form used	Label
*Laminated Glass	Bottle	A
Clear Glass	Bottle	B
Polyethylene terephthalate (PET)	Bottle	C
Transparent Linear Low-Density Polyethylene (LLDPE)	Bag	D

* This was added mainly for research purposes

The light source used for this determination was sunlight. The schematic diagram used for this measurement is given in Figure 18.



A – Sunlight

B – Optical fibre

C – Sample

D – Spectrometer

E – Computer

I – Incident light

I_O – Transmitted light

Figure 18: Schematic diagram used to determine UV transparency of materials

Part 2: Measurement of UV effect on Total Antioxidant Ability of Exposed Oils to Sunlight

The UV effect, if any, on the total antioxidant ability was determined for both the non-deodorised and deodorised oil samples by exposing them to sunlight. For the latter, the sample with the highest mass of deodorant was used. Accordingly, the oil samples used were REF (non-deodorised sample),

CD7, CF7 and L7. Two distinct exposures to sunlight were made, as described in Table 3.

Table 3: Description of how Exposure of Samples were made to Sunlight

Exposure	Description
1	Sample was put into a ceramic bowl and covered with laminated glass (Material A)
2	Sample was put into clear glass (Material B) and covered with , LLDPE (Material D)

The exposures using the descriptions in Table 3 were based on the findings of (Sackey et al., 2015). An equal volume of sample was used for each exposure. It could be deduced that a total of 8 exposures were made. Exposure time was from 9.00 am to 3.00 pm and continuously for 5 days, and the total antioxidant ability determined at the end of each day.

Chapter Summary

This chapter described the procedure for deodorizing the PKO used for the study. Secondly, details are given about the various experimental setups and techniques used to study the antioxidant behaviour of the PKO, and finally, the effect after exposure to sunlight.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

In this chapter, the results of various analytical measurements including absorbance, surface reflectance and LIF which were used to determine the effect of the different types of deodorants, their different levels of deodorization, as well as the effect on the antioxidant capacity of PKO when exposed to sunlight have been presented. A comparison has been made between the optical techniques and conventional methods used for the antioxidant measurements.

Absorbance Measurement

The absorbance measurements provided a degree of quantity of light absorbed and therefore gave the optical density or extinction of the sample. Figure 19 is a composite graph showing the absorbance spectra of the raw and refined deodorized PKO; (a) cinnamon dry leaves, (b) cinnamon fresh leaves and (c) lemon grass leaves.

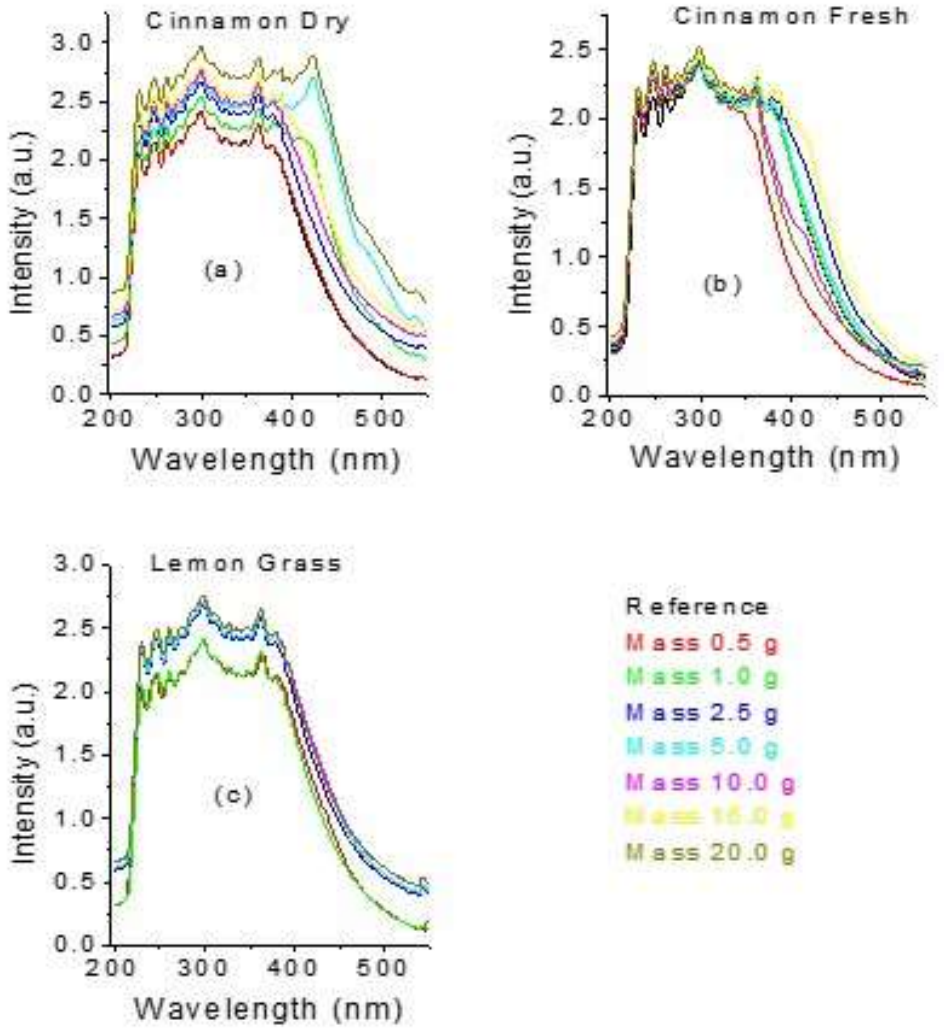


Figure 19: Absorbance spectra for the raw and refined deodorized PKO using; (a) cinnamon dry leaves; (b) cinnamon fresh leaves; (c) lemongrass leaves

From Figure 19 it can be observed that the absorbance intensity increased as the mass of the deodorant was increased in the PKO. This can be attributed to the increase in the concentration of the absorbing molecules in the deodorants. It was noted also that the intensity of light decreased as it travelled through the sample, allowing only a small amount of the light to be detected.

Surface Reflectance Measurements

Data for the surface reflectance measurements for each of the deodorants and their respective masses are presented in Figure 20, which is a composite graph for (a) cinnamon dry leaves, (b) cinnamon fresh leaves and (c) lemon grass leaves.

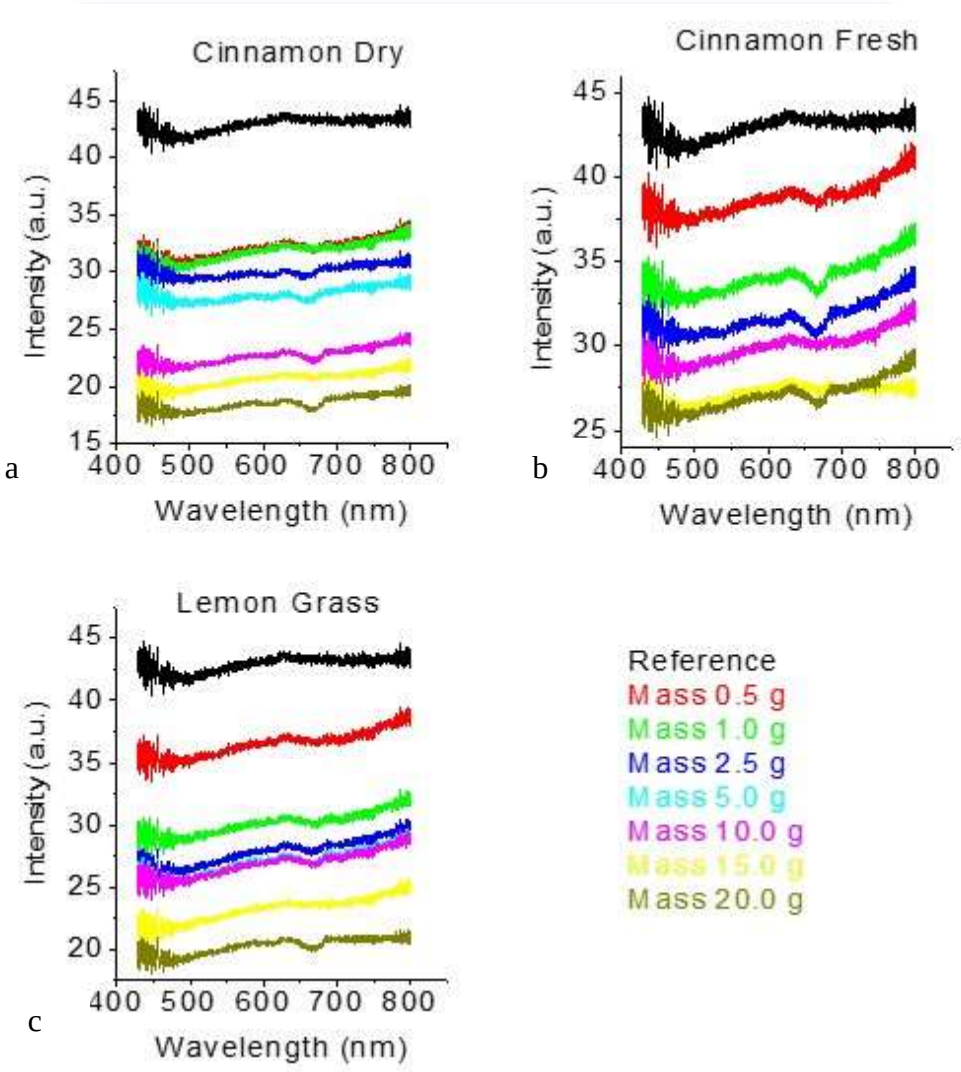


Figure 20: Surface reflectance measurements for the respective masses of deodorants (a) cinnamon dry, (b) cinnamon fresh and (c) lemon grass

From Figure 20 it could be deduced that an increase in deodorant mass resulted in a decrease in the amount of light reflected by the samples. This is because the absorption of radiation increased as the deodorant mass increased

(as depicted in Figure 19, thereby reducing the amount of light left to undergo reflection.

Empirical Relation between Surface Reflectance and Mass of Deodorant

An empirical relationship was established between the mass of the deodorant and the surface reflectance. The antioxidant regions, which are 500 600 nm (vitamin E) and 650 nm-730 nm (chlorophyll) (Kongbonga et al., 2011; Kyriakidis & Skarkalis, 2000; Sayago, Morales & Aparicio, 2001), were selected for this study. For each deodorant mass, the sum of surface reflectance was calculated within these two regions and plotted, as presented in Figure 21.

