A Selective Review of Lipid Autoxidation and Rancidity, Antioxidants, and Dithiooxamide

2. Abstract

Background: The authors had a research interest in antiaging, oxidation and antioxidants, and determined that lipids are some of the more common raw materials in foods, cosmetics, and humans, and would be an area to explore autoxidation, whether in retarding spoilage, or to slow aging.

Objective: The authors determined to select a few lipids and test them against common pro-oxidants, and the effects of common antioxidants as well as a proposed antioxidant candidate together with a short-term toxicity study.

Methods: The authors subjected the lipids on elevated temperatures, with a control and various pro-oxidants, as well as some commercial antioxidants and the antioxidant candidate. The authors subsequently tested the lipids for by-products of oxidation, and used mice for a short-term toxicity study.

Results: Lipids stored in iron, copper, and other heavy metals, or are contaminated with such metals, are prone to accelerated autoxidation. Aluminium seems to provide an induction period to lipids and does not accelerate autoxidation as other metals do. When compared to antioxidants such as vitamin E, BHA, BHT, and PG, the antioxidant candidate dithiooxamide seemed to be a proficient antioxidant and free from short-term toxicity in a small sample study, which were far below the LDLo and LD50 conducted on rats and mice using dithiooxamide in other published studies.

Conclusions: The authors determined that lipids should steer clear of iron, copper and other heavy metals, and suggest that the antioxidant candidate dithiooxamide in low quantities may be useful for external lipid applications.

2.1. Keywords

autoxidation, antioxidant, lipid, peroxidation, ferroptosis, dithiooxamide, toxicology

2.2. Introduction

Lipids, whether for consumption, or the broader context of beauty and lifestyle products, are important ingredients. Lipids are energy reserves for humans, and for insulation, restoration, transport substrates for fat-soluble nutrients, and cellular communication. Lipids in cuisine are useful in lending lubrication and richness in taste and are evident in global cuisines in all cultures.

Beyond consumption, lipids are useful in the manufacture of cosmetics, pharmaceuticals, health supplements, surfactants, finishings, and even as fuels.

And human bodies are also resplendent with lipid forming tissues throughout the body, being a critical part of human metabolism and existence. The study of lipids and their autoxidation can be a convergent journey to understand how foods, cosmetics and even human bodies, can stave off lipid spoilage and potentially retard the process of aging, since oxidative aging can be analogous to lipid autoxidation.

Studies have shown that metals such as iron can cause ferroptosis and lipid peroxidation, and in turn, ill effects including inflammation or worse. And since lipids can go through oxidation and degrade over time when external factors such as metals, oxygen, and temperature are applied, and can become less useful or even undesirable with the formation of degradation byproducts, lipids have traditionally been paired with antioxidants to retard their oxidative rancidity, and likewise, in the human body, the use of antioxidants such as α-tocopherol can retard ferroptosis or peroxidation. The authors explored some representative lipids, their degradation, some antioxidants, and a potential antioxidant candidate.

3. Rancidity, Autoxidation and Antioxidants

Rancidity, that of foods turning “bad”, with off-taste, off-colour, and off-odour, is a common household problem. Food rancidity can affect many foods, including carbohydrates, proteins (such as meats), fats and oils (lipids), and others. Natural unprocessed foods can turn rancid more quickly compared to preserved or highly processed foods (typically with preservatives and antioxidants), especially in tropical climates with higher ambient temperatures and higher humidity.

There are 2 types of rancidity, hydrolytic and oxidative rancidity [1].

3.1. Hydrolytic Rancidity

Glycerides are easily cleaved (i.e., go under division) into fatty acids and glycerol by heating in dilute alkali. The resulting salts of fatty acids are known as soaps, hence the name "saponification" [Section 3.4] is given to the hydrolytic cleavage of lipids, which is true for all esters.

The de-esterification of triglycerides is also catalysed by the enzyme lipase [2]. Lipase is present in all lipid-containing tissues in variable quantities and is also very widespread. Lipases from different sources of lipids always show varying degrees of specificity.

Lipases react in heterogenous systems such as emulsions of glycerides in aqueous media. From the consumer’s point of view, the most significant consequence of lipase activity in foods is the development of a harsh and acrid taste as a result of free fatty acid liberation. The short-chained volatile fatty acids (e.g., butyric acid, which gives off a characteristic bad smell in rancid milk) also contribute their characteristic odour to affected foods. This type of food deterioration, known as hydrolytic rancidity, is widespread in lipid-based food products, such as those containing palm oil, dairy, and lard. As lipase activity occurs in the unsaturated bonds of certain fatty acids, hydrolytic rancidity is more rapid in finely dispersed emulsions, such as homogenised milk and cream. A high free fatty acid level in edible oils and oil-containing foods are very objectionable and must be removed in the process of refining, which represents substantial loss in yield.

3.2. Oxidative Rancidity

The occurrence of off-flavours, generally described as rancidity, is a common observation in fat-containing foods. The principal source of rancidity in fatty foods is the autoxidation of the lipid components.

Autoxidation is described as the spontaneous oxidation of a substance in contact with molecular oxygen.

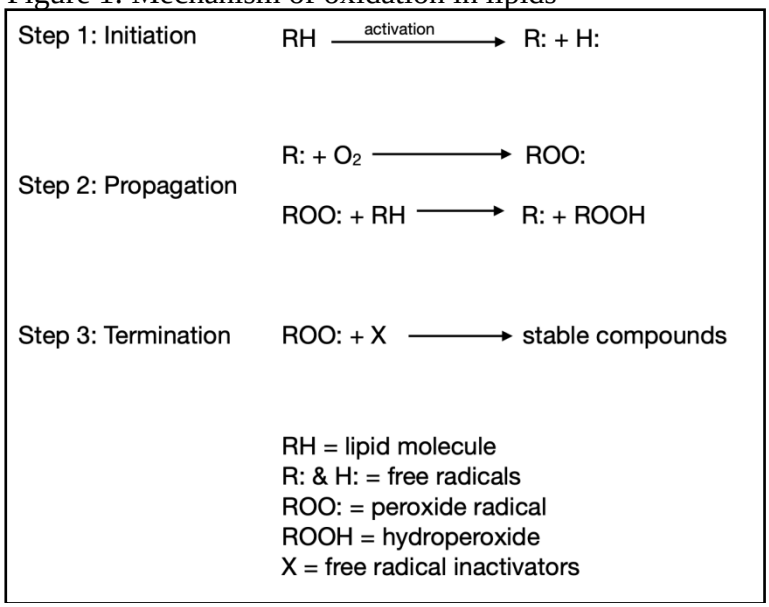
Although the onset of rancidity is the most significant consequence of lipid autoxidation, flavour deterioration is not the only damage suffered by foods in this process. The colour of the foods is also affected through accelerated browning reactions. The nutritional value is also impaired and toxicity may also be introduced. The texture may also change as a result of side reactions between proteins and the end-products of lipid oxidation. In short, oxidative deterioration of lipids contributes towards the spoilage factor affecting all aspects of food acceptability.

The lipid components most susceptible to autoxidation are the unsaturated fatty acids, especially those with more than 1 double bond. Although the process normally consists of a reaction between 2 molecular species (the lipid molecule and oxygen), the number of possible pathways increases enormously during the reaction. This will result in the formation of a very complex final system. In this respect, autoxidative deterioration of lipids resembles somewhat non-enzymatic browning [3].

3.3. Mechanism of Oxidation in Lipids

Although the hydroperoxide course was proposed by Farmer et al. [4], it evolved to be internationally accepted as the central mechanism of oxidation in lipids, where a free radical mechanism is responsible for the autoxidative reaction.

Figure 1: Mechanism of oxidation in lipids



In the first stage, a few molecules of the lipid RH are sufficiently activated by heat, light, metal ions, chlorophyll, etc, to decompose into unstable free radicals R: and H: [Section 3.5].

The free radical generation is not limited to lipids but can also occur in any organic compound. Usually, the generated free radicals would disappear quickly by recombination with RH, RR, H2, H2O, etc. However, in the presence of molecular oxygen, the possibilities include the combination between the free radical R: and O2, resulting in the formation of a peroxide radical ROO:. The peroxide radical ROO: will react with a free molecule of lipid RH, thus producing a hydroperoxide ROOH and a free radical R: through which the chain reaction is propagated. Now free radicals continue to be formed without the help of the initial activator through autocatalysis [5]. There may be intermediary compounds formed from ROOH to RO: and OH:. The reaction proceeds and more lipid molecules are transformed into hydroperoxides, the reaction is being terminated when free radicals combine with other free radicals or with free radical inactivators (represented by X), to yield stable compounds which accumulate in the system. The hydroperoxides enter a series of reactions leading to more free radicals and finally stable compounds. These final products include short-chained carboxylic compounds responsible for the rancid flavours and for side reactions leading to overall deterioration in lipid-containing foods.

3.3.1. Hydroperoxides and their Degradation

Hydroperoxides are important as the primary products of autoxidation since the formation of hydroperoxides, measured as the "Peroxide Value", indicates the progress of autoxidation, although not necessarily the appearance of rancidity in the affected foods.

Hydroperoxides are relatively unstable. As their concentration in the system increases, they begin to decompose [Sections 3.1, 3.2] and one of the possible reactions is the monomolecular decomposition of hydroperoxides into an alkoxy and an hydroxy radical, with various possibilities that exist for further reactions of the alkoxy radical to aldehydes, alcohols, ketones, and polymerisation.

If the alkoxy radical degrades to a short chain aldehyde with a free radical R: the free radical R: would start its own chain reaction. The short chain aldehyde itself may oxidise to an acid, be reduced to alcohol, react with amine groups, and so on. In the case of glycerides, the free radical R would remain attached to glycerol.

An alkoxy radical may react with another lipid molecule, generating an alcohol and a free radical R:, which again participates in the propagation of the chain.

An alkoxy radical may be oxidised by another free radical and terminates forming ketones. The monomolecular decomposition of hydroperoxides to alkoxy and hydroxy radicals seems to be the predominant route as long as the extent of oxidation is relatively low.

The formation of viscous, gum-like or even solid polymers (resins) is one of the consequences of lipid autoxidation, where direct recombination of free radicals occur. The "drying" of highly unsaturated oils used in paints is the result of such polymerisation.

3.4. Kinetic Aspects of Lipid Autoxidation

The course of autoxidation of lipids is experimentally followed by measuring the accumulation of peroxides, the rate of oxygen intake, the concentration of secondary reactions, or by organoleptic evaluation. Each one of these parameters may be indicative of a different aspect of oxidative deterioration.

Peroxide Value [6] is one of the most widely used concepts in lipid autoxidation. It is the measurement of the peroxide concentration of an oil, measured iodometrically or instrumentally, and is expressed as “milli equivalents of peroxide oxygen per 100 grams of oil or fat".

Iodine Value [7] is the amount of iodine in grams which can be fixed by 100 grams of oil or fat under investigation. This amount is proportional to the abundance of unsaturated fatty acid bonds.

