Effect of *Moringa oleifera* leaves extract on lipid peroxidation and sensory characteristics in cookies

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ผลของสารสกัดจากใบมะรุมต่อการเกิดออกซิเดชั่นของลิพิด และคุณลักษณะทางประสาทสัมผัสในผลิตภัณฑ์คุกกี้

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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เดบอราห์ โออูชัว แดนโซ : ผลของสารสกัดจากใบมะรุมต่อการเกิดออกซิเดชั่นของลิพิดและคุณลักษณะทางประสาทสัมผัสในผลิตภัณฑ์คุกกี้ (Effect of *Moringa oleifera* leaves extract on lipid peroxidation and sensory characteristics in cookies) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: สถาพร งามอุโฆษ, หน้า.

ผลิตภัณฑ์เบเกอรี่ที่มีไขมันสูงมักมีความไวต่อการเกิดการออกซิเดชั่นของไขมัน การเติมสารต้านอนุมูลอิสระที่มาจากผลิตภัณฑ์ธรรมชาติในเบเกอรี่จึงอาจเป็นแนวทางในลดการเกิดการออกซิเดชั่นของไขมันระหว่างกระบวนการผลิต มะรุมเป็นพืชที่รู้จักเป็นอย่างดีเนื่องจากมีคุณสมบัติในการต้านอนุมูลอิสระ ดังนั้นงานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาฤทธิ์ของสารสกัดจากใบมะรุมในการลดการเกิดการออกซิเดชั่นของไขมันในผลิตภัณฑ์คุกกี้ จากผลการศึกษาพบว่า คุกกี้ที่มีส่วนผสมของการสกัดจากใบมะรุมมีความแตกต่างของค่าความสว่าง ค่าความแดง และค่าสีโดยรวม และเมื่อทำการประเมินทางประสาทสัมผัสในคุกกี้ที่มีส่วนผสมของสารสกัดจากใบมะรุม 0.2%, 0.4%, 0.6%, 0.8% และ 1.0% พบว่า คะแนนการประเมินทางประสาทสัมผัสโดยรวม (overall acceptability) ในคุกกี้ที่มีส่วนผสมของสารสกัดจากใบมะรุม 0.2% และ 0.4% ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับคุกกี้กลุ่มควบคุม แต่คุกกี้ที่มีส่วนผสมของสารสกัดจากใบมะรุม 0.4% และ 0.6% มีคุณสมบัติในการต้านอนุมูลอิสระและการเกิดออกซิเดชั่นของไขมัน แตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ อีกทั้งยังมีคะแนนการยอมรับในผลิตภัณฑ์ที่สูง ดังนั้นคุกกี้ที่มีส่วนผสมของสารสกัดจากใบมะรุม 0.4% และ 0.6% จึงถูกนำไปศึกษาถึงคุณสมบัติดังกล่าวในการเก็บรักษานาน 4 สัปดาห์ และพบว่าคุกกี้ที่มีส่วนผสมของสารสกัดจากใบมะรุมมีความสามารถในการต้านอนุมูลอิสระและความสามารถในการลดการเกิดการออกซิเดชั่นของไขมันสูงกว่าคุกกี้กลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติตลอดระยะเวลา 4 สัปดาห์ นอกจากนี้คุกกี้ที่มีส่วนผสมของสารสกัดจากใบมะรุม 0.4% มีคะแนนความชอบในผลิตภัณฑ์โดยรวมไม่แตกต่างจากกลุ่มควบคุม และระยะเวลาเริ่มต้น (สัปดาห์ที่ 0) ในขณะที่คุกกี้ที่มีส่วนผสมของสารสกัดจากใบมะรุม 0.6% มีคะแนนความชอบในผลิตภัณฑ์มีคะแนนความชอบในผลิตภัณฑ์โดยรวมแตกต่างจากกลุ่มควบคุม แต่ไม่แตกต่างของคะแนนจากระยะเวลาเริ่มต้น (สัปดาห์ที่ 0) ดังนั้นสารสกัดจากใบมะรุมอาจใช้เป็นสารต้านอนุมูลอิสระจากธรรมชาติในการลดการเกิดออกซิเดชั่นของไขมันในผลิตภัณฑ์คุกกี้

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Bakery products with high fat content are highly susceptible to produce lipid peroxidation. Incorporation of bakery products with natural antioxidants is alternative approach for decreasing lipid peroxidation during food manufacturing process. *Moringa oleifera* is a well-known plant which has been demonstrated to possess antioxidant property. In this regard, the aim of this study was to investigate the effect of *Moringa oleifera* leaves extract (MOE) on prevention of lipid peroxidation in cookies. The results illustrated that the lightness, redness and E-index of the cookies were affected when fortified with MOE. After, sensory evaluation conducted in control and cookies fortified with MOE (0.2%, 0.4%, 0.6%, 0.8% and 1.0% MOE), 0.2% and 0.4% MOE cookies showed no significant difference in overall acceptability when compared to control cookies. However, the significant difference of antioxidant property and lipid peroxidation of 0.4% and 0.6% MOE fortified cookies with high score of overall acceptability were observed when compared with control cookies($p<0.05$). Thus, 0.4% and 0.6% MOE fortified cookies were chosen for further studies during 4 weeks of storage periods. The significant increase in antioxidant properties together with the reduction of lipid peroxidation of cookies fortified with MOE was observed throughout the storage time when compared to control cookies. In addition, overall acceptability of 0.4% MOE was not significantly different from control and also when compared to week 0 during the storage period whereas cookies fortified with 0.6% MOE were significantly difference when compared with control cookies ($p<0.05$) but had no change in overall acceptability during the storage period when compared to week 0. The findings indicated that *Moringa oleifera* leave extract may be used as a source of natural antioxidant to prevent lipid peroxidation in cookies.

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LIST OF ABBREVIATION

MOE – Moringa oleifera extract

AGEs – Advanced glycation end products

CD – Conjugated dienes

CT – Conjugated trienes

MDA – Malondialdehyde

BHA -Butylated hydroxyanisole

BHT - Butylated hydroxytoulene

DPPH - 2, 2-diphenyl-1-picrylhydrazyl

PV - Peroxide value

TBARS - Thiobarbituric acid Reactive Substance

a_w - Water activity

PVC - Polyvinylchloride

PA/PE - Polyamide/polyethylene

MAP - Modified atmosphere

CML - N^{ε}-carboxymethyl lysine

EPR - Electron spin resonance

ET - Electron transfer

AVRDC - The Asian Vegetable Research and Development Center (),

GK - Goto-Kakizaki

ALT - Alanine aminotransferase

AST - Aspartate aminotransferase

ALP - Alkaline phosphatase

BUN - Blood urea nitrose

TBA - Thiobabituric acid

QDA – Quantitative descriptive analysis

SEM – Standard error of mean

CHAPTER 1

INTRODUCTION

1.1 Background

One of the largest organized food industries in the world is the bakery industry, with many popular products like cakes, cookies and biscuits because of their convenience, ready to eat nature, and long shelf life [1-3].

Though bakery foods have good taste, texture, aroma and appearance; they may also undergo lipid peroxidation which might further go through series of reactions to form advanced glycation end products (AGEs). These are formed in the food products due to the ingredients used mainly the lipid content, temperature and time duration used in baking [4]. Even though, lipids bring texture and taste to food products, they are susceptible to oxidative processes in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and micro-organisms, giving rise to the development of off-flavors and loss of essential amino acids, fat-soluble vitamins, and other bioactives, this process in foods can also lead to changes in the color and texture of food products. The development of these off flavors is mostly due to oxidation in food products, which can be measured by determining the level of free fatty acids present. Lipids may undergo autoxidation, photo-

oxidation, thermal oxidation, and enzymatic oxidation under different conditions, most of which involve some type of free radical or oxygen species [5-7].

Lipid peroxidation is also a form of non-enzymatic browning reaction which occurs in food. Unsaturated fatty acids are generally the reactants affected by such reactions [8]. Oils and fats undergo oxidative changes during storage conditions which cause reduction in the nutritional quality and shelf life of them [9]. Oxidation of the lipids normally proceeds very slowly at the initial stage; the time to reach a sudden increase in oxidation rate is referred to as the induction period [10]. Lipid hydroperoxides have been identified as primary products of autoxidation; decomposition of hydroperoxides yields aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, known as secondary oxidation products. Lipidoxidation results into primary products such as free fatty acids, conjugated dienes (CD) or conjugated trienes (CT) and peroxides. Malondialdehydes (MDA) have been known to be the most toxic and most studied secondary product of lipid peroxidation from polyunsaturated fatty acids [11]. Previous studies showed that MDA can react with DNA to form mutagenic adduct in human cell [11, 12]. Recently, various polyphenol compounds, especially flavonoids, have received substantial attention because of their antioxidant activities in various *in vitro* systems [13]. Normally, the use of polyphenols in reducing lipid peroxidation typically aimed to harness their antioxidant activities to counteract the activities of pro-oxidation

factors which may initiate or propagate lipid peroxidation [14]. Although, synthetic antioxidants being used in food industries are good at increasing the shelf life of food products, some are also known to be toxic and carcinogenic [15, 16]. Therefore, there has been an increase in consumer preference for natural food additives than the synthetic ones and this has increased the search for more natural antioxidants from fruits, herbs, spices and vegetables as less harmful alternatives to synthetic antioxidants [17].

Therefore, there has been a recent development on the addition of some edible herbs and fruits into bakery products in order to make them healthier and have longer shelf life. This is due to the fact that these herbs and fruits contain polyphenols which have antioxidant properties. There have been studies on the addition of *Moringa oleifera* leaf extract, which is an edible herb and has many beneficial properties from having a high nutritive value to helping in reduce the risk of many diseases.

Even though, there have been reports on *Moringa oleifera* leaves extract (MOE) being used in bakery foods, there has been little or no information on the addition of MOE in the preparation of cookies. Therefore, in this research, cookies were enriched with MOE and its effect on the reduction of lipid peroxidation, and increase in antioxidant capacity and shelf life determined.

1.2 Objectives

- To investigate the effect of *Moringa oleifera* leaves extract on polyphenol content, the level of lipid peroxidation and antioxidant capacity in cookies.
- To determine the acceptability of cookies with *Moringa oleifera* leaves extract by consumers through sensory analysis.
- To determine the effect of storage conditions on the level of MDA, antioxidant capacity, conjugated dienes formation, peroxide value, acidity, polyphenol, and flavonoid content, color , texture, water activity and moisture within a period of 4 weeks on the *Moringa oleifera* leaves extract fortified cookies.

1.3 Hypothesis

- The level of polyphenol content and antioxidant capacity significantly increases as lipid peroxidation reduces significantly in cookies with added *Moringa oleifera* leaves extract.
- Acceptability of cookies with *Moringa oleifera* leaves extract not significantly different from control.
- The level of lipid peroxidation significantly reduces as change in antioxidant capacity, polyphenol, and flavonoid content, moisture, texture, water activity and color remains insignificant in cookies with *Moringa oleifera* leaves extract after the storage period of 4 weeks.

1.4 Research benefits

- This research will help in the development of a functional food product, which will help reduce the risk of developing metabolic diseases that may occur due to the prolong consumption of some bakery foods
- Enable the use of *Moringa oleifera* leaves extract in some bakery foods to increase their shelf life.

CHAPTER 2

LITERATURE REVIEW

2.1 Lipid peroxidation in foods

Intake of processed foods, sugar and fat diets have increased over the past three decades [18]. Maintaining the quality of these foods especially bakery products e.g. cookies, biscuits, bread, etc is economically important since they are kept over an extended period before consumption [19]. Fat contained in these foods are mostly triglycerides which comprise three esters of fatty acids and a glycerol backbone where the fatty acids can either be in the saturated or unsaturated form. The unsaturated form of fatty acids due to their double bond undergoes lipid oxidation and this leads to diets containing fat easily going rancid. The value of lipids in foods can decline during either the processing, handling or storage period of these food products.

Lipids present in food products can undergo either hydrolysis or oxidation. Hydrolytic rancidity of lipids occurs due to the hydrolysis of the ester linkages in the lipids leading to the formation of free fatty acids and glycerol. Factors that affect this reaction are high temperatures, acids, lipolytic enzymes and high moisture content. However, hydrolytic cleavage is not of great influence in the development of off flavors due to refining techniques in industries. Lipid oxidation reaction, however, are

more complicated than just the abstraction of hydrogen from fatty acid by oxygen. In terms of lipid deterioration, oxidation reaction frequently occurs and leads to rancidity, off flavor development, polymerization, reversion and other reactions that lead reduction in shelf life and loss of nutritive value of food products [20]. Lipid peroxidation can be divided into two categories. In the first category, the oxidation of highly unsaturated fat particularly polyunsaturated types results into polymeric end products whilst the second category deals with moderately unsaturated fats which leads to rancidity, reversion and other types of off flavors and odors [21]. Lipids are susceptible to oxidative processes in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and micro-organisms, giving rise to the development of off-flavors, colors and loss of essential amino acids, fat-soluble vitamins, and other bioactives. Lipids may undergo autoxidation, photo-oxidation, thermal oxidation and enzymatic oxidation under different conditions, most of which involve some type of free radical or oxygen species [5, 6]. The degree and rate of lipid oxidation is influenced by the composition of fatty acids, oxygen concentration present, temperature, surface area, water activity and presence of anti– and prooxidants [22]. Autoxidation is the most common process leading to oxidative deterioration and is defined as the spontaneous reaction of atmospheric oxygen with lipids [8]. The process can be accelerated at higher temperatures, such as those experienced during deep-fat frying (thermal oxidation) with increases in free fatty acid

and polar matter contents, foaming, color, and viscosity [23]. Autoxidation and thermal oxidation of unsaturated fatty acids occur via a free radical chain reaction that proceeds through three steps of initiation, propagation and termination [24] as shown in **Figure 1**.

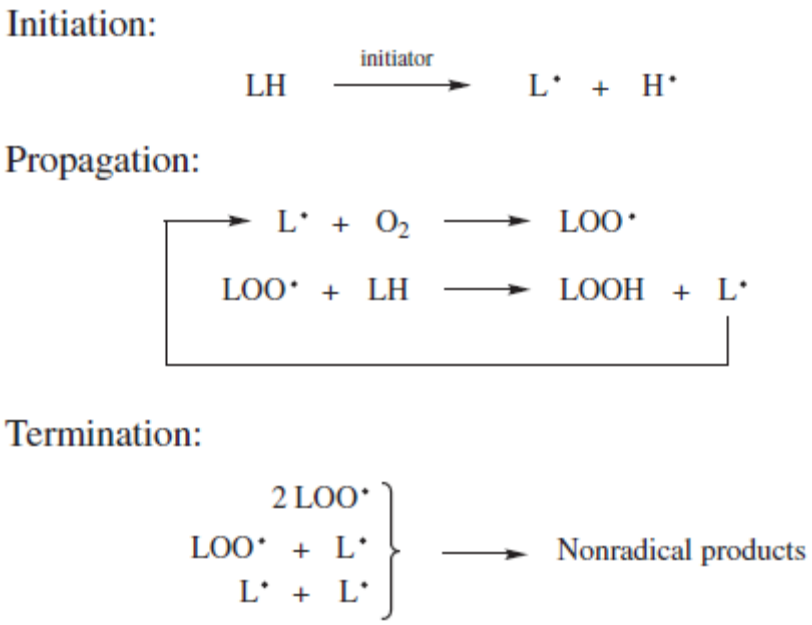


Figure 1. Lipid peroxidation mechanism [25].

2.1.1 Initiation stage of lipid peroxidation

During the initiation stage, molecular oxygen combines with unsaturated fatty acid to produce hydroperoxide and free radical of which both are reactive. For this stage to occur at a reasonable rate, some oxidative initiators must be present such as chemical oxidizers, transition metals (i.e. iron or copper) and enzymes such as

lipoxygenases. Additional factors that affect the initiation phase and other phases of lipid peroxidation are heat and light [26]. Lipid peroxidation is a chain reaction initiated by the hydrogen abstraction or addition of an oxygen radical, resulting in the oxidative damage of polyunsaturated fatty acids (PUFA). Since polyunsaturated fatty acids are more sensitive than saturated ones, it is obvious that the activated methylene (RH) bridge represents a critical target site. As shown in **Figure 2**, the presence of a double bond adjacent to a methylene group makes the methylene C-H bond weaker and therefore the hydrogen becomes more susceptible to abstraction. This leaves an unpaired electron on the carbon, forming a carbon-centered radical, which is stabilized by a molecular rearrangement of the double bonds to form a conjugated diene which then combines with oxygen to form a peroxyl radical. The peroxyl radical is itself capable of abstracting a hydrogen atom from another polyunsaturated fatty acid, starting a chain reaction [27]. At this stage, it leads to changes in the conformation of the double bonds in cis and trans configurations, with the more stable trans configuration predominating [28].

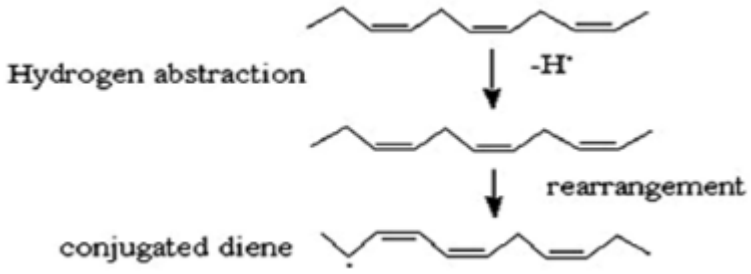


Figure 2. Initiation Stage of lipid peroxidation [29].

Molecular oxygen rapidly adds to the carbon-centered radicals (R.) formed in this process, yielding lipid peroxyl radicals (ROO.). The oxygen molecule that reacts at this stage exists in several states, and both the singlet and the triplet states are involved in the oxidation of lipids. The singlet oxygen ($^1O_2$) has an empty outer antibonding orbital which seeks to fill and this makes it a highly reactive electrophile which reacts with the unsaturated fatty acid [30]. The singlet oxygen is therefore considered as a substrate for lipid oxidation. In the triplet state ($^3O_2$), the two outer antibonding orbital contain a single electron each with the same spin direction. The oxygen is not able to abstract hydrogen because of its low energy. One of the available electrons interact with the alkyl radical in a diffusion-limiting rate to form a covalent bond and this leads to the formation of a high energy peroxyl radical (ROO˙) [22].

2.1.2 Propagation stage of lipid peroxidation

Decomposition of lipid peroxides is catalyzed by transition metal complexes yielding alkoxyl (RO.) or hydroxyl (HO.) radicals. These participate in chain reaction initiation that in turn abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. As shown in **Figure 3**, the formation of peroxyl radicals leads to the production of organic hydroperoxides, which, in turn, can abstract hydrogen from

another PUFA. This reaction is termed propagation, implying that one initiating hit can result into the conversion of numerous PUFA to lipid hydroperoxides. In sequence of their appearance, alkyl, peroxyl and alkoxyl radicals are involved. The resulting fatty acid radical is stabilized by rearrangement into a conjugated diene that retains the more stable products including hydroperoxides, alcohols, aldehydes and alkanes. Lipid hydroperoxide (ROOH) is the first, comparatively stable, product of the lipid peroxidation reaction [27]. Peroxides formed during the initial stage of oxidation are odorless and colorless and after further degradation into the secondary products such as aldehydes, ketones and alcohol, these are odiferous and detrimental which can be observed during sensory evaluation. Therefore, examining the level of peroxide in fat containing foods over a period of time is a way of evaluating its quality [31].

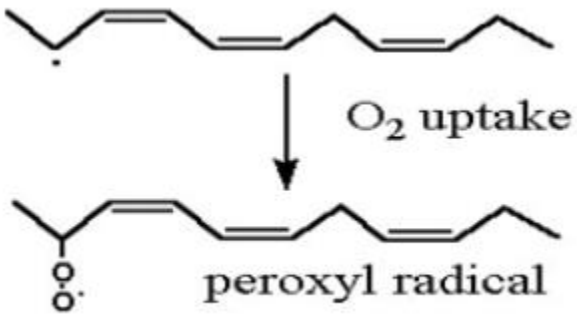


Figure 3. Propagation stage of lipid peroxidation [29].

Aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, which are formed from the decomposition of hydroperoxides, are known as secondary oxidation product [32]. Conjugated dienes are normally measured to determine the level of primary oxidation products since it has a good correlation with the peroxide value measurement [33]. White (1995), reported that when polyunsaturated fatty acids are oxidized, a shift in one of the double bonds occurs, producing a conjugated diene that can be measured by ultraviolet absorption [34]. Ultraviolet absorption at 232 nm shows an almost constant increasing diene content with progress of heating time through the process. Therefore, an increase in absorbance shows an increase in oxidation in the food product tested [35]. However the rate of increase in diene content in later stages of heating is expected to be lower, finally reaching a plateau due to the establishment of equilibrium between the rates of formation of conjugated dienes to that of polymers [36]. Peroxide value (PV) is also used to determine the rate of lipid peroxidation especially during the initial stage [37]. Lipid hydroperoxide is one parameter that is commonly measured in lipids to determine their oxidation state; however, iodometric titration is one preferred method for the determination of lipid hydroperoxide level as peroxide value (PV). Fat is normally termed rancid when its PV value is 10 $mEqO_2$/kg whilst a fresh and refined product should have a PV value below 1 $mEqO_2$/kg [38]. Lipid hydroperoxide can easily decompose under heat condition to form stable secondary

oxidation products [39]. Enzymes such as lipases and catalase cause oxidative reactions. These enzymes can then lead to the separation of fatty acids from fat to form free fatty acids which are substrates of oxidation. These free fatty acids cause a rise in acidity, which is an index of oxidation in fats [40, 41] as shown in **Figure 4**. Acid value is usually used to indicate the quality of frying oils, where a limit of 2 mgKOH/g oil is usually used. High level of acid value indicates fat breakdown/rancidity during refinery, storage or usage [31].

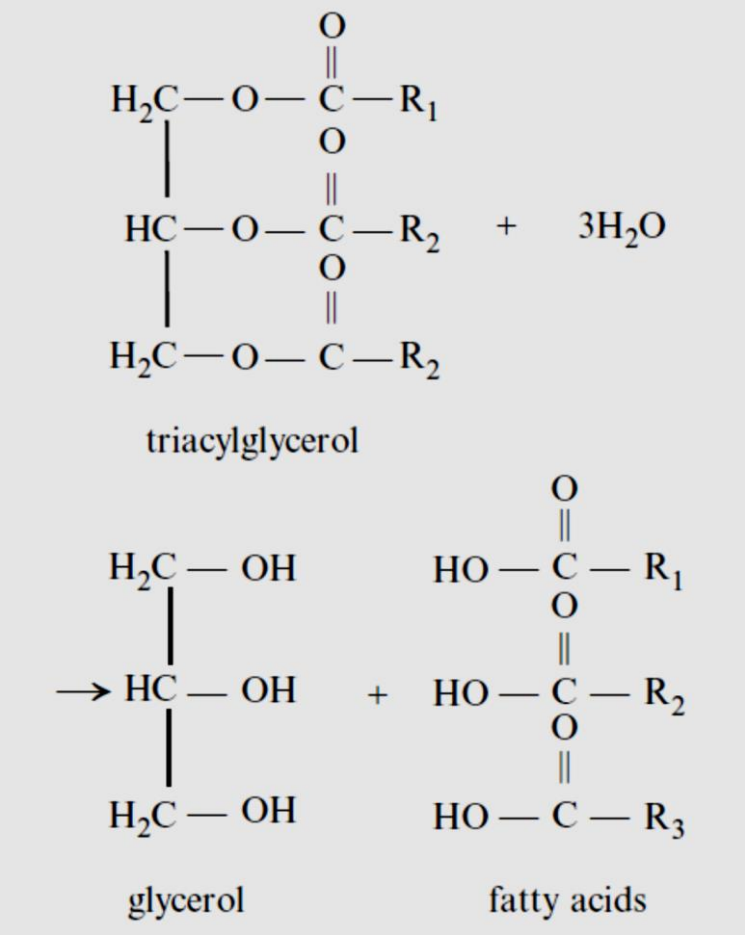


Figure 4. Hydroxylation of fatty acids from triacylglycerol [42].

2.1.3 Principle of Thiobarbituric acid Reactive Substance (TBARS) Assay

This was carried out to assess the level of lipid oxidation in the cookies, since it can occur by thermal oxidation of polyunsaturated oils. These are oxidized to form aldehyde and ketones which then reacts with amino acids to form brown pigments, as in the Maillard reaction. It is possible that peroxidation products induce the browning reaction of Amadori products [43].

2- thiobarbituric acid forms red-colored products with malondialdehyde (MDA), some polyunsaturated aldehydes, dioxolanes and furan derivatives. As shown in **Figure 5,** one molecule of MDA reacts with 2 molecules of 2- thiobarbituric acid via Knoevenagel- type condensation to yield a chromophore with absorbance maximum at 532 - 535nm.

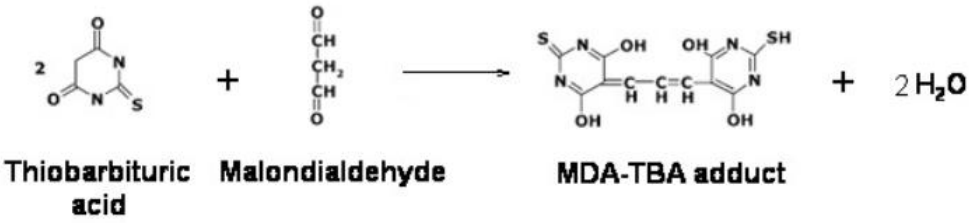


Figure 5. The reaction of MDA (malondialdehyde) and 2-thiobarbituric acid (TBA) [44]**.**

2.1.4 Factors that induce lipid peroxidation

There are several factors that induce lipid peroxidation in food. One of which is heat treatment; as it is for most other chemical reactions, the rate of oxidation increases with temperature. Thermally induced oxidation reactions can occur in both saturated and unsaturated lipids at temperatures encountered during processes such as deep frying [45]. Oxidation generally proceeds through the initial formation of hydroperoxides. The high temperature can cause many isomerization and scission reactions to take place, producing a myriad of secondary or breakdown products such as epoxides, dihydroperoxides, cyclized fatty acids, dimers and with scission reactions, aldehydes and ketones. Saturated fatty acids are relatively stable at the temperatures used in conventional canning operations, but unsaturated fats deteriorates, under the conditions of oxygen and heat to form a large number of volatile compounds, which give rise to both desirable and undesirable flavors [46].

Dehydration brings food component molecules into close proximity, thereby increasing the likelihood that they will interact [47]. Autoxidation of dried foods is accelerated by several factors, including exposure to light and elevated temperatures. Also, this reaction occurs most rapidly at very low water activity (a_w) levels, with higher levels producing a diminution in rates until the a_w 0.4-0.5 range is reached. Further increases in a tend to decrease oxidation rates, probably as a result of the dilution of oxidation components [48]. Water activity is an important factor in

determining the stability of a food product through lipid oxidation since it describes the level of boundness of water in food and its availability to act as a solvent and also to participate in chemical reactions [49]. During low level of water activity, the effect of antioxidants have been attributed to its binding with hydroperoxides, hydration of metal catalyst, whereby pro-oxidant effect on oxidation during high level of water activity (a_w) is due to increased mobility of reactants for oxidation reaction [50]. According to Karel, the effect of water on lipid oxidation is due to its effect on the concentration of initiating radicals or radicals that start the process of lipid oxidation in food, also its effect on the mobility of reactants and their level of contact and also the significance of radical transfer together with recombination of reactants [51]. Other studies conducted on the oxidation of methyl linoleate in the intermediate moisture content region showed that solution level of water affects the process of oxidation in several ways when its level exceeds that of the monolayer level of water and consequently increasing the rate of oxidation reaction. Thereby, as the level of water activity increases even though metal catalyst become less effective, they are easily mobilized to reaction sites in the aqueous environment. However, there is induction of new catalytic sites due to solubilization of precipitated crystals or swelling of bound surfaces [50, 52].

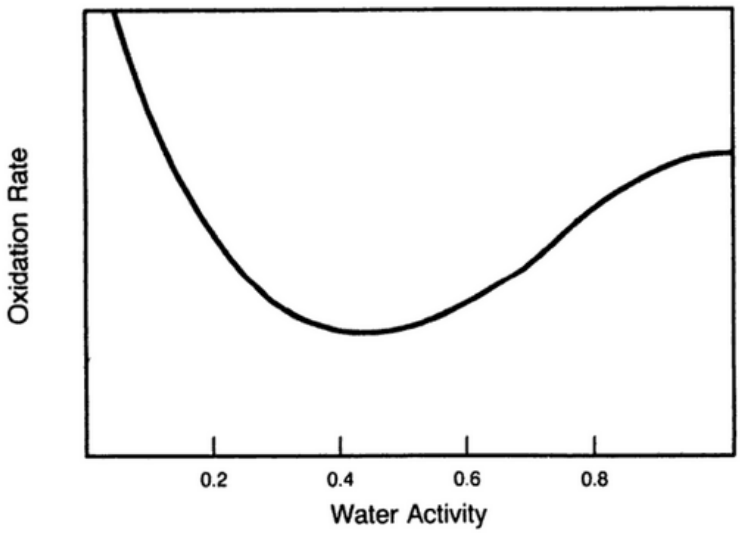


Figure 6. Rate of lipid oxidation as influenced by aw in food [49].

According to Labuza (1975), below the monolayer level of water, the rate of lipid oxidation reduces with increasing a_w. The reaction rate then reduces at the monolayer level/value and increases with further increase in a_w (**Figure 6**) [49].

Oxidation as a process which occurs in food is also affected by the type of food, relative concentration of oxidative components, relative reactivities of prooxidants and antioxidants in the food [20].

Transition metal ions are also promoting factors of the oxidation processes in food because they are good promoters of free radical reactions [53] due to single electron transfer during their change in oxidation states. In a review of the kinetics of metal-catalyzed lipid oxidation [54], transition metals with variable oxidation numbers (iron, Fe^{2+}, Fe^{3+}, copper as Cu^{+} or Cu^{2+}, Mn, Co, Ni) were known to increase the rate of

oxidation. This is due to the reduction of the activation energy at the initial stage. Decomposition of hydroperoxide is known to be the main initiation stage. A simply hemolytic cleavage of the weak O-O bond in hydroperoxides can lead to the formation of the free radicals that is responsible for the chain reaction [20]. Transition metal such as copper and iron can accelerate peroxidation by decomposing lipid hydroperoxide in both their lower [Eqn.1] and higher oxidation states [Eqn. 2] [27, 55, 56]. The alkoxyl and peroxyl radicals that are produced during this reaction can abstract hydrogen and continue the chain reactions [Eqn. 3] of lipid peroxidation [27, 55].

$$ROOH + Fe^{2+}\,(Cu^{+}) \xrightarrow{\text{Fast}} RO\cdot + Fe^{3+}\,(Cu^{2+}) + OH^{-}$$

(Alkoxyl Radical) Equation 1

$$ROOH + Fe^{3+}\,(Cu^{2+}) \xrightarrow{\text{slow}} ROO\cdot + Fe^{2+}(Cu^{+}) + H^{+}$$

(Peroxyl Radical) Equation 2

$$RO\cdot + RH \longrightarrow R\cdot + ROH$$ Equation 3

$$ROOH + RH \longrightarrow R\cdot + ROOH$$ Equation 4

These reactions may occur in cycles that even small amount of trace metals may be adequate for the generation of free radicals. Reducing agents like ascorbic acid or superoxide anion radical ($O_2^{\cdot-}$) can accelerate these metal ion dependent

reactions, as Fe^{2+} and Cu^{+} appear to react with hydroperoxides faster than Fe^{3+} and Cu^{2+} respectively [20].

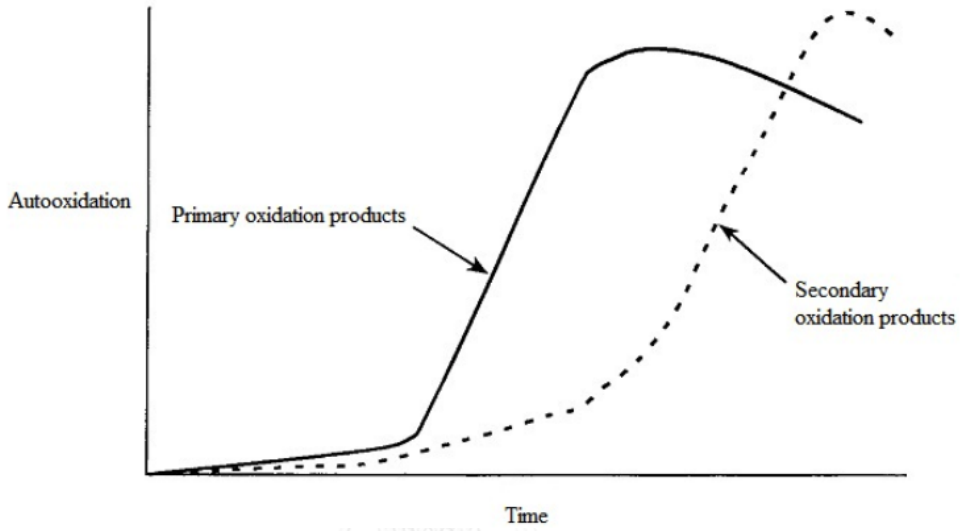


Figure 7. Theoretical development of primary and secondary oxidation products as a function of time in lipid oxidation [36].

MDA, which is a secondary product of lipid peroxidation can also be produced endogenously by oxidative breakdown of biological phospholipids occurs in most cellular membranes including mitochondria, microsomes, peroxisomes and plasma membrane. The toxicity of lipid peroxidation products in mammals generally involves neurotoxicity, hepatotoxicity and nephrotoxicity [57].

2.1.5 Health effects of lipid peroxidation products

Reactive oxygen species leads to a number of tissue malfunctioning and its destruction in a number of health conditions. Aldehydes and peroxides are products generated when reactive oxygen species react with lipids, with the aldehydes produced being very stable than the initial ROS and therefore migrating from their site of production and causing damage at other parts where they are deposited. End products from the lipid peroxidation are greatly reactive and cause many health defects especially when they are in high concentration causing changes in signaling of cells, protein and DNA damage and cytotoxicity. High level of the lipid peroxidation end products has been evident in diseases like cancer, atherosclerosis, Alzhiemer disease, heart failure, immunological disorders etc. In biological systems, lipid oxidation is a process that is related to the production of free radicals due to enzymatic control which leads to the production of lipid driven inflammatory mediators or by non-enzymatic control. The non-enzymatic process is linked to oxidative stress development which is mostly related to cellular damage, high variety of aldehydes are produced as a result lipid hydroperoxides degradation produced during oxidative stress among which malondialdehyde (MDA) is mostly common. An elevated level of MDA in the serum has been associated with diseases like hepatitis C virus in adults and pediatrics HIV. Oxidative stress has also been shown to play a role in diabetic neuropathy and also in alcohol intoxication induced polyneuropathy.