1

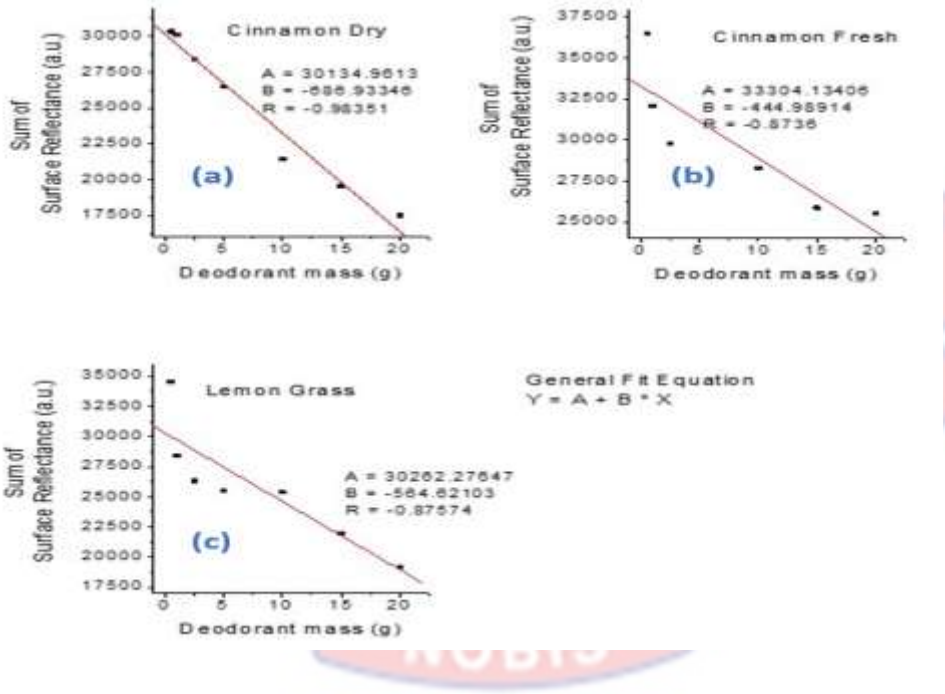


Figure 21: Plot of deodorant mass against the sum of surface reflectance intensity within the antioxidant region for (a) cinnamon dry leaves, (b) cinnamon fresh leaves and (c) lemon grass leaves.

From Figure 21, a linear relationship ($Y = A + B * X$) depicting a reduction in the surface reflectance intensity with deodorant mass was established for each of the deodorants. Using the empirical equations deduced,

the deodorant mass in a sample could be determined if the surface reflectance intensity is known.

Laser Induced Fluorescence (LIF) Measurements

The fluorescence emitted at longer wavelengths was measured at 90° to the excitation source when the samples were exposed to a laser light emitting at 445 nm. Figure 22 is a plot showing the variation of the fluorescence intensity with wavelength for the various masses for (a) cinnamon dry, (b) cinnamon fresh and (c) lemon grass deodorants. Figure22 (d) is a plot only for the 0.5 g and 20.0 g masses of the cinnamon fresh deodorant to depict the changes in fluorescence intensity at lower and higher wavelengths as the deodorant mass increases.

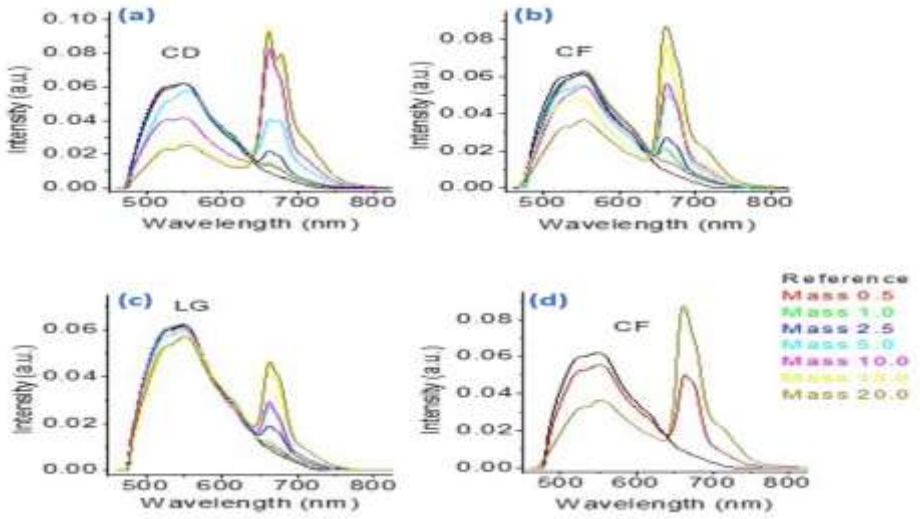


Figure 22: Variation of fluorescence intensity with wavelength for the various masses for (a) cinnamon dry, (b) cinnamon fresh and (c) lemon grass deodorants. (d) Plot for 0.5 g and 20.0 g masses only for cinnamon fresh deodorant

It would be observed that as the deodorant mass increased, the fluorescence intensity at lower wavelengths decreased while that at longer wavelengths increased. This is clearly depicted in Figure 23(d). An explanation for this observation has been provided later in the work.

De-convolution of Fluorescence Spectra

To separate overlapping peaks and reveal hidden ones, the fluorescence graphs were de-convoluted using PeakFit software. Three distinct regions were identified after the de-convolution using a Lorentzian profile, with the relative intensities of the lines being characteristic of the concentration of the emitting molecule within a region. The spectra of the samples in these regions were associated to the emission of oxidation products (Region I, 400 nm500 nm), vitamin E (Region II, 500 nm-600 nm) and chlorophyll (Region III, 650 nm-730 nm) (Kongbonga et al., 2011; Kyriakidis & Skarkalis, 2000; Sayago, Morales & Aparicio, 2001). Figure 23 shows the fluorescence graphs after the application of Peakfit software for the 0.5 g and 20.0 g masses of each deodorant.

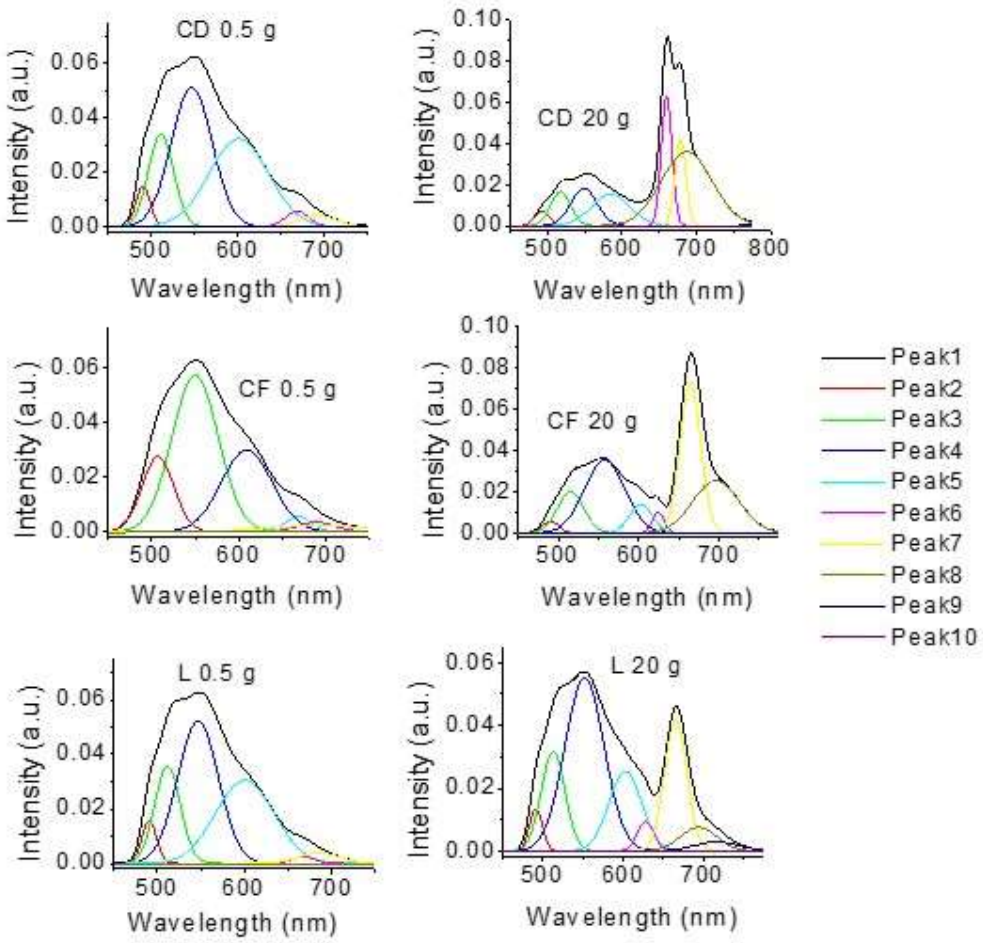


Figure 23: Fluorescence graphs after application of Peakfit software for 0.5 g and 20.0 g masses of each deodorant separating overlapping peaks and revealing hidden ones.

A total of 10 overlapping and hidden peaks were revealed for the various spectra. As was observed during the conventional measurements (to be presented later), an increase in the deodorant mass increases the antioxidant ability of the sample, and therefore an increase in both the vitamin E and chlorophyll content of the sample. The relatively higher intensities of the lines with deodorant mass at longer wavelengths as seen in Figure 24 (at the expense of shorter wavelengths, which corresponds to oxidation products) are therefore characteristic of an increase in the vitamin E and chlorophyll contents of the sample (Burton, Joyce & Ingold, 1983; Ferruzzi et al., 2002;

Hoshina, Tomita & Shioi, 1998).This therefore explains why an increase in deodorant mass resulted in a decrease in fluorescence intensity at lower wavelengths and increase at longer wavelengths.

The fluorescence spectra for the 0.5 and 20.0 g masses of the cinnamon dry deodorant are presented in Figure 24. The decrease in fluorescence intensity at lower wavelengths and increase at longer wavelengths as deodorant mass increases are clearly depicted. The three distinct regions associated with the emission of oxidation products, vitamin E and chlorophyll are also shown.

