

**Addis Ababa University**

**Faculty of Natural Science**

**Department of Food Science and Nutrition Center**



Level of Saturation and Antioxidant Value of Heat and Spice Treated Animal

Butter

**By:**

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The thesis submitted to the Graduate school of Addis Ababa University Faculty of Natural science in partial fulfillment of the requirements for the degree of Master of Science

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# **Addis Ababa University**

## **School of graduate studies**

### **Food science and nutrition center**

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## **Abstract**

*This study aimed to determine the effect of heat and spice treatment on the level of saturation and oxidative stability of both fresh and rancid animal butter. The spices used were ginger, garlic, and Korarima. Two different ratios, 80% animal butter: 20% spices and 90% animal butter:10% spices were prepared from each butter treatment and various spices. The treatments were packaged in a transparent plastic jar. One group of the treatments stored at 25°C and the other group stored at 65°C to accelerate the oxidation for 3 days while samples were taken for analysis at 0, 36 and 72 hours of storage. Samples were examined for bromine value, Peroxide value, p-anisidine value, and free fatty acid value. At 65°C free fatty acid value ranged from 1.22mg KOH/g - 3.44mg KOH/g and 1.72 mgKOH/g - 5.71mg KOH/g for fresh and rancid butter respectively after 72 hours while it ranges from 0.44 mgKOH/g - 0.94 mgKOH/g and 1.44 mgKOH/g -4.61 mgKOH/g for fresh and rancid butter respectively after 72 hours at 25°C. Spice treated butter exhibited lower values of FFA and PV when compared to untreated (UCB) samples for both fresh and rancid butter, but it showed higher bromine and p-anisidine value at 65°C. It was observed that bromine value of both the fresh and rancid decreased as storage days increased at 65°C. But the change for all values at 25°C is slow during storage time except for samples of Unclarified fresh butter (UCFB). Higher FFA, PV, p- Anisidine and totox values indicated the poor quality contributed by the type of butter and storage deterioration.*

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## Chapter 1

### 1. Introduction

#### 1.1 Background of the study

During the past two decades, the relationship between diet and health has been widely studied and increasing numbers of consumers have been encouraged to improve their eating habits. Moreover, fat consumption particularly that of saturated fat, is still considered to be excessive. According to the American Heart Association (2004), the daily ingestion of lipids for individuals with normal blood cholesterol levels, should be no more than 30% of the total calorie intake, that of saturated fat no more than 10% of the total calorie intake and cholesterol intake below 300 mg/day.

The butter deteriorates by auto-oxidation of fat, what leads to flavor defects after 1 month to 2 years under the cold storage conditions, depending on the temperature of storage, quality of original milk and the proceeding of processes involved in butter manufacturing. Generation of off flavors is accompanied with the formation of hydroperoxides which are harmful to human health. The process of fat oxidation can be prevented by adding natural or synthetic antioxidant substances. However, it was found that the artificial antioxidants, like BHT (butylated hydroxytoluene) or BHA (butylated hydroxyanisole) are not safe for human consumption (are suspected to have carcinogenic activity). On the other hand, increasing sensitivity of consumers to synthetic ingredients as well as their increasing awareness about the effect of diet on their health contributed to the increasing trend to use natural additives for the stabilization of fat-containing foodstuffs. A safe guarding fat against oxidation is normally done by restricting the access of oxygen or adding antioxidants. Therefore, attention is focused on natural antioxidants (*Walstra et al. (1999)*). Spices and herbs are known to possess a great potential as the natural

antioxidants in food. These antioxidants are polyphenol compounds (Helle & Grete, 1995; Yen, Chang, & Su, 2003), which are found in all plants and in all parts of the plants (tree bark, stalks, leaves, fruits, roots, flowers, pods and seeds) (Aruoma et al., 1995; Kim, Kim, Kim, & Heo, 1997).

Ginger and garlic are common spices in Ethiopia. They are a group of esoteric food adjuncts that have been in use for many years to enhance the sensory quality of foods. These spice ingredients impart characteristic flavor, aroma, or piquancy and color to foods. Several researches have documented the use of plant extracts or their essential oils as additives in food which demonstrated antioxidant properties (Banon *et al.*, 2007; Carpenter *et al.*, 2007; Ifesan *et al.*, 2009; Ifesan *et al.*, 2010).

## **1.2 Statement of the problem**

Butter is the major food item used in every part of Ethiopia. It contains important nutrients such as fat, vitamin, cholesterol, and carotene. Animal butter is highly appreciated by the people of Ethiopia. However, in general, it has cholesterol, total lipid and saturated fatty acid. Lipids play an important role in food product quality, making them more desirable by improving the organoleptic properties of flavor, color and texture. On the other hand, the lipid components are susceptible to attack by molecular oxygen, resulting in lipid oxidation which in turn results in fat deterioration.

Heat is the major factor that accelerates oxidation of fat. The fats of foods can be deteriorated by oxidation, especially affecting foods containing unsaturated fatty acids (British Department of Health, 1994). But the natural anti oxidants such as garlic, ginger and korarima can improve the oxidative stability of fats. In Ethiopia most rural and urban people treat butter commonly by heat

and spice for a long shelf life and good flavor. Thus this study aimed to determine the effect of heat and spice treatment on the level of saturation (alteration in the level of multiple bonds) and oxidative stability of animal butter.

### **1.3 Significance of the study**

It provides information/knowledge to the people/organization about the saturation level of different types of butter. It also provides an empirical data on the difference between raw, heat and spice treated animal butter. It will be the base for other researchers who want to work on similar study in the future.

### **1.4 Objective of the study**

The first objective of this study was aimed at determining and comparing level of saturation and oxidative stability of fresh and rancid butter. The hypothesis underlying this objective was that rancid butters will share similar chemical properties particularly the level of saturation and oxidative stability with fresh butter. The second objective of this study was to determine the level of saturation and oxidative stability of each butter type(fresh and rancid) when treated with and without some selected natural antioxidants, considering the butter is fat comprised and which are susceptible to oxidative degradation. The hypothesis underlying this objective was that selected antioxidants will prevent the oxidative degradation of butter and thus result in extended shelf-life than untreated butter.

## Chapter 2

### 1 Review of the Literature

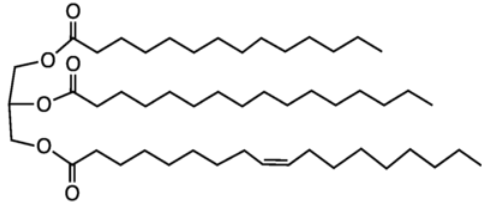
#### 2.1 Butter fat

One of the healthiest whole foods we can include in our diet is butter. Unfortunately, butter and dairy products are usually derived from cows that are confined indoors and fed grains. The diet of the cows used to produce butter can greatly influence its nutrient content (Hettinga, 2005)

Butter is a dairy product made by churning fresh or fermented cream or milk. It is generally used as a spread and a condiment, as well as in cooking, such as baking, sauce making, and pan frying. (Jacob, 2011)

Most frequently made from cows' milk, butter can also be manufactured from the milk of other mammals, including sheep, goats, buffalo, and yaks. Butter is a water-in-oil emulsion resulting from an inversion of the cream, an oil-in-water emulsion; the milk proteins are the emulsifiers. Butter remains a solid when refrigerated, but softens to a spreadable consistency at room temperature, and melts to a thin liquid consistency at 32–35 °C (90–95 °F). The density of butter is 911 g/L. It generally has a pale yellow color, but varies from deep yellow to nearly white. Its unmodified color is dependent on the animals' feed and is commonly manipulated with food colorings in the commercial manufacturing process, most commonly annatto or carotene. Unhomogenized milk and cream contain butterfat in microscopic globules. These globules are surrounded by membranes made of phospholipids (fatty acid emulsifiers) and proteins, which prevent the fat in milk from pooling together into a single mass. Butter is produced by agitating cream, which damages these membranes and allows the milk fats to conjoin, separating from the other parts of the cream. (Hettinga, 2005).

Butter is mainly a triglyceride (fat) derived from fatty acids such as myristic, palmitic, and oleic acids. (McGill, 1979; Gurr, 1992)



**Figure 1** Triglyceride. **Source:** [http://en.wikipedia.org/wiki/file Triglyceride. Dairy. Butter.png](http://en.wikipedia.org/wiki/file%20Triglyceride%20Dairy%20Butter.png).

Commercial butter is about 80% butterfat and 15% water; traditionally made butter may have as little as 65% fats and 30% water. Butterfat is a mixture of triglyceride, a tri-ester derived from glycerol and three of any of several fatty acid groups. Butter becomes rancid when these chains break down into smaller components, like butyric acid and di-acetyl (Free encyclopedia, 2012).

There is no such thing as a completely saturated or a completely unsaturated fat; every fat is a combination of both saturated and unsaturated fatty acids. Butter, beef suet, and tallow are about 50% saturated, but all other animal fats are more unsaturated than they are saturated. Scientists now know that, except in rare individuals, dietary cholesterol does not influence blood cholesterol, and fat from ruminants (animals that chew their cud) contains valuable nutrients that maintain health and prevent disease. (Douma, 2008)

It is important to remember that every cell in our body needs fat, and that dietary fat is a cornerstone of good health. Our brain and hormones rely on fat to function, and fat supports our immune system, fights disease, and protects our liver. Fat promotes clear skin and healthy hair, regulates our digestive system, and leaves us feeling sated after a meal. Fat is the body's preferred fuel, providing us with more than twice the amount of energy as the same quantity of carbohydrates and protein. It helps the body absorb nutrients, calcium, and the fat-soluble vitamins A, D, E, and K. (Douma, 2008)

Fat and protein are found together in nature because it's the fat that helps us digest the protein. So, it makes good sense to eat a well-marbled steak, or a roast chicken with crispy skin. Because fat is digested slowly, eating it leaves us sated and less likely to snack between meals. When you eat a moderate amount of good fat, you'll probably lose weight, but when you replace fat with sugar and carbohydrates, you'll likely gain weight.

Another group of fats, trans fats, is being increasingly scrutinized. These man-made fats are created when liquid or polyunsaturated fats are made solid by adding hydrogen. These fats are difficult for our body to process, so it stores them. They adversely affect our cholesterol levels by increasing bad cholesterol (LDL) and lowering good cholesterol (HDL), and they interfere with insulin production, promoting diabetes and obesity. As the dangers of these fats are being understood, efforts are underway to expose and remove them from our food. Food manufacturers must declare their presence on food labels, and several cities have moved to ban them from restaurants – creating fierce debate in these communities. Caught up in this campaign against trans fat is conjugated linoleic acid (CLA), a natural trans fat found in butter and the fat from pasture-fed ruminants. This trans fat is beneficial to our health, credited with fighting cancer, and preventing weight gain and heart disease. (Douma, 2008)

Humans have been eating butter and animal fat a lot longer than they have been abstaining from them. Good animal fat, like butter, plays an essential role in maintaining our health, as do quality ingredients and moderate consumption.

## 2.2 Oxidation of butter

The susceptibility of butter to oxidative reactions has been investigated. Emmons et al., 1986 showed that butter held frozen (-18 °C) and in the dark showed no evidence of oxidation after 1 year of storage, but there was some loss of butter quality after 14 weeks storage in the dark at 5 °C. However, stored butter in light, either in fluorescent in the cold (5 °C) or daylight at 22°C, evidence of lipid oxidation (cholesterol oxide production) was found. Their data showed that both singlet and free radical oxidation was occurring (Luby et al., 1986).

Temperature has a big influence on shelf-life, as the rate of reaction of oxygen with fats roughly doubles for every 10°C increase in temperature (Rossel, 1989).

Ambient shelf-life test is very time consuming and thus not generally practical. So several accelerating methods used for testing oxidative stability of fats and oils. Schaal oven test is commonly used method which involves simply keeping a sample of oil or fat in a loosely sealed glass container in an oven at 60-65°C. (Rossel, 1989).

Oxidative rancidity results from more complex lipid oxidation processes. The processes are generally considered to occur in three phases: an initiation or induction phase, a propagation phase, and a termination phase. In complex systems, the products of each of these phases will increase and decrease over time, making it difficult to quantitatively measure lipid oxidation. During the initiation phase, molecular oxygen combines with unsaturated fatty acids to produce hydroperoxides and free radicals, both of which are very reactive. For this phase to occur at any meaningful rate, some type of oxidative initiators must also be present, such as chemical oxidizers, transition metals (i.e., iron or copper), or enzymes (i.e., lipoxygenases). Heat and light also increase the rate of this and other phases of lipid oxidation. The reactive products of this

initiation phase will, in turn, react with additional lipid molecules to form other reactive chemical species. The propagation of further oxidation by lipid oxidation products gives rise to the term "auto-oxidation" that is often used to refer to this process. In the final, termination phase of lipid oxidation, relatively unreactive compounds are formed including hydrocarbons, aldehydes, and ketones. (Yildiz, et al., 2002)

In determining oxidative stability and quality of butter, the peroxide value reflecting the oxidation of fatty acids, the amount of free fatty acid and the degree of lipolysis are taken into consideration. The amount of free fatty acids (FFAs) in the medium is defined as the amount of KOH (mg) needed to neutralize FFAs in 1 g of butter. It is a measure of the degree of lipolysis. When the amount reaches 1.8 mg KOH/g fat, there is a perceptible off-flavour in butter. The relation between the amount of FFA and peroxide value against butter flavour was analyzed. Samples with FFAs exceeding 3.3 mg KOH/g fat were recognized as rancid or spoiled by 59% of the panelists. An off-flavour is also perceived in butter when peroxide values reach 2 meq O<sub>2</sub>/kg fat. Interestingly, the acceptable value is set at 10 meq O<sub>2</sub>/kg fat by the Food Regulations in Turkey. Atamer M (1993)

## **2.3 Antioxidants**

Spices and herbs are known to possess a great potential as the natural antioxidants in food. During the past few decades, the use of natural antioxidants and plant extracts have received increased interest due to the concerns about possible ill health effects generated by the use of synthetic antioxidants. The protective effects of antioxidants on products are generally determined by measuring several variables, including the induction period under normal storage conditions and oxidation accelerating conditions. Nothing can make the possibility of rancidity

go away completely, but anti-oxidants are huge weapons in fighting on ickiness! Free radicals are constantly running around in the fat(butter), seeking out electrons to fill its valence shell. When we add an anti-oxidant, we provide that free radical with those electrons. The radical is content with its electron shell and bothers us no more! (Abramovic and Abram, 2006; Kowalski, 2007; Azizkhani and Zandi, 2009).