Low or lack of an appreciable level of antioxidant capacity in the biological system can be related to health defects as it was observed in the cases of diabetic neuropathy and alcohol intoxication induced polyneuropathy, whereby lipid peroxidation products in the nerves exceeded that of gluthathione peroxidase activity in the same tissue. When serums of chronic hepatitis C patients were analyzed, levels of thiobarbituric acid substances and MDA concentrations increased before being treated with interferon α-2 which decreased their levels. Therefore, reducing the generation of lipid peroxidation products can lead to reduction of ROS and therefore prevent detrimental health effects.

2.1.6 Lipid peroxidation and sensory evaluation

During sensory evaluation, rancidity is described by the unpleasant odors and acid flavor in food as a result of deterioration of fat. An important assessment of rancidity must be by taste because it measures what the consumer perceives [26]. In sensory evaluation, descriptive analysis, which is the detection and description of food products in terms of its qualitative and quantitative sensory aspects is done by a panel because sensitivity of the off flavors varies between individuals [58]. In most food industries, taste and smell are the major ways of detecting oxidative off-flavors in foods and deciding whether a lipid containing food is no longer fit for consumption [8]. Moisture and water availability are known to affect textural acceptability. Food

products which are dry and crispy (e.g. potato chips and crackers) are considered as texturally unacceptable when gaining moisture content above 0.35-0.5 a_w [59].

2.1.6 Sensory shelf life test

The stability of food during its shelf life mostly depends on factors including quality of ingredients, product composition and structure, processing conditions during manufacture, packaging characteristics and the storage, handling and distribution conditions [60]. In knowing whether storage of the food product over a period of time affects its characteristics, sensory evaluation can be done. During storage period of lipid containing bakery food products, lipid peroxidation is one of the main factors that affect the food's quality. Lipid peroxidation is a slow process at room temperature; hence, does not fit in industrial shelf life studies [61]. Therefore, conditions such as temperature, moisture and light are normally used to increase the rate of oxidation in order to save time [62-64]. Food is stored in its original package or in food-grade containers. These food-grade materials are approved by the US Food and Drug Administration as not to contain or transfer hazardous chemicals to human health. The approved containers for food storage include glass and ceramic containers; plastic bags and rigid containers; and plastic, paper, and foil wraps whilst unapproved containers for storage of food include trash bag or plastic and fiberboard containers that have been used previously to hold non-food materials. The optimum

conditions for maintaining flavor and texture of some food products may differ from the optimum conditions for longer shelf life. An example is the storage of bread in the refrigerator which can help delay the growth of mold on the bread but becomes stale quickly. Another example is tomatoes which can be stored in the refrigerator for longer period but loses its flavor[65].

Difference test can be used if only the criteria for shelf life depends on the first detectable change in the food but then difference test detects only small changes which are of less relevance to shelf life. However, most sensory tests use quantitative measures of change that are more open to interpretations in consumer terms. Hedonic tests can also be used to determine consumer acceptability more directly [66]. In this sensory test, reference standard can be made available at each test session to reduce variability of sensory data. Unless highly trained panels are used, memory of sensory quality is unreliable for most shelf life testing especially for medium or long term storage periods, hence, reference samples should be provided for each test [67]. A new reference sample can be prepared for each test point and this procedure is valid only in circumstances whereby batch to batch variations are minimal; substantial variation will prejudice data interpretation [68]. Sensory tests provide information on whether changes are occurring, the nature of the changes and its magnitude. Criteria that can be used to interpret sensory shelf life data are grouped into three categories: first detectable change, measured attribute change

and change in consumer acceptability. The first detectable change (or just noticeable change) in product quality can be measured using difference test, assuming that suitable reference sample is available, but then difference test can be over sensitive to changes that have little relevance to sensory quality as perceived by consumers and give limited information on the degree of change [68].

2.1.7 Factors that affect bakery products during shelf life

The acceptability of bakery foods by the consumer is an important factor when it comes to market growth, therefore factors that affect its spoilage need to be considered and controlled [69]. Maintenance of both sensory and physical characteristics linked with freshness is used to define storage stability of bakery products [70]. Storage life of bakery foods is normally characterized with the food product not being fresh as it was and also gumminess that result from microbial spoilage. Other factor affecting shelf life are rancidity, crystallization, grittiness, synergies of jams and jellies, development of off flavors and odors other than rancidity, chocolate bloom, structural weakness, fade color or moisture migration. However, to prolong the shelf life of bakery foods, all these fore mentioned factors must be controlled by appropriate preservation methods. Recently, bakery consumers look out for new bakery products, better appeal, taste and convenience from bakery foods. Preservation in bakery means the retardation of spoilage including

the texture staling [71]. One serious physical spoilage problem in bakery product is staling. Staling has been defined as “almost any change, short of microbiological spoilage, which occurs in bread or other products, after the baking period, making it less acceptable to the consumer” [72]. The major changes that occur during this period are the redistribution of moisture, starch retrogradation, increased firmness, and loss of aroma and flavor, however low or intermediate moisture foods such as cookies and crackers are very slow in the staling process but are more susceptible to lipid oxidation and rancid flavor development [73].

Bread prepared with different sweeteners, showed higher rate of moisture loss when stored under ambient temperature than those stored under refrigerated condition. Type of sweetener, days of storage and type of packaging material also affect moisture content and water activity of the bakery product significantly. Increases in water activity in bread with polyol were less than the control group. Bread stored in low density polyethylene showed a higher rate of moisture loss than those packed in polypropylene packaging material, however, hardness of the bakery product increased over the storage period. Bread stored at ambient temperature and in different packaging materials showed statistically significant results in terms of free fatty acids. Bread packed in low density polyethylene was observed to have higher levels of free fatty acids than bread packed in polypropylene. This could have been because of the fact that polypropylene had less water vapor transmission rate as

compared to low density polyethylene. Increased level of moisture in the bakery product led to increase in oxidation [74].

Almond pastry cookies are known to have short shelf life due to the ingredients used and also type of packaging [75]. The qualitative decay due to internal hardening of paste is normally attributed to the redistribution of water that leads to recrystallization of sugar and as a result loss of water to the surrounding [76]. Hardening of cookies however, are caused as result of movement of water leading to recrystallization of sugar used in the preparation and also redistribution of moisture [77]. Water in cookies can also be lost into other components and as a result being lost in the cookies. In a study by permeability of film used for packaging and temperature had an influence on the browning reaction that occurred in cookies over a storage period, this observation was made by measuring the color intensity (chroma) in cookies stored in polyvinylchloride (PVC), aluminum foil (ALL) and plastic vessels sealed into a 170 mm film thickness of polyamide/polyethylene (PA/PE) under modified atmosphere (MAP) with N_2 under temperatures of 20 and 30°C. Samples showed higher level of browning reaction at 30°C. A positive correlation was observed between water activity and chroma in samples stored in MAP at 20°C, this could be due to the low permeability of the film and also oxygen contact. The physical characteristics of the cookie constituent especially lipids, however, did not change under 20°C [75]. The main causes depletion of quality in shelf stable foods

stored at ambient temperature are chemical and physical deterioration events. These events are activated applied thermal preservation or sanitation processes. Chemical deterioration processes which affect these products are mostly oxidation and non-enzymatic browning, which in its advanced stages leads to the development of off flavors which are easily acknowledged by consumers. Other 'silent' changes which do not affect the sensorial properties of the food are the evolution of potential toxic components and also the breakdown of nutrients and bioactive components. The non-enzymatic browning however, leads to the development of brown compounds accountable for color changes. Silent changes which occur during oxidation processes are also likely to occur here. Moisture absorption can also affect certain food sensory characteristics such as crispiness and texture. This change however, can affect series of physical and chemical events due to the modification of the molecular mobility [78].

2.1.8 Functional foods and antioxidants

There have been many types of healthy food products, of which functional foods are one of them. These functional foods are regular foods with functional ingredients and they can be taken regularly with added or fortified nutrients either extracted or synthesized from natural source [79]. Functional foods help in the reduction of disease risks related to food and also help to compensate for imbalanced diets [80].

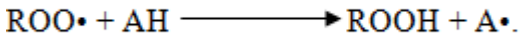
One of the most promising functional ingredients which are normally used is antioxidant [81]. Major sources of antioxidant are Cereals, vegetables, fruits, pulses, spices and other plant foods (e.g., isothiocyanates, phytic acids, flavonoids, phenolics, sterols) [82]. Recently, many plant extracts are being incorporated into food products or being used as medications due to their antioxidant capacities to reduce the production of lipid peroxidation products and Maillard reaction products. Peng *et al*., (2010) investigated the effect of grape seed extract fortified in bread and it was reported that the grape seed extract could reduce N^{ε}-carboxymethyl lysine (CML) in the bread and acted in a dose-dependent manner. Also except for an acceptable color change, addition of the extract also had little effect on the quality attributes of the bread [83]. Mildner-Szkudlarz *et al*., also reported the effect of muffins reformulated with grape by-product and this also showed that muffins enriched with 20 % grape by-products showed a lowering CML level and no significant changes in the sensory profile [32]. Lipid peroxidation in beef patties during cold storage was reported to be effectively controlled with the addition of freeze dried extracts from fenugreek seeds and ginger rhizome [19].

In order to produce a functional food product, the stability/shelf life should also be improved, since instability of fats and oils in food can undergo oxidation leading to the production of secondary oxidation products that can be detrimental to health. In a study by Ismail *et al*., (2014), cookies prepared with pomegranate peel

powder and control cookies without powder were kept at room temperature in air tight vials for 4 months and its stability determined by measuring free fatty acids at the initial stage, 2nd and 4th month. Free fatty acids in both the control and supplemented cookies increased progressively with increase in storage time. Levels of free fatty acids on termination of storage study in control (0.40 %) and 7.5 % pomegranate peel powder supplemented cookies (0.20 %) showed pomegranate peel powder supplemented cookies to have 50 % higher stability than control cookies [84].

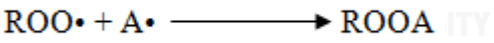
All these effects are believed to be possible due to the antioxidant capacities of phytochemicals (e.g. polyphenols) present in the plants. Plant phenolics are a diverse group of biochemical compounds, the majority of which originate from the phenyl propanoid pathway [15]. Phenolic antioxidants are substances that react with the initiating and propagating radicals to give harmless products and to extend shelf life until autoxidation finally takes place [85]. These polyphenols primarily consist of flavonoids including flavanols, flavones, isoflavones, flavonols, flavonones and anthocyanins and non-flavonoid polyphenolics including phenolic acids, lignans and stilbenes. Functions of polyphenols include the prevention of e.g. oxidative, inflammatory, microbial and viral assaults, and therefore have a potential of reducing chronic diseases [86, 87]. The mechanisms of antioxidant activity of polyphenols can be characterized by direct scavenging or quenching of oxygen free radicals and

inhibition of oxidative enzymes that generate reactive oxygen species [88]. One other mechanism of action of antioxidants is the chain breaking mechanism in which the primary antioxidant donates an electron to the free radical present in the system [89] as shown in Eqn.5.



Equation 5

The antioxidant radical formed is known to be unreactive due to the delocalization of the unpaired electron around the phenol group, making it unreactive to the fatty component. Therefore they are able to stop radical chain reactions by reacting with another fatty acid radical [90] as shown in Eqn.6.



Equation 6

Also antioxidants minimize discoloration and off flavor and contribute to emulsion stability that can be negatively affected by the degradation of the oil [89]. Natural antioxidants have been recognized as presenting varying levels of antioxidant activity [91]. Some of the commonest natural antioxidants are tocopherols, sterols and flavanoids from plant extracts. In a study by Zhang *et al*., (2014) even though dietary polyphenols used in the cookie model increased the health beneficial

antioxidants, changes were induced in cookie color, moisture content and pH. The fortification of the dietary polyphenols were also observed to reduce glycation effects in the cookie by lowering the levels of glyoxal and fluorescent AGEs which might be attributed to phenolics' free radical scavenging capacity. They also suggested that in future development of functional food by means of dietary polyphenol before processing, cautious efforts should be taken as to retain the structure of health beneficial bioactives such as polyphenols during thermal processing, since destruction of these primary structures can lead to alterations in the final food product's organoleptic properties and also biological activities [92].

2.1.9 Principle of methods used for antioxidant, polyphenol and flavonoid measurement

2.1.9.1 FRAP assay

The reducing power of a compound serves as an indicator of its antioxidant activity. Reducing power appears to be related to the degree of hydroxylation and extent of conjugation in polyphenols.

The presence of reductants such as antioxidant substances causes a reduction of Fe^{3+} / ferricyanide complex to Fe^{2+} / ferrous form. At low pH, reduction of ferric tripyridyltriazine (Fe III TPTZ) complex to ferrous form (which has an intense blue color) can be monitored by measuring the change in absorption at 593 nm (**Figure 8**).

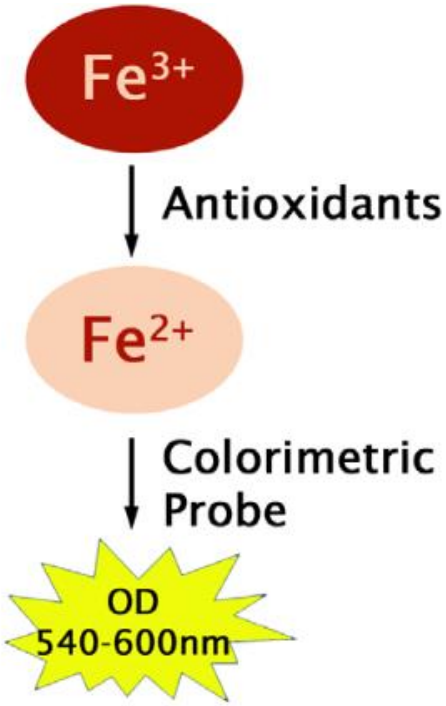


Figure 8. Principle of FRAP assay.

2.1.9.2 DPPH assay

This assay is based on the measurement of the reducing ability of antioxidants toward DPPH$^{\bullet}$. The ability can be evaluated by electron spin resonance (EPR) or by measuring the decrease of its absorbance. DPPH color can be lost via radical reaction (Hydrogen Atom Transfer) or reduction (Single Electron Transfer) as well as unrelated reactions, and steric accessibility is a major determinant of the reaction (**Figure 9**). The assay is not a competitive reaction because DPPH is both radical probe and oxidant [93].

(Purple) —— (DPPH) + (H-A) ——→ (Yellow)

Figure 9. Antioxidant activity using (DPPH) radical scavenging [94].

2.1.9.3 Folin-Ciocalteau's assay

This assay is used to determine polyphenol content. The underlying principle is the reduction of phosphomolybdic-phosphotungstic acid (Folin) reagent to a blue–colored complex in an alkaline solution which occurs in the presence of phenolic compounds. The method measures the number of potentially oxidizable phenolic groups. This assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes which are determined spectroscopically at 765 nm. The Folin-Ciocalteu method is an electron transfer (ET) based assay and gives reducing capacity [95].

2.1.9.4 Aluminium chloride colorimetric assay

The principle involved in aluminium chloride ($AlCl_3$) colorimetric method is that $AlCl_3$ forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, it also forms acid labile complexes with the ortho-dihydroxyl groups in the A-or B-ring of flavonoids (**Figure 10)** [96].

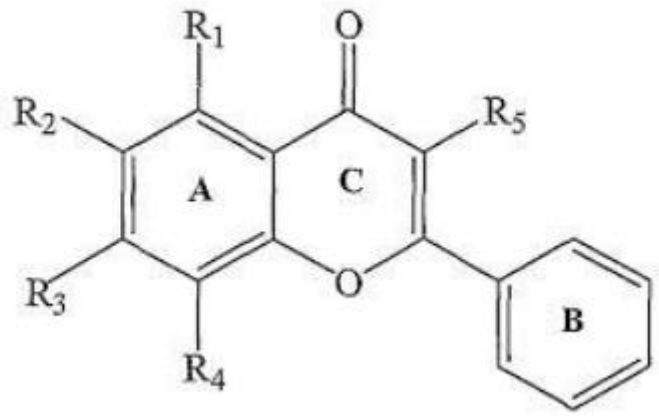


Figure 10. Basic structure of flavonoid.

2.2 Moringa oleifera and its uses

Moringa oleifera Lam (syn. M.pterygosperma; commonly known as "Miracle Tree", "Horseradish-tree", or "Ben oil tree") is also known to have an impressive range of medicinal uses with high nutritional values throughout the world (**Figure 11**). It is mainly found in Western and sub Himalayan tracts, India, Pakistan, Asia and Africa [97, 98]. It is a valued plant which has been consumed by humans and used for various domestic purposes such as alley cropping, animal forage, biogas, domestic

cleaning agents, etc. [99]. Besides these uses or benefits of *Moringa oleifera*, there have been other reports on the use of the different parts of the plant in the past [100, 101].

Figure 11. Moringa oleifera leaves.

Moringa oleifera has been used in functional food development and medicinal purposes worldwide. It's beginning to have much interest currently since studies have shown that herbal medicines have much benefit over conventional medicines. Current research works show *Moringa oleifera* to possess anti-inflammatory, anti-tumor, anti-ulcer, anti-oxidant, cholesterol lowering, anti-diabetic, anti-hypertensive and hepatoprotective activities property. Moreover, it has been used to treat skin infections, anaemia, anxiety, asthma, catarrh and many other

illnesses [102-104]. In a study by Sreelatha *et al*., (2009), it was observed that the aqueous extract of *Moringa oleifera* leave extract had potential DPPH antioxidant activity and thus further showed increase inhibition of lipid peroxidation in liver homogenates which was very prominent in the matured leaves than tender leaves extract [32].

2.2.1 Nutritional value and antimicrobial properties of *Moringa oleifera*

Moringa oleifera is a type of vegetable which belongs to the family Moringaceae and order Brassica [102]. Leaves of *Moringa oleifera* has been reported to be the source of both macro- and micronutrients. The leaves are rich sources of β-carotene, protein, vitamin C, calcium, potassium and good sources of natural antioxidants; and thus enhance the shelf life of fat containing foods [105, 106]. Fruit (pod)/drumsticks and leaves have been used in the fight against malnutrition especially among infants and nursing mothers for enhancing milk production [105, 106] and also regulate thyroid hormone imbalance [107, 108]. Fresh leaf juice has been reported to inhibit growth of pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* [109, 110]. Other parts of *Moringa oleifera* such as its seeds, flowers, fruit peel, roots and unripe pod have been described in a review to have antibacterial properties against some human bacterial pathogens Gram negative: *Shigellashinga*, *Pseudomonas aeruginosa, Shigellasonnei,* and *Pseudomonas spp*.

and also Gram-positive: *Staphylococcus aureus, Bacillus cereus, Streptococcus Bhaemolytica, Bacillus subtilis, Sarcinalutea* and *Bacillus megaterium* [100, 111]. The efficacy of aqueous and ethanolic *Moringa oleifera* leaf extract against the growth of Gram positive and negative bacteria was evaluated in a study conducted by Peixoto *et al*. It was found that the leaf extract was resistant against some strains of *E. coli, P. aureginosa*and *S. enteritidis*, but same extract was also most effective against *S. aureus, V. parahaemolyticus, E. faecalis* and *A.caviae* [112]. Other researchers also found that the aqueous and ethanolic *Moringa oleifera* leaf extract were effective against salmonella and this antibacterial effect was attributed to the constituents of saponin, tannic, phenolic and alkaloid phyto constituents in the leaf extract [113]. In a study conducted by Torondel, *et al*., (2014), 4g of *Moringa oleifera* leaf powder in wet and dried application had the same effect as non-medicated soap when used for hand washing. Saponin being one of the constituents of *Moringa oleifera* has been reported as a chemical compound which has detergent and surfactant properties and this explains its bacterial reduction properties [114]. *Moringa oleifera* flowers exhibit hepatoprotective properties due to the presence of quercetin [109-117]. At the Asian Vegetable Research and Development Center (AVRDC), many researchers reported that leaves of four Moringa species were rich in nutrients and antioxidants [118] but then the nutrients value varied with factors such as leaf age, harvest season and preparation methods, which also determine the potency of

Moringa oleifera leave extract as an antioxidant substance. Though most vegetables lose their nutritional content when cooked, but Moringa leaves maintains its nutritional value whether fresh, cooked or stored as dried powder for months without refrigeration [119] Boiled leaves resulted in three times more bio-available iron than the raw leaves and this was also observed in powdered Moringa leaves.

2.2.2 Pharmacological benefits of *Moringa oleifera* leaves

2.2.2.1 Hypoglycemic properties

Diabetes is one of the common metabolic diseases which are usually accompanied with either a stage of hyperglycemia or glucose tolerance impairment [120]. Many medicinal herbs have been reported to have beneficial in curing this disease, however, *Moringa oleifera* leaves is also known to have such beneficial pharmacological effects in curing diabetes and this has been strengthened by scientific data [121]. The glucose lowering effect of *Moringa oleifera* leaves has been attributed to the presence of terpenoids which has been reported to be involved in the stimulation of β-cells which triggers insulin secretion [122]. Effect of *Moringa oleifera* leaves in reducing blood glucose has been determined in many studies. In a study by Ndong *et al.* (2007) [123], OGTT and AUC reduces significantly in diabetic Goto-Kakizaki (GK) rats and non-diabetic Wistar rats administered with a dose of *Moringa oleifera* leaves after being given a dose of glucose solution when compared

with control rats. These results suggest that *Moringa oleifera* has a glucose intolerance ameliorating effect in both GK and Wistar rats, with a greater action in diabetic than in normoglycemic rats. In another study by Jaiswal *et al.,* (2009), [124], the effectiveness of aqueous extract of *Moringa oleifera* leaves on glucose homeostasis was tested in healthy and streptozotocin-induced sub, mild and severely diabetic Wistar rats (STZ, a cytotoxic drug that selectively destroys islet β cells). The dose of 200 mg/kg BW of leaves extract determined a maximum fall of 26.7 % in fasting blood glucose concentration and a maximum fall of 29.9 % in OGGT at 3 h after glucose administration.

2.2.2.2 Hypolipidemic properties

Lipid lowering effects of *Moringa oleifera* leaves has also been examined in rats fed with

a high-fat diet containing 20 % (*w/w*) fat for 30 days [125]. Animals were divided into two groups, one of which received a daily dose of 1 g/kg BW of aqueous extract of *Moringa oleifera* leaves. A significant lower level of serum cholesterol was observed in treated compared to untreated rats, but not in liver and kidney. Another study by Bais *et al.,* (2014) [126] also showed that a supplementation of 200 and 400 mg/kg bw/die of *Moringa oleifera* leaves extract exhibited anti-obesity effects in high-fat fed mice. This beneficial effect on lipid homeostasis, can however be linked to many

bioactive compounds. Polyphenols especially flavonoids has been suggested to play an important role in lipid homeostasis [127].

2.2.2.3 Hepato and Kidney Protective Properties

Effects of *Moringa oleifera* leaves on liver and kidney health have been reported by Oyagbemi *et al.,* (2013) [128] and Asiedu-Gyekye *et al.,* (2014) [129] who observed an increment in liver and kidney injury biomarkers such as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrose (BUN) and creatinine following an administration of the extract of *Moringa oleifera* leaves in mice. The authors concluded that the leaves might have made them liable to hepatic and kidney damage. However, histopathological examinations did not reveal any histological lesions in the sinusoids or central vein [129]. On the other hand, other studies [130-132] reported hepatic and kidney protective properties against several drugs, such as isoniazid, rifampicin, pyrazinamide, acetaminophen and gentamicin, attributable to *Moringa oleifera* leaves. The authors reported a reduction of serum ALT, AST, ALP [130, 131], BUN and creatinine [132] in animals treated with the extract of *Moringa oleifera* leaves.

2.2.2.4 Antioxidant properties

In order to decrease oxidative damage in tissues indirectly, polyphenols are the main phytochemicals in *Moringa oleifera* which brings about this effect by indirect development of a cell or by free radical scavenging [133]. The leaves are

reported to have antioxidant properties due to their polyphenol contents, with matured leaves having the highest level of antioxidant properties against free radicals and oxidative damage of biomolecules when compared to tender leaves [134, 135]. Different extract solvents of leaf samples (aqueous, ethanol, methanol) have exhibited good antioxidant activity. However, increasing concentration of all the extracts had significantly increased reducing power (FRAP) [136]. Similar results were observed by Vongsak *et al.,* (2013), [137] who measured antioxidant activity using various extraction methods on Thai *Moringa oleifera* leaves. It was concluded that the extract obtained macerating dried leaves with 70 % ethanol exhibited high DPPH-scavenging activity (EC50 = 62.94 g/mL) and the highest FRAP value (51.50 mmol $FeSO_4$ equivalents/100 g extract).

2.2.3 Moringa oleifera leaves extract in some food products

In a study by Luqman *et al*., (2012) it was reported that aqueous leaf extract of *Moringa oleifera* was able to increase the reduced glutathione (GSH) and reduce MDA level in a dose-dependent manner when administered to healthy Swiss albino mice [138]. Das *et al*., (2012) determined the effective utilization of *Moringa oleifera* mature leaves extract as an antioxidant in cooked goat meat patties during refrigerated storage as against butylated hydroxytoulene (BHT) which is a synthetic antioxidant. The Moringa leaves extract showed excellent antioxidant activity as determined by DPPH assay with an IC50 value of 18.54 µg/ml. Total phenolic content

(as gallic acid equivalent) significantly increased from 285.56 in control to 379.45 in patties with Moringa leaves extract. Meat with added 0.1 % *Moringa oleifera* leaf extract reduced lipid peroxidation of cooked goat meat patties as measured by TBARS number during refrigerated storage. Increase in TBARS number was very slow in extract samples and remained lowest (0.53 mg MDA/Kg sample) up to 15 days. The *Moringa oleifera* leaves extract at a level of 100 mg/100 g meat was sufficient to protect goat meat patties against oxidative rancidity for periods longer than the most commonly used synthetic antioxidant like BHT [139].

2.2.4 Safety evaluation of *Moringa oleifera*

Safety evaluation studies also showed no toxicity of the extracts up to a dose of 100 mg/Kg body weight. In a study by Adedapo *et al*., (2009), it was reported that *Moringa oleifera* is genotoxic at supra supplementation levels of 3000 mg/ Kg body weight. However, levels ≤ 1000 mg/Kg body weight are safe for intake when high levels and low levels of 1000 and 3000 mg/ Kg body weight respectively were administered to two groups of Sprague-Dawley rats and observed for 14 days. Acute toxicity test conducted also showed that *Moringa oleifera* extract of 2000mg/Kg dose showed no form of death in the rats used. This showed that the level of 2000 mg/Kg extract is safe for consumption and also for medicinal uses [140].

In one set of experiments, human peripheral blood mononuclear cells were exposed *in vitro* to graded doses of the extract and cytotoxicity was assessed.

Cytotoxicity occurred at 20 mg/kg, a concentration not achievable by oral ingestion [141]. LD50 of aqueous *Moringa oleifera* leaves was estimated to be 1,585 mg/ Kg in acute toxicity test conducted on Wistar albino rats who were orally administered an aqueous extract up to 6400 mg/ Kg and intraperitoneally up to 200 mg/Kg and also in a sub chronic toxicity test which was performed by daily administration with the extract at 250, 500 and 1500 mg/Kg orally for 60 days. The extract did not exhibit any significant difference in sperm quality, hematological and biochemical parameters in the treated rats compared to the control [142]. The leaves of *Moringa oleifera* have nutritional potential because they contain a high concentration of energy, nutrients, minerals and phenolic constituents, mainly flavanoids and phenolic acids, which represents a good source of natural antioxidant [143]. According to Asiedu-Gyekye *et al*., (2014), it was stated that consumption of *Moringa oleifera* should not exceed 70 g per day to prevent cumulative toxicity effect of some essential elements of the plant [129].

2.2.5 Moringa oleifera in bakery foods

Bakery products are consumed in every part of the world and this seems to be increasing because of their great taste, flavor and appearance. However, due to the high temperature, long baking time, high fat content that can lead to lipid peroxidation, sugar and protein content that can lead to formation of Maillard reaction products; there has been the need to reformulate these bakery products to

make them healthy. For instance, consumption of Maillard reaction products present in bakery foods over a long period of time can lead to the development of metabolic diseases. *Moringa oleifera*, which is known to have beneficial properties, has been supplemented in some bakery products. In a study by Ogunsina *et al*., (2010), where wheat flour was replaced by 10 %, 20 % and 30 % debittered Moringa seed grits, it was found that cookies with 20 % debittered Moringa seed grits had a nutty taste of Moringa seeds and were acceptable with high levels of protein, iron and calcium. Also in this study, bread with its wheat flour replaced with 10 % debittered Moringa seed flour obtained a high nutritive value and acceptable taste [144].

Sensory evaluation was also conducted in cookies which were prepared with blends of wheat flour and *Moringa oleifera* leaf powder and it was shown that there were significant difference in the different attributes that were evaluated such as in color, crispiness, taste, flavor and general acceptability. However, the best Moringa leaf powder substitution level for making the cookies were 10 % (90:10) and 20 % (80:20) [145]. *Moringa oleifera* extract concentration of 1-2 % was used as natural antioxidant for the production of biscuits and compared to that (biscuits) prepared with synthetic antioxidants such as BHA. *Moringa oleifera* extract fortified biscuits recorded no effect on the organoleptic properties of the biscuits during sensory evaluation when stored over a period of 6 weeks. During the storage period, biscuits

were observed to have lower percent (%) concentration of peroxide value and acid value compared with the control biscuits prepared without any addition of antioxidants and biscuits prepared with synthetic antioxidant (BHA) which were higher. The extract was therefore concluded to be a good form of natural antioxidant which can increase the shelf life of food products containing fats and oils [146].

CHAPTER 3

METHOD

3.1 Materials

3.1.1 Reagents

Aluminum chloride, hydrochloric acid, ethanol, hexane, dichloromethane, glacial acetic acid, potassium iodide (Merck, Darmstadt, Germany), Catechin, Folin-Ciocalteau reagent, 2,4,6-Tripyridyl-s-Triazine (TPTZ), , malondialdehyde (MDA), butylated hydroxyl toluene (BHT), thiobabituric acid (TBA), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich Co, St. Louis, MO, USA), gallic acid(Fluka, St. Louis, MO, USA), sodium carbonate, sodium hydroxide, ferric chloride, iron sulphate pentahydrate (Ajax finechem, Auckland, New Zealand), sodium nitrite, petroleum ether (Qrec Co Ltd, New Zealand), sodium thiosulfate. All reagents were of analytical grade.

3.1.2 Equipments

Spectrophotometer (Perkin Elmer, Waltham, MA, USA), pH meter (Thermo Scientific, Inc., Waltham, MA, USA), Spray dry machine (Eyela world, Tokyo, Japan), Vortex (Gemmy industrial corp., Taipei, Taiwan), oven (Nardi oven, NardiElettrodomestici S.p.A., Italy).

3.2 Method

3.2.1 Preparation of *Moringa oleifera* Leaves Extract

Fresh leaves of *Moringa oleifera* were obtained from Nongkhame district, Bangkok, and washed thoroughly with water. The washed leaves were air dried under shade, after which they were subjected to size reduction to a coarse powder by using a dry grinder. The dried plant material (250 g) was extracted using distilled water (3 L) at 90 °C for 2 h. The extract was filtered twice through a cheese cloth. The filtrate was further filtered twice using Whatman Grade 4 followed by Grade 1 filter papers, sequentially, under a vacuum pump. The aqueous solution was dried into the powdered form using a spray dryer (**Figure 3.1**). The conditions of the dryer were inlet temperature (165-168 °C) outlet temperature (51-78 °C), blower (0.70 - 0.79 m^3/min) [147].

Figure 12. *Moringa oleifera* leaves extract (MOE).

3.2.2 Preparation of Cookies

Information on the ingredients for the study was obtained from AACC method 10-50D with slight modifications [148] and is presented in **Table 1**.

Table 1. Ingredients used in cookie preparation.

Ingredient	Amount (g/100 g)
Cake flour	26.85
All-purpose flour	17.31
Sugar	20.58
Baking powder	0.63
Salt	0.63
Vanilla Flavor	0.63
Butter	25.09
Skimmed milk	8.03

Preparation of cookies was carried out according to the method by Hyun-Jung *et al*., with slight modification [165]. Butter (25.09 g/100 g) was blended with sugar (20.56 g/100 g) for 5 min. Skimmed milk powder (8.03 g/100 g), water (18 mL) and vanilla flavor (0.63 g/100g) were added and mixed. Cake flour (26.85 g/100 g), all-purpose flour (17.31g/100 g), baking powder and salt (0.63 g/100 g) were also added and mixed to form dough. The Cookie dough was wrapped with 3 layers of plastic wrap and put in the refrigerator for 30 min after which it is sheeted using a rolling pin

with side ring of height 0.6 cm. Sheeted cookie dough is then kept in the refrigerator for another 30 min, then cut in circular shapes using a cookie mold with diameter of 4 cm. Round shaped cookies were baked in a preheated oven (Nardi oven, NardiElettrodomestici S.p.A., Italy) at 180^{O}C for 16 min. Baked cookies were allowed to cooled at room temperature, then packed and sealed in aluminum packs filled with nitrogen. Cookies fortified with *Moringa oleifera* leave extract (MOE) were prepared by adding 0.2 %, 0.4 %, 0.6 %, 0.8 % and 1.0 % of the extract with respect to the weight of the cookie dough (**Figure 13**).

3.2.3 Addition of *Moringa oleifera* leaves extract

Amount of MOE extract added to each of the test groups (0.2 %, 0.4 %, 0.6 %, 0.8 % and 1 %) was made with respect to the weight of control cookie dough (since the amount of all the ingredients used in the preparation of both the control and test groups are the same apart from the extract). The weight of dough prepared from the ingredients provided was 219.58 g. Therefore, the amount of MOE added was estimated using the equation below:

Amount of MOE added = Amount of MOE × Weight of control cookie dough

Example; For 0.2 % Moringa cookies; Amount of MOE added

= (0.2 /100) × 219.58g

= 0.43916 g (439.16 mg)

Table 2. Amount of *Moringa oleifera* leave extract (MOE) in each group of cookie with respect to weight of control dough.