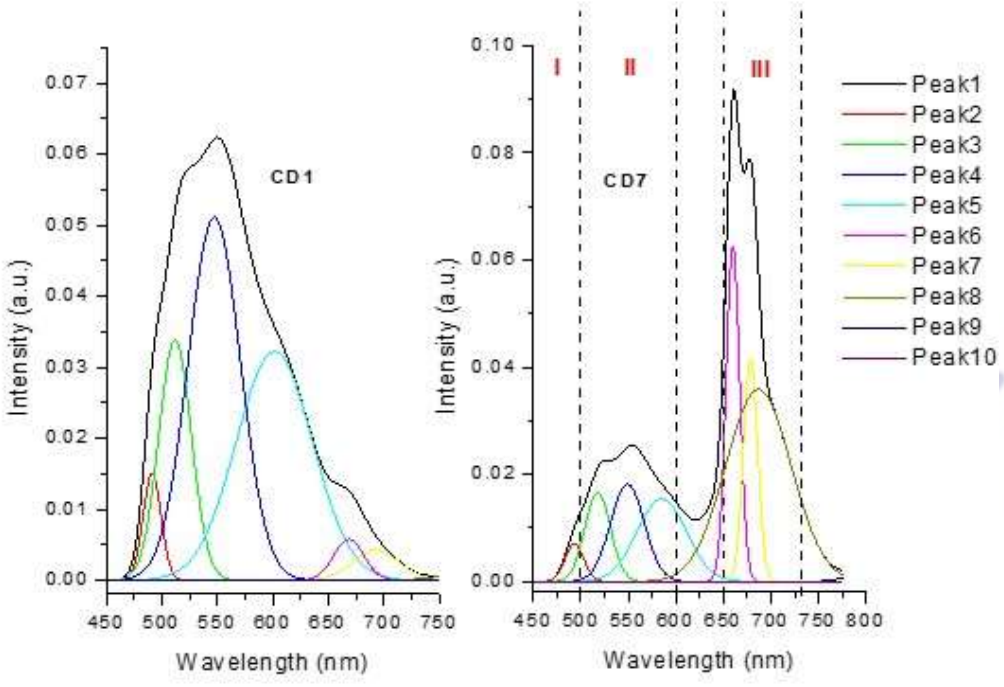


Figure 24: Fluorescence intensities for the 0.5 g (CD1) and 20.0 g (CD7) masses for the cinnamon dry deodorant. For the 20.0 g mass, the graph shows the three distinct regions associated with the emission of oxidation products, vitamin E and chlorophyll.

Deduction of Empirical Relations from De-convoluted Graphs

Two deductions were made from the de-convoluted graphs. These are (i) the deduction of an empirical relation between fluorescence intensity and mass of deodorant using Peak Intensity Ratios (PIR) and (ii) the deduction of an empirical relation between fluorescence intensity and mass of deodorant strictly within the antioxidant regions of the spectra.

Empirical Relation using Peak Intensity Ratios, PIR

This empirical relation was deduced using Figure 24. The PIR (P6/P4) were determined for each de-convoluted spectrum and a graph plotted. Figure 25 shows a plot of the deodorant mass against peak intensity ratio for the various deodorants.

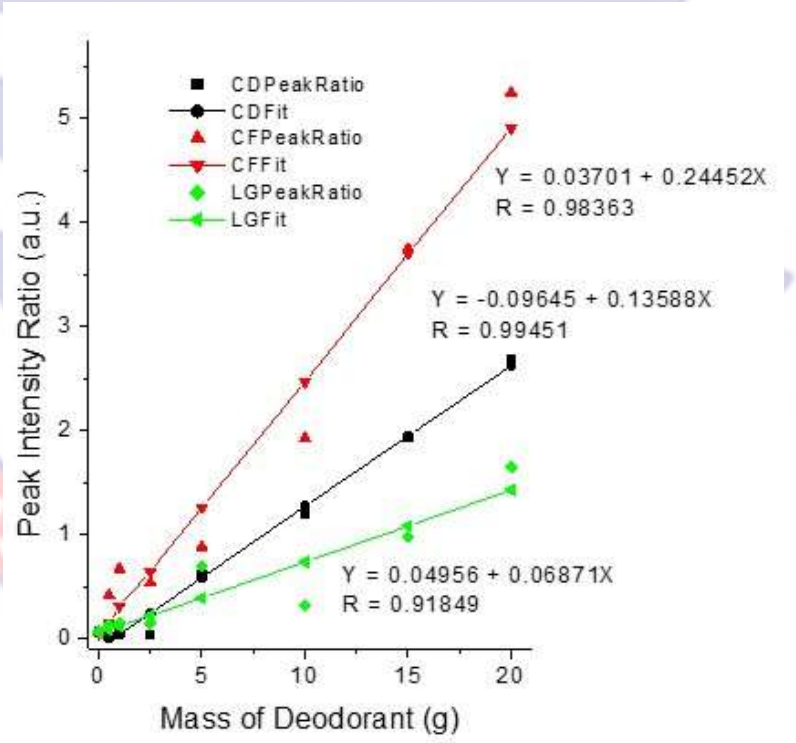


Figure 25: Plot of deodorant mass against peak intensity ratio for the deodorants showing a linear variation.

In plotting the graph, other peak ratios were first tried, but P6/P4 was used for the analysis because it gave the best R-value when plotted against the deodorant mass. As shown in Figure 25, a linear relationship was established for each deodorant, with which the mass of deodorant could be deduced when the peak intensity ratio is known.

Empirical Relation restricted within the Antioxidant Region

The empirical relation in this case was deduced using Figure 24. The antioxidant regions of the spectrum are the vitamin E and chlorophyll regions (Burton, Joyce & Ingold, 1983; Ferruzzi et al., 2002; Hoshina, Tomita &Shioi, 1998). These were selected for this analysis because these are the regions directly affected when the sample was deodorized. The sum of the fluorescence intensity within these regions was calculated and plotted against the deodorant mass. Figure 26 shows a plot of the sum of fluorescence intensity against the deodorant mass.

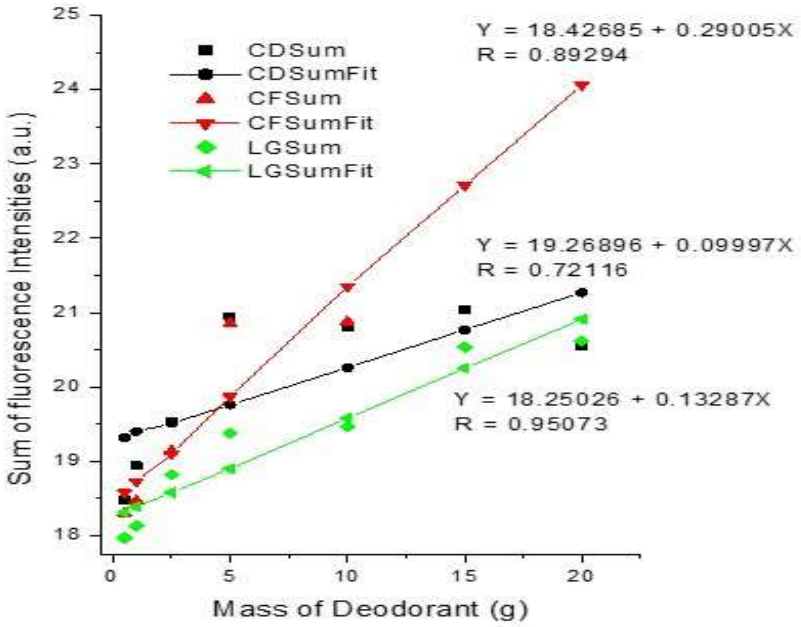


Figure 26: Sum of fluorescence intensity within the antioxidant regions against deodorant mass

A linear relation was deduced, and the constants associated with this relation for each deodorant are presented in Table 4.

Table 4: Linear Relation and constants Associated with each Equation Deduced from a plot of Mass against Sum of Fluorescence Intensity within the Antioxidant Regions.

Deodorant	Equation	Slope	Grading	R-Value
Cinnamon Dry	Y = 19.26896+ 0.09997X	0.09997	3rd	0.72116
Cinnamon Fresh	Y = 18.42685 + 0.29005X	0.29005	1st	0.89294
Lemon Grass	Y = 18.25026 + 0.13287X	0.13287	2nd	0.95073

With the individual equations, the mass of deodorant present in a sample could be determined when the sum of the fluorescence intensity within the antioxidant regions is known.

Additionally, useful information could be drawn from the values of the slopes of the individual graphs in Table 4. As has been established, the relative intensities of the lines obtained after de-convolution are characteristic of the concentration of the emitting molecule (Kongbonga, et al., 2011). The deduction therefore is that the cinnamon fresh deodorant with the highest slope yielded the highest/fastest concentration rate of antioxidant increase, and the cinnamon dry deodorant, the lowest. This deduction would be collaborated with actual antioxidant measurements carried out using the conventional techniques.

Determination of Total Antioxidant Capacity using Conventional Techniques

The total antioxidant capacity of each sample was determined using conventional techniques (phosphor molybdate method using ascorbic acid to measure antioxidant capacity). Figure 27 is a column graph showing the increase in total antioxidant capacity with deodorant mass for each sample. 'Ref' represents the refined sample without any deodorant, while the various deodorant masses are represented by 'Mass1' to 'Mass7'.

Figure 28 is a linear graph relating the deodorant mass and total antioxidant capacity in which an empirical equation was deduced between these two for each deodorant. The equations and the constants associated with each equation are presented in Table 5.

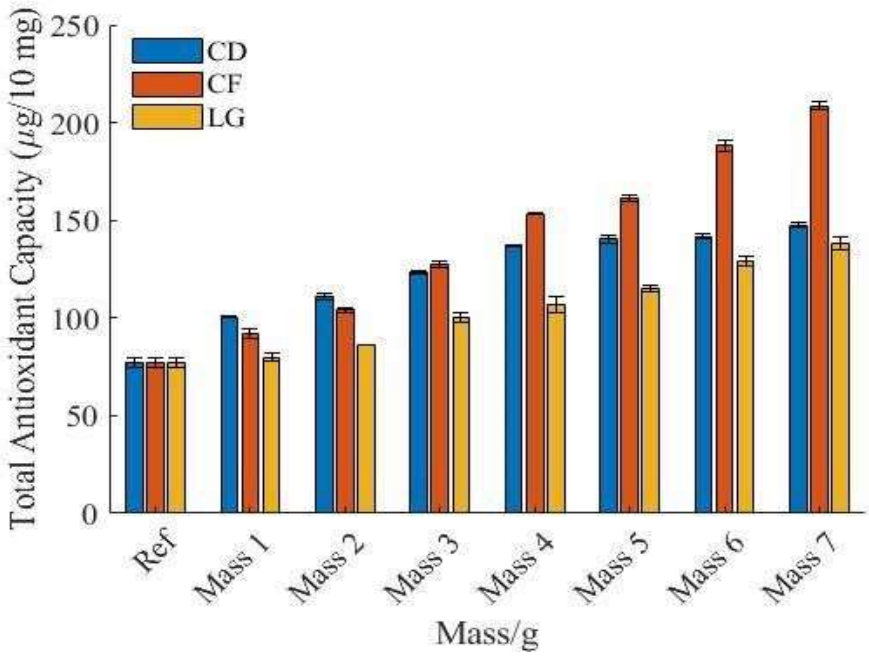


Figure 27: Column graph showing increase in total antioxidant capacity with deodorant mass for each sample. 'Ref' represents reference sample without any deodorant, and 'Mass1' to 'Mass7' represent various deodorant masses used