Antioxidants can act as primary chain breaking oxidants, or as secondary preventative antioxidants. They act as chain breakers by donating hydrogen atoms (Gorden, 1990). Secondary antioxidants reduce the rate of chain initiation by various mechanisms such as scavenging oxygen, decompose hydroperoxides to non-radical species, binding to metal ions, absorb UV radiation or deactivate single oxygen (Gorden, 1990)

The effectiveness of various antioxidants are generally determined under several conditions that include the induction period, normal storage conditions, and oxidation-accelerating conditions, such as high temperatures and airflow. The usual parameters measured in determining the effectiveness of antioxidants include sensory characteristics, volatile headspace oxidation products, peroxide values, conjugated dienes and trienes, p-anisidine values, thiobarbituric acid reactive substances (TBARS), and fatty acid composition (Pokorny *et al.*,2001; Judde *et al.*, 2003; Abramovic and Abram, 2006; Azizkhani and Zandim, 2009; Kowalski,2007; Jennings, et al., 2009). But the bromine value or Iodine value can also be a good parameter of indication of lipid oxidation, since there is a decline in unsaturation during oxidation

### **2.3.1 Ginger (*Zingiber officinale*)**

Ginger (*Zingiber officinale*) has been used as a spice for over 2000 years. Its roots and the obtained extracts contain polyphenol compounds (6-gingerol and its derivatives), which have a

high antioxidant activity. Although the digestion stimulating effect of this spice became known a long time ago, the stimulating effect on peptic juices, such as gastric juice, bile, pancreatic and intestinal juices, was discovered later. Bile acids play a major role in the uptake of fats and each upset in the metabolism of fats would impede food digestion as a whole, because the fatty particles cover the other food elements and make them inaccessible for the action of the digestive enzymes. (Bartley & Jacobs, 2000) Lipase is the other key factor which plays a vital role in fat digestion. When ginger was included in animal diets, it was found that there was a considerable increase in the pancreatic and intestine lipase (Platel & Srinivasan, 2000). Ginger is an indispensable component of many food additives. With regard to antioxidant properties of ginger, it can be successfully used as a component of curry powder, sauces, ginger bread and ginger flavoured carbonated drinks and in preparation of dietaries for its aroma and flavour. Many authors discuss the chemical composition of ginger, but no toxic effect was found. The ginger extract has an antioxidant activity comparable with that of BHT in inhibiting the lipid peroxidation both at 37°C, and at a high temperature of 80°C. Most inhibited was the stage of formation of secondary products of the auto-oxidation of fats. (Stoilov, 2007)

Ginger is commonly use in food as spice. The main components of ginger are 6-gingerol, 6-shogaol, 8-gingerol and 10- gingerol and these constituents had exhibited strong antioxidative activity (Kim et al., 2007)

**Table 1 Proximate Composition of ginger: (100g, edible portion)**

compositions	amount	compositions	amount
Water	9.4 g	Iron	12 mg
Food energy	347kcal	Magnesium	184 mg
Protein	9.1g	Phosphorus	148 mg
Fat	6.0g	Potassium	1342 mg
Totalcarbohydrate	70.8g	Sodium	32 mg
Fiber	5.9g	Zinc	5 mg
Ash	4.8g	Niacin	5 mg
Calcium	116mg	Vitamina	147 IU

(Farrell, 1985)

Several investigations in Japan reported that ginger or ginger extract added to lard or other foods showed reasonably strong antioxidant property (Saito et al., 1976). Ginger extract was effective in retarding the development of rancidity in salted pork patties and its effectiveness was directly related to the concentration (Lee et al., 1986). They concluded that extent of retardation of oxidation was proportionate to the amount of ginger extract added to the product. Shogaol and zingerone found in ginger exhibited strong antioxidant activities (Kikuzake and Nakatani, 1993)

### **2.3.2 Garlic (*Allium sativum*)**

Garlic had been known for centuries before the Christian era. Its color is brownish yellow and its odor is pungent and disagreeable. It has since long been recognized all over the world as a valuable condiment for foods and a popular remedy for various diseases and physiological

disorders. Pliny, Virgil and Hippocrates mentioned garlic as a treatment for a variety of ailments. However, not until the 1940's did scientific evidence appear to establish that garlic indeed did possess antimicrobial medicinal properties (Cavallito and Bailey, 1944).

Garlic is indigenous to Ethiopia and is cultivated worldwide for the fleshy segments of its bulbs, which are used as a condiment. Antioxidant potential of garlic in vivo and in vitro has been proved (Jackson et al., 2002). In addition to its antioxidant activity, it has antimicrobial, antibacterial, antiviral, antifungal, antiprotozoal properties and beneficial effects on the cardiovascular and immune systems (Harris, Cottrell, Plummer, & Lloyd, 2001). Garlic is rich in selenium and organosulphur compounds, which have pronounced antioxidant activity (Yin, Hwang, & Chan, 2002 ;). Garlic has been a favorite additive in food for many years in various cultures. It is known that garlic (*Allium sativum*) possesses antimicrobial, antiprotozoal, antimutagenic, antiplatelet and antihyperlipidemic properties. Allicin, the extract of garlic, has been presumed to be a very strong antioxidant. Allicin gives garlic its characteristic ungent smell. Also, it contains vitamins and minerals and trace elements (selenium and germanium). (Lawson *et.al.*, 1991)

According to Unani and Ayurvedic system as practiced in India, garlic is carminative and is a gastric stimulant. In modern allopathy, it is being used in a number of patented medicines and other preparations (Pruthi, 1976).

**Table 2 Proximate Composition of garlic: (100g, edible portion)**

compositions	amount	compositions	amount
Water	6.5g	Iron	3mg
Food energy	332 kcal	Magnesium	58mg
Protein	16.8 g	Phosphorus	419 mg
Fat	0.4g	Potassium	1101 mg
Total carbohydrate	77.6g	Sodium	26 mg
Fiber	1.9g	Zinc	3 mg
Ash	3.3 g	Other Vitamins	Insignificant
Calcium	55g	-----	-----
			-

(Farrell, 1985)

The extracts of garlic is identified as allicin or diallyl thiosulphinic acid. The constituents of the oil are 60% diallyl disulfide, 20% diallyl trisulfide, 6% allyl propyl disulphide and smaller quantities of diethyl, polysulfide, allinn and allicin. The true garlic odor is said to be derived from the diallyl disulfide. Commercial undiluted oil of garlic has 200 times the strength of dedhydrated garlic or 900 times the strength of fresh garlic (Farrell, 1985).

Garlic extract is also known as a food preservative (Krishnamurthy and Srinivasamurthy, 1956) and fresh garlic ground with meat prolongs the shelf life of fresh meat (Al-Delaimy and Barakat, 1971). Garlic oil has been found to have potent antioxidant activities in various oils (Shahidi and Wanasundara, 1992). Garlic products have been suggested to be beneficial to human health

(Dorant et al., 1993) by exerting antioxidant activity (Prasad et al.,1995). Consumption of garlic tablets (Kwai) has been reported to decrease the susceptibility of human low density lipoproteins to oxidation (Phelps and Harris, 1993). Aged garlic extract has recently been shown to protect vascular endothelial cells from H<sub>2</sub>O<sub>2</sub>-induced oxidant injury (Yamasake et al., 1994). Garlic has been shown to reduce radicals generated by the Fenton reaction (Torok et al., 1994).

### **2.3.3 Korarima(Aframomum Corrorima)**

Korerima is a milder spice similar to coriander, and is also highly popular for stews. Korerima is considered one of the few spices native to Ethiopia, where it is found growing in southern rain forests. Korerima is said to aid the digestive tract, and to cure common colds and upper respiratory infections. Generally roasted over an open fire, the smoky aroma adds a distinct flavor to Berbere sauces, clarified butter and other chili blends. Also it is used in condiment spice blends with Kitfo- Ethiopian Style Steak Tartar(Jansen, 1981)

Korarima (*Aframomum corrorima*) or the Ethiopian cardamom is a renowned spice and medicinal crop of the family Zingiberaceae native to Ethiopia. The dried fruits are part and parcel of the daily dishes of the Ethiopians. They are also used as a carminative, purgative and tonic in the traditional medicine (Jansen, 1981). korarima oil has similar chemical composition with that of its famous relative, the Indian cardamom (*Elettaria cardamomum*), except for its reduced content of terpinyl acetate, which is the major component in the latter (Sebsebe, 1993). Previously Ethiopia was well known for its considerable exports of korarima capsules to the world market, mainly as a substitute for the Indian cardamom.<sup>2</sup> However, the supply has greatly fluctuated during the past few decades that the total annual korarima export has decreased to less than 60 tones in the years 1994 - 1998, fetching only some 2.1 million USD (Chanyalew, 1999)

Korarima seed has a mild, sweet flavour and is less peppery or pungent than seed of *Aframomum melegueta* K.Schum (grain of paradise). The seeds contain essential oil which has a typical odour and is sometimes called 'nutmeg-cardamom'

Korarima (*Aframomum corrorima*) seeds, pods, leaves, rhizomes and flowers are used in traditional medicine and as spices in southern Ethiopia. The extracts and essential oils of korarima are potentially used as good sources of antioxidants. (Eyob et al., 2008).



Korarima with pod



Seeds of Korarima



Powder of Korarima seeds



Garlic



Garlic powder



Ginger



Ginger powder

**Figure 2. The spices and their powders used for butter treatment**

## **2.4 Preparation of antioxidants**

There is a big difference between the preparation of synthetic antioxidants and natural antioxidants for application in food products and processing. Synthetic antioxidants are produced as pure substances of constant composition, and are applied as such or in well defined mixtures with other pure substances. Application is thus relatively easy, requiring no substantial modifications of the recipe and processing conditions. On the contrary, natural antioxidants are available from raw materials of variable composition. Both the content of active substances (usually a mixture of several compounds) and the content of various other compounds, either inactive or possessing negligible activities, depend on the plant variety, agro technology, climatic conditions, degree of ripeness, and many other factors. Their composition should be determined in every batch, and if necessary, the procedure of their preparation or application, and the amount added to food products should be adapted according to analytical results. (Akguel A and Ayar A, 1993)

Many food components possessing antioxidant activities are used in their natural form, such as spices. The preliminary processing of such food components may be drying (in case of leaves or stems), milling of dried material (such as seeds), or some other mechanical treatment. Several ground spices (added in the amount less than 20%) were found to be active. (Akguel A and Ayar A, 1993)

## **2.5 Determination of fat oxidation**

### **2.5.1 Para-anisidine value and Peroxide value**

*Para*-anisidine is a reagent that reacts with aldehydes to give products that absorb at 350nm. The *p*-anisidine value is defined as the absorbance of a solution resulting from the reaction of 1 g fat in isooctane solution (100 ml) with *p*-anisidine (0.25 % in glacial acetic acid). The products

formed by reaction with unsaturated aldehydes (2-alkenals) absorb more strongly at this wavelength, and consequently the test is particularly sensitive to these oxidation products. Although the test does not distinguish between volatile and non-volatile products, the palate is generally more sensitive to unsaturated volatile aldehydes than to saturated volatile aldehydes, so the test is a reasonable way to assess secondary oxidation products. Measurements of *p*-anisidine value are commonly used together with peroxide value measurements in describing the total extent of oxidation by the Totox value, which equals the sum of the *p*-anisidine value plus twice the peroxide value. However, the Totox value is an empirical parameter since it corresponds to the addition of two parameters with different units.. Anisidine value is defined conventionally as the optical density measured at 350 nm, multiplied by 100 of the solution of 1g of fat in 100 ml of *p*-anisidine - acetic acid mixture. P-Anisidine number is correlated with the presence of aldehydes deriving from the second oxidation of fats. (Michael, nd)

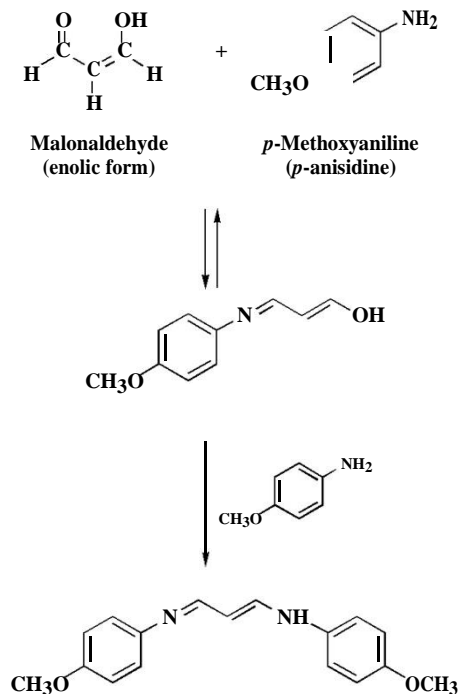
Peroxide value is one of the most widely used tests for oxidative rancidity; peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Milliequivalents of peroxide per kg of fat are measured by titration with iodide ion. Peroxide values are not static and care must be taken in handling and testing samples. It is difficult to provide a specific guideline relating peroxide value to rancidity. High peroxide values are a definite indication of a rancid fat, but moderate values may be the result of depletion of peroxides after reaching high concentrations. Oxidation reaction rates increase with temperature, and can increase more than twofold with every 10°C rise depending on the composition of the fat (Ihsan et al., 2002)

The oxidative process of oils and fats is one of the main causes of the deterioration of the principal organoleptic and nutritional characteristics of foodstuffs.

