Oxidative stability and shelf life of sunflower oil-in-water emulsions as affected by pro- and antioxidants and temperature

by

Jeanine Sainsbury
22006402

Submitted in partial fulfilment of the requirements for the degree

PhD Food Science

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

Pretoria

January 2019

DECLARATION

I, Jeanine Sainsbury, declare that the thesis herewith submitted for the degree PhD Food Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

…… ……..

J. Sainsbury Date

ETHICS STATEMENT

I, Jeanine Sainsbury, the author declares that I have observed the ethical standards required in terms of the University of Pretoria's code of ethics for researchers and the policy guidelines for responsible research.

……. ……..

J. Sainsbury Date

ACKNOWLEDGEMENTS

I hereby wish to express my sincere gratitude and appreciation to the following persons and institutions for their valuable contributions to the successful completion of this study:

My study leader, Prof. Henriëtte de Kock, for her patience, guidance, and encouragement and Prof. Gyebi Duodu, my co-supervisor, for support and assistance in designing the project

Dr. Hamed Faridi, Chief Science Officer, McCormick & Co., Inc for support and encouragement in the completion of this research

To the analytical and sensory laboratories of McCormick & Company, MD, USA, and their personnel for analytical and sensory analysis for the project,

My family and friends for their love, encouragement and support through these years.

Finally, I dedicate this study and my career to HIM who made it all possible.

ABSTRACT

Oxidative stability and shelf life of sunflower oil-in-water emulsions as affected by pro- and antioxidants and temperature

By

Jeanine Sainsbury

Supervisor: Prof. H. L. de Kock

Co-supervisor: Prof K. G. Duodu

Department of Food Science

Degree: PhD Food Science

Sunflower oil-in-water emulsions, like salad dressings and mayonnaise, have a shelf life of 6-12 months at room temperature. The shelf life of these emulsions is usually limited by lipid oxidation leading to the formation of undesirable components such as free radicals and reactive aldehydes which result in rancid notes. Various factors have been reported to induce lipid oxidation in emulsions during storage, thereby limiting the shelf life of oil-in-water emulsions. Antioxidants are added to emulsions to retard lipid oxidation. The effectiveness of these antioxidants can be evaluated over the 6-12 month shelf life period, but this has time and cost implications. There is a need for valid accelerated methods that can be used to measure the efficiency of antioxidants in commercial oil-in-water emulsions. This study is aimed at gaining a better understanding of how antioxidant levels influence oxidation of commercial sunflower oil salad dressing emulsions, when exposed to a combination of oxidation acceleration factors.

Therefore the first part of this study will focus on the effect of high and low concentrations of two antioxidants with different antioxidant mechanisms [gallic acid and ethylene diamine tetraacetate (EDTA)] in a commercial sunflower oil salad dressing emulsion (SOSDE) stored at accelerated deterioration and ambient conditions. SOSDEs stored at accelerated storage conditions contained four pro-oxidation factors (temperature increase to 32.2°C, addition of specific potential pro-oxidants $FeSO_4$ and ascorbic acid and adjustment of

pH to 5.5). The combination of these pro-oxidants and the their concentrations are based on research to optimise the development of lipid oxidation in oil-in-water emulsions. SOSDEs were examined by sensory (aroma evaluation by a trained sensory panel) and chemical analyses (anisidine value and peroxide value) daily for 20 days for accelerated stored products and bi-weekly for 24 weeks for ambient stored products. Sensory differences between products with high and low concentrations of antioxidants were more apparent for ambient stored than accelerated stored SOSDEs. Accelerated storage resulted in more aroma differences between SOSDEs with different types of antioxidants than between high and low antioxidant concentrations. Peroxide value (PV) differences between products with high and low concentrations of antioxidants were apparent for SOSDEs with EDTA stored under accelerated shelf life conditions, with higher concentrations resulting in significantly lower PV. Anisidine value (AV) differences existed between SOSDEs with different gallic acid concentrations stored in accelerated conditions (with AccLoGal (accelerated storage conditions, low concentration, gallic acid)) significantly lower AV than AccHiGal (accelerated storage conditions, high concentration, gallic acid) and ambient shelf life conditions (with AmbHiGal (ambient storage conditions, high concentration, gallic acid)) significantly lower AV than AmbLoGal (ambient storage conditions, low concentration, gallic acid), and SOSDEs with different EDTA concentrations stored in accelerated shelf life conditions (with AccHiEDTA (accelerated storage conditions, high concentration, EDTA)) significantly lower AV than AccLoEDTA (accelerated storage conditions, low concentration, EDTA). These results suggest that: 1) the accelerated storage model used in this study is more suitable to predict the shelf life changes of SOSDEs with metal chelator antioxidants such as EDTA, rather than free radical scavenging antioxidants such as gallic acid. This may be due to the addition of $FeSO_4$ as a potential pro-oxidant. 2) PV and AV are more sensitive oxidation markers than aroma analysis when using the accelerated storage model, and 3) PV, AV and aroma of SOSDEs stored in accelerated conditions as used in this study, do not clearly predict results for ambient stored SOSDEs.

The second part of the study focused on identifying key sensory attributes associated with the development of lipid oxidation and rancidity that can be used as predictors for end of shelf life during shelf life testing of oil-in-water emulsions. A multivariate accelerated shelf life test (MASLT) was applied to the aroma profiles of SOSDEs with different antioxidant treatments and storage conditions. Addition of antioxidants to the SOSDE decreased the rate constant of change in aroma of lipid oxidation compared to the SOSDE with no antioxidants added. Accelerated storage negatively affected the shelf life of SOSDEs, and all SOSDEs containing antioxidants, stored in accelerated storage conditions had a higher lipid oxidation

rate constant compared to those stored at ambient. Shelf life studies require the identification of an attribute that has the highest impact on the quality of the product or shows the most change over the shortest time period, through multivariate analysis it is concluded that pungent aroma is associated with early stage of ambient and accelerated storage and cardboard aroma is associated with later stages of ambient and accelerated storage and therefore the most probable predictors of shelf life in SOSDEs.

Although accelerated storage, using the storage conditions applied here, did not predict ambient storage of SODEs well enough, it seems that MASLT has the potential to screen potential effectiveness of antioxidants, including the most probable aroma shelf life predictors pungent and cardboard, in as little as 20 days before commencing with shelf life trial that may take up to two years. Analysis of more time intervals for longer periods during accelerated and ambient shelf life conditions may lead to better correlation and therefore predictability capacity of the accelerated storage.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

EDTA	Ethylene diamine tetraacetate
SOSDE	Sunflower oil salad dressing emulsion
PV	Peroxide Value
AV	Anisidine Value
MASLT	Multivariate accelerated shelf life testing
ASLT	Accelerated shelf life testing
BHA	Butylated hydro anisole
BHT	Butylated hydroxytoluene
TBHQ	Tert-butylhydroquinone
FA	Fatty acid
FFA	Free fatty acid
CV	Carbonyl Value
TBA	Thiobarbituric Acid
PCA	Principal Component Analysis
SDS	Sodium Dodecyl Sulphate
AmbNo	Ambient storage conditions, No antioxidants
AmbLoGal	Ambient storage conditions, Low concentration, Gallic acid
AmbHiGal	Ambient storage conditions, High concentration, Gallic acid
AmbLoEDTA	Ambient storage conditions, Low concentration, EDTA
AmbHiEDTA	Ambient storage conditions, High concentration, EDTA
AccNo	Accelerated storage conditions, No antioxidants
AccLoGal	Accelerated storage conditions, Low concentration, Gallic acid

AccHiGal	Accelerated storage conditions, High concentration, Gallic acid
AccLoEDTA	Accelerated storage conditions, Low concentration, EDTA
AccHiEDTA	Accelerated storage conditions, High concentration, EDTA

1

CHAPTER 1: INTRODUCTION

Sunflower oil emulsions, such as salad dressing and mayonnaises, are shelf stable with a typical shelf life of 6-12 months, unopened, at room temperature. These shelf life periods, demanded by consumers (Corrigan, *et al*., 2012), are often determined by quality changes rather than microbial safety of the product (Lewis and Heppel, 2000; Corrigan, *et al.*, 2012). These quality changes of emulsions are most often related to deterioration of the emulsifying agent that leads to oil separation (Rossel, 1999; Bou *et al.*, 2001) or oxidation of the unsaturated lipids in the oil (Gorji *et al.*, 2016). Oxidation of unsaturated lipids is actually the oxidation of unsaturated fatty acids, which can happen through autoxidation and enzyme catalysed oxidation (Sun *et al*., 2011). Autoxidation is the main process where oxygen interacts with unsaturated free fatty acids spontaneously without light or a catalyst (Sun *et al*., 2011) and subsequently form small volatile aldehydes, ketones, alcohols, furans, hydrocarbons or acids that produce the aroma associated with oxidative rancidity. These volatile compounds and their combinations are associated with the rancid off aroma (Hamilton, 1999) that can be determined through a variety of chemical and sensory tests. Release of these volatiles is affected by the food matrix (Hartvigsen *et al.*, 2000), and in oil-in-water emulsions, it is affected by oil content, headspace and lipophilic and/or hydrophilic nature of the flavour compounds (Tamaru, *et al*., 2018). Lipid oxidation is the general term used to describe a complex sequence of chemical interactions between unsaturated fatty acids with oxygen (Frankel, 1998; McClements & Decker, 2000; Min & Boff, 2002, Chaiyasit *et al.,* 2007). In this thesis the term lipid oxidation refers to the oxidation of free fatty acids through autoxidation.

Antioxidants are substances that improve shelf life by retarding lipid oxidation under specific circumstances through scavenging of free radicals and chelating with pro-oxidative metals (Chaiyasit *et al.*, 2007; Rodríguez-Rojo *et al.*, 2012). According to Act 54 of 1972, antioxidants are substances added to emulsions with the specific purpose to delay, retard or prevent the formation of rancid off notes or other deterioration due to oxidation, but does not include substances added to foodstuffs for purposes other than antioxidation nevertheless have an antioxidant action. The antioxidant effectiveness of phenolic compounds such as tocopherols, rosmarinic and carnosic acid (rosemary) (Rodríguez-Rojo *et al.*, 2012), methyl gallate (Asnaashari *et al.*, 2014), gallic acid, caffeic acid and catechin (Branco *et al.*, 2011) extracted from the stems, roots and flowers of plants (Jayasinghe *et al.*, 2013), is being researched extensively due to standards set by consumer (Chen *et al.,* 2011) and potential toxicological long term effects of some of the most effective free radical scavengers and metal chelator synthetic antioxidants such as butylated hydro anisole (BHA) and tert-butylhydroquinone (TBHQ) (Rodríguez-Rojo *et al.*, 2012; Asnaashari *et al.*, 2014). The first part of this study investigated the oxidative stability of an oil-in-water salad dressing emulsion as affected by different concentrations of the natural antioxidant, gallic acid and synthetic antioxidant, EDTA.

Accelerated shelf life tests are often used to determine shelf life and antioxidant efficacy due to time and cost implications associated with real time shelf life tests (Corrigan *et al.*, 2012). During accelerated shelf life tests, the product is exposed to factors (e.g. temperature, humidity and light, water activity, pro-oxidants and UV light) or combinations of factors that promote quality deterioration. Acceleration factors depend on the product and normal storage conditions for the product (Richards *et al.*, 2014). Most studies have evaluated the effects of one factor at a time (Branco *et al.*, 2011), for example the Arrhenius equation related to temperature of storage with reaction velocity (Córdova *et al.* 2011). However, shelf life is a dynamic process in which storage factors constantly interact with each other (Branco *et al.*, 2011) and therefore accelerated shelf life testing would be more accurate and predictive if the contributing factors could be modelled and evaluated simultaneously (Branco *et al.*, 2011).

Therefore the first part of this study focused on the effect of different concentrations of gallic acid (500 ppm and 1000 ppm) and EDTA (37.5 ppm and 75 ppm) antioxidants on a commercial formulation of a sunflower oil-in-water salad dressing emulsion stored at 1) ambient storage and 2) simultaneous combination of acceleration factors and storage

conditions (32.2°C, addition of potential pro-oxidants, adjusted pH), through descriptive sensory analysis and chemical analyses. Evaluation of lipid oxidation through descriptive sensory analysis formed the major part of the study as it is a more accurate indicator of end consumer responses.

The second part of the study focused on the development of an accelerated shelf life model for commercial oil-in-water emulsions through multivariate analysis, to identify the key sensory attributes associated with the development of lipid oxidation and rancidity that can be used as predictors for end of shelf life during shelf life testing of oil-in-water emulsions.

This study aimed at gaining a better understanding of how antioxidant levels influence oxidation of commercial sunflower oil salad dressing emulsions, when exposed to a combination of oxidation acceleration factors, thereby developing techniques or models to better predict oxidative stability of sunflower oil salad dressing emulsions consumed by consumers.

2

CHAPTER 2: LITERATURE REVIEW

Oxidation of oil-in-water emulsions is a complex process that differs from oxidation of bulk oils and is impacted by many factors. Emulsions have an oil and water phase which contains both pro-oxidants and antioxidants as well as an oil-water interface that impacts the interaction between these phases (Waraho *et al.*, 2011). This literature review includes a review of research on lipid oxidation in oil-in-water emulsions, factors affecting oxidation, measurements for lipid oxidation and health implications associated with consumption of oxidized products.

2.1 OIL-IN-WATER EMULSION

There are many definitions for an emulsion. According to Israelachvili (1994) the most authoritative and least controversial one was defined by IUPAC in 1972 as “a dispersion of droplets of one liquid in another one with which it is incompletely miscible”. In an oil-in-water emulsion, droplets of an oil are dispersed in the aqueous solution. Milk, margarine, salad dressing, mayonnaise, sauces and soups are some of the most common forms of oil-in-water emulsions in the food industry (McClements, 2004).

Sunflower oil is the most commonly used oil in emulsions in the food industry (Gan *et al.*, 2005). High linoleic content sunflower oil is used domestically and industrially, but may be more unstable in the food industry because of the high linoleic levels. Mid-oleic sunflower oil contains higher oleic acid levels and lower linoleic acid levels and is therefore more suitable for industrial uses. One of the most stable sunflower oils is high oleic sunflower oil, which is stable in industrial food applications (Gupta, 2014). Sunflower oil contains on average 0.41% sterols and 0.09% to 0.70% free fatty acids (Waraho *et al.*, 2011). Sunflower oil used in

emulsions undergoes a refining process (Table 2.1) to remove these major impurities with the least possible damage to the desirable constituents such as tocopherols.

Unfortunately, the refining process is not 100% selective and may remove some of the desirable antioxidant compounds such as tocopherols and not all the undesirable compounds such as trace metals and phospholipids. Lipid oxidation can also occur during the refining process of sunflower oil (Bou *et al.*, 2001).

Table 2.1: Refining stages of edible oils (modified from Chaiyasit *et al.*, 2007)

Refining stage	Compounds removed or reduced
Degumming	Phospholipids, trace metals, pigments, carbohydrates and proteins
Neutralisation	Free fatty acids, phospholipids, pigments, trace metals, sulphur and insoluble matter
Washing	Soap (can be free fatty acid or glycerol with sodium hydroxide)
Drying	Water
Bleaching	Pigments, oxidation products, trace metals, traces of soap
Deodorization	Free fatty acids, mono-and diacylglycerols, oxidation products, pigments, decomposition products, pesticides, sterols, sterol esters, tocopherols, other antioxidants
Physical refining	Free fatty acids, mono- and diacylglycerols, oxidation products, pigments, decomposition products, pesticides
Polishing	Any residual traces of oil insolubles

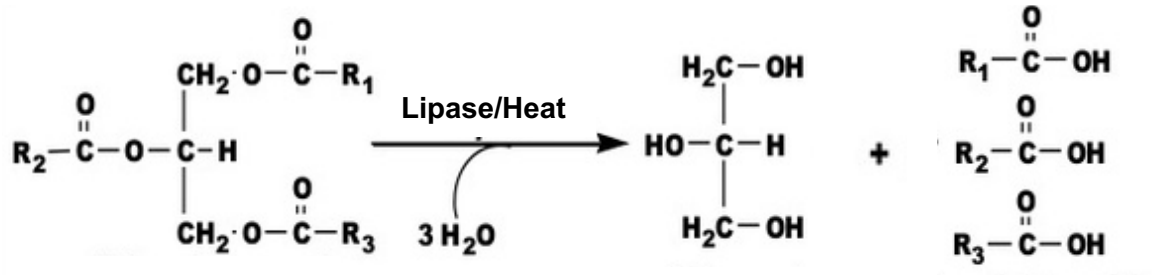
2.2 OXIDATION OF OIL AND OIL-IN-WATER EMULSIONS

Lipid oxidation is the major cause of shelf life reduction in emulsions as it leads to nutritional changes (Bou *et al.*, 2001; Chaiyasit *et al.*, 2007), the formation of off-flavours (Coupland and McClements, 1996), loss of colour as well as the accumulation of compounds which may be detrimental to the health of consumers (Addis, 1986). Mechanisms of lipid oxidation in bulk oils and the effect of pro-oxidants such as copper on bulk oils (Van der Merwe *et al.*, 2003) have been studied thoroughly but the mechanism of lipid oxidation in emulsions, the effect of pro-oxidants on oxidation in emulsions, as well as the effect of the two immiscible liquids and the interface on oxidation in emulsions is still not fully understood (Coupland and McClements, 1996) and is continuously researched (Waraho *et al.*, 2011; Tamaru *et al.*, 2018).

2.1.1 Oxidation of oil

Fatty acids in triacylglycerols and phospholipids usually do not contribute to the aroma of emulsions as they have low volatility. However, when these triacylglycerols and fatty acids (FAs) decompose during lipolysis (hydrolytic rancidity) and oxidation (oxidative rancidity), they form small volatile molecules that produce the off-aroma associated with rancidity (Chaiyasit *et al.*, 2007). Hydrolytic and oxidative rancidity can be enzymic or non-enzymic and present

individually or simultaneously during the shelf life of oil-in-water emulsion salad dressings which increases the complexity of testing rancidity indicators in a specific product. During hydrolytic rancidity the ester bonds between the glycerol and the fatty acids of the triacyclglycerol are hydrolysed and the free fatty acids are released in the presence of moisture and a catalyst. Hydrolytic rancidity can be enzyme catalysed by lipase or by heat and moisture (Fig 2.1). Fatty acids as well as free fatty acids (released during hydrolytic rancidity) can undergo rapid oxidative rancidity (Robards *et al.*, 1988; Hamilton, 1999).



Triacylglycerol Glycerol Free fatty acid

Figure 2.1: Molecular representation of hydrolytic rancidity

Oxidative rancidity is a general term used to describe the complex sequence of chemical interactions between fatty acids and atmospheric oxygen that limits the shelf life of foods as it is associated with the rancid off-flavour and aroma of the oil (Hamilton, 1999). Oxidative rancidity can be catalysed by the enzyme lipoxygenase which attacks the double bonds in the fatty acid or by reacting with oxygen. Oleic, linoleic and linolenic acids are the unsaturated fatty acids most often associated with oxidation (Hamilton, 1999). This process is also referred to as autoxidation, and is a chemical reaction with low activation energy (Hamilton, 1999). There is a good understanding of the mechanisms and factors involved in the development of rancidity in oil as this subject has been researched extensively (Coupland and McClements, 1996). The overall mechanism of autoxidation consists of three stages: initiation, propagation and termination (Hamilton, 1999; Chaiyasit *et al.*, 2007).

Initiation: $RH + O_2 \text{ -- } R^{\bullet} + {}^{\bullet}OOH$

RH - Unsaturated fatty acid

$R^{\bullet}$ - Lipid radical

During the initiation stage, a fatty acid radical ($R^{\bullet}$) is produced from the reaction between an unsaturated fatty acid (RH) and oxygen in the presence of a catalyst such as heat, light or a chemical compound (Hamilton, 1999). This step requires activation energy of 4-5 kcal/mol. The fatty acid radical continues to the propagation phase.

Propagation: $R^{\bullet} + O_2 \text{ -- } RO_2^{-}$

$RO_2^{-} + RH \text{ -- } ROOH + R^{\bullet}$

During the first step in the propagation stage, the fatty acid radical from the initiation stage, reacts with oxygen to form a lipid peroxy radical (RO_2^{-}). The lipid peroxy radical can further react to form hydroperoxide (ROOH) and a fatty acid radical (R^{-}) (Hamilton, 1999; Chaiyasit *et al.*, 2007). This is a self-propagating chain process and therefore the presence of a catalyst such as iron or copper can lead to the formation of many hydroperoxide molecules. The reaction continues until there is a depletion of oxygen or when the fatty acid radical reacts with a stable antioxidant or when two unstable radical react during the termination phase.

Termination: $R^{\bullet} + R^{\bullet} \text{ -- } R - R$

$RO_2^{-} + R^{\bullet} \text{ -- } ROOR$

Fatty acid hydroperoxides (ROOH) that are formed during the propagation phase undergo a natural decomposition pathway (Min and Boff, 2002) where the bond between the two oxygen molecules will split to form an alkoxyl (RO-) and a hydroxyl radical (-OH). The alkoxyl radical is very energetic and can enter into a number of different reaction pathways according to Chaiyasit *et al.*, (2007). The first pathway involves the alkoxyl radical attacking another unsaturated fatty acid and extracting hydrogen from the carbon-carbon bond on either side of the radical. The decomposition product on the carboxylic acid end of the fatty acid is then esterified to the glycerol or phospholipid. This molecule is not volatile and therefore would not contribute to rancidity unless it is further decomposed into compounds with low molecular weights. Volatile compounds produced through the cleavage of the hydrocarbon chain on the

methyl end of the fatty acid by alkoxyl radicals (Chaiyasit *et al.*, 2007) will interact with a variety of compounds to produce secondary lipid oxidation products (Hamilton, 1999). These secondary lipid oxidation products include aldehydes, ketones, alcohols, furans, hydrocarbons and acids (Chaiyasit *et al.*, 2007). Hamilton, (1999) described and illustrated the three stages involved in the formation of oxidative rancidity as a self-propagating cyclic process that can be stopped at the termination stage when two fatty acid radicals combine to form a product that does not feed the propagation reaction.

2.1.2 Oxidation of emulsions

Oil-in-water emulsions consist of two major components: oil (the dispersed phase), and water (the continuous phase) (Waraho *et al.*, 2011). The low pH and high fat (70-80%) content of oil-in-water emulsions such as mayonnaise makes them resistant to microbial spoilage (Depree and Savage, 2001) but oil-in-water emulsions are susceptible to deterioration due to oxidation of the unsaturated fats in the oil (Gorji *et al.*, 2016).

There has been a great focus on research to understand the mechanisms of lipid oxidation in emulsions over the last 20 years (examples are Coupland & McClements, 1996, McClement & Decker, 2000, Waraho *et al.*, 2011; Tamaru *et al.*, 2018) as the lipid oxidation in oil-in-water emulsions is more complex than in bulk oil systems (Jacobsen *et al.*, 2008). The mechanism of lipid oxidation in a particular food depends on the nature of the reactive species present in the foods as well as the physiochemical environment (temperature, humidity, pH) (Coupland and McClements, 1996). The strength of the interaction between oil droplets in an emulsion depends on van der Waals attractions (Depree and Savage, 2001). which are balanced by electrostatic and steric repulsions. The quality of the emulsion will depend on the balance between these forces (Depree and Savage, 2001). Due to the positive free energy required to increase the surface area between the oil and water phases, emulsions are thermodynamically unstable and require an emulsifier. Emulsifiers are surface-active molecules that form a protective membrane around the droplets to prevent the droplets from coming close enough to aggregate and flocculate based on the solubility of the molecules. Therefore, oil-in-water emulsions consist of three regions based on the solubility of the molecules. These three regions are the non-polar interior of the oil droplet, the polar water phase and the surface-active molecules at the interface. The interfacial layer has a thickness of a few nanometres and consists of a mixture of oil, water and emulsifier molecules. The molecular environment as well as the specific orientation of the lipid molecules at the interface

layer has a significant effect on the chemical reactivity (Chaiyasit *et al.*, 2007) of the product. The fatty acids in an emulsion become more polar during oxidation as these react with oxygen. This alters the physical properties of the emulsion which may lead to increased or decreased susceptibility to oxidation. It further appears that oxidation is initiated at the droplet interface, indicating that small droplet sizes promote the initiation of oxidation as it increases the surface area of the interface which in turn, allows more interaction within the aqueous phase (Depree and Savage, 2001; Waraho *et al.*, 2011). Once oxidation has been initiated, the propagation phase is independent of the droplet size (Jacobsen *et al.*, 2000). Numerous research studies have been conducted to find the factors influencing oxidation in oil-in-water emulsions and it is clear that the physical properties of the three regions in an emulsion play a critical role in lipid oxidation kinetics (Frankel *et al.*, 1994; Frankel, 1998).

Waraho *et al*., (2011) explained that the free fatty acids are pro-oxidative in emulsions due to their ability to concentrate at the emulsion droplet surface where they attract pro-oxidative transition metals that promote oxidation. A lower degree of unsaturated free fatty acids may decrease the ability of the free fatty acids to promote oxidation in emulsions (Waraho *et al.*, 2011). This explains why the lipid oxidation mechanisms in emulsions differ considerably from lipid oxidation in bulk oil.

Emulsions are more prone to oxidation than pure oils due to their large surface area that increases lipid interaction with water pro-oxidants (Cercaci *et al.*, 2007). Oxidation in emulsions is mainly found in the interface between the oil and water where pro-oxidants such as transition metals are able to come into close contact with the hydroperoxides located at the droplet surface (Chung and McClements, 2014). This oxidation can be minimised through antioxidants incorporated into the lipid, interfacial or water phases (Chaiyasit *et al.*, 2007). The effectiveness of antioxidants depends on the physical properties of the emulsion as well. It has been proven that lipophilic antioxidants are more effective in oil-in-water emulsions as it is retained in the oil droplet and scavenges any free radicals formed during oxidation of the fatty acids (Chaiyasit *et al.*, 2007).

Flavour deterioration due to lipid oxidation in emulsions is much more complex than in edible bulk oils as phytosterols and unsaturated fatty acids are oxidised faster in emulsions than in bulk oils (Coupland and McClements, 1996; Cercaci *et al.*, 2007). According to Cercaci *et al.*(2007), phytosterols at the emulsion droplet interface, are particularly susceptible to oxidation as their oxidative sensitive hydrophilic hydroxyl group and the double bond are

orientated towards the aqueous phase. Studies have been conducted to determine this surface activity through measurement of the interfacial tension as lower surface activity (lower interfacial tension) indicates lower susceptibility to oxidation (Cercaci *et al.*, 2007).

Except for the different mechanisms of lipid oxidation between emulsions and bulk oils, it is also not clear if the same secondary oxidation products responsible for rancid flavours are present in emulsions and bulk oils (Jacobsen *et al.*, 1999). These volatile compounds are furthermore distributed differently between emulsions and bulk oils. The volatile compounds in bulks oils are found and released from one phase, while the volatile compounds in emulsions are distributed and possibly released from several phases. The high surface area of the oil in an emulsion would allow more interaction with aqueous phase pro-oxidants (Cercaci *et al.*, 2007). Sensory perceptions are influenced by the food matrix, and the sensory threshold values of aldehydes appear to be significantly lower when released from water rather than oil possibly due to their solubility or K_{ow} which describes the equilibrium partition between octanol and water to define their relative lipophilicity and hydrophilicity (Heynderickx *et al.*, 2014). Therefore, the presence of secondary lipid oxidation products in bulk oils and emulsions at the same concentration is perceived in different ways, because a part of the compound in the emulsion will be released from the water phase with a higher volatility. Thus, it is assumed that oxidation products found in emulsions have a higher negative flavour impact than the same oxidation products at the same concentrations found in bulk oil (Jacobsen *et al.*, 1999). These oxidation products can be affected by numerous factors.

2.3 FACTORS AFFECTING THE DEVELOPMENT OF LIPID OXIDATION IN OIL-IN-WATER EMULSIONS

It is important to understand the factors that influences oxidative stability for oil-in-water emulsions as this will affect the shelf life.

All emulsion droplets have a lipid and aqueous phase as well as an interfacial phase that borders the lipid and aqueous phase. Factors affecting lipid oxidation in each of these phases are listed in Table 2.2. The oil-in-water interphase strongly influence oxidation kinetics due to interaction of aqueous phase pro-oxidants (e.g. metal ions) and lipid phase substrates (e.g. hydroperoxides).

Table 2.2: Factors capable of influencing lipid oxidation in oil-in-water emulsions (Waraho *et al.*, 2011).