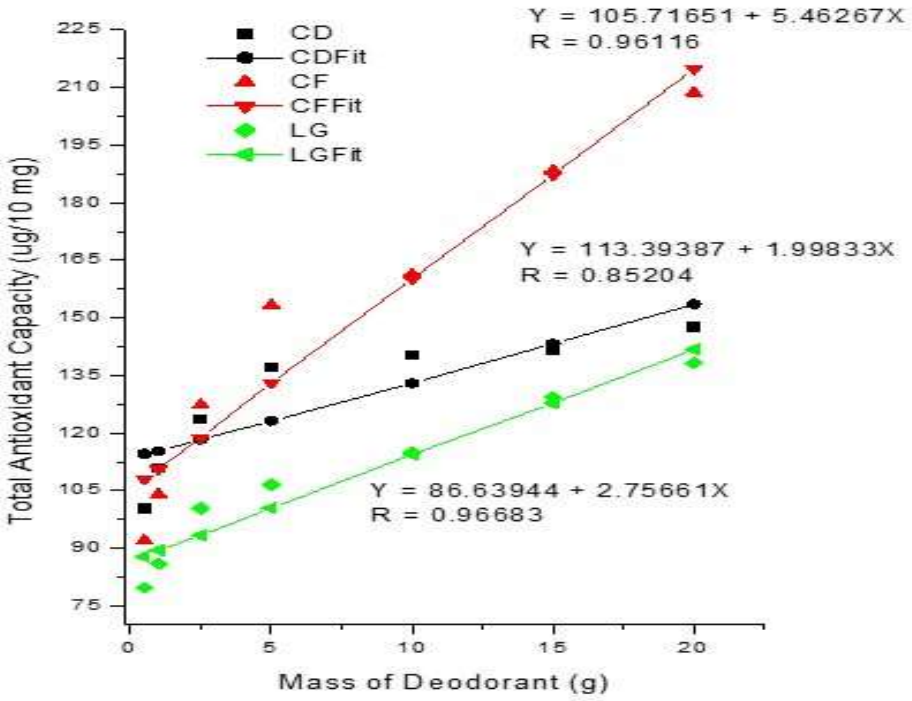


Figure 28: Linear of graph between the deodorant mass and total antioxidant capacity, with an empirical equation deduced between these two for each deodorant

Table 5: Linear Relation and Constants associated with each Equation for the Plot between the Deodorant Mass and Total Antioxidant Capacity.

Deodorant	Equation	Slope	Grading	R-Value
Cinnamon Dry	Y = 113.39387 + 1.99833X	1.99833	3rd	0.85204
Cinnamon Fresh	Y = 105.71651 + 5.46267X	5.46267	1st	0.96116
Lemon Grass	Y = 86.63944 + 2.75661X	2.75661	2nd	0.96683

Using the established individual equations, the total antioxidant capacity of a sample could be determined if the mass of deodorant is known.

The slopes in Table 5 represent the total antioxidant capacity per unit mass. The cinnamon fresh deodorant with the highest slope therefore means that it yielded the highest/fastest rate of total antioxidant capacity increase, and the cinnamon dry deodorant, the lowest. This deduction is in agreement with the use of optical techniques from the de-convoluted fluorescence graphs in Figure 26 and presented in Table 4.

Quantitative Analysis of Increase in Total Antioxidant Capacity

As presented in Figure 27, an increase in deodorant mass resulted in an average increase 15.4% in total antioxidant capacity of each sample. Table 6 shows the percentage increase in the total antioxidant capacity for each deodorant mass.

Table 6: Percentage Increase in the Total Antioxidant Capacity for each Deodorant Mass

	Percentage Increase		
Mass (g)	Cinnamon Dry	Cinnamon Fresh	Lemon Grass Leaves
0.5	29.74	18.96	3.01
1.0	43.53	34.48	11.20
2.5	59.91	64.65	29.74
5.0	77.15	98.27	37.93
10.0	81.46	108.62	48.70
15.0	83.18	143.53	67.24
20.0	90.94	169.82	78.87

Table 6 provides a quantitative analysis in terms of percentages, of the increase in total antioxidant capacity for each sample as the deodorant mass was increased.

Total antioxidant capacity as estimated as AAE (ascorbic acid equivalence) show a positive and strongly correlated results between the amount of deodorant treatment and total antioxidant capacity and this support the fact that deodorized samples exhibit high antioxidant capacity (refer to Figure 27 and 28). Refined non deodorized PKO (REFF) sample showed a lower total antioxidant capacity as a result of the fact that REFF was subjected to heat during its extraction and that its total antioxidant capacity is lower than 100 μ g/10mg as a result of degradation of some thermolabile phenol compounds. In general, all samples treated with plant sample extract i.e., cinnamon dry and fresh and also lemongrass leaves had strong positive correlation within the amount of deodorant used in refining. The results in this study have also shown that samples of PKO can be deodorized with respect to the deodorant used as it improves the quality, nutrition and medicinal properties of oil to ensure a pleasant appearance of the PKO.

Comparative analysis of Total Antioxidant Capacity Determined Using the Conventional and Optical Measurements

This analysis established a relationship between the total antioxidant capacity as measured using the conventional and optical techniques. For the optical technique, this was determined by summing the fluorescence intensities within the antioxidant regions, which are the vitamin E and chlorophyll regions.

Figure 29 is a double-y graph plotted to establish this relationship.

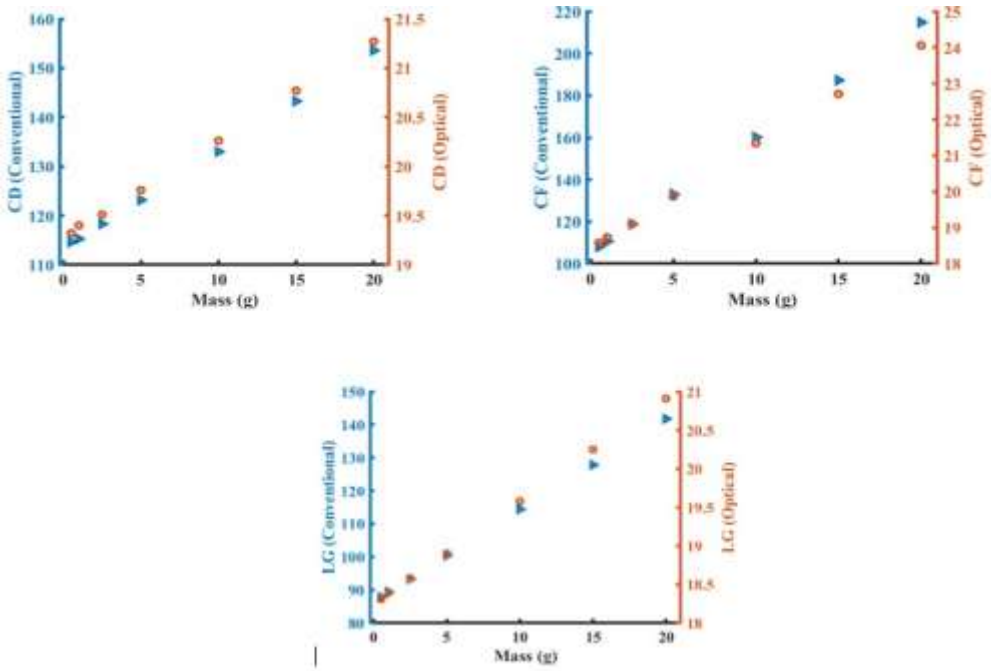


Figure 29: A comparative graph between conventional and optical (fluorescence intensity within the antioxidant regions) measurement on total antioxidant capacity.

Figure 29 clearly depicts conclusions already made, which are that, an increase in the deodorant mass increases the antioxidant capacity, as measured using the conventional and optical techniques. This confirms that these two techniques, though completely different, are in sync.

Measurement of Solar UV Effect on Total Antioxidant Capacity

UV Transparency of Materials

The spectra recorded after allowing sunlight through each of the materials, which gives the individual transmission and cut-off properties, are presented in Figure 30. This presentation was restricted for wavelengths within the UV (UVA and UVB) spectral regions. The materials were laminated glass (A), clear glass (B), polyethylene terephthalate (PET) (C) and transparent linear low-density polyethylene (LLDPE) (D). 'Sun' is the spectrum of the sun, which was used as the reference.

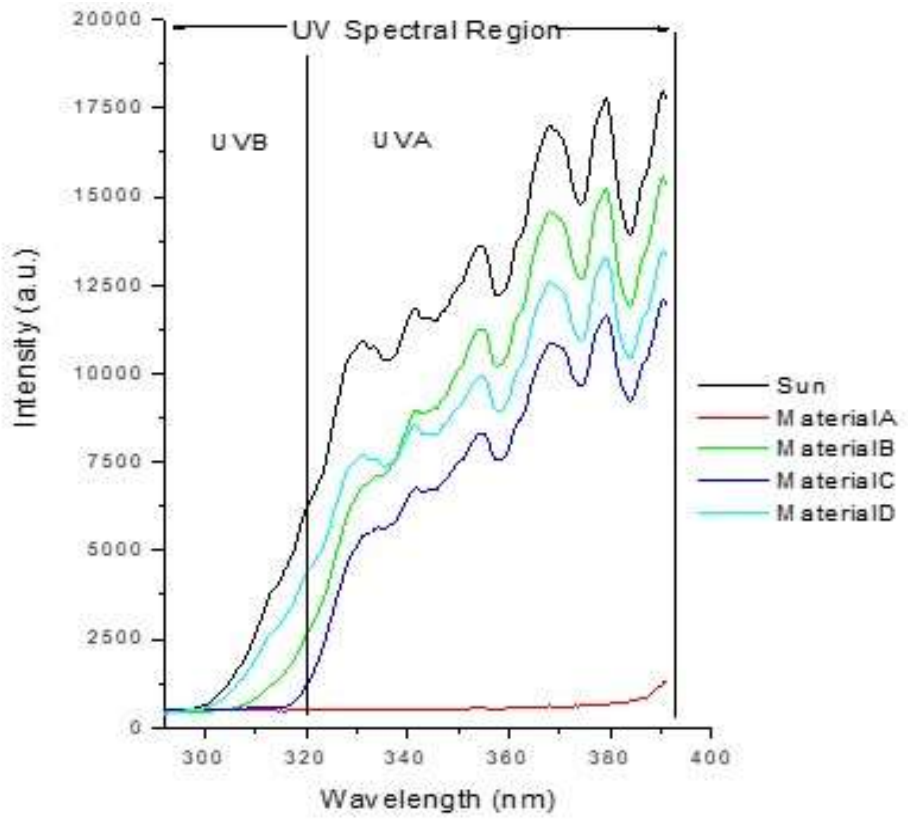


Figure 30: Spectra recorded after allowing sunlight through each of the materials investigated. 'Sun' represents spectrum of the sun, which was used as reference

Figure 30 shows the wavelengths of light that are not absorbed (transmitted wavelengths) when sunlight was incident on the materials. It could be deduced that the laminated glass (i.e., Material A) exhibited an opaque behaviour as it did not permit the transmission of any wavelengths within the UV region. It terminated all wavelengths within this region as it lay just above the '*0*' mark and run paralleled to the wavelength axis.