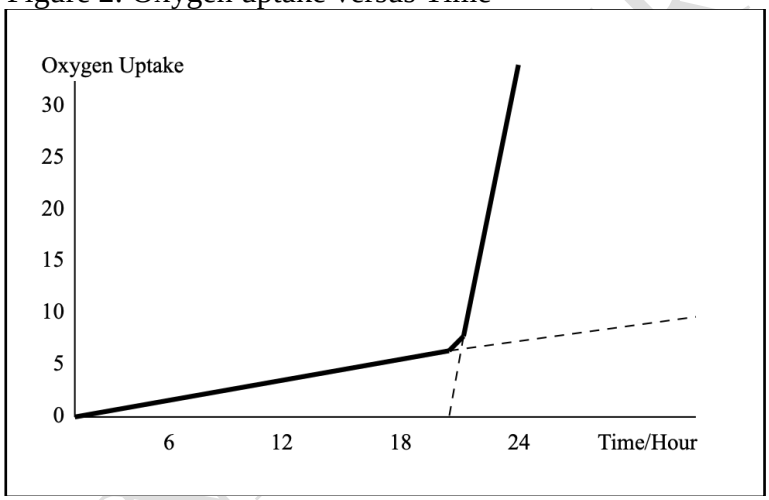
Saponification Value [8] is the amount of alkali (in milligram of potassium hydroxide or sodium hydroxide) needed to hydrolyse completely 1 gram of the oil or fat under investigation.

Rate of Oxygen Uptake is a more meaningful measure of the rate of oxidation. It is determined by somewhat complicated techniques similar to those used in the study of respiration in vitro.

Determination of decomposition products is meant principally to supply information on the build-up of flavoured compounds responsible for rancidity. These compounds can be identified by gas-liquid chromatography.

When the PV (or oxygen uptake) is followed, such a curve may be observed as illustrated in Figure 2:

Figure 2: Oxygen uptake versus Time



At first, PV increases at a slow, uniform rate. As soon as the PV reaches a critical value, depending on the system, a sudden and drastic increase in PV rate is recorded. The first slow phase is termed "Induction Period". The autocatalytic nature of this course, that is, the increase of the rate of the reaction as the reaction proceeds, can be inferred on the basis of the free radical chain reaction mechanism [Figure 1: Mechanism of Oxidation in lipids]. Rancidity usually begins to develop soon after the transition between the 2 phases. At this

stage, oxygen adsorption is a better measure than PV, since at this point considerable peroxide decomposition occurs simultaneously with new peroxide formation.

The behaviour of the graph shown in Figure 2 may be explained simplistically. During the induction period, initiation and propagation occur. Since for each radical which is transformed into a hydroperoxide one new free radical is formed, the reaction proceeds at a slow and uniform rate. As the concentration of hydroperoxides increases, hydroperoxide degradation occurs at an increasing rate. These reactions generate more free radicals than needed for propagation of the chain reaction at a constant rate. Consequently, the reaction becomes autocatalytic. The appearance of perceptible rancidity at or near such a transition point, may be explained by the decomposition of hydroperoxides to aldehydes and ketones which bear the characteristic rancid odour and taste.

3.5. Effect of Environmental Factors on Autoxidation

3.5.1. Temperature

The rate of autoxidation increases with temperature [9], since the energy of activation is strongly dependent on temperature. However, since high temperatures accelerate both the generation of free radicals and their disappearance, the rate-temperature relationship may be expected to pass through a maximum, especially at high oxidation levels and high temperatures.

Temperature may affect not only the rate of autoxidation but the reaction mechanism as well. At lower temperatures, the hydroperoxide course is the predominant mechanism with little to no loss of saturation. At higher temperatures, a considerable proportion of double bonds may undergo saturation.

3.5.2. Light

Fatty acids and their peroxides are colourless substances that do not absorb visible light. Thus, unless an accessory sensitiser is present, the effect of visible light on lipid autoxidation may be assumed to be unimportant.

However, ultraviolet light is strongly absorbed by unsaturated compounds, especially if the bonds are conjugated. Ultraviolet light may be a factor in the initiation of the chain reaction [10], but its principal effect is attributed to the acceleration of peroxide decomposition. In daily life, it is easy to find an "oxidised flavour" found in stale glass bottled milk than milk packed in opaque cartons. This can also explain why many photo-sensitive foods, including vitamins and health supplements, can sometimes be packed in opaque packaging or in dark coloured glass.

3.5.3. Oxygen

As long as oxygen is present in limited quantities, the rate of autoxidation increases with increasing oxygen pressure; until a constant oxidation rate is reached beyond a given pressure. At low oxygen pressures, the reaction rate is found to be proportional to the oxygen pressure. There is a linear relationship between the reciprocal of oxidation rate and the reciprocal of oxygen tension (otherwise known as partial pressure of oxygen, or PO_2).

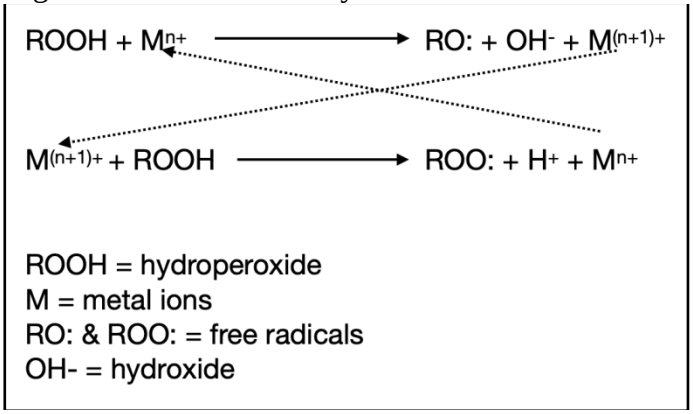
Exclusion of air, such as using vacuum or nitrogen packaging, from exterior packaging materials of low oxygen permeability, are among some of the more practical and common methods of packaging foods, especially fatty foods with oxidative deterioration, including foods such as nuts, coffee beans or ground coffee, canned or baked foods.

3.5.4. Catalysts (metallic ions and chlorophyll)

Ions of heavy metals (transitional) are powerful catalysts in lipid autoxidation. They shorten the induction period and increase the reaction rate. Most effective are metals with two or more oxidation states, and which can easily pass through from one state to another, e.g., iron, copper, manganese, etc. Most foods and even refined lipids contain these metal ions at concentration levels well above the amount needed for effective catalysis.

The main effect of these trace metals is to increase the rate of hydroperoxide decomposition and hence the rate of generation. This action may be represented in Figure 3.

Figure 3: Metals and catalysis

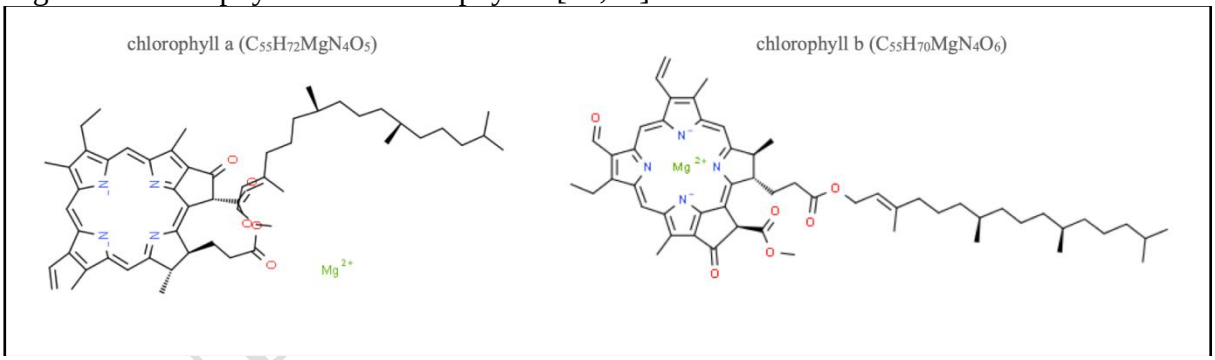


Thus, hydroperoxides are decomposed and the free radicals RO: and ROO: are formed as the metal (M) oscillates between its two oxidation states (represented by the dotted arrows).

The source of heavy metal ions in fatty foods may be contamination, such as equipment, piping, packaging, environmental contaminants, etc., or natural food components.

Similar reactions occur when metals are replaced by chlorophyll, where magnesium is attached to the porphyrin ring and is capable of free radical reactions forming peroxides.

Figure 4: Chlorophyll a and Chlorophyll b [11,12]

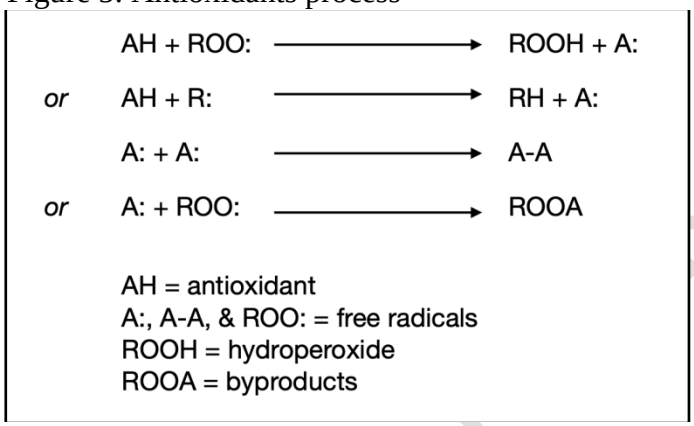


3.6. Antioxidants

Antioxidants are substances that retard autoxidation. In theory, a substance may act as an antioxidant in various ways. For example, such a substance may effect competitive binding with oxygen, retardation of the initiation step, blocking of propagation by destroying or binding free radicals, inhibition of the action of catalysts, or the stabilisation of hydroperoxides. All these and other mechanisms are found in food systems, but the most important one seems to be the blockage of propagation.

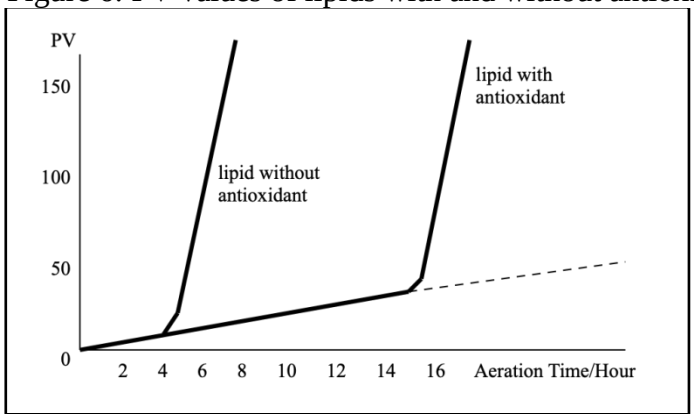
In this process, the antioxidant AH acts as a hydrogen donor to a free radical such as ROO: or R:. The antioxidant free radical A: is inactive, ie. it does not start a chain propagation process, but rather enters some termination process [13]:

Figure 5: Antioxidants process



For lipids undergoing PV tests, those with and without the introduction of antioxidants can show a difference in PV, as illustrated in Figure 6.

Figure 6: PV values of lipids with and without antioxidants



3.6.1. Phenolic Antioxidants

Phenolic antioxidants are typically the primary antioxidants, because they interfere directly with the free radical propagation process and they block the chain reaction. One phenolic antioxidant is vitamin E (alpha-tocopherol). Synthetic phenolic antioxidants approved for food use include: butylatedhydroxyanisole (BHA, $C_{22}H_{32}O_4$), butylatedhydroxytoluene (BHT, $C_{15}H_{24}O$), and n-propyl gallate (PG, $C_{10}H_{12}O_5$).

3.6.2. Secondary or Synergic Antioxidants

There exist a number of other substances which have little direct effect on the autoxidation of lipids but are able to enhance considerably the action of primary antioxidants. These substances are synergists or secondary antioxidants [14].

One of the best known and most widely used synergists is citric acid. In virtue of its polycarboxylic and alpha-hydroxylic structure, citric acid ($C_6H_8O_7$) is a potent metal chelating agent. There are secondary antioxidants such as sulphur and selenium containing compounds which can speed up the decomposition of peroxides to stable terminal compounds. Ascorbic acid (vitamin C) is another secondary antioxidant, typically used for boosting immunity and beauty products scavenging oxygen away from autoxidation.