The complex oxidation process can be summarized into two phases: in the first one fat acids react with oxygen and determine odorless compounds as peroxides; during the second phase the peroxides degrade into many substances as volatile aldehydes, responsible of the rancid odour and flavour, and in a non-volatile portion. The primary oxidation products are normally measured with Peroxide Value test (PV) and the secondary products with p-Anisidine test. Anisidine value (AnV) represents the level of non-volatile aldehydes, primarily 2-alkene present in the fat. On the contrary other tests consider the volatile portion of aldehydes and, due to their intrinsic variable nature, bring to results that are less reliable. (Velasco and Dobarganes, 2002)

The oxidative status of a fat should be evaluated considering both its primary and secondary oxidation. In fact it can happen that a fat that has initially a high peroxide value, kept in stock for a long time in absence of oxygen, endures a secondary oxidative process that determines the decrease of peroxide value but the increase of anisidine value. Performing only the analysis of peroxide it can happen that fats that are not fresh or damaged, are used as ingredients without having the chance to evaluate the consequences of secondary oxidations.

Generally, peroxide value indicates the actual oxidative status in fat matrix but anisidine value indicates its history from the oxidative point of you. That is the anisidine value test is used to assess the secondary oxidation of oil and fat, which is mainly aldehydes and ketones, and is therefore able to indicate the oxidation history of an oil or fat. These two values can be combined into the TOTOX number.  $Totox = AnV + (2 \times PV)$  (Velasco and Dobarganes, 2002)



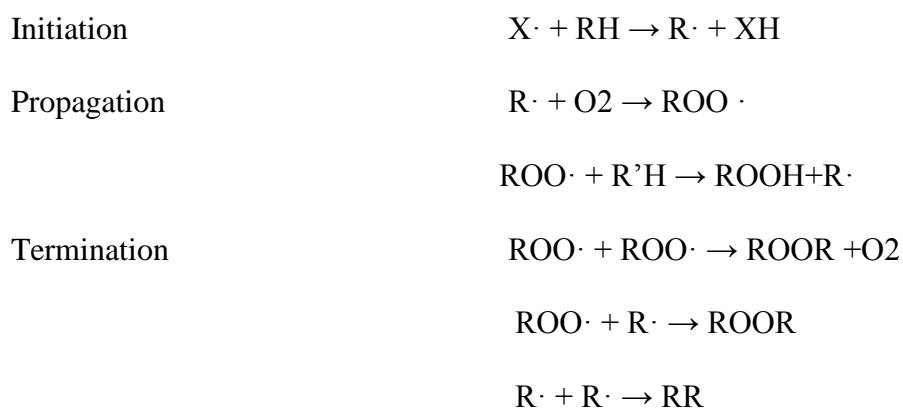
**Figure 3. Possible reactions between *p*-anisidine reagent and malonaldehyde.**

### 2.5.1.1 Mechanism of autoxidation in lipids

Once the free radical is formed, the propagation stage begins with the addition of oxygen atoms to the free radical to form peroxide (a peroxy radical: ROO•). Then the hydrogen removed from another unsaturated fatty acid attaches to the peroxide to form a hydroperoxide (ROOH). While the hydrogen is removed from the other unsaturated fatty acid, the unsaturated fatty acid forms a new free radical and the same procedure occurred in the first free radical is auto-catalytically repeated and thus this whole process is called autoxidation. Hydroperoxides can be also formed by the action of naturally occurring lipoxygenases on the polyunsaturated fatty acids before or during the extraction of oils (Pokorny *et al.*, 2001).

In the termination stage of oxidation, the autoxidation is ceased by the reaction of two radicals to form stable non-radical products such as ROOR (from peroxy and alkyl radicals), RR (from two alkyl radicals), and ROOR + O<sub>2</sub> (from two peroxy radicals).

With the presence of oxygen, lipid oxidation will be accelerated or delayed depending on the presence of pro- or anti-oxidants respectively. Lipid oxidation is facilitated under the high temperature and/or high water activity, and the presence of metals such as iron and copper, light, among others. (McWilliams, 2001; Damodaran *et al.*, 2008).



**Figure 4 Mechanism of lipid autoxidation (Pokorny *et al.*, 2001)**

**2.5.1.2 Decomposition of hydroperoxides and rancidity**

Hydroperoxides formed during lipid oxidation are not directly responsible for off-aroma and thus rancidity (Damodaran *et al.*, 2008). However, their further decomposition into alkoxy radicals (RO•) which have high energy causes β-scission, the cleavage of aliphatic chain of fatty acids into low molecular weight compounds that are responsible for perceived rancidity (Damodaran *et al.*, 2008). The low molecular weight compounds include volatile hydrocarbons, alcohols, and aldehydes and non-volatile alcohols and ketones. Volatile aldehydes are the most important contributor to off-aroma. Hexanal, heptanal, octanal, nonanal, decanal, *trans*-2-heptanal, *trans*-2-nonenal, *cis*-2-decenal, *trans,trans*-2,4-nonadienal, *trans-cis*-2,4-decadienal are some examples of volatile aldehydes that can be found in oxidized lipids including Shea butter (Pokorny *et al.*, 2001; Bail *et al.*, 2009).

### **2.5.2 Bromine value**

Bromine value is the amount of bromine in grams absorbed by 100 grams of a sample. The number indicates the degree of unsaturation. The Bromine Number is useful as a measure of aliphatic unsaturation in fat and oil samples. It is determined by the electrochemical titration of the fat or oil samples with the bromine which insures the bromination of olefins. Unsaturated fatty acids in the oils and fats of butter will decolorize bromine water. The reaction is quantitative. 1 mole of C=C bonds will react with 1 mole of bromine, Br<sub>2</sub>, and will self-indicate during a titration. At low concentrations of bromine in water a number of species are present, but at high concentrations most of the bromine is present as molecular bromine. The fats and oils in butter are not miscible with bromine water, but they will decolorize it if sufficiently vigorous shaking is used during titrations. (Nigel Saunders, 2003). Bromine value measures the degree of unstauration in a fat or vegetable oil and determines the stability of oils to oxidation (Daintith, 2008). Knowledge of the bromine value enables the combustion temperature of the oil to be evaluated (Roger *et al.*, 2010). The low bromine value for animal butter may be an indication that the butter is rich in saturated fatty acids, which ensures stability against oxidation and rancidification of foods prepared with the butter (Goh, 1994).

### **2.5.3 Free Fatty Acid Value**

The FFA determination measures the amount of hydrolytic activity that has occurred in the oil. Hydrolytic rancidity is generally caused by a combination of catalyst and moisture (Rossel, 1994). The amount of FFA present is of importance, not only because it indicates hydrolytic activity, but also because FFA has a provident effect, the intensity of which is related to FFA concentration (Frega et al, 1999).

## **Chapter 3**

### **3 Materials and methods**

#### **3.1 Sample collection**

The representative butter samples (3Kg of fresh butter and 3Kg of rancid butter) were randomly purchased from local farmers, in Wolaita zone Humbo woreda (the place where I live). and similarly the spices (ginger, korarima and garlic) were purchased from the local market in wolaita zone Humbo woreda. The reason for the selection of Wolaita zone for sample collection was that to eliminate the contamination of butter with other foreign matters. All butter samples were then stored in the refrigerator until they were needed for analysis.

#### **3.2 Preparation of spices**

Ginger and garlic were washed with water. The raw ginger and garlic and the seeds of korarima were placed in the air oven at a temperature about 50<sup>0</sup>C for two hours to dry. Then ginger and garlic were chopped and returned to the oven for further drying. After drying, the spices (the peeled garlic, unpeeled ginger and the seeds of korarima) were grounded into powdered form.

#### **3.3 Preparation of clarified butter (CB)**

Making clarified butter was involved slowly heating 400g of fresh and 400g of rancid unsalted butter to 100<sup>0</sup>C and letting it boiled until the water vaporized. Once the bubbling was stopped, three layers were remained: whey protein, liquid fat, and casein particles. After the skin of whey protein removed, the fat was poured off, which is the clarified butter.

#### **3.4 Preparation of spice treated butter**

##### **3.4.1 for fresh butter**

- 1) 400g of unclarified fresh butter (UCFBC)
- 2) 400g of clarified fresh butter (CFB)

- 3) 400g of 10% Ginger treated fresh butter (10%GTFB)
- 4) 400g of 20% Ginger treated fresh butter (20%GTFB)
- 5) 400g of 10% Garlic treated fresh butter (10%GrTFB)
- 6) 400g of 20% Garlic treated fresh butter (10%GrTFB)
- 7) 400g of 10% korarima treated fresh butter (10%KTFB)
- 8) 400g of 20% korarima treated fresh butter (20%KTFB)

### **3.4.2 For Rancid butter**

The peroxide value and free fatty acid value of the butter were determined before the preparation of spice treated and clarified butter. The rancid butter was the one with off-flavor, peroxide value greater than  $1.8\text{meqO}^2/\text{Kg}$  fat and FFA value greater than or equal to  $3.3\text{ mg KOH/g}$  fat. (Atamer and Sezgin, 1984). The samples were prepared as following

- 1) 400g of unclarified rancid butter (UCRB)
- 2) 400g of clarified rancid butter (CRB)
- 3) 400g of 10% Ginger treated rancid butter (10%GTRB)
- 4) 400g of 20% Ginger treated rancid butter (20%GTRB)
- 5) 400g of 10% Garlic treated rancid butter (10%GrTRB)
- 6) 400g of 20% Garlic treated rancid butter (10%GrTRB)
- 7) 400g of 10% korarima treated rancid butter (10%KTRB)
- 8) 400g of 20% korarima treated rancid butter (20%KTRB)

All the above mixture were then heated until it boils and a clear butter liquid was obtained (about 30 min.), which indicates the doneness of the products. The temperature did not exceed  $115^{\circ}\text{C}$  and at which the bulgur starts to gather and precipitate at the bottom of the container. Anhydrous butter fat was decanted and filtered through a piece of cheesecloth, filled in plastic jars and left to

solidify. Then from each of the above mixture 90g sample was stored at 25<sup>0</sup>C and 90g sample stored at 65<sup>0</sup>C.

### **3.5 Chemicals and reagents**

All the chemicals and the reagents obtained from Food science and Nutrition Centre, Addis Ababa. The experiments were conducted in the laboratories of “Food Science and Nutrition center”

**Chemicals:**-p-anisidine, potassium hydroxide, sodiumthiosalphate, Bromine water, starch, phenolphthalein indicator, distill water, ethanol, chloroform, isooctane, glacial acetic acid, potassium iodide, sodium hydroxide, ammonium hydroxide solution, ethyl alcohol, ethyl ether: petroleum ether solution.

### **3.6 Laboratory analysis**

Each laboratory determination was carried out on thirteen separate samples (in triplicate)

#### **3.6.1 Determination of fat content of butter**

Before analysis both fresh and rancid butter were clarified and heated to constant weight. Five gram of butter samples were taken and ammonium hydroxide solution was added in to the butter sample and mixed thoroughly. After that ethyl alcohol was added and the mixture was shaken for 1 minute. The fat was extracted from the butter sample three times using the mixture of ethyl ether: petroleum ether. After the extraction period the solvent were evaporated (Polish Standard, 2005). Then the fat content of butter was calculated as

$$\% \text{ of fat} = \frac{M_3 - M_1}{M_2 - m_1} \times 100$$

Where:

$M_1$  = weight of the container

$M_2$  = weight of the container plus crude fat

$M_3$  = weight of the sample plus weight of the container

### **3.6.2 Determination of moisture content**

Five grams of butter sample for both fresh and rancid butter were heated to constant weight in a vacuum oven at 102°C, for three hours. Then they were cooled in efficient desiccators, for 30 minute and weighed. Constant weight was attained when successive 1 hour draying periods showed additional loss, 0.05%. (Polish Standard, 2005). The moisture content was determined using:

$$\% \text{ of moisture} = \frac{w_1 - w_2}{S_w} \times 100$$

### **3.6.3 Determination of pH value**

50g of butter sample was accurately measured and transferred to the test tube. Then the sample was warmed in order to liquefy. The MP511 lab PH meter was calibrated with two standard buffer solutions having PH of 4.00 and 7.00. Then the electrode of the PH meter was inserted into the warmed solution. Between each test, the electrodes rinsed with warm distill water.

### **3.6.4 Determination of Bromine value**

0.5g of butter was weighed accurately in the conical flask and warmed gently to facilitate dissolution. Then 25ml of water added into the mixture whilst shaking the flask to disperse the butter and finally the mixture was titrated against 0.0625M bromine water until the bromine water retains its brown color. (Nigel Saunders, 2003)

### 3.6.5 Peroxide value determination

5g of butter was dissolved in 10ml chloroform in the Erlenmeyer flask and mixed with 15ml glacial acetic acid and 1ml saturated KI. Then the mixture were left in the dark at room temperature for 5 minutes. Then 75ml of distilled water was added and the mixture shaken vigorously. Finally 1ml of 1% starch solution was added and the resulting solution was titrated with 0.002N sodiumthiosalphate until the color become clear. The same procedures were followed for blank solution (without butter sample) preparation and determination (AOAC 1990). Then peroxide value was calculated as

$$PV = [(V_1 - V_0) N] / M$$

Where:  $V_1$  is the amount of  $Na_2S_2O_3$  used for titration (ml),

$V_0$  is the amount of  $Na_2S_2O_3$  used for the blank (ml)

$N$  is the normality of  $Na_2S_2O_3$  and

$M$  is the amount of sample (g).