Type of Cookies	Amount of MOE (mg)
Control Cookie	0
0.2 % MOE Cookie	439.16
0.4 % MOE Cookie	878.32
0.6 % MOE Cookie	1317.48
0.8 % MOE Cookie	1756.64
1 % MOE Cookie	2195.80

Cookie dough of weight 219.58 g produced 15 pieces of cookie with weight 12 g.

Hence, Moringa (mg) in 1 piece of cookie = (0.2/100) × 12

= 0.024 g (24 mg) of Moringa per cookie

Table 3. Amount of *Moringa oleifera* leaves extract (MOE) in a cookie (12 g).

Type of Cookie	Amount of Moringa extract /cookie (mg)
Control	0
0.2 % MOE Cookie	24
0.4 % MOE Cookie	48
0.6 % MOE Cookie	72
0.8 % MOE Cookie	96
1 % MOE Cookie	120

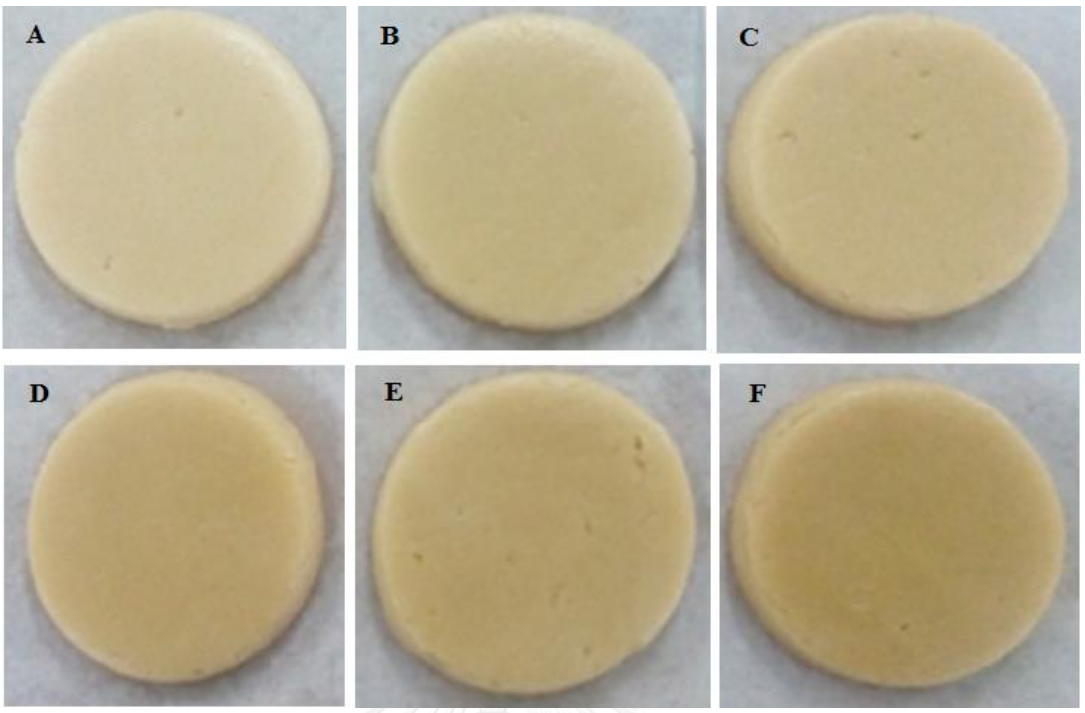


Figure 13. Cookie dough fortified with different amount of Moringa oleifera leave extract.

(A) cookies without MOE (control/0 % MOE), (B) cookies fortified with 0.2 % MOE, (C) cookies fortified with 0.4% MOE, (D) cookies fortified with 0.6% MOE, (E) cookies fortified with 0.8 % MOE and (F) cookies fortified with 1.0 % MOE.

Baked cookies to be used for analysis too were ground into powder and kept in air tight glass vials, wrapped in aluminum foil and stored at -20 $^{\circ}$C [149]. Some cookie dough from control and 0.2 – 1 % groups were kept in aluminum foil, packed in zip-lock bags filled with nitrogen gas and stored at -80 $^{\circ}$C. Cookies for lipid peroxidation assay were also packed in aluminum bags filled with nitrogen and stored at -80 $^{\circ}$C.

Cookies were prepared in four batches. Three replicates from each batch were used in the various assays/ tests and the mean taken. Chemical analysis in baked cookies was reported in terms of their dry weight basis.

3.2.4 Physical/physicochemical properties of cookies

Physical properties of the baked cookies were measured within 24 h after baking. These measurements were carried out to determine the quality of the cookies. These properties include weight, color, diameter, thickness, spread rate, moisture and pH.

3.2.4.1 Weight

Weight of cookies was measured using an electronic weighing balance (Sartorius, Scientific Promotion Co. Ltd, Germany).

3.2.4.2 Color

Color values were determined using colorimeter. The instrument was first calibrated using the black and white plate before colors of samples were determined. The CIE L*a*b* coordinates were measured using D65 illuminant. Color values L*, a*, b*, E index and Chroma as measures of lightness, redness-greenness, yellowness-blueness, color index and color intensity respectively were recorded for each sample and compared with control cookies.

$$\text{Chroma} = (a^2 + b^2)^{1/2}$$

$$\text{E index} = [(L)^2 + (a)^2 + (b)^2]^{1/2}$$

Five replicates of cookies were chosen randomly from the four batches of cookies prepared. Color was expressed as L* (100 = white, 0 = black), a* (positive = redness, negative = greenness), and b* (positive = yellowness, negative = blueness) values [150]. Color was measured from the surface of cookies.

3.2.4.3 Diameter

Diameter of each cookie was measured and again rotated 90° to obtain a second reading for that same cookie. This was repeated five times for each cookie and the mean taken. The total diameter of five cookies in a batch was measured in cm using a Vernier caliper [148].

3.2.4.4 Thickness/ Height

The height of each cookie was measured from two sides of the cookie. This was repeated five times for each cookie and the mean taken. The total height of five cookies in a batch was measured in cm using a Vernier caliper [148].

3.2.4.5 Spread ratio

This was estimated by using the equation below [148].

Spread ratio = Average diameter/ Average height

3.2.4.6 Moisture

This was determined using a moisture analyzer (Kett 610 Infrared Moisture Determination Balance FD 610) [111]. Cookies were weighed on a weighing balance and ground into fine powder. The temperature of the Moisture balance was set at 140 °C for 5 min and 5 g of ground cookie was then used to determine the moisture content of the cookie.

3.2.4.7 pH

Ground cookie of 0.2 g was dissolved with 10 mL of distilled water and vortex-mixed for 20 min. It was then kept at room temperature for 1 h, after which the supernatant was taken and pH measured using the pH meter. Readings were taken for triplicate sample cookies [92].

3.2.5 Sample preparation for the assays

For the MDA assay, sample extract was prepared by mixing 1 g of finely ground cookie sample with 3 mL of distilled water. The sample was homogenized using vortex for 30 min, and then centrifuged at 4500 rpm for 15 min [151]. Centrifugation process was repeated twice under the same conditions. Sample extracts for (FRAP antioxidant capacity assay, and Folin-Ciocalteu assay) and flavonoid content were prepared by adding 1g of finely ground cookie sample to 6 mL of distilled water and 2 g of powdered cookie in 4 mL of distilled water respectively under the same extraction conditions as that of MDA assay [151, 152].

3.2.5.1 Polyphenol and Flavonoid Assay

3.2.5.1.1 Folin-Ciocalteu Assay

This assay was used to determine the level of polyphenols in cookies fortified with Moringa leaves extract.

The assay was carried out based on the procedure by Singleton *et al*., with slight modifications. In brief, 10 µl of the sample extract was mixed with 75 µl of freshly prepared Folin-Ciocalteu reagent (10 times diluted). After incubation for 5 min at room temperature, 75 µl of sodium carbonate solution (10 %, w/v) was added and the solution mixed thoroughly and incubated for 30 min at room temperature. The absorbance was then measured using spectrophotometer at a wavelength of 765 nm. A suitable calibration curve was prepared using standard Gallic acid solution. All

the results were expressed as mg Gallic acid equivalents (GAE)/100 g of sample. A Gallic standard was prepared within the range of 0.02 – 0.75 mg/ml [95]. Standard curve for Gallic acid used as standard is shown in **Appendix 1.**

This assay was conducted in both cookie dough and baked cookie.

3.2.5.1.2 Total flavonoid assay

The level of total flavonoid was determined since it is one of the primary constituents of polyphenols that are known to have antioxidant activity.

Sample extract of 25 µl was mixed with 10 µl of $NaNO_2$ (5 %, w/v) and 100 µl of distilled water after which it is incubated for 5 min in the dark. After incubation, 15 µl of $AlCl_3.6H_2O$ (10 %, w/v) was added and incubated again for 6 min. NaOH (1M) solution of volume 50 µl was added to the reaction mixture and also 50 µl of distilled water. Absorbance of reaction mixture was measured immediately after shaken thoroughly at a wavelength of 510 nm. Catechin was used as standard within the range of 0.002–0.063 mg/ml [153]. Standard curve for catechin used as standard is shown in **Appendix 1.**

3.2.5.2 Antioxidant capacity assays

3.2.5.2.1 FRAP Assay

The assay is to determine the antioxidant capacity of the MOE fortified cookies. The reducing power of a compound serves as an indicator of its antioxidant activity. Reducing power appears to be related to the degree of hydroxylation and extent of conjugation in polyphenols.

The presence of reductants such as antioxidant substances causes a reduction of Fe^{3+}/ferrricyanide complex to Fe^{2+} / ferrous form. At low pH, reduction

of ferric tripyridyltriazine (Fe III TPTZ) complex to ferrous form (which has an intense blue color) can be monitored by measuring the change in absorption at 593 nm.

The oxidant in the FRAP assay consists of acetate buffer (pH 3.6), ferric chloride solution (20 mM) and TPTZ solution (10 mM TPTZ in 40 mM HCl) in a proportion of 10:1:1, respectively, and this was freshly prepared on the day of analysis. To obtain a FRAP value for antioxidant activity, 180 µl of FRAP solution warmed to 37 ℃ was added to 20 µl of the sample extract or standard. The sample reaction mixture was left at 37 ℃ for exactly 40 min and the absorbance was measured at 593 nm. A $FeSO_4.7H_2O$ standard curve was prepared from a 2000 µM stock solution for range of values 62.5 – 1000 µM. Calibration curve for $FeSO_4.7H_2O$ used as standard is shown in **Appendix 1.** It was further used to calculate the antioxidant capacity of the samples, which was expressed in µmol $FeSO_4$ equivalent/100 g of sample [95, 152]. FRAP solution used for blank consisted of acetate buffer (pH 3.6), distilled water and 40 mM HCl solution in a proportion of 10:1:1, respectively. This assay was conducted for both cookie dough and baked cookie.

3.2.5.2.2 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Antioxidant Assay

A second form of antioxidant assay was carried out to determine the antioxidant capacity of the cookies because FRAP leads to the production of reduced metals which are also propagators of radical chains through hydroperoxide reduction to RO.[93].

DPPH solution (0.2 mM) of 50 µl was added to 50 µl of sample/standard (ascorbic acid) followed by incubation for 30 min at room temperature in the dark. After incubation, the absorbance was measured at 515 nm using the spectrophotometer [119]. DPPH radical scavenging activity was reported as ascorbic acid equivalent antioxidant capacity.

3.2.5.3 Lipid Peroxidation Stability

3.2.5.3.1 Thiobarbituric acid Reactive Substance (TBARS) Assay

This was carried out to assess the level of lipid oxidation in the cookies, since it can occur by thermal oxidation of polyunsaturated oils. These are oxidized to form aldehyde and ketones which then reacts with amino acids to form brown pigments, as in the Maillard reaction. It is possible that peroxidation products induce the browning reaction of Amadori products [43].

Sample extract (250 µL) was transferred to a test tube. After which 250 µL of 20 % TCA solution was added and centrifuged twice. 12.5 µL butylated hydroxytoulene (7.2 %) and 250 µL TBARS–TCA solution (20 mM TBARS in 15 % TCA) were added to the test tube with supernatant of 250 µL and mixed thoroughly. Tubes were heated (105 °C) for 10 min, and cooled for 5 min. Absorbance of the supernatant was measured at 532 nm with a spectrophotometer. Calibration curve was prepared using MDA as a standard in the range of 1.88 - 40 µM. Results was expressed as nmol of MDA per g of sample [151].

This assay was conducted in the cookie dough and the baked cookie.

3.2.5.3.2 Lipid Extraction

A powdered cookie (20 g) was dissolved in 30 mL petroleum ether in the dark overnight (12 h). The solvent was filtered using filter paper over anhydrous sodium sulfate, followed by lipid extracted from petroleum ether by evaporation at 60 $^{\circ}$C [154]. The lipid extract was used for analysis of the conjugated dienes, peroxide value and acid value.

3.2.5.3.2.1 Conjugated Dienes

Extracted fat of 0.5 g was dissolved in 25 ml of hexane to make a mixture of 1 %. This solution was diluted 100 times since absorbance of the solution was more than 1. Absorbance of sample extracts was taken with a spectrophotometer at the wavelength of 234 nm using a quartz cuvette. Spectrophotometer was auto zeroed before any measurement was taken. Absorbance of mixture was expected to be between the absorbance of 0.1 – 0.8 [25, 42]. Conjugated diene value was expressed as µmol (CD) per g of sample. The pure solvent (hexane) was used as the blank.

3.2.5.3.2.2 Peroxide Value

This was done to know how much the *Moringa oleifera* leaves extract will be able to protect the cookies from oxidizing into the primary products of oxidation which will later breakdown into the secondary products.

Peroxide value is the initial product of oxidation of fat substances which does not lead to undesirable flavor and aroma directly but shows the degree of oxidation progress. Peroxide formation proceeds slowly in the initial stages. Peroxides present in the fat extract causes the release of iodine from the saturated potassium iodide added. The amount of iodine present was therefore determined by titrating with sodium thiosulfate with starch indicator used as an indicator [31].

Lipid extract (3 - 5 g) was dissolved in 50 mL neutral mixtures of absolute ethyl ether: absolute ethyl alcohol (2:1, v/v). Saturated solution of KI (0.5 mL) was added to the lipid extract solution. The mixture was shaken manually for 0.5min and kept in the dark for another 3min. After the addition of 30 mL distilled water, the mixture was titrated against sodium thiosulphate (0.002 M) until the yellow color almost disappears. Then, about 0.5 mL of starch indicator (0.05 %) solution was added. Titration was sustained until the blue color disappeared. A blank was also

determined under similar conditions. PV (mEq/kg) was calculated using equation below:

$$\text{PV (mEq/kg)} = \frac{\mathbf{V-Vo}}{\boldsymbol{m}} \times 1000$$

Where, C is the sodium thiosulphate concentration (M), V and V_o represent the volumes of sodium thiosulphate exhausted by the samples and the blank, respectively (mL), and *m* is the mass of sample used (g). This was done according to method proposed by GB/T 5538-2005 [172].

3.2.6 Sensory Analysis

This was undertaken to determine the acceptability of MOE fortified cookies by consumers through sensory analysis.

The sensory analysis was conducted in a sensory room with temperature and relative humidity of 22 – 25 $^{\circ}$C, 45-55 %'RH' respectively. There was no noise or odors and lighting in the room was also white. Each panel was provided with to rinse their mouths between each evaluation. Some considerations were taken into account before the commencement of the sensory analysis. These were;

- Panelists were prompted not to ingest any other food at least 1 h before the beginning of the analysis and also they should avoid chewing gums immediately before testing.
- Instructions provided to the panelist were clear and concise. It was given verbally to them on how to do the sensory evaluation before they entered their individual booths for analysis and this information was also provided in the first page of their score sheets.

- Sensory evaluation was carried out around mid-morning or mid-afternoon (i.e. 11 am or 3 pm respectively). This time period was used for the analysis because people are usually not overly hungry or full [155, 156].

  Inclusion criteria of panels involved in the sensory analysis were;

- Panels that were committed and were ready to be present at any session of the sensory analysis.
- Good health and free of illness related to sensory properties such as chronic cold, food allergies and diabetes.
- Non smokers
- Not color blind
- Have no strong dislikes for the food to be tested [156].
- Panels that have some knowledge about sensory analysis.

This was to determine the acceptability of cookies with *Moringa oleifera* leaf extract when compared to cookies without Moringa (control). It was done for two batches of cookies.

3.2.6.1 Acceptability test

This analysis was done using the 9 point hedonic scale to determine the degree of likeness for the food product. It assumes that the consumers' continuum and preference can be categorized on bases of likeness and dislike. The words used for the scale are equally spaced with numbers assigned to them. Also, the scale being horizontally or vertically assigned or likeness or dislike appearing first on the scale does not affect the evaluation. The scale used in this analysis should be equally spaced and this Parametric statistics are used for the analysis of data [157].

This was done with 30 semi-trained subjects [158, 159]. These subjects were obtained through an advertisement at the school premises. In this session, the panels were served with all samples randomly, one at a time (control, 0.2 %, 0.4 %, 0.6 %, 0.8 % and 1 % Moringa cookies) to taste. Samples were assigned a 3 digit code. The 9 point hedonic scale (9 = like extremely; 5 = neither like nor dislike; 1 = dislike extremely) was used by subjects to quantify the intensity of specific attributes such as appearance, color, hardness, saltiness, sweetness, aroma, flavor and determine the level of overall acceptability of the cookie samples [160]. Drinking water was given to panels to rinse their mouth between every taste of the cookies [161]. This session was done twice to ascertain the consistency of the assessment. Cookies to be used for sensory evaluation were cooled at room temperature after baking and packed in small size ziplock bags at room temperature 26-28 $^{\circ}$C until evaluated within 24 h [160]. Sensory questionnaire also contained questions to obtain demographic information about consumers such as gender, age and purchasing frequency of cookies.

3.2.7 Storage effect evaluation

This study was carried out to determine the effect of storage conditions on the level of lipid peroxidation (conjugated diene, acid value, peroxide value and MDA level), antioxidant capacity (FRAP antioxidant capacity, DPPH assay), polyphenol content, flavonoid content, color, moisture, texture and water activity within a period of 6 weeks. Analyses of these parameters were made weekly. Sensory evaluation was also conducted in cookies within the same period of time during storage. This session was conducted using the most acceptable cookie with *Moringa oleifera* leaf extract obtained from the previous initial sensory analysis.

3.2.7.1 Shelf life of cookies

This was done to determine the level of oxidative stability and acceptability during shelf life of the MOE fortified cookies over a period of 6 weeks. Polypropylene food containers were used for packaging because it has a moderate barrier to moisture, gases and odors. It has a high tensile strength and puncture resistant [162], due to these characteristics it is widely used to pack biscuits, snack and other dried foods. Polypropylene has a high barrier to water vapor which means that it is not affected by changes in humidity [163].

MOE fortified cookies with a high level of acceptability during the affective test and the control cookies (cookies without *Moringa oleifera* leaves extract) were prepared and sealed in polypropylene plastic food containers and kept 29 -30 $^{\circ}$C for a period of 6 weeks. Levels of lipid peroxidation (Conjugated dienes, peroxide value, acid value, MDA), antioxidant capacity (FRAP antioxidant capacity, DPPH assay), polyphenol content, total flavonoid content, color, moisture, texture and water activity were analyzed weekly. The level of conjugated dienes, peroxide value and acid value were measured during the storage period, since these shows the level of primary products produced during lipid peroxidation process [84, 164]. Parameters such as moisture, DPPH assay, MDA, conjugated diene, peroxide value, polyphenol content, flavonoid content and FRAP antioxidant capacity were measured.

3.2.7.2 Water Activity

Cookies were crushed into small pieces and a representative sample was placed into plastic cups and measured one at a time. Readings were taken for six sample cookies and was done using a water activity meter [160].

3.2.7.3 Hardness and fracturability of cookies

Cookie hardness was measured using a three-point bending test. The hardness of the cookies was indicated by the maximum peak force required to break the cookies. The texture analyzer, TA XT*Plus* Texture Analyzer (Texture Technologies Corp. Scarsdale, NY, USA), was fitted with sharp-blade probe, 6 cm long and 1 mm thick, and set to 'return to start' cycle, a pretest speed of 1.0 mm s−1, test speed of 2.0 mm s−1, post-test speed of 10 mm s−1, and a distance of 3.0 mm [165]. Fracturability of cookies was also obtained.

3.2.7.4 Acid Value

This was done to determine the extent of rancidity in the cookie during the storage period. The acid value measures free fatty acids and is usually considered to be one of the main parameters reflecting the quality of food during the storage period [166] Acid value is based on the principle of mg NaOH needed to neutralized free fatty acids hydrolyzed from 1g of fat or oil [31].

Lipid extract (3–5 g) was dissolved in 50 mL neutral mixtures of absolute ethyl ether: absolute ethyl alcohol (2:1, v/v) using 0.1ml phenolphthalein as indicator. The solution was titrated with 0.05 mol L^{-1} NaOH until reaching the end point (reddish). Acid values were calculated as follows:

$$\text{Acid Value (mg NaOH g}^{-1}) = \frac{V \times C \times 56.11}{m}$$

Where, *V* is the titration amount of standard volumetric NaOH solution used (mL); *C* is the concentration of the standard volumetric NaOH solution (mol L^{-1}), and *m* is the

weight of the sample (g).This was done according to method proposed by GB/T 5538-2005 [167].

3.2.8 Sensory analysis of baked cookies fortified with MOE during storage

Sensory evaluation during storage was done using quantitative descriptive analysis (QDA) technique. This was done by 10 panelists who included both undergraduate and graduate students in Chulalongkorn University, Department of food and nutrition.

Panelists were first trained in developing terms/attributes to describe cookies which were stored for 3 weeks. Attributes which were selected for the main analysis which took place for a period of 6 weeks included color, hardness, fracturability, moisture, dryness, rancid aroma, rancid flavor, acceptability of flavor and aroma and overall acceptability. Unstructured line scale of 15 cm with anchors at both ends indicating intensities (as shown in appendix) was used in this analysis. Analysis was done on weekly basis.

3.2.9 Data and statistical Analysis

Results were reported as mean of values ± SEM. ANOVA was performed using Tukey post hoc test to determine the significance difference between samples using SPSS statistical packages, version 17.0 software. Calculations were performed with Microsoft Excel 2010 from Microsoft Co. The graphs were generated with Sigma Plot, version 11.0 software. Correlation between instrumental measurement and sensorial parameters measured during storage period was done by Pearson correlation using SPSS statistical packages, version 17.0 software.

CHAPTER 4

RESULTS

4.1 Determination of polyphenol content and flavonoid content of *Moringa oleifera* leave extract

4.1.1 Percentage yield

The result in **Table 4** shows the percentage yield as 18.21 % from an equation as shown in **Appendix 1.**

4.1.2 Polyphenol content

This was determined using the Folin Ciocalteau assay in the *Moringa oleifera* leave extract (MOE) after spray drying. The content as shown in **Table 4** was 40.14 ± 0.56 mg Gallic acid/ g of extract.

4.1.3 Flavonoid Content

Flavonoid content was determined by the aluminium chloride colorimetric assay. Results showed that *Moringa oleifera* leave extract used contained 6.82 ± 0.14 mg Catechin/g of extract (**Table 4**).

4.1.4 Antioxidant capacity

Antioxidant capacity was determined by Ferric reducing antioxidant power (FRAP) capacity. Results showed that *Moringa oleifera* leave extract used contained 321.35 ± 9.45 mmol $FeSO_4$/g of extract (**Table 4**).

Table 4 Percentage yield, polyphenol, flavonoid content and FRAP antioxidant capacity in MOE**.**

Percentage yield (%)	Polyphenol Content (mg GAE/ g of extract)	Flavonoid Content (mg Catechin/ g of extract)	FRAP antioxidant capacity (mmol $FeSO_4$/ g of extract)
18.21	40.14 ± 0.56	6.82 ± 0.14	321.35 ± 9.45

Data are expressed as mean ± SEM, n=3.

4.2 Determination of physical and physicochemical properties of cookies

4.2.1 Weight, diameter, height, spread ratio, pH and moisture

Weight of cookies increased in a dose dependent manner when extract was added in a range of 11.21 g to 11.51 g in 0.2 %-1.0 % MOE fortified when compared to control cookies (11.11 g). In addition, weight of 0.6 % - 1.0% MOE fortified cookies were significantly different when compared to control whilst 1.0 % MOE fortified cookies was significantly different from 0.2 % MOE cookies as shown in **Table 5**.

Diameter of cookies also decreased in a dose dependent manner from 4.54 - 4.46 cm in 0.2 % - 1.0 %MOE fortified cookies when compared to control cookies which had a diameter of 4.56 cm (**Table 5**). A Significant difference was observed in 0.8 % – 1.0 % MOE fortified cookies when compared to control cookies and 0.2 % - 0.6 % MOE fortified cookies were significantly different when compared to 1.0 % MOE fortified cookies.

The height of MOE fortified cookies were slightly increased in a range of 0.91 - 0.93 cm in 0.2 % - 1.0 % MOE fortified cookies when compared to control cookies with height of 0.91 cm (**Table 5**). However, no significant differences were found among groups.

As shown in **Table 5**, a decrease of diameter together with a slight increase in height influenced the spread ratio of the cookies. The spread ratio of 0.2 % - 1.0% MOE fortified cookies was ranged from 5.02 - 4.72 which tended to decrease in a dose dependent manner among groups when compared to control (5.04). However, the significant reduction in spread ratio was observed only in 1.0 % MOE fortified cookies when compared to control ($p<0.05$).

The pH of fortified cookies decreased in a dose dependent manner ranged from 6.46 - 6.31 in 0.2 % to 1.0 % MOE fortified cookies when compared to control (6.63). The significant differences of pH were observed between control and cookie fortified with MOE as shown in **Table 5**.

An Increase in the level of moisture was observed when MOE was added to cookies in a dose dependent manner ranging from 5.81 % - 6.6 % in 0.2 % to 1.0 % MOE fortified cookies (**Table 5**). However, the significant different was observed only when cookies fortified with 1.0 % MOE compared to the control cookies ($p<0.05$).

Table 5. Physical and Physicochemical Properties of baked cookies fortified with MOE

Physical And Physicochemical Properties	Control	0.2% MOE	0.4% MOE	0.6% MOE	0.8% MOE	1.0% MOE
Weight (g)	11.11 ± 0.08^{a}	11.21 ± 0.11^{a,b}	11.35 ± 0.02^{a,b,c}	11.41 ± 0.01^{b,c}	11.46 ± 0.01^{b,c}	11.51 ± 0.01^{c}
Diameter (cm)	4.56 ± 0.02^{a}	4.54 ± 0.00^{a}	4.52 ± 0.00^{a}	4.51 ± 0.01^{a}	4.50 ± 0.00^{b}	4.46 ± 0.00^{b}
Height (cm)	0.91 ± 0.00	0.91 ± 0.01	0.91 ± 0.00	0.91 ± 0.01	0.92 ± 0.02	0.93 ± 0.02
Spread Ratio	5.04 ± 0.02^{a}	5.02 ± 0.00^{a}	4.93 ± 0.01^{a}	4.87 ± 0.01^{a}	4.83 ± 0.08^{a}	4.72 ± 0.10^{b}
pH	6.63 ± 0.02^{a}	6.46 ± 0.01^{b}	6.42 ± 0.01^{b,c}	6.37 ± 0.00^{c,d}	6.36 ± 0.01^{d}	6.31 ± 0.01^{d}
Moisture (%)	5.42 ± 0.15^{a}	5.81 ± 0.11^{a}	6.06 ± 0.26^{a}	6.17 ± 0.29^{a}	6.51 ± 0.28^{a}	6.61 ± 0.32^{b}

Control, 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE prepared with 0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% of *Moringa oleifera* leave extract, respectively. Data are expressed as means ± SEM, n = 3. Means with different letter within a row are significantly different ($p<0.05$) according to Tukey HSD test.

4.2.2 Color

All color data were expressed by Hunter L, a, and b values corresponding to lightness (L*), redness (a*) and yellowness (b*), respectively. Lightness level of cookies fortified with MOE was 68.64 - 62.43 in 0.2 % to 1.0 % MOE fortified which was lower than control (69.90) (**Table 6**). The significant reduction of lightness level in a dose dependent manner was observed in cookies fortified with MOE when compared to control (p<0.05) as shown in **Table 6.** The redness of cookies fortified with MOE was in the range of 9.70 - 10.98 in 0.2 % to 1.0 % MOE fortified cookies. The redness of 0.8 % (10.93) to 1 % (10.98) MOE fortified cookies significantly increased when compared to control (9.24). However, no significant difference in the level of yellowness was found among groups.

The color intensity/ Chroma of cookies fortified with MOE were not significantly difference when compared with control cookies. However, Overall color (E index) was reduced in cookies fortified with MOE. The E index in 0.2 %-1 % MOE fortified cookies (79.46 - 73.89) were significantly reduced when compared to control (80.73) (p<0.05) as shown in **Table 6**.

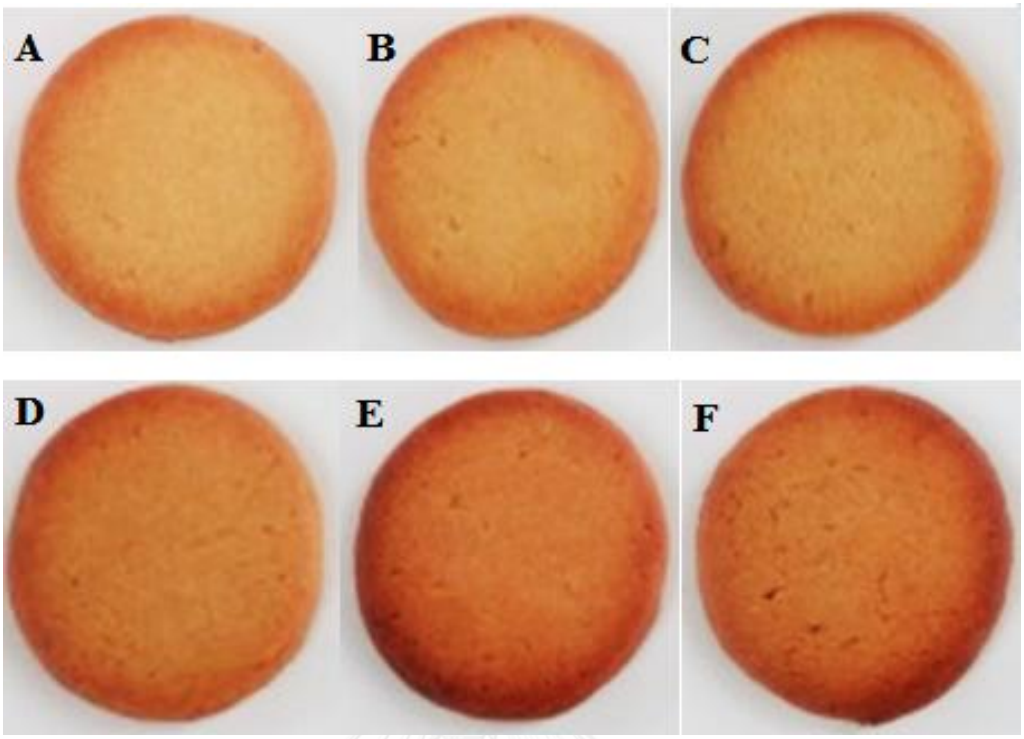


Figure 14 Baked cookies fortified with different amount of *Moringa oleifera* leave extract (MOE).

(A) cookies without MOE (control/0 % MOE), (B) cookies fortified with 0.2 % MOE, (C) cookies fortified with 0.4 % MOE, (D) cookies fortified with 0.6% MOE, (E) cookies fortified with 0.8 % MOE and (F) cookies fortified with 1.0 % MOE

Table 6. Color characteristics in baked cookies fortified with MOE.

Color	Control	0.2% MOE	0.4% MOE	0.6% MOE	0.8% MOE	1.0% MOE
L^*	69.90 ± 0.06^a	68.64 ± 0.15^b	66.70 ± 0.33^c	64.26 ± 0.01^d	63.73 ± 0.10^d	62.43 ± 0.13^e
a^*	9.24 ± 0.56^a	9.70 ± 0.37^a	9.76 ± 0.17^a	10.54 ± 0.24^a	10.93 ± 0.22^b	10.98 ± 0.18^b
b^*	39.15 ± 0.26^a	38.83 ± 0.46^a	38.86 ± 0.61^a	39.11 ± 0.45^a	38.26 ± 0.32^a	38.23 ± 0.45^a
Chroma	39.84 ± 0.25^a	40.02 ± 0.50^a	40.06 ± 0.63^a	40.51 ± 0.37^a	40.31 ± 0.46^a	40.29 ± 0.20^a
E index	80.73 ± 0.20^a	79.46 ± 0.23^b	77.76 ± 0.21^c	75.96 ± 0.24^d	75.05 ± 0.20^d	73.89 ± 0.19^e

Control, 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE prepared with 0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% of *Moringa oleifera* leave extract, respectively. Data are expressed as means ± SEM, n = 3. Means with different letter within a row are significantly different (p<0.05) according to Tukey HSD test. L*: Lightness, a*: redness, b*: yellowness.

4.3 Determination of polyphenol and flavonoid content in cookie dough and baked cookie fortified with MOE.

As shown in **Table 7**, polyphenol content in cookie dough of control and cookie doughs fortified with 0.2% - 1.0 % MOE were 57.04 mg GAE/100g dough and in the range of 62.31-86.19 mg GAE/100g dough, respectively. Polyphenol content in cookie doughs fortified with 0.2% - 1.0 % MOE were increased in a dose dependent manner with 9.24%, 20.55%, 30.17%, 41.08% and 51.10% were observed in cookie doughs fortified with 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE, respectively. The significant increase in polyphenol content was observed in cookie doughs fortified with 0.6%-1.0% MOE when compared to control as shown in **Table 7** ($p<0.05$). Polyphenol content after addition of 0.2 - 1.0 % MOE in baked cookies (dry basis) was shown in **Table 7**. Polyphenol content after addition of 0.2 - 1.0 % MOE in baked cookies (dry basis) were increased in a dose dependent manner ranging from 71.53 to 106.95 mg GAE/100g dry basis when compared to control (63.69 mg GAE/100g dry basis) in the percentage of 12.31%, 28.97%, 42.82%, 47.26% and 67.92% in 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE cookies, respectively. However, a significant increase in polyphenol content was found when 0.4 - 1.0 % of the extract was added in the cookies (**Table 7**).