The spectra for materials B, C and D all rose above the '*0*' mark, just like the spectrum of the sun, and showed intensity values. This indicates the transparency of the materials within the UV spectral region. A summary of the findings from Figure 30 that recap the extent of transparency for the materials investigated is given in Table 7. These findings are in agreement with a previous study (Sackey et al., 2015).

Table 7: Summary of Transparency of the Materials Studied

Material	UV Transparency
Laminated glass	Terminates
Clear glass	Transparent
Polyethylene terephthalate (PET)	Transparent
Transparent linear low-density polyethylene (LLDPE)	Transparent

Exposure of Oil Samples to Sunlight under Different Conditions

Analysis using data obtained by conventional techniques

Results are presented for Exposure 1, where the sample were put into a ceramic bowl and covered with laminated glass (i.e., Material A). For this, the samples were UV-protected as the ceramic bowl did not allow light to pass through, while the laminated glass which allowed sunlight to pass through, terminated the UV. Measurements for these would be identified using 'P' (i.e., Protected).

Results are also presented for Exposure 2, where the samples were exposed to UV as both the clear glass (i.e., Material B) in which the samples were put, and the LLDPE (i.e., Material D) used to cover the samples, are transparent to UV. Measurements for these will be identified using 'NP' (i.e., Not Protected). Figure 31 are column graphs showing the reduction in total antioxidant capacity with days when the samples were exposed to sunlight under the two different exposure procedures.

This is presented for both the non-deodorised (REF) and deodorised oil samples.

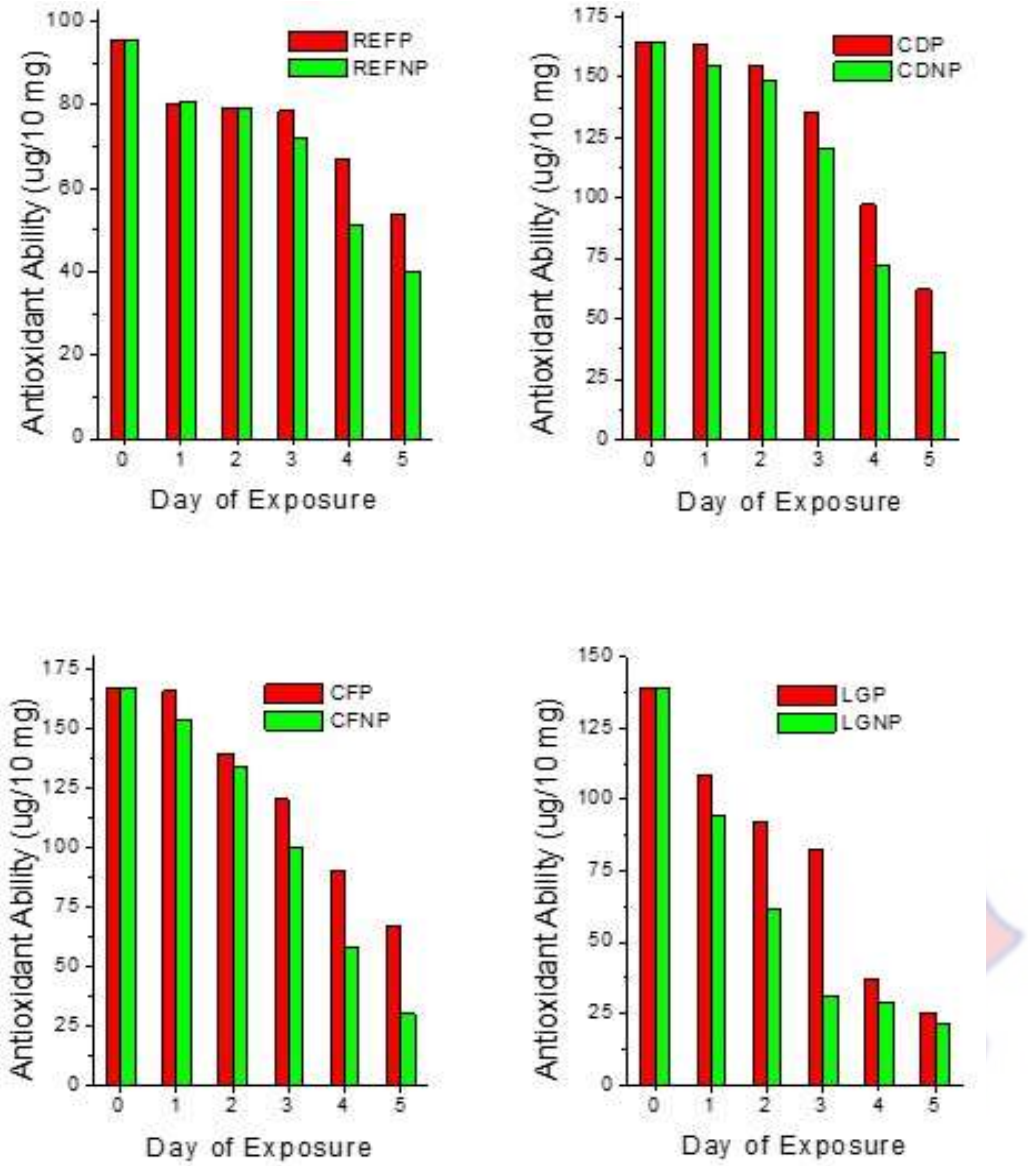


Figure 31: Column graph showing the reduction in total antioxidant capacity with days with samples exposed to sunlight under the two different exposure procedures. REF represents the non-deodorised RPKO sample.

Figure 31 clearly depicts a reduction in the total antioxidant ability with days for both samples; P and NP from UV. However, a greater reduction was observed for the sample not protected from UV radiation from the sun. This suggests that a prolonged exposure to the sun, even if protected from the

UV, leads to a reduction in the antioxidant capacity of the sample due to the sample being exposed to high temperature. Figure 32 is a plot showing the temperature variations, within the day, on the days the samples were exposed to sunlight. The times of measurement were 9.00 am, 12.00 noon and 3.00 pm.

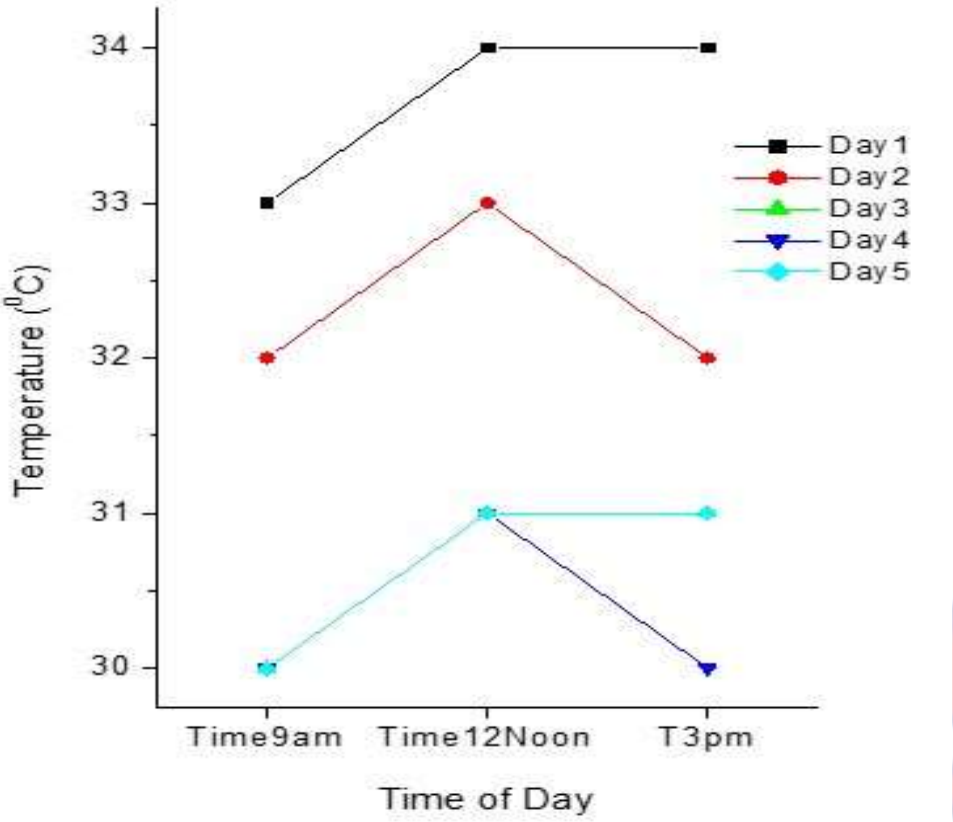


Figure 32: Plot showing temperature variations, within the day, on days the samples were exposed to sunlight.

To accurately determine the different rates of reduction in total antioxidant capacity within the period of exposure, a line graph was plotted and the slopes calculated. Figure 33 are line graphs showing the reduction in total antioxidant capacity with days when the samples were exposed to sunlight under the two different exposure procedures. The line of best fit was

plotted, out of which an equation was established. This is presented for both the non-deodorized (REF) and deodorised PKO samples.