3.6.3. Requirements of an ideal antioxidant

Higgins and Black [15] suggested the following requirements that an ideal antioxidant should exert no harmful physiological effect on the recipients, with no objectionable flavour, odour, or colour to the fats or foods made with this lipid, can carry through and effectively protect the foods made with lipids from rancidity, be sufficiently fat-soluble so that it can be added to fats with ease, be effective in low concentrations, be readily available in adequate amounts, and be reasonable in costs.

3.6.4. Dithiooxamide as an synergic antioxidant candidate

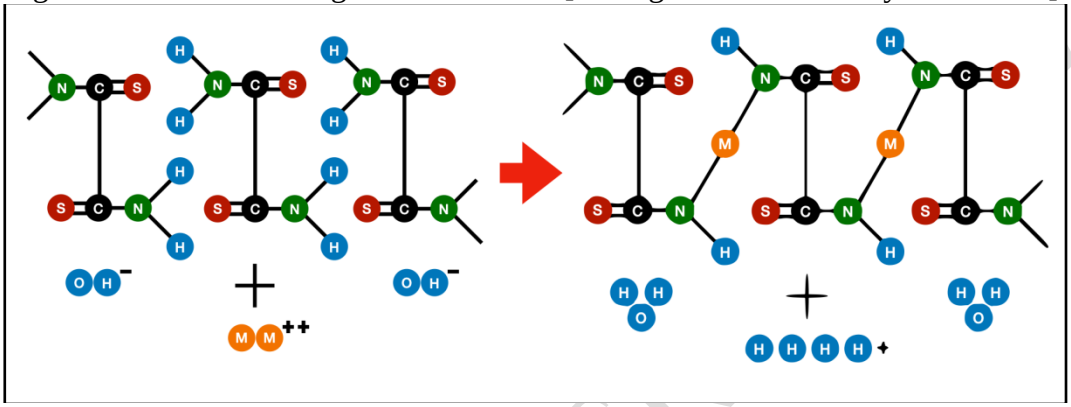
Dithiooxamide [16], also known as Rubeanic Acid or Ethanedithioamide, is a sulphur compound and a metal scavenger terminating in chelate compounds.

Ito, Hirose, Imaida [14] classified antioxidants under primary antioxidants that stop free radical chain reactions, secondary antioxidants that contain sulphur and selenium that decompose peroxides to stable terminal products, compounds that scavenge available oxygen

away from the autoxidation process, enzymes that digest oxidative substances, and chelating agents. By this inference, dithiooxamide being both a sulphur compound and a chelating agent, would fit the mould of a secondary antioxidant.

The H+ ions formed during chelation by dithiooxamide can combine with the OH- ions derived from autoxidation to form water, inhibiting the free radical process. Although not like phenolic primary antioxidants like natural tocopherols or synthetic BHA and BHT, dithiooxamide can potentially act as a secondary synergist.

Figure 7: Chelation through dithiooxamide [an original illustration by the authors]



3.7. Lipid Peroxidation and Ferroptosis

The human body exists in a state of equilibrium, where everything is finely balanced for the body to function normally, without physiologically failing or succumbing to external stimuli or trauma. This represents homeostasis [17].

Closely parallel to lipid autoxidation is the lipid peroxidation and ferroptosis processes in the human body [18]. In the human body and many other living things, ferroptosis, the process of oxidation that works in conjunction with iron ions, is attributed to cell death, also known as “programmed cell death”, which are associated with problematic symptoms in the human body such as inflammation, neurological degeneration, and ishchemia (constriction of blood flow in parts of the body) [19]).

There are studies to infer the use of fat-soluble antioxidants such as α-Tocopherol or derivatives can retard ferroptosis [19, 20], closely mimicking the same autoxidation processes in lipids.

4. Food Lipids

The consumption of fatty food products has increased to a considerable extent. Many bakeries and food products like milk, cooking oils, butter, salad cream, margarine, cookies, etc. contain lipids in one form or another. These items are constantly used as shelf items and are commonly stored for a considerable amount of time. To meet these commercial requirements adequately, especially in the warm equatorial weather in ASEAN, antioxidants and other additives are often added to such processed foods containing lipids, to retard oxidative rancidity. Oxidative rancidity has been attributed to a cause for bad odours and taste in fatty foods [21].

Malaysia is one of the most prominent producers of palm-related products, including lipids and biofuel [22], with projected exports from 2006 to 2020 at RM 78.8 billion, with an average annual growth of 7.6% for the same period.

Manufacturers, particularly of edible oil industries, consume large quantities of palm and palm kernel oil, and use standards in examining the quality of palm oils. One of the standards is the degree of oxidation, which gives the buyer an indication of its stability or keeping quality. This degree of oxidation will determine the shelf life of the end-products, such as margarine and shortening.

Suppression of oxidation will increase bleachability, another factor for quality control. Difficulty in the bleachability of oils and fats was found to be due to the accumulation of oxy-carotenoids, by-products of the process of autoxidation. This can be prevented using antioxidants.

4.1. Palm Oil

Palm oil, when extracted from the fruit, is brownish-red in colour and contains substances other than pure triglycerides. Such substances include air, dissolved moisture, colouring matter (eg. beta-carotene), free fatty acids (f.f.a.), odoriferous substances, sterols, alcohols, phosphatides, tocopherols and other impurities [table 1]. Although not all substances are undesirable (some are even highly desirable, eg. tocopherol which is an antioxidant), the majority of them are objectionable and have to be removed. This is done by refining and deodorisation. Refining removes free fatty acids, phosphatides (mucilaginous material, ie. adhesives made out of vegetable origin), air (a component of autoxidation), and colouring matter, etc. The most common method of refining is alkali refining whereby the oil

is chemically treated with sodium hydroxide, converting the free fatty acids into removable soap.

Palm oil has an amount of triglycerides (neutral oils) of different melting points. Triglycerides are esters of long-chain carboxylic acids on the triol glycerol, such as Tristearin (1,2,3-Trioctadecanoylglycerol, $C_{57}H_{110}O_6$).

Triglycerides in palm oil are rather stable and therefore they do not take part in the process of autoxidation. Triglycerides are usable, edible, and beneficial substances used by higher biological systems.

Free fatty acids are food substances which are essential in cell function and formation, with minorities in enzyme formation. Since they cannot be synthesised in the body, they must be ingested as such in fatty foods. Fatty acids of vegetable origin are normally straight-chained unsaturated fatty acids having 18 carbon atoms with one double-bond at the middle of the hydrocarbon chain. If other double bonds are present, they lie closer to the carboxyl group. The double bond cannot be rotated and so there are two distinct geometries possible - the cis and trans orientation. The most abundant of all fatty acids is Oleic acid ($C_{18}H_{34}O_2$). The vast majority of olefinic (unsaturated) linkages in lipids are cis-oriented.

The polyunsaturated fatty acids are those having more than one double bond and they have the general formula: CH2(CH2)x(CH=CHCH2)y-(CH2)zCOOH.

Several polyunsaturated fatty acids include: Stearic acid (saturated, $C_{18}H_{36}O_2$), Oleic acid (monounsaturated, $C_{18}H_{34}O_2$), Linoleic acid (polyunsaturated, $C_{18}H_{32}O_2$), α-Linolenic acid (polyunsaturated, $C_{18}H_{30}O_2$), octadeca-6,9,12-trienoic acid (polyunsaturated, $C_{18}H_{30}O_2$), Arachidonic acid (polyunsaturated, $C_{20}H_{32}O_2$). Linoleic acid, α-Linolenic acid, and Octadeca-6,9,12-trienoic acids are usable by the human body for further introduction of double bonds, while Arachidonic acid is important for higher animals, including humans.

Although polyunsaturated fats or oils are essential to animals, these lipids are only present in plants. Therefore, animals can only obtain polyunsaturated lipids through the ingestion of plant materials. Polyunsaturated lipids are the building blocks for prostaglandins and help to lower the cholesterol level in the human body effectively.

Carotenoids are a class of terpenes, of which the molecules are derived from an arrangement of 8 isoprene units. They are red or yellow pigments widely distributed in small

quantities in many varieties of plants. They owe their colour to the large number of conjugated double bonds of the structure: -C=C-C=C-C=C-.

There are three types of carotenoids. Carotenes are hydrocarbons having the general formula of: $C_{40}H_{56}$. Xanthophylls are almost similar to carotenes but contain oxygen in the form of hydroxyl or carbonyl groups. Carotenoid Acids are similar to carotenes but with oxygen joined in the form of carboxylic acid groups.

Some carotenes include: Lycopene ($C_{40}H_{56}$), α-carotene ($C_{40}H_{56}$), and β-Carotene ($C_{40}H_{56}$). Lycopene is abundant in tomatoes, and carotene can be found in carrots.

Tocopherols ($C_{29}H_{50}O_2$) are organic compounds of the phenol group. Phenols contain a hydroxyl group attached directly to an aromatic nucleus and have a general formula ArOH, where Ar is the aromatic nucleus. Like alcohols, phenols can be monohydric or polyhydric depending on the number of hydroxyl groups that they may contain. Tocopherols are reddish, dense liquids which are soluble in lipids and fatty acids. They are natural antioxidants and are usually present in some crude vegetable oils.

Sterols of vegetable origin are usually phytosterols (phyto - plant in Greek), although sterols of animal origin (similar to phytosterols), such as Cholesterol ($C_{27}H_{46}O$) are also present in palm oil. Cholesterol is not fat chemically but is related to bile acids, reproductive hormones, and vitamin D.

Table 1: Malaysia plantation crude palm oil composition

Glycerides - Mole (%)	#
Tripalmitin	5.5
Dipalmitostearin	1.0
Dipalmitin (unsaturated)	29.5
Palmitostearin (unsaturated)	13.5
Palmitodiunsaturated	44.5
Triunsaturated	6.0
Fatty Acids - Average Composition (%)	
Caprylic Acid	0.005
Capric Acid	0.005
Lauric Acid	0.075
Myristic Acid	1.270
Palmitic Acid	39.600
Palmitoleic Acid (9-monoene)	0.130
Stearic Acid	6.170
Oleic Acid (9-monoene)	39.300
Linoleic Acid (9,12-diene)	12.180
Arachidic Acid	0.370
Linolenic Acid (9,12,15-triene)	0.370
Iodine Value	(54.800 to 64.200)
Alcohol Content (unsaponifiable)	Approx. 800 ppm
Triterpenic Alcohols	80%
Aliphatic Alcohols	20%
Unsaponifiable Matter - Average Composition	
Cartotenoids	500 - 700 ppm
Alpha-carotene	36.2%
Beta-carotene	54.4%
Gamma-carotene	3.3%
Lycopene	3.8%
Xanthophylls	2.2%
Tocopherols	500 - 800 ppm
Alpha-tocopherol	35.0%
Gamma-tocopherol	35.0%
Delta-tocopherol	10.0%
E+n-tocopherol	20.0%
Sterols	Approx. 300 ppm
Cholesterol	4.0%
Campesterol	21.0%
Stigmasterol	21.0%
Beta-sitosterol	63.0%
Phosphatides	500 - 1000 ppm

4.2. Palm Kernel Oil

Palm kernel oil is extracted from the kernel of the oil palm fruit [Figure 8]. The oil is a sweet-smelling, dense, and yellow coloured liquid. Although obtained from the same fruit, palm kernel oil is almost entirely different from palm oil in composition and physical appearance. Palm kernel oil has a saturation percentage of 82.5% while palm oil has only a saturation percentage of 52%. This explains why palm kernel oil is more stable compared to palm oil.