Increase in polyphenol content in cookies reflected a relative increase in the flavonoid content of cookies. Flavonoid content in cookie dough of control and cookie dough fortified with 0.2 % - 1.0 % MOE were 1.69 mg Catechin/ 100g dough and in the range of 3.14-9.17 mg Catechin/100g dough, respectively. Flavonoid content in cookie doughs fortified with 0.2 % - 1.0 % MOE was higher than control with 85.80 %, 260.95 %, 312.43 %, 388.17 % and 442.60 % in 0.2 % MOE, 0.4 % MOE, 0.6 % MOE, 0.8 % MOE and 1.0 % MOE cookies, respectively. However, the significant difference was observed in 0.4 to 1.0 % MOE fortified cookies (6.10 to 9.17 mg

Catechin/100g cookie) when compared to control (**Table 8**). In addition, flavonoid content after addition of 0.2 - 1.0 % MOE in baked cookies (dry basis) were increased in a dose dependent manner ranging from of 1.22 – 4.13 mg Catechin/ 100g dry basis when compared to control (0.39 mg Catechin/ 100g dry basis) with percentage increment of 212.82 %, 412.82 %, 692.31 %, 800 % and 958.97 % in 0.2 % MOE, 0.4 % MOE, 0.6 % MOE, 0.8 % MOE and 1.0 % MOE, respectively (**Table 8**). The significant difference was found in 0.2 to 1.0 % MOE fortified cookies when compared to control cookie ($p<0.05$). Percentage increase of flavonoid content in cookie dough was observed to be higher in the groups of cookies when compared to the baked cookies (dry basis), 76.92%, 61.15%, 67.21%, 55.67%, 57.45% and 54.96% increase was however observed in control, 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE, respectively. Moreover, percentage increase of polyphenol in baked cookies (dry basis) in comparison with dough is 11.66 %, 14.80 %, 19.46 %, 22.51 %, 16.55 % and 24.09 % for control, 0.2 %, 0.4 %, 0.6 %, 0.8 % and 1 % respectively. The increase in polyphenol and flavonoid content in baked cookies (dry basis), when compared to cookie dough was however, significantly different in all the sample groups (control cookies to 1% MOE fortified cookies).

Thus, MOE added in cookies can increase the amount of polyphenol and flavonoid content in cookie dough and these active compounds were remained after baking.

Table 7. Polyphenol content in baked cookie (dry basis) and cookie dough fortified with MOE.

	Control	0.2% MOE	0.4% MOE	0.6% MOE	0.8% MOE	1.0% MOE
Polyphenol content (mg GAE/100g dough)	57.04 ± 1.42^{a}	62.31 ± 0.70^{a,b}	68.76 ± 1.51^{a,b,c}	74.25 ± 2.23^{b,c,d}	80.47 ± 4.67^{c,d}	86.19 ± 3.86^{d}
Polyphenol content (mg GAE/100g dry basis)	81.14 ± 1.53^{a}	90.35 ± 1.70^{a,b}	103.99 ± 1.79^{b,c}	113.96 ± 2.24^{c,d}	118.09 ± 4.07^{c,d}	127.51 ± 5.65^{d}

Control, 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE prepared with 0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% of *Moringa oleifera* leave extract, respectively. Data are expressed as means ± SEM, n = 3. Means with different letters (A-B: comparison between cookie dough and baked cookie dry basis) and (a-e: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

Table 8. Flavonoid content in baked cookies (dry basis) and cookie dough fortified with MOE.

	Control	0.2% MOE	0.4% MOE	0.6% MOE	0.8% MOE	1.0% MOE
Flavonoid content (mg Catechin/ 100g dough)	1.69 ± 0.27^{a}	3.14 ± 0.38^{a}	6.10 ± 0.77^{b}	6.97 ± 0.26^{b}	8.25 ± 0.48^{b,c}	9.17 ± 0.42^{c}
Flavonoid content (mg Catechin/100g dry basis)	0.50 ± 0.10^{a}	1.55 ± 0.08^{b}	2.52 ± 0.07^{c}	3.80 ± 0.11^{d}	4.32 ± 0.22^{e}	5.18 ± 0.11^{f}

Control, 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE prepared with 0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% of *Moringa oleifera* leave extract, respectively. Data are expressed as means ± SEM, n = 3. Means with different letters (A-B: comparison between cookie dough and baked cookie dry basis) and (a-f: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

4.4 Determination of antioxidant capacity (FRAP antioxidant capacity and DPPH radical scavenging activity) in cookie dough and baked cookies (dry basis) fortified with MOE

The effect of MOE on the antioxidant capacity of cookie dough and baked cookies (dry basis) fortified MOE are shown in **Tables 9**. **Table 9** demonstrated the Ferric reducing antioxidant power (FRAP) level of antioxidant capacity in cookie dough fortified with 0.2 % – 1.0 % MOE was dramatically increased in a range of 173.46 – 422.89 μmol $FeSO_4$/100g dough when compared with control cookies (114.38 μmol $FeSO_4$/100g dough) (p<0.05). The percentage increase of FRAP level was 51.65 %, 96.28 %, 154.63 %, 224.92 % and 269.72 % in 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE, respectively. In addition, FRAP level of baked cookies (dry basis) containing 0.2 – 1.0% MOE was gradually increased from 277.23 to 524.58 μmol $FeSO_4$/100g dry basis when compare to the control cookies with 240.52 μmol $FeSO_4$/100g dry basis. Fortification of MOE in baked cookies (dry basis) increased FRAP capacity by 15.26%, 51.70%, 89.77%, 98.10% and 118.10% in 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8 % MOE and 1.0 % MOE cookies, respectively (**Table 9**). However, the significant difference in FRAP level was found in baked cookies (dry basis) fortified with 0.4 to 1.0 % MOE when compared to control. Although, FRAP capacity in cookie dough and baked cookies (dry basis) were increased dose dependently but this increase was less in cookie dough than that observed in baked cookies (dry basis). The percentage increase in FRAP capacity among the cookie groups was higher in the baked cookies (dry basis) than cookie dough in a range of 59.82%, 62.53%, 56.72%, 28.20% and 24.05% in 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE cookies, respectively. The increase in FRAP antioxidant capacity in baked cookies (dry basis), when compared to cookie dough was however, significantly different in all the sample groups (control cookies to 1% MOE fortified

cookies). Therefore, as MOE fortification increased, FRAP antioxidant capacity in baked cookies (dry basis) also increased dose dependently.

DPPH radical scavenging antioxidant capacity in cookie dough fortified with 0.2 % – 1.0 % MOE was dramatically increased in a range of 345.50 – 790.36 μg AEAC/g dough when compared with control cookies (221.31μg AEAC/g dough) (p<0.05). The percentage increase of DPPH radical scavenging activity level was 59.09 %, 104.55 %, 150.00%, 177.27% and 259.09% in 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE respectively. In addition, DPPH radical scavenging activity of baked cookies (dry basis) containing 0.2 – 1.0% MOE was gradually increased from 376.78 to 825.61 μg AEAC/g dry basis when compare to the control cookies with 239.80 μg AEAC/g dry basis. Fortification of MOE in baked cookies (dry basis) increased DPPH radical scavenging activity by 57.12%, 94.15%, 143.30%, 182.84% and 244.29% in 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8 % MOE and 1.0 % MOE cookies, respectively (**Table 9**). However, the significant difference in DPPH radical scavenging activity was found in baked cookies (dry basis) fortified with 0.2 to 1.0 % MOE when compared to control. Although, DPPH radical scavenging activity in cookie dough and baked cookies (dry basis) were increased dose dependently but this increase was less in cookie dough than that observed in baked cookies (dry basis). The percentage increase in DPPH radical scavenging activity among the cookie groups was higher in the baked cookie (dry basis) than cookie dough in a range of 9.05%, 8.10%, 6.27%, 11.78% and 4.46% in 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE cookies, respectively. The increase in DPPH radical scavenging activity in baked cookies (dry basis), when compared to cookie dough was however, not significantly different in all the sample groups (control cookies to 1% MOE fortified cookies).

Table 9. Antioxidant capacity in cookie dough and baked cookies (dry basis) fortified with MOE.

	Control	0.2% MOE	0.4% MOE	0.6% MOE	0.8% MOE	1.0% MOE
Antioxidant capacity						
FRAP (μmol $FeSO_4$/100g dough)	$114.38 \pm 7.85^{A,a}$	$173.46 \pm 10.71^{A,b}$	$224.50 \pm 4.87^{A,c}$	$291.25 \pm 7.25^{A,d}$	$371.65 \pm 6.91^{A,e}$	$422.89 \pm 15.87^{A,f}$
DPPH (μg AEAC/g dough)	$221.31 \pm 7.77^{A,a}$	$345.50 \pm 16.97^{A,b}$	$430.69 \pm 20.37^{A,c}$	$549.02 \pm 1.33^{A,d,e}$	$606.79 \pm 33.45^{A,e}$	$790.36 \pm 15.43^{A,f}$
Antioxidant capacity						
FRAP (μmol $FeSO_4$/100g dry basis)	$240.52 \pm 5.75^{B,a}$	$277.23 \pm 6.22^{B,a}$	$364.88 \pm 12.63^{B,b}$	$456.44 \pm 10.26^{B,c}$	$476.46 \pm 10.19^{B,c}$	$524.58 \pm 7.61^{B,d}$
DPPH (μg AEAC/g dry basis)	$239.80 \pm 10.55^{A,a}$	$376.78 \pm 5.87^{A,b}$	$465.56 \pm 18.21^{A,c}$	$583.44 \pm 11.98^{A,d}$	$678.25 \pm 3.33^{A,e}$	$825.61 \pm 6.32^{A,f}$

*AEAC = Ascorbic acid equivalent antioxidant activity. Control, 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE prepared with 0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% of *Moringa oleifera* leave extract, respectively. Data are expressed as means ± SEM, $n = 3$. Means with different letters (A-B: comparison between cookie dough and baked cookie dry basis) and (a-f: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

4.5 Determination of lipid peroxidation products (conjugated diene, peroxide value and malondialdehyde) in cookie dough and baked cookies (dry basis) fortified with MOE.

In this study, conjugated diene and peroxide value, which are primary products of lipid oxidation, were measured to demonstrate the effect of addition of MOE against lipid oxidation in the cookies. The reduction of conjugated diene and peroxide value in cookie dough were gradually increased when an increase amount of MOE was added to cookies (**Table 10**). However, no significant difference in conjugated diene was observed between MOE fortified cookie groups and control cookies as percentage reductions of 1.76 %, 2.53 %, 5.54 %, 5.74 % and 10.63 % in 0.2 %, 0.4 %, 0.6 %, 0.8 % and 1.0 % MOE cookies, respectively whereas the level of peroxide value in cookie dough fortified with 0.2 to 1 % MOE were significantly different when compared to control cookies ($p<0.05$) with percentage reduction of 14 %, 29.11 %, 42.05 %, 52.83 % and 57.68 % in 0.2 %, 0.4 %, 0.6 %, 0.8 % and 1.0 % MOE cookies, respectively as shown in **Table 10**.

Baked cookies (dry basis) fortified with 0.2 to 1 % MOE showed a reduction in conjugated diene and peroxide value in a dose dependent manner from 46.69 to 39.25 μmol/g of lipid and 2.95 to 1.30 mEq/Kg of lipid, respectively as shown in **Table 10**. A significant difference in reduction of conjugated diene (6.02 % to 19.6 %) and peroxide value (27.38 % to 60.00 %) was observed in cookies fortified with 0.4 to 1.0 % MOE when compared to the control ($p<0.05$).

The percentage increase of conjugated diene and peroxide value in cookie dough were higher than baked cookies (dry basis) a range of 3.93%, 4.79%, 6.36%, 7.93% and 11.22% and 7.23%, 10.27%, 17.67%, 16.57% and 17.20%, respectively in 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE cookies. The reduction in conjugated diene and peroxide value content in baked cookies (dry basis), when

compared to cookie dough was however, significantly different in 0.8% - 1% MOE fortified cookies and control – 0.6% MOE fortified cookies, respectively.

The results indicated that MOE has a potential to inhibit the production of primary products of lipid oxidation before and after baking cookies (dry basis). However, the higher potent was observed after baking the cookies.

Malondialdehyde (MDA), a secondary product of lipid oxidation, was determined by Thiobarbituric acid reactive substance (TBARS) assay. The effectiveness of MOE in reducing MDA concentration in cookies was shown in **Table 10**. In cookie dough fortified with MOE, MDA was reduced in a range of 22.73-20.95 nmol/g dough (2.0 % - 1.0 % MOE fortified cookies) when compared to control cookies (23.50 nmol/ g dough) with percentage reductions of 3.28 %, 6.60 %, 9.45 %, 10.85 % and 14.51 % in 0.2 % MOE, 0.4 % MOE, 0.6 % MOE, 0.8 % MOE and 1.0 % MOE cookies, respectively. Significant reduction was observed in 0.4 to 1.0 % MOE fortified cookie dough when compared to control ($p<0.05$).

MDA concentration in baked cookies (dry basis) fortified with MOE was reduced in a dose dependent manner in a range of 22.84 – 19.04 nmol/g of dry basis 0.2 % - 1.0 % MOE fortified cookies when compared to control (23.79 nmol/g of dry basis) as shown in **Table 10**. Percentage reduction in MDA concentration in baked cookies (dry basis) fortified with MOE ranged in 3.99 %, 6.98 %, 9.33 %, 11.56 % and 19.97 % in 0.2 % MOE, 0.4 % MOE, 0.6 % MOE, 0.8 % MOE and 1.0 % MOE, respectively. Significant reduction was observed in 0.4 to 1.0 % MOE fortified cookies when compared to control ($p<0.05$).

The percentage increase of MDA value in baked cookies (dry basis) when compared to cookie dough was 0.48%, 0.82%, 1.36%, 0.43% and 5.23% in 0.2%, 0.4%, 0.6%, 0.8% and 1% MOE cookies, respectively. The reduction in

malondialdehyde in baked cookies (dry basis), when compared to cookie dough was however, significantly different in all the sample groups (control cookies to 1% MOE fortified cookies). Therefore, as MOE fortification was increased, MDA formation in baked cookies (dry basis) decreased in a dose dependent manner.

Table 10 Lipid peroxidation products in cookie dough and baked cookies (dry basis) fortified with MOE

	Control	0.2% MOE	0.4% MOE	0.6% MOE	0.8% MOE	1.0% MOE
Lipid peroxidation in dough						
Conjugated diene (μmol/ g lipid)	$49.47 \pm 2.22^{A,a}$	$48.60 \pm 2.27^{A,a}$	$48.22 \pm 2.2^{A,a}$	$46.73 \pm 2.06^{A,a}$	$46.63 \pm 0.55^{A,a}$	$44.21 \pm 1.40^{A,a}$
Peroxide value (mEq/Kg lipid)	$3.71 \pm 0.05^{A,a}$	$3.47 \pm 0.09^{A,b}$	$2.63 \pm 0.12^{A,c}$	$2.15 \pm 0.08^{A,c,d}$	$1.75 \pm 0.07^{A,d,e}$	$1.57 \pm 0.08^{A,e}$
MDA (nmol/g dough)	$23.50 \pm 0.01^{A,a}$	$22.73 \pm 0.14^{A,a,b}$	$21.95 \pm 0.04^{A,b,c}$	$21.28 \pm 0.16^{A,c,d}$	$20.95 \pm 0.26^{A,d,e}$	$20.09 \pm 0.31^{A,e}$
Lipid peroxidation in baked cookie						
Conjugated diene (μmol/ g lipid)	$48.85 \pm 0.84^{A,a}$	$46.69 \pm 0.58^{A,a,b}$	$45.91 \pm 0.85^{A,a,b}$	$43.76 \pm 0.37^{A,b,c}$	$42.93 \pm 0.58^{B,c}$	$39.25 \pm 0.33^{B,d}$
Peroxide value (mEq/Kg lipid)	$3.25 \pm 0.15^{B,a}$	$2.95 \pm 0.16^{B,a,b}$	$2.36 \pm 0.09^{B,b,c}$	$1.77 \pm 0.11^{B,c,d}$	$1.46 \pm 0.11^{A,d}$	$1.30 \pm 0.19^{A,d}$
MDA (nmol/g dry basis)	$23.79 \pm 0.41^{A,a}$	$22.84 \pm 0.35^{A,a,b}$	$22.13 \pm 0.33^{A,b,c}$	$21.57 \pm 0.31^{A,b,c}$	$21.04 \pm 0.16^{A,c}$	$19.04 \pm 0.40^{A,d}$

Control, 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE prepared with 0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% of *Moringa oleifera* leave extract, respectively. Data are expressed as means ± SEM, n = 3. Means with different letters (A-B: comparison between cookie dough and baked cookie dry basis) and (a-e: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

4.5 Sensory evaluation of baked cookies (Acceptability test)

Table 11 and Figure 15 showed the effect of MOE added in the cookies on sensory attributes. Likeness score of saltiness, sweetness and hardness did not affect when MOE was added in the cookies whereas likeness score of color, aroma, flavor, appearance and over all acceptability were gradually decreased when an increase concentration of MOE was added in the cookies (**Table 11**). The likeness score of color was significantly reduced in 0.4 to 1.0 % MOE fortified cookies whereas aroma, flavor, appearance and over all acceptability score were significantly reduced in 0.6 to 1.0 % MOE fortified cookies when compared to control ($p<0.05$). Although the 0.2 % MOE fortified cookies obtained the highest score for most attributes, antioxidant capacity and inhibition of lipid oxidation were not different in 0.2 % MOE fortified cookies when compare to control cookies. According to overall acceptability score and chemical properties, 0.4 % MOE fortified cookies was chosen for further study. In addition, 0.6% MOE cookies were also chosen for further studies due to its high antioxidant capacity, polyphenol and flavonoid content which was significantly higher than control cookies. Cookies fortified with 0.6% MOE also reduced lipid peroxidation products significantly when compared with control cookies. From this sensory evaluation, cookies fortified with 0.6% MOE had its sweetness level not significantly different from control cookies which had no extract. Aroma, flavor and overall likeness were not also found significantly different in 0.6% MOE cookies when compared with 0.4% MOE cookies.

Table 11. Sensory evaluation of baked cookies fortified with MOE using 9 point hedonic scale.

	Control	0.2% MOE	0.4% MOE	0.6% MOE	0.8% MOE	1.0% MOE
Saltiness	6.63 ± 0.16^{a}	6.70 ± 0.17^{a}	6.33 ± 0.16^{a}	6.52 ± 0.15^{a}	6.38 ± 0.16^{a}	6.20 ±0.16^{a}
Sweetness	6.82 ±0.18^{a}	7.02 ± 0.15^{a}	6.77 ± 0.13^{a}	6.58 ±0.15^{a}	6.40 ±0.15^{a}	6.57 ± 0.16^{a}
Color	7.62 ± 0.10^{a}	7.32 ± 0.14^{a}	6.83 ± 0.15^{b}	6.08 ± 0.17^{c}	6.02 ± 0.19^{c}	5.78 ± 0.19^{c}
Hardness	6.02 ± 0.23^{a,b}	6.05 ± 0.21^{a,b}	6.13 ± 0.20^{a,b}	6.33 ±0.20^{a}	5.48 ± 0.21^{b}	5.95 ± 0.19^{a,b}
Aroma	7.07 ± 0.16^{a}	6.82 ± 0.17^{a}	6.47 ± 0.17^{a,b}	6.02 ± 0.19^{b,c}	5.65 ± 0.21^{c}	5.53 ± 0.20^{c}
Flavor	7.27 ± 0.14^{a}	7.03 ± 0.15^{a,b}	6.73 ± 0.16^{a,b}	6.52 ± 0.14^{b,c}	5.90 ± 0.19^{c}	5.90 ± 0.21^{c}
Appearance	7.50 ± 0.12^{a}	7.25 ± 0.15^{a}	6.93 ±0.14^{a}	6.23 ± 0.16^{b}	6.25 ±0.18^{b}	6.22 ± 0.18^{b}
Overall likeness	7.18 ± 0.15^{a}	7.10 ±0.15^{a}	6.68 ± 0.13^{a,b}	6.40 ± 0.15^{b,c}	5.82 ± 0.18^{c}	5.97 ± 0.18^{c}

Control, 0.2% MOE, 0.4% MOE, 0.6% MOE, 0.8% MOE and 1.0% MOE prepared with 0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% of *Moringa oleifera* leave extract, respectively. Data are expressed as means ± SEM, n = 30. Means with different letter within a row are significantly different (p<0.05) according to Tukey HSD test. Scores used were 1= dislike extremely, 2= dislike very much, 3= dislike moderately, 4=dislike slightly, 5= neither like or dislike, 6= like slightly, 7= like moderately, 8= like very much, 9= like extremely.

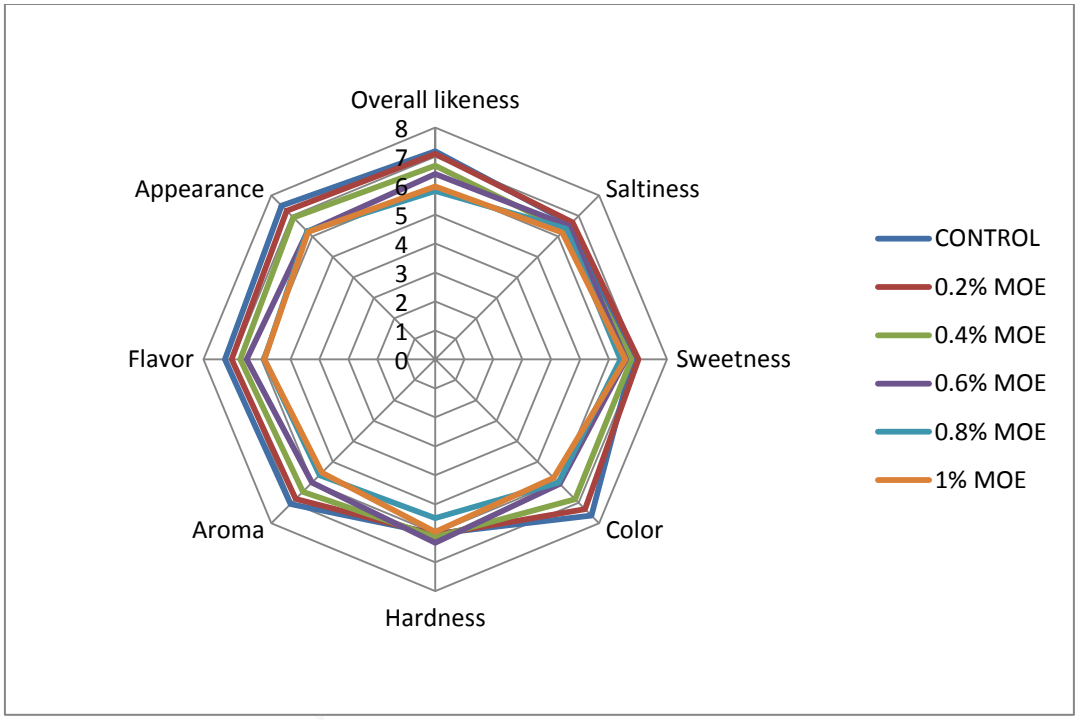


Figure 15. Radar plot of sensory attributes in baked cookies fortified with MOE.

4.6 Storage effect evaluation

4.6.1 Physical and physicochemical properties of cookies during storage

4.6.1.1 Water activity and Moisture in baked cookies fortified with MOE during storage time

The effect of addition of MOE into the cookies on water activity was shown in **Figure 16 and Table 12**. Water activity of cookie fortified with 0.4%-0.6% was significantly reduced when compared to control cookie throughout the period of storage time ($p<0.05$). However, no change of water activity during 4 weeks of storage time was observed in 0.4%-0.6% cookie fortified cookies whereas water activity of control cookie was increased at week 4 of storage time when compared to week 0-3 of storage time ($p<0.05$). From the results, MOE fortification (0.4% MOE and 0.6% MOE) helped to maintain the level of water activity during the storage period more than control cookies. Interaction between storage weeks and sample groups (control, 0.4% MOE and 0.6% MOE cookies) on water activity during the storage period showed statistically significant interaction with p=0.000.

The effect of addition of MOE into the cookies on moisture content was shown in **Figure 17 and Table 12**. Moisture of cookies was significantly higher in test groups when compared to control in weeks 1 to 3 ($p<0.05$). During 4 weeks of storage time, no significant difference of moisture content was observed in 0.6% MOE fortified cookies throughout the periods ($p<0.05$). Interaction between storage weeks and sample groups (control, 0.4% MOE and 0.6% MOE cookies) on moisture during the storage period showed statistically significant interaction with p=0.031.

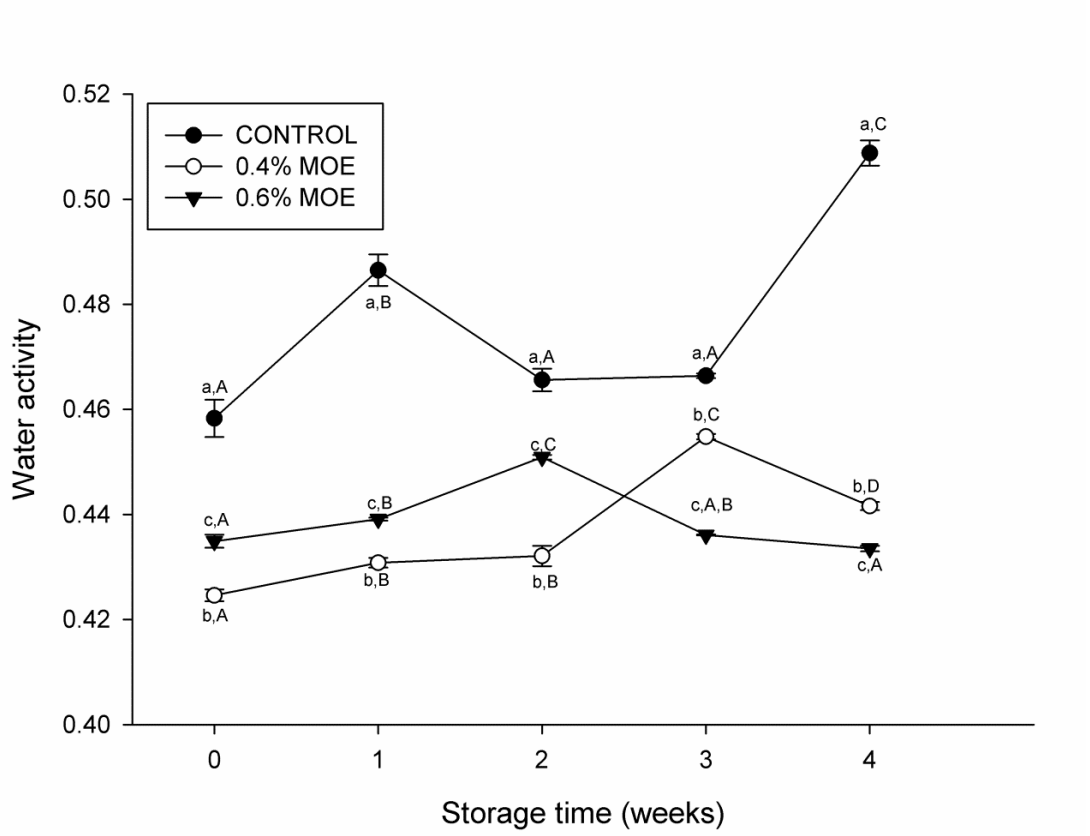


Figure 16. Water activity in baked cookies during 4 weeks of storage time at 30 ± 1°C. Control, 0.4% MOE and 0.6% MOE prepared with 0%, 0.4% and 0.6% of *Moringa oleifera* leave extract, respectively.

Data are expressed as means ± SEM, n=3. Means with different letters (A-D: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

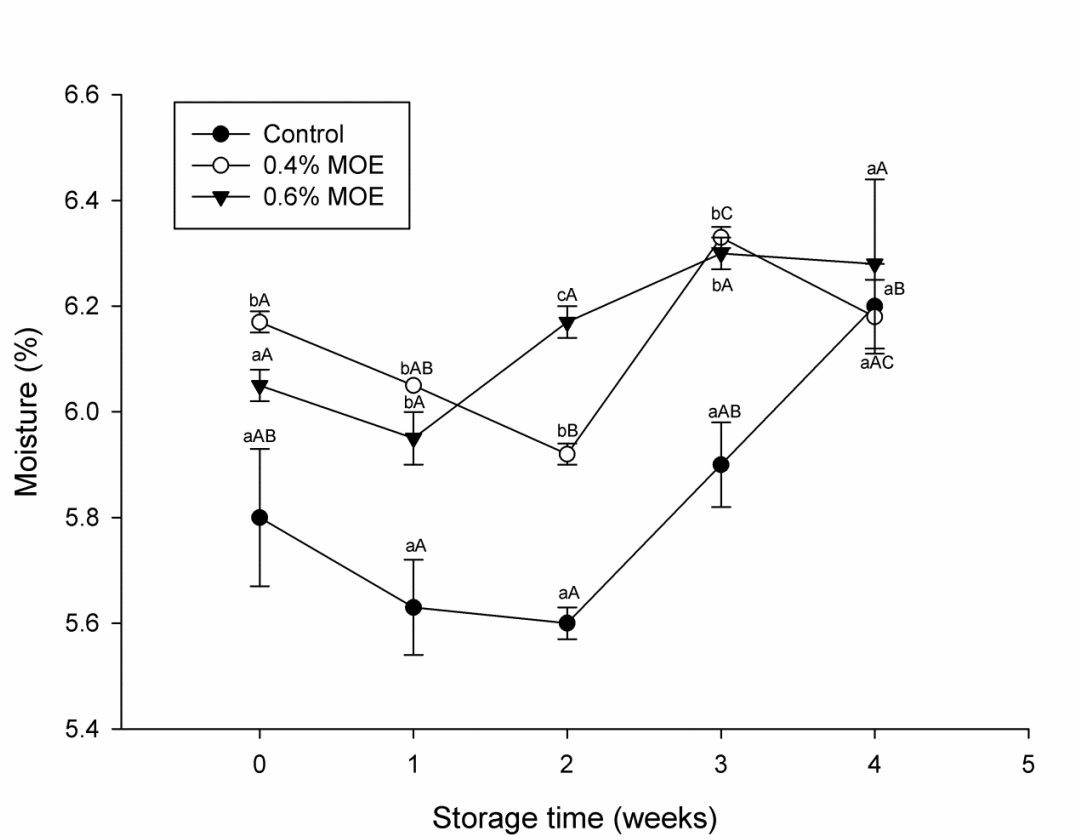


Figure 17. Moisture content in baked cookies during 4 weeks of storage time at 30±1°C. Control, 0.4% MOE and 0.6% MOE prepared with 0%, 0.4% and 0.6% of *Moringa oleifera* leave extract, respectively.

Data are expressed as means ± SEM, n=3. Means with different letters (A-C: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

Table 12. Moisture content and water activity in baked cookies fortified with MOE during 4 weeks of storage time at 30 ± 1 °C.

		Storage time (weeks)				
	Sample	0	1	2	3	4
Moisture (%)	Control	$5.80 \pm 0.13^{a,A,B}$	$5.63 \pm 0.09^{a,A}$	$5.60 \pm 0.03^{a,A}$	$5.90 \pm 0.08^{a,A,B}$	$6.20 \pm 0.08^{a,B}$
	0.4% MOE	$6.17 \pm 0.02^{b,A}$	$6.05 \pm 0.00^{b,A,B}$	$5.92 \pm 0.02^{b,B}$	$6.33 \pm 0.02^{b,C}$	$6.18 \pm 0.07^{a,A,C}$
	0.6% MOE	$6.05 \pm 0.03^{a,A}$	$5.95 \pm 0.05^{b,A}$	$6.17 \pm 0.03^{c,A}$	$6.30 \pm 0.03^{b,A}$	$6.28 \pm 0.16^{a,A}$
Water Activity	Control	$0.46 \pm 0.00^{a,A}$	$0.49 \pm 0.00^{a,B}$	$0.47 \pm 0.00^{a,A}$	$0.47 \pm 0.00^{a,A}$	$0.51 \pm 0.00^{a,C}$
	0.4% MOE	$0.42 \pm 0.00^{b,A}$	$0.43 \pm 0.00^{b,B}$	$0.43 \pm 0.00^{b,B}$	$0.46 \pm 0.00^{b,C}$	$0.44 \pm 0.00^{b,D}$
	0.6% MOE	$0.43 \pm 0.00^{c,A}$	$0.44 \pm 0.00^{c,B}$	$0.45 \pm 0.00^{c,C}$	$0.44 \pm 0.00^{c,A,B}$	$0.43 \pm 0.00^{c,A}$

Data are expressed as means ± SEM, n=3. Means with different letters within a row (A-E: comparison between storage time) and in a column (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

4.6.1.3 Determination of color characteristics in baked cookies fortified with MOE during storage

The effect of MOE on color characteristics of cookies were demonstrated in **Table 13 and 14.** Lightness, redness, yellowness, chroma (color intensity) and E index (overall color) of cookies in control and 0.4% MOE cookies did not change throughout of the 4 weeks of storage time (p >0.05) whereas yellowness, chroma and E index of 0.6 % MOE cookies were significantly decreased at week 1 to 4 when compared to initial week (p<0.05). In the initial week, the addition of 0.4% and 0.6% of MOE in the cookies significantly affected on lightness, redness, yellowness, chroma and E index of cookies as shown in **Table 13 and 14.** Addition of 0.4% MOE and 0.6% MOE in the cookies were significantly reduced E index (overall color) throughout the storage time when compared to control (p<0.05). At week 4, E index of control cookies and cookies fortified with 0.4% and 0.6% MOE were significantly difference when compared to initial week of storage time (p<0.05). Interaction between storage weeks and sample groups (control, 0.4% MOE and 0.6% MOE cookies) on lightness, redness, yellowness, chroma and E index during the storage period showed statistically significant interaction with p=0.000.