Characteristic	Property	Factors
Lipid phase	Composition	Degree of unsaturation Pro-oxidant impurities (e.g. hydroperoxides) Inherent antioxidants Added antioxidants
	Physical state (solid/crystal)	Solubility, partitioning and diffusion of antioxidants and pro-oxidants
	Physical properties	Rheology determines diffusion of antioxidants and pro-oxidants Polarity determines partition coefficient
Aqueous phase	Composition – pH, ionic strength, solutes	Pro-oxidant impurities (e.g. transition metals) Inherent antioxidants (e.g. chelators) Added antioxidants (e.g. chelators) Micelles may alter location of antioxidants and pro-oxidants Reducing agents that can redox cycle pro-oxidant metals
	Physical state – ice crystal structure and location	Solubility, partitioning and diffusion of reactants and products
	Physical properties	Rheology determines diffusion of antioxidants and pro-oxidants Polarity determines partition coefficients
Interfacial Phase	Composition	Anti-/pro-oxidant activity Impurities (hydroperoxides)
	Thickness	Steric hindrance of interactions between water- and oil-soluble components
	Charge	Electrostatic attraction/repulsion of antioxidants and pro-oxidants
	Permeability	Diffusions of antioxidants and pro-oxidants in lipid and aqueous phase
Structural Organization	Emulsion	Droplet concentration Droplet size distribution
	Spray dried products	Porosity Exposed lipid levels
	Hydrogel particles	Emulsion droplet characteristics upon rehydration Hydrogel composition, structure and properties

As seen in Table 2.2, there are factors in the composition, thickness, charge and permeability of the interfacial phase that influence lipid oxidation in oil-in-water emulsions. Two other factors that affect the lipid oxidation of oil-in-water emulsions are pro-oxidants and antioxidants. Eliminating pro-oxidants will reduce the induction period which can increase the shelf life of the emulsion, and incorporating antioxidant delays the lipid oxidation (Gorji *et al.*, 2016).

2.3.1 Pro-oxidants

During lipid oxidation, the carbon-carbon double bond is attacked by oxygen. Therefore, oils with a higher proportion of unsaturated double bonds in the triglyceride fatty acids (e.g. olive oil and corn oil (Gatti *et al.*, 1992)) are more prone to autoxidation. Other factors that may have a pro-oxidative influence on the development of lipid oxidation in food products are temperature, light, metals, packaging and pH (Merril *et al.*, 2008).

Temperature

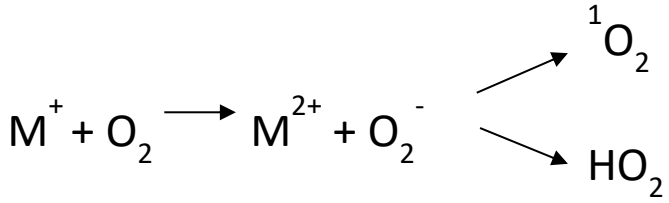
In theory, high temperatures increase lipid oxidation (Frankel, 1998), and various studies have shown results in agreement with this theory. The breakdown of hydroperoxides is increased by increased temperatures (Waraho *et al.*, 2011) as the rate of reaction of oxygen with fats roughly doubles for every 10°C increase in temperature (Rossel, 1999) and higher totox values and peroxide values were recorded for mayonnaise stored at 25°C compared to samples stored at 4°C (Li *et al.*, 2014). Morales-Aizpurua and Tenuta-Filho (2005) concluded that decreased storage temperature suppressed oxidation of cholesterol in mayonnaise after conducting research on oxidative stability of cholesterol in commercial mayonnaise.

Light

Light promotes lipid autoxidation through one of two pathways of photooxidation: photolytic autoxidation or photosensitized oxidation. Photolytic autoxidation occurs when free radicals are produced in lipids due to exposure to ultraviolet radiation while photosensitized oxidation occurs in the presence of visible light, when singlet oxygen is produced in the presence of photosensitisers such as riboflavin and chlorophyll (Bradley and Min, 1992; Chaiyasit *et al.*, 2007). This singlet oxygen reacts 1500 time faster with methyl linoleate than does triplet oxygen, and singlet oxygen further reacts directly with double bonds by addition at either end of the double bond to form allylic hydroperoxides (Hamilton, 1999). Product packaging is often developed to limit the presence of light, and reduced oxidation rates have been noticed with the use of amber instead of clear glass bottles (Tekin *et al.*, 1995).

Metals

According to Kellerby *et al*. (2006) transition metal ion-based catalysis is the primary cause of lipid oxidation in food emulsions. According to Frankel (1980), metal ions catalyse the formation of singlet oxygen and peroxy radicals as shown:



Both products formed are good chain initiators with singlet oxygen reacting about 1500 times faster than $^3O_2$ with unsaturated double bonds (Frankel, 1980).

Hydroperoxides, produced during the propagation phase, produce additional radicals that could exponentially increase the oxidation rate (Chaiyasit *et al.*, 2007; Richards & Golding, 2011). The breakdown of hydroperoxides is increased by the presence of transition metals. There have been various research studies on the pro-oxidative potential of metals, and it is reported that copper and iron are the most pro-oxidative in oil (Garrido *et al.*, 1994) with iron being the most important pro-oxidative metal in emulsions (Waraho *et al.*, 2011) due to its high solubility and reactivity (Mei *et al.*, 1998). Iron will also promote hydroperoxide degradation when located at the emulsion interface because of electrostatic interactions between the droplet surface and the charged metal ion (Richards and Golding, 2011). More information on iron as pro-oxidant is discussed under pro-oxidant interaction.

Packaging

Packaging preserves the food product from environmental stresses caused by different agents (Manzini *et al.*, 2017) by providing light and air barrier properties (Barden & Decker, 2016). Different aspect of the packaging affect the shelf life of the product. Different packaging materials have different effects on the oxidation of the oil-in-water emulsion. Glass packaging is a great barrier against oxygen, while polyester materials filter out ultraviolet radiation to different degrees (Lennersten and Lingnert, 2000). Packaging enclosure can also affect the integrity of the product from physical and flavour deterioration (Manzini *et al.*, 2017). The size of the packaging further may affect the aroma of oil-in-water emulsions (Martinez *et al.*, 1998), with smaller headspace leading to reduced oxidation (Hsieh and Regenstein, 1991).

pH

An oil-in-water emulsion stabilised with egg yolk is more stable when the pH is closer to the isoelectric point of the egg proteins as this has minimum charge on the proteins (Depree and Savage, 2001). According to Kiosseoglou and Sherman (1983) the ideal pH in an emulsion is 3.9. However, decreasing pH from neutral to 4.0 breaks bridges between egg yolk proteins and iron, subsequently releasing iron to be more accessible as an oxidation initiator (Jacobsen *et al.*, 1999). The distribution of volatile compounds is also influenced by the pH, and Takai *et al.* (2003) found that carbonyl compounds can migrate from the liquid phase to the gas phase which affects the stability of the flavour of the emulsion.

Pro-oxidant interaction

There has been a focus on the effect of the pro-oxidants described above on food emulsions, however most studies evaluate one factor at a time, e.g. metal ions (Branco *et al.*, 2011) even though oxidation is a dynamic process, and the pro-oxidative factors (e.g. pH, metal ions) interact with each other (Chaiyasit *et al.*, 2007). Chen *et al.* (2011) concluded in their research on minor components in food oils, that it is important to look at how combinations of minor components impact lipid oxidation. They further concluded that there are very few studies that have looked at combinations rather than isolated pro-oxidants. According to Branco *et al.*, (2011) the contribution of studies that investigated factors with a fixed variation range would be much greater if the interactions could be evaluated simultaneously. This would be especially beneficial when evaluating the efficacy of natural antioxidants on shelf life of oi-in-water emulsions. The acceleration of the oxidative reaction is useful to screen the efficacy of potential antioxidants. Branco *et al.*, (2011) illustrated this by evaluating the effects of seven pro-oxidant factors (temperature, pH, iron, copper, ascorbyl palmitate, ascorbic acid and sodium chloride concentrations) on the oxidative stability of emulsions. Table 2.3 illustrates the Plackett-Burman Design that Branco *et al.*, (2011) applied to these seven factors in order to determine which factors have the largest impact on increasing lipid oxidation.

Table 2.3: Plackett-Burman Design applied for the screening of significant factors that influences the oxidation (Branco *et al.*, 2011)

	Factors [a] (coded values)						
	Temp (°C)	Fe^{2+} (mmol/L)	Cu^{2+} (mmol/L)	AP (mmol/L)	AH (mmol/L)	NaCl (%)	pH
assay							
1	+1	-1	+1	-1	-1	-1	+1
2	+1	+1	-1	+1	-1	-1	-1
3	-1	+1	+1	-1	+1	-1	-1
4	+1	-1	+1	+1	-1	+1	-1
5	+1	+1	-1	+1	+1	-1	+1
6	+1	+1	+1	-1	+1	+1	-1
7	-1	+1	+1	+1	-1	+1	+1
8	-1	-1	+1	+1	+1	-1	+1
9	-1	-1	-1	+1	+1	+1	-1
10	+1	-1	-1	-1	+1	+1	+1
11	-1	+1	-1	-1	-1	+1	+1
12	-1	-1	-1	-1	-1	-1	-1
13	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0
True value [b]							
(-1)	30	0.00	0.0	0.0	0.0	0.0	3.0
0	45	0.25	0.5	0.5	0.5	0.5	5.0
(+1)	60	0.50	1.0	1.0	1.0	1.0	7.0

[a] Factors are designated as temperature (temp), concentration of iron (Fe^{2+}), copper (Cu^{2+}), ascorbyl palmitate (AP), ascorbic acid (AH) and sodium chloride (NaCl) and pH. Coded values: (+1), (0) and (-1) correspond to highest, intermediate, and lowest values of each factor. [b] Corresponds to the value adopted for each factors in the emulsion formulation. For example, assay 1 was performed under the following conditions: 60°C, iron absence, 1.0 mmol/L copper, ascorbyl palmitate absence, ascorbic acid absence, salt absence, and pH 7.0

The objective of the study referred to in Table 2.3 was to achieve higher values for the oxidation markers, therefor results (not illustrated) focussed on the impact of a combination of factors on lipid oxidation rather than each individual factor at each level. According to the results from this study the combination of the four factors temperature, pH, ascorbic acid and iron (Fe^{2+}) concentrations had the largest impact on lipid oxidation. These four factors were used in the research conducted as part of this thesis. The pH of oil-in-water emulsions has a significant influence on the formation of lipid oxidation products (Branco *et al.*, 2011). An increase in pH from 3.0 to 7.0 reduces the formation of hydroperoxides, while a pH lower than 5 initiates the release of iron ions (if present) in the product which in turn decomposes pre-existing lipid hydroperoxides located at the oil-water interface in the emulsion (Thomsen *et al.*, 2000), which enters the natural decomposition pathway of the termination phase of oxidation (Min and Boff, 2002). Ascorbic acid is water soluble and often used as an antioxidant in products such as beer and soft drinks rather than lipids (Coppen, 1999). Ascorbic acid also has pro-oxidative abilities in the presence of transition metals where it reduces ferric ions

(Fe $^{3+}$) into ferrous ions (Fe $^{2+}$) at a specific concentration. Branco *et al* (2011) has researched this optimal concentration and concluded that an ascorbic acid concentration of 1.70 mmol/L is the optimal concentration to reduce ferric ions (Fe $^{3+}$) into ferrous ions (Fe $^{2+}$) in the specific combinations tested. Ferrous ions decomposes lipid hydroperoxides quickly (Jayasinghe *et al.*, 2013) to form secondary lipid peroxide products such as aldehydes, ketones, alcohols and furans (Chaiyasit *et al.*, 2007) which produces the aroma associated with oxidative rancidity (Hamilton, 1999).

Lipid oxidation in oil-in-water emulsions are controlled by applying different techniques, including the control of oxidation substrates, control of pro-oxidants and the addition of antioxidants (Jayasinghe *et al.*, 2013).

2.3.2 Antioxidants

Antioxidants on the other hand are factors that retard lipid oxidation under specific conditions through different mechanisms (Merril *et al.*, 2008; Rodríguez-Rojo *et al.*, 2012). There are other means of preventing oxidation such as vacuum or controlled atmosphere packaging or packing in an inert gas to exclude oxygen or/and refrigeration/freezing. These methods greatly reduce the rate of oxidation. Unfortunately, these methods are not always effective, and it is seldom realised how little oxygen present can initiate the oxidative process. Equipment to remove the last traces of oxygen from a product is also very costly. Antioxidants however, are inexpensive, generally effective and easily applied. According to Coppen (1999) the addition of an antioxidant to a food product might increase the cost of the product by less than half a percent. Therefore, antioxidants are generally used in isolation or with other methods to prevent lipid oxidation.

Antioxidants can be added to the bulk oil after the refining process, or directly to the foodstuff (e.g. salad dressing or mayonnaise). The efficacy of an antioxidant is influenced by its ability to be located at the interface, where oxidation takes place (Coupland and McClements, 1996). As antioxidants are added in very small quantities, it is important to ensure that the antioxidant is evenly dispersed in the product. Efficient agitation is needed to ensure that the antioxidant is evenly dispersed, but care should be taken as the agitation may lead to the inclusion of air which will affect the oxidation of the product (Coppen, 1999; Elias *et al.*, 2008).

Antioxidants are classified as primary, secondary or multiple-function antioxidants depending on the specific mechanism of action (Coupland and McClements, 1996; Chaiyasit *et al.*, 2007). Primary antioxidants are free radical scavengers as they deactivate free radicals by binding with the hydroperoxyl to form hydroperoxides and with alkoxyl radicals to form alcohols. In both cases, low energy antioxidant radicals that do not readily promote the oxidation of unsaturated fatty acids are formed (Warner *et al.*, 1986). This takes place before the free radicals can attack the unsaturated fatty acid. Secondary antioxidants decrease the rate of oxidation by acting as chelators, oxygen scavengers or singlet oxygen quenchers.

Chelators. Transition metals act as pro-oxidants and accelerate lipid oxidation as mentioned in the pro-oxidant section earlier. Chelating antioxidants such as Ethylenediaminetetraacetic acid (EDTA) have the ability to bind to transition metals thereby reduce or inactivate their activity. Some chelators may act as a pro-oxidant under certain conditions by increasing metal solubility or altering the redox potential of the metal.

Oxygen Scavengers. Antioxidants such as ascorbic acid, erythorbic acid and sodium erythorbate act as a reductant by scavenging and reacting with oxygen and eliminating it from food.

Singlet Oxygen Quenchers. Singlet oxygen is formed by enzymatic reactions in the presence of a photosensitizer, light and triplet oxygen, Singlet oxygen is an excited state of oxygen that can be deactivated through chemical and physical pathways by tocopherols, carotenoids and polyphenols (Chaiyasit *et al.* (2007).

Antioxidants can have multiple functions, such as free radical scavenging and metal chelation. Ascorbic acid, a multifunctional antioxidant can quench singlet oxygen, reduce free radicals and remove singlet oxygen in the presence of metal ions (Chaiyasit *et al.,* 2007; Decker *et al.*, 2002).

According to Coppen (1999) an ideal antioxidant should be safe to use, should impart no odour, flavour or colour, should be effective at low concentrations, should be easy to incorporate and should survive the cooking process such as baking. Coppen (1999) further

stated that there are very few antioxidants that meet these criteria. This explains the reason for the focussed research on new antioxidants for bulk oils and emulsions and the research on antioxidant synergism (Merril *et al.*, 2008).

Antioxidants used for lipid oxidation retardation in bulk oils are usually effective in emulsions as well; however, the level of effectiveness may differ considerably. Predominantly, non-polar antioxidants are more effective in an oil-in-water emulsion than in bulk oil, while polar antioxidants are more effective when used in bulk oil (Coupland and McClements, 1996). This refers to the 'polar paradox', a theory that illustrates the paradoxical behaviour of antioxidants in different media and rationalizes the fact that polar antioxidants are more effective in less polar media, such as bulk oils, while nonpolar antioxidants are more effective in relatively more polar media, such as oil-in-water emulsions or liposomes (Zhong and Shahidi, 2011). There has also been a focus on when the antioxidant should be added to the food product. It is believed that the earlier the antioxidant is added to the product the better. Some studies indicate that an antioxidant is more effective if the antioxidant is mixed with the oil after the refining process (Coppen, 1999). Combinations of antioxidants may retard lipid oxidation more effectively than in isolation (Choueiri *et al.*, 2012).

It is important to understand that antioxidants cannot improve the flavour of poor quality oils. They cannot improve oil in which oxidative rancidity has already developed, and they cannot prevent microbial decay, hydrolytic rancidity, ketonic rancidity or flavour reversion (Coppen, 1999). At the very best, antioxidants can only maintain an oil in its original condition. Although antioxidants cannot prevent microbial spoilage, it has been found that phenolic antioxidants may have some antimicrobial effect (Jayasinghe *et al.*, 2003). Antioxidants cannot prevent the formation of free fatty acids through hydrolytic rancidity. Hydrolytic rancidity usually takes place during high temperature frying processes where water is present (Coppen, 1999).

Natural antioxidants

There has been an international focus in the food industry on the use of natural flavours, colourants, flavour enhancers and antioxidants. Natural antioxidants are being researched intensively due to the potential toxicological long-term effects of synthetic antioxidants (Rodríguez-Rojo *et al.*, 2012). Food companies are changing their products to

adhere to the standards set by consumers. Using natural antioxidants is challenging as they do not always provide the same shelf life as a synthetic antioxidant (Chen *et al.*, 2011).

Research showed that some phytochemical antioxidants have unpredictable effects and can act as antioxidants or pro-oxidants depending on their concentration (Choueiri *et al.*, 2012). Pectin, a natural antioxidant, has also been reported to cause emulsion instability (Celus *et al*., 2018). This adds to the challenges of using natural antioxidants (Choueiri *et al.*, 2012). Natural antioxidants often used in the food industry include proteins, tocopherols, polyphenols to name a few.

Proteins

Proteins such as β-lactoglobulin have been shown to inhibit oxidative deterioration of lipids in a wide range of food systems including oil-in-water emulsions (Faraji *et al.*, 2004; Elias *et al.*, 2008). Aromatic amino acids (tyrosine, tryptophan, phenyl-alanine) as well as sulphur-containing amino acids (free cysteine, methionine) can act as natural antioxidants in foods through different mechanisms such as scavenging of peroxyl radicals and chelation of transition metals (Elias *et al.*, 2008). Proteins furthermore protect endogenous antioxidant enzymes (Elias *et al.*, 2008) and can also act as antioxidants by reducing iron's reactivity by means of interfering with its redox cycling capacity and oxidizing ferrous ions to the ferric state. Oxidized iron migrates to the protein's cavity where it nucleates and aggregates to form a ferric hydroxide core that is unavailable to participate in lipid decomposition (Elias *et al.*, 2008). Proteins can be added to the aqueous phase of oil-in-water emulsions to improve oxidative stability, however the use of proteins in oil-in-water emulsions is limited as they could impact the texture, colour and flavour of oil-in-water emulsions (Elias *et al.*, 2008).

Tocopherols

Tocopherols and tocotrienols are important minor components in most edible oils as they are naturally present antioxidants and a source of vitamin E (Chaiyasit *et al.*, 2007). Tocopherols found in sunflower oil are mainly in the α-tocopherol form, as illustrated in Fig 2.2. Tocopherols are naturally present in oil, but may be removed during the refining and deodorising process. According to Coppen (1999) there is very little benefit in adding natural tocopherols to a vegetable oil in addition to the natural tocopherols already present in an attempt to improve the shelf life. However, the natural tocopherols present in oil are not very

heat stable and may not survive food processing operations. Therefore, the naturally occurring tocopherol may be supplemented with synthetic tocopherols. A concern often raised with the use of the tocopherols as antioxidants relates to the pro-oxidant effects at elevated levels. It has been proposed that tocopherols are not pro-oxidants, but may act synergistically with pro-oxidants in the presence of copper and ascorbate. This is probably due to the recycling of tocopherols by ascorbate through reactions with tocopheroxyl radical. Merril *et al.* (2008) found that the antioxidant effect of tocopherols is maintained as long as ascorbate is present.

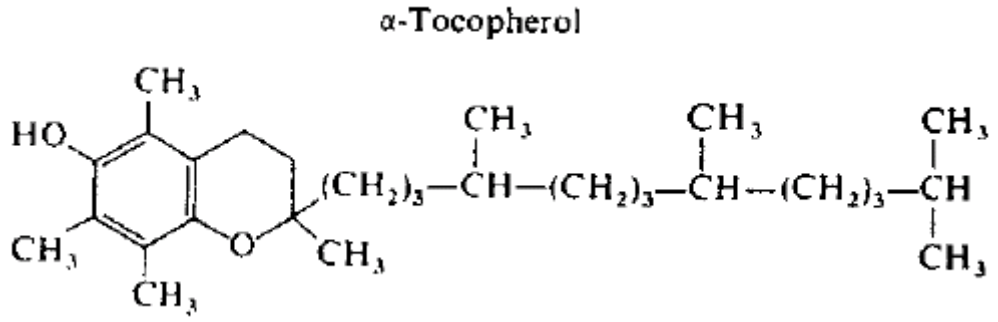


Figure 2.2: Molecular structure of α-tocopherol (Coppen, 1999)

Phenolic compounds

Phenolic compounds, naturally found in plant extracts have been reported to show good antioxidant activity (Hinnenberg *et al.*, 2006; Jayasinghe *et al.*, 2013), however their effect on the oxidative stability of lipid dispersions can be mixed with both antioxidant and pro-oxidant activities reported (Huang and Frankel, 1997). Jayasinghe *et al.* (2013) conducted extensive research on the antioxidative effect of phenolic compounds found in the fruit of Indian gooseberry fruit (*Emblica officinallis*) and the leaves of sweet basil (*Ocimum basilicum* L.) in oil-in-water emulsions and concluded that these phenolic compounds have antioxidative activity. These phenolic compounds are found in the chloroplasts in the leaves as well as the stems, roots and flowers of plants (Rodríguez-Rojo *et al.*, 2012) and may have a bitter and astringent taste (Gomes *et al.*, 2016). Hydro distillation removes the flavour of the herbs which enables the use of phenolic compounds as antioxidants. The number of phenolic hydroxyl groups as well as their position and the presence of other functional groups in the whole molecule plays an important role in antioxidant activity (Rice-Evans *et al.*, 1996). The total phenolic content is expressed as gallic acid equivalents which are equal to (mg gallic acid)/(g extract) (Hinnenberg *et al.*, 2006). The total phenolic content of rosemary extract is 162 mg gallic acid/g extract with the major active phenolic compound being rosmarinic acid (Fig 2.3) and carnosic acid (Fig 2.4) (Erkan *et al.*, 2008). Sweet basil has 579.3 mg gallic acid/g extract

equivalent (Jayasinghe *et al.*, 2013) with the major active compounds being rosmarinic acid (Jayasinghe *et al.*, 2003). Rosmarinic acid with four phenolic hydroxyl groups (Fig 2.3) is expected to have higher antioxidant power compared to carnosic acid, for example, with only two phenolic hydroxyl groups (Fig 2.4).

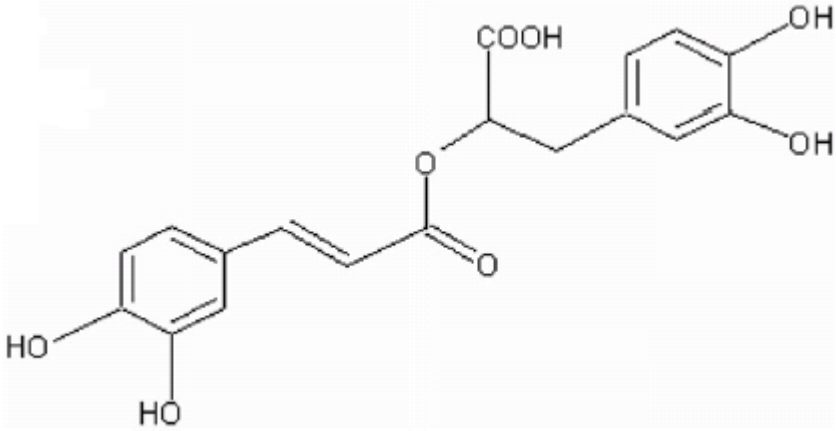


Figure 2.3: Molecular structure of rosmarinic acid (Erkan *et al.*, 2008)

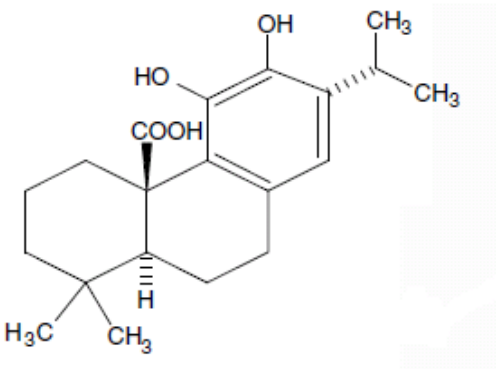


Figure 2.4: Molecular structure of carnosic acid (Erkan *et al.*, 2008)

Predominant antioxidant mechanisms of phenolic compounds are metal chelation (Fang and Wada, 1993) and radical scavenging, as they bind to free radicals formed during the initiation phase of lipid oxidation (Erkan *et al.*, 2008). The concentration of phenolic compounds as well as their location in different phases affect their antioxidative effectiveness in food (Jayasinghe *et al.*, 2013). The solvent used in the extraction process may also affect the antioxidant activity of the phenolic compounds (Liu *et al.*, 2007; Erkan *et al.*, 2008).

Pro-oxidant activities have also been reported for phenolic compounds in oil-in-water emulsions (Huang and Frankel, 1997). A possible explanation for this pro-oxidative activity proposed by Zhou and Elias (2012) includes the nonenzymatic, metal-catalysed oxidation of polyphenols, which results in the formation of hydrogen peroxide (Choueiri *et al.*, 2012). The hydrogen peroxide forms hydroxyl radicals through metal-catalysed reduction and they can react with organic matter (Zhou and Elias, 2012).

Gallic acid, a phenolic acid present in many fruits and vegetables, has strong antimicrobial and antioxidant properties (Asnaashari *et al.*, 2014; Sun *et al.*, 2014). Gallic acid and its derivatives are widely used in the pharmaceutical and food industry as free radical scavengers (Asnaashari *et al*, 2014). The radical scavenging activity of phenolic acids depends on the number of electron donor hydroxyl and methoxy substitutions. As seen in Fig 2.5 gallic acid has three hydroxyl groups and one carboxyl group compared to its derivative methyl gallate (Fig 2.6) with three hydroxyl groups and one carboxylic acid group (Asnaashari *et al.*, 2014) and research by Asnaashari *et al.*, (2014) indicates that gallic acid is a stronger radical scavenger in oil-in-water emulsions compared to methyl gallate. The carboxylic acid ester group of methyl gallate may furthermore hinder the scavenging effect of the hydroxyl groups by intra- or intermolecular hydrogen bonding (Asnaashari *et al.*, 2013). However, the antioxidant activity of an antioxidant depends on chemical structure as well as its interactions in emulsions, and according to Schwarz *et al.*, (2000) the antioxidant activity of gallic acid derivatives in oil-in-water emulsions decreases as their polarity increases, leading to the least polar derivative (dodecyl gallate) being the most affective antioxidant. Gallic acid is a more polar compound compared to its derivatives and according to Schwarz *et al.*, (2000) exhibited pro-oxidant activity in oil-in-water emulsions. According to Feng *et al.*, (2009), gallic acid increases emulsion stability by changing the polarity of the phases, leading to increased solubility between them or penetration of the interfacial film which causes increased flexibility of the interface.

Gomes *et al.*, (2016) conducted research on the incorporation of gallic acid in oil-in-water emulsions and found that increasing concentration of gallic acid decreased the pH. Gomes *et al.*, (2016) further concluded that the water saturation point of gallic acid is 1.3% w/w. Gallic acid has also been shown to act as a pro-oxidant in a study on fish oil mayonnaise where it slightly decreased the droplet size and increased the rate of lipid oxidation by reducing metal ions to their more active form. This led to an increased intensity of fishy, rancid and metallic off flavour (Jacobsen *et al.*, 2000).

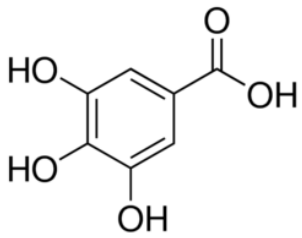


Figure 2.5: Molecular structure of gallic acid (Asnaashari *et al.*, 2014)

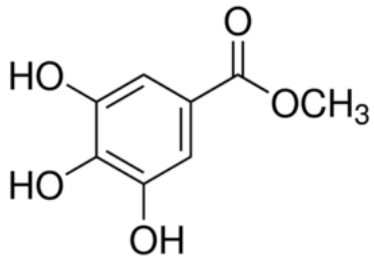


Figure 2.6: Molecular structure of methyl gallate (Asnaashari *et al.*, 2014)

Ascorbic Acid

Ascorbic acid can bind with trace quantities of metals which would otherwise promote oxidative reactions and can act together with tocopherols (vitamin E) in biological tissues to remove free fatty radicals generated in polyunsaturated fatty acid radicals. Antioxidant synergism has also been seen between ascorbic acid, tocopherol and phospholipids (Hamilton, 1999).