Figure 8: Oil palm [an original illustration by the authors]

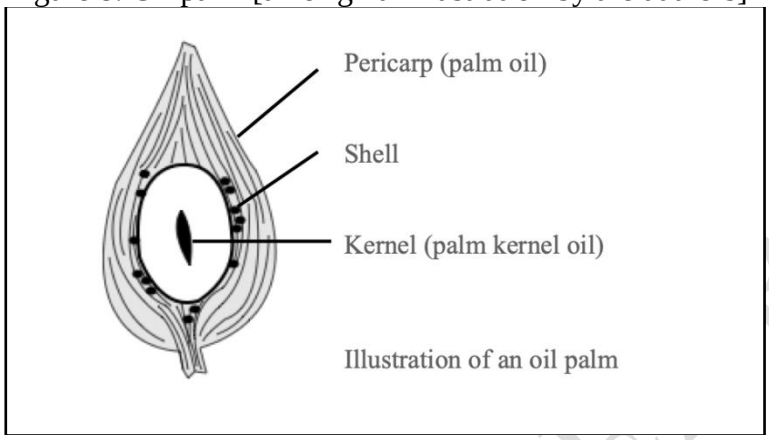


Table 2 : Palm Kernel Oil

Fatty Acids (Weight %)	#
Total Saturated Fatty Acids	82.5%
Caproic Acid	0.2
Caprylic Acid	4.8
Capric Acid	6.6
Lauric Acid	44.1
Myristic Acid	15.4
Palmitic Acid	8.5
Stearic Acid	2.7
Arachidic Acid	0.2
Total Unsaturated Fatty Acids	17.5%
Oleic Acid	16.1
Linoleic Acid	1.4

4.3. Milk (Cow)

Milk has traditionally been a popular food with good nutritional value.

Cow's milk is an oil/water emulsion containing 3.5 to 4% of fat (or phospholipids). In addition, the fat phase contains fat-soluble vitamins and the aqueous phase contains proteins, mineral salts, lactose, and water-soluble vitamins.

Fresh cow's milk is an important source of calcium and riboflavine but is deficient in iron, nicotinic acid, vitamin D, and ascorbic acid (vitamin C).

The fat of milk comes in the form of minute droplets of diameters between 5 to 10 microns. This facilitates the digestion of milk fat by organisms. Milk fat, also known as butter fat, is a mixture of glycerides, free fatty acids, cholesterol, lecithin, and other substances existing in minute amounts [table 3].

The fatty acids have varying degrees of solubility and volatility. Here are some fatty acids in decreasing order of water solubility:

A. butyric acid (most soluble)
B. caproic acid
C. caprylic acid
D. capric acid
E. lauric acid (almost insoluble)

The phenomena of solubility and volatility explain the disagreeable taste and odour of the 4 volatile soluble fatty acids. The fatty acids are all soluble in alcohol, thus distinguishing them from glycerides. From the alcoholic solutions, the solid acids separates in crystalline form:

A. capric, myristic & stearic acids (plate crystals)
B. lauric & palmitic acid (needle crystals)
C. caprylic acid (in both forms)

Table 3 : Cow's milk

Fatty Acids (Composition %)	#
Butyric Acid	3.2
Caproic Acid	1.8
Caprylic Acid	0.8
Capric Acid	2.1
Lauric Acid	3.8
Myristic Acid	10.4
Palmitic Acid	28.0
Stearic Acid	10.0
Oleic Acid	34.6
Total Soluble Acids	7.9%
Total Insoluble Acids	92.1%
Total	100%

4.4. Butter

Butter is the fat extracted from cow's milk. Therefore, its fat composition is similar to that of milk's [table 3].

4.5. Lard

Lard is prepared by melting pork's fat. It is approximately 100% pure fat. Natural lard is a low-melting fat. During the 1970s, lard was popular due to its low cost and the improvement of its property. Lard is also frequently used in Chinese (Taiwan and mainland China), as well as Japanese and South Korean cuisine. Lard is peculiar among fats in that the saturated fatty acids (mainly palmitic) are found predominantly in the middle position of the triglyceride molecules.

Table 4 : Lard

Fatty Acids - Average Composition (%)	#
Oleic Acid	56%
Palmitic Acid	28%
Stearic Acid	8%
Linoleic Acid	5%
Myristic Acid	1%
Lauric, Caprylic, Caproic, Capric, and Arachidonic Acids	2%
Iodine Value	Approx. 57

5. Lipid Autoxidation, Identification and Short-Term Toxicity

The authors conducted experiments circa 1981 to 1982, which included the action of pro-oxidants and antioxidants on selected lipids, identification of by-products such as aldehydes, and short-term toxicity studies that involved a suggested secondary antioxidant candidate.

5.1. Materials and Methods: Pro-oxidants and Antioxidants in Lipids

The apparatus required for the experiments included: Boiling tube, test tube, conical flask (250ml), burette (50 ml), volumetric flask (250 ml), measuring cylinder (10ml, 50ml and 1 litre), beaker (100 ml, 250 ml, and 1 litre), pipette (l ml, 2 ml, 10 ml, and 25 ml), Pasteur pipette, retort stand, tripod stand, droppers, glass rods, delivery tubes, burners, wire gauze, wash bottles (for distilled water), rubber stoppers, and tubes.

The following equipment and accessories were also used: Thermometers (0 to 100^{o}C), stop watches, digital balance (accuracy up to 0.0001g), white tiles (for titrimetric analysis), and fresh grass (for chlorophyll extraction).

These chemicals were used: Copper (II) sulphate, Zinc (II) sulphate, Manganese sulphate, Cobalt chloride, Aluminium sulphate, Nickel sulphate, Ferrous ammonium sulphate, Potassium iodide, Ethanoic acid (glacial), Chloroform, Sodium thiosulphate, Sodium carbonate, Sodium hydroxide, Manganese dioxide, Hydrogen peroxide (20 volume), Ethanol (laboratory grade), Fehling's reagent (CuH_2O_4S), Schiff Reagent ($C_{19}H_{21}N_3S_2O_7 \cdot 4H_2O$), 2,4-dinitrophenylhydrazine (2,4-DNPH), alpha-tocopherol (commercial grade), BHA/BHT/PG mixture (commercial grade), and dithiooxamide.

5.1.1. Preparation of the lipids used

Palm oil and palm kernel oil were the only two out of the tested lipids required no special preparation. Lard and butter were first melted before being used and are used while still in the molten state.

As milk contains a large percentage of other substances besides phospholipids, special preparation is needed before it can be used. 300 ml of milk was added to an Ethanol-Chloroform solvent (150:150, v/v). The resulting solution was shaken vigorously for 10 minutes. Then the organic liquid section (bottom layer of the shaken liquid section was extracted using a Pasteur pipette. The organic section was shaken with an equal volume of the

solvent used. This was repeated for 8 times and the extracted liquid after the repeated processes was used in the experiments in Section 5.1.

5.1.2. Preparation of the various solutions

The various metallic salt solutions were all concentration 0.001 mol.dm-3 . All metallic salts were weighed accurately using the electronic balance:

0.0624g of copper sulphate ($CuSO_4.5H_2O$)

0.0719g of zinc sulphate ($ZnSO_4.7H_2O$)

0.0703g of nickel sulphate ($NiSO_4.7H_2O$)

0.0305g of cobalt chloride ($CoCl_2$)

0.0378g of manganese sulphate ($MnSO_4$)

0.1665g of aluminium sulphate ($Al_2(SO_4)_3.18H_2O$)

0.0980g of ferrous ammonium sulphate ($FeSO_4(NH_4)SO_4.6H_2O$)

The carefully weighed salts were dissolved in 250 ml of distilled water (in separate volumetric flasks) except ferrous ammonium sulphate. Ferrous ammonium sulphate is not a chemically stable compound and therefore, it was first dissolved in 50 ml of dilute sulphuric acid and then the mixed solution was dissolved thoroughly with 200 ml of distilled water.

0.2 ml of the chlorophyll extract from ground grass was dissolved in 250 ml of ethanol.

0.1 mg of dithiooxamide was first dissolved in 50 ml of ethanol and then the solution was thoroughly mixed with 200 ml of distilled water. 0.02g each of vitamin E and the commercial antioxidant mixtures of BHA, BHT, and PG were dissolved in 50 ml of ethanol separately.

The Ethanoic Acid-Chloroform reagent was prepared by mixing 200 ml of ethanoic acid with 200 ml of chloroform (50:50, v/v).

0.25g of potassium iodide (KI) was dissolved in 250 ml of distilled water to make up a 0.1% solution.

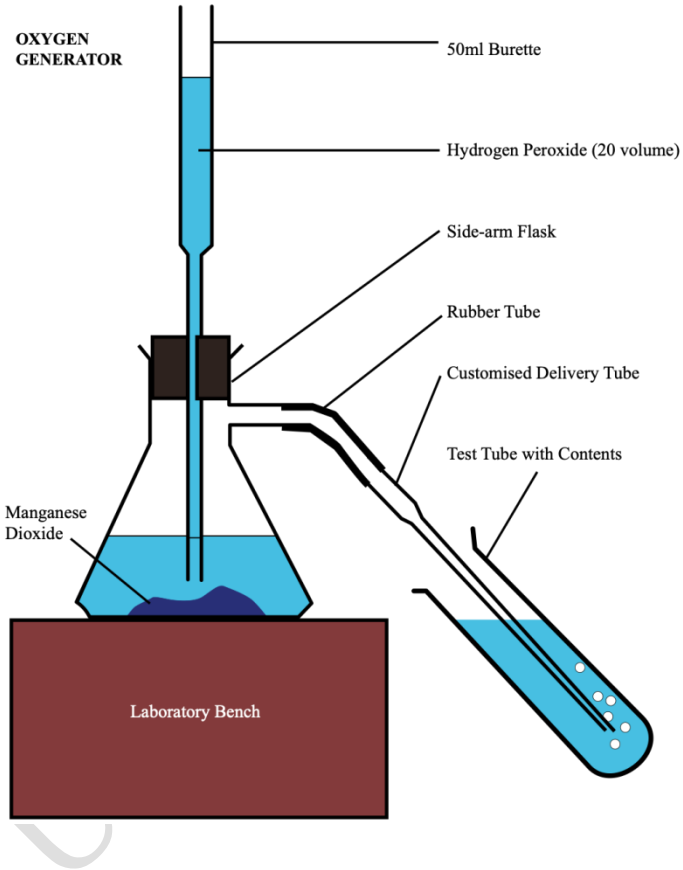
The reagent required for this section was 0.005N sodium thiosulphate solution. This was prepared by dissolving 0.31g of sodium thiosulphate crystals in 250 ml of distilled water.

5.1.4. Procedure of the experiment

12 boiling tubes each containing 20 ml of crude palm oil were prepared. Tube 1 was chosen as a control. Tubes 2 to 8 each contained 0.2 ml of the prepared zinc, manganese, cobalt, copper, iron, nickel, and aluminium salt solutions, respectively. Tube 9 contained 0.2 ml of the chlorophyll solution. Tube 10 contained 0.2 ml of the prepared dithiooxamide solution. Tube 11 contained 0.2 ml of the standardised vitamin E solution and Tube 12 contained 0.2 ml of the standardised phenolic mixture of BHT, BHA, and PG.

All boiling tubes were aerated with oxygen for 5 minutes using a simple "oxygen generator" as illustrated in Figure 9.