### 3.6.6 P-Anisidine Value and Totox Value determination

1g sample of butter was dissolved in 25ml of isooctane, (test solution (a)). Then 5ml of test solution (a) was mixed with 1ml p-anisidine (0.25% in glacial acetic acid), (test solution (b)). To prepare the reference solution, 1.0 ml of a 2.5 g/l solution of p-anisidine in glacial acetic acid was added to 5.0 ml of isooctane, and the solution shaken well (IUPAC, 1987). Then the absorbance of both test solutions and reference solution were measured at 350 nm. The p-anisidine value was calculated as

$$\text{P-anisidine value} = \frac{25(1.2A_1 - A_2)}{m}$$

where:-

$A_1$  = absorbance of test solution (b) at 350 nm,

$A_2$  = absorbance of test solution (a) at 350 nm,

$m$  = mass of the substance to be examined in test solution (a), in grams.

Finally the totox value was calculated and which was equal to the sum of the p-anisidine value plus twice the peroxide value.

### **3.6.7 Determination of Free Fatty Acid Value**

5g of butter and 30ml of ethanol were mixed in Erlenmeyer flask. Then few drops of phenolphthalein indicator were added and the mixture was warmed to promote dissolution. The solution was titrated with 0.1NKOH until the pink color was stable at least for 15 seconds. (AOCS International, 2005).

### **3.7 Statistical analysis**

Each determination was carried out in triplicate and results were reproduced as an average value (mean  $\pm$  standard deviations). Data was analyzed by using one way Analysis of Variance (ANOVA) SPSS version 15.0 model. Differences between treatments were determined by using Duncan's multiple comparison tests. Statistical significance was set at  $P < 0.05$ .

## Chapter 4

### 4 Results and discussions

The rancid butter is the one with off-flavor, peroxide value greater than 1.8meqO<sup>2</sup>/Kg fat and FFA value greater than or equal to 3.3 mg KOH/g fat (Atamer and Sezgin, 1984). But the values of each parameter for fresh animal butter were highly less than that of rancid butter except AnV, PH and moisture value.

**Table 3 Initial information of the Butter**

Parameters	Fresh butter	Rancid butter
Peroxide value	0.23±0.1 meqO <sub>2</sub> /Kg	2.2± 0.6meqO <sub>2</sub> /Kg
Bromine value	29.88±0.7mg bromine/100gfat	29.5 ±0.6mg bromine/100g fat
Free fatty acid value	0.65 ±0.1mgKOH/g fat	3.72± 0.6mgKOH/g fat
P-anisidine value	0.33±0.1	4.6±0.1
Totox-value	0.76±0.5	9±0.1
Moisture content	23.6%±0.7	12.8%±0.5
Fat content	75.4%±0.1	83.7%±0.1
PH value	6.2±0.1	5.3±0.1

The values are expressed in mean ± standard deviation.

#### 4.1 Moisture content

High moisture content in fats and oils usually leads to increase in microbial load as well as lipid oxidation resulting in rancidity.

Moisture contents of animal butter in this study were 23.6% and 12.8% in fresh and rancid butters respectively (Table 3). These values are comparable with the values reported by Food and Agriculture Organization of United Nations, 2011 in which it ranges from 12.7- 26. The moisture content of rancid butter was very less than fresh butter. This may be due to evaporation of some amount of moisture from the butter during the storage time.

#### **4.2 Free Fatty Acid, PH and peroxide value of butter**

In determining the storage stability and quality of butter, the peroxide value reflecting the oxidation of fatty acids, the amount of free fatty acids and the degree of lipolysis are taken into consideration. When the amount reaches 1.8mgKOH/g fat, there is a perceptible off flavor in butter. Sample with FFA value exceeding 3.3 mgKOH/g fat were recognized as rancid by 59% of the panelists. An off flavor is also perceived in butter when peroxide value reaches 2 meqO<sub>2</sub>/Kg fat (Atamer and Sezgin, 1984).

The peroxide values were 0.23 meqO<sub>2</sub>/Kg and 2.2 meqO<sub>2</sub>/Kg for the fresh and rancid butter respectively. The FFA values of fresh and rancid butter were 0.65 mgKOH/g and 3.72 mgKOH/g respectively. These values were comparable with the values reported by Atamer and Sezgin which ranges from 0 to 1.8 for peroxide value and 0 to 3.3 for free fatty acid value. Even though the FFA value and peroxide value of the respective butter showed that the rancid butter may be spoiled, when compared to the Turkey's Food Regulations (10 meqO<sub>2</sub>/Kg of fat), it was in well acceptable amount. The pH value of a food is a direct function of the free hydrogen ions present in that food. Acids present in foods release these hydrogen ions, which give acid foods their distinct sour flavor. The pH value of fresh butter, 6.2 was higher than that of rancid butter, 5.3. Values were comparable with the values reported by Atamer and Sezgin which ranges from 4.1 to 6.9. This may be because of the low free fatty acids in fresh butter than rancid butter. (Atamer and Sezgin, 1984).

**The Summary table of the analysis showing the oxidation took place after 72 hours**

At 25 °C					At 65 °C			
Bromine value	Peroxide value	P-anisidine value	FFA value	Totox value	Bromine value	Peroxide value	P-anisidine value	FFA value
29.54±0.01 <sup>a</sup>	0.33±0.01 <sup>a</sup>	0.4±0.01 <sup>a</sup>	0.94±0.06 <sup>a</sup>	1.06±0.1 <sup>a</sup>	7.89±0.01 <sup>a</sup>	1.86±0.06 <sup>a</sup>	6.8±0.006	3.44±0.06 <sup>a</sup>
24.47±0.05 <sup>b</sup>	0.25±0.06 <sup>b</sup>	0.4±0.06 <sup>a</sup>	0.49±0.01 <sup>b</sup>	0.9±0.07 <sup>b</sup>	15.32±0.06 <sup>b</sup>	1.8±0.01b	5.4±0.001	1.76±0.01 <sup>b</sup>
24.47±0.01 <sup>b</sup>	0.18±0.01 <sup>c</sup>	0.3±0.01 <sup>b</sup>	0.46±0.01 <sup>c</sup>	0.66±0.1 <sup>c</sup>	15.32±0.05b	0.8±0.06 <sup>c</sup>	2.3±0.006	1.29±0.01 <sup>c</sup>
24.42±0.01 <sup>c</sup>	0.18±0.06 <sup>c</sup>	0.3±0.06 <sup>b</sup>	0.44±0.01 <sup>d</sup>	0.66±0.07 <sup>c</sup>	23.76±0.05 <sup>c</sup>	0.6±0.06 <sup>d</sup>	2.6±0.006	1.26±0.01 <sup>d</sup>
24.46±0.06 <sup>b</sup>	0.18±0.06 <sup>c</sup>	0.3±0.06 <sup>b</sup>	0.45±0.06e	0.66±0.07 <sup>c</sup>	23.79±0.01 <sup>d</sup>	0.7±0.06 <sup>e</sup>	2.5±0.001	1.31±0.06 <sup>e</sup>
24.45±0.05 <sup>d</sup>	0.19±0.06 <sup>d</sup>	0.3±0.06 <sup>b</sup>	0.45±0.06 <sup>e</sup>	0.68±0.1 <sup>d</sup>	23.77±0.05 <sup>e</sup>	0.6±0.06 <sup>d</sup>	2.6±0.001	1.22±0.06 <sup>f</sup>
24.42±0.01 <sup>e</sup>	0.18±0.06 <sup>c</sup>	0.3±0.06 <sup>b</sup>	0.46±0.01 <sup>c</sup>	0.66±0.07 <sup>c</sup>	23.76±0.05 <sup>c</sup>	0.6±0.06 <sup>d</sup>	2.5±0.006	1.29±0.01 <sup>c</sup>
24.46±0.06 <sup>b</sup>	0.18±0.06 <sup>c</sup>	0.3±0.06 <sup>b</sup>	0.44±0.01 <sup>d</sup>	0.66±0.1 <sup>c</sup>	23.79±0.01 <sup>d</sup>	0.7±0.06 <sup>e</sup>	2.6±0.001	1.26±0.01 <sup>d</sup>
29.47±0.01 <sup>f</sup>	2.75±0.06 <sup>e</sup>	4.8±0.06 <sup>c</sup>	4.61±0.01 <sup>f</sup>	10.3±0.1 <sup>e</sup>	7.71±0.01 <sup>f</sup>	4.46±0.06 <sup>f</sup>	20.2±0.006	5.71±0.06 <sup>g</sup>
25.9±0.005 <sup>g</sup>	1.95±0.06 <sup>f</sup>	4.5±0.06 <sup>d</sup>	1.65±0.06 <sup>g</sup>	8.4±0.07 <sup>f</sup>	6.6±0.006 <sup>g</sup>	2.89±0.01 <sup>g</sup>	16.1±0.001	2.19±0.06 <sup>h</sup>
25.91±0.05 <sup>h</sup>	1.85±0.06 <sup>g</sup>	4.2±0.06 <sup>e</sup>	1.63±0.01 <sup>h</sup>	7.9±0.07 <sup>g</sup>	25.22±0.01 <sup>h</sup>	2.29±0.06 <sup>h</sup>	9.2±0.006	1.76±0.06 <sup>b</sup>
25.89±0.05 <sup>i</sup>	1.82±0.06 <sup>h</sup>	4.2±0.06 <sup>e</sup>	1.64±0.01 <sup>i</sup>	7.84±0.1 <sup>h</sup>	25.27±0.05 <sup>i</sup>	2.25±0.06 <sup>i</sup>	9.2±0.001	1.73±0.01 <sup>i</sup>
25.91±0.06 <sup>h</sup>	1.83±0.01 <sup>i</sup>	4.3±0.01 <sup>f</sup>	1.63±0.06 <sup>h</sup>	7.96±0.1 <sup>i</sup>	25.21±0.01 <sup>j</sup>	2.18±0.06 <sup>j</sup>	8.6±0.006	1.72±0.01 <sup>j</sup>
25.90±0.06 <sup>g</sup>	1.82±0.06 <sup>h</sup>	4.3±0.06 <sup>f</sup>	1.45±0.01 <sup>j</sup>	7.94±0.07 <sup>j</sup>	25.31±0.05 <sup>k</sup>	2.17±0.05 <sup>k</sup>	8.9±0.001	1.75±0.01 <sup>k</sup>
25.92±0.06 <sup>j</sup>	1.83±0.01 <sup>i</sup>	4.3±0.01 <sup>f</sup>	1.63±0.06 <sup>h</sup>	7.96±0.1 <sup>i</sup>	25.21±0.01 <sup>j</sup>	2.18±0.06 <sup>j</sup>	10.1±0.006	1.72±0.01 <sup>j</sup>
25.90±0.06 <sup>g</sup>	1.82±0.06 <sup>h</sup>	4.4±0.06 <sup>g</sup>	1.44±0.01 <sup>k</sup>	8.04±0.07 <sup>k</sup>	25.31±0.05 <sup>k</sup>	2.17±0.05 <sup>k</sup>	9.9±0.001	1.75±0.01 <sup>k</sup>

The values are expressed in mean ± standard deviation. The samples with the different superscript in the same column is significantly different (at p< 0.05).

UCFB = unclarified fresh butter, CFB = clarified fresh butter, 10%GTFB = 10% ginger treated fresh butter, 20%GTFB = 20% ginger treated fresh butter, 10%GrTFB = 10% garlic treated fresh butter, 20%GrTFB = 20% garlic treated fresh butter, 10%KTFB = 10% korarima treated fresh butter, 20%KTFB = 20% korarima treated fresh butter, UCRB = unclarified rancid butter, CRB = clarified rancid butter, 10%GTRB = 10% garlic treated rancid butter, 20%GTRB = 20% ginger treated rancid butter, 10%GrTRB = 10% garlic treated rancid butter, 20%GrTRB = 20% garlic treated rancid butter, 10%KTRB = 10% korarima treated rancid butter, 20%KTRB = 20% korarima treated rancid butter

### 4.3 Bromine value

Table 4 shows the average value of each parameter of the butter oxidation after 72 hours storage time. It was observed that bromine value of all the heat and spice treated butter and untreated butter decreased very slowly as storage days increased for the samples stored at 25°C. But for samples stored at 65°C the bromine values were decreased amazingly. This was probably due to a higher oxidation took place at 65°C temperature. The treatment had effect on the amounts of bromine value. Reduction of bromine value might be an important measure of the decrease in unsaturated fats and thus resulted in rancidity of butter. Reduction of bromine value was due to oxidation of unsaturated fats and may get promoted by acceleration oxidation of fat with heat (Frega, Mozzon, & Lercker, 1999). Bromine value went on decreasing with the increase in storage period for all the samples, but no regular pattern of decrease could be observed (Table 5). Unclarified butter (UCB) exhibited the highest bromine value, UCFB = 29.54 and UCRB = 29.5 at 25°C after 72 hours storage time, while 20%GTFB and 10%KTFB were exhibited the least at 25°C. After 72 hours storage time at 65°C, UCFB = 7.89 and CRB = 6.6 observed to have the least bromine value. The decreases were 3.8 fold for UCFB and 3.89 for CRB. This might be due to the higher amount of moisture content of UCFB butter and the higher content of hydroperoxides in CRB which may resulted in prooxidant effect and so that the butter was significantly oxidized. Garlic treated and Korarima treated butter samples were showed comparable bromine values in reverse proportions for fresh samples and in direct proportions for rancid butter. The increase in bromine value of all samples at 25°C was slow for both types of butter, but it is significantly increased for all samples at 65°C. Spice treated samples showed interesting resistance to the decrease in bromine value than untreated one after 2-3 days of storage at 65°C. The higher bromine value of butter means it was less exposed to the phenomenon of rancidification.

Generally, 10%GrTFB and 20%KTFB for fresh butter samples and 20%GrTRB and 20%KTRB for rancid samples were showed the highest oxidative stability and so that had highest unsaturated fats.