Table 13. Color characteristics in baked cookies fortified with MOE during 4 weeks of storage time at 30 ± 1°C.

		Storage time (weeks				
	Sample	0	1	2	3	4
L^*	Control	$70.97 \pm 0.02^{a,A,}$	$71.05 \pm 0.08^{a,A,}$	$71.13 \pm 0.31^{a,A,}$	$71.53 \pm 0.15^{a,A,}$	$72.31 \pm 0.14^{a,B}$
	0.4% MOE	$67.90 \pm 0.08^{b,A,C}$	$67.72 \pm 0.14^{b,A,B}$	$68.16 \pm 0.04^{b,A,C}$	$67.36 \pm 0.19^{b,B,}$	$68.38 \pm 0.17^{b,C}$
	0.6% MOE	$63.70 \pm 0.06^{c,A}$	$63.44 \pm 0.19^{c,A}$	$63.55 \pm 0.06^{c,A}$	$63.67 \pm 0.17^{c,A}$	$63.73 \pm 0.08^{c,A}$
a^*	Control	$9.84 \pm 0.04^{a,A,B}$	$9.96 \pm 0.03^{a,A}$	$9.75 \pm 0.21^{a,A,B}$	$9.74 \pm 0.12^{a,A,B}$	$9.50 \pm 0.07^{a,B}$
	0.4% MOE	$9.92 \pm 0.09^{a,A,B}$	$9.71 \pm 0.05^{a,A}$	$9.53 \pm 0.02^{a,A}$	$10.28 \pm 0.16^{b,B}$	$9.76 \pm 0.07^{a,A}$
	0.6% MOE	$11.81 \pm 0.08^{b,A}$	$11.73 \pm 0.21^{b,A}$	$11.46 \pm 0.06^{b,A}$	$11.43 \pm 0.12^{c,A}$	$11.90 \pm 0.13^{b,A}$
b^*	Control	$39.04 \pm 0.06^{a,A}$	$38.24 \pm 0.43^{a,A,B}$	$37.18 \pm 0.65^{a,B}$	$37.85 \pm 0.33^{a,A,B}$	$38.32 \pm 022^{a,A,B}$
	0.4% MOE	$38.05 \pm 0.04^{b,A}$	$37.82 \pm 0.13^{a,A}$	$38.07 \pm 0.09^{a,A}$	$39.15 \pm 0.16^{b,B}$	$38.04 \pm 0.19^{a,A}$
	0.6% MOE	$40.81 \pm 0.19^{c,A}$	$39.67 \pm 0.28^{b,B}$	$39.18 \pm 0.19^{b,B}$	$39.00 \pm 0.06^{b,B}$	$39.28 \pm 0.09^{b,B}$

Data are expressed as means ± SEM, n=3. Means with different letters within a row (A-C: comparison between storage time) and in a column (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test. L*: Lightness, a*: redness, b*: yellowness.

Table 14. Color characteristics in baked cookies fortified with MOE during 4 weeks of storage time at 30 ± 1°C (Cont'n).

		Storage time (weeks)				
	Sample	0	1	2	3	4
Chroma	Control	40.27 ± 0.06^{a,A}	39.51 ± 0.42^{a,A,B}	38.43 ± 0.69^{a,B}	39.09 ± 0.34^{a,A,B}	39.48 ± 0.22^{a,A,B}
	0.4% MOE	39.33 ± 0.06^{b,A}	39.05 ± 0.14^{a,A}	39.25 ± 0.09^{a,A}	40.48 ± 0.19^{b,B}	39.27 ± 0.18^{a,A}
	0.6% MOE	42.49 ± 0.20^{c,A}	41.37 ± 0.32^{b,B}	40.82 ± 0.20^{b,B}	40.64 ± 0.08^{b,B}	41.04 ± 0.12^{b,B}
E index	Control	81.59 ± 0.03^{a,A}	81.31 ± 0.27^{a,A,B}	80.90 ± 0.06^{a,B}	81.52 ± 0.18^{a,A}	82.39 ± 0.15^{a,C}
	0.4% MOE	78.47 ± 0.04^{b,A,B}	78.18 ± 0.05^{b,A}	78.65 ± 0.07^{b,A,B}	78.60 ± 0.07^{b,A,B}	78.86 ± 0.22^{b,B}
	0.6% MOE	76.57 ± 0.12^{c,A}	75.75 ± 0.17^{c,B}	75.54 ± 0.15^{c,B}	75.54 ± 0.11^{c,B}	75.81 ± 0.07^{c,B}

Data are expressed as means ± SEM, n=3. Means with different letters within a row (A-C: comparison between storage time) and in a column (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

4.6.2 Determination of polyphenol and flavonoid content in baked cookies fortified with MOE during storage time.

The effect of MOE on polyphenol and flavonoid content in cookies were shown in **Table 15**. The significant difference of polyphenol was found between cookies fortified with MOE (0.4% and 6% MOE) and control cookies throughout the storage time ($p<0.05$). Level of polyphenol and flavonoid contents progressively reduced throughout the storage periods. Polyphenol in control cookies was gradually decreased in the percentage of 5.86%, 9.25%, 11.56% and 16.40% at week 1, 2, 3 and 4, respectively when compared to initial week of storage time. At week 3 and 4, the significant difference of polyphenol was found in control cookies compared to initial week of storage time ($p<0.05$). Polyphenol in cookies fortified with 0.4% MOE was slightly decreased in the percentage of 1.73%, 4.68%, 5.39% and 5.58% at week 1, 2, 3 and 4, respectively; however, no significant difference was observed throughout the storage time.

Cookies fortified with 0.6 % MOE showed significant reduction of 9.07%, 10.43%, 12.40% and 12.66% in polyphenol content at week 1, 2, 3 and 4, respectively ($p<0.05$) **(Figure 18).** The results demonstrated that cookies fortified with MOE were able to maintain a high level of polyphenol content throughout the storage period compared to control cookies.

Flavonoid content in control cookies was not detected within the storage weeks whereas it was found in the range of 1.19-1.51mg Catechin/100 g cookie in 0.4%MOE cookies and 1.83-2.54 mg Catechin/100 g cookie in 0.6%MOE cookies during 4 weeks of the storage time as shown in **Table 15**. Additionally, Flavonoid content of cookies fortified with 0.4% and 0.6% MOE was slightly reduced throughout the storage weeks ($p >0.05$). Percentage reduction of flavonoid content in 0.4% MOE fortified cookies during the storage period was 3.29%, 10.53%, 17.76% and 21.71% at week 1,2,3 and

4, respectively whereas those in 0.6% MOE fortified cookies during the storage period was 6.69%, 16.93%, 20.87% and 27.95% at week 1,2,3 and 4, respectively.

Table 15. Polyphenol and flavonoid content in baked cookies fortified with MOE during 4 weeks of storage time at 30 ± 1°C.

		Storage time (weeks)				
	Sample	0	1	2	3	4
Polyphenol (mg GAE/100g cookie)	Control	60.19 ±0.82^{a,A}	56.66 ± 0.29^{a,A,B}	54.62 ±0.98^{a,A,B,C}	53.23 ±0.48^{a,B}	50.32 ±0.45^{a,C}
	0.4% MOE	71.28 ±0.49^{b,A}	70.05 ± 1.12^{b,A}	67.94± 1.20^{b,A}	67.44 ±0.67^{b,A}	67.30 ±0.86^{b,A}
	0.6% MOE	86.54 ±0.36^{c,A}	78.69± 0.36^{c,B}	77.51 ± 0.56^{c,B}	75.81 ± 1.74^{c,B}	75.58 ±0.92^{c,B}
Flavonoid (mg Catechin/100g cookie)	Control	ND	ND	ND	ND	ND
	0.4% MOE	1.52±0.06^{a,A}	1.47±0.19^{a,A}	1.36±0.10^{a,A}	1.25±0.09^{a,A}	1.19±0.12^{a,A}
	0.6% MOE	2.54±0.22^{b,A}	2.37±0.16^{b,A}	2.11±0.30^{b,A}	2.01±0.19^{a,A}	1.83±0.14^{b,A}

Data are expressed means ± SEM, n=3. Means with different letters within a row (A-C: comparison between storage time) and in a column (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

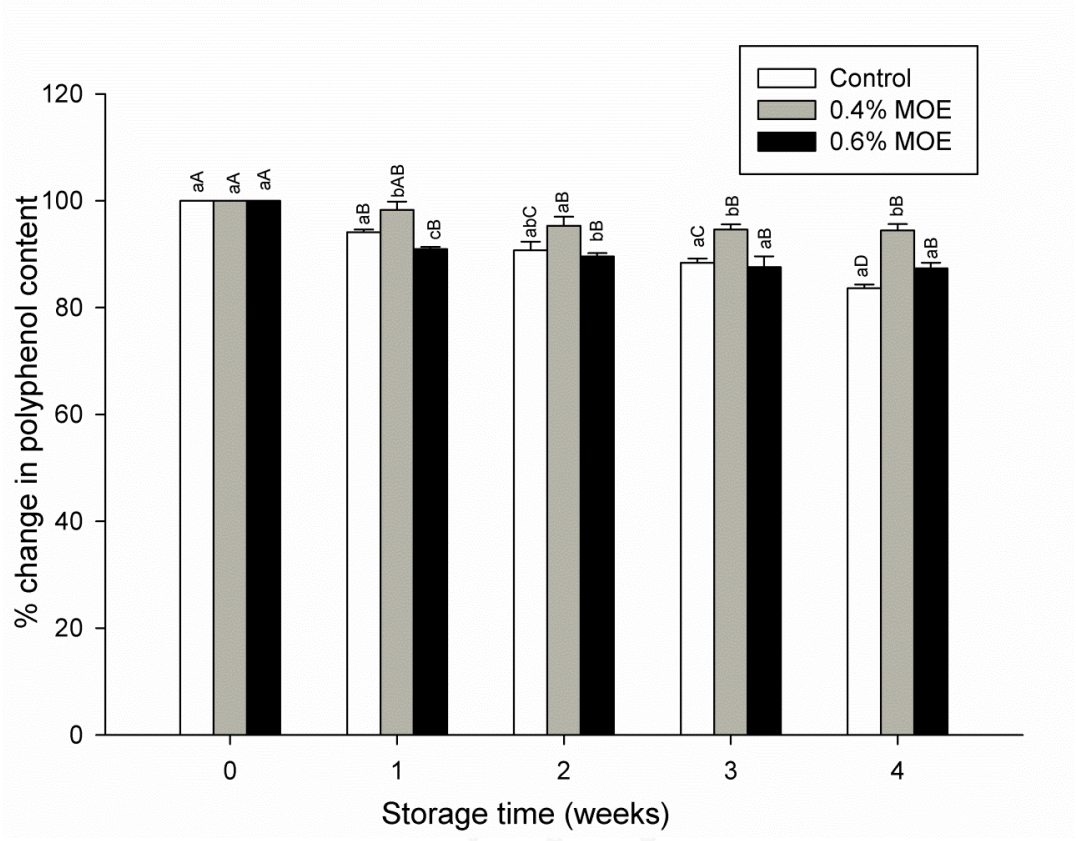


Figure 18. Polyphenol content normalized to week 0 (%) in cookies fortified with MOE then stored for 4 weeks at 30 ± 1°C. Data are expressed as means ± SEM, n=3. Means with different letters (A-B: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

4.6.3 Determination of antioxidant capacity (FRAP and DPPH) in baked cookies fortified with MOE during storage time

The effect of MOE on Ferric reducing antioxidant power (FRAP) was shown in **Table 16.** The significant increase of FRAP value was observed in cookies fortified with MOE (0.4% and 6% MOE) when compared to control cookies throughout the storage time as shown in **Figure 19** ($p<0.05$). In addition, FRAP value of control cookies was dramatically decreased throughout the storage time ($p<0.05$) whereas those of cookies fortified with MOE (0.4% and 6% MOE) were slightly decreased throughout the storage time. FRAP value in control cookies was decreased in the percentage of 16.95%, 33.00%, 35.40% and 35.84% at week 1, 2 ,3 and 4, respectively when compared to initial week of storage time. Percentage reduction of FRAP value observed in 0.4% MOE cookies were 2.16%, 10.08%, 11.32% and 12.27% at weeks 1, 2, 3 and 4, respectively whereas those of 0.6% MOE cookies were 1.80%, 2.70%, 3.27% and 4.39% at week 1, 2 ,3 and 4, respectively (**Figure 21**).

From the results, it was indicated that cookies fortified with MOE was able to maintain the level of FRAP antioxidant capacity during the storage period than control cookies which showed drastic reduction in antioxidant capacity during the storage period.

Interaction between storage weeks and sample groups (control, 0.4% MOE and 0.6% MOE cookies) on FRAP capacity during the storage period showed statistically significant interaction with p=0.000.

DPPH radical scavenging decreased throughout the storage period. Cookies fortified with 0.6 % MOE showed the highest amount of ascorbic acid equivalent throughout the storage period followed by 0.4 % MOE fortified cookies and then control cookies (**Table 16, Figure 20**). Cookies fortified with 0.4% and 0.6% MOE were also significantly higher when compared with control cookies. Cookies fortified with 0.6 % MOE showed significant difference throughout the storage weeks when compared to 0.4 % MOE cookies in DPPH radical scavenging activity. Percentage

decrease in cookies fortified with 0.4% MOE cookies was observed to be higher than 0.6% MOE cookies with reductions of 1.92%, 7.67%, 7.67% and 11.54% in week 1, 2, 3 and 4 respectively for 0.4% MOE cookies and reductions of 3.23, 4.84%, 6.45% and 8.06% in week 1, 2, 3 and 4 respectively for 0.6% MOE cookies. Drastic reduction in DPPH radical scavenging activity was however, observed in control cookies throughout the storage period with percentage reduction of 21.74%, 39.13%, 47.83%, 52.17% in week 1, 2, 3 and 4, respectively (**Figure 22**). This result shows that MOE helped to maintain DPPH radical scavenging activity in fortified cookies than control cookies throughout the storage period.

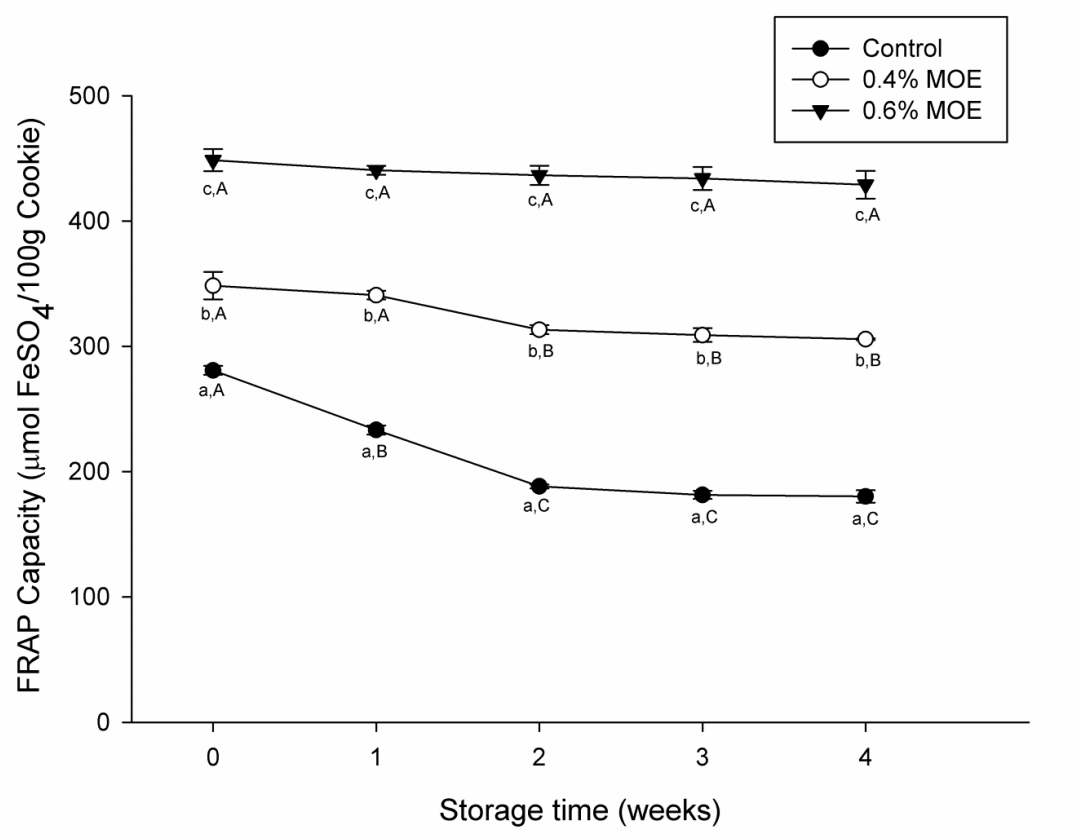


Figure 19. FRAP antioxidant capacity in baked cookies during 4 weeks of storage time at 30 ± 1°C. Control, 0.4% MOE and 0.6% MOE prepared with 0%, 0.4% and 0.6% of *Moringa oleifera* leave extract, respectively.

Data are expressed as means ± SEM, n=3. Means with different letters (A-C: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

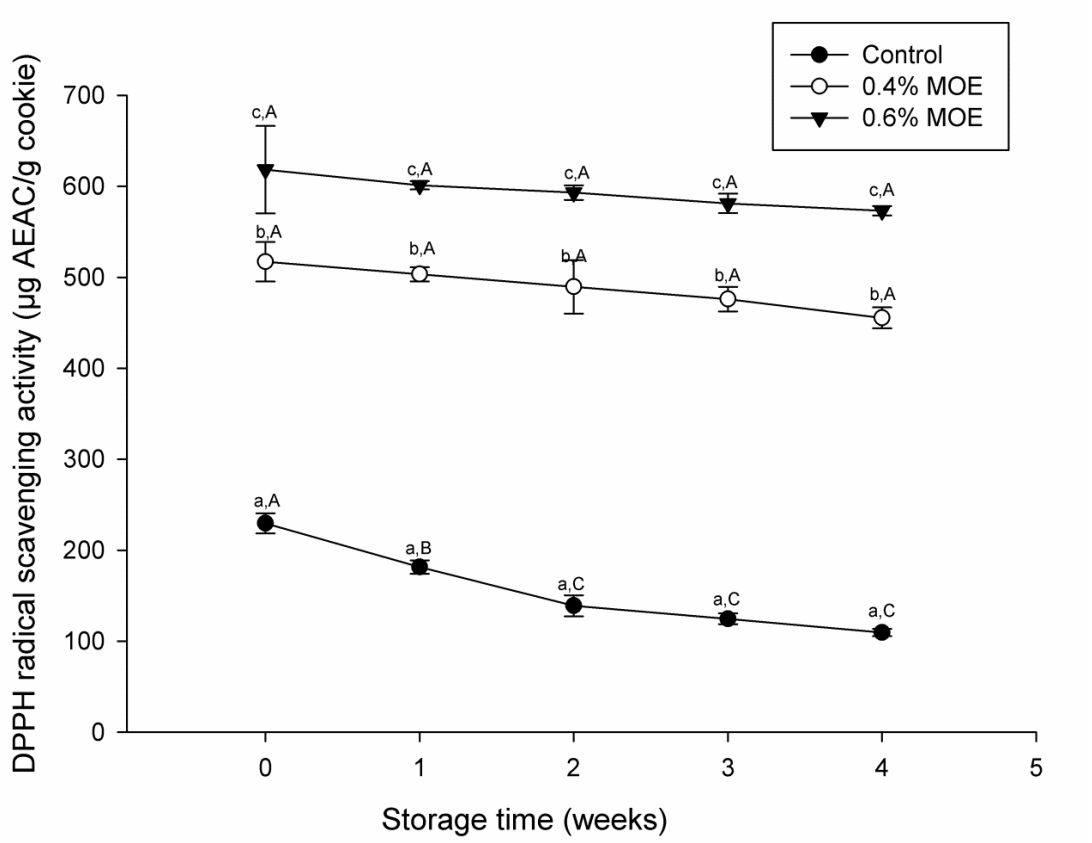


Figure 20. DPPH radical scavenging activity in baked cookies during 4 weeks of storage time at 30 ± 1°C. Control, 0.4% MOE and 0.6% MOE prepared with 0%, 0.4% and 0.6% of *Moringa oleifera* leave extract respectively.

Data are expressed as means ± SEM, n=3. Means with different letters (A-C: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

Table 16. Antioxidant capacity in baked cookies fortified with MOE during 4 weeks of storage time at 30 ± 1°C.

		Storage time (weeks)				
	Sample	0	1	2	3	4
	Control	280.82 ± 3.47^{a,A}	233.22 ± 3.51^{a,B}	188.15 ± 1.64^{a,C}	181.42 ± 3.23^{a,C}	180.18 ± 4.96^{a,C}
FRAP	0.4% MOE	348.38 ± 10.93^{b,A}	340.85 ± 3.35^{b,A}	313.25 ± 3.66^{b,B}	308.95 ± 5.49^{b,B}	305.65 ± 0.71^{b,B}
(μmol $FeSO_4$/100g cookie)	0.6% MOE	448.56 ± 8.81^{c,A}	440.50 ± 3.64^{c,A}	436.44 ± 7.66^{c,A}	433.91 ± 9.31^{c,A}	428.87 ± 11.11^{c,A}
	Control	229.46 ± 10.69^{a,A}	181.44 ± 7.39^{a,B}	138.96 ± 11.59^{a,C}	124.55 ± 5.93^{a,C}	109.49 ± 4.06^{a,C}
DPPH radical scavenging	0.4% MOE	517.01 ± 21.63^{b,A}	503.28 ± 7.78^{b,A}	489.46 ± 29.37^{b,A}	475.91 ± 13.50^{b,A}	455.34 ± 11.51^{b,A}
(μg AEAC/g cookie)	0.6% MOE	618.35 ± 47.98^{c,A}	601.13 ± 4.58^{c,A}	593.06 ± 7.95^{c,A}	581.15 ± 10.74^{c,A}	573.18 ± 5.21^{c,A}

Data are expressed as means ± SEM, n=3. Means with different letters within a row (A-C: comparison between storage time) and in a column. (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

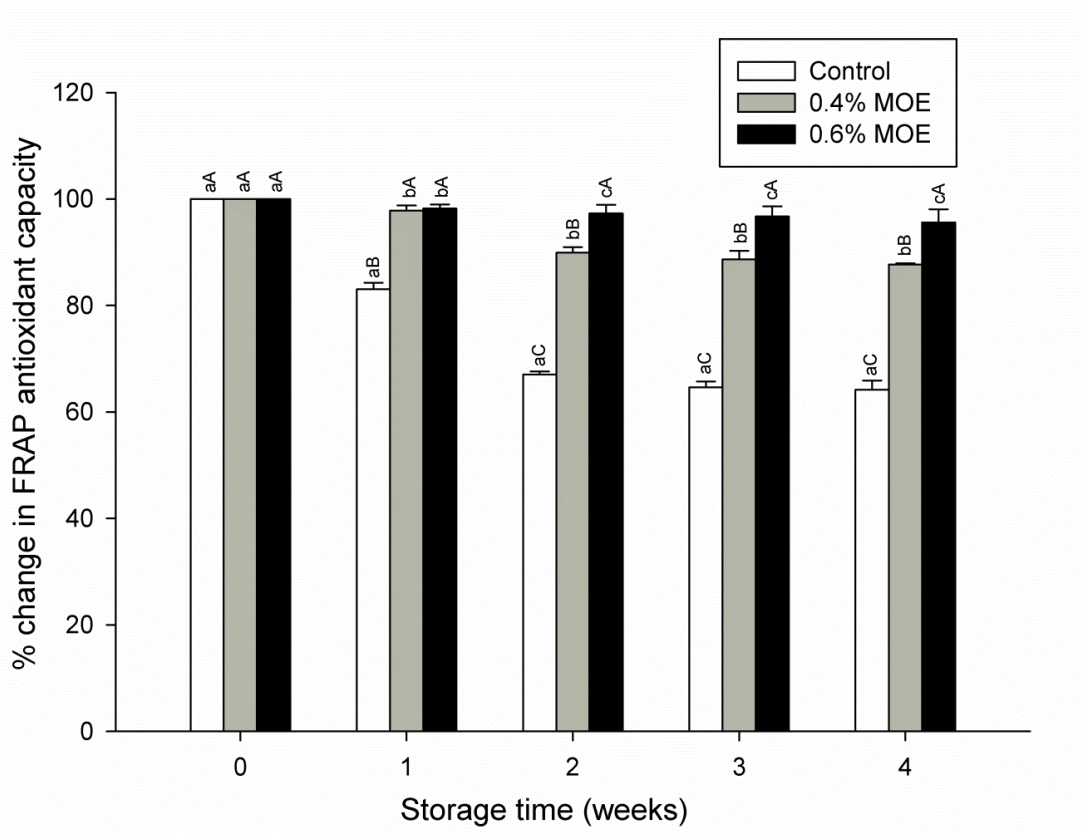


Figure 21. FRAP antioxidant capacity normalised to week 0 (%) in cookies fortified with MOE then stored for 4 weeks at 30 ± 1°C. Data are expressed as means ± SEM, n=3. Means with different letters (A-C: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

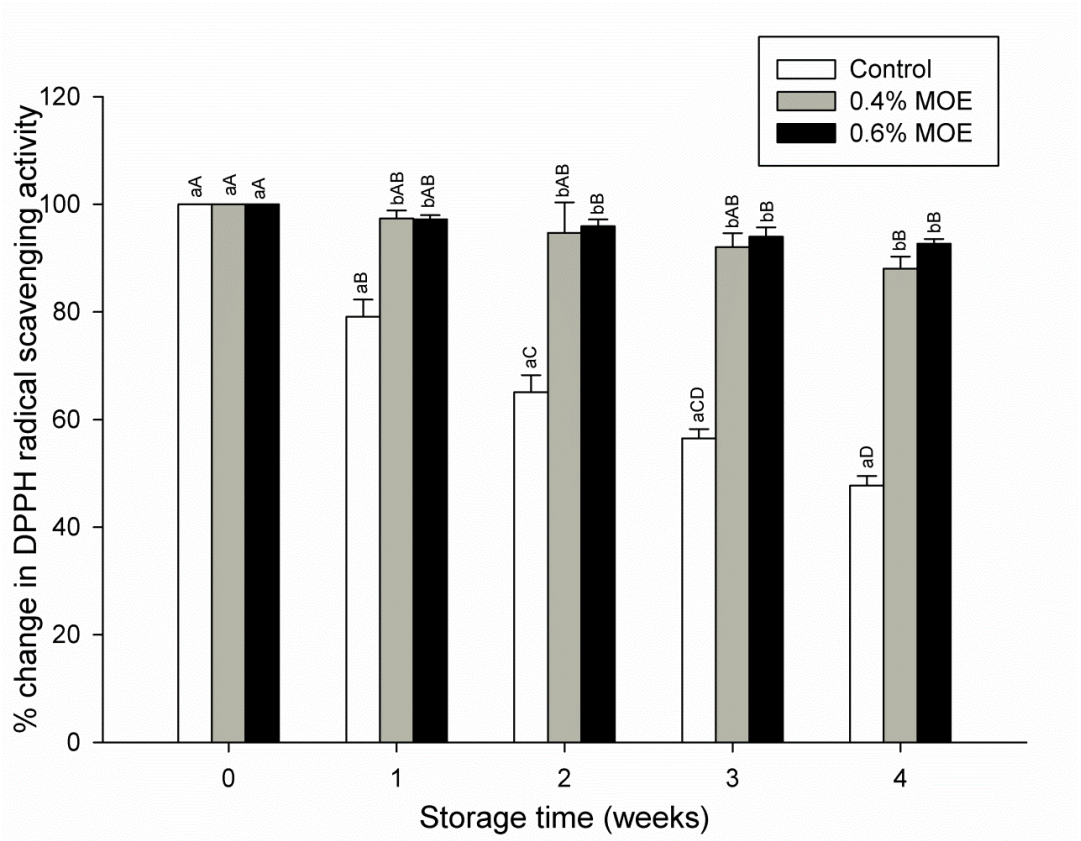


Figure 22. DPPH radical scavenging activity normalised to week 0 (%) in cookies fortified with MOE then stored for 4 weeks at 30 ± 1°C. Data are expressed as means ± SEM, n=3. Means with different letters (A-D: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

4.6.4 Determination of lipid peroxidation products (Conjugated diene, peroxide value, MDA) in baked cookies fortified with MOE during storage time.

The effect of MOE in the cookies on primary products of lipid peroxidation (conjugated diene and peroxide value) was shown in **Table 17, Figure 23, 24.** The significant reduction of conjugated diene and peroxide value were observed in cookies fortified with MOE (0.4% and 6% MOE) when compared to control cookies throughout the storage time ($p<0.05$). In addition, conjugated diene and peroxide value in control cookies and cookies fortified with MOE (0.4% and 6% MOE) were gradually increased throughout the storage time ($p<0.05$). Conjugated diene and peroxide values increased in the control group in percentages of 5.56%, 12.22%, 18.33% and 28.33% in weeks 1, 2, 3 and 4, respectively for peroxide value and 1.58%, 3.51%, 4.76% and 10.44% in weeks 1, 2, 3 and 4, respectively for conjugated diene . Cookies fortified with 0.4 % MOE had a progressive increase in conjugated diene and peroxide value with percentage increase of 3.42%, 7.21%, 7.92% and 8.48% in weeks 1, 2, 3 and 4, respectively for conjugated diene and 2.26%, 5.26%, 15.04% and 24.06% in weeks 1, 2, 3 and 4, respectively for peroxide value. Cookies fortified with 0.6 % MOE had a progressive increase in conjugated diene and peroxide value from week 1 to week 4 with percentage increase of 0.17%, 4.94%, 7.32% and 8.44% in weeks 1, 2, 3 and 4, respectively for conjugated diene and 22.45%, 16.33%, 43.88%, 52.04% in weeks 1, 2, 3 and 4, respectively for peroxide value **(Figure 26, 27).**

The effect of MOE in the cookies on secondary products of lipid peroxidation (Malondialdehyde; MDA) was shown in **Table 17, Figure 25.** The significant reduction of MDA was observed in cookies fortified with MOE (0.4% and 6% MOE) when compared to control cookies throughout the storage time ($p<0.05$). Malondialdehyde (MDA) in fortified cookies (0.4% and 0.6% MOE) increased progressively during the

storage period, with control cookies having the highest increment followed by 0.4 and 0.6 % MOE fortified cookies. MDA level of control cookies was significantly higher at week 4 and when compared with week 0 whereas those of 0.4% and 0.6% MOE added in the cookies were significantly higher at week compared to week 0 (**Table 17 and Figure 25**). Control cookies showed percentage increment of MDA from 5.01%, 7.13%, 20.22% and 43.78% at week 1, 2, 3 and 4, respectively. In addition, MDA of 0.4% MOE cookies was increased by 2.40%, 6.98%, 31.90% and 49.35 at weeks 1, 2, 3 and 4, respectively whereas MDA of 0.6% MOE cookies was observed an increase in 0.23%, 2.28%, 12.19% and 36.10% at weeks 1, 2, 3 and 4, respectively as shown in **Figure 28**. Interaction between storage weeks and sample groups (control, 0.4% MOE and 0.6% MOE cookies) was not significantly different with p=0.76. The results illustrated that cookies fortified with MOE had more potential to reduce primary and secondary products of lipid oxidation during the storage period in a dose dependent manner than control cookies.

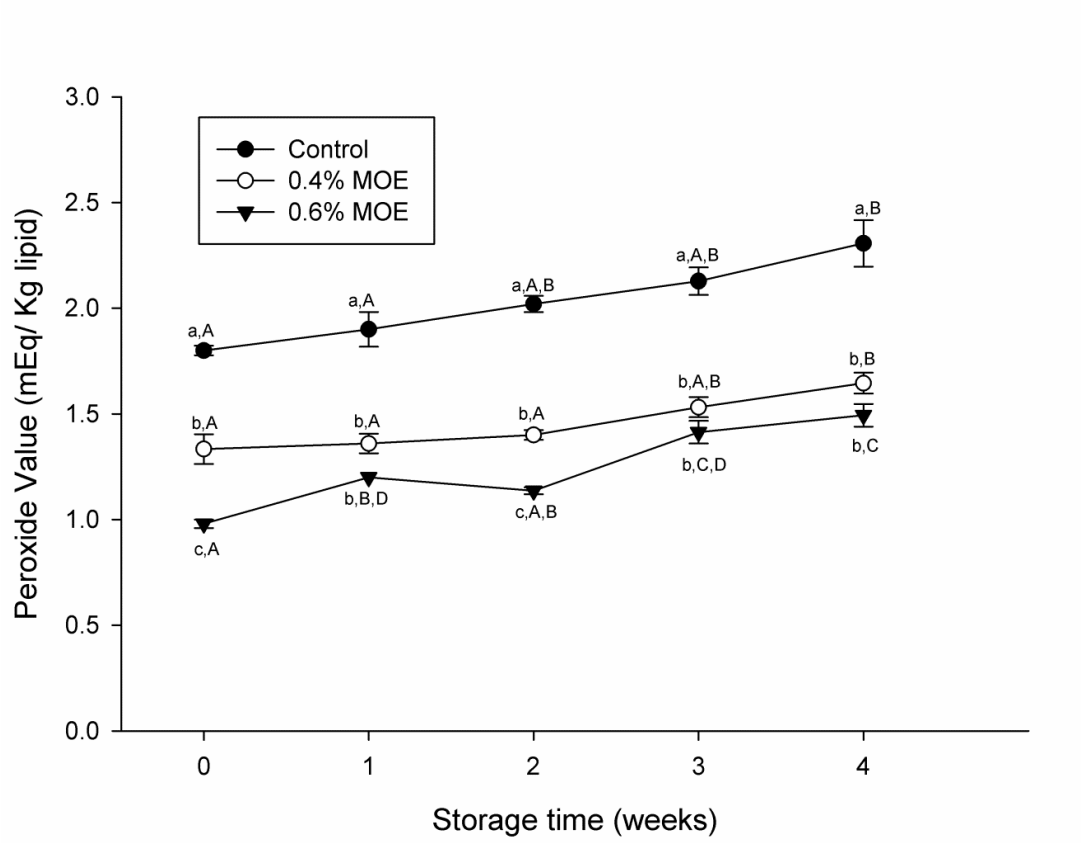


Figure 23. Peroxide value in baked cookies during 4 weeks of storage time at 30 ± 1°C. Control, 0.4% MOE and 0.6% MOE prepared with 0%, 0.4% and 0.6% of *Moringa oleifera* leave extract respectively.