Figure 9: Simple “oxygen generator”



Each boiling tube, which was oxygenated, was then placed in a water bath maintained at a constant temperature of 70^{o}C. The peroxide value of each of the treatments was determined at zero time and subsequently at regular 20-minute intervals using 0.5 ml of palm oil (treated or control) pipetted with a l-ml pipette; 2 ml of the ethanoic acid/chloroform

reagent and 0.5g of solid potassium iodide were added to the 0.5 ml of palm oil in a conical flask. The conical flask was then heated for 1 minute in a steady 100^{o}C water bath. After that, 10 ml of the 0.1% potassium iodide solution and 5 drops of starch indicator solution were added. The iodine liberated (brown) was titrated with 0.005N sodium thiosulphate solution until the cloudy brown solution turned clear and the peroxide value estimated from the volume of the sodium thiosulphate solution used (milli equivalents per kilogram of palm oil). Each experiment was repeated 5 times and the average values taken.

The whole experiment was repeated for palm kernel oil, lard, butter, and the extracted phospholipids of milk.

5.1.5. Results and Discussion

The experimental results for the rate of oxidation of crude palm oil in the presence of pro-oxidants and antioxidants are shown in the pages to follow.

Table 5 : Average peroxide value in milli equivalents per kilogram of palm oil

Palm Oil - Average Peroxide Value in milli equivalents per kilogram						
Treatment/Time (minute)	0	20	40	60	80	100
Control	0.00	0.30	0.20	0.15	0.50	0.60
Zinc	0.00	0.40	0.10	0.15	0.40	0.40
Manganese	0.00	0.20	0.40	0.20	0.50	0.40
Cobalt	0.00	0.10	0.20	0.40	0.50	0.30
Copper	0.00	0.60	0.30	0.60	0.50	0.35
Iron	0.00	0.15	0.30	0.20	0.25	0.55
Nickel	0.00	0.40	0.20	0.60	0.30	0.60
Aluminium	0.00	0.05	0.05	0.05	0.10	0.00
Chlorophyll	0.00	0.50	0.30	0.30	0.35	0.40
Dithiooxamide	0.00	0.05	0.00	0.00	0.00	0.05
Alpha-tocopherol	0.00	0.15	0.10	0.10	0.05	0.05
BHA/BHT/PG	0.00	0.25	0.15	0.10	0.10	0.15

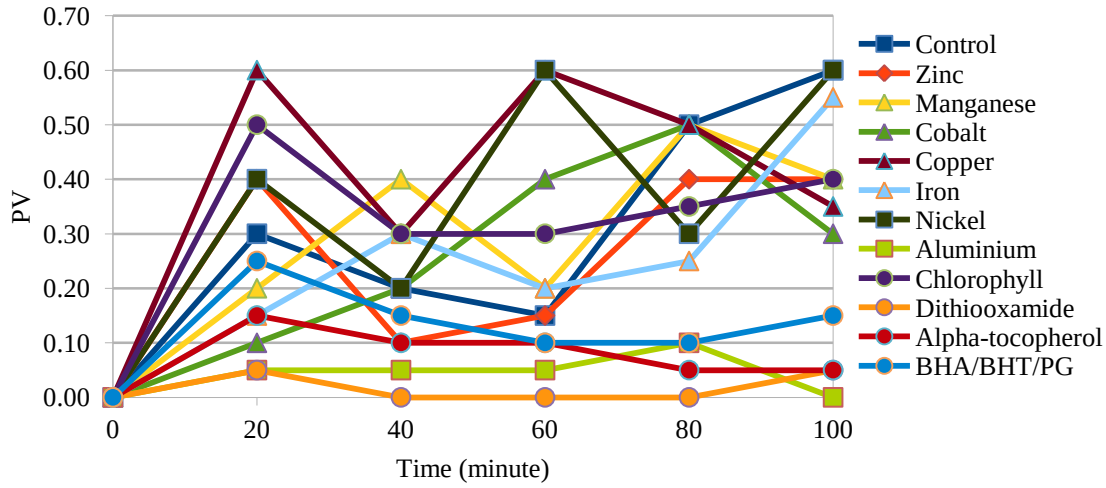


Figure 10 illustrates the effects of different pro-oxidants on crude palm oil. As the graph shows, copper, zinc, and chlorophyll give the highest PV in the initial 40 minutes. And from this we can conclude that copper, zinc, and chlorophyll can give the most trouble if present in crude palm oil. Beyond 60 minutes, metals such as iron gave high PV as well.

From the graph, we can also see that in the presence of aluminium, palm oil is not quite oxidised; in other words, aluminium gives an induction period and is the best material to store crude palm oil.

Table 5 also shows that peroxides are very unstable. This is seen from the fact that after the initial 40 minutes, the PVs become very unstable and therefore only the initial 40 minutes period can be taken into account.

The experimental results for the rate of oxidation of crude palm kernel oil in the presence of pro-oxidants and antioxidants are shown in Table 6:

Table 6: Average peroxide value in milli equivalents per kilogram of palm kernel oil

Palm Kernel Oil - Average Peroxide Value in milli equivalents per kilogram						
Treatment/Time (minute)	0	20	40	60	80	100
Control	0.00	0.10	0.10	0.15	0.20	0.20
Zinc	0.00	0.20	0.10	0.10	0.20	0.20
Manganese	0.00	0.15	0.15	0.10	0.25	0.30
Cobalt	0.00	0.25	0.10	0.20	0.20	0.15
Copper	0.00	0.30	0.20	0.30	0.25	0.30
Iron	0.00	0.15	0.20	0.10	0.10	0.20
Nickel	0.00	0.25	0.20	0.30	0.15	0.20
Aluminium	0.00	0.00	0.05	0.00	0.05	0.10
Chlorophyll	0.00	0.20	0.10	0.20	0.15	0.25
Dithiooxamide	0.00	0.00	0.00	0.00	0.00	0.00
Alpha-tocopherol	0.00	0.05	0.05	0.10	0.05	0.10
BHA/BHT/PG	0.00	0.20	0.10	0.05	0.10	0.05

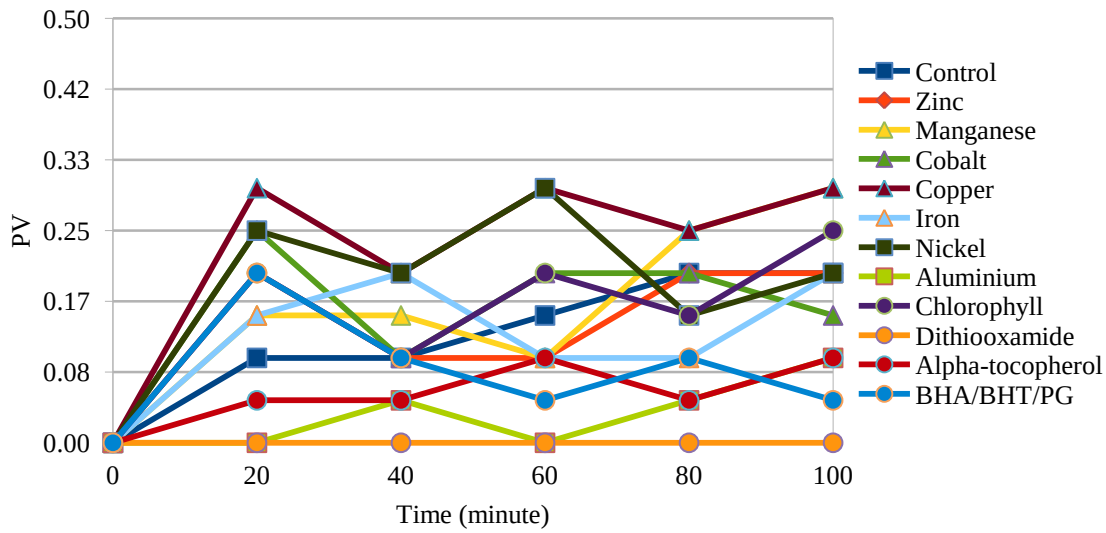


figure 11: Average peroxide value in milli equivalents per kilogram of palm kernel oil

From Figure 11, we can draw the same conclusions as from the graph for palm oil. In fact, all lipids the authors experimented showed almost the same results relatively. As palm

kernel oil has a higher saturation percentage than palm oil, the recorded values for palm kernel oil are lower than those for palm oil. This was expected as palm kernel oil is more stable than palm oil and thus is less oxidised.

It will be noted that the chlorophyll factor is only present in palm oil and palm kernel oil. This is logical since only both of these oils come into a great amount of contact.

The experimental results for the rate of oxidation of commercial butter in the presence of pro-oxidants and antioxidants are shown in Table 7:

Table 7: Average peroxide value in milli equivalents per kilogram of butter

Butter - Average Peroxide Value in milli equivalents per kilogram						
Treatment/Time (minute)	0	20	40	60	80	100
Control	0.00	0.15	0.10	0.25	0.15	0.15
Zinc	0.00	0.30	0.45	0.40	0.55	0.10
Manganese	0.00	0.35	0.25	0.25	0.30	0.10
Cobalt	0.00	0.20	0.30	0.40	0.45	0.30
Copper	0.00	1.30	1.45	1.05	0.80	1.30
Iron	0.00	0.70	0.60	0.75	0.80	1.30
Nickel	0.00	0.20	0.60	0.30	0.20	0.35
Aluminium	0.00	0.00	0.05	0.10	0.10	0.05
Chlorophyll						
Dithiooxamide	0.00	0.00	0.00	0.00	0.05	0.00
Alpha-tocopherol	0.00	0.05	0.10	0.20	0.00	0.05
BHA/BHT/PG	0.00	0.10	0.05	0.15	0.15	0.00

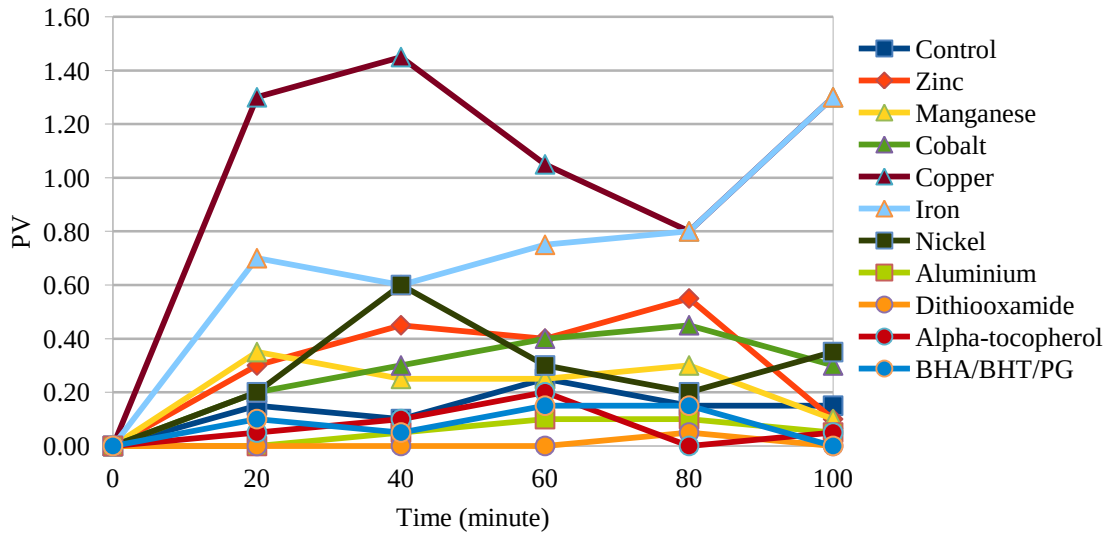


Figure 12 : Average peroxide value in milli equivalents per kilogram of butter

Butter demonstrated a very peculiar behaviour in the presence of copper. This can only mean that butter is greatly oxidised in the presence of copper. The rest of the metals show sufficiently normal behaviour compared to copper. The effect of copper on butter was not expected as we did for other lipids and their rates of oxidation. However, beyond 60 minutes, iron catches up with copper and is undesirable in the context of lengthened time.