Ascorbic acid is water soluble and is therefore more often used as an antioxidant in beer and fruit juice rather than fats and oils (Coppen, 1999). To increase its solubility in oil, it is often synthesised as ascorbyl palmitate. However, its solubility is still very low and it is usually necessary to use it in combination with a solubilising agent such as a monoglyceride (Coppen, 1999). Ascorbyl palmitate has an amphiphilic nature which is very beneficial when used in animal products as it is transported through the meat and fatty tissue cell walls into the fat phase where it acts as an antioxidant. Ascorbyl palmitate has a hydrogen-donating potential, which allows it to function as a chain-breaking antioxidant (Perricone *et al*., 1999). It can act as an independent free radical scavenging antioxidant and, when used in combination with tocopherols, it can regenerate tocopherol from the oxidized tocopheroxyl radicals formed during oxidation (Frankel *et al.,* 1994). According to Coppen (1999), ascorbic acid (including

ascorbyl palmitate) is a sequestering agent and not a true antioxidant as it doesn't exhibit any antioxidant activity in a fat from which all trace metals haven been removed.

Ascorbic acid also has pro-oxidative potential as it is able to reduce transition metals. As discussed earlier, transition metals such as iron are pro-oxidative in its reduced form (Fe^{2+}). At high concentrations of ascorbic acid, the regeneration of metal ions may override the antioxidative properties (Let *et al.*, 2007).

Synthetic antioxidants

EDTA

EDTA (Fig 2.7), a synthetic antioxidant that belongs to the aminopolycarboxylic acid family of ligands and is typically used in emulsions to retard lipid oxidation (Furia, 1964) by chelating iron to form FeEDTA (MacPhail *et al.*, 1994). EDTA usually binds to a metal through its two amines and four carboxylates as seen in Fig 2.8 (Kirchner and Gyarfas, 1957). Iron bound to EDTA does not act as a pro-oxidant in emulsions and therefore does not accelerate the oxidation process. However, this FeEDTA complex has been reported to still participate in oxidation reactions. Because EDTA binds so well with iron, FeEDTA is often added to food to increase the iron bioavailability of the food product (MacPhail *et al.*, 1994). EDTA can chelate metal ions even in an acidic medium, but shows higher chelating preference for ferric than ferrous iron (Bou *et al.*, 2001).

EDTA is inexpensive and highly effective at the maximum permitted dose of 75 ppm in the USA (Coppen, 1999) but studies have found the effectiveness of EDTA is not concentration dependent (Let *et al.*, 2007). EDTA has also been shown to stimulate the oxidation of ascorbic acid and phenolic compounds in the presence of trace metals (Zhou and Elias, 2012). Stabilisers used in the oil-in-water emulsion may also influence the antioxidant effectiveness of EDTA. Bou *et al.*, (2011) undertook research on two oil-in-water emulsions with similar properties (including pH and EDTA concentrations) but different stabilisers. The stabilisers used were Tween 20, a non-ionic surfactant and sodium dodecyl sulphate (SDS), an anionic surfactant. In the SDS stabilised oil-in-water emulsion, EDTA inhibited lipid oxidation through metal chelating at the emulsion droplet interface, however EDTA did not inhibit lipid oxidation in the oil-in-water emulsion stabilised with Tween 20. Bou *et al.*, (2011) concluded that EDTA's higher iron chelating capabilities towards ferric ions may explain its pro-oxidative effect in Tween 20 stabilised oil-in-water emulsions as it leads to increased interaction of ferrous iron.

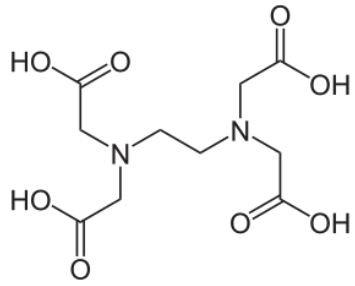


Figure 2.7: Molecular structure of EDTA (Kirchner and Gyarfas, 1957)

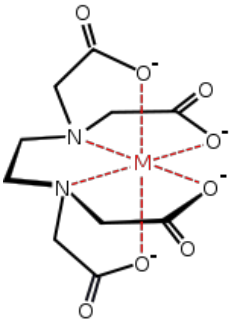


Figure 2.8: Molecular structure of metal-EDTA chelate (Kirchner and Gyarfas, 1957)

Butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT)

BHA (Fig 2.9), a volatile antioxidant, is a very effective antioxidant for animal fat and has very good stability under food processing operations (Coppen, 1999). BHA is effective at levels as low as 11 ppm (Coppen, 1999). BHT is very similar to BHA and exerts a synergistic action with BHA (Coppen, 1999).

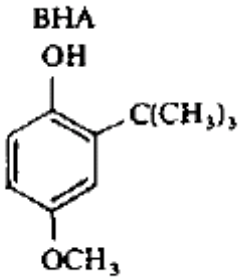


Figure 2.9: Molecular structure of BHA (Coppen, 1999)

t-butylhydroquinone (TBHQ)

TBHQ (Fig 2.10) was approved for use in the USA in 1972 already. Although the use of TBHQ has been authorised and used in many countries, it was only authorised as safe to use in Europe in 2015 (Food Standards Agency, 2015). This antioxidant is stable at high temperatures, soluble in oil, does not discolour and is less volatile than BHA. TBHQ added to crude oil results in an increased shelf life and stability of the oil (Coppen, 1999).

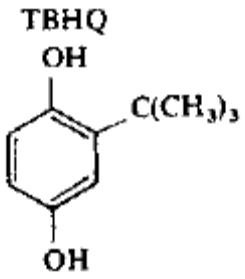


Figure 2.10: Molecular structure of TBHQ (Coppen, 1999)

Antioxidant synergism

Most antioxidants are effective in delaying lipid oxidation through one main mechanism but it has been reported that some antioxidants delay lipid oxidation through more than one mechanism (Erkan *et al.*, 2008). Oxyacids such as citric acid, mallic acid and tartaric acid have very little antioxidant activity that act as chelating agents. When used in combination with a radical scavenger antioxidant such as tocopherols, the oxyacid may improve the antioxidant activity of the tocopherol. Mixtures of tocopherols and rosemary extracts have also showed stronger antioxidant activity than either of these antioxidants in isolation (Fang and Wada, 1993). The same synergism is illustrated in Fig 2.11 for BHA and BHT (Coppen, 1999) where the combination of 0.01% BHA and 0.01% BHT added to a fat decreased lipid oxidation more effectively than 0.02 % BHA or 0.02% BHT in isolation. According to Coppen (1999) not all combinations of antioxidants display synergism and the mechanism of this synergism is not really known. However Coppen (1999) emphasizes the fact that there are a number of free radical reactions that occur during the oxidation process and it is possible that antioxidants differ in their efficiency in dealing with the different mechanisms.

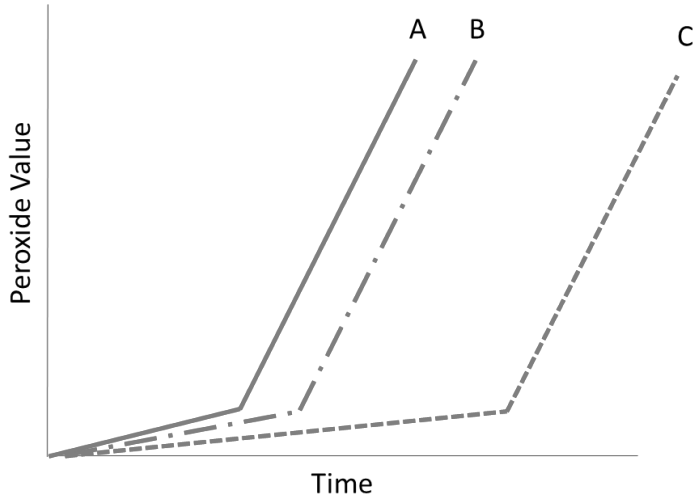


Figure 2.11: Synergism in typical fat (A) with 0.02% BHA, (B) with 0.02% BHT, (C) with 0.01% BHA + 0.01% BHT (Coppen, 1999).

2.4 MEASURING OXIDATION

Many sensory chemical and instrumental methods are available for measuring rancidity in different products (Robards *et al.*, 1988). Lipid oxidation is a dynamic process and a number of these methods are unreliable in determining the rancidity at a specific time, which remains a crucial aspect (Haumann, 1993). The methods can be complex and often involve sample preparation by pulverisation or maceration followed by cold extraction which should be executed correctly as they will influence the results (Robards *et al.*, 1988). Different methods are used when measuring primary oxidative products compared to measuring secondary oxidative products and changes, and no one method can be used to detect changes during the entire process (Yoon *et al.*, 1985; Crapiste *et al.*, 1999).

2.4.1 Sensory methods

Sensory evaluation is the application of knowledge and skills of various scientific disciplines such as food science, psychology, physiology, mathematics and statistics and it relies on humans to assess and measure the sensory properties of a product. No instruments can replace the human response, therefore emphasising the importance of sensory evaluation in a quality assessment of food products (Malcolmson, 1995). Sensory evaluation consisted of two distinct focus areas based on the objective :

1) Subjective measurements by consumers or users of the product.

   Subjective panels, also referred to as consumer panels measure the acceptability and preference of a product by evaluating the hedonic and diagnostic questions in relations to a product evaluated. These findings are instrumental in the development of new products and the prediction of market success. A high number of consumers is often used to increase the statistical validity of the results.

2) Objective measurements from trained panellists.

   Analytical panels consist of 8-10 highly trained individuals, that takes part in discrimination testing and descriptive testing. Discrimination testing such as triangle test, duo trio or paired comparison measures the presence and the size of differences between samples. The objective of descriptive testing on the other hand is to measure the presence or intensity of attributes present in a product.

As rancidity describes the odour and flavour in foods resulting from the deterioration of oil through oxidative and hydrolytic rancidity, objective sensory evaluation is the preferred method for the measurement of rancidity (Rossel, 1999). Descriptive sensory terms used to describe flavours caused by lipid oxidation are not clearly defined and often have similar meanings (Bou *et al.*, 2001). Sensory terms often used to describe rancidity in different products include stale, oxidized, painty and off odour, however, more general terms such as rancid aroma and rancid flavour are also used in sensory studies (Bou *et al.*, 2001).

FFAs (free fatty acids) that are formed during oxidation can be responsible for the rancid aroma and flavour associated with oxidised emulsions. Research by Chale-Rush, Burgess and Mattes (2007) indicated that degradation of FFAs can be described by attributes such as sour, astringent, pungent and burning bitter. The study concluded that the detection of FFAs remained a challenge as FFA presence cannot be definitely determined. Product flavour can also mask the rancid aroma and flavour during the early stages of oxidation, making it difficult to measure the rancidity accurately. Trained panels may describe the rancid attribute with different words as the different structures of the short-chain aldehydes lead to different odours and flavours (Malcolmson *et al.*, 1996). Instrumental or chemical analyses such as Gas Chromatography (GC), PV and others can be correlated with sensory data (Warner, 1995). Table 2.4 illustrates the different odour descriptors as defined by a trained sensory panel associated with the specific secondary product volatiles formed during lipid oxidation.

Odumosu *et al* (1979) completed a study comparing chemical and sensory methods in the evaluation of thermally oxidised groundnut oil and found a 0.92 correlation coefficient between peroxide value (PV) and sensory flavour evaluation. Research completed by Hedegaard *et al* (2006) compared sensory analysis and chemical analysis for oxidative changes in milk. In this study they found a correlation between cardboard flavour and aroma and metallic flavour and the specific chemical marker for oxidation hexanal. In the same study, TBARS measured at absorbance 450nm showed no correlation with sensory descriptors while TBARS measured at absorbance 532nm was found to correlate with sensory descriptors. A concern with sensory analysis for measuring rancidity remains the fact that different studies and different panels generate different words and terms to describe the rancid aroma and flavour. Nevertheless, a review on rancidity measurements indicated that sensory evaluation remains the ultimate measure of rancidity as it measures what the consumer perceives in the product (Heiniö *et al.*, 2002; Malcolmson *et al*., 1996).

Table 2.4: Odour descriptors of individual volatile compounds related to lipid oxidation (Malcolmson *et al.*, 1996)

Volatile	Reported odour threshold in oil (mg/kg)	Reported odour descriptor
Hydrocarbons		
Pentane	340	-
Hexane	-	-
Saturates		
Butanal	0.025	-
Pentanal	0.070	Painty, herbal
Hexanal	0.120	Fatty, green, fruity, cut grass, herbal, rancid, painty, crushed weeds
Heptanal	0.055	Weeds, green, sour, sweaty, herbal, painty, rancid
Octanal	1.50	Lime, grassy, citrus, sharp, heavy, candle-like, crushed weeds
Nonanal	1.00	Green, soapy, rubbery, beany,
Decanal	-	Fruity, candle-like
Monounsaturates		
Propenal	-	-
2-Pentenal	1.00	-
3-Hexenal	0.003	Green, apple-like
2-Heptenal	1.50	-
2-Nonenal	0.15	Green, fatty, tallowy
2-Decenal	2.10	Metallic
Polyunsaturates		
2,4-Hexadienal	-	-
2,4-Heptadienal	0.04	Fatty, nutty
2,4-Octadienal	2.40	-
2,4-Decadienal	0.135	Waxy, fatty, green

Sensory evaluation remains an expensive method and is therefore often replaced or supplemented with objective methods for cost saving initiatives. Electronic nose analyses have been used to supplement human sensory panels in the determination of rancidity (Shen *et al.*, 2011). The electronic nose is an objective method with an array of chemical sensors, where each sensor has partial specificity to a wide range of aroma molecules. Research studies indicate that electronic nose measurement correlate with sensory evaluation and analytical methods (Shen *et al.*, 2011).

2.4.2 Chemical analytical methods

Peroxide Value (PV)

The lipid hydroperoxide concentration in oil is generally expressed as PV. There are numerous analytical procedures to measure the PV of foods. The most common methods are based on iodometric titration where the PV of oil is determined by iodine liberated from potassium iodide by the peroxides present (Rossel, 1990) or the addition of iodine and subsequent titration against sodium thiosulphate (Pearson, 1970). There are two principal sources of error associated with these methods which include the absorption of iodine at unsaturated bonds in the fatty acids and the liberation of additional iodine by oxygen present in the solution. An alternative method recommended for PV determination is a variation of the iodometric method which includes a colorimetric aspect based on the oxidation of ferrous to ferric ion and the determination of the latter as ferric thiocyanate.

The PV of oil is often used as a good indicator for the initial stages of oxidation (Mariod *et al.*, 2010) and is very useful in predicting the onset of rancidity (Depree and Savage, 2001). The PV of commercial oil can range from less than 1 to over 15 milli equivalents/kg (meq/kg) while the average PV for sunflower oil is 3.99 meq/kg (Gan *et al.*, 2005). Research indicates that these peroxide levels increase as oxidation commences until the hydroperoxide decomposition rate exceeds the rate of hydroperoxide formation so that the PV reaches a maximum and then declines (Wills and Cheong, 1979). Wills and Cheong (1979) found that PV indicated the potential shelf life rather than the presence of rancidity as the maximum PV was perceived before the onset of rancidity. A decreasing PV indicates that rancidity would soon occur in the emulsion (Cercaci *et al.*, 2007). PV does not correlate well with rancid flavour scores obtained from sensory evaluation. This may be due to the fact that rancid flavours will only be perceived once PV has reached a maximum and starts decreasing (Robards *et al.*,

1988; Depree and Savage, 2001). PV is therefore a good shelf life predictor but does not aid in measuring the immediate rancidity presence in a product.

Anisidine Value (AV)

AV measures the secondary oxidation products that occur after primary oxidation products (hydrogen peroxides) have been degraded to secondary oxidation products of which aldehydes are one of the main contributors to off aromas. The test involves a condensation reaction between the conjugated dienals or alk-2-enals in the sample and the reagent *p*-anisidine. This reaction is followed by spectrophotometric measurement at 350 nm. The absorption maximum shifts to longer wavelengths with increasing unsaturation and additionally the colour intensity is greater with 2:4dienals than with 2-enals. This method correlates well with PV, although it shows low specificity and does not correlate with sensory evaluation scores (Robards, Kerr and Patsalides, 1988). According to Robards *et al*., (1988) separation of the various condensation products through HPLC may improve the selectivity of this method while Hudson & Gordon (1999) reported that this method is straightforward and a useful indicator of the actual state of rancidity in an oil. It is important to note that the absorption maximum as well as intensity of absorption complexes varies from oil to oil, and therefore the value obtained is only comparable within each type of oil (Rossel, 1999). Generally, well refined oils have AV between 1.0 – 10.0 mmol/kg sample (White, 1995), while oils with high levels of polyunsaturated fatty acids might have an AV higher than 10.0 even when fresh due to the increased potential for the formation of 2-alkenals.

According to Rossel (1999), AV is an effective measurement for abused products with low peroxide levels. Crapiste *et al.* (1999) found AV to be a useful parameter to study oxidative deterioration as it remains practically constant during the initial stages of oxidation and increase rapidly during the decomposition of peroxides.

Totox value

Totox value refers to the total oxidation value which is calculated with the PV and AV: totox value = 2 PV + AV. This equation is based on data indicating 1 PV unit decomposes to give an increase of 2 AV units as peroxides have two oxygens per molecule while aldehydes have only one (Rossel, 1999). Rossel (1999) further stated that the totox value is useful as it combines information on the history of the oil in the AV, and that of the current state in the PV.

Free Fatty Acids (FFA)

The FFA method measures the hydrolytic rancidity that has occurred in a product (Van der Merwe *et al.*, 2003). According to Sonntag (1979) the presence of FFA is equal to the amount of sodium hydroxide required to neutralise the FFA.

Carbonyl Value (CV)

The carbonyl group is a functional group composed of a carbon atom double-bonded to an oxygen atom as seen in aldehydes, ketones and carboxylic acid, and the CV is a measurement of the presence of the carbonyl groups present. CV increases during the development of oxidation but with a different onset and rate when compared to the PV. The CV continues to increase throughout lipid oxidation. Therefore, Wills and Cheong (1979) concluded that CV should not be used to predict rancidity but rather to measure the degree or severity of rancidity present.

Thiobarbituric acid (TBA)

The TBA method is used to measure the product decay caused by lipid oxidation (Haumann, 1993). TBA reacts with malondialdehyde in the sample to produce a chromogen which is determined through spectrophotometry and expressed as mg malonaldehyde per kg of sample (Robards *et al.*, 1988). It is possible that other aldehydes react with TBA as well which exaggerates the impact of lipid oxidation (Robards *et al.*, 1988; Haumann, 1993). HPLC indicates that the TBA method overestimates the quantity of malonaldehyde produced, which is why Addis recommended in 1986, that TBA should not be used as a procedure for malonaldehyde quantification, and the results should not be expressed as ppm malonaldehyde (Addis, 1986).

Iodine Value (IV)

The IV test measures the overall unsaturation of fatty acids in a product and can be used in the determination of rancidity. IV decreases with the proportion of unsaturated fatty acids to total fatty acids and therefor decreases during oxidation as unsaturated fatty acids become more saturated. IV results have shown to correlate well with sensory scores on the development of rancidity indicating the accuracy of this method (Robards *et al.*, 1988). This correlation was only found in drastically accelerated oxidation situations and is not likely to be of interest in shelf life conditions not extensively oxidised (Hudson and Gordon, 1999).

Volatile compounds

Volatile compounds are directly responsible for the rancid aroma and flavour associated with lipid oxidation. Liquid column chromatography is used to separate polar and non-polar material in oils and measures the concentrations of thermally labile peroxides, hydroperoxides as well as volatile and non-volatile secondary oxidation products. Pentane is one of the major volatile hydrocarbons released through thermal decomposition of oxidised intermediates that correlates well with sensory results (Rossel, 1999). Head space hexanal and 7-keto derivatives of phytosterols can be measured through gas chromatography to determine the extent of lipid oxidation in emulsions (Cercaci *et al.*, 2007).

Kreis test

The Kreis test is also known as the Rancidity Index Test. This method is rapid and also gives an indication of incipient rancidity. This test involves the formation of a red colour when phloroglucinol reacts with epoxy aldehydes or their acetals formed during oxidative rancidity in the sample. The red colour is reported in units. A colour up to 3 Red units is considered to indicate incipient rancidity while 3-9 Red units indicate rancidity towards the end of the induction period. Colour readings of over 8 are considered to indicate definite rancidity. Rossel (1999) reported that food additives and flavours can interfere with this test, however this can be overcome by means such as using extracted oil or evaluating the product at the onset of the study for a baseline reading.

2.4.3 Other tests

Rancimat

According to Mendez *et al.* (1996) the most common method used to determine the oxidative stability of oil is the Rancimat test. This method involves an oil sample being heated under atmospheric pressure, and then air is allowed to bubble through the oil at a selected temperature. These high temperature testing conditions differ from normal storage conditions and according to Mendez *et al.* (1996) the oxidation mechanism at high temperatures may be different from those at lower temperatures therefore the results from the Rancimat method should mainly be used as a shelf life predictive model and not correlated with room temperature storage.

2.4.4 Accelerated shelf life testing (ASLT)

ASLT methods are not standardised and differ for different products and between different scientists. ASLT mainly relies on increased and decreased temperatures. These temperature changes can have unnatural effects on the fat tested like melting and denaturation of proteins and enzymes, which subsequently alter the pro-oxidative potential of the product (Rossel, 1999). The Schaal Oven Test is an ASLT that has been used to predict the oxidative stability in baked products (Ragnarsson and Labuza, 1977). Other methods used for predicting oxidative stability include Oxygen Absorption Method, Active Oxygen Method, Oxygen Bomb Method, Thin Layer Test (Ragnarsson and Labuza, 1977) and Weight-Gain Technique (Sherwin, 1968). These ASLT methods are more accurate in predicting the oxidative stability of pure vegetable oils than oil-in-water emulsions (Ragnarsson and Labuza, 1977). In a study conducted on canola oil it was concluded that one day in storage at 60°C was equivalent to 1 month at ambient storage (Vaisey-Genser *et al.*, 1994). Many different studies have focused on accelerated methods to determine shelf life in emulsions (Berton *et al.*, 2012; Branco *et al*., 2011; Jayasinghe *et al.*, 2013). These studies accelerated the shelf life through different conditions, which often included addition of pro-oxidants such as iron and copper (Fomuso *et al.*, 2002) and haem proteins (Baron and Andersen, 2002), and/or increased temperatures (Berton *et al.*, 2012; Branco *et al*., 2011; Jayasinghe *et al*., 2013). The acceleration of the oxidation process is beneficial as it enables the screening of potential antioxidants, however it is important to note that acceleration factors may react differently between products and the levels of pro-oxidants, adjusted temperatures and pH levels should be determined for the specific product at the onset of the study. This may add cost and time to the research but will also increase the validity of the results.

2.5 HEALTH IMPLICATIONS ASSOCIATED WITH RANCIDITY

Lipid oxidation negatively impacts the sensory attributes of food. In addition to the development of off-flavours it may give rise to the formation and accumulation of undesirable products which can be of health concern (Addis, 1986). Lipid oxidation can produce toxic products that can have a negative impact on the biological tissue (Chen *et al.*, 2011) and therefore it is important to consider the health implications associated with these changes in the FAs during the development of hydrolytic and oxidative rancidity. Hydrolytic rancidity which involves the FA splitting from the glyceride is very important in the formation of rancid off-flavours but do not generally have any health effects as fats are in any case hydrolysed in the small bowel before absorption can take place (Sanders, 1999). Oxidative rancidity, on the other hand, can influence the food safety of a product (Gotoh *et al.*, 2006) and may pose

health implications as it leads to the formation of indigestible compounds such as lipid peroxides, hydroxy FAs, carbonyl compounds, cyclic monomers, malonaldehyde, polycyclic aromatic compounds and oxidised sterols (Sanders, 1999).

According to Sanders (1999) these indigestible or poorly digested compounds may affect the absorption of fat-soluble vitamins, while oxidised sterols may possess hormone like activity. Oxidized palm oil has been shown to induce reproductive toxicity and organotoxicity of the kidneys, lungs, livers and hearts of rodents (Ebong *et al.*, 1999). High intake of oxidized lard and cod liver caused impaired fertility in female rats and increased incidence of morphologically abnormal spermatozoa in male rats (Zidkova *et al.*, 2004). Lipid peroxides produced during oxidative rancidity, decrease the nutritional quality of food by reacting with the disulphide bonds in proteins which decreases the sulphur amino acid content and ultimately the protein quality (Sanders, 1999). The long term consumption of oxidised oils can also be detrimental to human health as it exposes the human body to significant levels of oxidative stress due to the imbalance between the oxidative defence system and the formation of oxidising substances. This has a severely weakening effect on the human antioxidant defence system which can lead to many chronic diseases such as hypertension, diabetes and coronary heart diseases (Goswami *et al.*, 2015). According to Chen *et al.* (2011) the consumption of oxidized lipids should be avoided whenever possible due to the negative health impacts associated with their consumption.

Linoleic acid peroxides and secondary products of linoleic acid oxidation are absorbed into the circulatory system and incorporated into the liver. This can lead to detrimental effects such as liver hypertrophy, increased serum transaminase activities and cleaved hepatic lipid peroxide levels which in turn can cause coronary heart disease (Addis, 1986). Oxidised and unoxidized linolenic acid are esterified and absorbed equally well in the intestines of rats (Penumetcha *et al.*, 2000). However, according to Esterbauer (1993) more than 40 research studies conducted on rats and mice from 1965 to 1992 indicate that heavily oxidized oils consumed orally are not acutely toxic. Human absorption of oxidized products is estimated at 100 times lower than that of a rat, and therefore it seems unlikely that consumption of oxidized lipids represents an acute toxicological risk (Esterbauer, 1993). Orally consumed lipid oxidation products are detoxified by glutathione-dependent enzymes to less toxic lipid alcohols in the human gut (Esterbauer, 1993). Esterbauer (1993) further explains that oral toxicity of oxidized lipids is unexpectedly low as oxidation products are not well absorbed in the human intestine and therefore do not reach the blood. This is contradicting to research by Addis

(1986) who found that lipid oxidation products from dietary sources are absorbed into the blood, internal organs and tissues.

It has also been found that cell damage by oxygen radicals and lipid peroxidation play a crucial role in the pathogenesis of several chronic diseases (Esterbauer, 1993). Smooth muscle cells are more resistant to attack by hydroperoxides than endothelial cells (Addis, 1986). Lipid oxidation products can further cause inflammation in the body and play a role in diseases such as arthritis, atherosclerosis, heart diseases (initiation, progression and termination) and breast and colon cancer (Haumann, 1993). It is known that free radicals, such as benzoyl peroxides and hydroperoxy fatty acids participate in tumorigenesis but the effects of lipid peroxides on cancer are complicated by the effect of antioxidant levels (Addis, 1986).

It has also been shown that primary lipid oxidation products, as well as malonaldehyde, a secondary product of lipid oxidation can cross link proteins, deactivate ribonuclease and react with DNA (Addis, 1986). There is still uncertainty whether these undesirable compounds are formed before, during or after the development of a recognisable rancid flavour (Hudson and Gordon, 1999), but it is clear that a high PV is associated with the significant increase in plasma lipid peroxides (Haizman *et al.*, 2016).

Cholesterol lipid oxidation products have similar qualitatively toxic effects to phytosterol lipid oxidation products, but higher concentrations of phytosterol lipid oxidative products are required to have similar levels of toxicity (Cercaci *et al.*, 2007). Ironically, research has also indicated that oxidised lipid products might destroy cancer cells in the body (Haumann, 1993), while some scientists believe these compounds cannot survive the gastric juices of the human stomach (Haumann, 1993). These food safety concerns emphasise the importance of correctly determining rancidity and the development of rancidity in products (Hudson and Gordon, 1999).