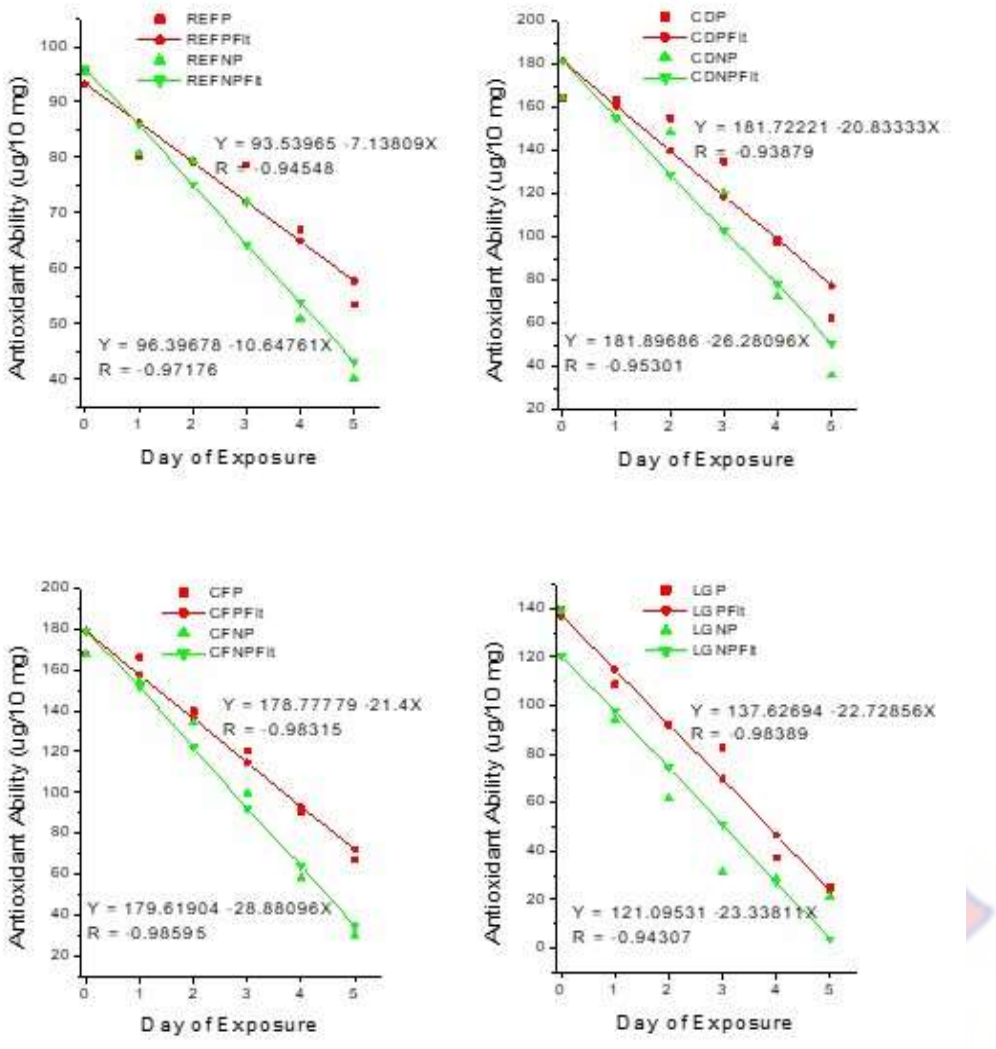


Figure 33: Line graphs showing reduction in total antioxidant capacity with days when samples were exposed to sunlight under the two different exposure procedures. Line of best fit was used to establish an equation between days of exposure and total antioxidant capacity.

The linear relation between the days of exposure and total antioxidant capacity, and their associated constants are presented in Table 8.

Table 8: Linear Equations between the days of Exposure and Total Antioxidant Capacity and Associated Constants for each Sample after being subjected to the two Exposure Procedures.

Sample	Equation	Slope	Rate	R-Value
Reference				
UV Protected	Y = 93.53965 -7.13809X	-7.13809	2nd	-0.94548
UV Not Protected	Y = 96.39678 -10.64761X	-10.64761	1st	-0.97176
Cinnamon Dry				
UV Protected	Y = 181.72221 -20.83333X	-20.83333	2nd	-0.93879
UV Not Protected	Y = 181.89686 -26.28096X	-26.28096	1st	-0.95301
Cinnamon Fresh				
UV Protected	Y = 178.77779 -21.4X	-21.4	2nd	-0.98315
UV Not Protected	Y = 179.61904 -28.88096X	-28.88096	1st	-0.98595
Lemon Grass				
UV Protected	Y = 137.62694 -22.72856X	-22.72856	2nd	-0.98389
UV Not Protected	Y = 121.09531 -23.33811X	-23.33811	1st	-0.94307

It could be deduced from Table 8 that for each of the samples, the slope of the linear fit, which represents the rate of reduction in antioxidant capacity with days of exposure, was greater for the samples Not Protected from UV radiation. This suggests that exposure of the deodorized oil to UV resulted in a reduction in its antioxidant capacity. Samples protected from UV also experienced a reduction with days of exposure. This suggests that a prolonged exposure to the sun, even if protected from the UV, resulted in a reduction in the antioxidant ability. This finding would be collaborated using the optical measurements taken.

Quantitative Analysis of Decrease in Total Antioxidant Capacity

Figure 31 clearly depicts a reduction in the total antioxidant ability with days for both samples after exposure to sunlight; Protected (P) and Not Protected (NP) from ultraviolet radiation. Table 9 shows the percentage decrease in the total antioxidant capacity for each deodorant mass after exposure to sunlight protected and not protected from ultraviolet radiation from the sun.

Table 9a: The Percentage Decrease in the Total Antioxidant Capacity for each Deodorant Mass Protected and Not Protected from Ultraviolet Radiation after Exposure to Sunlight.

	Percentage Decrease			
Day	RefP	RefNP	CDP	CDNP
1	76.69	76.89	86.12	87.61
2	76.82	76.93	86.76	87.72
3	76.91	76.96	87.41	87.77
4	77.05	77.06	87.57	87.79
5	77.10	77.16	87.68	87.84

Table 9b: The Percentage Decrease in the Total Antioxidant Capacity for each Deodorant Mass Protected and Not Protected from Ultraviolet Radiation after Exposure to Sunlight.

Day	Percentage Decrease			
	CFP	CFNP	LGP	LGNP
1	90.51	91.32	86.89	87.10
2	90.92	91.38	86.96	87.12
3	91.14	91.39	86.97	87.14
4	91.18	91.39	86.98	87.17
5	91.28	91.41	87.03	87.27

Table 9 provides a quantitative analysis, in terms of percentages, of the decrease in total antioxidant capacity for each sample after exposure to sunlight protected and not protected from the ultraviolet radiation.

Analysis using Data obtained from Optical Analysis of Oil Samples

As shown in Figure 33, an increase in the mass of deodorant, which resulted in an increase in the antioxidant capacity, caused a decrease in fluorescence intensity at lower wavelengths and an increase at longer wavelengths. It is therefore expected that there would be changes in the spectra after exposure to sunlight due to the reduction in total antioxidant capacity. Figure 34 is a composite graph for the samples under study. For each composite graph, a comparison was made after the first day of exposure between UV Protected and UV Not Protected Figure 34 and between the spectra of the first and fifth days for the protected exposure (right graphs).

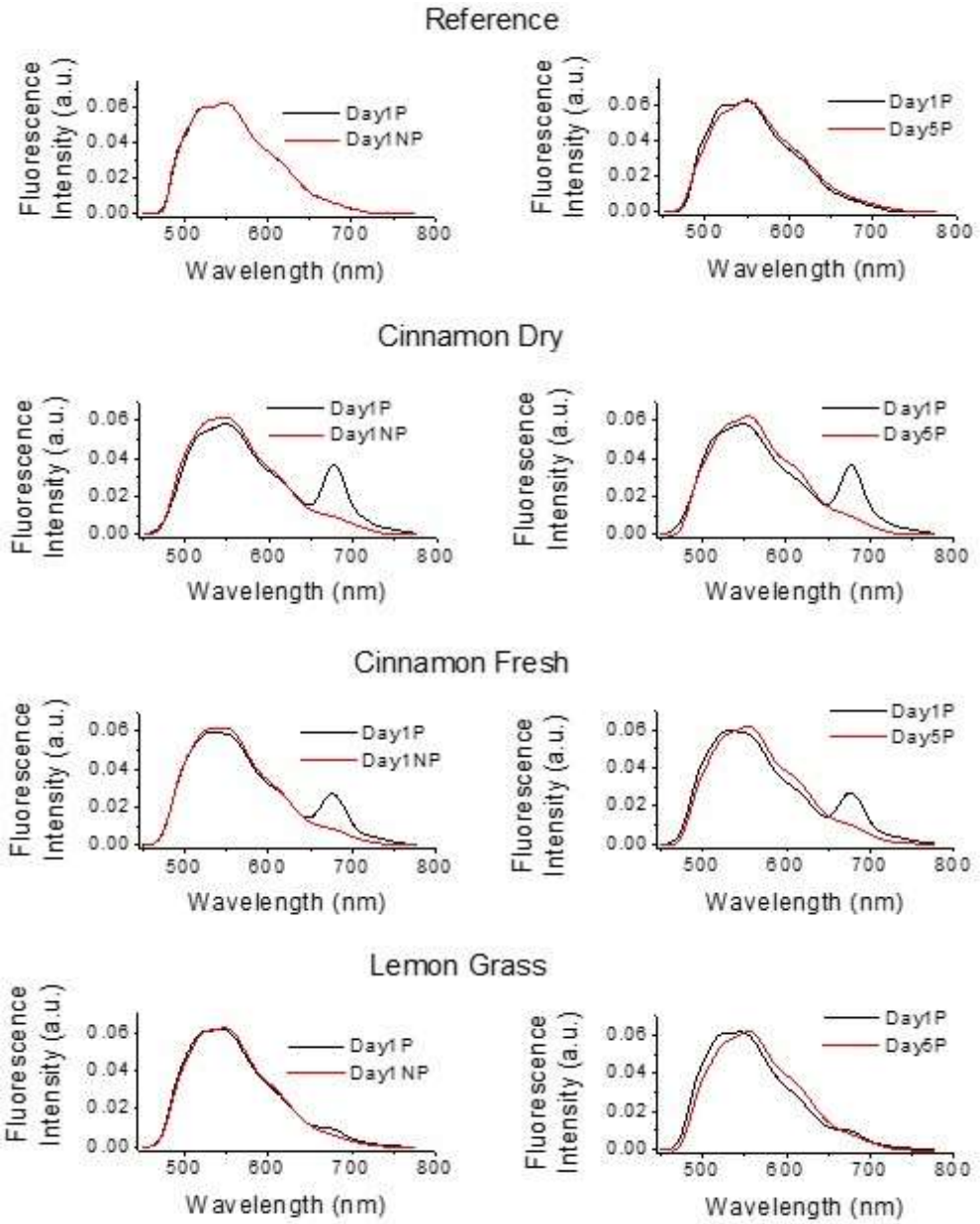


Figure 34: Composite graph showing the spectra after the first day of exposure between UV Protected and UV Not Protected (left graphs) and between the spectra of the first and fifth days for the Protected exposure (right graphs)

It's could be observed that after the first day of exposure (for graphs on the left), the spectra of the samples Not Protected from UV showed an increase in fluorescence intensity at shorter wavelengths and a reduction at longer wavelengths. This was exactly opposite what was observed when the samples were deodorized. This therefore suggests a decrease in the total

antioxidant capacity (vitamin E and chlorophyll content) of the sample Not Protected from UV. The antioxidant region also recorded a peak in the antioxidant region for the Protected sample, but was absent for the sample which was Not Protected.