Data are expressed as means ± SEM, n=3. Means with different letters (A-E: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

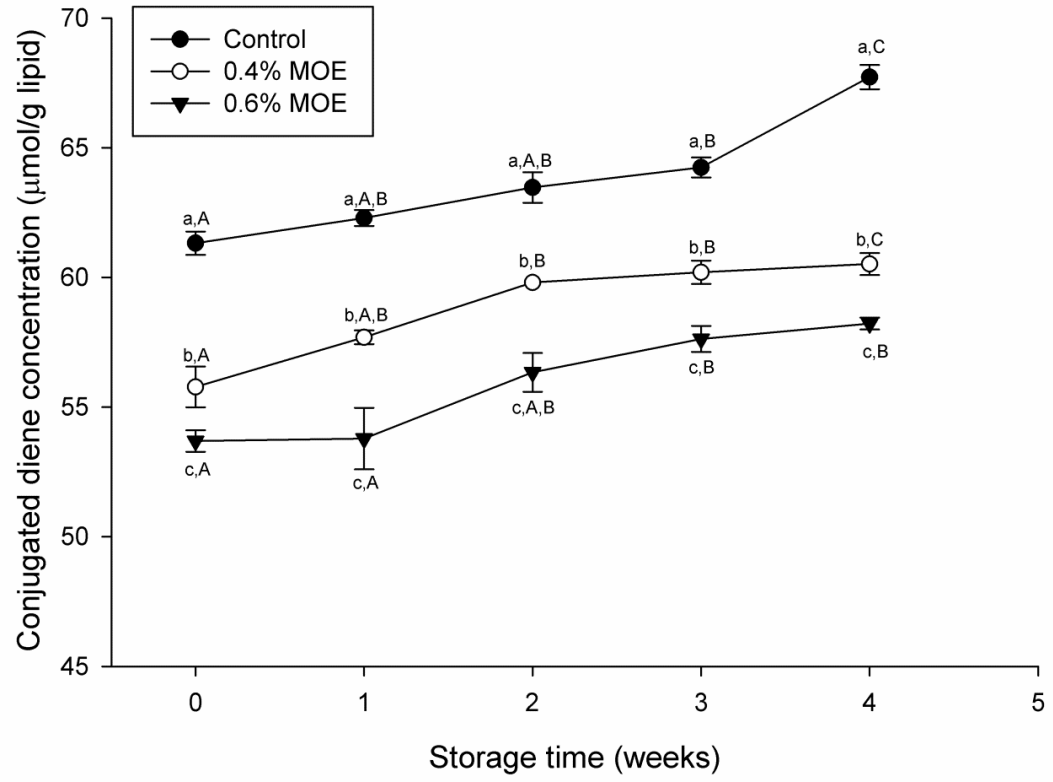


Figure 24. Conjugated diene content in baked cookies during 4 weeks of storage time at 30 ± 1°C. Control, 0.4% MOE and 0.6% MOE prepared with 0%, 0.4% and 0.6% of *Moringa oleifera* leave extract respectively.

Data are expressed as means ± SEM, n=3. Means with different letters (A-C: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

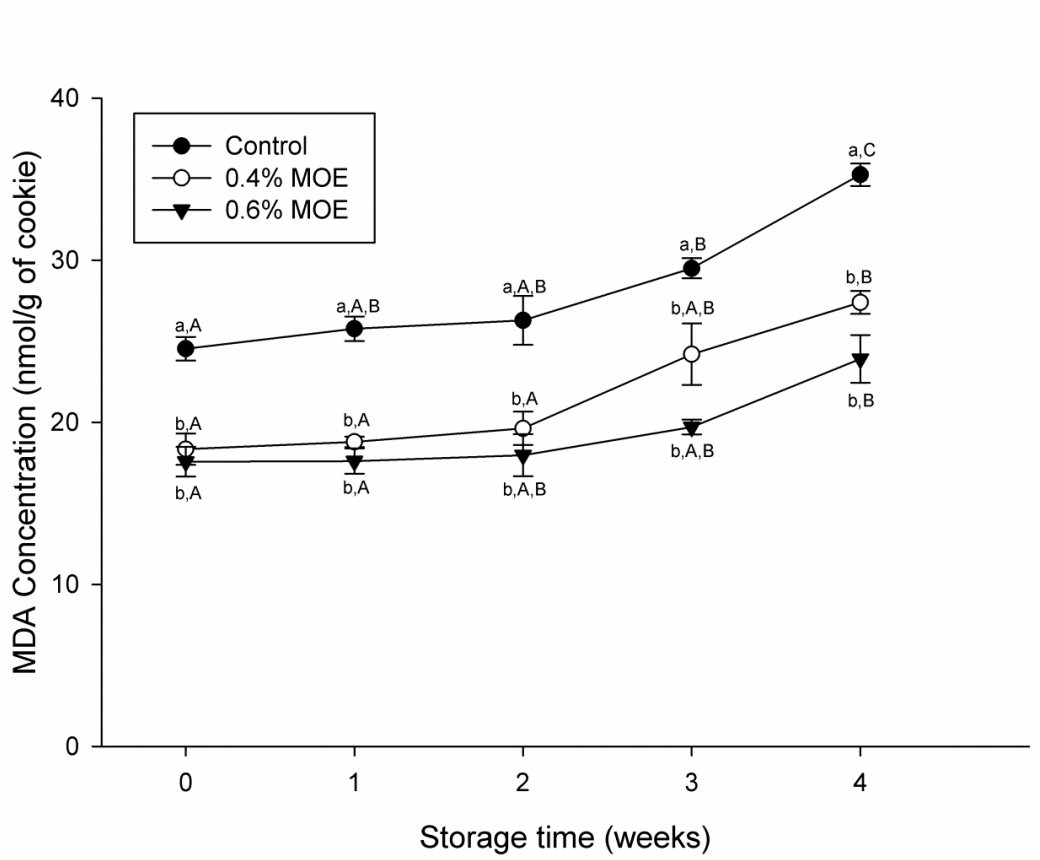


Figure 25. MDA concentration in baked cookies during 4 weeks of storage time at 30 ± 1°C. Control, 0.4% MOE and 0.6% MOE prepared with 0%, 0.4% and 0.6% of *Moringa oleifera* leave extract respectively.

Data are expressed as means ± SEM, n=3. Means with different letters (A-C: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

Table 17. Lipid peroxidation products in baked cookies fortified with MOE during 4 weeks of storage time at 30 ± 1°C.

		Storage time (weeks)				
	Sample	0	1	2	3	4
	Control	1.80±0.02^{a,A}	1.90±0.08^{a,A}	2.02±0.04^{a,A,B}	2.13±0.06^{a,A,B}	2.31±0.11^{a,B}
Peroxide value	0.4% MOE	1.33±0.07^{b,A}	1.36±0.05^{b,A}	1.40±0.02^{b,A}	1.53±0.05^{b,A,B}	1.65±0.05^{b,B}
(mEq/Kg lipid)	0.6% MOE	0.98±0.02^{c,A}	1.20±0.00^{b,B,D}	1.14±0.02^{c,A,B}	1.41±0.05^{b,C,D}	1.49±0.05^{b,C}
	Control	61.32±0.45^{a,A}	62.29±0.31^{a,A,B}	63.47±0.59^{a,A,B}	64.24±0.38^{a,B}	67.72±0.47^{a,C}
Conjugated diene	0.4% MOE	55.78±0.79^{b,A}	57.69±0.26^{b,A,B}	59.80±0.00^{b,B}	60.20±0.45^{b,B}	60.51±0.42^{b,C}
(µmol/ g lipid)	0.6% MOE	53.69±0.41^{c,A}	53.78±1.18^{c,A}	56.34±0.75^{c,A,B}	57.62±0.50^{c,B}	58.22±0.23^{c,B}
	Control	24.53±0.73^{a,A}	25.76±0.76^{a,A,B}	26.28±1.51^{a,A,B}	29.49±0.62^{a,B}	35.27±0.69^{a,C}
MDA	0.4% MOE	18.34±0.96^{b,A}	18.78±0.32^{b,A}	19.62±1.02^{b,A}	24.19±1.89^{b,A,B}	27.39±0.71^{b,B}
(nmol/g cookie)	0.6% MOE	17.56±0.92^{b,A}	17.60±0.78^{b,A}	17.96±1.29^{b,A,B}	19.70±0.45^{b,A,B}	23.90±1.47^{b,B}

Data are expressed means ± SEM, n=3. Means with different letters within a row (A-D: comparison between storage time) and in a column (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

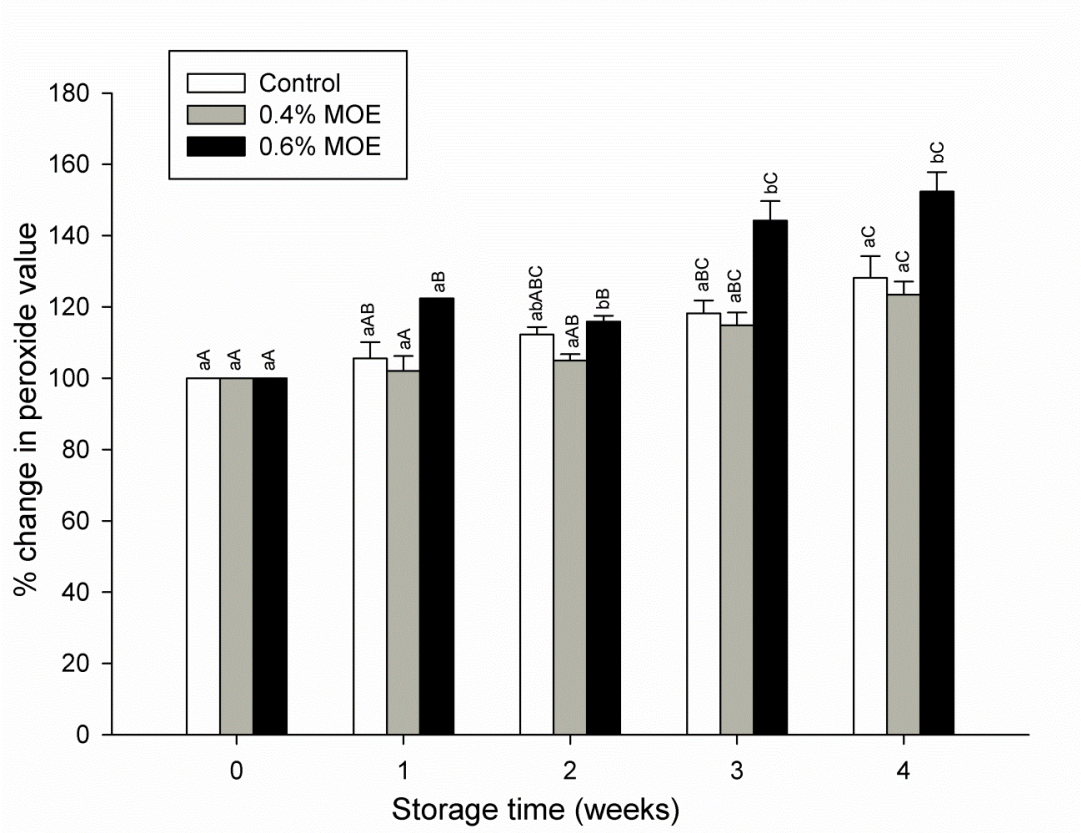


Figure 26. Peroxide value normalised to week 0 (%) in cookies fortified with MOE then stored for 4 weeks at 30 ± 1°C. Data are expressed as means ± SEM, n=3. Means with different letters (A-B: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

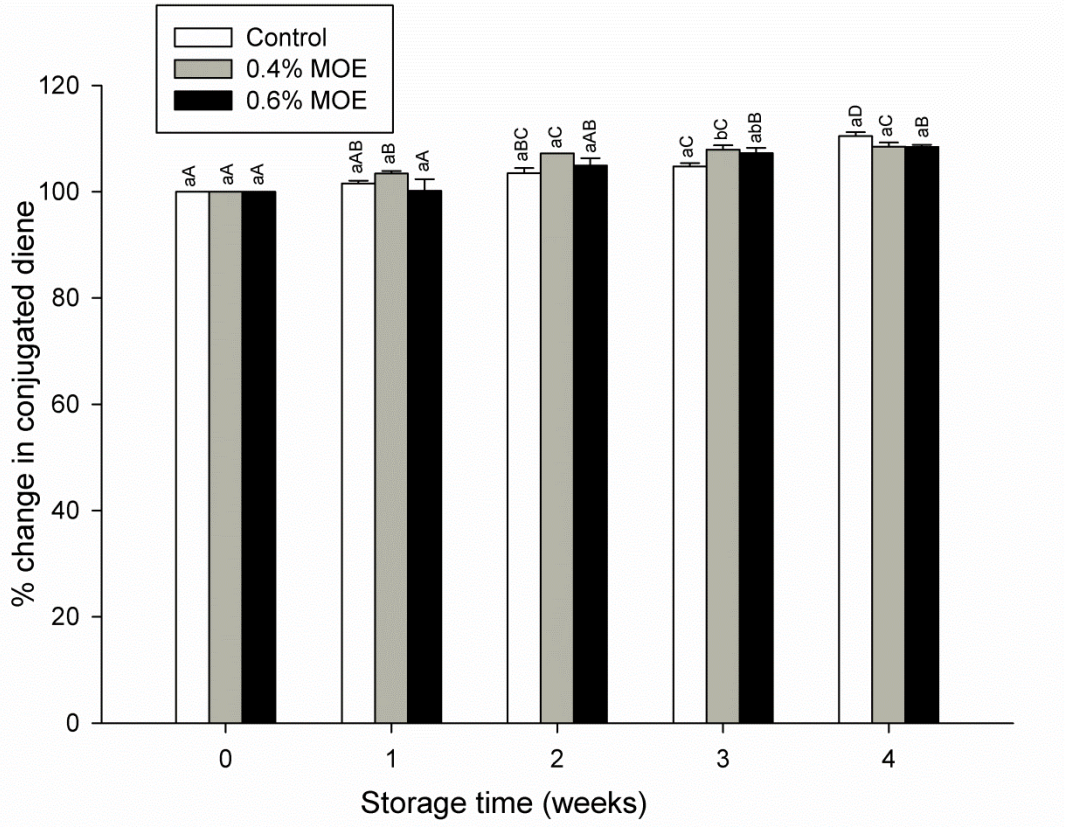


Figure 27. Conjugated diene concentration normalised to week 0 (%) in cookies fortified with MOE then stored for 4 weeks at 30 ± 1°C. Data are expressed as means ± SEM, n=3. Means with different letters (A-D: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

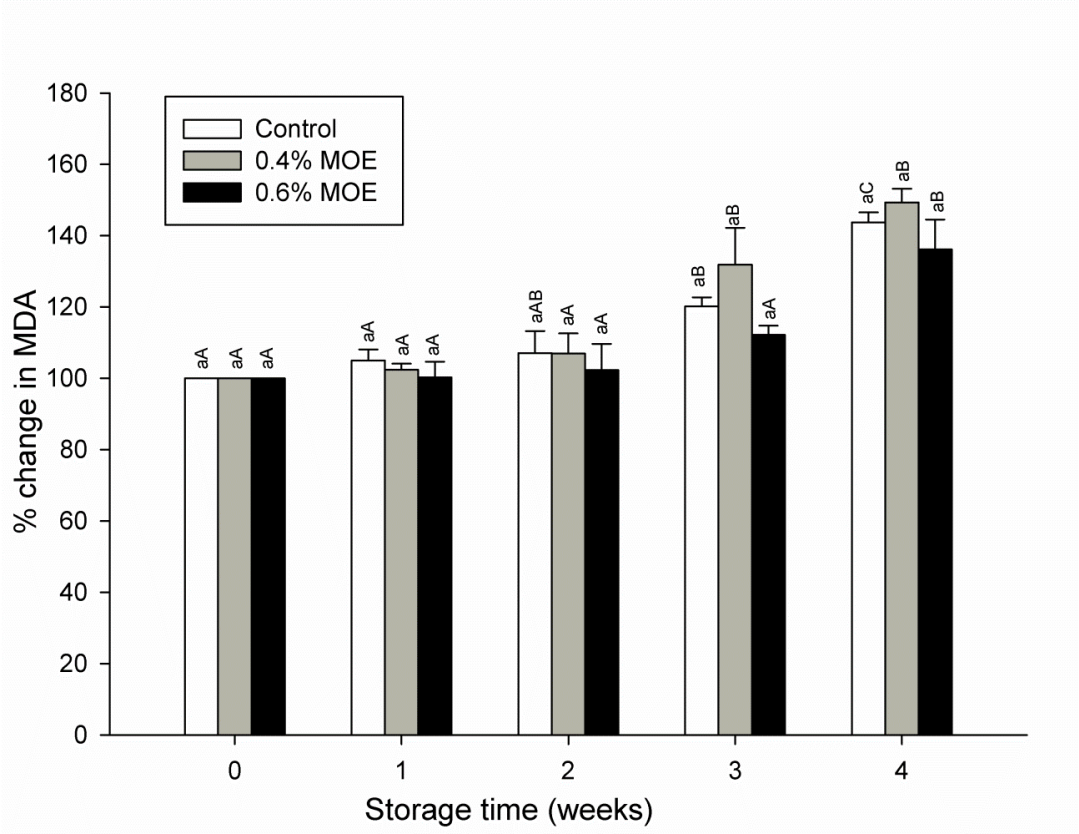


Figure 28. MDA concentration normalised to week 0 (%) in cookies fortified with MOE then stored for 4 weeks at 30 ± 1°C. Data are expressed as means ± SEM, n=3. Means with different letters (A-B: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

4.6.4.1 Determination of fat Hydrolysis in baked cookies fortified with MOE during storage time.

The effect of MOE in the cookies on hydrolysis of fat, measured as acid value, was shown in **Table 18.** Hydrolysis of fat, measured as acid value, increased progressively in all the groups (control, 0.4% MOE and 0.6% MOE cookies) during the storage period with control cookies having the highest increment followed by 0.4 and 0.6 % MOE fortified cookies. Cookies fortified with 0.6 % MOE were significantly lower from control cookies in week 0 to 2 and week 4. Control cookies, however, were significantly higher in acid value from week 2 to 4 when compared with week 0, and whereas 0.6 % MOE fortified cookies were significantly higher from week 3 to 4 when compared to week 0. The 0.4 % MOE fortified cookies were significantly different on week 4 when compared to control cookies (**Figure 29**). Acid value increased during storage, control cookies increased by 2.68%, 10.71%, 13.39% and 16.64%, from the initial amount in weeks 1, 2, 3 and 4, respectively. An increment of acid value of cookies fortified with 0.4% MOE was observed at week 2-4 with percentage of 6.42%, 6.42% and 7.34%, respectively. Acid value of cookies fortified with 0.6% MOE increased by 9.09%, 14.77%, 17.05% and 18.18%, at weeks 1, 2, 3 and 4, respectively (**Figure 30**).

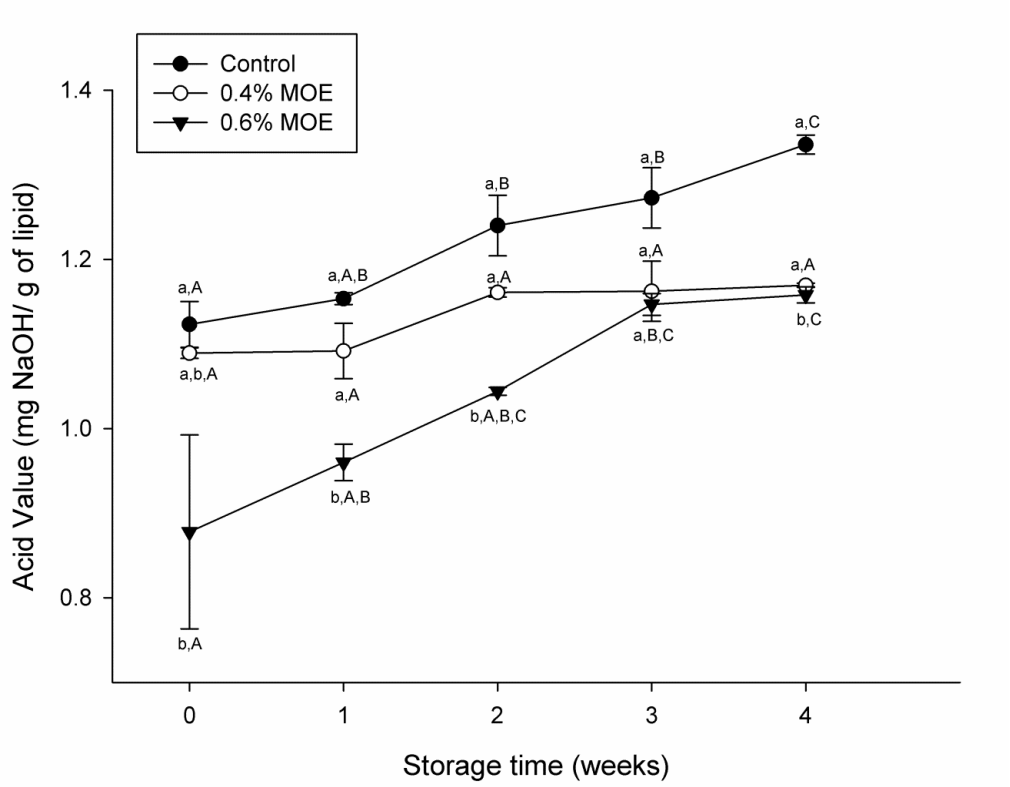


Figure 29. Acid value in baked cookies during 4 weeks of storage time at 30 ± 1°C. Control, 0.4% MOE and 0.6% MOE prepared with 0%, 0.4% and 0.6% of *Moringa oleifera* leave extract respectively.

Data are expressed as means ± SEM, n=3. Means with different letters (A-C: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

Table 18. Lipid peroxidation products in baked cookies fortified with MOE during 4 weeks of storage time at 30 ± 1 °C.

		Storage time (weeks)				
	Sample	0	1	2	3	4
Acid Value (mg NaOH/Kg lipid)	Control	1.12±0.03^{a,A}	1.15±0.01^{a,A,B}	1.24±0.04^{a,B}	1.27±0.04^{a,B}	1.34±0.01^{a,C}
	0.4% MOE	1.09±0.01^{a,b,A}	1.09±0.03^{a,A}	1.16±0.01^{a,A}	1.16±0.04^{a,A}	1.17±0.00^{a,A}
	0.6% MOE	0.88±0.11^{b,A}	0.96±0.02^{b,A,B}	1.01±0.03^{b,A,B,C}	1.03±0.05^{a,B,C}	1.04±0.00^{b,C}

Data are expressed means ± SEM, n=3. Means with different letters within a row (A-C: comparison between storage time) and in a column (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

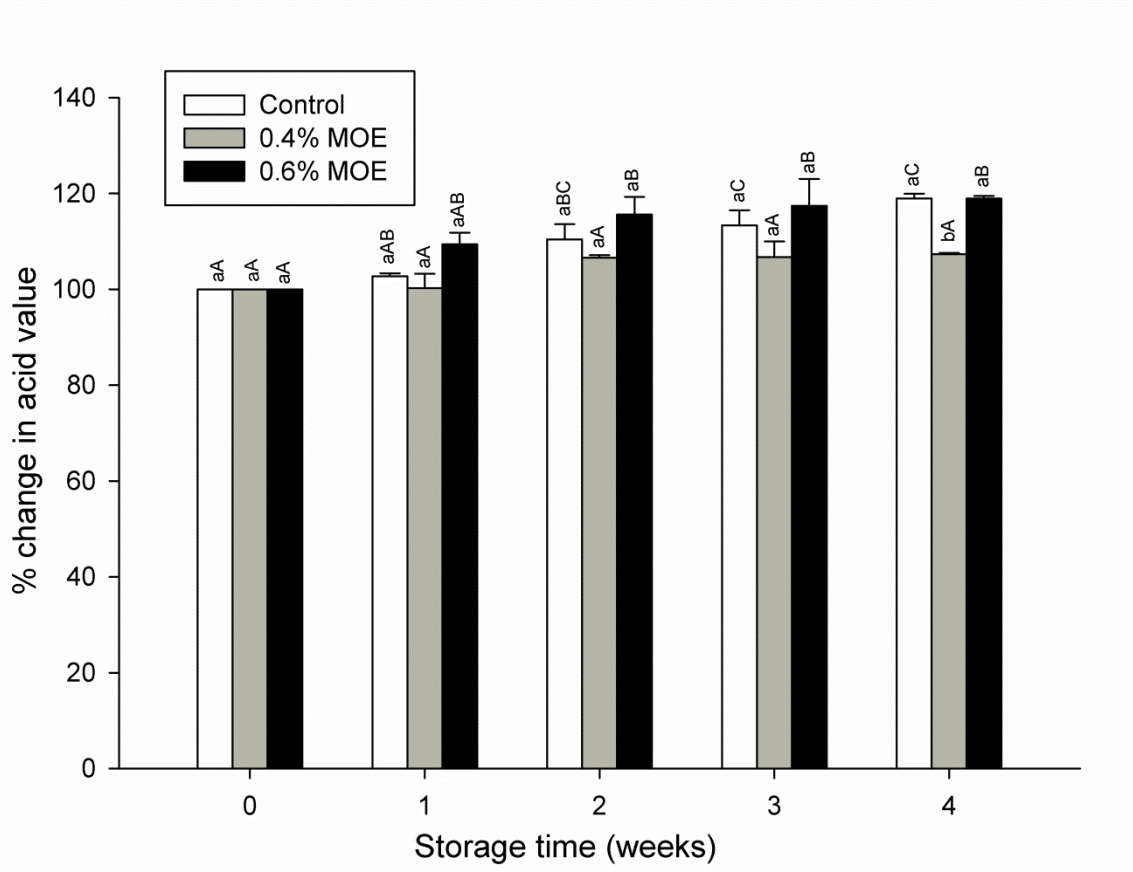


Figure 30. Acid value normalised to week 0 (%) in cookies fortified with MOE then stored for 4 weeks at 30 ± 1°C. Data are expressed as means ± SEM, n=3. Means with different letters (A-C: comparison between storage time) and (a-c: comparison between samples) indicate significant difference ($p < 0.05$) according to Tukey HSD test.

4.6.5 Sensory evaluation of baked cookies fortified with MOE during storage using quantitative descriptive analysis (QDA)

Panelist evaluated cookies on weekly basis; color of cookies reduced significantly in sample groups from week 0 to week 4 and was also significant between sample groups. Hardness, fracturability, moisture and dryness were not significantly different between groups and throughout the storage period. Cookies fortified with 0.4% and 0.6% MOE showed no significance difference within the storage weeks (**Table 19**). Oxidation of fat and other factors that might have affected the stability of cookies led to an increase in rancid aroma and flavor which was significant during the storage weeks. This furthermore reduced the acceptability of aroma, flavor and overall acceptability significantly different among groups during the storage period **(Table 20).**

Table 19. Sensory evaluation of baked cookies fortified with MOE during 4 weeks storage time at 30 ± 1 ^{o}C using QDA

		Storage time (weeks)				
	Sample	0	1	2	3	4
Color	Control	6.49 ± 0.80^{a,A}	4.09 ± 0.45^{a,B}	3.86 ± 0.34^{a,B}	4.24 ± 0.48^{a,B}	6.03 ± 0.50^{a,A,B}
	0.4%MOE	5.45 ± 0.56^{a,A}	6.70 ± 0.59^{b,A}	5.93 ± 0.52^{b,A}	5.54 ± 0.62^{a,b,A}	5.99 ± 0.48^{a,A}
	0.6%MOE	9.32 ± 0.45^{b,A}	7.77 ± 0.45^{b,A,B}	8.42 ± 0.42^{c,A,B}	6.64 ± 0.41^{b,B}	8.38 ± 0.35^{b,A,B}
Fracturability	Control	6.14 ± 0.88^{a,A,B}	8.51 ± 0.65^{a,A}	7.38 ± 0.76^{a,A,B}	6.98 ± 0.54^{a,A,B}	6.56 ± 0.54^{a,A,B}
	0.4%MOE	6.46 ± 0.8^{a,A}	8.75 ± 0.60^{a,A}	7.37 ± 0.68^{a,A}	6.78 ± 0.59^{a,A}	6.61 ± 0.89^{a,A}
	0.6%MOE	6.86 ± 0.96^{a,A}	8.49 ± 0.50^{a,A}	6.62 ± 0.63^{a,A}	5.50 ± 0.88^{a,A}	6.52 ± 0.83^{a,A}
Hardness	Control	9.59 ± 0.82^{a,A}	6.29 ± 0.93^{a,A}	6.62 ± 0.47^{a,A}	6.78 ± 0.84^{a,A}	6.92 ± 0.66^{a,A}
	0.4%MOE	8.17 ± 0.56^{a,A}	6.12 ± 0.59^{a,A}	6.83 ± 0.72^{a,A}	7.39 ± 0.45^{a,A}	7.97 ± 0.79^{a,A}
	0.6%MOE	7.04 ± 1.07^{a,A}	7.41 ± 0.77^{a,A}	7.85 ± 0.81^{a,A}	5.87± 0.74^{a,A}	5.9 ± 0.53^{a,A}
Moisture	Control	5.96 ± 0.74^{a,A}	6.11 ± 0.68^{a,A}	6.4 ± 0.55^{a,A}	6.62 ± 0.76^{a,A}	6.97 ± 0.88^{a,A}
	0.4%MOE	7.13 ± 0.74^{a,A}	6.92 ± 0.49^{a,A}	6.61 ± 0.60^{a,A}	6.80 ± 0.60^{a,A}	6.46 ± 0.53^{a,A}
	0.6%MOE^{a,A}	7.13 ± 0.83^{a,A}	6.79 ± 0.51^{a,A}	6.94 ± 0.66^{a,A}	7.14 ± 0.65^{a,A}	7.01 ± 0.87^{a,A}
Dryness	Control	10.02 ± 0.87^{a,A}	6.54 ± 0.75^{a,A,B}	8.59 ± 0.39^{a,A,B}	6.55 ± 0.76^{a,B}	8.67 ± 0.76^{a,A}
	0.4%MOE	7.78 ± 0.61^{a,A}	6.21 ± 0.52^{a,A}	7.71 ± 0.82^{a,A}	7.05 ± 0.76^{a,A}	7.80 ± 0.79^{a,A}
	0.6%MOE	9.09 ± 1.02^{a,A}	7.68 ± 0.59^{a,A}	7.20 ± 0.64^{a,A}	6.78 ± 0.87^{a,A}	6.92 ± 0.72^{a,A}

Data are expressed as means ± SEM, n=10. Means with different letters within a row (A-B: comparison between storage time) and in a column (a-c: comparison between samples) indicate significant difference ($p< 0.05$) according to Tukey HSD test. Scores used; 1: very low level of attribute, 15: very high level of attribute.

Table 20. Sensory evaluation of baked cookies fortified with MOE during 4 weeks storage time using QDA (cont'n)

		Storage time (weeks)				
	Sample	0	1	2	3	4
Acceptability flavor	Control	9.42 ± 0.54^{a,A}	9.39 ± 0.32^{a,A}	9.13 ± 0.47^{a,A}	9.09 ± 0.42^{a,A}	8.91± 0.38^{a,A}
	0.4% MOE	8.92 ± 0.36^{a,A}	8.46 ± 0.42^{a,A}	8.19 ± 0.38^{a,A}	8.04 ± 0.45^{a,b,A}	8.03 ± 0.45^{a,A}
	0.6% MOE	8.77 ± 0.6^{a,A}	7.79 ± 0.60^{a,A}	7.59 ± 0.64^{a,A}	7.39 ± 0.43^{b,A}	7.33 ± 0.54^{a,A}
Acceptability aroma	Control	10.07 ± 0.46^{a,A}	9.71 ± 0.31^{a,A,B}	8.98 ± 0.63^{a,A}	8.60 ± 0.56^{a,A}	8.52 ± 0.55^{a,A}
	0.4% MOE	9.52 ± 0.38^{a,A}	8.80 ± 0.42^{a,A}	8.71 ± 0.52^{a,A}	8.29 ± 0.60^{a,b,A}	7.90 ± 0.55^{a,A}
	0.6% MOE	9.10 ± 0.91^{a,A}	8.58 ± 0.69^{a,A}	8.03 ± 0.38^{a,A}	7.5 ± 0.51^{b,A}	7.10 ± 0.71^{a,A}
Overall acceptability	Control	9.51 ± 0.53^{a,A}	9.15 ± 0.42^{a,A}	9.03 ± 0.49^{a,A}	8.91 ±0.62^{a,A}	8.51 ± 0.54^{a,A}
	0.4% MOE	7.68 ± 0.60^{a,A}	7.32 ± 0.47^{ab,A}	7.35 ± 0.76^{a,b,A}	7.24 ± 0.66^{a,b,A}	6.66 ± 0.60^{a,b,A}
	0.6% MOE	7.14 ± 0.53^{b,A}	6.70 ± 0.45^{b,A}	6.66 ± 0.57^{b,A}	6.45 ± 0.92^{b,A}	6.35 ± 0.46^{b,A}

Data are expressed as means ± SEM, n=10. Means with different letters within a row (A-B: comparison between storage time) and in a column (a-c: comparison between samples) indicate significant difference ($p< 0.05$) according to Tukey HSD test. Scores used; 1: very low level of attribute, 15: very high level of attribute.

CHAPTER 5

DISCUSSION

5.1 Phytochemical constituents of MOE

With a percentage yield of 18.21%, polyphenol and flavonoid content were measured to be 40.14mg GAE/g of extract and 6.82mg Catechin/g of extract respectively, which is comparably higher than aqueous extract from Nicaragua with percentage yield of 31.70%, phenolic content of 7.43 GAE and flavonoid content of 10.83 as equivalent to rutin. This change in phenolic content in the same plant extract could be as a result of difference in origin and also environmental conditions. Phytochemical constituents such as quercetin, kaempferol, ferulic acid, ellagic acid, Catechin etc were also reported to be present in the aqueous MOE [136].