2.5 CONCLUSION

It is clear that significant research has been dedicated to the mechanism of lipid oxidation and factors impacting lipid oxidation in both bulk oil and emulsions. Pro-oxidants and antioxidants are factors present in the food product or added to the food product and influence

the development of lipid oxidation. Pro-oxidants such as metals, light and temperature accelerate lipid oxidation while antioxidants retard the lipid oxidation and formation of peroxides. Pro-oxidants and antioxidants are often researched in isolation in order to better understand the mechanism of the specific factor in question in a model emulsion. Although these studies have been beneficial, they are not a true reflection of the dynamic lipid oxidation process taking place in a commercial emulsion, in the presence of commercial ingredients where there is more than one interacting factor affecting the development of lipid oxidation. The effect of antioxidants (natural and artificial) added to commercial type oil-in-water emulsions containing a combination of lipid oxidation factors needs to be investigated in order to more accurately screen the efficiency of antioxidants used in shelf stable retail oil-in-water emulsions. Therefore the first part of this study focused on the effect of different concentrations of two antioxidants (gallic acid (500 ppm and 1000 ppm) and EDTA (37.5 ppm and 75 ppm)) on a commercial formulation of a sunflower oil-in-water salad dressing emulsion stored at two storage conditions: 1) ambient storage and 2) accelerated storage (simultaneous combination of acceleration factors and storage conditions (32.2°C, addition of potential pro-oxidants, adjusted pH)), and lipid oxidation was measured through descriptive sensory analysis and chemical analyses. There are food safety concerns as well as consumer dissatisfaction associated with the consumption of rancid food products. This emphasises the importance of selecting an acceleration methodology to predict in a short time, the onset and rate of lipid oxidation. The methodology used to develop a predictive tool should correlate with actual shelf life storage to deliver acceptable consumer products and improve the eating experience.

Sensory and chemical methods (e.g. PV, AV, FFA and TBA) have been used to measure rancidity in food products. Sensory analysis is often the preferred method as it measures rancidity related attributes from the deterioration of oil, and it is more representative of what the consumer perceives. Different sensory terms have been used in different studies to describe the rancid aroma and flavour as there appears to be no standard lexicon to be used. Developing a standard rancidity lexicon and identifying key attributes associated with the development of rancidity in oil-in-water emulsions will greatly benefit future research in this field. The second part of the study focused on the development of an accelerated shelf life model for commercial oil-in-water emulsions through multivariate analysis, to identify the key sensory attributes associated with the development of lipid oxidation and rancidity. This rancidity lexicon was used to help identify predictors for end of shelf life during shelf life testing of oil-in-water emulsions that could potentially support the food industry.

Published December 2016:
Sainsbury, J., Grypa, R., Ellingworth, J., Duodu, K. G. and de Kock, H.L. (2016) 'The effects of antioxidants and shelf life conditions on oxidation markers in a sunflower oil salad dressing emulsion (SOSDE).' *Food Chemistry,* 213, pp. 230-237.

3

CHAPTER 3: THE EFFECTS OF ANTIOXIDANTS AND SHELF LIFE CONDITIONS ON OXIDATION MARKERS IN A SUNFLOWER OIL SALAD DRESSING EMULSION (SOSDE)

Jeanine Sainsbury[1,2], Roman Grypa[2,] John Ellingworth[2], Kwaku G Duodu[1], Henriëtta L de Kock[1]

1. Department of Food Science, University of Pretoria, Pretoria Private Bag X20, Hatfield 0002, South Africa
2. McCormick & Company Inc., 204 Wight Avenue, Hunt Valley, MD 21030, USA
*Corresponding author: Henriëtta L de Kock, riette.dekock@up.ac.za

Abstract

The effects of high and low concentrations of antioxidants (gallic acid and EDTA) in a SOSDE stored at accelerated deterioration and ambient conditions were examined. SOSDEs stored at accelerated shelf life conditions contained pro-oxidation factors (higher temperature, pH 5.5, iron and ascorbic acid). SOSDEs were examined by sensory (aroma) and chemical analyses (AV and PV) daily for 20 days for accelerated stored products and bi-weekly for 24 weeks for ambient stored products. Sensory differences between products with high and low concentrations of antioxidants were more apparent for ambient stored than accelerated stored SOSDEs. Accelerated storage resulted in more aroma differences between SOSDEs with different types of antioxidants than antioxidant concentrations. PV differences between products with high and low concentrations of antioxidants were apparent for SOSDEs with EDTA stored under accelerated shelf life conditions. AV differences existed between SOSDEs with different gallic acid concentrations stored in accelerated and ambient shelf life conditions, and SOSDEs with different EDTA concentrations stored in accelerated shelf life conditions.

These results suggest that: 1) the accelerated storage model used in this study is more suitable for SOSDEs with metal chelator antioxidants such as EDTA, rather than free radical scavenging antioxidants such as gallic acid because EDTA retarded lipid oxidation as measured through PV and AV compared to SOSDE with no antioxidants added. 2) PV and AV are more sensitive oxidation markers than aroma analysis when using the accelerated storage model, while sensory analysis differentiated more between low and high concentrations of antioxidants during ambient storage. 3) PV, AV and aroma of SOSDEs stored in accelerated conditions as used in this study, do not clearly predict results for ambient stored SOSDEs but can be used to screen the efficiency of antioxidants.

3.1 INTRODUCTION

Consumers demand high-quality shelf stable products with shelf lives of several weeks or months (Corrigan *et al.*, 2012). Sunflower oil emulsions, like salad dressings and mayonnaise, have a shelf life of 6-12 months, unopened, at room temperature. The shelf life of these products is often determined by quality changes rather than microbial safety of the product (Lewis and Heppel, 2000; Corrigan *et al.*, 2012). Spoilage of emulsions is related to lipid oxidation or deterioration of the emulsifying agent that leads to oil separation (Rossel, 1999; Bou *et al.*, 2001). Antioxidants added to emulsions retard lipid oxidation and the formation of rancid off notes (Rodríguez-Rojo *et al.*, 2012). Lipid oxidation is the term used to describe the complex sequence of chemical interactions in oils where the fatty acids react with oxygen to form volatile aldehydes, ketones, alcohols, furans, hydrocarbons or acids. These volatile compounds and combinations are associated with the rancid off aroma (Hamilton, 1999) that can be determined through a variety of chemical and sensory tests. Flavour release of volatiles is strongly affected by the food matrix (Hartvigsen *et al.*, 2000). The efficacy of natural antioxidants like tocopherol and rosemary extract (Rodríguez-Rojo *et al.*, 2012) has been researched extensively due to potential toxicological long term effects of synthetic antioxidants such as BHA and TBHQ (Rodríguez-Rojo *et al.*, 2012; Asnaashari *et al.*, 2014).

Accelerated shelf life tests are often used to determine shelf life due to time and cost constraints associated with ambient shelf life tests (Corrigan *et al.*, 2012). During accelerated shelf life tests, the product is exposed to factors or combinations of factors that promote quality deterioration e.g. higher temperatures, above normal humidity and light or specifically UV light exposure, higher water activity and/or addition of potential pro-oxidants. Acceleration factors depend on the product and normal storage conditions for the product (Richards *et al.*, 2014). Most studies evaluate the effects of one factor at a time (Branco *et al.*, 2011), for example the

Arrhenius equation was used to relate temperature of storage with reaction velocity (Córdova *et al.*, 2011). However, shelf life is a dynamic process in which storage factors constantly interact with each other (Branco *et al.*, 2011), and therefore a model based on combinations of lipid oxidation acceleration factors, studied by Branco *et al.* (2011), was applied in this study. A limiting factor in the study by Branco *et al.* (2011) was the use of a model oil-in-water emulsion that is not fully representative of the product manufactured in the food industry. In order to predict lipid oxidation in oil-in-water emulsions more accurately, this study was completed on a more commercial-like oil-in-water emulsion instead of a model oil-in-water emulsion.

The objective of this study was to determine the effect of different concentrations of the natural antioxidant, gallic acid and the synthetic antioxidant, EDTA on aroma attributes, AV and PV of a SOSDE. Emulsions were stored at ambient shelf life conditions as well as at accelerated shelf life conditions as proposed by Branco *et al.*, (2011) who developed an accelerated shelf life model for oil-in-water emulsions to optimize conditions to evaluate natural antioxidants. Ambient shelf life and accelerated shelf life are the terms used throughout this thesis to describe the addition or absence of the combination of factors added or altered with regards to temperature, potential pro-oxidants ascorbic acid and $FeSO_4$ and pH.

3.2. MATERIALS AND METHODS

Branco *et al*. (2011) evaluated oil-in-water emulsions to evaluate seven factors that could be used to extend the variation range of oxidation markers measured by PV and TBARS. This research concluded that four factors; temperature (32.2°C), ascorbic acid (1.70 mmol/L), $FeSO_4$ (1.0 mmol/L Fe^{2+}), and pH 5.5 would be the optimal emulsion conditions giving the largest variation range of these oxidative markers. The shelf life model as proposed by Branco *et al.* (2011) with the simultaneous addition of factors (temperature, pH, ascorbic acid, iron) with modification to timing intervals of analyses was used for the accelerated study component of this study.

3.2.1 Emulsion Preparation

Table 3.1 shows the preparation and storage of 8.5% oil-in-water emulsions (sunflower oil, water, white sugar, vinegar, egg yolk powder, stabilisers, salt, mustard powder, black pepper). The dry ingredients consisting of sodium benzoate (2.39 g), potassium sorbate (2.39 g), ascorbic acid (2.39 g 1.70 mmol/L)(potential pro-oxidant), $FeSO_4$ (0.86 g 0.885 mmol/L)

and antioxidants (gallic acid or EDTA) were dissolved in water (600 g) by mixing the solution for 2 minutes with an electric mixer. Salt (79.8 g), white sugar (997.9 g), mustard powder (47.9 g), ground black pepper (0.8 g) and egg yolk powder (159.7 g) were mixed into the solution. Additional water (2813.2 g) was added. The NaOH (673 g 0.1 M) was only added to products that were stored in accelerated storage conditions. The quantity of 0.1 M NaOH added to alter the pH to 5.5 was determined in a pre-study. After the addition of NaOH and water, the mixture was mixed for 5 minutes. Independently sunflower oil (479 g) was mixed with the stabiliser Ultra Tex (199.6 g) obtained from Ingredion Inc. (Westchester, IL, USA) and Visco Xan (8.8 g) obtained from Archer Daniels Midland (Decatur, IL, USA) for 3 minutes in Artisan Kitchen Aid electric mixer and then left to rest for 5 minutes. After the resting period, the solution of sunflower oil and stabilisers was added to the initial solution and mixed for 2 minutes. After 2 minutes, sunflower oil (117.7 g) was gradually added while mixing continued. Lemon concentrate (4.0 g), vinegar (780.8 g) (10%) and lactic acid (16 g) were added to the solution and mixed for 5 minutes. Accelerated stored products contained potential pro-oxidants as proposed by Branco *et al.*, (2011) for optimal accelerated lipid oxidation. The products contained either no antioxidants (No) or low or high levels of gallic acid (LoGal and HiGal) or EDTA (LoEDTA or HiEDTA) as shown in Table 3.1.

Table 3.1: Preparation and storage conditions for 10 sunflower oil salad dressing emulsions (SOSDE) treatments

	Sunflower oil salad dressing emulsions (SOSDE)									
Products	AmbNo	AmbLoGal	AmbHiGal	AmbLoEDTA	AmbHiEDTA	AccNo	AccLoGal	AccHiGal	AccLoEDTA	AccHiEDTA
Treatment	Ambient					Accelerated				
Potential pro-oxidants added (0.885 mmol/L $FeSO_4$, 1.70 mmol/L Ascorbic acid). pH 5.5	No					Yes				
Antioxidant	None	Gallic Acid		EDTA		None	Gallic Acid		EDTA	
Antioxidants added	None	500ppm	1000 ppm	37.5 ppm	75ppm	None	500ppm	1000 ppm	37.5ppm	75ppm
Frequency of aroma analysis	Bi-weekly					Daily				
Frequency of chemical analysis	Week 0,2,4,8,12,16,20 and 24					Day 0,1,2,4,6,8,11,14,17 and 20				
Storage temperature	25°C					32.2°C				
Storage period	24 weeks					20 days				

All products were prepared using the same batch of raw materials and using the same equipment. The pH of each emulsion was measured (pH 3.24-3.32) and the pH of accelerated products was adjusted to 5.5 using 0.01 M HCl or 0.1 M NaOH, and then homogenised using a Scott Turbon Mixer (LA1.3, Adelanto, California. USA). Each emulsion was poured into four clear glass containers (80 ml emulsion per 100 ml container (20 ml head space)) for each treatment time interval. All samples were stored in the dark. Ambient shelf life SOSDEs (stored at 25°C) were collected every 2 weeks (14 days) and analysed for aroma properties, PV and AV. Accelerated shelf life products (stored at 32.2°C) were collected every 24 hours and analysed for aroma properties, PV and AV. Selected glass bottles were stored in a freezer (in dark conditions) at -18.9°C until time of evaluation. Bottles were removed from the freezer 4 hours before analyses to reach a temperature of 22-24 °C. Two samples were used for sensory evaluation and two samples were used for chemical analyses.

3.2.2. Evaluation of the aroma of SOSDEs

Sensory evaluation was performed by 8-12 trained sensory panellists whose selection was based on their performance in screening tests. The screening tests included recognition of rancidity and rancidity related aromas such as cardboard, plastic and paint (Jacobsen,

1999) as well as other general aroma attributes (e.g. pungency, eggy and dairy aromas) in oil-in-water emulsions. As an initial guideline for this study, attributes, references and definitions from a previous study on lipid oxidation were used (Arancibia *et al.*, 2011). During four orientation sessions (2 hours each), panellists received representative SOSDE products and determined which attributes best described the emulsions. Due to potential health implications associated with the consumption of oxidised food products (Chen *et al.*, 2011) the panel evaluated aroma attributes only. Lerma-García *et al.* (2010) conducted research on defects in oils and noted that the most important properties of oil are represented by their aroma. Clear definitions and references were finalised for the 13 aroma attributes evaluated (Table 3.2). An application for ethical approval for the inclusion of humans as panellists in the research was not made because the sensory panel that evaluated the samples were sourced and employed by the specific food company involved. The panellists have signed contracts that adhere to the ethics, intellectual property and confidentiality as prescribed by McCormick & Company.

Table 3.2: Definitions for attributes developed in the aroma evaluation of sunflower oil salad dressing emulsions (SOSDE)

Aroma Attributes	Definition
Pungent	Sharp, piercing, somewhat irritant odour that gives a recoil response. Tactile stinging sensation.
Vinegar	Aroma associated with white vinegar
Eggy	Aroma associated with boiled egg. Sulphur like.
Citrus	Aroma associated with acidic fruity notes of the citrus family
Musty	Aroma associated with attic smell, mouldy, damp, reminiscent of air in an unventilated room.
Dairy	Aroma associated with non-fermented dairy products such as cow's milk
Green	Aroma associated with freshly cut leaves, grass or green vegetables.
Oil (fresh)	Aroma associated with fresh oil
Earthy	Aroma associated with moistened soil
Metallic	Aroma associated with metals
Plastic	Aroma associated with a waxy oxidised off note (associated with a plastic container)
Cardboard	Aroma associated with wet cardboard boxes (associated with oxidised fats and oils)
Painty	Aroma associated with wet paint (associated with oxidised fats and oils)
Rancid	Aroma associated with oxidised fats or oils

Evaluation was conducted under red light to mask any colour differences between products (Meilgaard *et al.*, 2007). All data recording was completed on computer using Compusense Five version 5.2 (Compusense Inc., Guelph, Canada). Products (80 ml) were

served in 100 ml glass containers with lids, labelled with random 3-digit codes. Due to low detection levels of rancidity related attributes expected in products and the complexity of aroma evaluation, panellists had to merely indicate the presence or absence of an attribute rather than rating the intensity of the attribute. In order to ensure that panellists were not influenced in any way, no information regarding the nature of the experiment (shelf life testing) was provided. Five products were analysed per day. All products were evaluated in duplicate by using two of the four samples stored for each treatment time interval.

3.2.3 Chemical analysis of SOSDEs

Chemical analyses were performed on the oil extracted from the stored oil-in-water emulsion samples. To measure the lipid hydroperoxide concentrations (PV) of SOSDEs at each storage period (Table 3.1), the iron-based spectrophotometric peroxide method (IDF74A: 1991), described by Shanta & Decker (1994) was followed. The extracted oil sample was mixed in disposable glass tube with 9.8ml chloroform-methanol and mixed, after which ammonium thiocyanate and iron(II) solution was added separately. After 5 minute incubation at room temperature, the absorbance of the sample was determined at 500nm. The entire process was conducted in subdued light and completed within 10 minutes. The *p*-anisidine values of the products were determined through the condensation reaction between conjugated dienals or alk-2-enals and the reagent *p*-anisidine followed by spectrophotometric measurement at 350 nm in duplicate according to the AOCS Official Method Cd 18-90 (AOCS, 1999). Chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA).

3.2.4 Statistical analyses

The detection of aroma attributes in the SOSDE products was expressed as a binomial function of the proportion of the panellists who detected the attribute. Statistical analysis was conducted using GenStat®. The generalized linear mixed model (Payne, 2012) analysis was applied to the sensory data with the binomial distribution and logit link function. The fixed effects were specified as products and storage period (days or weeks) and the random effect as replication. Mean proportions of presence/absence (between 0 and 1) were separated with Fisher's protected least significant difference (FPLSD) test at the 5% level. Standard deviations of means were considered when comparing treatments. The linear mixed model analysis, or REML, was applied to chemical analyses (Payne, 2012). The fixed effects were specified as products, days and the products x days interaction. FPLSD test was used to

compare means at the 1% level as the treatment variances were heterogeneous. Separate analyses were conducted for ambient and accelerated stored SOSDEs.

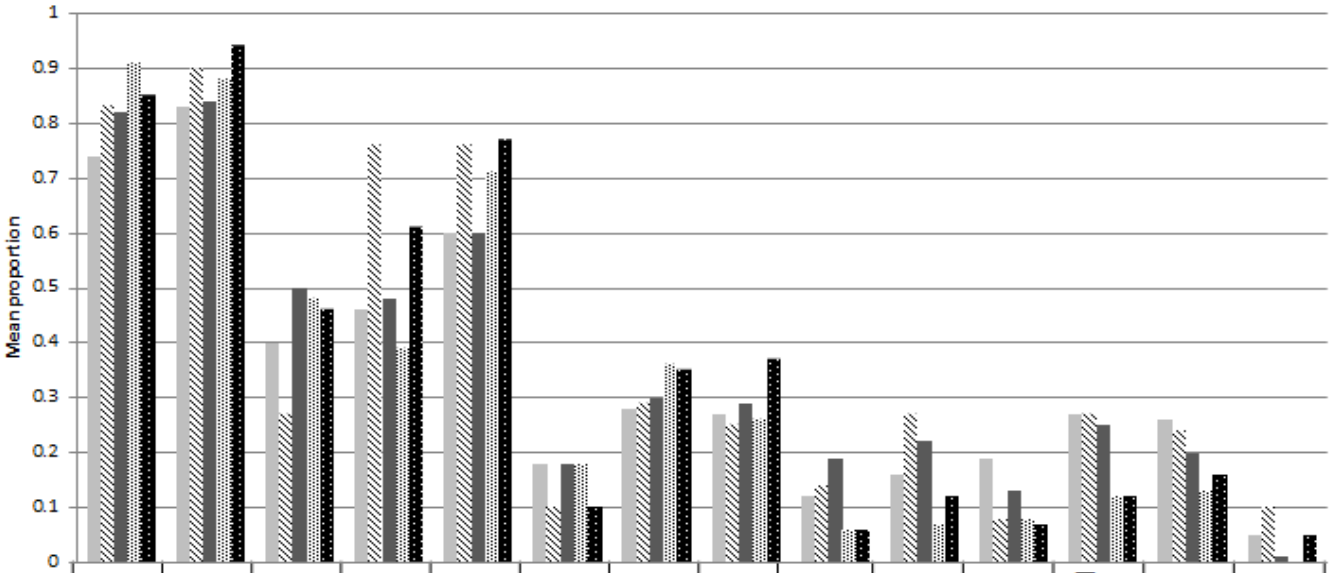
3.3. RESULTS

3.3.1 Sensory and chemical analyses of SOSDEs with different levels of antioxidants and stored under ambient shelf life conditions

The average effects of no antioxidant and low and high concentrations of antioxidants on perception of aroma attributes of SOSDEs stored at ambient storage as evaluated by trained sensory panellists over 24 weeks are shown in Figure 3.1. The mean proportion refers to the percentage panellists allocating a 0 (not detected) or a 1 (detected) to each attribute for each SOSDE treatment. A mean proportion of 1 indicates all panellists detected the attribute while a mean proportion of 0.5 indicates only 50% of the panellists detected the specific attribute. There was no significant difference in mean proportion of pungent or green aromas between SOSDEs with high and low concentrations of either antioxidant, however differences in musty were apparent when high and low concentration of both antioxidants were applied to the SOSDE. For SOSDEs with EDTA, vinegar aroma was significantly more noted at the higher concentration. Citrus aroma was less noted in AmbHiGal. Differences were not noted for EDTA treated SOSDEs. Oil aroma in AmbHiEDTA was detected by 10% fewer panellists compared to AmbLoEDTA. Although the oil aroma attribute was not associated with rancidity during training sessions, oil and fried oil are sensory terms often used to describe the formation of aldehydes e.g. benzene acetaldehyde, trans,trans-2,4-octadienal, trans,cis-2,4 nonadienal, trans,trans-2,4-nonadienal, trans,cis-2,4-decadienal, trans,trans-2,4-decadienal (Hartvigsen *et al.*, 2000). It is possible that the perceived oil aroma was due to the presence of these aldehydes during oxidation and may therefore be an indication of lipid oxidation, but further tests would be needed to confirm the specific aldehydes in the SOSDE. Of the oxidation related aroma attributes; plastic, painty, cardboard and rancid (Hudson and Gordon, 1999), were the only attributes that showed differences between SOSDEs with high and low concentration of antioxidants (Fig 3.1) even though detected by less than 10% panellists in general. Rancid aroma was detected by a significantly higher percentage of panellists in AmbLoGal compared to AmbHiGal. In contrast, rancid aroma was detected by significantly fewer panellists in AmbLoEDTA compared to AmbHiEDTA. Even though it was expected that more panellists would detect rancid aroma in the SOSDE with lower concentrations EDTA, Let *et al.* (2007) found no concentration-dependent effect of EDTA in fish oil enriched salad dressings as measured with aroma analysis and PV. No significant effect on cardboard and painty aroma was detected among the SOSDEs with no antioxidant and gallic acid, indicating

the minimal effect of low and high concentrations of gallic acid on retarding development of oxidation related aroma attributes.

Upon comparison of different antioxidants, EDTA applied to the SOSDE decreased the presence of earthy and metallic aromas compared to SOSDEs containing no antioxidant and gallic acid, respectively (Fig 3.1). In shelf life studies of emulsions it was found that these two sensory attributes (earthy and metallic) could be related to the development of ketone 1-hexen-3-one during oxidation (Hartvigsen *et al.*, 2000). However, according to Jacobsen (1999) it is impossible to conclude which volatiles relate to which descriptor without testing specifically. Further analysis is needed in order to determine the presence of 1-hexen-3-one and its relation to earthy and metallic aroma in the SOSDE.



	Pungent	Vinegar	Citrus	Musty	Eggy	Dairy	Green	Oil	Earthy	Metallic	Plastic	Cardboard	Painty	Rancid
p-value	0.001	0.004	<0.001	<0.001	<0.001	0.006	0.045	0.026	<0.001	<0.001	0.001	<0.001	0.006	0.003
AmbNo	c	c	a	c	b	a	c	b	b	bc	a	a	a	ab
AmbLoGal	b	ab	b	a	a	b	bc	b	ab	a	bc	a	ab	a
AmbHiGal	bc	bc	a	bc	b	a	abc	ab	a	ab	ab	a	abc	bc
AmbLoEDTA	a	bc	a	c	a	a	a	b	c	d	bc	b	c	c
AmbHiEDTA	ab	a	a	b	a	b	ab	a	c	c	c	b	bc	ab

abc – for a specific aroma attribute, products with different letters were significantly different at the 95% confidence level

Figure 3.1: The effect of low and high concentrations of antioxidants or none added on aroma attributes for SOSDEs stored at ambient conditions for 24 weeks with measurements taken every 2 weeks (see Table 3.1 for detail of product code names).

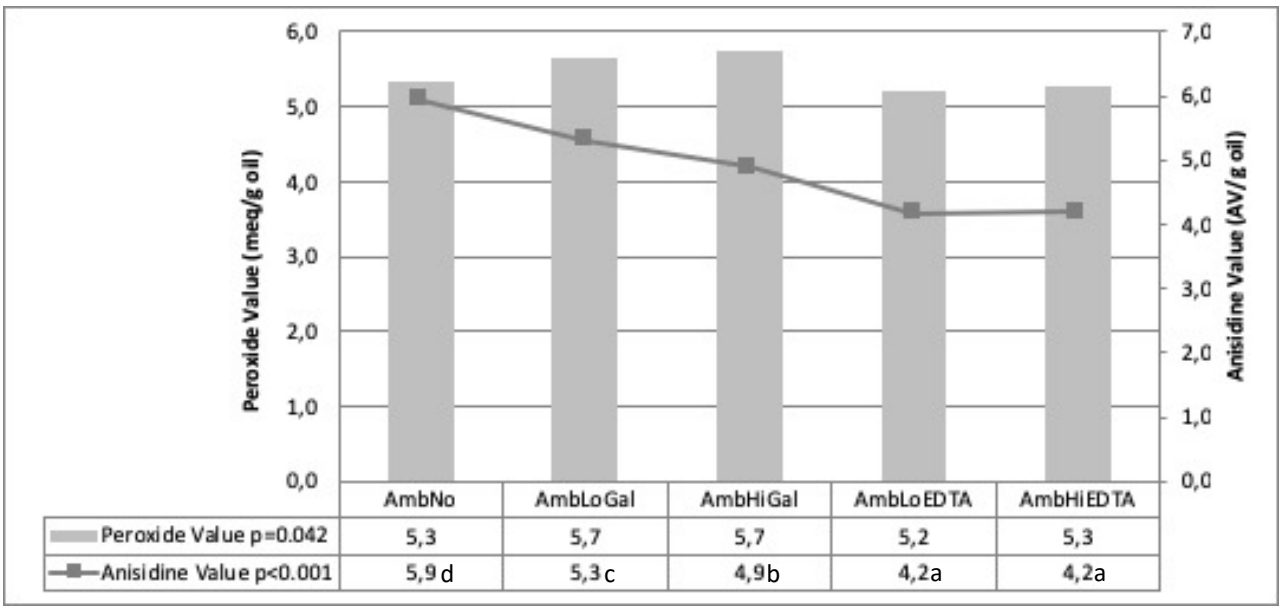
To better understand how different concentrations of the antioxidants gallic acid and EDTA influenced oxidation of SOSDE, PV and AV were determined in duplicate for each SOSDE during ambient storage, and these average values are seen in Figure 3.2. Lipid peroxide formation, measured by PV, was not affected significantly (at 99% confidence level) by different concentrations of different antioxidants ($p=0.042$, Fig 3.2i) and did not show differences over the 24-week ambient storage time either ($p=0.423$, Fig 3.2ii). PV was below 6 for all SOSDEs stored in ambient conditions throughout 24 weeks. It is hypothesised that continuing with this study for a longer period may lead to increased PV where differences caused by addition of antioxidants will be observed. AV increased significantly over time and showed significant increases from week 0 to week 2, week 10 to 12, and week 20 to 24 (Fig 3.2ii) and increased more rapidly towards the end of the shelf life period. Alamed *et al.* (2006) reported research on emulsions with different concentrations of EDTA and found that higher concentrations of EDTA retarded lipid oxidation over longer periods of time. Similarly, Bou *et al.*, (2001) found that increasing concentrations of free radical scavenging antioxidants (gallic acid and propyl gallate) decreased lipid oxidation, measured through relative lycopene concentrations over time. This was in accordance with results from this study, where SOSDE with higher concentrations gallic acid had a significantly lower AV compared to SOSDE with low concentration gallic acid ($p<0.001$, Fig 3.2i).

However, SOSDEs with high concentration EDTA compared to the low concentration of EDTA did not have lower AV in this study (Fig 3.2i), possibly because the low concentration EDTA was optimally chelating with metals and therefore higher concentrations EDTA would be not more effective. Similar to results from this study where SOSDE with addition of EDTA had lower AV than SOSDE with no antioxidant or SOSDE with addition of gallic acid, Let *et al.* (2007) also found EDTA to be more effective in inhibition of volatiles as measured through AV rather than the inhibition of PV. This supports the hypothesis that transition metals catalyse the degradation of lipid hydroperoxides and thus can be prevented by chelating of these metals by EDTA.