A similar deduction could be made looking at the spectra for Days 1 and 5 for the Protected samples (graphs on the right), which showed an increase in fluorescence intensity at shorter wavelengths and a reduction at longer wavelengths. This therefore suggests that a prolonged exposure to the sun, even if protected from the UV, leads to a reduction in the antioxidant capacity of the sample. The peak within the antioxidant region which was present after the first day was completely absent by the fifth day.

To further investigate these findings, the antioxidant region (vitamin E Region, 500 -600 nm and chlorophyll Region 650 -730 nm) was selected, the sum of fluorescence intensity determined and plotted. Figure 35 is a composite graph showing a plot of the day (s) of exposure against the sum of fluorescence intensity. The graphs on the left are Day 1 intensities for the Protected and Not Protected samples, while those on the right are intensities after the first and fifth days of exposure for the protected sample.

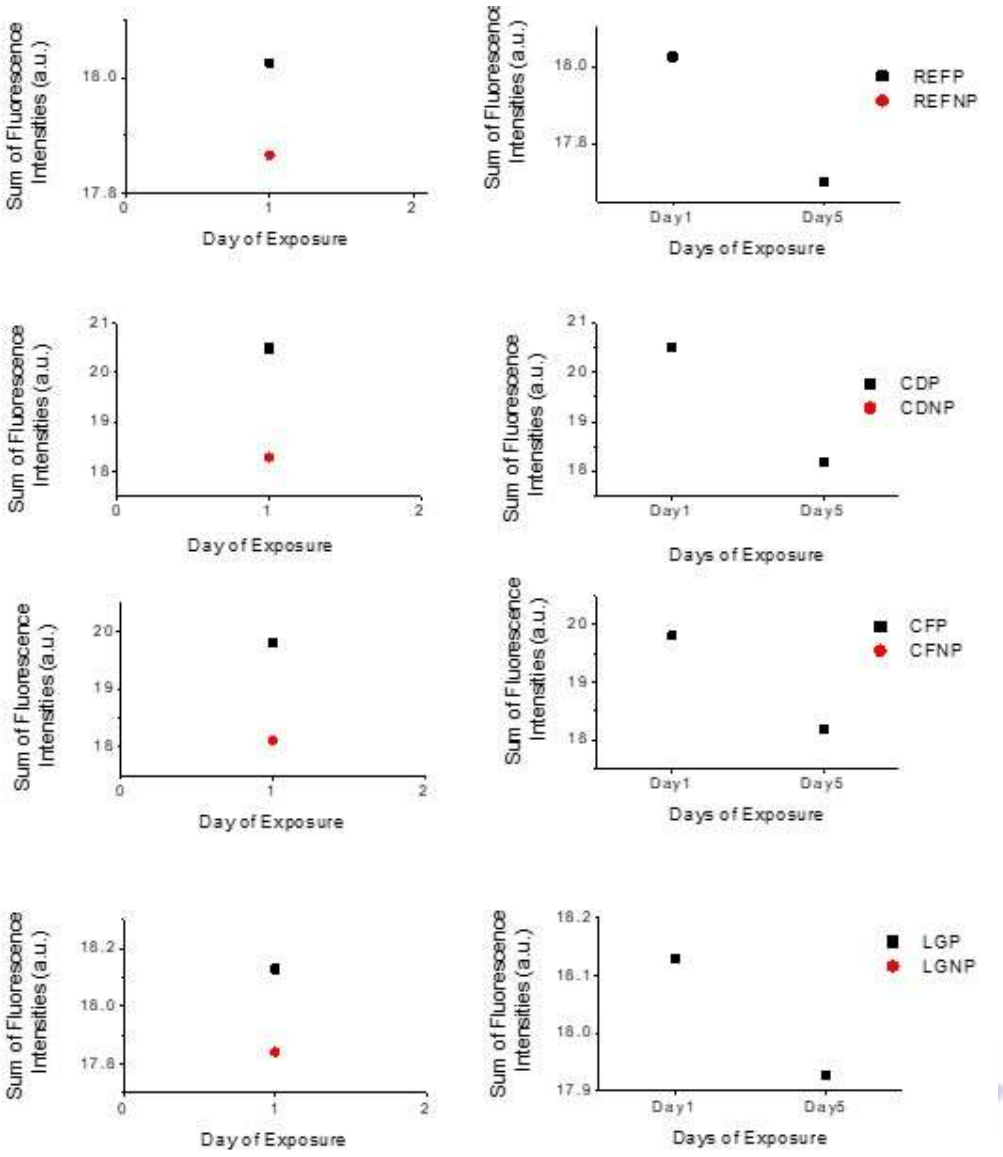


Figure 35: Composite graph showing sum of fluorescence intensity plots after Day 1 of exposure for the Protected and Not Protected samples (left plots), and sum of fluorescence intensity plots after the first and fifth days of exposure for the protected sample (right plots)

From Figure 35, the plots on the left show a reduction in fluorescence intensity, within the antioxidant region, after Day 1 of exposure for the sample Not Protected from UV. The plots on the right also show a reduction in fluorescence intensity after the fifth day of exposure even for the protected samples. This suggests that a prolonged exposure to the sun, even if protected from the UV, leads to a reduction in the fluorescence intensity. As has been

established, that the relative intensities of the lines were characteristic of the concentration of the emitting molecule within a region (Kongbonga et al., 2011; Kyriakidis & Skarkalis, 2000; Sayago, Morales & Aparicio, 2001), it could therefore be stated that the exposure to sunlight without any protection from UV resulted in a reduction in the antioxidant capacity of the sample. Prolonged exposure to sunlight, even when protected from UV, also resulted in a decrease in the antioxidant capacity of the sample.

The ability of a product to act as donor (antioxidant) is indicated by its ability to scavenge or inhibit free radicals. Higher antioxidant inhibition ability indicates that the product has high scavenging ability towards free radicals and vice versa. They are capable of reducing the antioxidant capacity of foods, oils and other antioxidant sources. The antioxidant inhabitation ability of refine PKO exposed to the sun was lower compared to that of the refined PKO opaque to UV. This could be observed on the graph on the graph in Figure31 and 33. For example the percentage decrease value in day 1 for CFP was 90.5 % as compared to CFNP which was 91.32 %. the reduction in inhibition might be attributed to the fact that solar UV induced free radicals into the refined PKO that was transparent to UV and might had reacted with the antioxidants of the refined PKO. Antioxidants are capable of donating hydrogen atom to the free radical species to curtail their deleterious activities. This clearly showed that the refined PKO opaque to solar UV is better in protecting antioxidant than refined PKO transparent to UV.

Prolong exposure to sunlight had immense effect on the refined PKO by drastically reducing the antioxidant capacity. For 5 days exposure of refined PKO transparent to UV saw an increase in percentage decrease in antioxidant

from 91.3 % in day 1 to 91.4 % by day 5 for CFNP this result was similar for the different deodorized plant extract refined PKO. There was a reduction in ant oxidant capacity for CFP which was opaque to UV the percentage decrease was from 90.5 % in day 1 to 91.2 % in day 5. This meant that solar UV induced more free radicals in to refine PKO.

Principal Component Analysis (PCA)

A PCA algorithm was developed and implemented in MATLAB software (MATLAB R2017a, Math's Works Inc). This was used to transform the fluorescence spectral of the samples from a high dimensional to a low dimensional space called the principal components (PCs), thereby not altering any special information contained in it. It allowed for the detection of any patterns present in the LIF spectra data, and the information was used in discriminating the refined deodorized and refined non-deodorized (Reference, REF) palm kernel oil samples. PCA was computed using all the fluorescence spectra data (normalized) from the oils (deodorized and non-deodorized) in order to determine the distribution pattern of the samples. Figure 36 shows the eigenvalue/scree plot for the cinnamon dry deodorant. The first and second PC together described 89.16 % variability in the fluorescence data.

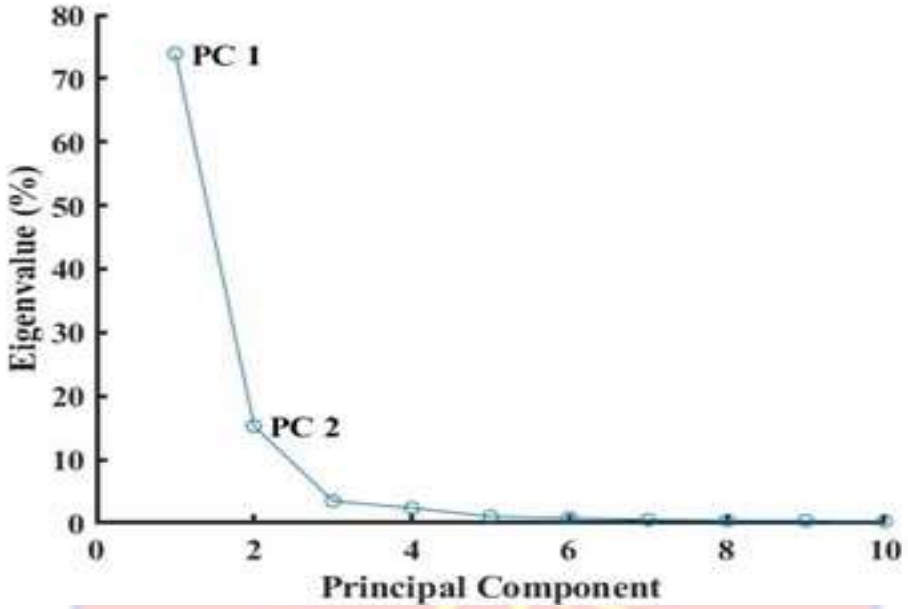


Figure 36: Eigenvalues (%) of the PCs which represent the weight of each principal component

Figure 37 shows the score plot of the non-refined PKO and refined deodorized PKO using dry cinnamon leaves as the deodorizer. The figure shows a cluster grouping between the non-deodorized (RFF) and deodorized oil samples. The cluster separation for the nondeodorized and deodorized oil occurs along both the PC1 and PC2. In order to generate a model to determine whether the oil is deodorized or not, linear discriminant analysis was performed to determine the boundary between the

REF and CD in Figure 35. The model equation is expressed in equation (5) as:

$$K + L(1)\,PC1 + L(2)\,PC2 = 0 \quad (5)$$

where K = 4.44e15, L (1) = 9.52 and L (2) = -3.49.