The experimental results for the rate of oxidation of phospholipids of milk in the presence of pro-oxidants and antioxidants are shown in Table 8:

Table 8: Average peroxide value in milli equivalents per kilogram of milk

Milk - Average Peroxide Value in milli equivalents per kilogram						
Treatment/Time (minute)	0	20	40	60	80	100
Control	0.00	0.20	0.10	0.20	0.15	0.10
Zinc	0.00	0.25	0.30	0.30	0.35	0.25
Manganese	0.00	0.25	0.15	0.20	0.30	0.10
Cobalt	0.00	0.05	0.15	0.35	0.50	0.30
Copper	0.00	2.15	1.30	1.15	1.00	1.45
Iron	0.00	0.30	0.40	0.60	0.55	0.70
Nickel	0.00	0.15	0.25	0.40	0.20	0.20
Aluminium	0.00	0.00	0.00	0.05	0.10	0.05
Chlorophyll						
Dithiooxamide	0.00	0.00	0.05	0.00	0.05	0.00
Alpha-tocopherol	0.00	0.05	0.10	0.20	0.10	0.10
BHA/BHT/PG	0.00	0.10	0.15	0.15	0.05	0.05

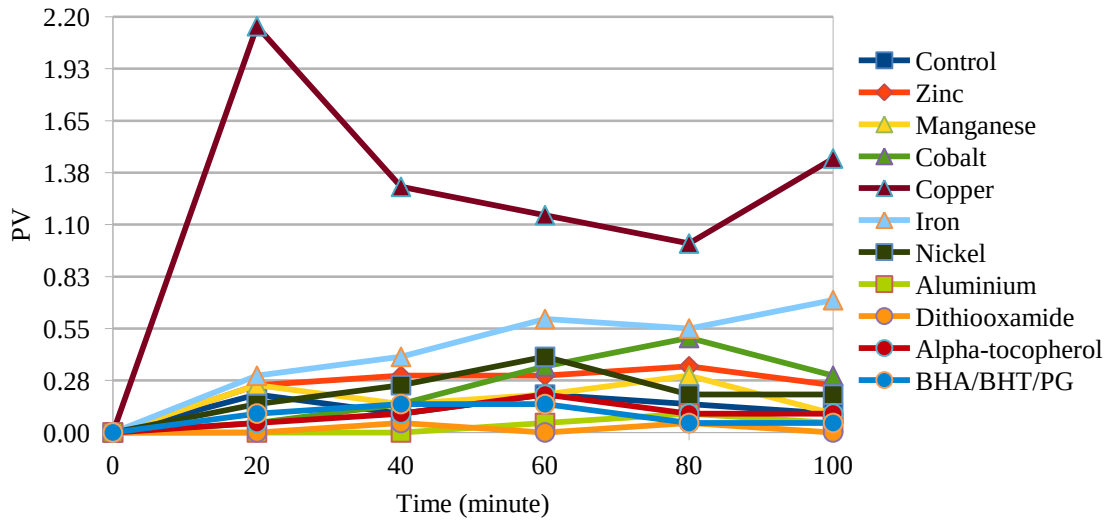


Figure 13: Average peroxide value in milli equivalents per kilogram of milk

The rate of oxidation of milk was almost proportional to the rest of the tested lipids except for copper. Again, copper caused an abnormally high rate of oxidation. The next metal causing the most trouble for milk was iron, faring second in the highest PV over time, especially beyond 60 minutes under these conditions.

The experimental results for the rate of oxidation of lard in the presence of pro-oxidants and antioxidants are shown in Table 9

Table 9: Average peroxide value in milli equivalents per kilogram of lard

Lard - Average Peroxide Value in milli equivalents per kilogram						
Treatment/Time (minute)	0	20	40	60	80	100
Control	0.00	0.05	0.10	0.10	0.00	0.05
Zinc	0.00	0.05	0.10	0.30	0.05	0.10
Manganese	0.00	0.30	0.10	0.40	0.20	0.20
Cobalt	0.00	0.25	0.10	0.50	0.10	0.40
Copper	0.00	2.10	1.30	1.30	2.20	2.10
Iron	0.00	0.20	0.30	0.90	1.20	0.60
Nickel	0.00	0.30	0.20	0.20	0.25	0.30
Aluminium	0.00	0.00	0.05	0.10	0.05	0.05
Chlorophyll						
Dithio-oxamide	0.00	0.00	0.00	0.00	0.00	0.05
Alpha-tocopherol	0.00	0.20	0.10	0.05	0.05	0.05
BHA/BHT/PG	0.00	0.30	0.10	0.10	0.20	0.10

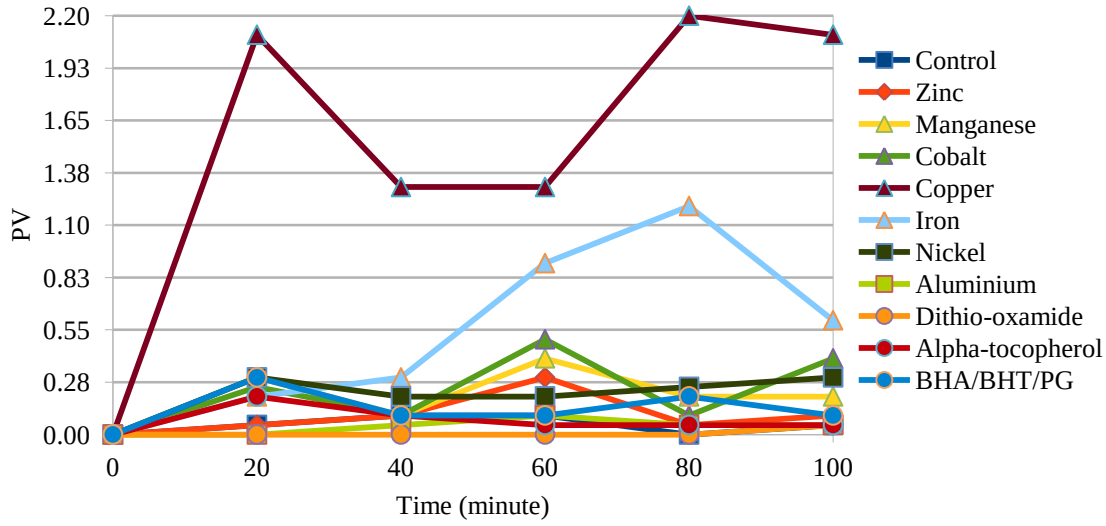


Figure 14 : Average peroxide value in milli equivalents per kilogram of lard

The peroxide values for the oxidation of lard are generally higher compared to palm oils and may be due to its instability. Copper ranked worst with the highest PV, and iron was second.

Table 10: Antioxidants in the oxidation of Palm Oil

Antioxidants on the Oxidation of Palm Oil (Average PV in milli equivalents per kilogram)						
Treatment/Time (minute)	0	20	40	60	80	100
Control	0.00	0.30	0.20	0.15	0.50	0.60
Dithiooxamide	0.00	0.05	0.00	0.00	0.00	0.05
Alpha-tocopherol	0.00	0.15	0.10	0.10	0.05	0.05
BHA/BHT/PG	0.00	0.25	0.15	0.10	0.10	0.15

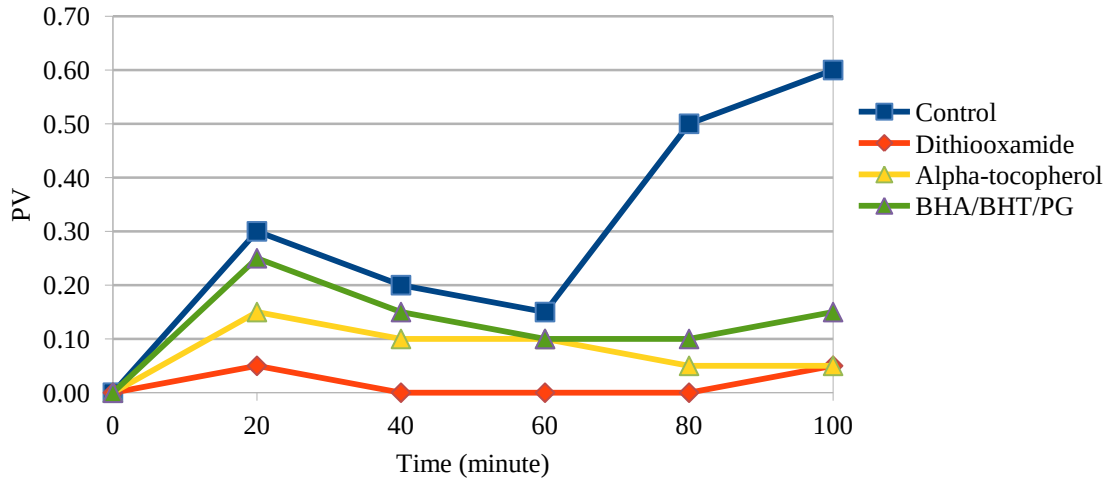


Figure 15 : Antioxidants in the oxidation of Palm Oil

In table 10, 11, 12, 13, and 14, we can conclude that dithiooxamide is the most effective antioxidant compared to the other two, and that palm oil seems to degrade beyond one hour under the tested conditions.

Table 11: Antioxidants on the oxidation of Palm Kernel Oil

Antioxidants on the Oxidation of Palm Kernel Oil (Average PV in milli equivalents per kilogram)						
Treatment/Time (minute)	0	20	40	60	80	100
Control	0.00	0.10	0.10	0.15	0.20	0.20
Dithiooxamide	0.00	0.00	0.00	0.00	0.00	0.00
Alpha-tocopherol	0.00	0.05	0.05	0.10	0.05	0.10
BHA/BHT/PG	0.00	0.20	0.10	0.05	0.10	0.05

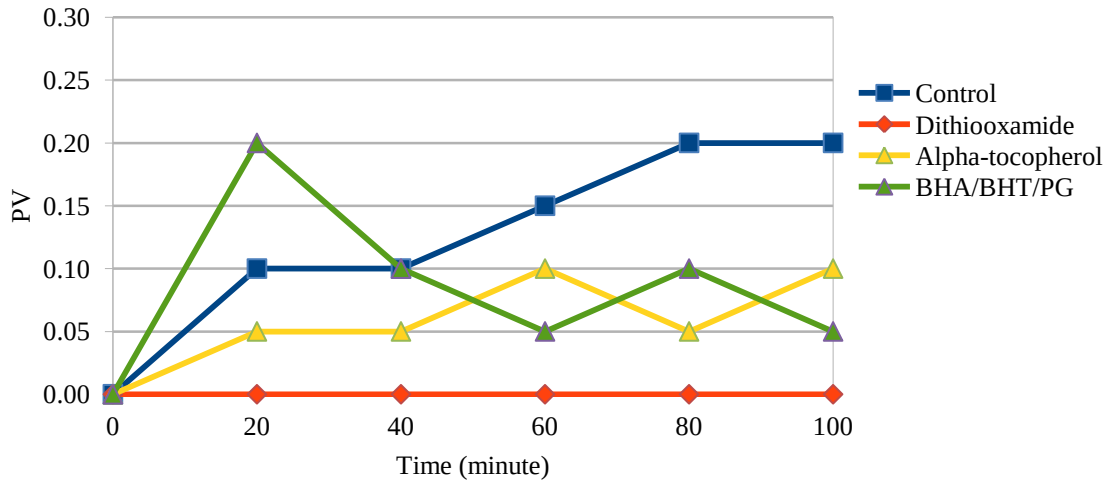


Figure 16: Antioxidants on the oxidation of Palm Kernel Oil

In table 11 it is observed that dithiooxamide lies on the time axis and therefore, if it is present in the oil, it will be very effective in preventing autoxidation.