In this study, the addition of EDTA had a higher retardation effect than the addition of gallic acid and no antioxidants on the development of secondary oxidation products as measured by AV (Fig 3.2i). Boon, *et al* (2008) also concluded that the addition of 100 µM EDTA into an emulsion, reduced lipid oxidation compared to no addition of antioxidants. EDTA retards oxidation by chelating iron, a pro-oxidant, to form FeEDTA (MacPhail *et al.*, 1994). A pH lower than 5 initiates the release of iron in oil-in-water emulsions, which in turns decomposes pre-existing lipid hydroperoxides located at the oil-water interface *(*Thomsen *et*

al, 2000). The pH of SOSDEs in this study were 3.24 - 3.32 indicating the possibility of release of iron. With EDTA binding to the iron it retards the decomposition of pre-existing lipid hydroperoxides explaining why the SOSDE with EDTA had a lower AV (Fig 3.2i). Bou *et al.*, (2001) found contradictory results for the effectiveness of EDTA but concluded that metal chelators such as EDTA are effective when used with anionic surfactants. Therefore, Visco Xan, a stabiliser used in the preparation of all SOSDEs is classified as an ionic surfactant, may have impacted the effectiveness of EDTA as metal chelator in this study.

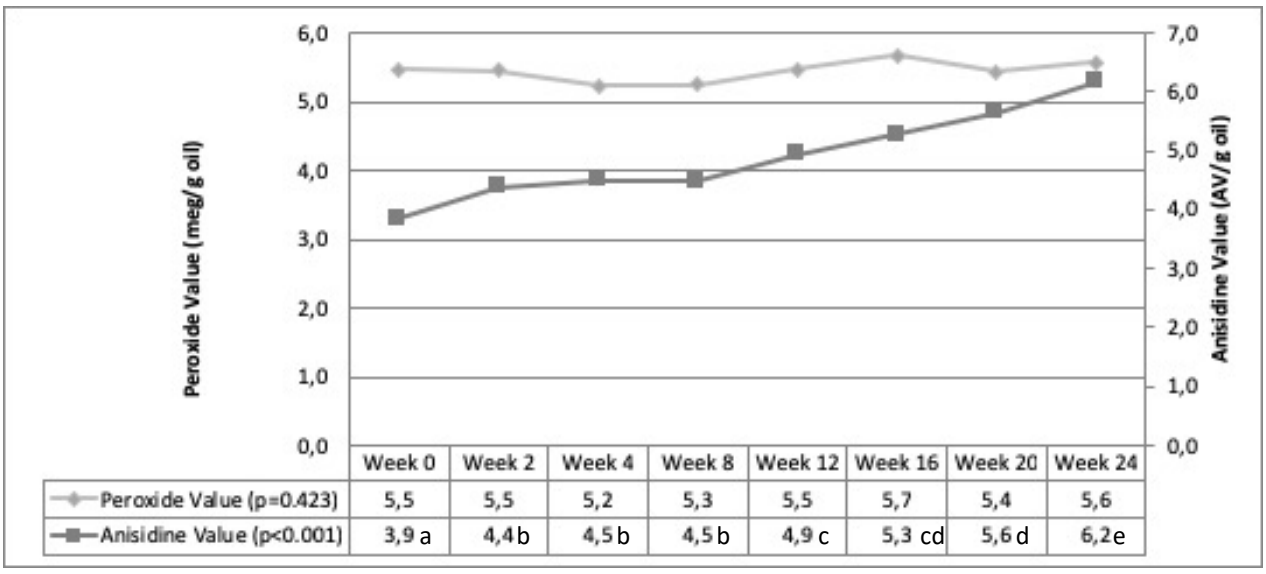
i)



	AmbNo	AmbLoGal	AmbHiGal	AmbLoEDTA	AmbHiEDTA
Peroxide Value p=0.042	5,3	5,7	5,7	5,2	5,3
Anisidine Value p<0.001	5,9 d	5,3 c	4,9 b	4,2 a	4,2 a

abcd - products with different letters indicate significant differences at 99% confidence level
*Standard error peroxide value: 0.1042, Standard error anisidine value: 0.1080

ii)



	Week 0	Week 2	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Peroxide Value (p=0.423)	5,5	5,5	5,2	5,3	5,5	5,7	5,4	5,6
Anisidine Value (p<0.001)	3,9 a	4,4 b	4,5 b	4,5 b	4,9 c	5,3 cd	5,6 d	6,2 e

abcde - values for weeks with different letters indicate significant differences at 99% confidence level

Figure 3.2: Chemical analyses (PV and AV) for oil extracted from SOSDEs stored under ambient shelf life conditions for 24 weeks, illustrated for (i) different concentrations of EDTA and gallic acid antioxidants and (ii) intervals over a 24-week time period.

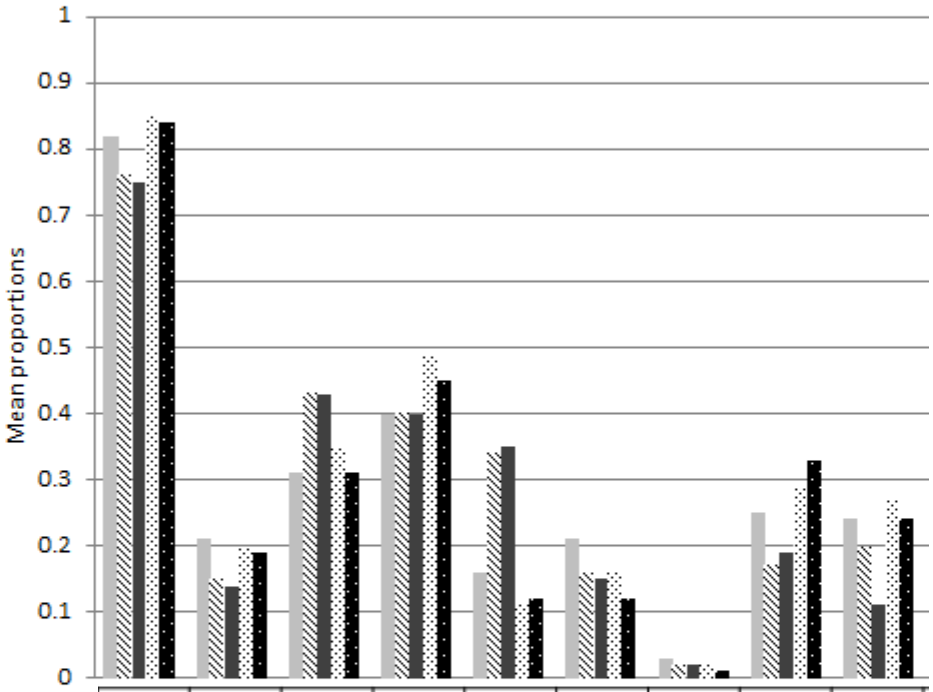
Comparing the results from sensory and chemical analyses for ambient stored SOSDEs, the high concentration of gallic acid significantly decreased AV (Fig 3.2i) and rancid aroma (Fig 3.1) compared to the low concentration gallic acid and the addition of EDTA to SOSDE, reduced ($p < 0.01$) the AV (Fig 3.2i) as well as the cardboard aroma (Fig 3.1). The lower AV was therefore associated with lower detection of rancid aroma and cardboard aroma. Cardboard and rancid aroma are both attributes associated with the development of lipid oxidation. Similar findings were observed by Hudson and Gordon (1999) who concluded that AV is a useful indicator of the actual state of rancidity in oil containing products.

3.3.2 Sensory and chemical analyses of SOSDE with different levels of antioxidants and stored under accelerated shelf life conditions

Fig 3.3 shows the average aroma profiles of SOSDEs with low and high concentrations of antioxidants stored under accelerated shelf life conditions. Painty aroma was the only attribute that showed significant differences in detection between SOSDEs with high and low concentrations of gallic acid. Painty aroma in AccLoGal was perceived by 10% more panellists compared to AccHiGal.

Gallic acid applied to the SOSDE, reduced the detection of eggy and dairy aromas significantly, while it increased the detection of green and earthy aromas compared to SOSDEs with EDTA and no antioxidants. The aroma attributes for the individual antioxidants, gallic acid and EDTA, were not analysed and it is possible that the green aroma is associated with gallic acid. Metallic aroma was perceived by significantly ($p=0.008$) more panellists in SOSDE with no antioxidants compared to SOSDE with gallic acid or EDTA. Cardboard aroma was significantly ($p<0.001$) more noted in SOSDEs with EDTA compared to gallic acid. Plastic aroma was perceived by a very small proportion (less than 5 %) of panellists when compared to the other attributes (Fig 3.3). It is clear that plastic was not a differentiating attribute of lipid oxidation in this study. In other studies panellists had difficulty describing, rating or identifying specific rancidity related attributes. Serfert *et al.* (2010) and Böttcher *et al.* (2015) reported the difficulty panellists may experience with describing, rating or identifying rancidity related attributes and recommended the use of overall rancidity, rather than specific descriptive attributes of rancidity during sensory analyses. Different rancidity related attributes were used in this research due to the objective associated with the identification of aroma attributes used to predict lipid oxidation in oi-in-water-emulsions. Results from this study clearly indicate

differences associated with the detection of these rancidity related attributes in the SOSDE treatments.



	Eggy	Dairy	Green	Oil	Earthy	Metallic	Plastic	Cardboard	Painty
p-value	<0.001	<0.001	<0.001	0.002	<0.001	0.008	0.020	<0.001	<0.001
AccNo	a	a	b	b	b	a	a	b	ab
AccLoGal	b	b	a	b	a	b	b	c	b
AccHiGal	b	b	a	b	a	b	ab	c	c
AccLoEDTA	a	a	b	a	c	b	b	ab	a
AccHiEDTA	a	a	b	ab	bc	b	b	a	ab

abc – for a specific aroma attribute, products with different letters were significantly different at the 95% confidence level

Figure 3.3: The effect of low and high concentrations of antioxidants or none added on aroma attributes for SOSDEs stored at accelerated conditions for 20 days with measurements taken every 24 hours (see Table 3.1 for detail of product code names).

To better understand how different concentrations of the antioxidants gallic acid and

EDTA influenced oxidation of SOSDEs, PV and AV were determined for each SOSDE every second day during accelerated storage of 20 days, and these averages are seen in Figure 3.4. EDTA had a significant effect on the development of peroxides (PV) as well as secondary peroxide products (AV), with PV and AV for AccHiEDTA being significantly lower (Fig 3.4i), indicating that the high concentration of EDTA had a greater effect on retarding lipid oxidation.

This effect was also noted by Coppen (1999) in a study with 200 ppm and 2500 ppm polymeric antioxidant in vegetable oil, where the higher concentration of polymeric antioxidant had a greater effect on oxidation. Contradictory to this, SOSDE with a low gallic acid concentration had a significantly lower AV compared to SOSDE with a high gallic acid concentration (Fig 3.4i). The reason for this finding is unknown however it might be attributed to pro-oxidant interaction or location of the antioxidant. The SOSDEs containing gallic acid as the antioxidant had higher PV compared to the SOSDEs with EDTA as the antioxidant. The decline in PV from day 0 to day 1 was unexpected and may be due to a measurement error. PV showed no differences from day 1 to day 8, with significant increases at day 14 and again at day 17 (Fig 3.4 ii) due the development of peroxides. As measurements were only taken until day 20, it is not known if the PV changed after day 20. According to Robards *et al.* (1988) as the PV reached a peak, it could be expected to see an increase in AV as the peroxides decomposed into volatiles. This expectation, was based on the negative correlation found between AV and PV in edible oil (Robards *et al.* 1988).

Comparing chemical analysis with sensory analysis for SOSDEs stored at accelerated conditions, EDTA addition to SOSDE reduced the PV but increased the detection of painty and cardboard aromas compared to SOSDE with gallic acid (Fig 3.4 i) (Fig 3.3). Similar results were seen with AccHiGal having a higher AV (Fig 3.4i) and a less detected painty aroma (Fig 3.3) compared to AccLoGal. This came as no surprise as peroxides and hydroperoxides as measured by PV have been reported as hardly detectable (Hudson and Gordon, 1999) and did not correlate with sensory evaluations (Frankel, 1998). However, their presence was a potential indicator of the development of rancid off-flavour as they inevitably break down to volatile compounds, including, but not limited to, alcohols, aldehydes, ketones and carbonyl compounds that are responsible for the off flavours associated with rancidity (Hudson and Gordon, 1999; Cercaci *et al.*, 2007).

To better understand oxidative stability of emulsions, sensory and chemical results from SOSDEs at ambient storage were compared to results from SOSDEs in accelerated

storage. Reviewing the sensory results (Fig 3.1 and Fig 3.3) first, earthy aroma was perceived by significantly ($p<0.001$) more panellists in SOSDE with gallic acid compared to SOSDE with EDTA across both storage conditions. The earthy aroma may be associated with the mere presence of gallic acid in the SOSDE rather than lipid oxidation. No literature on descriptive analysis of gallic acid could be found and therefor no association between earthy aroma and gallic acid could be made. Further descriptive research on gallic acid is needed to better understand its contribution to the aroma and flavour profile. There were no other parallels made between the high and low concentrations of the antioxidants EDTA and gallic aid in the ambient and accelerated storage conditions.

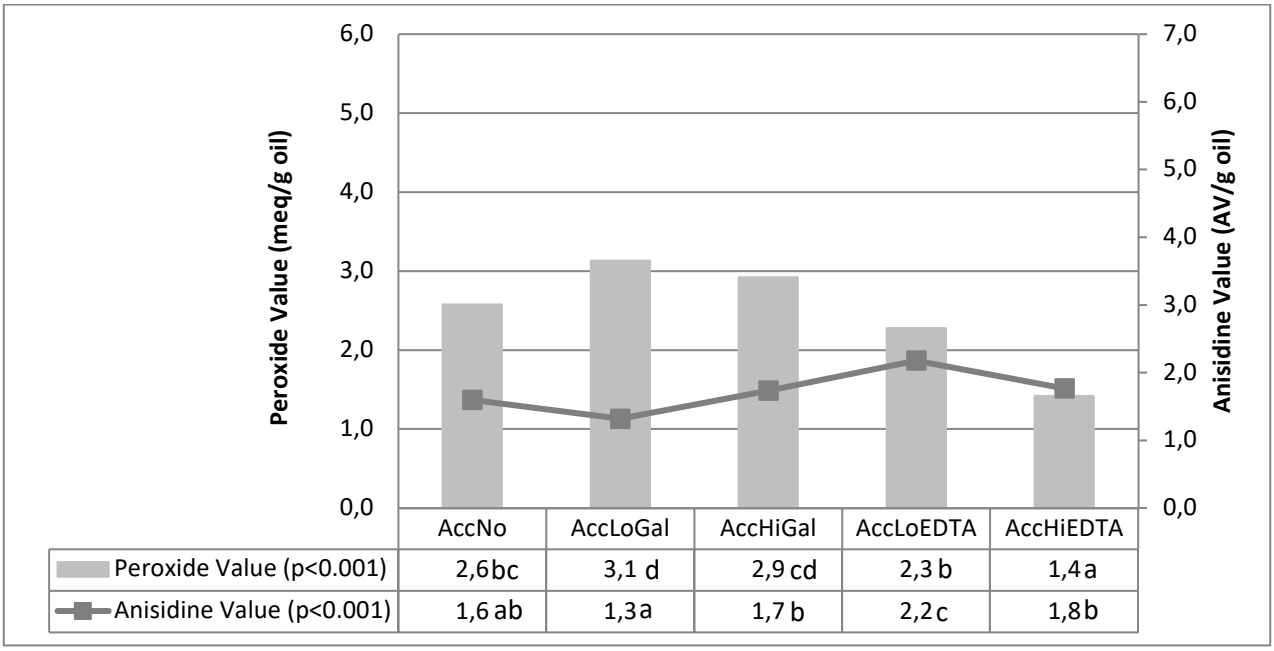
By comparison of the average results of chemical analyses for SOSDEs at ambient storage (Fig 3.2) with SOSDEs in accelerated storage (Fig 3.4), interesting findings were made. With pro-oxidants $FeSO_4$ and ascorbic acid added to SOSDEs it was expected that PV for SOSDEs in accelerated storage would be higher than PV for SOSDE in ambient storage (Branco *et al.*, 2011). However, the average PVs for SOSDEs from accelerated storage was lower 1.42 – 3.13 meq/g (Fig 3.4i), compared to ambient stored SOSDEs which ranged from 5.20 – 5.74 meq/g (Fig 3.2i). This was inconsistent with findings by Jayasinghe *et al.* (2013) who demonstrated that the presence of a transition metal, led to higher rates of hydro peroxides formed which is accelerated by the addition of ascorbic acid as it reduced the $Fe^{3}+$ (Jayasinghe *et al.*, 2013).

The reason for the contradictory findings is not clear but it is hypothesized that one day accelerated storage may not be equivalent to seven days storage at ambient conditions. The acceleration ratio of the accelerated storage to ambient storage may be less than 1:7 days. Changes in AV over time were inconsistent between accelerated and ambient stored SOSDEs. AccHiGal had higher AV compared to AccLoGal (Fig 3.4i), while AmbHiGal had lower AV than AmbLoGal (Fig 3.2i). The same was found for SOSDEs with EDTA, where AccHiEDTA had lower AV than AccLoEDTA (Fig 3.4i), with no differences between AmbHiEDTA and AmbLoEDTA SOSDEs (Fig 3.2i). This may be due to the fact that the SOSDEs in both storage systems were oxidised, but through different mechanisms due to the addition of pro-oxidants.

The transition metal iron, added as a pro-oxidant in the SOSDE samples, stored in accelerated conditions, chelated with the antioxidant EDTA to form FeEDTA (MacPhail *et al.,* 1994) that does not accelerate lipid oxidation. The presence of naturally occurring transition

metals was not measured in the ambient SOSDE samples. It is hypothesized that EDTA can only decrease lipid oxidation in the presence of transition metals and may be ineffective when used as an antioxidant in a product with no transition metal present.

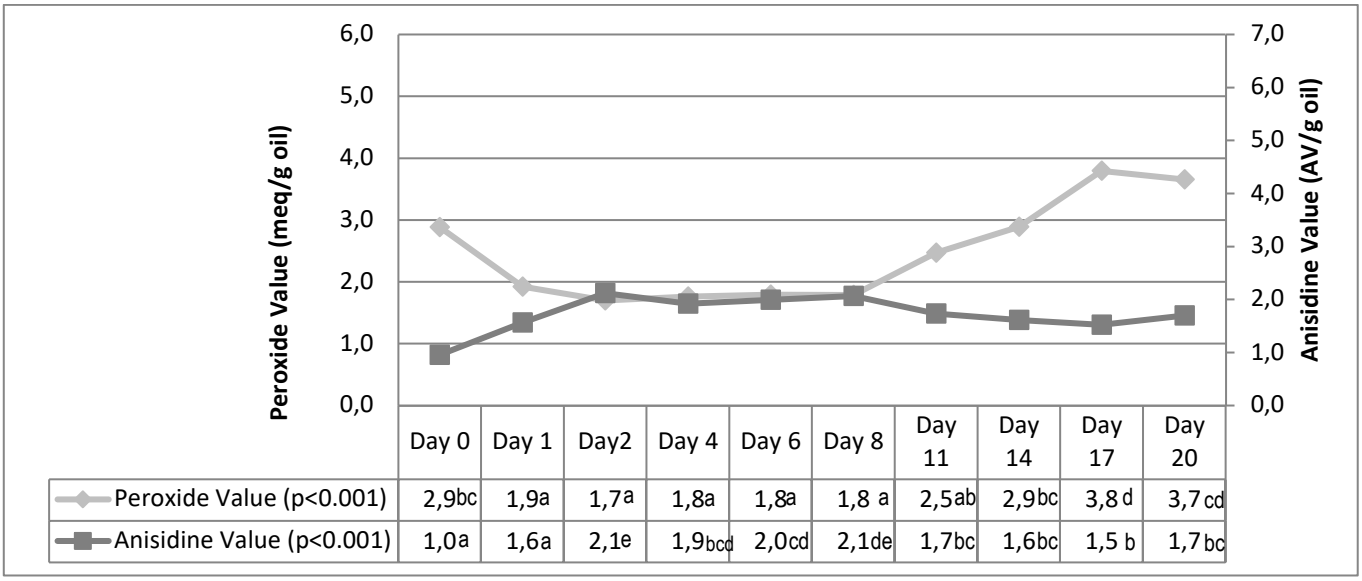
i)



	AccNo	AccLoGal	AccHiGal	AccLoEDTA	AccHiEDTA
Peroxide Value (p<0.001)	2,6 bc	3,1 d	2,9 cd	2,3 b	1,4 a
Anisidine Value (p<0.001)	1,6 ab	1,3 a	1,7 b	2,2 c	1,8 b

abcd - values among products with different letters indicate significant differences at 99% confidence level
*Standard error peroxide value: 0.1393 Standard error anisidine value: 0.07586

ii)



	Day 0	Day 1	Day2	Day 4	Day 6	Day 8	Day 11	Day 14	Day 17	Day 20
Peroxide Value (p<0.001)	2,9 bc	1,9 a	1,7 a	1,8 a	1,8 a	1,8 a	2,5 ab	2,9 bc	3,8 d	3,7 cd
Anisidine Value (p<0.001)	1,0 a	1,6 a	2,1 e	1,9 bcd	2,0 cd	2,1 de	1,7 bc	1,6 bc	1,5 b	1,7 bc

abcde - days in a row with different letters indicate significant differences at 99% confidence level
*Standard error peroxide value: 0.1983, Standard error anisidine value: 0.1073

Figure 3.4: Chemical analyses (PV and AV) for oil extracted from SOSDEs stored under accelerated shelf life conditions for 20 days illustrated for (i) average of different concentrations of EDTA and gallic acid for the storage period and (ii) average at time intervals over a 20 day time period.

3.4. CONCLUSIONS

Addition of EDTA to the SOSDE retarded lipid oxidation as measured by aroma and chemical analysis (PV and AV) compared to the SOSDE with no antioxidants added. Increased lipid oxidation retardation was noted for the high concentration of EDTA as assessed by chemical analysis during accelerated storage and the high concentration of gallic acid by AV analysis and rancid aroma detection during ambient shelf life. Sensory differences between products with high and low concentrations of antioxidants were more apparent during the ambient storage, while PV and AV differentiated samples more clearly stored under accelerated conditions. Limited parallels were made between the high and low concentrations of the antioxidants EDTA and gallic acid in the ambient and accelerated storage conditions which may be attributed to the acceleration ratio of accelerated storage:ambient storage of less than 1:7.

These results suggest that: 1) the accelerated storage model used in this study is more suitable for SOSDEs with metal chelator antioxidants such as EDTA, rather than free radical scavenging antioxidants such as gallic acid because EDTA retarded lipid oxidation as measured through PV and AV compared to SOSDE with no antioxidants added. 2) PV and AV are more sensitive oxidation markers than aroma analysis when using the accelerated storage model, while sensory analysis differentiated more between low and high concentrations antioxidants during ambient storage. 3) PV, AV and aroma of SOSDEs stored in accelerated conditions as used in this study, do not clearly predict results for ambient stored SOSDEs but can be used to screen the efficiency of antioxidants.

Further research focused on identifying the sensory attributes that could be used to predict end of shelf life of SOSDE would be very valuable.

4

CHAPTER 4: MULTIVARIATE ACCELERATED SHELF LIFE TEST OF SUNFLOWER OIL IN WATER EMULSIONS

4.1 INTRODUCTION

Accelerated shelf life tests have become increasingly important because of rapidly changing technologies, more elaborate food products, higher consumer demands for quality and the need for rapid product development (Labuza, 1982; Corrigan *et al.*, 2012). The information from accelerated shelf life tests has often been extrapolated to obtain shelf life estimates at lower, usual levels of the accelerating variables (Hough, Garitta and Gomez, 2006), by evaluating the reaction velocity profile (Pedro and Ferreira, 2006). Although the accelerated shelf life methods enable the calculation of suitable shelf life estimations (Corradini and Peleg, 2004; Derossi *et al.*, 2016) it cannot assure agreement between what is estimated and what is observed experimentally which may lead to significant discrepancies (Derossi *et al.*, 2016). Another concern in accelerated shelf life testing occurs when several variables are studied, for example a number of sensory characteristics, where it is not easy to state which attribute is most relevant for defining the expiry date of the product (Pedro and Ferreira, 2006).

Pedro and Ferreira (2006) introduced a new approach for determining shelf life by simultaneously considering many quality attributes, known as the multivariate accelerated shelf life testing (MASLT). MASLT has been used with tomato paste (Pedro and Ferreira, 2006), dried apple snacks (Saavedra *et al.*, 2013), UHT milk (Richards et al, 2014) and fresh

cut lettuce (Derossi *et al.*, 2016). MASLT is based on Principal Component Analysis (PCA), which calculates new axes in the multivariate space to improve the description of the experimental data structure. An important assumption in MASLT is that the degradation reactions are the main sources of variation in the data set, given that the PCA is driven by the time-related phenomena (Pedro and Ferreira, 2006). By using this method, the PC scores may be used to build a multivariate kinetics chart that reflects the degradation information of the studied attributes (Derossi *et al.*, 2016). The MASLT method has never been reported for salad dressing emulsions, where the quality is directly affected by lipid oxidation during storage.

The first part of the study (Chapter 3) focussed on the effects of high and low concentrations of antioxidants on oxidation markers in a sunflower oil salad dressing emulsion (SOSDE) stored at accelerated deterioration and ambient conditions through univariate analysis (analysing one aroma attribute at a time).

The second part of the study focused on the multivariate shelf life tool analysis (MASLT) of SOSDE data (objective 1). The fundamentals of the MASLT method has previously only been used on accelerated storage conditions, so it was interesting to determine how multivariate analysis could be used to analyse the SOSDE shelf life data from ambient storage conditions through multivariate analysis as proposed by Pedro and Ferreira (2006).

The second objective of this chapter, was to identify the sensory attributes that could be used as predictors for end of shelf life of SOSDEs during accelerated storage through MASLT and to compare data sets from the two shelf life conditions.

Reference will be made to results from univariate analysis in Chapter 3 to understand the context and the benefit of the additional research as analysed through MASLT.

4.2 MATERIALS AND METHODS

The products used are referred to as AmbNo, AmbLoGal, AmbHiGal, AmbLoEDTA, AmbHiEDTA, AccNo, AccLoGal, AccHiGal, AccLoEDTA, AccHiEDTA. The antioxidant that was added as well as the product name used for the specific level and type of antioxidant is listed in Table 4.1.

These products were stored under 2 different shelf life conditions: ambient shelf life (25°C) and accelerated shelf life (0.885 mmol/L $FeSO_4$, 1.70 mmol/L ascorbic acid, pH 5.5, 32.2°C). The terms “Amb” for ambient shelf life, and “Acc” for accelerated shelf life were added to the product names (Table 4.1) to identify the storage conditions of the specific products. For example, AccLoGal, contains 500 ppm Gallic acid and was stored under accelerated shelf life conditions (pH 5.5, 32.2°C, 0.885 mmol/L $FeSO_4$, 1.70 mmol/L ascorbic acid).

Table 4.1. Antioxidant types and levels added to the SOSDE.

Product name	Antioxidant added
No	None
LoGal	500 ppm Gallic Acid
HiGal	1000 ppm Gallic Acid
LoEDTA	37.5 ppm EDTA
HiEDTA	75 ppm EDTA

SOSDEs in ambient storage was stored for 24 weeks and samples were evaluated bi-weekly, while samples stored in accelerated conditions were stored for 20 days and samples were evaluated daily.

The shelf life model as proposed by Branco *et al*., (2011) with modification to timing intervals of analyses was used for the accelerated study component. The sensory data collected relates to the same materials and methodology that were described in section 3.2.

4.2.1 Statistical analyses

Statistical analysis was conducted using XLSTAT®. Repeated measure ANOVA was performed independently on the two data sets a) sensory profiling data of SOSDEs that were stored at ambient deterioration conditions and b) sensory profiling data of SOSDEs that were stored under accelerated deterioration conditions. Aroma attributes that showed significant ($p < 0.05$) changes over time for each of the five products in the two sets were determined. All the aroma attributes that showed time related significant changes for at least one SOSDE within a set (Ambient or Accelerated) were then included in a PCA to allow a simplified visual interpretation of the similarities and differences of SOSDEs. The PC1 scores obtained from the PCA were further used in the multivariate accelerated shelf-life test (MASLT) method for shelf-life assessment by plotting these values against time. MASLT is based on compressing the space spanned by the original variables (sensory attributes) via PCA and then using the scores as properties for further shelf-life assessment (Pedro and Ferreira, 2006; Richards *et al.*, 2014).