**Table 5 Average Bromine Value of Butter during the storage time**

Treatment	At 25 °C			At 65 °C		
	Storage Time			Storage Time		
	0	36	72	0	36	72
UCFB	29.87±0.01	29.63±0.006	29.54±0.01	30±0.005	16.3±0.005	7.89±0.01
CFB	24.53±0.001	24.49±0.01	24.47±0.005	24.33±0.006	19.3±0.006	15.32±0.006
10%GTFB	24.53±0.01	24.49±0.005	24.47±0.01	24.33±0.005	19.3±0.005	15.32±0.005
20%GTFB	24.44±0.005	24.43±0.001	24.42±0.01	24.45±0.005	24.22±0.01	23.76±0.005
10%GrTFB	24.48±0.01	24.46±0.006	24.46±0.006	24.47±0.01	24.18±0.005	23.79±0.01
20%GrTFB	24.46±0.001	24.45±0.005	24.45±0.005	24.46±0.02	24.18±0.01	23.77±0.005
10%KTFB	24.44±0.005	24.43±0.001	24.42±0.01	24.45±0.005	24.22±0.01	23.76±0.005
20%KTFB	24.48±0.01	24.46±0.006	24.46±0.006	24.47±0.01	24.18±0.005	23.79±0.01
UCRB	29.5±0.01	29.45±0.006	29.47±0.01	29.5±0.01	17±0.01	7.71±0.01
CRB	25.93±0.006	25.91±0.01	25.9±0.005	25.68±0.006	19.8±0.006	6.6±0.006
10%GTRB	25.92±0.01	25.92±0.005	25.91±0.005	25.92±0.005	25.34±0.005	25.22±0.01
20%GTRB	25.91±0.005	25.89±0.01	25.89±0.005	25.91±0.01	25.32±0.01	25.27±0.005
10%GrTRB	25.92±0.01	25.91±0.006	25.91±0.006	25.92±0.006	25.41±0.001	25.21±0.01
20%GrTRB	25.91±0.001	25.91±0.006	25.90±0.006	25.91±0.006	25.39±0.006	25.31±0.005
10%KTRB	25.92±0.01	25.91±0.006	25.92±0.006	25.92±0.006	25.41±0.001	25.21±0.01
20%KTRB	25.91±0.001	25.91±0.006	25.90±0.006	25.91±0.006	25.38±0.006	25.31±0.005

The values are expressed in mean ± standard deviation (at p< 0.05)

UCFB = unclarified fresh butter, CFB = clarified fresh butter, 10%GTFB = 10% ginger treated fresh butter, 20%GTFB = 20% ginger treated fresh butter, 10%GrTFB = 10% garlic treated fresh butter, 20%GrTFB = 20% garlic treated fresh butter, 10%KTFB = 10% korarima treated fresh butter, 20%KTFB = 20% korarima treated fresh butter, UCRB = unclarified rancid butter, CRB = clarified rancid butter, 10%GTRB = 10% garlic treated rancid butter, 20%GTRB = 20% ginger treated rancid butter, 10%GrTRB = 10% garlic treated rancid butter, 20%GrTRB = 20% garlic treated rancid butter, 10%KTRB = 10% korarima treated rancid butter, 20%KTRB = 20% korarima treated rancid butter

#### 4.4 Peroxide value

According to the data (Table 6), peroxide values continuously increase during the storage period in all of the butter samples. The highest values were produced by UCRB in the samples, 4.46 meq O<sub>2</sub>/Kg fat at 65°C. This may be due to the higher initial peroxide value of this sample rather than indicating its oxidative stability. The same was true for the samples stored at 25°C.

Christensen and Holmer reported similar results in their study in which they stated that the PV value of butter increases as storage time increase. The data reported in Table 6 shows the progress of butter oxidation during storage at 65°C. As in the case of fresh butter, peroxide values were very small at the onset of storage period, ranging from 0.16 to 0.23, which may indicate the good quality of the products. But the case was not true for rancid butter which ranges from 1.81 to 2.2. Furthermore, the heat treatment used during the preparation of clarified butter (CB) had little or no influence on PV. It was evident that the addition of the spices exhibited significant effect in depressing the progress of butter oxidation throughout storage period. Significant differences between the untreated butter and the other treatment in their PV were observed after 1 day of storage time. Peroxide value was in the range 0.6–0.8 meq/kg for spice treated fresh samples after storage up to 3 days at 65°C, while maximum values of PV for UCFB and CFB sample was 1.86meq/ kg and 1.8 meq/ kg respectively. But the peroxide value was in the range 2.17-2.29 meq/kg for spice treated rancid butter samples after storage up to 3 days at 65°C, while maximum value of PV for UCRB sample was 4.46 meq/kg. At all stages, highest PV was observed for UCRB sample followed by CRB, 10%GTRB, 20%GTRB, 10%GrTRB, 10%KTRB, 20%KTRB and 20%GrTRB respectively. Garlic treated butter at both the concentrations and ginger treated butter at 10% concentration for fresh and rancid sample stored at 65°C, controlled peroxide value appreciably; revealing good antioxidant efficacy in stabilization of butter. But in 20% concentration garlic was less effective than ginger for rancid butter stored at 65°C. A regular increase in PV as a function of storage time was observed for all the samples at all intervals. Initially, the difference in peroxide content of unclarified butter (UCB) and spice treated butter samples was not noticeable; it became significant ( $p < 0.05$ ) just after heating up to one day. After the 3<sup>rd</sup> day, there was a tremendous rise in PV of UCB sample.

Peroxide content for all the spice treated samples also increased but this increase was very slow. Peroxide value of UCFB increased by 8.3 fold after 3 days at 65°C, which is extremely greater than that of UCRB which increased by 2 fold. Similarly, CFB increased by 8 fold while CRB increased by 1.3 fold. The peroxide values of animal butter samples are below the maximum acceptable value of 10 meq KOH/g set by the Codex Alimentarius Commission for groundnut seed oils (Abayeh *et al.*,1998). The low peroxide values could be an indication that the butter would not easily go rancid when properly stored in a container free from atmospheric oxygen and other contaminants

**Table 6 Average Peroxide Value of Butter during storage time**

Treatment	At 25 °C			At 65 °C		
	Storage Time			Storage Time		
	0	36	72	0	36	72
UCFB	0.23±0.006	0.28±0.006	0.33±0.001	0.23±0.005	1.2±0.006	1.86±0.006
CFB	0.22±0.006	0.24±0.01	0.25±0.006	0.22±0.006	1.2±0.01	1.8±0.01
10%GTFB	0.18±0.006	0.19±0.006	0.18±0.01	0.18±0.006	0.3±0.01	0.8±0.006
20%GTFB	0.16±0.006	0.17±0.01	0.18±0.006	0.16±0.006	0.21±0.006	0.6±0.006
10%GrTFB	0.16±0.006	0.16±0.006	0.18±0.006	0.16±0.006	0.2±0.006	0.7±0.006
20%GrTFB	0.16±0.006	0.18±0.006	0.19±0.006	0.16±0.006	0.21±0.006	0.6±0.006
10%KTFB	0.16±0.006	0.17±0.01	0.18±0.006	0.16±0.006	0.21±0.006	0.6±0.006
20%KTFB	0.16±0.006	0.16±0.006	0.18±0.006	0.16±0.006	0.2±0.006	0.7±0.006
UCRB	2.2±0.006	2.45±0.006	2.75±0.006	2.2±0.006	3.49±0.006	4.46±0.006
CRB	1.92±0.006	1.93±0.006	1.95±0.006	1.92±0.006	2.29±0.01	2.89±0.01
10%GTRB	1.84±0.006	1.85±0.006	1.85±0.006	1.84±0.006	1.96±0.01	2.29±0.006
20%GTRB	1.82±0.006	1.82±0.006	1.82±0.006	1.82±0.01	1.94±0.006	2.25±0.006
10%GrTRB	1.82±0.006	1.82±0.006	1.83±0.01	1.82±0.006	1.94±0.01	2.18±0.006
20%GrTRB	1.81±0.01	1.81±0.006	1.82±0.006	1.81±0.006	1.89±0.02	2.17±0.057
10%KTRB	1.81±0.006	1.81±0.006	1.83±0.01	1.81±0.006	1.95±0.01	2.18±0.006
20%KTRB	1.81±0.01	1.81±0.006	1.82±0.006	1.81±0.006	1.89±0.02	2.17±0.057

The values are expressed in mean ± standard deviation (at p< 0.05)

UCFB = unclarified fresh butter, CFB = clarified fresh butter, 10%GTFB = 10% ginger treated fresh butter, 20%GTFB = 20% ginger treated fresh butter, 10%GrTFB = 10% garlic treated fresh butter, 20%GrTFB = 20% garlic treated fresh butter, 10%KTFB = 10% korarima treated fresh butter, 20%KTFB = 20% korarima treated fresh butter, UCRB = unclarified rancid butter, CRB = clarified rancid butter, 10%GTRB = 10% garlic treated rancid butter, 20%GTRB = 20% ginger treated rancid butter, 10%GrTRB = 10% garlic treated rancid butter, 20%GrTRB = 20% garlic treated rancid butter, 10%KTRB = 10% korarima treated rancid butter, 20%KTRB = 20% korarima treated rancid butter

#### 4.5 Free Fatty Acid value

The amounts of FFAs determined in butter samples during a storage period of 3 days are presented in Table 10. During storage time, the levels of FFAs in samples kept at 65°C temperatures were higher than in those stored at 25°C temperatures. The differences were found to be statistically significant at the level of  $P < 0.05$ . This was probably due to a higher lipolytic activity at 65°C temperature (Ahmet, 2010). The butter type had effect on the amounts of FFAs. However, the amounts increased during storage, and the differences were found to be significant between different storage times at 65°C ( $P < 0.05$ ). Interaction of storage temperature with storage period was significant at a level of  $P < 0.05$ , and interaction of butter type with storage temperature was significant at a level of  $P < 0.05$ . Formation of free fatty acids might be an important measure of rancidity of foods. FFAs were formed due to hydrolysis of triglycerides and may get promoted by reaction of fat with moisture (Frega, Mozzon, & Lercker, 1999). FFA content went on increasing with the increase in storage period for all the samples, but no regular pattern of increase could be observed. UCB (UCFB and UCRB) exhibited the highest FFA in all storage time and storage temperature for both fresh and rancid samples, while 20%GTFB, 0.83, 20%GrTFB, 0.83 and KTFB in both proportion, 0.83 exhibited least for fresh butter at 65°C. Garlic treated and Korarima treated butter have comparable. Fresh butter was not showed significant increase in FFA of treated samples at both storage temperature but, after 2-3 days of storage, an increase was observed for samples stored at 65°C. The lower the acid value of an oil, the fewer free fatty acids it contains which makes it less exposed to the phenomenon of rancidification (Roger et al., 2010).

**Table 7 Average Free Fatty Acid Value of Butter**

Treatment	At 25 °C			At 65 °C		
	Storage Time			Storage Time		
	0	36	72	0	36	72
UCFB	0.65±0.01	0.81±0.01	0.94±0.06	0.65±0.01	2.42±0.006	3.44±0.006
CFB	0.44±0.006	0.46±0.001	0.49±0.01	0.44±0.01	1.1±0.006	1.76±0.01
10%GTFB	0.43±0.006	0.45±0.006	0.46±0.01	0.43±0.01	0.86±0.006	1.29±0.01
20%GTFB	0.42±0.006	0.43±0.001	0.44±0.01	0.42±0.006	0.83±0.006	1.26±0.01
10%GrTFB	0.43±0.006	0.44±0.006	0.45±0.06	0.43±0.01	0.84±0.01	1.31±0.006
20%GrTFB	0.42±0.006	0.42±0.006	0.45±0.06	0.42±0.006	0.83±0.01	1.22±0.006
10%KTFB	0.43±0.006	0.45±0.006	0.46±0.01	0.43±0.01	0.86±0.006	1.29±0.01
20%KTFB	0.42±0.006	0.43±0.001	0.44±0.01	0.42±0.006	0.83±0.006	1.26±0.01
UCRB	3.72±0.01	4.13±0.01	4.61±0.01	3.71±0.006	4.65±0.006	5.71±0.006
CRB	1.46±0.006	1.56±0.006	1.65±0.06	1.46±0.01	1.75±0.01	2.19±0.006
10%GTRB	1.43±0.006	1.52±0.006	1.63±0.01	1.43±0.006	1.51±0.006	1.76±0.006
20%GTRB	1.44±0.006	1.53±0.006	1.64±0.01	1.44±0.005	1.48±0.006	1.73±0.01
10%GrTRB	1.43±0.006	1.53±0.006	1.63±0.06	1.43±0.006	1.49±0.006	1.72±0.01
20%GrTRB	1.44±0.006	1.44±0.01	1.45±0.01	1.44±0.005	1.49±0.01	1.75±0.01
10%KTRB	1.43±0.006	1.54±0.006	1.63±0.06	1.43±0.006	1.48±0.006	1.72±0.01
20%KTRB	1.44±0.006	1.44±0.01	1.44±0.01	1.44±0.005	1.49±0.01	1.75±0.01

The values are expressed in mean ± standard deviation (at p< 0.05)

UCFB = unclarified fresh butter, CFB = clarified fresh butter, 10%GTFB = 10% ginger treated fresh butter, 20%GTFB = 20% ginger treated fresh butter, 10%GrTFB = 10% garlic treated fresh butter, 20%GrTFB = 20% garlic treated fresh butter, 10%KTFB = 10% korarima treated fresh butter, 20%KTFB = 20% korarima treated fresh butter, UCRB = unclarified rancid butter, CRB = clarified rancid butter, 10%GTRB = 10% garlic treated rancid butter, 20%GTRB = 20% ginger treated rancid butter, 10%GrTRB = 10% garlic treated rancid butter, 20%GrTRB = 20% garlic treated rancid butter, 10%KTRB = 10% korarima treated rancid butter, 20%KTRB = 20% korarima treated rancid butter