Table 12: Antioxidants on the oxidation of Butter

Antioxidants on the Oxidation of Butter (Average PV in milli equivalents per kilogram)						
Treatment/Time (minute)	0	20	40	60	80	100
Control	0.00	0.15	0.10	0.25	0.15	0.15
Dithiooxamide	0.00	0.00	0.00	0.00	0.05	0.00
Alpha-tocopherol	0.00	0.05	0.10	0.20	0.00	0.05
BHA/BHT/PG	0.00	0.10	0.05	0.15	0.15	0.00

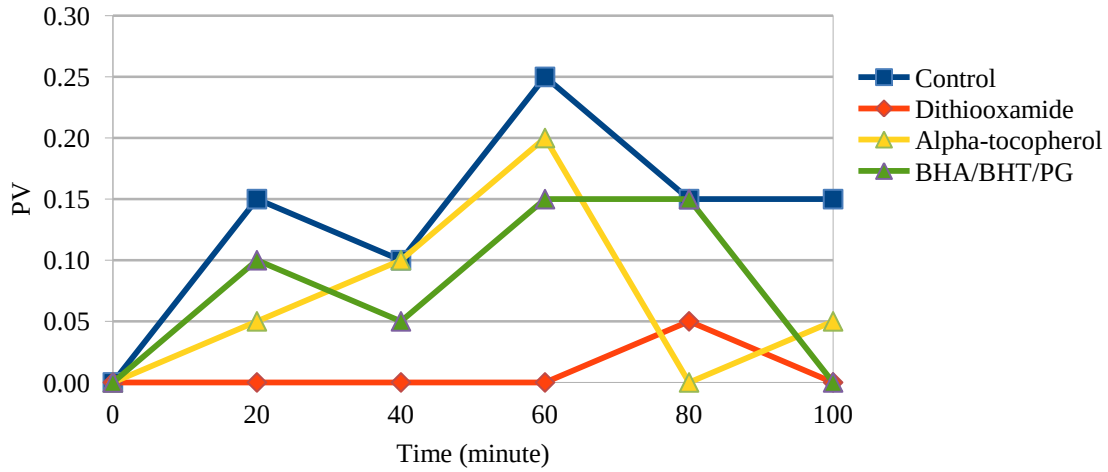


Figure 17: Antioxidants on the oxidation of Butter

Table 13 : Antioxidants on the oxidation of Milk

Antioxidants on the Oxidation of Milk (Average PV in milli equivalents per kilogram)						
Treatment/Time (minute)	0	20	40	60	80	100
Control	0.00	0.20	0.10	0.20	0.15	0.10
Dithiooxamide	0.00	0.00	0.05	0.00	0.05	0.00
Alpha-tocopherol	0.00	0.05	0.10	0.20	0.10	0.10
BHA/BHT/PG	0.00	0.10	0.15	0.15	0.05	0.05

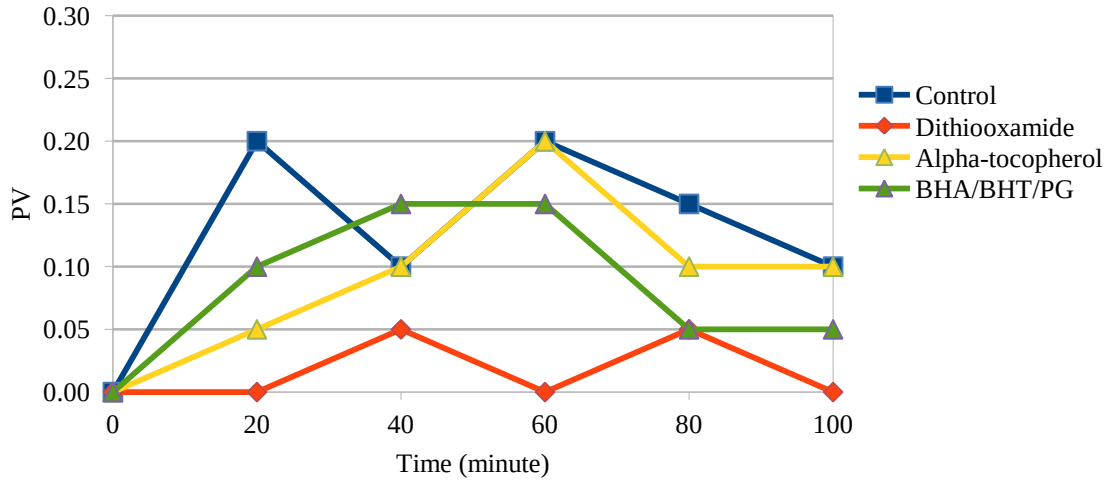


Figure 18: Antioxidants on the oxidation of Milk

Because the lipids in milk and butter are closely related, the effects of vitamin E on both these lipids for the first 40 minutes are the same.

Table 14 : Antioxidants on the oxidation of Lard

Antioxidants on the Oxidation of Lard (Average PV in milli equivalents per kilogram)						
Treatment/Time (minute)	0	20	40	60	80	100
Control	0.00	0.05	0.10	0.10	0.00	0.05
Dithiooxamide	0.00	0.00	0.00	0.00	0.00	0.05
Alpha-tocopherol	0.00	0.20	0.10	0.05	0.05	0.05
BHA/BHT/PG	0.00	0.30	0.10	0.10	0.20	0.10

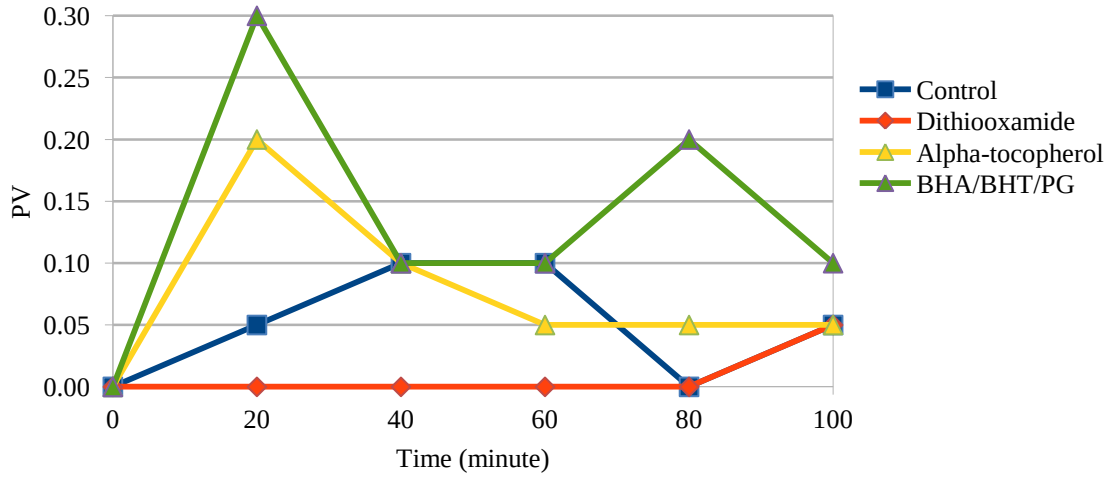


Figure 19 : Antioxidants on the oxidation of Lard

Even dithiooxamide is not effective after the 80 minute period for lard as it is very unstable as shown in Table 14.

5.2. Identification of Aldehydes, Ketones and Acids

Simple tests were conducted on the oxidised lipid samples to determine the presence of byproducts such as aldehydes, ketones, and acids, which are undesirable in lipids.

5.2.1.1. Schiff Reagent Test

Method: To the oxidised sample of the lipid, Schiff Reagent ($C_{19}H_{21}N_3S_2O_7 \cdot 4H_2O$) was added drop by drop.

5.2.1.2. 2,4-DNPH Test

Method: To the oxidised lipid sample, 2,4-dinitrophenylhydrazine (2,4-DNPH) was slowly added.

5.2.1.3. Fehling's Reagent Test

Method: To the above treated solution, Fehling's reagent (CuH_2O_4S) was added and this was heated in a water bath for about 3 minutes.

5.2.1.4. Sodium Carbonate Test

Method: Sodium carbonate solution was added to the above treated solution.

5.2.1.5. Neutral Ferric Chloride Test

Method: Sodium hydroxide solution was added drop by drop to a test tube with a quarter full of ferric chloride solution until a permanent precipitate of ferric hydroxide were obtained. The precipitate was filtered off and the filtrate is called NEUTRAL ferric chloride. To the oxidised sample of the lipid, the NEUTRAL ferric chloride was added and the mixture boiled.

Observation: A reddish-brown precipitate appeared.

Deduction: Acid group (-COOH) present.

From the Schiff Reagent tests, a reddish violet colour appeared in the treated oxidised lipid samples, showing that aldehydes were present.

In the 2,4-DNPH test, a dark brown precipitate appeared in the treated lipid samples showing that either aldehydes or ketones were present.

From the Fehling's reagent tests, a yellow precipitate appeared initially, which turned orange, and finally to a red precipitate, in all treated lipid samples, showing the presence of aldehydes.

In the Sodium Carbonate tests, effervescence was observed with a gas turning lime water milky, showing the presence of -COOH acid groups in the treated lipid samples.

From the Neutral Ferric Chloride tests, a reddish brown precipitate appeared in all treated lipid samples, showing the presence of -COOH acid groups.

Table 15 illustrates the results from the brief experiments in 5.2.1.1, 5.2.1.2, 5.2.1.3, 5.2.1.4, and 5.2.1.5.

Table 15: Identification of Aldehydes, Ketones and Acids

Identification of Aldehydes, Ketones and Acids				
	Palm Oil	Palm Kernel Oil	Milk	Butter
2,4-DNPH	Aldehydes or Ketones	Aldehydes or Ketones	Aldehydes or Ketones	Aldehydes or Ketones
Schiff's Reagent	Aldehydes	Aldehydes	Aldehydes	Aldehydes
Fehling's Reagent	Aldehydes	Aldehydes	Aldehydes	Aldehydes
Sodium Carbonate	Acid (-COOH)	Acid (-COOH)	Acid (-COOH)	Acid (-COOH)
Neutral Ferric Chloride	Acid (-COOH)	Acid (-COOH)	Acid (-COOH)	Acid (-COOH)

5.3. Short-term Toxicity Experiments

For orally consumed chemicals and additives, they should be free from toxicity. Even for chemicals and additives applied externally, it should be free from toxicity as well. A simple short-term toxicity study was conducted with dithiooxamide.

The usual measure for acute oral toxicity of a substance is the LD50, which is the dose necessary to kill, on average, 50% of a group of test animals (usually rats or mice). This dose is expressed as milligrams of substance per kilogram of body weight. The lower the figure, the more toxic the substance will be.