4.3 RESULTS AND DISCUSSION

4.3.1 Multivariate analysis of SOSDEs with different levels of antioxidants and stored under ambient conditions

The first step in the MASLT used involves identifying the aroma attributes that showed significant ($p < 0.05$) changes over time for each of the five products. The effects of no antioxidant and low and high concentrations of antioxidants on perception of aroma attributes of SOSDEs as measured every second week over 24 weeks ambient storage are shown in Table 4.2. AmbNo showed a significant difference ($p < 0.05$) in musty aroma over time. SOSDEs with gallic acid showed significant differences ($p < 0.05$) in six aroma attributes (pungency, citrus, green, vinegar, dairy and cardboard) over time and SOSDEs with EDTA showed significant differences ($p < 0.05$) in two aroma attributes (citrus and earthy) over time. The PCA (Fig 4.1 i) included the 9 aroma attributes that showed significant changes over time for at least one product. Eggy, oil, metallic, plastic, painty and rancid aromas did not present significant changes over time in any SOSDE and were excluded from the PCA. Oil and rancid aromas did not show significant differences over time in any of the SOSDEs (Table 4.2), however univariate analysis (Chapter 3) of oil aroma differentiated SOSDEs with low and high concentrations of EDTA and indicated oil aroma in AmbHiEDTA was detected by 10% fewer panellists compared to AmbLoEDTA. As explained in Chapter 3, the oil aroma attribute was not associated with rancidity during training sessions, but oil and fried oil are sensory terms often used to describe the formation of aldehydes associated with lipid oxidation (Hartvigsen

et al., 2000). Further to this, by univariate analysis, rancid was the only attribute that showed differences between SOSDEs with high and low concentration of antioxidants. This information relating to oil and rancidity as analysed in Chapter 3 through univariate analysis is not presented in the multivariate analysis as these attributes did not differentiate products over time.

Table 4.2: p-values from ANOVA to illustrate significant changes of sensory attributes over 24 weeks storage at ambient storage conditions.

	AmbNo	AmbLoGal	AmbHiGal	AmbLoEDTA	AmbHiEDTA
Pungency	0.09	<0.01	0.59	0.09	0.60
Vinegar	0.56	0.09	<0.01	0.14	0.87
Eggy/Sulphur	0.84	0.73	0.16	0.11	0.81
Citrus	0.10	<0.01	0.38	0.02	0.09
Musty	0.04	0.41	0.28	0.54	0.33
Dairy	0.19	0.66	0.01	0.58	0.39
Green	0.35	<0.01	0.47	0.26	0.22
Oil (fresh-like)	0.38	0.30	0.87	0.27	0.23
Earthy/Dirty	0.66	0.31	0.10	0.32	0.04
Metallic	0.79	0.06	0.42	0.88	0.94
Plastic	0.30	0.85	0.23	0.43	0.36
Cardboard	0.18	0.70	<0.01	0.10	0.25
Painty	0.84	0.06	0.08	0.09	0.17
Rancid	0.70	0.59	0.67	0.49	0.07

Product codes – see Table 3.1 in Chapter 3

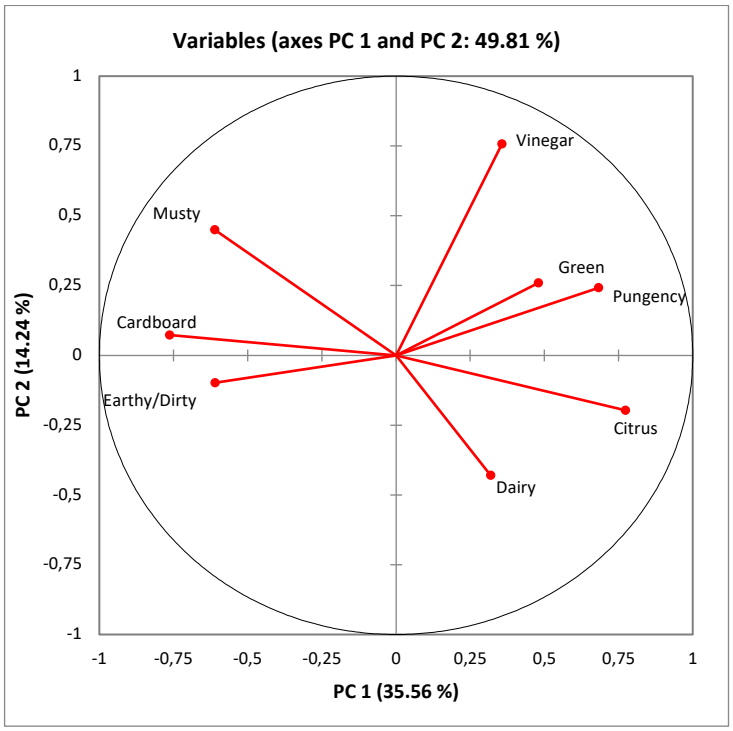
Shaded cells indicate significant differences at the 95% confidence level

In the second step of the MASLT, all aroma attributes that showed significant ($p<0.05$) differences over time for at least one treatment (Table 4.2) were included in a PCA. The PCA plot in Figure 4.1 summarizes the differences in aroma attributes over time and explains in one plot the differences and similarities between the SOSDEs with different levels of antioxidants stored at ambient storage over 24 weeks. In the PCA plot (Fig 4.1a), PC 1 explains 36% of the variation in the sensory aroma profiles of the SOSDE products. It separates SOSDEs in the early stages of ambient storage (weeks 0 – 8), which had more intense citrus, green and pungent aromas on the right of the plot. Results from univariate analysis (Chapter 3) indicated no significant difference present in pungent or green aromas between SOSDEs with high and low concentrations of either antioxidant. Results from univariate analysis explained differences in pungent and green aromas perceived between different treatments, while results from multivariate analysis explained differences apparent over time for the same aroma attributes.

Samples on the left side of the plot represented samples stored at ambient conditions for longer periods (weeks 9 – 24) with a higher intensity of cardboard and earthy aroma.

SOSDE AmbHiEDTA performed slightly different from other SOSDEs and even during later stages of ambient shelf life (weeks 18 - 24) AmbHiEDTA treatment is located on the right side of the plot and more intense in green, pungent and citrus aroma than in cardboard and earthy aromas. Although gas chromatogram olfaction (g.c.o) was not completed on these samples, the attributes of green, pungent and citrus aromas have been attributed to the volatile compounds hexanal, heptanal, octanal and nonanal (Malcolmson *et al.*, 1996). PC 2 separating samples at the top of the plot with more vinegar aroma from those at the bottom of the plot with more dairy aroma; explain an additional 14% of the sensory variation in SOSDEs stored at ambient deterioration conditions.

i)



ii

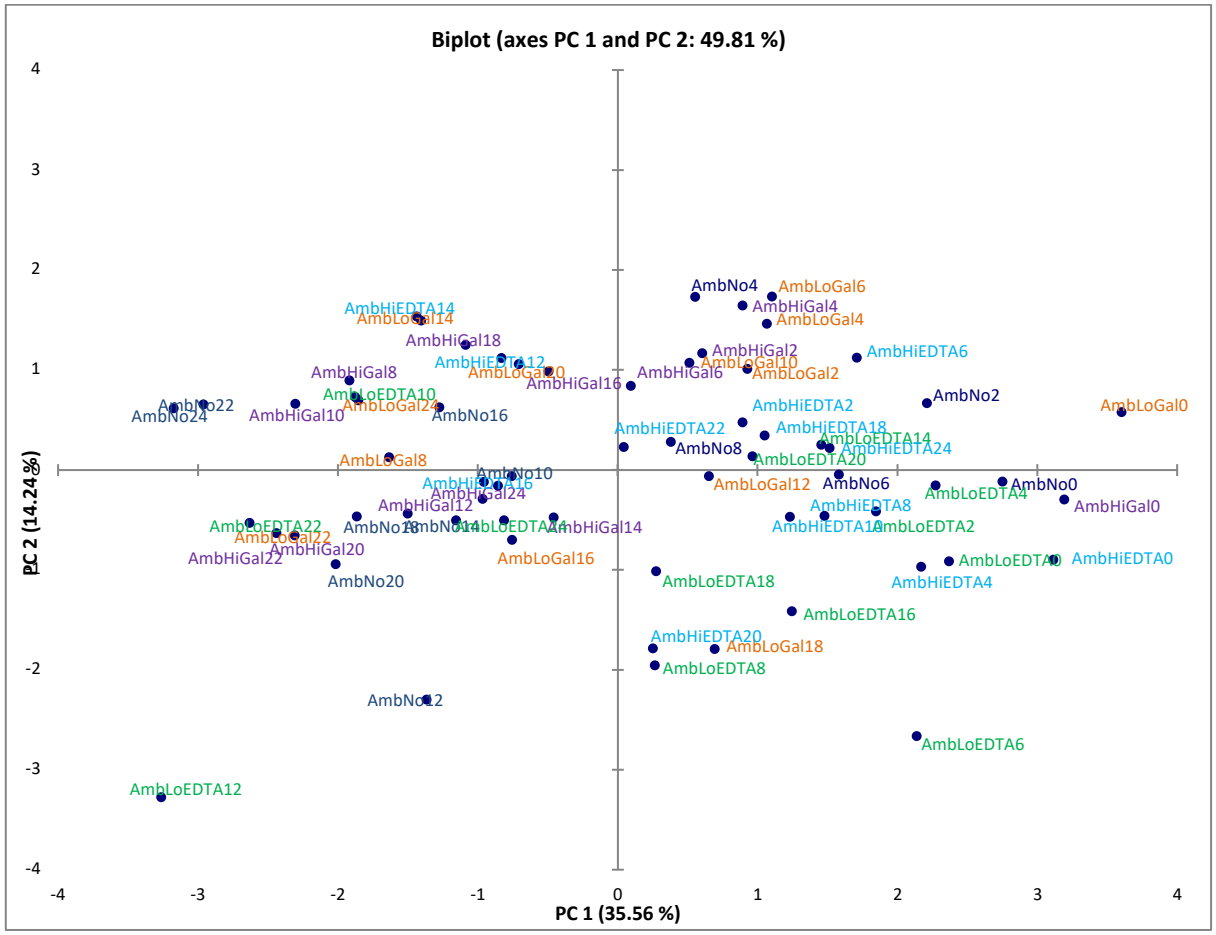


Figure 4.1: Principal component analysis of SOSDEs stored under ambient storage conditions for 24 weeks as evaluated by trained sensory panel: i) scores for sensory aroma attributes, ii) the correlation loadings describing the relationship among SOSDEs with different colours (different colours represent different treatments)

Product codes – see Table 3.1 in Chapter 3
Number in product code indicates number of weeks in ambient storage

The importance of the independent variables, also called the variable's power provides useful information on how well the principal components, model that specific variable (Derossi *et al.*, 2016). Fig 4.2 shows the power of independent variables from Figure 4.1i on the first PC (PC 1) and the PC model (PC 1 + PC 2). The power of each variable increased as the number of components considered increased. PC1 mainly modelled the pungent, citrus, green, cardboard and earthy aromas of the SOSDEs. The addition of PC2 in the PC model increased the power of the variables vinegar, musty and dairy. According to the power values of variables (Fig 4.2) the second plane mainly explains differences in vinegar, musty and dairy aromas of SOSDEs. PC 2 (Fig 4.1i) distinguishes the SOSDEs at the top with more vinegar and musty aromas, and those at the bottom with more dairy aroma. According to Derossi *et al.* (2016) attributes with power values below 0.4 indicate that they were not significant in the definition of the PC model and did not add useful information to explain the variance of the experimental data.

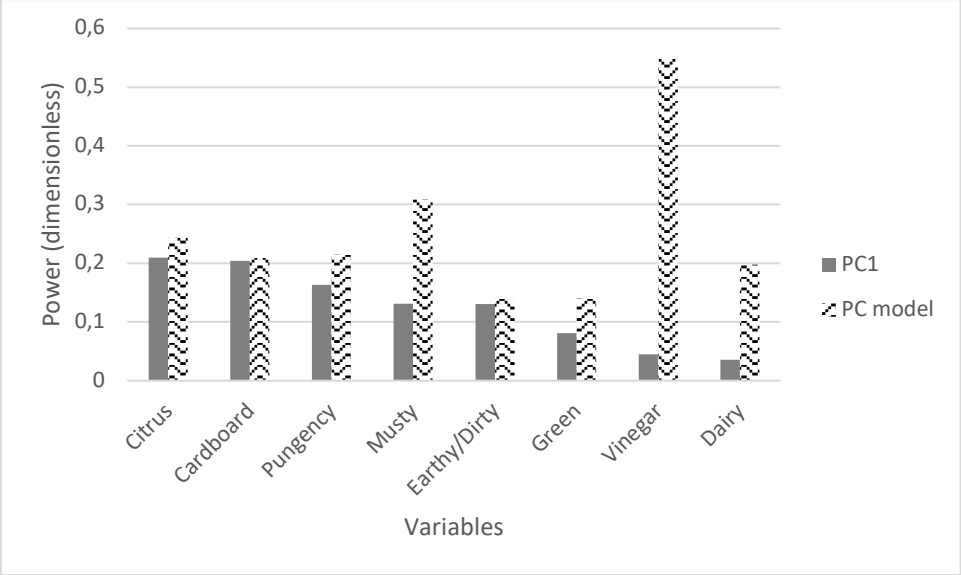


Figure 4.2: Power values of aroma variables on the PC model and on PC1 for SOSDEs stored under ambient storage conditions

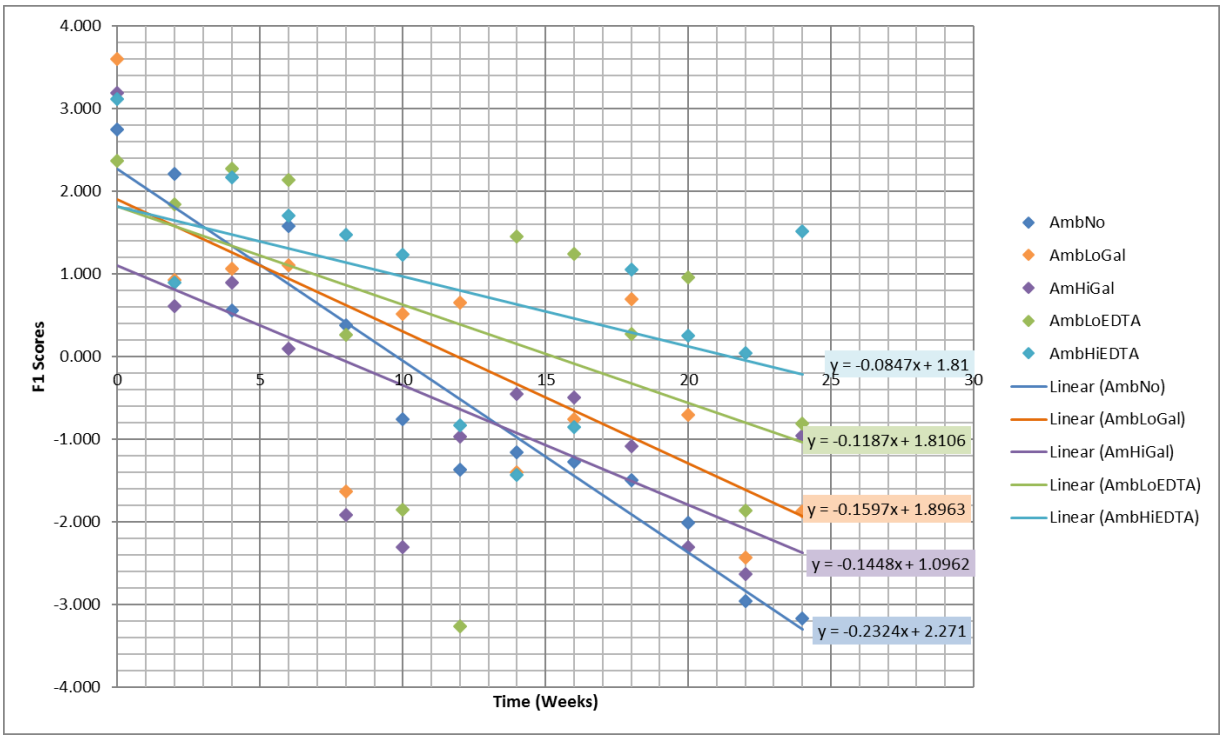


Figure 4.3: Multivariate kinetic chart and regression lines for SOSDEs with different concentrations of antioxidants stored at ambient conditions for 24 weeks.

Product codes – see Table 3.1 in Chapter 3

The PC1 scores obtained from the PCA were further used in the third step of the MSALT by plotting these values against time. Fig. 4.3 shows the Factor 1 scores for all the SOSDEs versus time plots. Linear regression lines were then fitted for each SOSDE. PC1 was time structured and all SOSDEs seemed to have changed from the positive side (associated with pungency and citrus aroma) to the negative side on PC1 (associated with cardboard and earthy aroma) over time. The coefficient of determination (R^2) in Table 4.3, was 0.9266 for AmbNo indicating that differences observed in AmbNo was mainly time related. This was expected as not antioxidants or pro-oxidants were present in this sample. R^2 for SOSDEs stored under accelerated storage ranged from 0.2003 to 0.6945 indicating that time is not the only factor affecting the changes observed in these products. The rate of change was also different among the SOSDEs. The rate constant (linear regression line equation from Fig 4.3) (Table 4.3) was the highest for AmbNo. AmbNo had no antioxidant retarding lipid oxidation and therefor it was expected that AmbNo would oxidise at a faster rate compared to the other products (Merril *et al.*, 2008; Rodríguez-Rojo *et al.*, 2012). Rate constant values were lower for high concentrations of the antioxidants compared to low concentrations indicating that higher concentrations of antioxidants slowed aroma changes of the SOSDEs more effectively. Furthermore, the rate constant was lower for SOSDEs with EDTA as antioxidant

compared to gallic acid as antioxidant, demonstrating the higher effectiveness of EDTA in retarding lipid oxidation. This is supported by results from Fig 4.1 that indicated AmbHiEDTA was on the right-hand side of the of the plot throughout ambient storage indicating higher green and pungent aroma and lower cardboard and earthy aroma, attributes typically associated with fresh non oxidised SOSDE.

Table 4.3: Multivariate parameters for SOSDEs stored under ambient conditions for 24 weeks and accelerated conditions for 20 days.

	Rate constants		Coefficient of determination (R^2)	
	Ambient storage	Accelerated storage	Ambient storage	Accelerated storage
No	0.2324	0.1030	0.9266	0.2003
LoGal	0.1597	0.2715	0.5716	0.6945
HiGal	0.1448	0.1793	0.4971	0.4054
LoEDTA	0.1187	0.1640	0.2581	0.4168
HiEDTA	0.0847	0.1914	0.2539	0.6058

Product codes – see Table 3.1 in Chapter 3

By comparison of results as analysed through univariate analysis (reported in part 3.2.4) and multivariate analysis (reported in 4.2.1) in Table 4.4, it is concluded that univariate analysis mainly described differences between products with different antioxidants and high and low levels of antioxidants, while multivariate analysis, mainly described differences over time. Attributes pungency, dairy, green and cardboard did not show differences between products when analysed through univariate analysis, however multivariate analysis of these attributes indicated differences over time. In addition, multivariate analysis indicated that pungency, citrus and green aromas were more intense during weeks 0-8, while earthy and cardboard were more intense during the later stages of shelf life (weeks 9-24).

Table 4.4: Summary of findings from univariate and multivariate statistical analysis for each aroma attribute used to evaluate SOSDEs with and without antioxidants stored under ambient conditions

Aroma attribute	Univariate analysis	Multivariate analysis
Pungency	No differences between low and high concentrations antioxidants	More intense during weeks 0-8
Vinegar	More noted for SOSDE with higher concentration EDTA	Highest power value from PC model
Eggy/Sulphur	Less noted in AmbNo and AmbHiGal	No differences in SOSDEs over time
Citrus	Less noted in AmbHiGal. No differences for SOSDE with EDTA	More intense during weeks 0-8
Musty	More noted in AmbLoGal	Difference over time in AmbNo
Dairy	No differences between products	Differences over time in AmbHiGal
Green	No differences between low and high concentrations antioxidants	More intense during weeks 0-8
Oil (fresh like)	Differentiated between AmbLoEDTA and AmbHiEDTA	No differences in SOSDEs over time
Earthy	Less noted in SOSDEs with EDTA	Higher intensity during weeks 9-24
Metallic	Less noted in SOSDEs with EDTA	No differences in SOSDEs over time
Plastic	No differences	No differences in SOSDEs over time
Cardboard	No differences	Higher intensity during weeks 9-24
Painty	No differences	No differences in SOSDEs over time
Rancid	Differentiated between high and low levels antioxidants in SOSDEs	No differences in SOSDEs over time

4.3.2 Multivariate analysis of SOSDEs with different levels of antioxidants and stored under accelerated shelf life conditions

The data on the effects of no antioxidant added and low and high concentrations of antioxidants on perception of aroma attributes of SOSDEs as measured daily over 20 days under accelerated storage are shown in Table 4.5. The first step in the MASLT used, involved identifying the aroma attributes that showed significant ($p < 0.05$) changes over time for each of the five products. All SOSDEs showed significant differences ($p < 0.05$) in vinegar, oil and cardboard aromas over time. The PCA included the 12 attributes that showed significant changes over time in at least one treatment. Musty and earthy did not present significant changes ($p > 0.05$) over time for any SOSDE and were excluded from further MASLT analysis.

Table 4.5: p-values from ANOVA to determine the effect of time of 20 days under accelerated storage on the sensory attributes of SOSDE stored in accelerated storage conditions.

	AccNo	AccLoGal	AccHiGal	AccLoEDTA	AccHiEDTA
Pungency	0.05	<0.01	0.05	0.02	0.09
Vinegar	0.03	<0.01	0.01	0.04	<0.01
Eggy/Sulphur	0.10	0.14	<0.01	0.33	0.73
Citrus	0.07	0.33	0.61	<0.01	<0.01
Musty	0.25	0.21	0.15	0.07	0.46
Dairy	0.14	0.06	<0.01	0.01	0.91
Green	<0.01	0.21	0.04	<0.01	0.25
Oil (fresh-like)	<0.01	<0.01	<0.01	<0.01	<0.01
Earthy/Dirty	0.45	0.33	0.44	0.27	0.21
Metallic	0.10	0.45	0.02	0.04	0.45
Plastic	0.02	0.86	0.42	0.46	0.79
Cardboard	<0.01	<0.01	<0.01	<0.01	<0.01
Painty	0.87	0.38	0.02	0.02	0.26
Rancid	0.38	0.17	<0.01	0.71	<0.01

Product codes – see Table 3.1 in Chapter 3
Shaded cells indicate significant differences at the 95% confidence level

Fig. 4.4 summarizes the sensory aroma differences of all the attributes that showed significant differences over time (according to Table 4.5) that were included in the second step of the MASLT. Cardboard aroma was the only rancidity related aroma attribute that showed statistically significant differences over the 20 day storage (p<0,01) for all 5 treatments. Other rancidity related attributes such as plastic aroma changed significantly over the storage time for the AccNo treated products only while painty aroma was statistically significant over time for the AccHiGal and AccLoEDTA and rancid aroma changed significantly over time in AccHiGal and AccHiEDTA treatments. In the PCA (Fig 4.4), PC1 explains 23% of the variation in the sensory profiles of the SOSDE products. As stated, the importance of the dependent variables (aroma attributes) are called the variable's power and provides useful information indicating how well the principal components model that specific variable (Derossi *et al.*, 2016). The power of variables increases with increasing number of components considered as more information is included in the analysis. Power values for the variables (aroma attributes) in Fig 4.4 are illustrated in Fig 4.5. Power values were used to determine which variables should be included in the multivariate analysis. Power values below 0.4 are not significant in the definition of the PC (Derossi *et al.*, 2016), while power values smaller than 0.2 are not included in the next step of the MASLT (Richards *et al.*, 2014).

Citrus, green, eggy and metallic had power values (PC1) smaller than 0.2 (Fig 4.5) and were therefore excluded from further multivariate analysis as recommended by Richards *et al.* (2014).

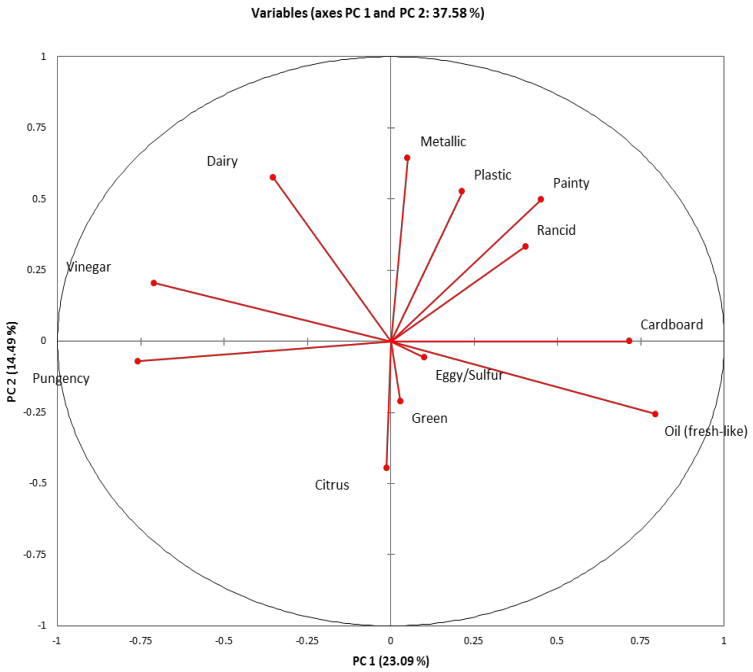


Figure 4.4: Principal component analysis of the 12 attributes that differed significantly over time in SOSDEs stored under accelerated storage conditions for 20 days as evaluated by a trained sensory panel

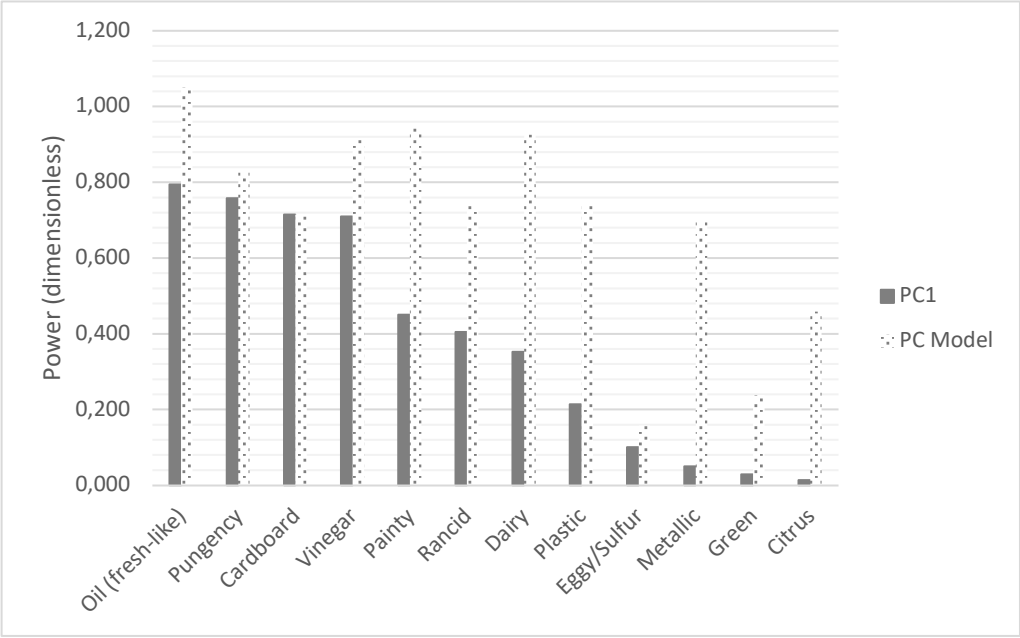


Figure 4.5: Power values of 12 variables that differed significantly over time on the PC1 and PC model (PC1 + PC2) for SOSDEs stored under accelerated storage conditions.

A new PCA (Fig 4.6) was performed which included attributes that contributed to PC1 (Fig.4.4 and Fig 4.5) and attributes eggy, metallic, green and citrus that had power values lower than 0.2 on PC1 (Fig 4.5) were excluded. In this second PCA (Fig 4.6 and Fig 4.7), PC

1 explained 39% of the variation in the sensory aroma profiles of the SOSDEs. It separated SOSDEs in the early stages of accelerated storage (day 0 - day 14), which had more intense pungent, vinegar and dairy aromas on the left of the plot, from SOSDEs on the right. The latter treatments stored under accelerated storage conditions for longer periods (day 15 – day 20) had higher intensities of cardboard, oil, rancid and painty aromas. This is similar to results from multivariate analysis of ambient stored SOSDEs where the PCA (Fig 4.1) separated SOSDEs in early stages of ambient shelf life with higher green, pungent and citrus aromas from SOSDEs stored at ambient for longer periods (weeks 9 - 24) with higher intensities of cardboard, musty and earthy aromas. Cardboard is the one attribute that was identified in samples during the later stages of both ambient and accelerated storage.

Attributes that contributed to PC1 were included in further MASLT analysis. Although PC2 explained an additional 19% of the sensory variation in SOSDEs stored at accelerated conditions, it didn't contribute significantly to the MASLT and will not be discussed in more detail.