The model finally is expressed as

$$9.52PC1 - 3.49PC2 = -4.44e\text{-}15 \quad (6)$$

It could also be observed in Figure 36 that there were two outliers for the REF in the CD.

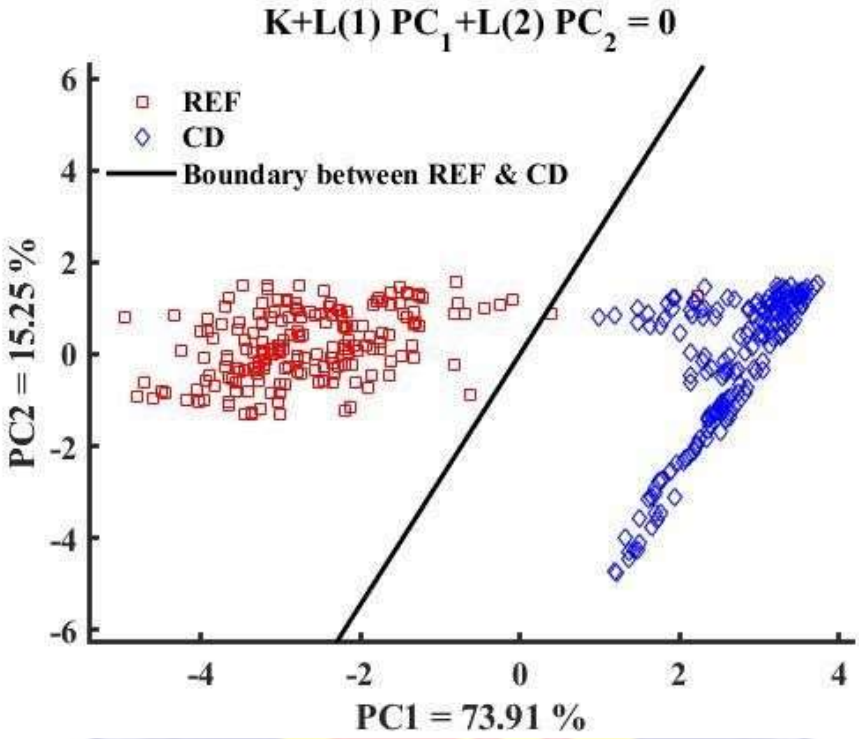


Figure 37: Score plot of the non-refined PKO and refined deodorized PKO using dry cinnamon leaves as the deodorizer

Figures 38 and 39 shows respectively the score plot for non-refined PKO and refined deodorized PKO using fresh cinnamon leaves and lemon grass leaves as the deodorizer. The first two PCs gave a cumulative percentage of 92.12 and 88.78 total variability in the fluorescence data as seen in Figures 38 and 39 respectively. As explained in Figure 38, a linear discriminant analysis was performed to determine the boundary between the REF and CF and that of the REF and LG in Figures 38 and 39. The models generated from the linear discriminant analysis were as follows:

$K + L(1) PC1 + L(2) PC2 = 0$ (7) where

$K = -7.11*10^{-15}$, L (1) = 3.18 and L (2) = 9.98 for CF,

$K + L(1)\,PC1 + L(2)\,PC2 = 0$ (8) where

$K = 3.55*10^{-15}$, L (1) = 7.62 and L (2) = 1.93 for LG.

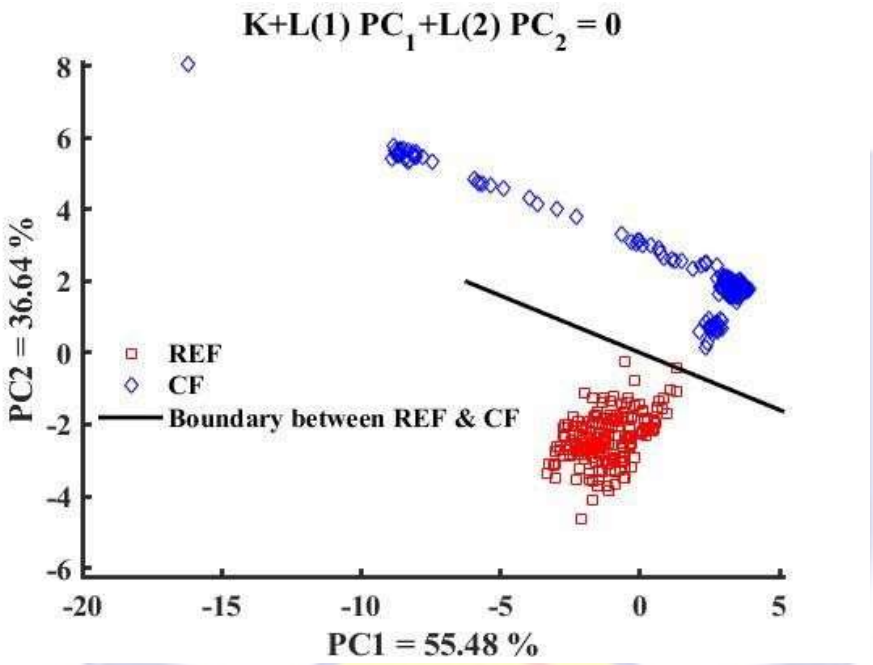


Figure 38: Score plot of the non-refined PKO and refined deodorized PKO using fresh cinnamon leaves as the deodorizer

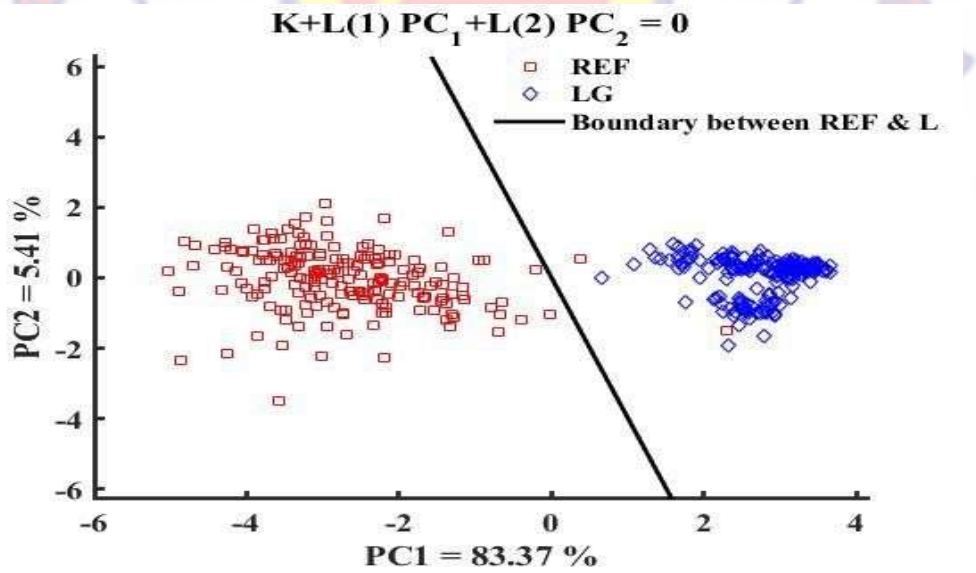


Figure 39: Score plot of the non-refined palm kernel oil and refined deodorized palm kernel oil using lemon grass leaves as the deodorizer

The individual equations and constants associated with the equations are presented in Table 10.

Table 10: Individual Equations and Constants Associated with Equations from PCA

Deodorant	Equation	K-value	L1-value	L2-value
CD	K + L (1) PC1 + L (2) PC2 = 0	$4.44*10^{-15}$	9.52	-3.49
CF	K + L (1) PC1 + L (2) PC2 = 0	$-7.11*10^{-15}$	3.18	9.98
LG	K + L (1) PC1 + L (2) PC2 = 0	$3.55*10^{*-15}$	7.62	1.93

Chapter Summary

In this chapter, the results of various analytical measurements including absorbance, surface reflectance and laser induced fluorescence which were used to determine the effect of the different types of deodorants, their different levels of deodorization, as well as the effect on the antioxidant capacity PKO when exposed to sunlight was described. A comparison was made between the optical techniques and conventional methods used for the antioxidant measurements.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Overview

This chapter presents the summary of the thesis, conclusions drawn, and suggested recommendations for future work.

Conclusions

In this thesis after the crude PKO was deodorized with the medicinal plant i.e., cinnamon fresh, cinnamon dry leaves and lemongrass there was an improvement in Flavor of the refined PKO. The medicinal plant used as deodorant enhanced the smell there aided in removing the pungent smell associated with the crude PKO.

The optical and conventional techniques used in this study to measure the antioxidant capacity of the PKO after deodorization showed an increase in the antioxidant capacity of PKO with increasing mass of the deodorant. Fresh cinnamon leaves deodorized palm kernel oil had the highest antioxidant ability followed by cinnamon dry and lemongrass leaves.

The result drawn from the optical measurement of antioxidant capacity of the refined PKO showed that the equation of the line of best fit of this experiment could be used to determine any mass of the deodorants used in deodorizing PKO. This could therefore enable one to know the level of deodorization of the refined PKO.

The equation of line of best fit for the refined PKO deodorized with cinnamon dry leaves, $y = 0.1579x + 0.044$ with R^2 value of 0.9839, refined PKO deodorized with cinnamon fresh leaves, $y = 0.975x + 0.237$ with R^2 value of 0.9632 and refined PKO deodorized with lemongrass leaves, $y =$

$0.0299x$ + 0.240 with R^2 value of 0.8546. The refined palm kernel oil deodorized with cinnamon dry leaves gave the highest R^2 value with the lemongrass leaves deodorized palm kernel oil giving the least. With the equation y been the fluorescence intensity ratio and x the mass of the deodorant used and c the intercept. When the y, m and c are know you can find the mass of deodorant used in the refined PKO.

Principal component analysis (PCA) was used to differentiate between refined palm kernel and deodorized palm kernel oil. The PCA model was generated from the laser induced fluorescence spectra of the refined deodorized palm kernel oil.

The refined deodorized palm kernel oil was exposed to the sun for five days, laser induced fluorescence spectral indicated a decrease in the total antioxidant capacity of the deodorized palm kernel oil as the days go by.
The rate of reduction is greater when the PKO is exposed to UV radiation.

Recommendations

It is recommended that the equation obtained from the experiment to determine the mass of deodorants used in refined deodorized palm kernel oil with the help of a laser source of wavelength 445 nm can be used by Food and Drugs Authority (FDA) to determine the level of antioxidant in refined PKO deodorized with cinnamon or lemongrass leaves.

Further studies should be done using the cold pressed deodorized palm kernel oil using different light sources of different wavelengths to determine the level of deodorization.

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