PubChem's Compound Summary on dithiooxamide [16] showed this substance to be an "irritant", with an oral LDLo in rats of 500 mg/kg, or an oral LD50 in mice at 350 mg/kg, causing the likes of somnolence (similar to lethargy), convulsions, seizures, and changes in motor activity. The lowest dosage tested was intravenous injection LD50 at 56 mg/kg in mice by the U.S. Army Armament Research & Development Command. However, these dosages are considered very high and not applicable to the tests we conducted. Our tests were prepared by dissolving 0.1mg of dithiooxamide in 50ml of ethanol and then the solution was thoroughly mixed with 200 ml of distilled water. This would yield only 0.4 mg of dithiooxamide in 1 litre of water (equivalent to 1 kg), far below LDLo and LD50 designated in PubChem for laboratory rats and mice.

The authors 'short-term toxicity study predated the Animal Welfare Act from the Guide published by the National Research Council and the Institute for Laboratory Animal Research in 1985, and is exempt, and compatible with the Guide in 1985, as rats and mice were excluded from the Animal Welfare Act.

10 mice, 2 large cages with water feeders, and mouse pellets were used. The pellets were ground for easier handling.

5 mice was placed in each cage to be fed and weighed daily. The first cage would be Group A, and the second cage, Group B. Group A served as the control where their ground pellets and water would be free from dithiooxamide. Group B would have their ground pellets infused with dithiooxamide, but not their water. Both groups were placed in the same ambient setting for light, room temperature, and air.

The mouse pellets to be used for the toxicity study were first ground to powder, apportioning for 10 grams of pellets per day for all mice in Group B. A 50 ml dithiooxamide solution was prepared, and mixed with the ground pellets. The ground pellets infused with dithiooxamide were then dried in a low-temperature oven to evaporate the ethanol and water before the food pellets could be accepted by the mice.

Table 16 shows the weights (in grams) of the mice on the 8 days of the study.

Table 16: Weight (grams) of mice per day

Group A (Control) - Weight (grams)/day								
	1	2	3	4	5	6	7	8
A1	23.5	23.5	23.5	23.0	22.5	22.0	22.0	21.0
A2	25.5	25.5	25.0	24.5	24.5	24.0	23.0	23.5
A3	27.0	27.0	27.0	27.5	26.0	25.5	25.0	25.5
A4	27.5	27.0	26.0	26.5	26.5	25.5	25.0	25.0
A5	21.0	22.5	22.0	22.0	22.0	22.5	23.0	24.0
Group B (Experimental) - Weight (grams)/day								
	1	2	3	4	5	6	7	8
B1	23.5	23.5	24.0	23.0	23.5	22.0	22.0	22.5
B2	28.5	29.5	30.0	30.5	29.0	29.0	27.5	27.0
B3	27.0	28.0	29.0	28.5	27.0	27.0	26.5	27.0
B4	24.0	25.5	26.0	24.5	24.0	25.0	25.0	24.0
B5	27.5	28.0	29.0	29.5	29.0	28.5	28.0	27.5

None of the mice showed any physical difference in behaviour and none of them died, showing that dithiooxamide in that concentration is free from short-term toxicity. To determine more about the short term effects of dithiooxamide, the mice were dissected and some slides of micro-sectioned organs were prepared.

Figure 20, 21, 22, and 29 are scanned photographs of the slides. None of the organs seem to be damaged. The lung in Group B [Figure 21] was destroyed due to aspiration pneumonia (entering of food particles into the lungs by accident).

Figure 20: Brain cells

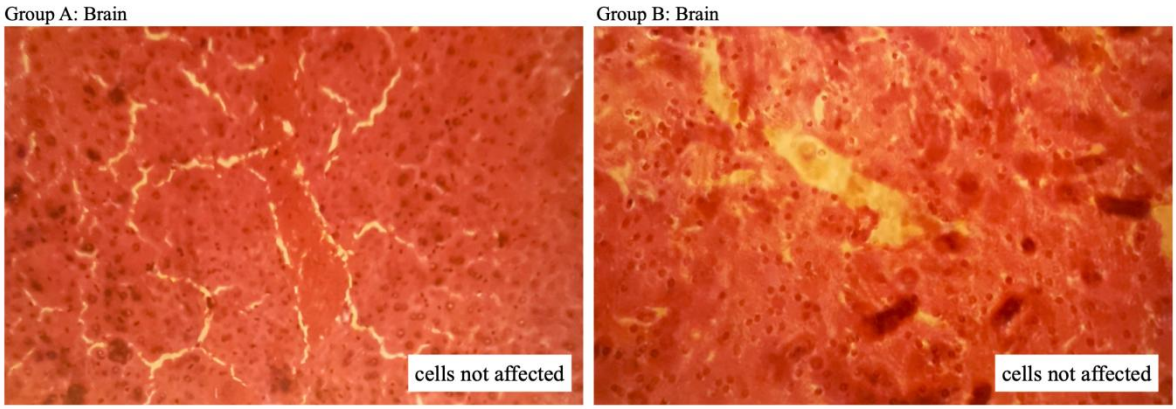


Figure 21: Lung cells

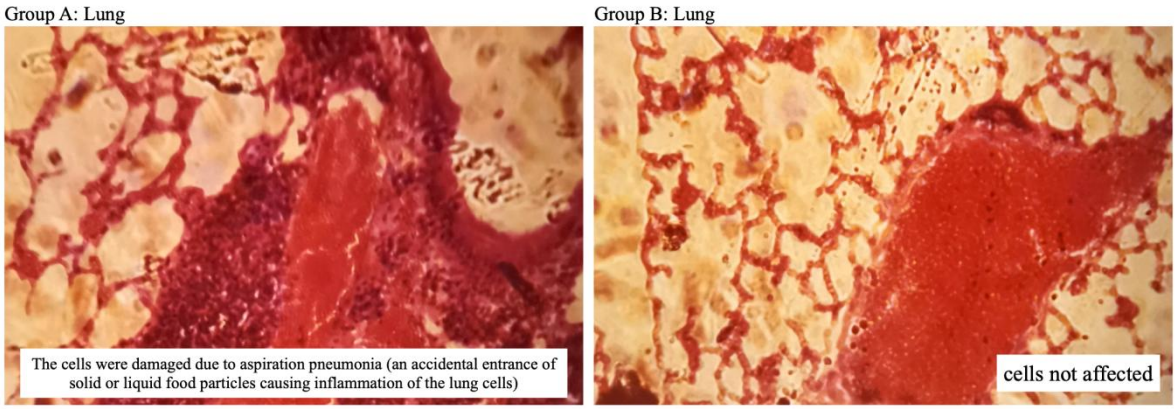


Figure 22: Liver cells

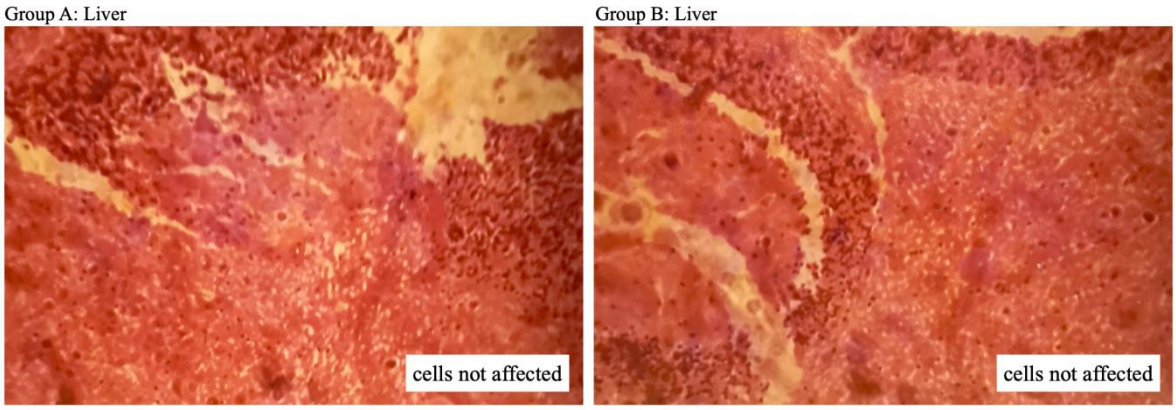
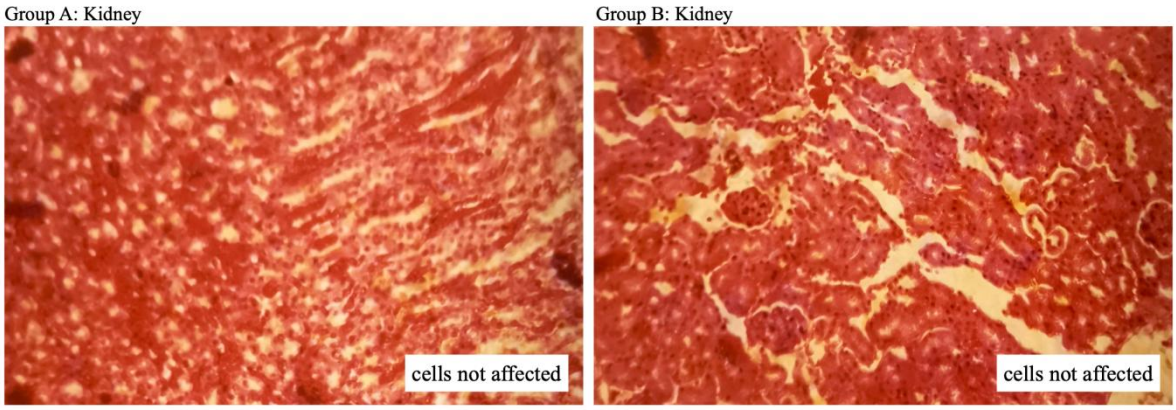


Figure 23: Kidney cells



6. Conclusions

In the short study, during the autoxidation of lipids, aldehydes, and ketones were formed as expected, which may be undesirable for consumption. Lipids stored in copper and other heavy metals, or are contaminated with such metals, are prone to accelerated autoxidation. Conversely, aluminium seems to provide an induction period to lipids and does not accelerate autoxidation as other metals such as copper do. When compared to antioxidants such as vitamin E, BHA, BHT, and PG, dithiooxamide seemed to be a proficient secondary antioxidant candidate, and was found to be free from short-term toxicity in the small amounts used in the study, which are far below the LDLo and LD50 conducted on rats and mice using dithiooxamide compared to other published studies.

A study of spoilage in animal and vegetable fatty food materials due to the contact with molecular oxygen in the air and the possibility of dithiooxamide as an antioxidant. In the study, the authors studied the background information of the causes, mechanism, and prevention of oxidative rancidity, on the kinetic aspects of oxidative rancidity on a representative range of lipids, such as palm oil, palm kernel oil, butter, milk phospholipids, and lard.

The authors examined metals and chlorophyll as pro-oxidants on the lipids investigated. It was also observed that the catalytic capacity of the pro-oxidants during the initial 40 minutes of the experiment was in the decreasing order of:

Cu > Chlorophyll > Zn > Mg > Ni > Fe > Co > Al.

The authors found that peroxides were very unstable, and so the peroxide value (PV) of an oil or fat cannot be solely regarded as an index of oxidation, but as an indication. The functional groups in the oxidation products were expectedly found to be mainly aldehydes, ketones, and acids.

The authors compared the antioxidant properties of alpha-tocopherol, a commercial mixture of BHT/BHA/PG, and dithiooxamide as a candidate, with palm oil, palm kernel oil, milk, butter, and lard.

In the study, dithiooxamide provided an induction period, as it may act as a hydroperoxide destroyer and a metal-chelating agent. The authors found the degree of efficiency of the antioxidants tested in an order of:

Dithiooxamide (more effective) > Alpha-tocopherol > BHA/BHT/PG (less).

A short-term toxicity study on experimented mice showed dithiooxamide to be free from short-term toxicity, which is consistent with latter-day studies on LDLo and LD50 studies of this compound.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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