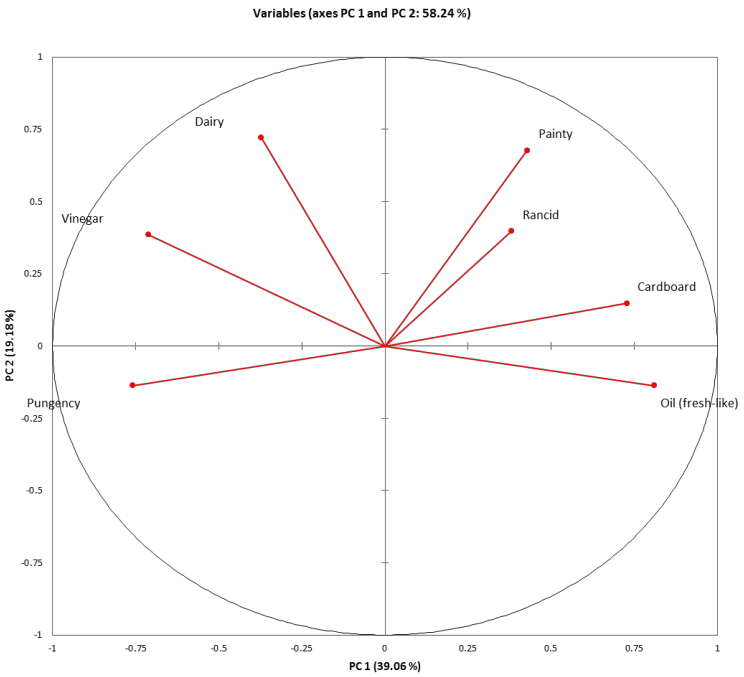


Figure 4.6: Principal component analysis of attributes (pungency, vinegar, dairy, painty, rancid, cardboard, oil) that showed significant difference over time and contributed to PC1 in SOSDEs stored under accelerated storage conditions for 20 days as evaluated by trained sensory panel.

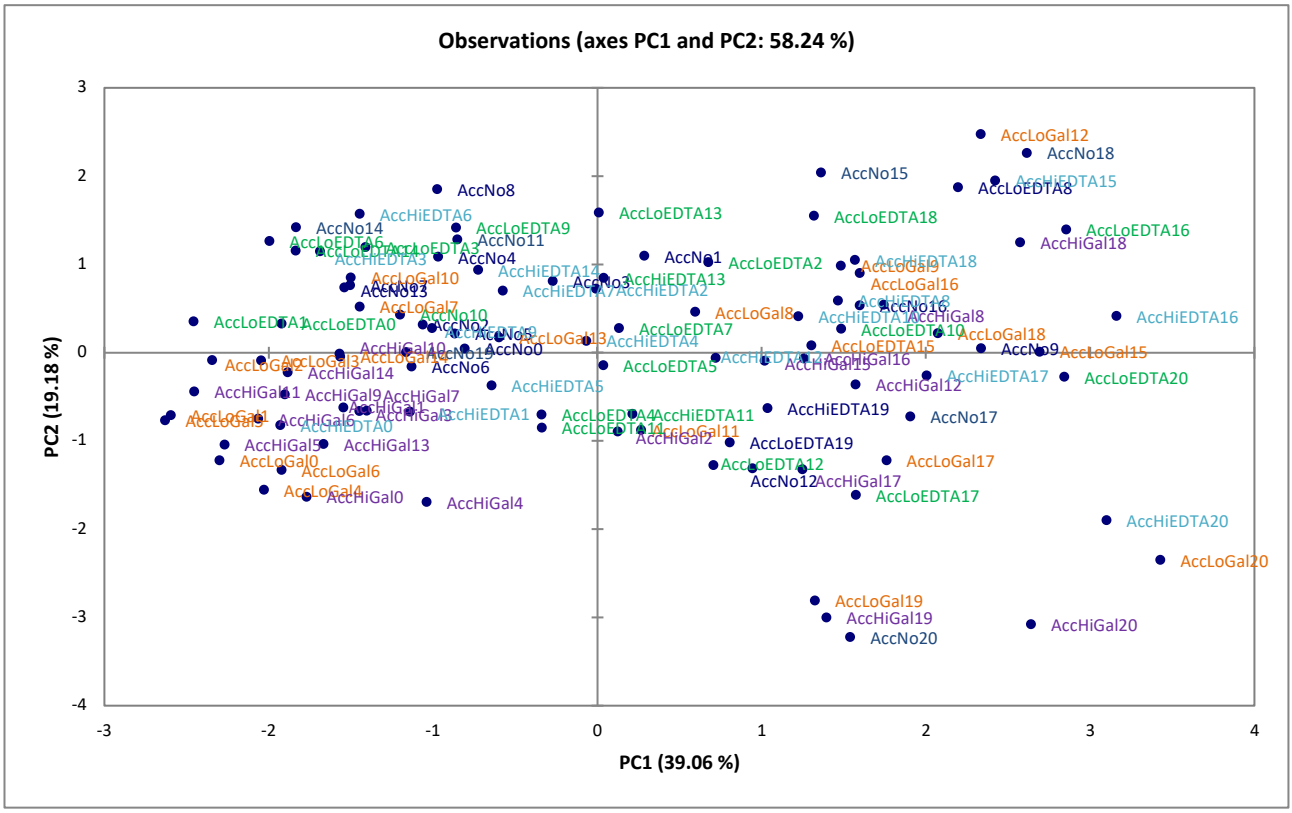


Figure 4.7: Correlation loadings for principal component analysis (Fig 4.5) of SOSDEs stored under accelerated storage conditions for 20 days as evaluated by trained sensory panel. (Different colours represent different SOSDE treatments)

Product codes – see Table 3.1 in Chapter 3
Number in product code indicates number of days in accelerated storage

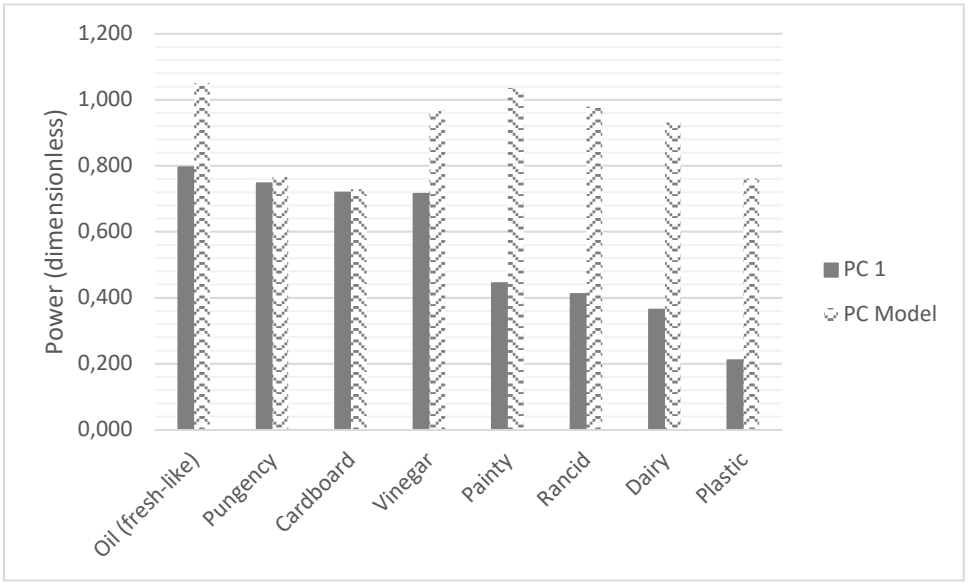


Figure 4.8: Power values of variables (pungency, vinegar, dairy, painty, rancid, cardboard, oil) that showed significant difference over time and contributed to PC1 (Fig 4.6), on PC1 and the PC model (PC1 +PC2) for SOSDEs stored under accelerated storage conditions.

Product codes – see Table 3.1 in Chapter 3
Number in product code indicates number of weeks in ambient storage

The power or importance of the independent variables on the first PC (PC1) and the PC model (PC1+PC2) is shown in Fig 4.8 and provides useful information on how well the principal components, model that specific variable (Derossi *et al.*, 2016). The power of each aroma attribute increased with the number of components considered. PC1 mainly modelled the pungent, vinegar, oil and cardboard aroma differences while PC2 modelled the variables dairy, painty and rancid aromas in SOSDEs distinguishing the SOSDEs at the top with more dairy and painty aromas. Scores for dairy, painty and rancid aromas influenced the PC model (PC1+PC2) with lowest projection on PC1 and, on the contrary, the highest influence on the positive axes of the PC2. According to the power values (Fig 4.8) the second plane of the PCA mainly explains differences in painty, rancid, dairy and plastic. Dairy and plastic aroma had power values lower than 0.4 on PC1 indicating that they were not significant in the definition of PC1 (Derossi *et al.*, 2016).

Fig. 4.9 shows following MASLT step, the PC1 scores versus time plots together with linear regression lines for SOSDEs stored in accelerated conditions. There are many independent variables involved in the commercial SOSDE that lead to wide variations and relatively lower degree of fit of the linear regression line. This was taken into account during the interpretation of the results. PC1 was time structured and all SOSDEs seemed to change from the negative side of the PCA (associated with attributes vinegar, pungency and dairy) to the positive side of the PCA (attributes cardboard, rancid, oily, painty) over time. However, the rate of change was different between SOSDEs. The rate constant (regression line equation from Fig 4.9) (Table 4.3) was the lowest for AccNo, the SOSDE with no antioxidant added stored at accelerated conditions. AccNo contains no antioxidant and was expected to change at a faster rate compared to SOSDEs with the addition of antioxidants, stored under accelerated condition (Merril *et al.*, 2008; Rodríguez-Rojo *et al.*, 2012) as it didn't contain any antioxidants to limit the lipid oxidation. Plastic aroma changed significantly over time in AccNo but this attribute was not included in the final steps of the MASLT due to its low contribution to PC1. Plastic aroma didn't change significantly over time in other treatments. While painty aroma that changed significantly over time in treatments AccHiGal and AccLoEDTA only were included in all the steps of MASLT and impacted the regression line. Upon interpretation of the multivariate kinetic chart it is important to also consider which attributes were included in the final step of the MASLT. The lower rate constant for AccNo may be due to the fact that plastic aroma, an aroma attribute that changed significantly over time in this treatment was not included while aromas painty and rancid that didn't show significant differences over time for this treatment were included.

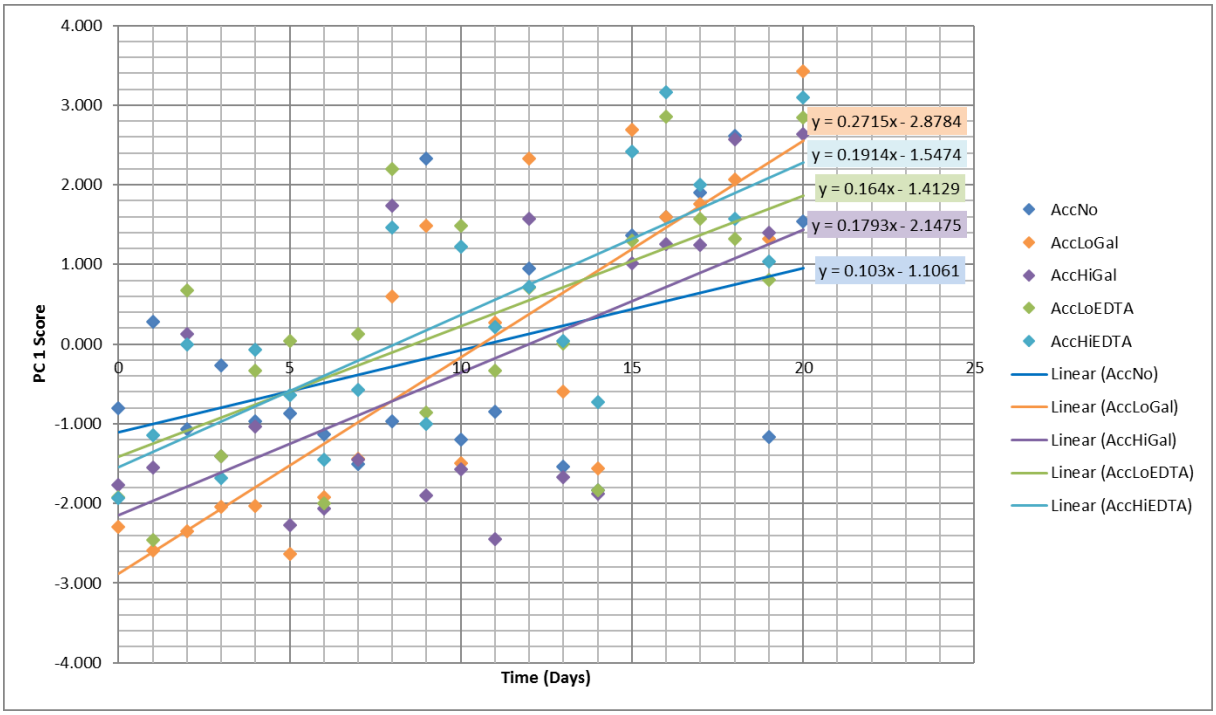


Figure 4.9: Multivariate kinetic chart of SOSDEs with different concentrations of antioxidants stored at accelerated conditions

Table 4.6: Summary of findings from univariate analysis and multivariate analysis for each attribute for SOSDEs stored under accelerated conditions

Aroma attribute	Univariate analysis	Multivariate analysis
Pungency	Differentiated SODEs with high and low levels gallic acid	Difference in AccNo, AccLoGal and AccLoEDTA over time. Associated with early stages of storage (day 0-day 14)
Vinegar	No differences among SOSDEs	Differences for all SOSDEs over time
Eggy/Sulphur	Less detected in SOSDE with gallic acid	Differences in AccHiGal over time but did not contribute to PC1 and therefor excluded for further analysis
Citrus	No differences among SOSDEs	Differences in AccHiGal over time but did not contribute to PC1 and therefor excluded for further analysis
Musty	No differences among SOSDEs	No differences in SOSDEs over time
Dairy	Less detected in SOSDE with gallic acid	Differences in AccHiGal and AccLoEDTA; power value lower than 0.4 on PC1
Green	More detected in SOSDE with gallic acid	Differences in AccHiGal over time but did not contribute to PC1 and therefor excluded for further analysis
Oil (fresh like)	No differences among SOSDEs	Differences for all SOSDEs over time; Differentiate between products stored more than 15 days.
Earthy	More detected in SOSDE with gallic acid	No differences in SOSDEs over time
Metallic	More detected in SOSDE with no antioxidant	Differences in AccHiGal over time but did not contribute to PC1 and therefor excluded for further analysis
Plastic	Perceived by less than 5% panellists	Difference in AccNo over time; Power value lower than 0.4 for PC1
Cardboard	More noted in SOSDE with EDTA	Differences for all SOSDEs over time. Differentiate between products stored more than 15 days.
Painty	No differences among SOSDEs	Differences in AccHiGal and AccLoEDTA over time.
Rancid	No differences among SOSDEs	Differences in AccHiGal and AccHiEDTA over time; Differentiate between products stored more than 15 days.

Table 4.6 compares results for each attribute as analysed through univariate analysis (given in section 3.2.4) and multivariate analysis (given in section 4.2.1 in Table 4.4). Univariate analysis mainly describes the differences between SOSDEs with different antioxidants and high and low levels of antioxidants, while multivariate analysis mainly describes differences of these attributes in a specific SOSDE treatment over time. Univariate analysis differentiated SOSDEs with high and low levels of gallic acid based on pungency aroma, while multivariate analysis indicated pungency aroma differences over time in AccNo, AccLoGal and AccHiGal. Based on the multivariate analysis, it was clear that pungency aroma was associated with the early stages of storage. There were no vinegar aroma differences among SOSDEs (univariate analysis), while multivariate analysis indicated differences in vinegar aroma in all five SOSDEs over time. Musty aroma didn't show significant differences between SOSDEs (univariate) or over the 20 days storage in any SOSDEs (multivariate)

indicating that musty aroma was not a differentiating aroma in the analysis of these SOSDE treatments. Oil aroma didn't differentiate among SOSDEs (univariate) but showed significant differences overtime in all five SOSDE treatments (multivariate) and furthermore differentiated between products stored more than 15 days. Earthy aroma was more detected in SOSDE with gallic acid (univariate), but didn't change significantly over time in any SOSDE (multivariate). Cardboard aroma was more noted in SOSDEs with EDTA (univariate) while rancid aroma didn't show significant difference among SODEs (univariate). Both cardboard and rancid aroma differentiated between products stored more than 15 days. It is clear univariate analysis provides important information upon comparison of the five SOSDEs while multivariate provided additional information on aroma changes over time and differentiated between products in early and later stages of storage.

Findings from multivariate analysis of SOSDEs in both ambient and accelerated storage are summarised in Table 4.7 to determine attributes that can be used as predictors for end of shelf life in both ambient and accelerated storage. Similarities and differences were identified and attributes eggy, musty, dairy, metallic, and plastic did not show differences and/or had power values lower than 0.4 for both ambient and accelerated storage. These attributes contributed to univariate analyses, but it was concluded that they were not predictors of end of shelf life as these aroma attributes did not change over time.

Table 4.7: Summary of findings from multivariate analysis for each attribute for SOSDEs stored under ambient and accelerated conditions

Aroma attribute	Ambient deterioration storage	Accelerated deterioration storage
Pungency	More often detected during weeks 0-8	Difference in AccNo, AccLoGal and AccLoEDTA over time. Associated with early stages of storage (day 0-day 14)
Vinegar	Highest power value for PC model, but did not contribute to PC1	Differences for all SOSDEs over time. Associated with early stages of storage (day 0-day 14)
Eggy/Sulphur	No differences in SOSDEs over time	Differences in AccHiGal over time but did not contribute to PC1 and therefor excluded for further analysis
Citrus	More often detected during weeks 0-8	Differences in AccHiGal over time but did not contribute to PC1 and therefor excluded for further analysis
Musty	Difference over time in AmbNo	No differences in SOSDEs over time
Dairy	Differences over time in AmbHiGal	Differences in AccHiGal and AccLoEDTA; power value lower than 0.4 on PC1
Green	More often detected during weeks 0-8	Differences in AccHiGal over time but did not contribute to PC1 and therefor excluded for further analysis
Oil (fresh like)	No differences in SOSDEs over time	Differences for all SOSDEs over time; Differentiate between products stored more than 15 days.
Earthy	Higher intensity during weeks 9-24	No differences in SOSDEs over time
Metallic	No differences in SOSDEs over time	Differences in AccHiGal over time but did not contribute to PC1 and therefor excluded for further analysis
Plastic	No differences in SOSDEs over time	Difference in AccNo over time; Power value lower than 0.4 for PC1
Cardboard	More often detected during weeks 9-24	Differences for all SOSDEs over time. Differentiate between products stored more than 15 days.
Painty	No differences in SOSDEs over time	Differences in AccHiGal and AccLoEDTA over time.
Rancid	No differences in SOSDEs over time	Differences in AccHiGal and AccHiEDTA over time; Differentiate between products stored more than 15 days.

Pungent aroma was associated with early stage of ambient storage (weeks 0-8) and accelerated storage (days 0-15) and cardboard aroma was associated with later stages of ambient storage (weeks 9-24) and accelerated storage (more than 15 days). Cardboard as a predictor of shelf life for oil-in-water emulsions was unexpected as Bou *et al.*, (2001) recommended to use overall rancidity as measurement rather than specific descriptive attributes. It is important to keep in mind that aroma attributes as shelf life predictors for oil-in-water emulsion is dependent on oil volume. Tamaru *et al.*, (2018) studied the released flavour concentration of lipophilic and hydrophilic flavour compounds in oil-in-water emulsion and

found that the oil volume in the oil-in-water emulsion affected the release concentration in the head space of oil in water emulsion systems.

4.4. CONCLUSIONS

Univariate analysis mainly describes the differences of aroma attributes among SOSDEs with different antioxidants and high and low levels of antioxidants, while multivariate analysis mainly describes differences of these attributes over time. The PC model yielded more information on the relationship between the independent variables, as well as their individual contribution to the shelf life of SOSDEs. Attributes citrus and musty did not show difference among SOSDEs stored under accelerated conditions when analysed through univariate analysis and didn't change over time in SOSDEs or didn't contribute to PC1 in MASLT, but both these aroma attributes showed differences among products (univariate analysis) and over time (multivariate analysis) during ambient storage. No differences were noted in plastic aroma among SOSDEs stored in ambient condition when analysed through univariate and multivariate analysis, but differences in plastic aroma were noted over time in AccNo stored in accelerated conditions. The map of aroma attributes on the PC1-PC2 plane permitted the identification of aroma attributes painty and rancid, as well as vinegar and pungency that were correlated. Carboard aroma was the only aroma attribute in the ambient storage PCA map and the accelerated PCA map associated with lipid oxidation and rancidity indicating the importance of including cardboard in the lexicon of shelf life studies on emulsions.

Addition of EDTA or gallic acid to the SOSDE stored in ambient conditions, reduced the rate constant of lipid oxidation compared to the SOSDE stored in ambient conditions with no antioxidants added. Furthermore, the higher concentration of both antioxidants had a greater lipid oxidation retardation effect in ambient storage conditions compared to the SOSDE with low concentrations antioxidant. Addition of EDTA to the SOSDEs stored in ambient conditions retarded lipid oxidation more effectively compared to SOSDE with gallic acid. All treatments with added antioxidants had a higher rate constant in accelerated storage compared to ambient storage. AccNo had a lower rate constant than AmbNo and a lower rate constant than all SOSDEs with antioxidants stored in accelerated storage conditions possibly due to the exclusions of plastic aroma, an aroma attribute that changed significantly over time in AccNo, in the multivariate analysis. Although accelerated storage does not predict ambient

storage it is hypothesized that the acceleration ratio of the accelerated storage to ambient storage may be less than 1:7 days.

Shelf life studies require the identification of an attribute that has the highest impact on the quality of the product, or shows the most change over the shortest time period (Hough *et al.*, 2003; Curia and Hough, 2009). Pungent aroma was associated with the early stage of ambient storage (weeks 0-8) and accelerated storage (days 0-15) and cardboard aroma was associated with later stages of ambient storage (weeks 9-24) and accelerated storage (more than 15 days) and it has been concluded that pungent aroma and cardboard aroma are the most probable predictors of shelf life in SOSDEs.

5

CHAPTER 5: *GENERAL DISCUSSION*

The general discussion critically examines the experimental methodologies used in the accelerated shelf life studies of SOSDEs and discusses some future considerations when using these methodologies. The sensory and chemical analysis of SOSDEs stored under ambient and accelerated storage conditions will be considered based on the findings and the application of the results. A critical evaluation of the MASLT of SOSDE used in the current study and the commercial potential of MASLT will be provided and recommendations for future studies on shelf life studies of oil-in-water emulsion products and analyses by MASLT will finally be discussed.

5.1 SHELF LIFE METHODOLOGIES

During this study the sensory and chemical changes that occurred in SOSDEs with different antioxidants, at low and high levels, during storage at ambient and accelerated shelf life conditions (exposed to a combination of oxidation acceleration factors), as evaluated by a trained sensory panel, and chemical analysis, were used in the multivariate accelerated shelf life test (MASLT) to identify the key sensory attributes, associated with the development of lipid oxidation and rancidity to gain a better understanding of how antioxidant levels influence oxidation of commercial sunflower oil salad dressing emulsions, when exposed to a combination of oxidation acceleration factors.

Consumers demand high quality shelf stable products with a shelf life of several months. In order to meet consumers‘ expectation, the need exists for food industries to conduct shelf life studies that often include the assessment of an array of analytical and sensory properties. Accelerated studies are often used for products with a longer shelf life. With accelerated shelf life tests the product is stored in relatively severe storage conditions and may be altered to include acceleration factors that will increase the rate of deterioration and therefore shorten the shelf life of the product. There are however pitfalls associated with accelerated shelf life tests.

5.1.1 Pitfalls associated with accelerated shelf life tests

- *Masked rejection mode*

Different sensory attributes develop at different rates during normal and elevated temperatures, due to different activation energies. Critical attributes at room temperature can therefore be masked at the accelerated condition, which can lead to over or under estimation of the shelf life. The impact of the acceleration factors and their deterioration mechanisms should be considered and compared to those that would occur under normal storage conditions to ensure the applicability of the accelerated tests. The following steps were taken to reduce this risk, as described by Meeker and Escobar (1998).

- *Simultaneous interaction of acceleration factors*

Lipid oxidation is a dynamic process with multiple factors interacting continuously. The accelerated shelf life model used, included different factors that simultaneously interact and affect the stability of an oil-in-water emulsion. The factors or potential pro-oxidants included in the accelerated deterioration in this research were 1.70 mmol/L AH (ascorbic acid), and 0.885 mmol/L $FeSO_4$. The pH of the products was adjusted to 5.5 as proposed by Branco *et al*. (2011) as this pH level was found to have the most significant influence on the formation of lipid oxidation products. An increase in pH from 3.0 to 7.0 reduces the formation of hydroperoxides, while a pH lower than 5 initiates the release of iron (if present) in the product which in turn decomposes pre-existing lipid hydroperoxides located at the oil-water interface in the emulsion (Thomsen *et al.*, 2000).

- *Commercial oil-in-water emulsions*

More often than not, model systems are used in shelf life studies of oil-in-water emulsions, where the oil used in the emulsion preparation is stripped of its minor components (Branco *et al.*, 2011), the latter materials may affect lipid oxidation (Chaiyasit *et al.*, 2007). Sunflower oil is rich in linoleic acid and the commercial sunflower oil used in this research was not stripped of its minor components before using it in the emulsion. Sunflower oil is reported to contain naturally occurring antioxidants tocopherols and tocotrienols. The chemical composition of the sunflower oil used in this study was not determined and it is recommended to analyse the chemical composition of sunflower oil in future studies to determine and measure the presence of any antioxidants and/or pro-oxidants such as water or metal ions. Alternatively stripping the sunflower oil of its minor components could have been an option, however this was not done in the current study because the emulsion used was to be as representative of a commercial salad dressing emulsion product as possible in order for the results and learnings to be directly transferable to commercial emulsions produced in the food industry.

Apart from sunflower oil and water, the product in the current study contained other ingredients; vinegar, mustard, black pepper, salt and egg yolk typically found in a commercial salad dressing oil-in-water emulsion. These ingredients were added for flavour and appearance purposes and may have affected the rate of lipid oxidation. For example, strong antioxidant activity has been reported for egg yolk in emulsions (Yamamoto *et al.*, 1990). Research has also been conducted on herbs and spices as natural antioxidants and results indicated varied levels of antioxidant activity of different herbs and spices in different products. For example, clove was more effective as an antioxidant in oil-in-water emulsions while rosemary and sage were the most effective in lard (Yanishlieva *et al.*, 2006). Similarly, black pepper has been shown to have antioxidant activity (Nakatani *et al.*, 1986). Therefore, it is possible that the black pepper added to the oil-in-water emulsion in the current study may have had an antioxidant effect retarding lipid oxidation as measured in the current study. These effects of ingredients on lipid oxidation in the SOSDE used in the current study are complex and add more variability when compared to research using a model emulsion, however, it also emphasizes the benefits of using a commercial product formulation in accelerated shelf life studies as it is more representative of what happens to products in the consumer market.

To understand the effect of masked rejection, it is important to include some means of validating the findings of results obtained from accelerated tests. In the current study, each SOSDE exposed to accelerated shelf life conditions was also evaluated at ambient storage without the addition of any potential pro-oxidative factors. This enabled the comparison between ambient and accelerated storage condition for each treatment. A sample with no antioxidants added was also included in the sample design of the ambient and accelerated shelf life storage conditions. SOSDE with no antioxidants and no potential pro-oxidants (AmbNo) had a PV of 5.3 meq/g oil and an AV of 5.9 AV/g oil after 24 weeks compared to the SOSDE with no antioxidant but added potential pro-oxidants (AccNo) that had a PV of 2.6 meq/g oil and AV of 1.6 AV/g oil after only 20 days. Considering these results, it is hypothesized that the lower PV and AV is attributed to the sampling times of the accelerated shelf life study and increasing the storage time of the accelerated shelf life to 6 weeks or 42 days may result in better correlation between the results for SOSDEs stored under ambient and accelerated shelf life conditions.