#### 4.6 P-anisidine Value

The amount of secondary oxidation products is expressed in the pAV. For a good quality fat/oil the pAV should be lower than 10 (Rossell, 1994). It is clear that all the fats had a pAV lower than 10 except the p-anisidine value of UCRB, 14.1 and CRB, 12.2 after 72 hours at 65°C. But at 25°C, all samples had p-anisidine value highly less than 10

From the data, p-anisidine values were increased during the storage period in all of the butter samples at 65°C. The highest values were produced by UCRB followed by CRB and UCFB which are 14.1±0.7, 12.2±0.7 and 5.8±0.7 respectively, which means that oxidative stability was

lowest in this type of butter. But for the samples stored at 25°C room temperature it was very low and goes in the same way as the one stored at 65°C. The effects of butter type, storage temperature and storage times were found to be significant (at  $P < 0.05$ ). Christensen and Holmer, 1996 reported similar results in their study which indicated the increase of p-anisidine value as a function of storage temperature and time. The results of the study was showed that the progress of butter oxidation during storage at 65°C. As in the case of fresh butter, p-anisidine values were very small at the beginning of the experiment. This may indicate the quality of the butter samples. Furthermore, the heat treatment used during the preparation of clarified butter (CB) had little or no influence on it. It was evident that the addition of the spices exhibited significant effect in depressing the progress of butter oxidation throughout storage period. Significant differences between the butter samples in their p-anisidine value were observed after 72 hours of storage. P-anisidine value was in the range 1.3–5.8 for spice treated fresh samples after storage up to 72 hours at 65°C. But the p-anisidine value was in the range 4.6-14.1 for spice treated rancid butter samples after storage up to 3 days at 65°C, while maximum value of p-anisidine value for UCRB sample was 14.1. At all stages, highest p-anisidine value was observed for UCRB sample followed by CRB, UCFB, 10%GTRB, 20%GTRB, CFB, 10%GrTRB, etc respectively. Spice treated butter at both the concentrations for fresh and rancid sample stored at 65°C, controlled p-anisidine value appreciably when compared to un treated butter; revealing good oxidative stability of butter. But in both concentrations garlic is more effective than ginger for rancid butter stored at 65°C. A regular increase in p-anisidine value as a function of storage time was observed for all the samples at all intervals. Initially, the difference in p-anisidine value of unclarified butter (UCB) and spice treated butter samples was not noticeable; it became significant ( $p > 0.05$ ) just after heating up to one day. After the 72 hours, there was a radical rise

in p-anisidine value of UCRB and CRB samples. But having the higher p-anisidine value of UCRB and CRB could not indicate the better oxidative stability of that butter rather than it may be due to the higher initial value and more saturation of the butter. Some authors have indicated that anisidine values are comparable only within each oil type because the initial value varies among oil sources (Guillén and Cabo, 2002). P-anisidine value for all the spice treated samples also increased but this increase was gradual. The low p-anisidine value could be an indication of the effectiveness of spices in retarding the secondary product formation or that the fats or oils would not easily go rancid and also its good quality.

**Table 8 Average P-anisidine Value of Butter during storage time**

Treatment	At 25 °C			At 65 °C		
	Storage Time			Storage Time		
	0	36	72	0	36	72
UCFB	0.42±0.001	0.38±0.006	0.4±0.001	0.4±0.006	5.2±0.001	6.8±0.006
CFB	0.21±0.006	0.29±0.001	0.4±0.006	0.37±0.01	4.3±0.006	5.4±0.001
10%GTFB	0.26±0.006	0.28±0.006	0.3±0.01	0.35±0.001	1.2±0.006	2.3±0.006
20%GTFB	0.28±0.001	0.29±0.001	0.3±0.006	0.36±0.006	1.2±0.001	2.6±0.006
10%GrTFB	0.28±0.006	0.29±0.001	0.3±0.006	0.35±0.001	1.3±0.006	2.5±0.001
20%GrTFB	0.26±0.001	0.28±0.006	0.3±0.006	0.37±0.006	1.2±0.006	2.6±0.001
10%KTFB	0.28±0.006	0.29±0.006	0.3±0.006	0.36±0.001	1.2±0.001	2.5±0.006
20%KTFB	0.3±0.006	0.3±0.001	0.3±0.006	0.37±0.001	1.2±0.006	2.6±0.001
UCRB	4.6±0.001	4.7±0.001	4.8±0.006	4.6±0.001	13.1±0.006	20.2±0.006
CRB	4.5±0.001	4.5±0.001	4.5±0.006	4.5±0.001	11.8±0.001	16.1±0.001
10%GTRB	4.2±0.006	4.2±0.001	4.2±0.006	4.2±0.006	4.8±0.006	9.2±0.006
20%GTRB	4.0±0.001	4.1±0.006	4.2±0.006	4.0±0.001	4.9±0.006	9.2±0.001
10%GrTRB	4.3±0.006	4.3±0.001	4.3±0.01	4.3±0.006	4.4±0.001	8.6±0.006
20%GrTRB	4.1±0.001	4.2±0.006	4.3±0.006	4.1±0.001	4.2±0.001	8.9±0.001
10%KTRB	4.1±0.006	4.2±0.001	4.3±0.01	4.1±0.006	5.1±0.006	10.1±0.006
20%KTRB	4.1±0.006	4.3±0.006	4.4±0.006	4.1±0.006	4.7±0.001	9.9±0.001

The values are expressed in mean ± standard deviation (at p< 0.05)

UCFB = unclarified fresh butter, CFB = clarified fresh butter, 10%GTFB = 10% ginger treated fresh butter, 20%GTFB = 20% ginger treated fresh butter, 10%GrTFB = 10% garlic treated fresh butter, 20%GrTFB = 20% garlic treated fresh butter, 10%KTFB = 10% korarima treated fresh butter, 20%KTFB = 20% korarima treated fresh butter, UCRB = unclarified rancid butter, CRB = clarified rancid butter, 10%GTRB = 10% garlic treated rancid butter, 20%GTRB = 20% ginger treated rancid butter, 10%GrTRB = 10% garlic treated rancid butter, 20%GrTRB = 20% garlic treated rancid butter, 10%KTRB = 10% korarima treated rancid butter, 20%KTRB = 20% korarima treated rancid butter

#### **4.7 Totox value**

As p-anisidine values (Table 4), totox values were also high for UCRB (10.3 at 25°C and 29.12 at 65°C) and CRB (8.4 at 25°C and 21.88 at 65°C). At 65°C, 10%GTFB and 10%GrTFB, 20%GTFB and 20%GrTFB were not significantly different, but all the rests are significantly different (at  $p < 0.05$ ). But at 25°C, the difference between 10%GTFB, 20%GTFB, 10%GrTFB, 10%KTFB and 20%KTFB was not significant (at  $p < 0.05$ ) after 3 days. So generally totox value was a good indicator of oxidative stability of butter samples.

Table 3 and 4 shows that the totox value of UCFB increased by 13.84 fold after 3 days at 65°C, which is extremely greater than that of UCRB which increased by 3.23 fold. Similarly, CFB increased by 11.11 fold while CRB increased by 2.62 fold (Table 4, 6 and 8). The low totox value could be an indication that the butter would not easily go rancid when properly stored in a container free from atmospheric oxygen and other contaminants. Generally, fresh butter samples were more susceptible to oxidation than rancid butter. This might be due to the more saturation of rancid butter than that of fresh butter samples. Because saturated fat has better resistance toward oxidation. Table 4 showed that 10%KTFB and 10%GrTRB were the first two oxidation stable samples for fresh and rancid butter respectively.

## Chapter 5

### 5 Conclusions and recommendations

#### 5.1 Conclusion

This study showed variation in the quality characteristics of animal butter samples. Some samples (UCFB and CFB from fresh butter and UCRB and CRB from rancid butter) showed very high levels of rancidity during storage time at 65°C, while 10%KTFB and 10%GrTRB, for fresh and rancid butter respectively showed the lowest levels of deterioration (Table 4). These results suggest that korarima treated and garlic treated butter has better oxidation resistance in the specified proportion so that decreased deterioration. Where as untreated raw butter was extremely susceptible to oxidation. Relatively low relationship between moisture, free fatty acid, and peroxide value were found, which implies all the parameters should be considered collectively in quality control of animal butter.

- While it was observed, the addition of natural antioxidants can delay the oxidation of animal butter and thus extend its shelf life.
- The heat treatment used during the preparation of clarified butter (CB) had little or no influence on PV.
- The clarified butter is more resistant to oxidation than that of raw butter.
- Fresh butter is more susceptible to oxidation than rancid butter.
- The fresh butter has more unsaturated fats than that of rancid butter.
- Storage of butter for a long period of time results in rancidity or deterioration.
- In the future the research should focus on how to treat butter for oxidative stability without the involvement of heat treatment for better oxidative stability.

## 5.2 Recommendation

- Butter should be clarified and treated with spices in order to have better oxidative stability and so that longer shelf-life.
- Since heat treatment results in oxidation of butter fat, it is better to clarify butter within a few minutes and the temperature should be controlled.
- Using rancid butter ( yebesele kibe) should be strongly avoided because it is more saturated and contains compounds which may harm our health, such as aldehyde compounds.

## References

- Abayeh, O.J. – Aina, E.A. – Okounghae, C.O., 1998. Oil content and oil quality characteristics of some Nigerian oils seeds. In *Journal of Pure and Applied Sciences*, vol. 1, 1998, no. 1, p.1723.
- Abramovic, H. And V. Abram., 2006. Effect of added Rosemary extract on oxidative stability of *Camelina sativa* oil. *Acta Agriculturae Slovenica* 87:255-261.
- Abuzaytoun R.; Shahidi F., 2006. Oxidative Stability of Flax and Hemp Oils. *J Am Oil Chem Soc.* 83:855-861.
- Ahmet Ayar, Durmus Sert, Derya Arselan and Mehmet Musa Ozcan, 2010. The effect of some spice extracts on storage stability of “ Yayik Butter”. *World Applied Science Journal* 11(9): 1114-1123,2010. ISSN1818-4952.
- Akguel A and Ayar A., 1993 ‘Antioxidant effects of Turkish spices’. *Doga Turkish J Agric Forestry*, 1993 **17** 1061–8.
- Akingbala J.; Falade K.; Adebese E.; Baccus-Taylor G.; Lambert I., 2006. Effect of Processing Conditions on Yield, Physical and Chemical Properties of Shea Butter. *W Indian J Eng.* 29: 7380.
- Alander, J.; Andersson, A.C. 2002., *The Shea Butter Family – the Complete Emollient Range for Skin Care Formulations. Cosmetics and Toiletries Manufacture Worldwide*, 2002: 28–32.
- Alander J. 2004., *Shea Butter- a Multi Functional Ingredient for Food and Cosmetics. Lipid Technol.* 16:202-205.
- Al-Delaimy, K.S. and Barakat, M.M.F., 1971. *J. Sci. Food Agric.*, 22: 96-98.
- Allen J C., 1994. Rancidity in dairy products. In *Rancidity in Foods* (4th edn), pp 179–190.

American Heart Association., 2004. Heart and Stroke Encyclopedia. Dietary Guidelines for Healthy American Adults. Cholesterol. Fat. Retrieved August 23, 2004 from World Wide Web: [http:// Www.americanheart.org](http://Www.americanheart.org).

Anon., 1990. *G'da Maddeleri Tüzüpi*. Hukuk Dizisi No.21(39). Istanbul: Bayrak Matbaacylyk.

American Shea Butter Institute (ASBI), 2011. [www.sheainstitute.com](http://www.sheainstitute.com) (accessed on 02/06/13)

AOAC 1990. Official methods of analysis, 15th ed.; Association of Official Analytical Chemists: Arlington, Virginia.

AOCS International, 2005. Official Method Ce 11-05. Determination of Cocoa Butter Equivalents in Cocoa Butter and Plain Chocolate. Champaign, AOCS Press, 8p.

Atamer M., 1993.*Tereya@'Teknolojisi*. Ankara Üniversitesi Ziraat Fakültesi YayynlaryNo.1313. Ankara: Ankara Üniversitesi Ziraat Fakültesi Halkla Y liokiler ve Yay y n Ünitesi.

Atamer M and Sezgin E (1984) Tereyaolarynda Lipolitikve Oksidatif Bozulmalaryn Saptanmasynda Yararlanylän Asit ve Peroksid De2erleri Yle Aroma Arasyndaki Yli6ki.*G'da* 9 329–334.

Azizkhani, M. And P. Zandi. 2009. Effects of some Natural antioxidants mixtures on margarine Stability. World Academy of Science, Engineering and Technology 49:93-96

Badifu G.I.O. 1989. Lipid Composition of Nigerian *Butyrospermum paradoxum* Kernel. J Food Comp Anal. 2:238-244.

Bail S.; Krist S.; Masters E.; Unterweger H.; Buchbauer G. 2009. Volatile Compounds of Shea Butter Samples Made under Different Production Conditions in Western, Central and

Eastern Africa. *J Food Comp Anal.* 22: 738-744.

Banon, S. – Diaz, P. – Rodriguez, M. – Garrido, M.D. – Price, A. 2007. Ascorbate, green tea and grape seed extracts increase the shelf life of low sulphite beef patties. In *MeatScience*, vol.4,2007, no. 77, p. 626–633.

Bartley, J., & Jacobs, A., 2000. Effects of drying on flavour compounds in Australian-grown ginger (*Zingiber officinale*). *Journal of the Science of Food and Agriculture*, 80, 209–215

Baydar N.G.; Özkan G.; Cetin E.S., 2007. Characterization of Grape Seed and Pomace Oil Extracts. *Grasas Aceites.* 58:29-33

Baysal, A., 1992. Diyet ya lar ve sa l m z: Son görü ler. *Beslenme ve Diyet Dergisi.* 21, 5-16.