5.2 Physical and physicochemical properties of baked cookies fortified with MOE

5.1.1 Moisture, Weight, Diameter, Height, Spread ratio and pH

In the production of cookies; weight, dimensions and moisture are known to be very important physical characteristic properties that describe the quality of the cookie product. Moisture content of regular cookies is between the range of 2.5 – 3% [168]. In this study, increase in moisture content could have been due to water absorption capacitiy of the extract due to its mode of extraction and also some phytochemical constituents. Spray dried extracts prepared by aqueous extraction can be hygroscopic in nature [169]. The high hygroscopicity of the extracts has been commonly attributed to the presence of the significant amounts of hydrophilic compounds including carbohydrates, glycosides, organic acids, phenolics, amino

acids, proteins [169, 170]. Major phytochemicals reported in *Moringa oleifera* are quercetin, glycosides, rutin, kaempferol glycosides and chlorogenic acids [136]. These however, might have increase the water absorption property of the extract. Spray dryer conditions used in drying the MOE might have also had an effect on its water absorption property. Inlet temperature of 160^{o}C which was used during spray drying of the extract preparation during this study has also been reported to produce powder with high moisture properties. Higher heat temperature used during spray drying, causes increase of heat transfer to particles leading to greater level of moisture evaporation [171]. Similar effect was observed when MOE was added to dough in cookie preparation, in determining the farinograph characteristics of wheat flour, water absorption increased from 59.2 to 66.7 % when 0 to 15 % (85 g – 95 g) MOE was added [172].

This study also took into consideration the changes in moisture content during the storage period since it could lead to textural changes and bring about chemical and microbiological spoilage in low and intermediate moisture products. During the storage period, similar effect of MOE on the moisture content of cookies was also observed as fortification increased in a dose dependent manner. Water vapor transmission through the packaging used during the storage period might have also been a cause for the increase in moisture level during the storage period even though polypropylene is known to have a high barrier to water vapor [163]. Biscuits packed in polypropylene gauge pouches during storage studies showed an increase in moisture from the initial period up to 30 days and remained stable throughout the rest of the 120 days of storage. This effect was, however, reported to have been as a result of the water vapor transmission rate of the packaging material which was 4.5 g/m^{2}/day [173].

From the results, weights of cookies with *Moringa oleifera* leave extract showed an increase as the dose of the extract also increased. This could be as a result of increase level in moisture in cookies fortified with MOE.

The diameter of cookies reduced in a dose dependent manner with MOE fortification. According to Miller and Hoseney, 1997, diameter of cookies is affected by the type of flour used in baking. Soft flour is reported to increase diameter of cookies significantly when compared with hard flour [174]. However, this was contrary to what was observed during this study even though amount of soft flour (cake flour) used exceeded that of hard flour. Similar results of decrease in diameter and increase in height of cookies which resulted in reduced spread ratio was reported by Dachana *et al*., when wheat flour used in cookie production was supplemented with *Moringa oleifera* leave powder (85 g – 95 g) [172]. A study conducted by Gupta, *et al*. (2011) reported a decrease in spread ratio of cookies when wheat flour was supplemented with non-wheat flours [165]. This has been reported to be probably due to the effects of composite flours that form aggregates with increased numbers of hydrophilic sites found within the oligosaccharides, polysaccharides and protein which increased competing for the limited free water in cookies dough [175]. In addition, use of composite flour has been known to cause an increase in dough viscosity and forms aggregates by competing with limited free water available in cookie dough [176]. The spread ratio of cookies made with high protein flour reduce during baking, as non-wheat high protein flours used in biscuits exhibit greater water retention than those made from wheat flour [177]. Absorption of free water by the hydrophilic sites tends to reduce water in the system making it inadequate to dissolve sugar present in the bakery product during baking and thereby reducing the spreadability due to increase in viscosity [178].

It is therefore shown in this study that small amount of MOE added to cookies can affect its dimensions.

Spread ratios in quality cookies are known to be high [174]. The spread ratios, which is the diameter divided by height (D/H) of the cookies were observed to have their spread ability decreasing in cookies fortified with MOE, with 1% MOE fortified cookies when compared to control (**Table 5**). Diameter of cookies positively correlates with its spread ratio. However, even though moisture content in fortified cookies increased dose dependently, this did not increase diameter of cookies. This was also observed when cookies were prepared with barley flour [165]. Spread ability of cookies is influenced by the gluten development and also increase amount of fat in the product [160]. This was however, not the case in this present study since the amount of fat and wheat flour (which helps in gluten formation) was the same in all the cookie groups.

pH is an important factor that plays a role in the preservation of food together with certain factors such as water activity. Microorganisms that cause spoilage of food are also known to have minimum, optimum or maximum pH levels for their growth in foods. According to U.S. FDA, 2012, the pH of biscuits is in a range of 7.1 – 7.3 [179]. However, in this study, cookies which are the soft forms of biscuits had its pH reduced in a dose dependent manner as MOE was fortified in cookies. This could be due to the formation of acidic compounds when polyphenol contents are added to cookies. Polyphenol contents such as chlorogenic acid, *p*-coumaric acid, ferulic acid and gallic acid [136] which have been reported to be present in MOE used in this study, could have influenced the reduction in pH level in the cookies due to their acidic nature. In a study in which different dietary polyphenols were supplemented in cookies, reported decreased pH level within a range of 9.49 to 8.88 when compared to control cookies which had the highest pH (9.62) [92]. pH values around

7.0 serves as an optimum condition for growth of microorganisms while few grow below 4.0.

5.1.2 Color

With the use of plant extracts in food to produce functional food products, one of the physical properties which is affected and could later influence consumer acceptability is color [92, 160]. This effect could be due to the presence of polyphenols in these plants extract and how they are affected by the thermal processing during baking and also new products which are formed as a result of interaction between polyphenol and some of the cookie ingredients [92]. Sponge cakes fortified with green tea had the color of their crust affected by being darker when the amount of green tea powder replacing cake flour increased as measured by the colorimeter. The crumb of sponge cakes with green tea powder became darker redder and less yellow as the fortification increased. This was, however, reported to be as a result of the tea pigments and polyphenol contents undergoing oxidation and also the caramelization of the sucrose content in the cake [180], which could have also been a factor for the induction of color change by the reduction in lightness in the MOE fortified cookies. This change in lightness is however, observed in other studies which had flaxseed flour and dried roselle (*Hibiscus sabdariffa* L.) seed flour added to cookies, decreasing in fortified flaxseed flour cookies when compared to control cookies [160, 180].

In this study, redness of the cookies increased significantly in 0.6 to 1 % Moringa fortified cookies. Maillard reaction which occurs between carbohydrate and protein components of food in the presence of thermal processing could be a factor which induces redness of the cookies. Maillard reaction has been reported to occur at temperatures above 120°C [181] and this could be a factor for the increased redness in fortified cookies than control cookies. Yellowness of the cookies increased

but not significantly in the fortified cookie groups when compared to the control but reduced in the 0.2 % MOE fortified cookies. With protein content of 27.1g/100g leaf powder and carbohydrate content in both the leaf (38.2g/100g) [182] and also the cookie, Maillard reaction could have effectively occurred under thermal processing to induce the red color in cookies with increased amount of MOE.

Chroma which is the color intensity of cookies, which is the dullness and vividness of a color increased with the addition of the *Moringa oleifera* leave extract, when compared to control cookies, however there was no significant difference between the Moringa fortified cookies and control cookies. The difference in values in b* and chroma could be attributed to uneven exposure of cookies during thermal processing of food [183]. In addition, the E index which classifies the overall color of the cookies reduced significantly when MOE fortification increased. This parameter measured took into consideration the lightness, redness and yellowness of the cookies unlike chroma which took into consideration only the yellowness and redness of the cookies. Caramelization and Maillard reaction have been reported to induce color change in cookies [183].

There was no change in color during the storage period. An increase in redness has been reported to be associated with browning reaction, however, among the groups of cookies redness did not increase during the storage period. The color of the cookies could have been influenced by the amount of polyphenol in each group of cookie, since polyphenols in bakery products could form colored compounds due to the effect of thermal energy. Lightness of cookies in this study, was observed to have a strong positive correlation with E-index throughout the storage period (r=0.97, 0.99, 1.00, 1.00, 1.00 in week 0 to 4, respectively).

5.2 Polyphenol and flavonoid content in cookies dough and baked cookies fortified with MOE

In the present study, the content of polyphenol and flavonoid significantly increased in *Moringa oleifera* leaves extract (MOE) fortified cookies in a concentration-dependent manner since MOE has high content of polyphenol and flavonoid as shown in the results. Our finding, consistent with other studies shows an increase in natural antioxidant compounds in cookies similar to *Hibiscus sabdariffa* and pomegranate peel addition. In this research, the amount of polyphenol in the extract added to form the cookie dough in 0.2 % to 1 % MOE cookies ranged from 0.96 to 4.82 mg per cookie prepared. This amount of polyphenol reduced when measured in the baked cookie even though it was higher than that of the unbaked cookie dough. Mixing of ingredients during the preparation of cookie dough could be a factor in reducing the polyphenol amount from 0.96 to 0.62 mg/ g of dough in the amount of extract added in the dough preparation for 0.2 % MOE cookies and also from 4.82 mg per cookie in 1 % MOE cookies to 0.86 mg/g cookie. This reduction could be due to the phenolic antioxidants binding with the protein components of the wheat flour through hydrogen bonding during the dough preparation [184]. Phenolic antioxidants added to bakery products can also interact with polysaccharides through hydrogen bonding and hydrophobic interactions [185-187]. However, this was not observed when red raspberry was used in the preparation of muffins as mixing time had no significant effect on the polyphenol content. The percentage recovery in this research for the cookie dough therefore reduced as more Moringa extract was added with the range of 73.33 % in 0.2 % MOE cookies to 21.74 % in the 1 % MOE cookies. This shows the aqueous extraction used for sample extract preparation for analyzing polyphenol content could have affect its amount measured.

The amount of polyphenol content in the baked cookies was also observed to be higher than the cookie dough, even though it had undergone thermal processing by baking and together with other factors such as type of substrate and extraction time which could have led to its loss. Similar result was reported; when polyphenol content were increased in biscuits with mango peel powder [188]. Apple or lemon fiber used to make cookies also had no effect on their phenolic content when baked [189]. There are several mechanisms that affect the level of phenolic contents of foods when exposed to high temperatures, these include bound polyphenols being released, phenolic compound derivatives being released from partial degradation of lignins and the breakdown of phenolic compounds at the beginning of thermal processing [190]. The high level polyphenol in the baked cookies when compared to that measured in the cookie dough might have been due to the mechanism of the bound polyphenols being released when exposed to high temperature. Reported studies by both Ross *et al.,* (2011) and Jeong *et al.,* (2004) [191, 192], showed that, subjection of grape seed flour and citrus peel to a temperature of 150°C and rice hull heated for 10 min, led to increase level of total polyphenol content [192]. Jeong *et al.,* concluded that this could be due to insoluble polyphenol content been transformed to extractable soluble polyphenol [191]. In the present study, *Moringa oleifera* leaves extract (MOE) was used as a natural source of antioxidant, since high content of polyphenol, especially flavonoid content such as kaempferol, quercetin and rutin have been found in it [187]. This result demonstrated that the content of polyphenol significantly increased when adding MOE to cookies in a concentration-dependent manner which is consistent with other studies where an increase in polyphenol content was observed in *Hibiscus sabdariffa* and pomegranate peel fortified cookies [188, 190]. Moreover, different varieties of garden eggs supplemented in cookies also showed a dose dependent

increase in polyphenol level [193]. Heating of onions which contain similar phytochemical constituent as MOE (quercetin and kaempferol) at 120^{o}C for 30 mins increased the total polyphenol level [194]. The higher content of polyphenol in the MOE fortified cookies may be due to the active compounds as previously described. Polyphenol content also increased in control cookies when baked due to its phenolic contents (ferulic acid, vanillic acid, *p*-coumaric acid) that attaches with proteins to form complexes through hydrophobic bonds. These bonds are however, broken down with the effect of heat releasing the polyphenol and increasing the antioxidant capacity of the bakery product [186].

The molecular size, solubility, degree of polymerization etc. are factors that affect the bioavailability of polyphenols/phenolic compounds [195]. The most abundant polyphenols in diet are flavonoids, with quercetin being the main flavonol in our diets such as fruits, vegetables as well as beverages [196]. This is also present in *Moringa oleifera* used in this study. Flavonoids are one of the potent plant antioxidants due to their possession of one or more of these structural attributes; the o-diphenolic group, a conjugated 2-3 double bond with the 4- oxo function and the presence of hydroxyl groups at the 3 and 5 positions (**Figure 34**). This degree of hydroxylation at the 3 and 5 positions and also the presence of reduced amount of sugar moiety determine potency of flavonoid as an antioxidant [14, 197].

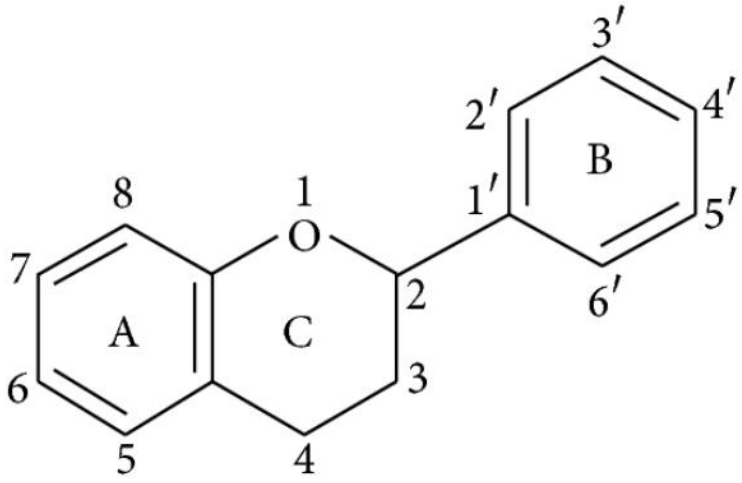


Figure 31. Basic Structure of Flavonoid **[193]**.

With the effect of heat on grape seed flour, the total flavonoid, significantly reduced when temperature increased from 150°C and above with increase in time for 10 min or longer [192]. In using different temperatures and time normally used in baking, it was observed that, the level of catechin reduced at temperatures ≥ 180°C and for longer times together with the antioxidant capacity [192]. Heating results in oxidation, thermal breakdown and draining out of bioactive compounds of which flavonoids are no exception, and depending on their morphology and nutritional function, the effect of heat can be either positive or negative [197]. Effect on flavonoid level after heating at a certain temperature led to the decrease in flavonoid, this indicated that some flavonoids were destroyed during the heating process. However, total phenolics increased. Flavonoids in fruits and vegetables contain C-glycoside bonds existing as dimers and oligomers, heating and other food processes can therefore lead to the formation of monomers by hydrolysis of the C- glycoside bonds [198]. Heating for 3 hrs at 150°C reduced the level of flavonoids [199].

In this research, the level of flavonoid concentration reduced in the baked cookies when compared to its concentrations in the unbaked cookie dough, but

increased significantly in a dose dependent manner when compared to the control baked cookie. This reduction, however, could be as a result of the thermal processing (baking) that the cookie had to undergo.

One factor that affects the polyphenol content of food is storage. Several studies have shown that polyphenol content of food change during storage, since they are easily oxidized. Reactions involving oxidation result in the formation of more or less polymerized substances, which lead to changes in the quality of foods, particularly in color and organoleptic characteristics [200]. During the 4 weeks storage period, polyphenol content was maintained in 0.4% MOE cookies whiles significant reduction was observed in 0.6% MOE cookies from week 1. Flavonoid content was also maintained in both fortified groups throughout the storage period. Storage and heating has been also been reported not to cause significant differences in total flavonol content in red onion cultivars. However, significant change was observed in quercetin and isorhamnetin glucosides during storage and heating. Quercetin and kaempferol (one of the main flavonoids in *Moringa oleifera* leave extract) in vegetable tissue also have thermal instability [201].

5.3 Antioxidant capacity and lipid peroxidation in cookie dough and baked cookies fortified with MOE

In this study, *Moringa oleifera* leaves extract was used as a natural source of antioxidant, due to its high polyphenol content, especially flavonoid such as kaempferol, quercetin, rutin, ferulic acid, (+)-catechin, etc. However, Kaempferol and quercetin were reported in previous studies as the two main active compounds with concentration ranges of 633.5 to 926 mg 100 g-1 and 104.7 to 225.4 mg 100 g-1, respectively. The presence of these phytochemicals does not only promote the leave of *Moringa oleifera* having pharmacological importance but also makes it to be

considered as a potential ingredient for functional foods. Antioxidant capacity of foods is linked with many health effects as in reducing the risk of cancer, cardiovascular diseases etc [202, 203]. Baked muffins prepared with raspberry juice increased the level of antioxidant capacity when compared to the batter (not baked) [204][203]. Similar trend was observed in this study as antioxidant level in the baked cookie increased compared to the unbaked cookie dough when FRAP antioxidant capacity was determined, and this could have been as a result of the liberation of polyphenols during the baking process.

According to Michalska *et al.,* (2008), several factors influence the level of antioxidant capacity in final bakery foods, some of which are the intrinsic polyphenol in wheat flour, added ingredient which contains polyphenol, other ingredient used in baking that contain phenolics, phenolic compounds generated during the bakery process (example through Maillard reaction), heat induced breakdown products etc. [205, 206]. The development of sulfhydryl groups in some ingredients used for the bakery product can also lead to the increase in antioxidant activity. Increase of heat processing in skim milk has been reported to increase its antioxidant capacity and thereby promoting oxidative stability due to the formation of sulfydryl groups [207, 208]. Disulphide bonds can also form within protein components in flour during dough development and mobilized through disulphide interactions [209-211]. Heating up to 75^{o}C of gluten proteins are known to increase the formation of sulfhydryl-disulphide interactions [212]. According to Sreelatha *et al*., (2009), *Moringa oleifera* leave extract exhibited some level of antioxidant capacity, with its aqueous extract exhibiting strong effect on DPPH free radical which was comparable to trolox which was used as a positive control [213]. This effect, however, could have been due to hydrogen donating ability of the *Moringa oleifera* leave extract. For the FRAP antioxidant capacity in the cookies, baked MOE fortified cookies obtained significantly

higher level of antioxidant capacity from 0.4 to 1 % when compared to the control, this was consistent with the previous studies that addition of pomegranate peel extract in the cookies and apple skin powder in the muffins showed higher in FRAP value. These effects may be due to polyphenol in the extract, especially kaempferol and quercetin in pomegranate peel while apple skin contains chlorogenic acid and quercetin [84, 206]. Unbaked cookie dough also had lower levels of the antioxidant capacity when compared to the baked cookies but were significantly different in the 0.2 to 1 % MOE fortified cookies when compared to the unbaked control cookie dough.

Reduction in polyphenol and flavonoid content influenced the reduction of antioxidant capacity during the storage period when FRAP assay and DPPH radical scavenging activity were measured. Despite the reduction in polyphenol and flavonoid contents in cookie groups during the storage period, a dose dependent increase in antioxidant capacity was obtained.

5.4 Lipid peroxidation in cookie dough and baked cookies fortified with MOE

The quality of cookie is affected by factors such as oxidation of lipid components, moisture loss, change in texture of bakery product and its long term storage which contributes to spoilage [214]. However, the use of synthetic forms of antioxidants that retard lipid oxidation in most food industries, but then these antioxidants are reported to be toxic and carcinogenic [19]. There has been much concern now about using natural antioxidants from herbs, fruits, vegetables and spices which will have potent antioxidant effect and not lead to making the bakery products undesirable.

Therefore, in this study small amount of plant extract was added to determine its effect on level of lipid peroxidation in the bakery product. According to a research conducted by Sreelatha *et al.,* (2009), aqueous *Moringa oleifera* leave extract showed a good radical scavenging property which was similar to reference antioxidants and also was able to inhibit lipid per oxidation in goat liver homogenate [213]. This extract was therefore used in this research to determine how well it's able reduce both primary and secondary peroxidation products, since the main mechanism of polyphenols is to inhibit the initial stage of lipid peroxidation [103]. Removal of pro-oxidant such as free fatty acids, the removal of oxygen or substituting with oxygen scavengers and removal of metals or by protecting food products from light are also various ways of reducing the rate of oxidation. However, antioxidants are added to retard oxidation rate since they are able to significantly prevent or slow down the rate of oxidation of oxidizable substrates even when added in small amounts to food compared to high amounts of lipids and proteins [215, 216].

Thermal processing of bakery foods lead to lipid oxidation which is similar to autoxidation of lipids, whereby hydrogen is abstracted from stable lipid to form lipid radical which later reacts with triplet oxygen under normal oxygen pressure to form lipid peroxyl radical. The peroxyl radical removes hydrogen from another lipid to form lipid hydroproxide and a new lipid radical. The removal of hydrogen from a lipid which results in the double bond adjacent the carbon to be shifted resulting in conjugated diene formation. This reaction tends to be faster in thermal of oxidation and this leads to the breakdown of unstable products of oxidation such as lipid hydroperoxide, peroxyl radical, conjugated diene etc. to secondary oxidation products such as aldehydes and ketones [217, 218]. Polyphenol, which are known to have antioxidant properties are able to prevent or reduce the rate of lipid

peroxidation by acting as radical scavengers, whereby they are able to donate hydrogen to free radicals in order to stabilize them and as a result producing non-reactive antioxidant radicals which lead to the retardation of the initial stage of lipid peroxidation [219].

Cookies prepared in this research had relatively high level of fat as it was 25 % w/w of the total amount of ingredients used in preparation. There have been many studies recently on the use of natural extract to reduce lipid peroxidation in bakery foods. Cookies enriched with different levels of chokeberry polyphenols was able to reduce primary product (peroxide value) and secondary products (TBARS) of lipid peroxidation and suggested that may be used in extending shelf life. With respect to the oxidation degree, margarine cookies containing 100 and 250 mg of chokeberry extract were concluded to be safe for consumption within 9 weeks and 1000 mg of chokeberry polyphenol safe in butter cookies for not more than 9 weeks [220]. *Moringa oleifera* leave extract used in this case was able to reduce primary (conjugated diene, hydroperoxides) and secondary (malondialdehyde) lipid peroxidation products even in thermal processed cookies. Conjugated diene products reduced significantly in the 0.4 % baked Moringa cookies to 1 % baked MOE fortified cookies.

PV is a defined indicator of state of oxidation but particularly in the early stage of oxidation it serves as a good tool for the measurement of degree of oxidation. Peroxide value (PV) measurement is a procedure used in measuring the level of hydroperoxides present in lipid being analyzed and expressed as the milli equivalent of peroxide oxygen per kilogram of lipid. Peroxide values which are ≤ 1.0 mEq/Kg are mostly found in deodorized or refined fresh oil [221]. A fat is considered as rancid when the PV value is about 10 mEq/kg [222]. Even though PV is used in evaluating the rate of lipid peroxidation in food products, the accurate level of

oxidative deterioration cannot be assessed because peroxides formed in the early stages of peroxidation may be unstable and as a result be converted into stable aldehydes, ketones and alcohol following the free radical termination mechanism [223]. In a reported study by Halvorsen and Blomhoff, 2011, 12 types of vegetable oils were analyzed together with other brands of fish oil to determine the level of primary oxidation products (PV) and secondary oxidation products (alkenals) before and after subjecting them to heat at 225°C for 25 min. The PV of the vegetable oils before being subjected to heat ranged from 0.60 – 5.33 mEq/kg. After heating, 9 of the vegetable fat had their peroxide values increased whiles 3 had reduced. The range of peroxide value, however, now ranged from 0.07- 5.8 mEq/kg after heating. The secondary oxidation product which in that case was the level of alkenal measured also increased in the oil when heated in all the oil [224]. The level of PV measured in the oil before being exposed to heating could be due to stages of manufacturing process it had to go through, storage, antioxidants added and also due to the presence of metals and light [222]. PV in rice crackers increased slowly during the initial stage of 18 days storage period but increased rapidly during the later stages of storage. Increase in PV was reported to be an indication of later stages of lipid peroxidation. Fortification of crackers with *Cratoxylum formosum* extract caused much less increase in PV when compared to control cookies [225].

In this present study, *Moringa oleifera* leave extract had a reducing effect on the level of PV in the cookies both before and after baking. MOE fortified cookies baked at 180°C for 16 min showed a reduced range of 2.95 - 1.30 mEq/kg when compared to the control baked cookie which had a value of 3.25 mEq/kg. Unbaked cookie dough also showed a range of 3.18 – 1.57 mEq/kg in 0.2 - 1% MOE fortified cookies when compared to the control unbaked cookie which had a PV of 3.71 mEq/kg.

However, increase in antioxidant capacity in baked cookies due to high polyphenol and flavonoid content was able to reduce the primary products of lipid peroxidation in the baked cookies by its radical scavenging and hydrogen donating activity more than that measured in the cookies dough.

Secondary products of oxidation which in this experiment was malondialdehyde (MDA) measured also showed a dose dependent reduction when *Moringa oleifera* leave extract was added to the cookies. Baked cookies showed significant difference in 0.4 to 1 % MOE cookies compared to control when measured for MDA. Similar reduction and significance difference was also observed from 0.4 – 1 % MOE fortified cookie dough. However, the concentrations of MDA were lower in the cookie dough than that measured in the baked cookies; this could have been as a result of the baked cookies exposed to thermal processing which resulted in rapid degradation of the primary oxidation products (peroxides and conjugated dienes) to the secondary products (MDA) of oxidation. Crackers were highly susceptible during 18 day storage due to its large surface area exposed to air and presence of iron in the product; this was however retarded in crackers fortified with *Cratoxylum formosum* extract during the storage period. MDA was reported to have been significantly reduced ($p<0.001$) in *Cratoxylum formosum* extract fortified crackers and crackers fortified with BHT and tocopherol when compared with control cookies without any antioxidant fortification as it (MDA) increased rapidly during the storage period [225]. *Moringa oleifera* leaf powder contains iron (28.2mg/100g) [182] and this could have contributed to increased level of lipid peroxidation in the cookies since iron acts as a catalyst in radical formation reactions [226]. Effective antioxidant capacity of *Moringa oleifera* could have however reduced the rate of lipid peroxidation in the fortified cookies.

Over the 4 weeks storage period, MDA, conjugated diene, peroxide value and acid value were relatively lower in cookies fortified with MOE when compared with control cookies. Obtained results were in accordance with the results of polyphenol, flavonoid content and antioxidant capacity recorded in this study. In cookies, the addition of vita plant extract was reported to have good antioxidant effect and oxidative stability during a storage period of 6 weeks [227].

Cookies in this study consist of wheat flour, fat, sugar, salt and flavoring agents, which are predominantly sensitive to oxygen reaction. The large surface area of cookies increase the exposure of oxygen and light and this may increase the possibility of oxidation during the early stage of the storage period before it was packaged. Relative humidity (RH) has also been reported to affect the development of peroxide value. In a study by Kumar *et al.,* (2014), [173], successive increase in RH led to the decomposition of peroxides in cookies, with maximum decomposition being observed at RH of 56 %. RH of 11 % also showed significant increase in peroxides after 90 days of storage.

5.4 Sensory evaluation of baked cookies (Acceptability test)

Sensory evaluation was done by 30 panelists. Questionnaire used for evaluation included demographic information which included the sex, age and cookie purchase frequency. Panels included 4 males and 26 females between the age differences of 18 to 60 yrs. 17 panels purchased cookies less than once per month, 9 panels had purchasing frequency at least once per month and 3 panels purchased cookies at least once per week.

Results from the sensory analysis showed a reduction in the overall likeness, color and appearance as the amount of MOE also increased. This is however, in

agreement with studies done by Dachana *et al.*, (2010), with MOE fortified cookies. It was reported that the overall quality of cookies reduced in a dose dependent manner from 54 in control cookies to 20 in 15 % MOE fortified cookies. Also, cookie crust and crumb also became greener with increased level of extract. Gritty mouthfeel and bitterness was also recorded for cookies with 15 % MOE. The decrease in color and appearance in this study corresponded to the increase in redness level in cookies which was in a dose dependent manner. This effect could be as a result of Maillard reaction between the protein components in the MOE and lactose in the skimmed milk used for the cookie production or as a result of the phenolic content of the extract [172]. Likeness for aroma and flavor in the cookies ranged from 'like moderately' to 'like slightly' in control to 0.6 % MOE fortified cookies, whilst 0.8 % and 1 % MOE fortified cookies were ranked as 'neither like nor dislike'. Control cookies and all cookie groups fortified with MOE were ranked as 'like slightly' for sweetness, except 0.2 % MOE fortified cookies which were ranked as 'like moderately'. Addition of 15 % *Moringa oleifera* powder was also reported to negatively affect the texture of cookies, as was also observed in this study, as likeness of cookies in terms of hardness reduced as MOE fortification in cookies increased.

Overall likeness in 0.2% and 0.4% fortified cookies were not significantly different from control cookies. From the results, cookies fortified with 0.4 and 0.6 % MOE can be termed as acceptable since most of the attributes tested for were ranked as' like slightly' on the nine-point hedonic scale and also showed significance level in antioxidant capacity and reduction in lipid peroxidation products when compared to the control cookies in previous chemical analysis conducted. Effect of green tea powder on color and also taste led to the decrease in likeness during sensory evaluation in cakes with the highest amount of green tea powder [180].

Partial replacement of wheat flour with pumpkin seed flour into muffins also affected the sensory characteristics when analyzed by experts and children. Sensory rating decreased significantly in terms of buttery taste, color, texture, buttery aroma and overall quality [220].

5.5 Storage effect evaluation

Overall likeness of cookies decreased in a dose dependent manner during sensory evaluation of baked cookies fortified with MOE. Evaluated samples with mean readings below 5.0 are rated as poor quality samples or foods which felt strange to observers, whiles mean readings which are above 7.5 are regarded as samples with good quality [228]. However, during sensory evaluation in this present study, cookies fortified with 0.2% had the highest level acceptability which was not significantly different from the control cookies.

After sensory evaluation, 0.4% MOE and 0.6% MOE were the amounts of extract chosen to fortify cookies used for the storage. Reasons or factor considered for this selection were due to the mean reading of these groups in terms of overall likeness, aroma, flavor, appearance and sweetness during the sensory evaluation and also the level of effectiveness of these groups during the chemical analysis (reduction of lipid peroxidation products, increase in levels of polyphenol, flavonoid and antioxidant capacity). Even though, the mean readings of 0.4% and 0.6% MOE fortified cookies were lower than 7.5 which is the acceptable level in literature for good quality samples during sensory evaluation, 0.4% MOE fortified cookies were significantly different when compared with control cookies which had no extract. Levels of antioxidant capacity, polyphenol and flavonoid contents were significantly higher whiles lipid peroxidation products also reduced significantly in 0.4% and 0.6% MOE cookies when compared to control cookies. The storage period (4 weeks) in this

study involved a weekly measurement of polyphenol and flavonoid content, antioxidant capacity (FRAP, DPPH assay), lipid peroxidation (conjugated diene, peroxide value, acid value and TBARS assay), color, hardness and physicochemical properties (water activity, moisture content). The effect of several conditions that makes food less palatable during consumption causes spoilage of bakery products and this can be divided into physical spoilage (which might be caused by factors such as moisture loss, staling), chemical spoilage (caused by rancidity) and microbiological spoilage (caused by mold, yeast and bacterial growth). Rancidity/lipid peroxidation which is a major cause of chemical spoilage normally leads to change in reduction in the quality of nutritive value, color, safety, flavor and texture.

5.5.1 Physicochemical properties

5.5.1.1 Water activity in baked cookies fortified with MOE during storage time.

The conditions/factors which promote the spoilage process are basically storage temperature, relative humidity, level of preservatives, pH, packaging material, gaseous environment surrounding product and especially moisture content and water activity (aw).

Like many processed foods, bakery foods are subjected to physical, chemical and microbiological spoilage, however, physical and chemical spoilage problems limit the shelf life of low and intermediate moisture bakery products like cookies [73]. Furthermore, classifying products in terms of their pH and water activity is helpful in knowing the spoilage and safety potential of bakery products [73]. Water activity is used to determine the accessibility of water molecules to enter into microbial, enzymatic or chemical reactions. It has been used as a reliable concept for assessment of the microbial growth and chemical stability of foods after their

manufacture. Water activity of low moisture foods which includes cookies has been reported to have water activity of < 0.6 [179]. The relation between moisture content and its water activity in a food product does not assume the linear relation but rather in the sigmoid form termed as the sorption isotherms [229].

Multiplication of microorganisms is suppressed or stopped when water level is reduced in food. Microorganisms do not grow below water activity of 0.6 but however, remain viable for long period of time. In this study, water activity remained within this region between 0.42 and 0.51, rendering cookies fresh and void of any microbial growth. Most pathogenic microorganisms tend to have the favorable water activity for growth to be from 0.8 and above. However, the limit for mold and yeast growth is 0.6 [73]. Water activity has been used to control lipid oxidation in susceptible food products. Lipid oxidation is accelerated at both very high and very low water activities [160]. At low levels of water activity, oxidation is increased by high accumulation of molecules that were unreactive during low water activities by being trapped and encapsulated in unreactive food components; water alters the matrix and makes it permeable to reactants and catalysts [51]. Autoxidation of lipids is diminished in the water activity within range of 0.3–0.5, while below or above this range the autoxidation of lipids increases rapidly [230]. In this present study, with a range of 0.42 – 0.51, cookies are however, not susceptible to oxidation.

Strong positive correlation was observed between water activity and the primary products of lipid peroxidation throughout the storage period except week 2 (**Appendix, Table 24 – 28**). During the storage period of cookies in polypropylene food containers, the level of water activity was observed to be very high in the control cookies during the initial period than the test groups (0.4 and 0.6 % MOE fortified cookies).

5.5.4 Hydrolysis of fat (Acid value)

Acid value measured in cookies during the storage period was to evaluate the rate of fatty acid hydrolysis from its glycerol backbone. They are therefore measured as the free fatty acid. This however, is responsible for the undesirable aroma, flavor and bitterness in food products. The level of hydrolysis however is as a result of the presence of enzymes eg lipase and moisture [173]. These catalyze the oxidation of unsaturated fats, producing peroxides and heat stable compounds that survive the baking process [73].

From this study, it was observed that the level of acid value increased during the storage period, indicating hydrolysis of fat content in the cookies. MOE protected cookies from increased level of hydrolysis and this could be attributed to its antioxidant capacity which helps in minimizing the rate of oxidation caused by the presence of moisture and lipase enzyme. Pomegranate peel supplementation (37.5g) in cookies reduced free fatty acid by 50% at the end of 4 months storage period when compared with control cookies [84], however, in this study 0.26g (0.6% MOE fortification) reduction was about 18.18% at the end of the 4 weeks storage period.