Bouaziz M.; Fki I.; Jemai H.; Ayadi M.; Sayadi S., 2008. Effect of Storage on Refined and Husk Olive Oils Composition: Stabilization by Addition of Natural Antioxidants from Chemlali Olive Leaves. *Food Chem.* 108:253-262

Carpenter, R.O. – Grady, M.N. – O’collaghan, Y.C. – O’brien, J.P. –Kerry,J.P., 2007. Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. In *Meat Science*, vol. 4, 2007, no. 76, p. 604–610.

Chalfin B., 2004. *Shea Butter Republic*. Routledge. New York, NY. Intro, Ch1.

Chanyalew Z., 1999. The role of spices in the nationaleconomy of Ethiopia. Paper presented on the curriculum development workshop for diploma and degree program in horticulture at the Jimma College of Agriculture. 6 – 7, August 1999, Jimma, Ethiopia (Inpress).

Chipault, J. R, Mizuno, G. R., Hawkins, J. M., & Lundberg, W. O., 1952. The antioxidant properties of natural spices. *Food Research*,17, 46–55.

- Christensen T C and Holmer G., 1996. GC/MS analysis of volatile aroma components in butter during storage in different catering packaging. *Milchwissenschaft* **51** 134–138.
- Cogan T M. 1972. Modification of the Prill and Hammer method for determining diacetyl. *Journal of Dairy Science* **55** 382–384.
- Damodaran S.; Parkin K.L.; Fennema O.R., 2008. Fennema's Food Chemistry. 4th edn. CRC Press. Boca Raton, FL. Ch. 4.
- Department of Health., 1994. Nutritional aspects of cardiovascular Disease. Report on Health and Social Subjects no. 46, London.
- Deeth H C and Fitz-Gerald C H., 1976. Lipolysis in dairy products: a review. *Australian Journal of Dairy Technology* **31** 53–64.
- Dhara R.; Bhattacharyya D.K.; Ghosh M. 2010. Analysis of Sterol and Other Components Present in Unsaponifiable Matters of Mahua, Sal, and Mango Kernel Oil. *J. Oleo Sci.* **59**:169-176.
- Di Vincenzo D.; Maranz S.; Serraiocco A.; Vito R.; Wiesman Z.; Bianchi G., 2005. Regional Variation in Shea Butter Lipid and Triterpene Composition in Four African Countries. *J. Agric. Food Chem.* **53**: 7473-7479.
- Dorant, E. et al., 1993. *Br. J. Cancer*, **67**: 424-429.
- Douma, M., curator., 2008. *Butter through the Ages*. Retrieved April 20 2013, from <http://www.webexhibits.org/butter>
- Duncan S E, Christen G L and Penfield M P., 1991. Rancid flavor of milk: relationship of acid degree value, free fatty acids, sensory perception. *Journal of Food Science* **56** 2.
- East African Standard, 2006. Butter. Methods of chemical analysis Part 7: Determination of pH of the serum - Potentiometric method. EAS 80-7 (2006)

- Emmons, D B, Froehlich, D A, Paquette, G J, Beckett, D C, Modler, H W, Butler, G, Brackenridge, P and Daniels, G., 1986. 'Flavor stability of butter prints during frozen and refrigerated storage.' *J. Dairy Sci.* 1986 69:2451-7.
- Esuoso K.O.; Lutz H.; Bayer E.; Kutubuddin M., 2000. Unsaponifiable Lipid Constituents of Some Underutilized Tropical Seed Oils. *J. Agric. Food Chem.* 48: 231-234.
- Eyob S, Appelgren M, Rohloff J, Tsegaye A, Messele G., 2008. Traditional medicinal uses and essential oil composition of leaves and rhizomes of korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen) from southern Ethiopia. *S. Afr. J. Bot.* 74: 181-185.
- Farrell, K.T., 1985. *Spices, Condiments and Seasonings*. Ed. 2. Aspen Publication, Maryland. pp. 15-253.
- Ferris R.S.B.; Collinsom C.; Wanda K.; Jagwe J.; Wright P., 2001. Evaluating the Market Opportunities for Shea Nut and Shea Nut Processed Products in Uganda. Submitted to USAID, The United States Agency for International Development (available at [http://www.foodnet.cgiar.org/Projects/Sheanut\\_Rep.pdf](http://www.foodnet.cgiar.org/Projects/Sheanut_Rep.pdf), accessed on 02/05/13)
- Firestone D., 1990. *Official Methods and Recommended Practices of the American Oil Chemists' Society*. 4th edn. American Oil Chemists' Society. Champaign, Ill.
- Fix G J., 1993. Diacetyl: formation, reduction, and control. <http://www.brewingtechniques.com/library/backissues/issue1.2/fix.html>.
- Food Advisory Committee., 1990. Report on review of food labelling and advertising, London.
- Frankel E.N., 1993. In Search of Better Methods to Evaluate Natural Antioxidants and Oxidative Stability in Food Lipids. *Trends Food Sci Tech.* 4:220-225.
- Frega, N., Mozzon, M., & Lercker, G., 1999. Effect of free fatty acids on the oxidative stability of vegetable oils. *Journal of American Oil Chemists Society*, 76, 325-329.

- Gan H.L.; Che Man Y.B.; Tan C.P.; NorAini I.; Nazimah S.A.H., 2005. Characteristics of Vegetable Oils by Surface Acoustic Wave Sensing Electronic Nose. *Food Chem.* 89:507-518.
- Gaertner, Karl Friedrich von., 1807. *Supplementum Carpologiae*. Leipzig. Cited in Tropicos.org. Missouri Botanical Garden. (<http://www.tropicos.org>, accessed on 12/05/13).
- Global Shea., 2011. <http://www.globalshea.com/about-us> (accessed on 02/05/13)
- Goreja. W.G., 2004. *Shea Butter: The Nourishing Properties of Africa's Best-Kept Natural Beauty*. Amazing Herbs Press. New York, NY
- Gordon, M. H., 1990. The mechanism of antioxidant action in vitro. In: *Food antioxidant*. B. J. F. Hudson, (Ed), Elsevier Science Publishers, London. p 1
- Guardiola, F., Codony, R., Addis, P. B., Rafecas, M., & Boatella, P., 1996. Biological effects of oxysterols: Current status. *Food Chemistry and Toxicology*, 34, 193–198.
- Guillén M. D. and Cabo N. 2002. Fourier transform infrared spectra data versus peroxide and anisidine value to determine oxidative stability of edible oils. *Food Chemistry*, 77,503-510.
- Gurr,M.I.,1992.. Dietary lipids and coronary heart disease: old evidence, new perspective. *Prog.Lipid Res.* 31:195-243.
- Gutfinger T.; Letan A., 1974. Studies of Unsaponifiables in Several Vegetable Oils. *Lipids.* 9:658-663.
- Harris, J. C., Cottrell, S. L., Plummer, S., & Lloyd, D., 2001. Antimicrobial properties of *Allium sativum* (garlic). *Applied Microbiology and Biotechnology*, 57, 282–286
- Hartman, L., & Lago, R. C. A., 1973. Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practices*, 22, 475–481.
- Hettinga D., 2005, *Bailey's Industrial Oil and Fat Products*, Sixth Edition, Six Volume Set.

- Ifesan, B.O.T. – Siripongvutikorn, S. – Voravuthikunchai, S.P., 2009. Application of Eleutherine americana Crude Extract in Homemade Salad Dressing. In Journal of Food Protection, vol. 72, 2009a, no. 3, p. 650–655.
- Ifesan, B.O.T. – Siripongvutikorn, S. – Voravuthikunchai, S.P., 2009. Evaluation of the Ability of Eleutherine americana Crude Extract as Natural Food Additive in Cooked Pork. In Journal of Food Science, vol. 74, 2009b, no 7, p. 353-357.
- Ihsan B, Serafettin Ç., and Cihat Ö., 2002. The effects of commercial starter culture and storage temperature on the oxidative stability and diacetyl production in butter. Vol 55, No 4 November 2002 International Journal of Dairy Technology.
- IUPAC, 1987. Standard Methods for the Analysis of oils, Fats and Derivatives, 7th ed; Blackwell Scientific Publications: Oxford, UK, 1987.
- Jackson, R., McNeil, B., Taylor, C., Holl, G., Ruff, D., & Gwebu, E. T., 2002. Effect of aged garlic extract n casepase-3 activity in vitro. Nutritional Neuroscience, 5, 287–290.
- Jacob A., 2011. Butter. : retrieved from <http://www.livestrong.com/article/531175-nutrients-in-pasture-butter/#ixzz1u4aEo5AA>.
- Jansen PCM., 1981 Spices, Condiments and Medicinal Plants in Ethiopia: Their taxonomy and agricultural significance. Agricultural Research Reports 906, pp. 10-20 Center for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- Jennings, B.H. and C.C. Akoh., 2009. Effectiveness of Natural versus synthetic antioxidants in a rice Bran oil-based structured lipid. Food Chem. 114:1456-1461
- Judde, A., P. Villeneuve, A. Rossignol-Castera, and A. Le Guillou., 2003. Antioxidant effect of soy Lecithins on vegetable oil stability and their Synergism with

- tocopherols. *J Am Oil Chem Soc.* 80:1209-1215.
- Kesava-Rao, V., Kowale, B. N., Babu, N. P., & Bisht, G. S., 1996. Effect of cooking and storage on lipid oxidation and development of cholesterol oxidation products in water buffalo meat. *Meat Science*, 43, 179–185.
- Kikuzake, H. and Nakatani, N., 1993. *J. Food Sci.*, 58(6): 1407-1410.
- Kim, J.K., Kim, Y., Na, K.M., Surh, Y.J. and Kim, T.Y. 2007. [6]-Gingerol prevents UVB induced ROS production and COX-2 expression *in vitro* and *in vivo*. *Free Radical Research* 41: 603-614
- Kowalski, R., 2007. GC analysis of changes in the Fatty acid composition of sunflower and olive Oils heated with quercetin, caffeic acid, Protocatechuic acid and butylated hydroxyanisole. *Acta Chromatogr.* 18:15-23.
- Krishnamurthy, K. and Srinivasamurthy, V., 1956. *Garlic Bull. Cent. Food Technol. Res. Inst. Mysore.*, 5: 264.
- Kubow, S., 1990. Toxicity of dietary lipid peroxidation products. *Trends Food Science Technology*, 1, 67–74.
- Kurt A, Çakmakçy S and Ça2lar A., 1996. *Süt ve Mamulleri Muayene ve Analiz Metodlar' Rehberi* (genioletilmip 6. basky). Atatürk Üniversitesi Yayynlary No. 252/D Erzurum: Atatürk Üniversitesi Ziraat Fakültesi Ofset Tesisi.
- Lawson, L.D., Wang, Z. J and Hughes, B. G., 1991. Identification and HPLC quantitation of the sulfides and dialk(en)yl thiosulfinates in commercial garlic products. *Planta Medica*57(4):363 70.
- Lee, Y.B. et al., 1986. *J. Food Sci.*, 56(1): 20-23.
- Li, N., Oshima, T., Shozen, K., Ushio, H., & Koizumi, C., 1994. Effects of the degree of

- unsaturation of coexistent triglycerols on Cholesterol oxidation. *Journal American Oil Chemistry and Society*, 71, 623–627.
- Luby, J M, Gray, J I and Harte, B R., 1986. 'Effects of packaging and light source on the oxidative stability of cholesterol in butter.' *J. Food Sci.* 1986 51:908–11.
- Luby, J M, Gray, J I, Harte, B R and Ryan., 1986. T C. 'Photooxidation of cholesterol in butter.' *J. Food Sci.* 1986 51:904–7.
- Michael H. Gordon, nd. *Measuring antioxidant activity.* The University of Reading
- Phelps, S. and Harris, W.S.,1993. *Lipids*, 28: 475-477.
- McGill, H.C. 1979. The relation of dietary cholesterol to serum cholesterol concentration and to atherosclerosis in man. *Am.J.Clin.Nutr.*32:2664-2702.
- McWilliams M., 2001. *Foods: Experimental Perspectives.* 4th edn. Prentice Hall, Upper Saddle River, NJ. Ch.11.
- Moharram H.; Ray J.; Ozbas S.; Juliani H.; Simon J., 2006. *Shea Butter: Chemistry, Quality, and new Market Potentials* (Wang M.; Shengmin S.; Hwang L.S.; Ho C.T. 2006. *Challenges in Chemistry and Biology of Herbal Research.* American Chemical Society Washington DC. USA. Ch. 25. Pp. 326-340)
- Mortensen B K and Danmark H., 1982. Manufacturing of cultured butter using lactic concentrates. *Milchwissenschaft* **37** 402–404.
- Munro D S, Cant P A E, MacGibbon A K H, Illingworth D, Kennett A and Main A J., 1992. Concentrated milk fat products. In *The Technology of Dairy Products*, pp 117–145. Early R, ed. New York: VCH.
- Murphy, E. W., Criner, P. E., & Gray, B. C., 1975. Comparison of methods for calculating retentions of nutrients in cooked foods. *Journal of Agricultural and Food Chemistry*,

23, 1153–1157.

Nehdi I.; Omri S.; Khalil M.I.; Al-Resayes S.I., 2010. Characteristics and Chemical Composition of Date Palm (*Phoenix canariensis*) Seeds and Seed Oil. *Ind Crop Prod.* 32:360-365.

Nielsen S.S. 2010. *Food Analysis*. Springer, New York, NY. Ch 14.

Nigel Saunders, 2003. Comparing the degree of unsaturation of margarine with that of butter.

Copyright © 2003 Nigel Saunders N-ch3-10

Nikiéma J.B.; Vanhaelen-Fastré R.; Vanhaelen M.; Fontaine J.; De Graef C.; Heenen M., 2001.

Effect of Antiinflammatory Triterpenes Isolated from *Leptadenia hastata* Latax on Keratinocyte Prolifereation. *Phytother. Res.* 15: 131-134.

Njoku O.U.; Eneh F.U.; Ononogbu I.C.; Adikwu M.U., 2000. Compositional and Toxicological Studies on Shea Butter. *J Nutraceut Function Med Foods*(Currently *J Diet Suppl.*) 2: 3339.

Osawa C.C.; Goncalves L.A.G.; Ragazzi S., 2007. Correlation between Free Fatty Acids of Vegetable Oils Evaluated by Rapid Tests and by the Official Method. *J Food Compos Anal.* 20:523-528.

Otuki M.F.; Ferreira J.; Lima F.V.; Meyre-Silva C.; Malheiros A.; Muller L.A.; Cani G.S.;

Otuki M.F.; Lima F.V.; Malheiros A.; Yunes R.A.; Calixto J.B., 2005. Topical Anti inflammatory Effects of the Ether Extract from *Protium kleinii* and  $\alpha$ -amyrin Pentacyclic Triterpene. *Eur J Pharmacol.* 507: 253-259.

Olaniyan A.M., Oje K., 2007. Quality Characteristics of Shea Butter Recovered from Shea Kernel through Dry Extraction Process. *J. Food Sci Technol.* 44: 404-407.

Paquot C.; Hautfenne A., 1987. *Standard Methods for the Analysis of Oils, Fats, and Derivatives*.

7th rev. and enl. edn. International Union of Pure and Applied Chemistry. Blackwell

Scientific Publications. Boston. MA.

Pedernera A.M.; Guardia T.; Calderón C.E.G.; Rotelli A.E.; de la Rocha N.E.; Saad J.R.; Verrilli M.A.L.; Aseff S.G.; Pelzer L.E., 2010. Anti-inflammatory Effect of Acacia visco extracts in animal models. *Inflammopharmacol.* 18:253-260.

Pokorny, J., N. Yanishlieva, and M. Gordon., 2001. *Antioxidants in Food*. CRC Press LLC. Boca Raton, Florida.

Polish standard, 2005. Oleje Ithuszcze recelinne oraz zwierzecze. Oznaczanie liczby kwasowej I Kwasowosci. PN-EN ISO, 660:2005

Prasad, K. et al., 1995. *Mol. Cell Biochem.*, 148: 183-189.

Rižnar K., Čelan Š., Knez Ž., Škerget M., Bauman D., Glaser R., 2006: Antioxidant and antimicrobial activity of rosemary extract in chicken Frankfurters. *Food Chemistry and Toxicology*, 71, 7, C425-C429.

Robert J. Gordon., 1990. "The Measurement of Durable Goods Prices," NBER Books, National Bureau of Economic Research, Inc, number gord90-1, August.

Rodriguez-Estrada, M. T., Penazzi, G., Caboni, M. F., Bertacco, G., & Lercker, G., 1997. Effect of different cooking methods on some lipid and protein components of hamburgers. *Meat Science*, 45, 365–375..

Roger, A.B., Rebecca, R.A., Georges, A., Mathias, I.O., 2010. Chemical characterization of oil form Germinated nuts of several coconut cultivars (*Cocosnuciferi* L.). In *European urnal of ScientificResearch*, vol. 391, 2010, no.4, p. 514 - 522.

Rossel, J. B., 1989. In J. C. Allen & R. J. Hamilton (Eds.), *Rancidity in foods* (pp. 45–49). England: Elsevier Science Publishers Ltd..

ROSSEL, J.B., 1994. Measurement of rancidity. In: Allen, J.C. & Hamilton, R.J. (eds.)

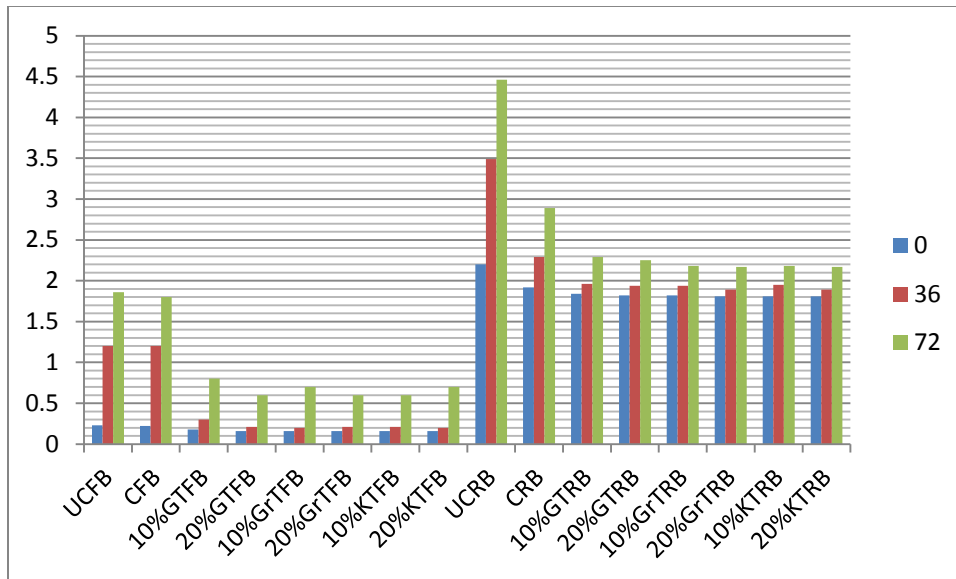
- Rancidity in foods. Gaithersburg, Aspen Publishers Inc., 22-48.
- Saito, Y. et al., 1976. *Eiyo to shokuryo*, 29(9): 505. (In Chem. Abstr., (1977) 81: 150314r.)
- Santos A.R.S.; Yunes R.A.; Calixto J.B., 2005. Antinociceptive Properties of Mixture of  $\alpha$  Amyrin and  $\beta$ -Amyrin Triterpenes: Evidence for Participation of Protein Kinase C and Protein Kinase A Pathway. *J. Pharmacol. Exp. Ther.* 313:310-318.
- Sebsebe D., 1993. A description of some essential oil bearing plants in Ethiopia and their indigenous uses. *J Ess Oils Res* 5,465-79.
- Shahidi, F. and Wanasundara, P.K.J.P.D., 1992. *Crit. Rev. Food Sci. Nut.*, 32: 67-103.
- Stoilova ,I., Krastanov ,A., Stoyanova ,A., Denev P., Gargova ,S.,2007. Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chemistry* 102 (2007) 764–770
- Tchobo F.P.; Natta A.K.; Barea B.; Barouh N.; Piombo G.; Pina M.; Vileneuve P.; Soumanou Tella A., 1979. Preliminary Studies on Nasal Decongestant Activity from the Seed of Shea Butter. Tree, *Butyrospermum parkii*. *Br. J. clin. Pharmac.* 7:495-497.
- Torok, B. et al., 1994. *Arzneim-Forseh, Drug Res.*, 5: 608-611.
- Torres, E., Pearson, A. M., Gray, J. I., & Ku, P. K., 1989. Lipid oxidation in charqui (salted and dried beef). *Food Chemistry*, 32, 257–268.
- Van Hoed V.; Depaemelaere G.; Villa Ayala J.; Santiwattana P.; Verhé R.; De Greyt W., 2006. Influence of Chemical Refining on the Major and Minor Components of Rice Bran Oil. *J Amer Oil Chem Soc.* 83: 315-321
- Velasco J.; Dobarganes C., 2002. Oxidative Stability of Virgin Olive Oil. *Eur J Lipid Sci Technol.* 104: 661-676
- Vuković-Gačić, Knežević-Vukčević J., Jankov R.M., Simić D., 1996: Antimutagenic activity of plant extracts. II. Bioantimutagenic effect of Various fractions of

- cultivated sage (*Salvia officinalis* L.). Abstracts/Mutation Research, 360, 6-22, 270.
- Walsh B and Cogan T M., 1974. Separation and estimation of diacetyl and acetoin in milk.  
*Journal of Dairy Research* **41** 25–30.
- Walstra p., Geurts T.J., Noomen A., Jellema A., van boekel M.A.J.S., 1999: Dairy Technology: Principles of Milk Properties and Processes. Marcel Dekker, Inc., New York, USA.
- Walter S.; Cole D.; Kathe W.; Lovett P.; Paz Soldan M., 2003. Impact of Certification on the Sustainable use of NWFP: Lessons-learnt from Three Case Studies. (Paper submitted for presentation at the International Conference on Rural Livelihoods, Forests and Biodiversity 19-23 May 2003, Bonn, Germany)
- Waraho T.;Cardenia V.; Rodriguez-Estrada M.T.; McClements D.J.; Decker E.A., 2009. Prooxidant Mechanisms of Free Fatty Acids in Stripped Soybean Oil-in-Water Emulsions. *J. Agric. Food Chem.* 57:7112-7117.
- Wiedner M.S., 2008. Novel Composition Containing Extracts of *Butyrospermum Parkii* and the Use of Such a Composition for Preparing a Medicament or a Dietary Supplement for the Treatment or Prevention of Inflammation Hypersensitivity or Pain. United States Patent Application Publication (US 2008/0124413 A1).
- Wijendran, V., & Hayes, K. C., 2004. Dietary n-6 and n-3 fatty acid Balance and cardiovascular health. *Annual Review of Nutrition*, 24, 579–615.
- Wikipedia., 2012. The free encyclopedia.
- Yamasaki, T. et al., 1994. *Phytother. Res.*, 8: 408-412.
- Yanishlieva-Maslarova N.V., Heinonen I.M., 2001: Rosemary and Sage as antioxidants. In: Peter K.V. ed. Handbook of herbs and spices. CRC Press, Woodhead Publishing Limited, Cambridge, England.

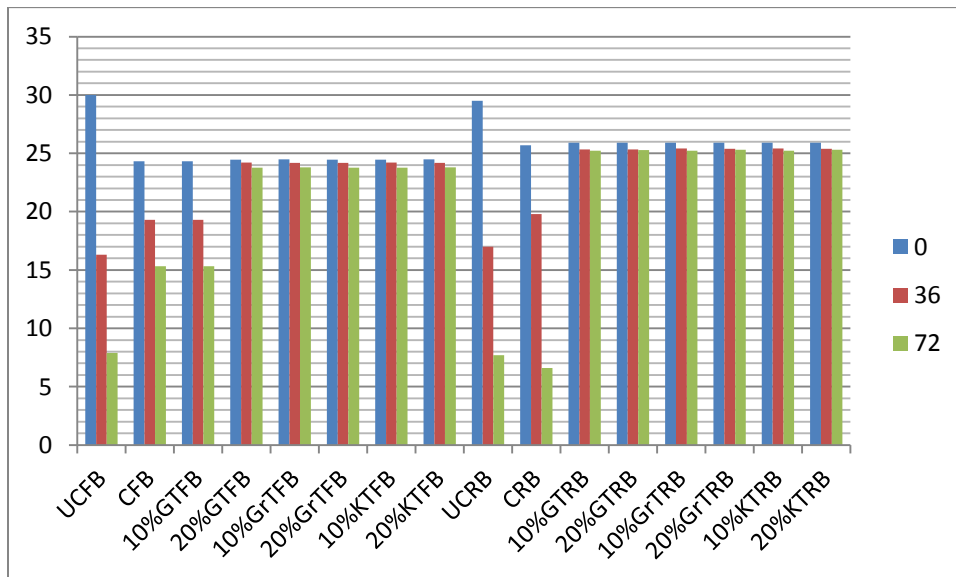
Yildiz, G.,R.L. Wehling and S.L. Cuppett.,2002. Monitoring in corn and soyabean oils by NIR Spectroscopy. J. Am. Oil Chem. Soc., 11:1085-1089

Yin, M., Hwang, S., & Chan, K., 2002. Nonenzymatic antioxidant activity of four organosulfur compounds derived from garlic. Journal of Agricultural and Food Chemistry, 50(21), 6143–6147.

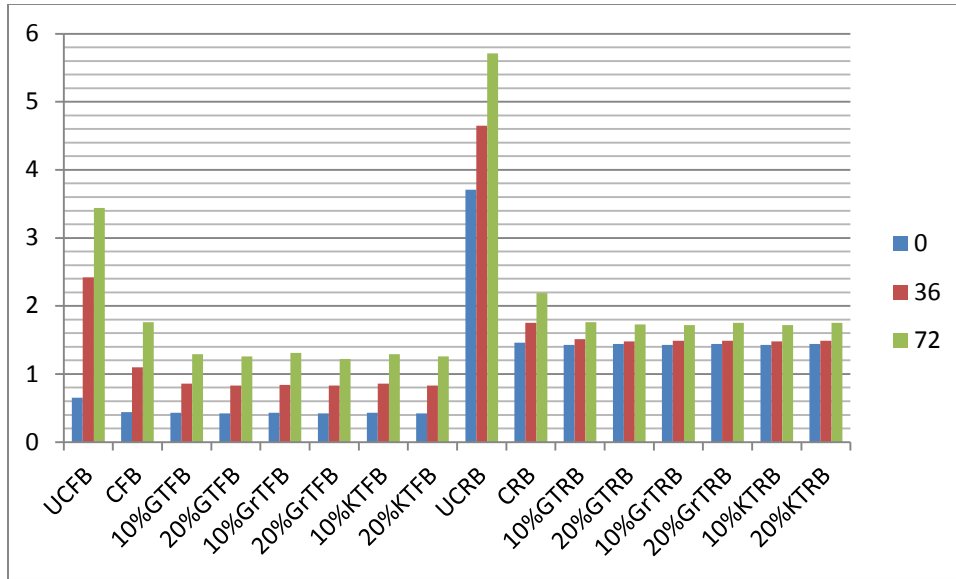
**Appendix II Peroxide value of butter during 72 hours when accelerated at 65°C**



**Appendix III Bromine value of butter during 72 hours when accelerated at 65°C**



**Appendix IV FFA value of butter during 72 hours when accelerated at 65°C**



**Appendix V P-anisidine value of butter during 72 hours when accelerated at 65°C**