5.5.5 Sensory evaluation during storage using QDA

Quality of food products has a significant effect on consumer behavior when it comes to the selection of food during purchase and consumption [231].

Descriptive analysis which in this study involved quantitative descriptive analysis (QDA) was used during the storage period, since it is used for determining the perceived intensity of well-defined food sensory attributes. When this methodology is well applied, food sensory attributes are actually analytical indicators, similar to what is obtained by any other instrumental analysis. The QDA method of analysis as

well allows the evaluation of the interactions between food and consumers to be monitored during storage. Such interactions are actually the result of overall food sensory attributes affecting consumer acceptability or preference [78].

Flavor has been one major attribute when it comes to food quality when considering the consumption of a food product; this fact has been established by consumption statistics of highly developed countries [232]. Primary flavor compounds can undergo wide range of changes during handling of raw materials, processing into a product and also during storage and can eventually lead to their lost and result in the formation of the secondary ones affecting the sensory quality of foods [233]. Oxidation which occurs in fat containing foods leads to the development of off flavors and odors which in turn leads to the poor quality of food product [234].

Other parameters such as fracturability, hardness, moisture and dryness were not significantly different when compared within weeks and between groups of cookies. Hardness and fracturability of cookies during storage were not significantly different between the different groups of cookies (control, 0.4% and 0.6% MOE fortified cookies) and also within the storage weeks in 0.4% and 0.6% MOE cookies. Hardness of cookies reduced in control and 0.4% fortified cookies at the end of the storage period. Reduced hardness of cookies during storage can be attributed the level of moisture absorption. Instrumental measurement of moisture had a positive correlation with sensorial scores for moisture given by panelist (r=0.95, 1.00, 0.98, 0.72 and 0.70 in week 0 to 4, respectively during the storage period) (**Appendix, Table 24 – 28**). Moisture scores given by panelist during sensory analysis correlated with fracturability scores with r=0.83, 0.57, -0.93, -0.98, -0.92 in week 0 to 4, respectively and hardness scores with r=-0.90, 0.25, 0.97, -0.73 and 0.90 in week 0 to 4, respectively (**Appendix, Table 24 – 28**).

Color of cookies were observed to be high in cookies fortified with 0.6% MOE when compared to the other groups of cookies and this could be as a result of the polyphenol extract in the cookies.

Food products with PV range of 10 -20 mEq/kg have been reported to be considered rancid but acceptable to consume, whereas PV of more than 20 mEq/kg are unacceptable when consumed. In this study, all samples were considered not rancid and still acceptable since their PV's were below 10 mEq/kg [235]. Acceptability of flavor and aroma, however, decreased throughout the storage period. Fortified cookie groups had lower level of acceptability when compared to control cookies with 0.6% MOE fortified cookies having the lowest acceptability for aroma and flavor, this decrease might have been due to the influence of the extract even though MOE fortified cookies had its level of lipid peroxidation reduced significantly. This negative effect of the MOE on sensorial qualities of fortified cookies also led to a relative reduction in its overall acceptability. Overall acceptability was determined on the basis of quality scores obtained from the evaluation of all the sensory attributes in the cookies. As a whole, maximum scores were obtained in the sample groups (control, 04% MOE and 0.6% MOE fortified cookies) during week 0 which gradually decreased with storage days **(Table 20)**. In a study by Elahi *et al*., (1997), a gradual decrease in overall acceptability of biscuits during storage was observed and this was attributed to moisture absorption and increase in peroxide value and free fatty acid content in the biscuits [236].

CHAPTER 6

CONCLUSION

Moringa oleifera leave extract in this study was indicated to have high amount of polyphenol, flavonoid and antioxidant capacity. Findings from this research show that 0.4% to 1% MOE fortified cookies, had an appreciable level of polyphenol and flavonoid content and at the same time possess antioxidant capacity which was significantly higher when compared to control cookie. Furthermore, MOE addition improved oxidative stability of the cookies dose dependently and this was significant when compared to the control cookies also in 0.4% and 0.6% MOE fortified cookies. Cookies fortified with 0.2% fortified cookies had good sensorial qualities which were comparable to control and had highest acceptability level when compared to the other fortified groups. Cookies fortified with 0.4% MOE were also not significantly different from control cookies, in terms of overall acceptability. Fortified cookies (0.4% and 0.6% fortified) showed shelf life stability when cookies were stored at a temperature of 30 ± 1°C for a period of 4 weeks. Moisture and water activity of fortified cookies were maintained. Overall acceptability of fortified cookies were not significantly different in 0.4% MOE cookies from control cookies ($p<0.05$). Fortified cookies maintained their overall acceptability level throughout the storage period together with the control cookies.

After all the experiments, cookies fortified with 0.4% MOE can be accepted due to its good sensorial property and its ability to reduce lipid peroxidation products because of its high antioxidant property. This study confirms the use of *Moringa oleifera* leave extract as a functional food ingredient in bakery products

which has been demonstrated with cookies due to its high antioxidant capacity and its ability to reduce lipid peroxidation products.

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APPENDIX

Appendix 1. Percentage yield of MOE

Percentage yield was measured using the formula:

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{Weight of plant material}} \times 100$$

$$= (509.467 / 2797.96) \times 100$$

$$= 18.21\ \%$$

Appendix 2. Standard curves for polyphenol, flavonoid content and FRAP antioxidant capacity.

Table 21. Values used for standard curve of Gallic acid.

Gallic acid concentration (mg/ml)	Absorbance
0.75	1.83 ± 0.01
0.38	0.97 ± 0.01
0.19	0.50 ± 0.00
0.09	0.27 ± 0.00
0.05	0.13 ± 0.00
0.02	0.06 ± 0.01

Data expressed as mean ± SEM, n=3

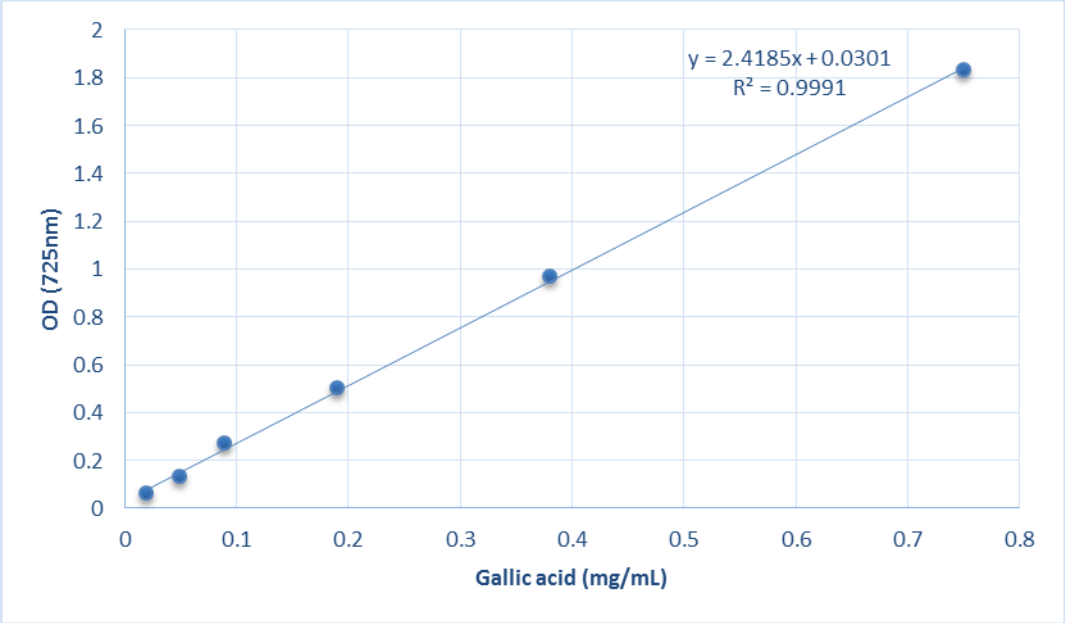


Figure 32. Standard curve of gallic acid used to determine polyphenol content.

Table 22. Values used for standard curve of Catechin.

Catechin concentration (mg/ml)	Absorbance
0.06	0.15 ± 0.00
0.03	0.07 ± 0.00
0.02	0.04 ± 0.00
0.01	0.018 ± 0.00
0.004	0.01 ± 0.00
0.002	0.001 ± 0.00

Data expressed as mean ± SEM, n=3

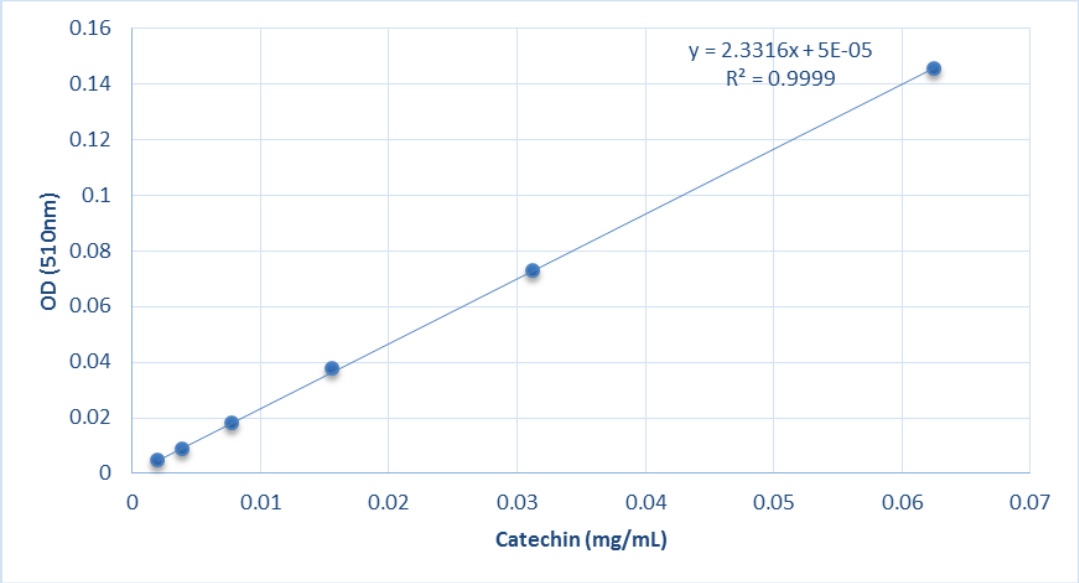


Figure 33. Standard curve of catechin used to determine flavonoid content.

Table 23. Values used for standard curve of $FeSO_4$.

Catechin concentration (mg/ml)	Absorbance
1000.00	1.30 ± 0.03
500.00	0.68 ± 0.03
250.00	0.35 ± 0.02
125.00	0.19 ± 0.02
62.50	0.11 ± 0.02

Data expressed as mean ± SEM, n=3

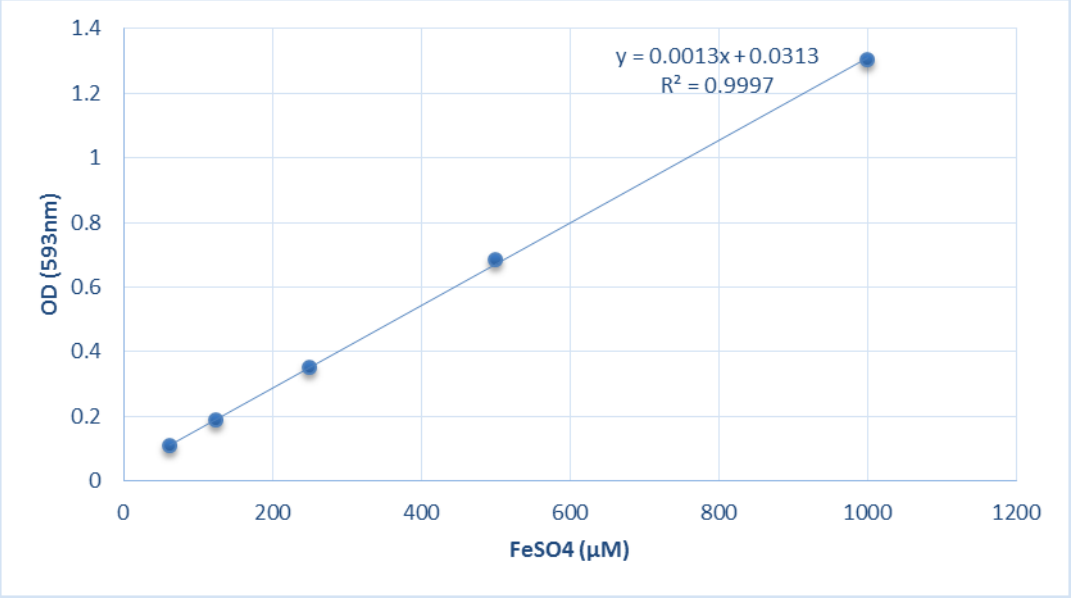


Figure 34. Standard curve of $FeSO_4$.

Appendix 3. Determination of diameter, height, moisture and color of cookies

Figure 36. Diameter of cookies

Figure 35. Height of cookies

Figure 37. Color measurement using moisture analyzer.

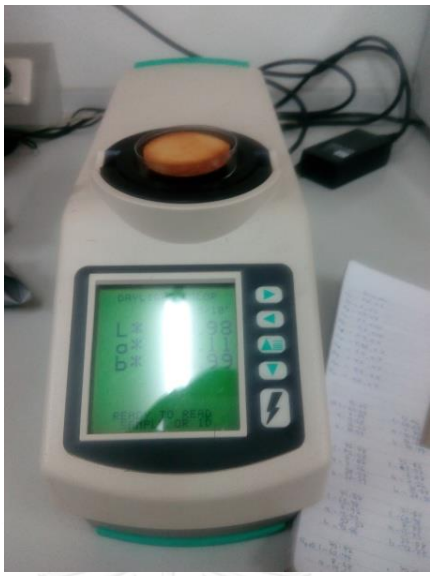
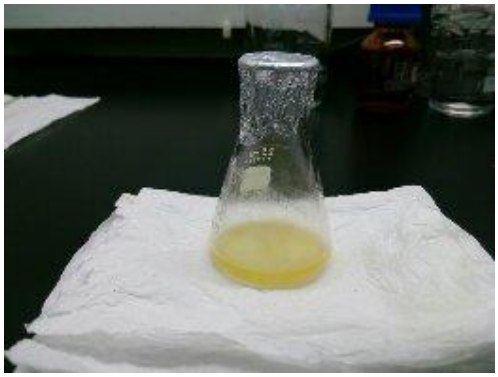


Figure 38. Color measurements from top side of cookies using colorimeter.

Appendix 4. Determination of peroxide value in cookies.



Fat dissolved in solvent mixture (ethanol:dichloromethane)

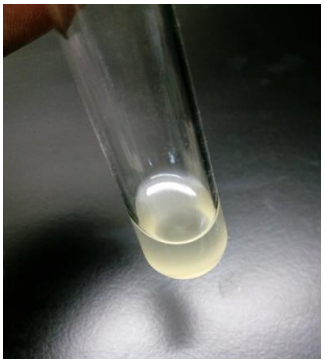
Mixture kept in the dark for 3 mins after addition of KI

Addition of starch indicator to mixture. Blue black color appears.

Sodium thiosulphate titrated against mixture until blue black color disappears.

Appendix 5. Determination of hydrolysis of fat (Acid value).

Solvent mixture (diethyl ether : acetic acid) neutralized by adding phenolphthalein and titrating against sodium hydroxide (NaOH).



Sample fat dissolved in solvent mixture

Addition of phenolphthalein to develop pink color.

Sodium hydroxide titrated against mixture until pink color disappears.

Appendix 6 Questionnaire for descriptive sensory analysis

Name of Panel: **Date:**

Quantitative Descriptive Analysis of Cookies

Please fully describe the sample cookies with codes under these main attributes by listing terms/words that appropriately describes the cookie.

List of Attributes

- ❖ Appearance Characteristics
- ❖ Skin feel Characteristics
- Fat/Moisture Parameters
- ❖ Aroma Characteristics
- Olfactory Sensation
- ❖ Flavor Characteristics
- Olfactory Sensation
- Taste Sensation
- Oral feeling Factors
- ❖ Texture/Fracturability
- ❖ After taste

* Please remember to write the code of each cookie that you describe on the sheet.

Sample Code: .....

❖ Appearance Characteristics

❖ Skin feel Characteristics

- Fat/Moisture Parameters

❖ Aroma Characteristics

- Olfactory Sensation

❖ Flavor Characteristics

- Olfactory Sensation

- Taste Sensation

- Oral feeling Factors

❖ Texture/Fracturability

❖ After taste

Appendix 7 Questionnaire used for acceptability test (9 point hedonic scale)

PANEL NUMBER:

CONSUMER SENSORY ANALYSIS OF COOKIES USING 9 POINT HEDONIC SCALE

*Please tick ( ) the appropriate choice

Demographic Questions

Gender:

Male ☐ Female ☐

Age (yr):

18-25 ☐ 26-35 ☐ 36-45 ☐

45-60 ☐ >60 ☐

Cookie purchasing frequency

Never ☐

Less than once per month ☐

At least once per month ☐

At least once per week ☐

About once per day ☐

More than once per day ☐

Key For Scale

1-Dislike extremely 2-Dislike very much

3-Dislike moderately 4- Dislike slightly

5-Neither like or dislike 6-Like slightly

7-Like moderately 8-Like very much

9-Like extremely

ATTRIBUTES

NOTE:

*Please tick ( ) the appropriate choice

*Please analyze the cookie samples according to the following attributes listed below. Indicate how much you like or dislike each of the samples by checking the appropriate phrase.

SAMPLE CODE: .....

1. Appearance

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

2 Color

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

3 Hardness

4 Aroma

5 Flavor

6 Sweetness

7 Saltiness

8 Overall likeness

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

Appendix 8 Questionnaire used for QDA during storage period

NAME:

DATE:

QUANTITATIVE DESCRIPTIVE ANALYSIS USING UNSTRUCTURED LINE SCALE

NOTE:

*Please analyze the cookie samples according to the following attributes listed below by **MARKING THE APPROPRAITE POINT ON THE LINE SCALE** indicating the intensity of the attribute.

SAMPLE CODE: .....

1. Color

Very Light — Very Dark

2. Fracturability (The brittleness status during chewing).

Crumbly — Brittle

3. Hardness (The resistance of the cookie structure against the biting).

Soft — Hard

4. Moisture

None Much

5. Dryness

Oily Dry

6. Rancid aroma

None Much

7. Rancid flavor

None Much

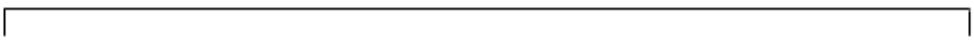
8. Acceptability of Flavor

Not Acceptable Much Acceptable

9. Acceptability of Aroma

Not Acceptable Much Acceptable

10. Overall acceptability



Not Acceptable Much Acceptable

Parameter	L*	a*	b*	Chroma	E index	Moisture	Water activity	Peroxide value	Conjugated diene	Acid value	Polyphenol	Color	Fracturability	Hardness	Moisture	Dryness	Acceptability of flavor	Acceptability of aroma	Overall Acceptance
L*	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
a*	-0.92	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b*	-0.70	0.92	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chroma	-0.75	0.95	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E index	0.97	-0.81	-0.52	-0.58	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moisture	-0.59	0.23	-0.16	-0.09	-0.76	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
Water activity	0.61	-0.25	0.14	0.07	0.77	-1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-
Peroxide value	0.99	-0.84	-0.57	-0.62	1.00	-0.72	0.73	1.00	-	-	-	-	-	-	-	-	-	-	-
Conjugated diene	0.94	-0.74	-0.42	-0.48	0.99	-0.83	0.84	0.98	1.00	-	-	-	-	-	-	-	-	-	-
Acid value	0.95	-1.00	-0.88	-0.91	0.86	-0.32	0.34	0.89	0.80	1.00	-	-	-	-	-	-	-	-	-
Polyphenol	-1.00	0.92	0.70	0.75	-0.97	0.59	-0.61	-0.99	-0.94	-0.95	1.00	-	-	-	-	-	-	-	-
Sensory																			
Color	-0.77	0.96	1.00	1.00	-0.60	-0.06	0.04	-0.65	-0.50	-0.93	0.77	1.00	-	-	-	-	-	-	-
Fracturability	-1.00	0.91	0.68	0.73	-0.98	0.61	-0.63	-0.99	-0.95	-0.95	1.00	0.75	1.00	-	-	-	-	-	-
Hardness	0.99	-0.85	-0.58	-0.64	1.00	-0.71	0.72	1.00	0.98	0.90	-0.99	-0.66	-0.99	1.00	-	-	-	-	-
Moisture	-0.82	0.53	0.16	0.23	-0.93	0.95	-0.95	-0.90	-0.96	-0.61	0.82	0.26	0.83	-0.90	1.00	-	-	-	-
Dryness	0.33	0.06	0.44	0.38	0.54	-0.96	0.95	0.48	0.63	0.03	-0.33	0.35	-0.35	0.47	-0.81	1.00	-	-	-
Acceptability of flavor	0.92	-0.70	-0.37	-0.44	0.99	-0.86	0.87	0.98	1.00	0.77	-0.92	-0.47	-0.93	0.97	-0.98	0.67	1.00	-	-
Acceptability of aroma	0.99	-0.84	-0.57	-0.63	1.00	-0.72	0.73	1.00	0.98	0.89	-0.99	-0.65	-0.99	1.00	-0.90	0.48	0.98	1.00	-
Overall Acceptance	0.92	-0.70	-0.37	-0.43	0.99	-0.86	0.87	0.98	1.00	0.76	-0.92	-0.46	-0.93	0.97	-0.98	0.67	1.00	0.97	1.00

Table 24 Correlation between instrumental, chemical measurements and sensory analysis at week 0.

Parameter	L*	a*	b*	Chroma	E index	Moisture	Water activity	Peroxide value	Conjugated diene	Acid value	Polyphenol	Color	Fracturability	Hardness	Moisture	Dryness	Acceptability of flavor	Acceptability of aroma	Overall Acceptance
L*	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
a*	-0.85	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b*	-0.79	1.00	1.00	-															
Chroma	-0.80	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E index	0.99	-0.76	-0.69	-0.71	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moisture	-0.68	0.18	0.08	0.10	-0.78	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
Water activity	0.74	-0.27	-0.17	-0.19	0.83	-1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-
Peroxide value	0.93	-0.59	-0.51	-0.53	0.97	-0.90	0.94	1.00	-	-	-	-	-	-	-	-	-	-	-
Conjugated diene	0.99	-0.78	-0.71	-0.73	1.00	-0.76	0.82	0.97	1.00	-	-	-	-	-	-	-	-	-	-
Acid value	0.99	-0.91	-0.86	-0.87	0.96	-0.58	0.65	0.87	0.97	1.00	-	-	-	-	-	-	-	-	-
Polyphenol	-0.98	0.73	0.65	0.67	-1.00	0.81	-0.86	-0.98	-1.00	-0.95	1.00	-	-	-	-	-	-	-	-
Sensory																			
Color	-0.95	0.64	0.56	0.58	-0.99	0.87	-0.91	-1.00	-0.98	-0.90	0.99	1.00	-	-	-	-	-	-	-
Fracturability	0.14	-0.65	-0.72	-0.71	-0.00	0.63	-0.56	-0.23	0.02	0.27	0.06	0.17	1.00	-	-	-	-	-	-
Hardness	-0.84	1.00	1.00	1.00	-0.75	0.17	-0.26	-0.58	-0.77	-0.91	0.72	0.64	-0.66	1.00	-	-	-	-	-
Moisture	-0.74	0.26	0.16	0.18	-0.83	1.00	-1.00	-0.93	-0.81	-0.64	0.85	0.91	0.57	0.25	1.00	-	-	-	-
Dryness	-0.79	0.99	1.00	1.00	-0.69	0.08	-0.17	-0.50	-0.71	-0.86	0.65	0.56	-0.72	1.00	0.16	1.00	-	-	-
Acceptability of flavor	0.99	-0.75	-0.67	-0.69	1.00	-0.79	0.84	0.98	1.00	0.96	-1.00	-0.99	-0.02	-0.74	-0.84	-0.67	1.00	-	-
Acceptability of aroma	0.92	-0.56	-0.47	-0.50	0.97	-0.92	0.95	1.00	0.96	0.86	-0.98	-1.00	-0.27	-0.55	-0.95	-0.47	0.97	1.00	-
Overall Acceptance	0.94	-0.61	-0.53	-0.55	0.98	-0.89	0.93	1.00	0.97	0.89	-0.99	-1.00	-0.21	-0.60	-0.92	-0.53	0.98	1.00	1.00

Table 25 Correlation between instrumental, chemical measurements and sensory analysis at week 1

Parameter	L*	a*	b*	Chroma	E index	Moisture	Water activity	Peroxide value	Conjugated diene	Acid value	Polyphenol	Color	Fracturability	Hardness	Moisture	Dryness	Acceptability of flavor	Acceptability of aroma	Overall Acceptance
L*	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
a*	-0.88	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b*	-1.00	0.85	1.00	-															
Chroma	-1.00	0.90	0.99	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E index	1.00	-0.86	-1.00	-1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moisture	-0.98	0.77	0.99	0.97	-0.99	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
Water activity	0.39	0.10	-0.44	-0.34	0.42	-0.57	1.00	-	-	-	-	-	-	-	-	-	-	-	-
Peroxide value	0.94	-0.65	-0.96	-0.92	0.95	-0.99	0.69	1.00	-	-	-	-	-	-	-	-	-	-	-
Conjugated diene	0.99	-0.80	-1.00	-0.98	0.99	-1.00	0.52	0.98	1.00	-	-	-	-	-	-	-	-	-	-
Acid value	1.00	-0.90	-0.99	-1.00	1.00	-0.97	0.34	0.92	0.98	1.00	-	-	-	-	-	-	-	-	-
Polyphenol	-0.98	0.75	0.99	0.96	-0.98	1.00	-0.58	-0.99	-1.00	-0.96	1.00	-	-	-	-	-	-	-	-
Sensory																			
Color	-100	0.84	1.00	0.99	-1.00	0.99	-0.45	-0.96	-1.00	-0.99	0.99	1.00	-	-	-	-	-	-	-
Fracturability	0.93	-0.99	-0.90	-0.95	0.91	-0.84	0.01	0.74	0.86	0.94	-0.82	-0.90	1.00	-	-	-	-	-	-
Hardness	-0.97	0.97	0.96	0.98	-0.96	0.91	-0.16	-0.83	-0.93	-0.98	0.90	0.95	-0.99	1.00	-	-	-	-	-
Moisture	-1.00	0.88	1.00	1.00	-1.00	0.98	-0.39	-0.94	-0.99	-1.00	0.98	1.00	-0.93	0.97	1.00	-	-	-	-
Dryness	0.96	-0.71	-0.98	-0.95	0.97	-1.00	0.63	1.00	0.99	0.95	-1.00	-0.98	0.79	-0.87	-0.96	1.00	-	-	-
Acceptability of flavor	0.97	-0.73	-0.98	-0.95	0.98	-1.00	0.61	0.99	0.99	0.96	-1.00	-0.98	0.80	-0.88	-0.97	1.00	1.00	-	-
Acceptability of aroma	0.99	-0.93	-0.98	-1.00	0.99	-0.95	0.28	0.89	0.97	1.00	-0.94	-0.98	0.96	-0.99	-0.99	0.92	0.93	1.00	-
Overall Acceptance	0.94	-0.65	-0.96	-0.92	0.95	-0.99	0.69	1.00	0.98	0.92	-0.99	-0.96	0.73	-0.83	-0.93	1.00	0.99	0.89	1.00

Table 26 Correlation between instrumental, chemical measurements and sensory analysis at week 2

Parameter	L*	a*	b*	Chroma	E index	Moisture	Water activity	Peroxide value	Conjugated diene	Acid value	Polyphenol	Color	Fracturability	Hardness	Moisture	Dryness	Acceptability of flavor	Acceptability of aroma	Overall Acceptance
L*	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
a*	-0.97	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b*	-0.83	0.67	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chroma	-0.92	0.81	0.98	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E index	1.00	-0.98	-0.80	-0.90	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moisture	-0.85	0.70	1.00	0.99	-0.83	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
Water activity	0.98	-1.00	-0.68	-0.81	0.98	-0.71	1.00	-	-	-	-	-	-	-	-	-	-	-	-
Peroxide value	0.95	-0.84	-0.97	-1.00	0.93	-0.98	0.85	1.00	-	-	-	-	-	-	-	-	-	-	-
Conjugated diene	1.00	-0.95	-0.88	-0.95	0.99	-0.90	0.95	0.97	1.00	-	-	-	-	-	-	-	-	-	-
Acid value	1.00	-0.99	-0.78	-0.89	1.00	-0.81	0.99	0.92	0.99	1.00	-	-	-	-	-	-	-	-	-
Polyphenol	-0.99	0.94	0.89	0.96	-0.99	0.91	-0.94	-0.98	-1.00	-0.98	1.00	-	-	-	-	-	-	-	-
Sensory																			
Color	-1.00	0.97	0.84	0.93	-1.00	0.86	-0.97	-0.95	-1.00	-1.00	1.00	1.00	-	-	-	-	-	-	-
Fracturability	0.91	-0.98	-0.52	-0.68	0.93	-0.55	0.98	0.72	0.87	0.94	-0.85	-0.90	1.00	-	-	-	-	-	-
Hardness	0.57	-0.75	-0.01	-0.21	0.61	-0.05	0.74	0.27	0.49	0.63	-0.47	-0.56	0.86	1.00	-	-	-	-	-
Moisture	-0.98	1.00	0.69	0.82	-0.99	0.72	-1.00	-0.86	-0.96	-0.99	0.95	0.98	-0.98	-0.73	1.00	-	-	-	-
Dryness	-0.49	0.27	0.89	0.79	-0.45	0.87	-0.28	-0.75	-0.57	-0.42	0.59	0.50	-0.08	0.44	0.30	1.00	-	-	-
Acceptability of flavor	1.00	-0.94	-0.88	-0.96	0.99	-0.90	0.95	0.97	1.00	0.98	-1.00	-1.00	0.86	0.48	-0.95	-0.58	1.00	-	-
Acceptability of aroma	0.96	-1.00	-0.64	-0.78	0.97	-0.67	1.00	0.82	0.93	0.98	-0.92	-0.96	0.99	0.77	-1.00	-0.23	0.93	1.00	-
Overall Acceptance	0.99	-0.92	-0.91	-0.98	0.98	-0.93	0.92	0.99	1.00	0.97	-1.00	-0.99	0.82	0.42	-0.93	-0.63	1.00	0.90	1.00

Table 27 Correlation between instrumental, chemical measurements and sensory analysis at week 3

Parameter	L*	a*	b*	Chroma	E index	Moisture	Water activity	Peroxide value	Conjugated diene	Acid value	Polyphenol	Color	Fracturability	Hardness	Moisture	Dryness	Acceptability of flavor	Acceptability of aroma	Overall Acceptance
L*	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
a*	-0.93	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b*	-0.77	0.95	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chroma	-0.83	0.98	0.99	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E index	1.00	-0.89	-0.71	-0.78	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moisture	-0.79	0.96	1.00	1.00	-0.73	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
Water activity	0.90	-0.67	-0.41	-0.51	0.93	-0.43	1.00	-	-	-	-	-	-	-	-	-	-	-	-
Peroxide value	0.93	-0.72	-0.47	-0.57	0.96	-0.50	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-
Conjugated diene	0.94	-0.76	-0.51	-0.60	0.97	-0.54	0.99	1.00	1.00	-	-	-	-	-	-	-	-	-	-
Acid value	0.99	-0.88	-0.68	-0.76	1.00	-0.70	0.95	0.97	0.98	1.00	-	-	-	-	-	-	-	-	-
Polyphenol	-0.97	0.81	0.59	0.68	-0.99	0.61	-0.98	-0.99	-1.00	-0.99	1.00	-	-	-	-	-	-	-	-
Sensory																			
Color	-0.88	0.99	0.98	1.00	-0.84	0.99	-0.58	-0.64	-0.68	-0.82	0.74	1.00	-	-	-	-	-	-	-
Fracturability	0.49	-0.77	-0.93	-0.89	0.41	-0.92	0.05	0.12	0.17	0.37	-0.26	-0.84	1.00	-	-	-	-	-	-
Hardness	0.53	-0.81	-0.95	-0.91	0.46	-0.94	0.11	0.18	0.22	0.42	-0.31	-0.87	1.00	1.00	-	-	-	-	-
Moisture	-0.11	0.47	0.72	0.64	-0.02	0.70	0.34	0.27	0.22	0.01	-0.13	0.57	-0.92	-0.90	1.00	-	-	-	-
Dryness	1.00	-0.91	-0.74	-0.81	1.00	-0.76	0.92	0.94	0.96	1.00	-0.98	-0.86	0.45	0.50	-0.07	1.00	-	-	-
Acceptability of flavor	0.99	-0.88	-0.69	-0.77	1.00	-0.71	0.94	0.96	0.98	1.00	-0.99	-0.82	0.38	0.44	0.00	1.00	1.00	-	-
Acceptability of aroma	1.00	-0.94	-0.79	-0.85	0.99	-0.80	0.89	0.92	0.94	0.99	-0.96	-0.89	0.51	0.56	-0.14	1.00	0.99	1.00	-
Overall Acceptance	0.91	-0.69	-0.43	-0.52	0.94	-0.45	1.00	1.00	1.00	0.95	-0.98	-0.60	0.07	0.12	0.32	0.92	0.95	0.89	1.00

Table 28 Correlation between instrumental, chemical measurements and sensory analysis at week 4

VITA

Deborah Owusua Danso was born in 1st October, 1988 at Akuse in the Eastern Region, Ghana and graduated from high school in Akosombo. She attended University of Cape Coast for undergraduate degree in the department of Biochemistry from Faculty of Biological Science. She graduated with her Bachelors of Science (Biochemistry) with second class honor in July, 2011. Afterwards, with much concerns about nutrition in her country, she was really interested in finding new knowledge and experiences in graduate degree with regards to this area. Thus, she entered to study in the master degree at Faculty of Allied Health Sciences, Chulalongkorn University in the department of Nutrition and dietetics and the major of applied nutrition. Her research focused on the effect of Moringa oleifera leave extract on lipid peroxidation and sensory characteristics in cookies and her thesis advisor was Dr. Sathaporn Ngamukote.