- *Multiple deterioration modes*

Accelerated storage can induce changes in the food product, and different mechanisms of deterioration that would not occur under normal storage conditions. The mechanism of lipid oxidation in a particular food depends on the nature of the reactive species present in the foods as well as the physicochemical environment consisting of temperature, humidity and pH (Coupland & McClements, 1996). The breakdown of hydroperoxides is increased by increased temperatures (Waraho *et al.*, 2011) and increased temperatures may affect the stability of the antioxidant, which would not occur under ambient temperature storage. The SOSDEs stored in accelerated shelf life conditions included different factors that simultaneously affected the lipid oxidation and enlarged the variation range of lipid oxidation (Branco *et al.,* 2011) as measured through PV, AV and sensory analysis. It is therefore important to include some means of validating the findings of results obtained from accelerated tests. In the current study, samples stored at ambient condition without any acceleration factors were included to compare with the results obtained from the accelerated study.

The rate constant of change in aroma, as calculated in Section 4.1, was 0.2324 for AmbNo compared to the rate constant of 0.1030 for AccNo indicating a decreased change in aroma attribute production measured by MASLT in accelerated shelf life storage. A possible

theory for this finding may be attributed to storage time, storage temperature, sensory method and analysis methods used, which is further discussed in section 5.1.2 below. The exact rate of acceleration in comparison to ambient storage conditions could not be determined in this study due to a lack of correlating time intervals between the accelerated and ambient stored SOSDEs. During the initial phases of this research, an accelerated study was completed with hourly measurements as proposed in the research by Branco *et al. (*2011). Although these time intervals correlated with ambient storage (daily in accelerated storage and weekly in ambient storage), no differences were noted in the sensory or chemical analysis of the SOSDEs. The time intervals were adjusted to be measured over a longer period of time in order to detect differences in the lipid oxidation markers, but this affected the correlation of time intervals.

Even with this adjustment of time intervals to daily for 20 days, it appears that the degree of lipid oxidation is still not as high as reported in the study completed by Branco *et al.* (2008). The lack of correlating time intervals between accelerated and ambient storage and the lower lipid oxidation levels as measured through PV and AV is a limitation in this research that should be addressed in future research to enable correlation between accelerated and ambient storage.

- *Rejection and degradation affected by unforeseen variables*

Actual factors such as ingredient distribution and storage conditions may be more complicated than a simple relationship between the shelf life of a product and the accelerated variables. Oil-in-water emulsions consist of three regions with different solubility of the molecules. Distribution of ingredients as well as pro-oxidants and antioxidants in these phases will affect the lipid oxidation in accelerated shelf life studies. Steps were taken in the production of the SOSDEs for this research to keep the process as identical to commercial production as possible. In studies completed by Richards and Golding (2011) on lipid oxidation in an oil-in-water emulsion, the researchers analysed the droplet size of the emulsions after 5 passes through homogenization and still saw small differences in droplet sizes. Droplet size impacts the perceived sensory characteristics and hedonics (Let *et al*., 2016). Analysing the molecular environment, particle size and specific orientation of lipid molecules and other ingredients at the interface layer will therefore be beneficial as this has a significant effect on the chemical reactivity (Chaiyasit *et al*., 2007) and sensory perception of the product (Lett *et al*., 2016).

The 20 ml head space in the glass containers of the ambient and accelerated stored samples in the current study probably affected the rate and extent of lipid oxidation, as it increased the oxygen interaction on the surface. According to Hamilton (1999) oxygen is required in the initiation and propagation phases of oxidation of oil. Therefore, the larger head space of the stored samples in the current study (20 ml) contained more oxygen that could have promoted oxidation. The head space in the current study is similar to headspace found in commercial salad dressing emulsion products.

Antioxidant are used in very low quantities and doesn't contribute to sensory qualities of the product, therefor the aroma attributes associated with the specific antioxidant gallic acid and EDTA used were not determined in this research (Coppen, 1999; Elias *et al.*, 2008).. Therefore, it is not possible to analyse the aroma differences associated with the addition of any specific antioxidant. In order to accurately identify aroma attributes as predictors for lipid oxidation in commercial oil-in-water emulsions, it is recommended to complete descriptive analysis to understand the aroma attributes associated with each antioxidant individually.

5.1.2 Sensory evaluation

Sensory evaluation has been described as the preferable method for the measurement of rancidity (Rossel, 1999). The frozen samples used in the current study were removed from the freezer 4 hours prior to sensory analysis. Samples reached an internal temperature of 22-24°C before they were presented to the trained panel for sensory analysis. Research from the 1970's concluded maximal taste intensity of various food products are observed when served at room temperature (Pangborn *et al.*, 1970; McBurney *et al.*, 1973; Moskowitz, 1973). More recent research by Engelen *et al.* (2003) on the effects of product temperatures (10, 22 and 35°C) of oil-in-water emulsions (mayonnaise) found that product temperature influenced perceived flavour intensities, and higher product temperatures increased flavour intensity. Although the current study only included aroma evaluation, the samples were evaluated between 22-24°C. It is possible that the intensities of sensory attributes would increase even more when evaluated at 35°C. Research by Engelen *et al*. (2003) on effect of temperature on mayonnaise indicates that aroma attributes increased with increasing temperatures, possibly because at higher temperatures the aroma compounds became more volatile, resulting in a higher concentration of these compounds reaching the receptors in the nose. Similar results with increased aroma-related volatiles with increased temperatures were found in fruit (Dixon and Hewett, 2000; Zhang *et al.*, 2011) and cheese (Rehman *et al.*, 2000). The effect of serving temperature was not investigated in the current study as the product studied was stored in the fridge (4-10°C) after opening and would therefore be more often consumed at lower

temperatures, however the SOSDE may be used in warm applications and therefore it is recommended that evaluation temperature of SOSDE be researched in a future study.

Most sensory studies conducted on lipid oxidation of oils and emulsions included the evaluation of flavour and aroma attributes (Malcolmson *et al.*, 1996; Bou *et al.*, 2001; Chale-Rush *et al.*, 2007). Aroma descriptors associated with volatile secondary lipid oxidation products were listed in Chapter 2 and included: painty, fatty, soapy, metallic, green, apple like and nutty to name a few. Other attributes used to describe the degradation of free fatty acids (FFA) are astringent and burning (Chale-Rush *et al.*, 2007). These attributes refer to mouthfeel and are perceived during the consumption and flavour evaluation of a product. These attributes would not be detected through orthonasal aroma evaluation only. This emphasized the importance of evaluating aroma, flavour and mouthfeel of oxidised products.

However, due to potential health risks such as cell damage, inflammation and liver hypertrophy (see discussion in section 2.4 Chapter 2) associated with the consumption of indigestible oxidised food products such as lipid peroxides, hydroxy FAs, carbonyl compounds, cyclic monomers, malonaldehyde, polycyclic aromatic compounds and oxidised sterols (Sanders, 1999; Chen *et al.*, 2011) the panel evaluated orthonasal aroma only and did not taste or consume the SOSDEs. The sensory evaluation was conducted by identification of presence or absence of specific aroma qualities in a sample. This method was an adaptation of the method referred to as “Check All That Apply” methodology (CATA) and there have been a number of research studies evaluating CATA as a profiling method (Dooley *et al.*, 2010; Meyners *et al.*, 2013; Reinbach *et al.*, 2014; Ares *et al.*, 2015). CATA is arguably not the best methodology to use when only subtle changes are present as it does not measure attribute intensity (Ares *et al.*, 2015), however due to the complexity of the SOSDE and the low intensities associated with rancidity related aroma attributes during the onset of lipid oxidation, indicating the presence or absence of an aroma attribute was already challenging and required significant training for the approach used. Significant differences were reported for some of the aroma attributes for SOSDEs stored in accelerated and ambient storage conditions as discussed in Chapter 3. Results from Chapter 3 further indicated that more PV and AV differences compared to aroma differences were reported in SOSDEs stored in accelerated and ambient storage conditions over time. As rancidity relates to presence of volatiles it was expected that these rancidity related attributes would be identifiable through aroma analysis. Because of this and the possible health risk associated with the consumption of oxidised products, the decision was made to analyse aroma attributes only in the current study. For

future studies it may be worthwhile to consider the following three approaches to increase sensory sensitivity: 1) scaling aroma attribute intensities rather than CATA whichmay be more sensitive as panellists have to assign an intensity score for each attribute rather than just indicating presence or absence, 2) expectorate products after evaluation of flavour and mouth feel to ensure that there is no or limited health risk associated with the evaluation, 3) analyse the formation of potential toxic products that can have a negative impact on biological tissue (Chen *et al.*, 2011) before exposing the panel to the stored SOSDEs.

Sensory evaluation of the SOSDEs was conducted under red light to mask any potential colour differences in the samples (Meilgaard *et al.*, 2007). It may be possible that colour differences or phase separation, thinning or thickening occurred, but no colour, texture or visual tests were included in the study of the SOSDEs. According to Barden and Decker (2016), lipid oxidation can alter textural properties and adversely affect colour and nutrition of food products. Stability of emulsions is affected by storage temperature but remains relatively stable between 0°C to 40°C. As these samples were stored frozen at -20°C prior to analysis, the emulsion structure may have been compromised and it may be valuable to consider visual and texture evaluation by human panel and/or instrumental techniques in future studies.

All SOSDEs were evaluated in duplicate. It is recommended that future research in this field complete sensory evaluation in triplicate to increase statistical power and identify variation between assessors (Lawless & Heymann, 2010).

Gas Chromatogram Olfaction (G.C.O) was not completed in this research. It was originally not deemed necessary based on the objective of this research. Upon analysis of sensory results, it was unclear whether the metallic and/or earthy aroma detected was due to the presence of the lipid oxidation related volatile 1-hexen-3-one or if it was related the antioxidant gallic acid. Although this will not affect the results of this research, it may be beneficial to include G.C.O in future studies in this field, to define and describe specific volatiles associated with the development of lipid oxidation.

5.1.3 Chemical analysis

In this study, PV and AV was compared to orthonasal aroma analysis and were found to be more discriminating oxidation markers for the SOSDE using the 20 days of accelerated storage. Aroma analysis was performed every day for SOSDEs stored in accelerated storage conditions, however the chemical analyses were performed at day 0, 1, 2, 4, 6, 8, 11, 14, 17 and 20 due to constraints. Due to the discrimination of PV and AV in measuring oxidation markers in SOSDEs stored in accelerated storage conditions, it is recommended to conduct PV and AV at intervals the same time as the sensory analysis (daily) in order to analyse results simultaneously and compare directly.

5.2 MULTIVARIATE ACCELERATED SHELF LIFE TESTING (MASLT)

Although accelerated shelf life methods enable the calculation of shelf life estimations (Corradini and Peleg, 2004; Derossi *et al.*, 2016), they cannot assure agreement between what is estimated and what is observed experimentally which may lead to significant discrepancies (Derossi *et al.*, 2016). Several variables are often studied in accelerated shelf life studies, for example sensory characteristics and chemical analysis (current study). In such cases it is not easy to determine which parameter is most relevant for defining the end of shelf life of the product. Pedro and Ferreira (2006) introduced a new approach for determining shelf life by simultaneously taking into account many quality attributes, known as the MASLT.

MASLT incorporates not only the critical descriptors, but all descriptors that showed significant change over time and contributes to PC1. Using MASLT reduces the risk of establishing product shelf life based on one critical attribute but may exclude attributes that showed significant changes over time in only one product. Plastic aroma changed significantly over time in AccNo but not for any other treatments stored under accelerated storage. Due to plastic aroma's low contribution to PC1, this attribute was not included in the final steps of the MASLT. Painty aroma on the other hand changed significantly over time in treatments AccHiGal and AccLoEDTA and was included in all the steps of MASLT and impacted the regression line and rate constant. It is hypothesised that the inclusion and exclusion of specific attributes that changed over time in different products significantly impacts the multivariate kinetic chart and rate constant which may lead to incorrect interpretation when comparing rate constants of all the products. Therefore it is crucial to interpret the multivariate kinetic chart in combination with the attributes included in the PCA map. According to Labuza and Dugan (1971), each phase in lipid oxidation (initiation, propagation and termination) has its own rate constant, and Sullivan *et al.* (2011) concluded that data fit first order kinetics only within certain temperature ranges.

MASLT has been used on various food products over the last 8 years and has also been applied to this study (Chapter 4). Results from MASLT in this study clearly showed the changes that occurred in the SOSDE products over time. Interpreting the MASLT results with the aid of the power values of variables has proved to be beneficial in understanding the relationships between attributes over time. Some modifications to this method could also be considered.

The experimental design of other MASLT studies predicting the shelf life of concentrated tomato paste (Pedro & Ferreira, 2006), UHT milk (Richards *et al*., 2014), lettuce (Derossi *et al*., 2016), hamburger buns and orange juice (Gimenez *et al*., 2017) were based on the fact that the rate constant was temperature dependent. Therefore, in those studies, three different temperatures were used as the only acceleration factor. The acceleration factors used in the shelf life model proposed by Branco *et a*l. (2011) included several aspects such as elevated temperature (32.2°C), pH 5.5, 1.70 mmol/L ascorbic acid and 0.885 mmol/L $FeSO_4$. It is important to note that the shelf life model proposed by Branco *et al*. (2011) was followed, as oxidation in food emulsions is a dynamic process in which factors interact with each other. Branco and co-worker research further included six to seven sampling times of one treatment over periods of up to 6 months while this shelf life study on SOSDEs included 5 different treatments evaluated over 10 sampling times over 20 days. It was expected that significant lipid oxidation would occur within the 20 days under accelerated storage conditions as Branco *et al*. (2011) reported differences in similar emulsion products within a few days and Celus *et al*. (2018) reported differences in lipid oxidation of oil-in-water emulsions after 2 weeks storage at 35°C. However, it may be beneficial to consider an experimental design of three temperatures over longer periods in a future study in order to compare the findings with other MASLT studies.

5.2.1 Survival analysis

The MASLT studies discussed above included a point of rejection in the multiple kinetic chart that was either determined as part of the research through survival analysis (Richards *et al*., 2014; Gimenez *et al*., 2017) or by using rejection points based on previous research (Derossi *et al*., 2016). Determining the 'point of rejection' or 'end of shelf life' through survival analysis with consumers may be very useful to understand when consumers reject a product and subsequently determining the probability of a consumer rejecting the product as the cut-off point for MASLT. Hough *et al.* (2003) describes the process of survival analysis and determining the point of rejection in detail. In this study it was assumed that all attributes had equal importance to the shelf life of SOSDEs. Some of these attributes may, however, have

more significance to shelf life of SOSDEs than others, and higher weights can be given to the loadings of attributes with more significance. In order to conduct survival analysis for the SOSDEs, they will have to be stored in reverse storage (where SOSDEs will be prepared and start shelf life at different times in order to end shelf life at the same time) and be presented to consumers in order for consumers to indicate if they will or will not consume the product stored for a certain time period. Survival analysis is resource intensive and although not suitable for regular checks on the shelf life of a product it is recommended for once off shelf life estimations or validations. Establishing the 'point of rejection' for SOSDE will aid in the shelf life estimation and confirmation and should be considered for future studies (Garitta *et al.*, 2015).

5.3 DISCUSSION OF RESULTS

Fig 5.1 – Fig 5.3 depicts the possible lipid oxidation processes within the SOSDE stored under accelerated shelf life conditions with and without antioxidants added. During ambient and accelerated storage (Fig 5.1), in the SOSDE with no antioxidants, hydroperoxides and free radicals formed during the initiation phase, react to form carbonyl compounds. These reactions take place at a faster rate in the SOSDEs stored in accelerated shelf life though the acceleration factor has not been determined yet.

In the presence of gallic acid, a free radical scavenger antioxidant, it binds to free radicals formed during the initiation phase of oxidation to form more stable lipid peroxides (Fig 5.2) that do not continue to the propagation phase of the oxidation process. SOSDEs with gallic acid as antioxidant (AmbHiGal and AmbLoGal) had a significantly lower AV and rancid aroma compared to AmbNo, indicating the effective retardation of lipid oxidation during the propagation phase as depicted in Fig 5.2. Further to this, the sensory results indicate that AmbLoGal had a higher rancid aroma detected compared to AmbHiGal which indicates higher effectiveness of increased concentration of gallic acid in the SOSDE.

As illustrated in Fig 5.3, EDTA chelates to metals found in the SOSDE that accelerate lipid oxidation. In the current study the iron added as $FeSO_4$ acted as a pro-oxidant and accelerated the oxidation process. In the presence of EDTA, the iron binds to EDTA, to form FeEDTA (MacPhail *et al.*, 1994). FeEDTA does not act as a pro-oxidant in emulsions and therefor does not accelerate the oxidation process. Results from the accelerated shelf life storage indicated SOSDE with EDTA as antioxidant had a significantly lower metallic aroma

compared to SOSDE with no antioxidants. This is probably due to the EDTA chelating with the iron to form FeEDTA, or a decreased development of lipid oxidation products (1-hexen-3-one) with metallic aroma attributes (Hartvigsen *et al.*, 2000). EDTA also had a significant effect on the development of peroxides (PV) as well as secondary peroxide products (AV) in the SOSDE, with PV and AV for AccHiEDTA being significantly lower indicating that the higher concentration of EDTA had a greater effect on retarding lipid oxidation in the SOSDE. EDTA binds to the iron that accelerates lipid oxidation throughout shelf life and therefore the lipid oxidation is retarded compared to SOSDE with no antioxidants. Based on the results and the depictions in Fig 5.3 it is estimated that once the EDTA binds to iron, the EDTA is bound in the FeEDTA form and cannot react with another iron ion. Therefore, the higher the concentration of EDTA, the more iron ions can be chelated into FeEDTA.

According to the multivariate analysis, cardboard aroma is associated with the later stages of lipid oxidation in the SOSDEs stored under ambient and accelerated conditions. These aroma changes relate to the development of volatiles during the decomposition of hydroperoxides and does not affect the lipid oxidation process and are therefore not included in the figures below.

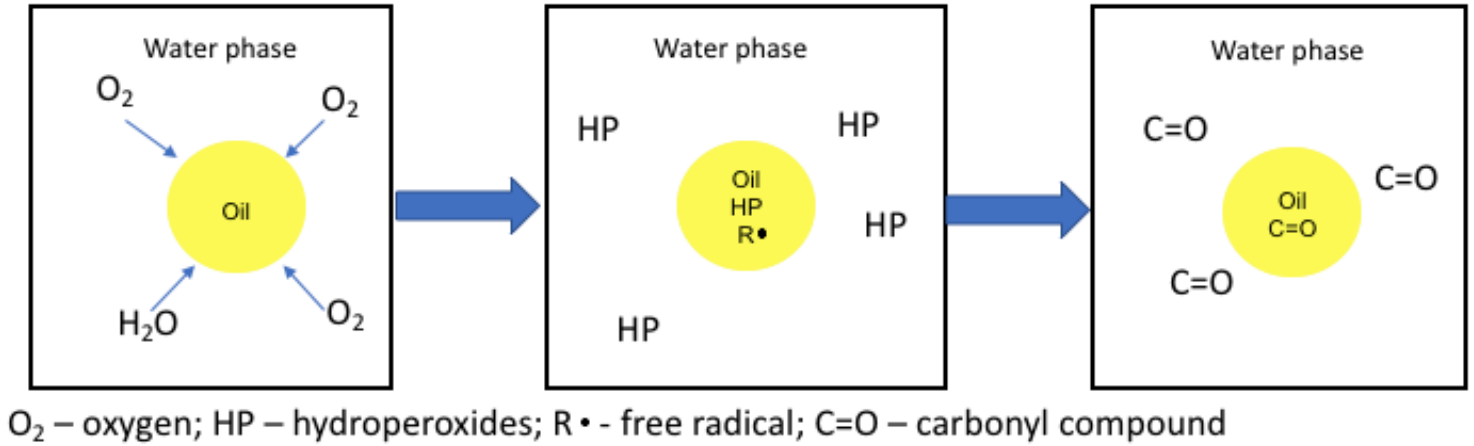


Figure 5.1: Depiction of possible lipid oxidation process within the SOSDE in the presence of no antioxidants during accelerated storage conditions.

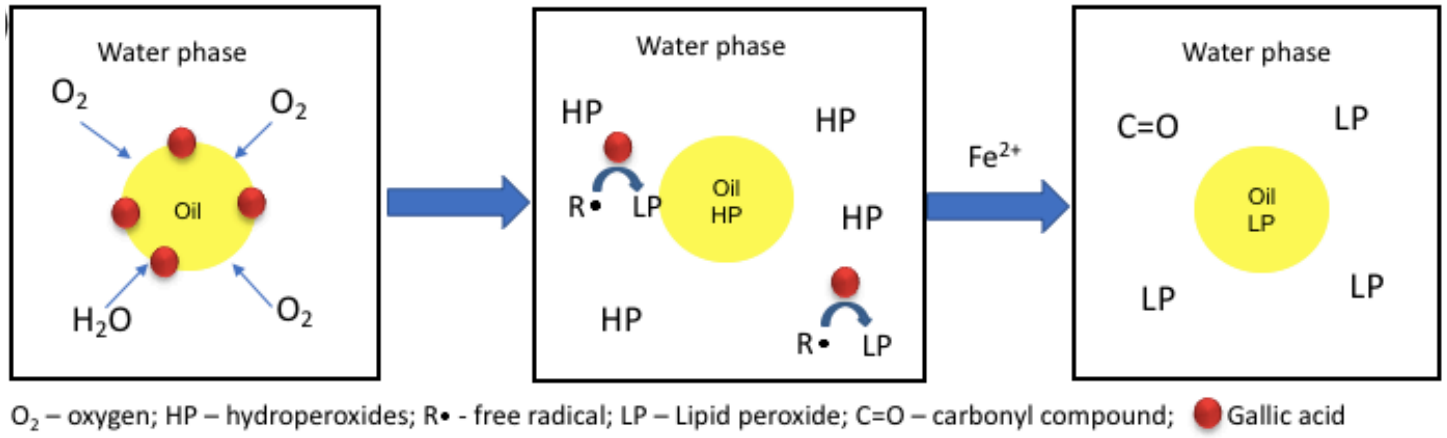


Figure 5.2: Depiction of possible lipid oxidation process within the SOSDE in the presence of Gallic acid as an antioxidant during accelerated storage conditions.

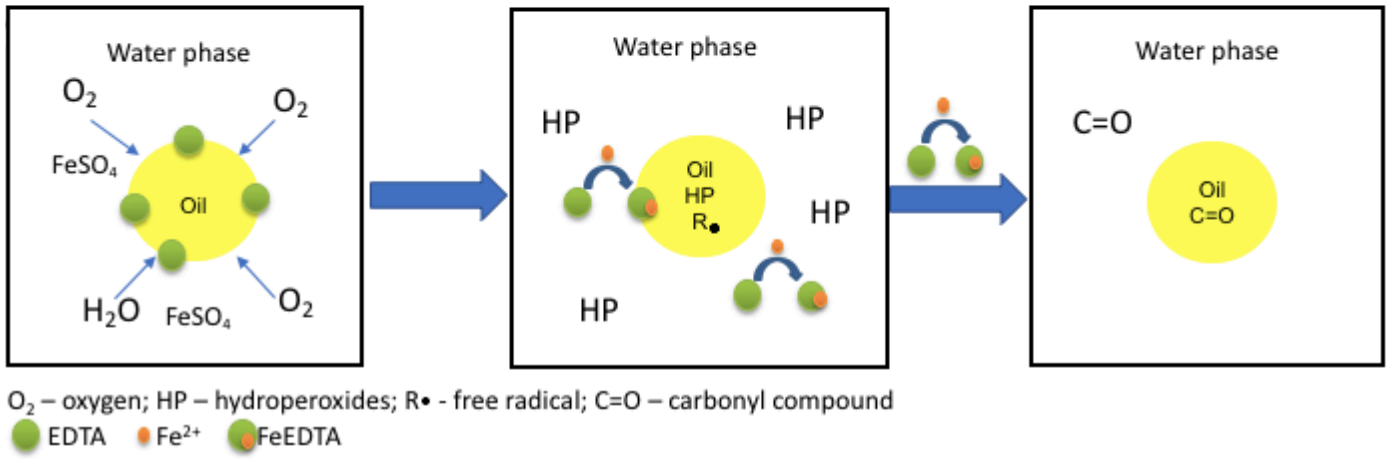


Figure 5.3: Depiction of possible lipid oxidation process within the SOSDE in the presence of EDTA as an antioxidant during accelerated storage conditions.

The current study included two concentrations of each antioxidant (gallic acid and EDTA) (Table 5.2). This was valuable in helping to understand the concentration effect of the same antioxidant on the deterioration of the SOSDE. Results reported in Chapter 3 indicated that aroma differences, as evaluated by the sensory panel, between SOSDEs with different concentrations of antioxidants were more apparent for ambient than accelerated stored SOSDEs. PV differences were apparent between SOSDEs with high and low EDTA concentrations that were stored in accelerated shelf life conditions and AV differences existed between SOSDEs with different gallic acid concentrations for both storage conditions, and for accelerated stored SOSDEs with different EDTA concentrations. Including more than one concentration of an antioxidant gives the opportunity to study concentration effect and understand sensory attributes associated with the antioxidant and should be considered in future research.

Interactions may occur between antioxidants which may in turn affect the oxidation of a product. The reason for these interactions are unknown but it is possible that antioxidants differ in their efficiency in dealing with different mechanisms in the oxidation process (Coppen, 1999). It is important to note that not all combinations of antioxidants display synergistic effects. In the current study, one antioxidant was evaluated at a time which provides limited information. It may be beneficial to research combinations of antioxidants with different mechanisms (e.g. metal chelator and free radical scavenger) in future studies when determining efficiency of antioxidants in oil-in-water emulsions in order to better screen antioxidants.

Upon comparison of the emulsion preparation, antioxidants used, potential pro-oxidants included, sample storage, sample collection and analysis between the current study in this thesis and the model study as described by Branco *et al.* (2011) there were clear differences and similarities. This was a first attempt at reporting the measurement of lipid oxidation markers in a commercial SOSDE stored under accelerated and ambient shelf life conditions. Although results indicate that PV, AV and aroma of SOSDEs stored in accelerated conditions as used in this study, do not clearly predict results for ambient stored SOSDEs, MASLT as applied in this model is valuable in screening effectiveness of antioxidants in relative short time periods.

6

CHAPTER 6: CONCLUSIONS

The first novel contribution of this study includes the simultaneous application of shelf life acceleration factors on a commercial sunflower oil-in-water salad dressing emulsion (SOSDE). Previous research has been conducted on a simplified model system that didn't contain ingredients associated with commercial oil-in-water salad dressing emulsion which may affect the oxidative stability of the product. The addition of antioxidant EDTA (low and high concentration) to the SOSDE stored at ambient conditions, retarded lipid oxidation as measured through aroma and chemical analysis (PV and AV), compared to the SOSDE with no antioxidants added. During accelerated storage conditions, SOSDEs with the high concentration (75 ppm) of EDTA increased lipid oxidation retardation compared to the low concentration of EDTA (37.5 ppm) as assessed by chemical analysis. The antioxidant concentration effect was also observed with addition of gallic acid, where lipid oxidation retardation was greater during ambient storage of SOSDE with 1000 ppm gallic acid compared to 500ppm gallic acid added as measured by AV and rancid aroma detection.

Both sensory analysis and chemical analysis contributed to the examination of SOSDEs stored at ambient and accelerated conditions, with sensory differences more differentiating among SOSDE products stored at ambient storage conditions and chemical analysis more differentiating among SOSDEs stored under accelerated conditions. The determination of antioxidant effectiveness in a commercial oil-in-water emulsions under accelerated conditions is a valuable contribution to the industry, since it can be used to screen antioxidants effectiveness in a representative product in a relatively short time period.

The second novel contribution of this study is the successful application of the multivariate accelerated shelf life test (MASLT) and the identification of possible predictors for end of shelf life for commercial oil-in-water emulsions. Addition of EDTA or gallic acid to the SOSDE lowered the rate constant of lipid oxidation compared to the SOSDE with no antioxidants added. All SOSDEs with added antioxidants, had a higher rate constant in accelerated storage compared to ambient storage, indicating the effectiveness of the accelerated storage model. Pungent aroma was associated with early stage of ambient storage (weeks 0-8) and accelerated storage (days 0-15) and cardboard aroma was associated with later stages of ambient storage (weeks 9-24) and accelerated storage (more than 15 days) and it was concluded that pungent aroma and cardboard aroma were the most probable predictors of shelf life in SOSDEs. No differences were noted in plastic aroma among SOSDEs stored under ambient condition when analysed through univariate and multivariate analysis however significant increases in plastic aroma were found over time in the SOSDE with no added antioxidant added stored under accelerated storage conditions (AccNo). Further research will be needed before plastic aroma can be eliminated from the lexicon. It is recommended that the following attributes and definitions be included in the lexicon development of oil-in-water emulsions shelf life studies:

Aroma attribute	**Definition**
Pungent	Sharp piercing somewhat irritant odour that gives a recoil response. Tactile stinging sensation.
Plastic	Aroma associated with waxy oxidised off notes (associated with plastic container)
Cardboard	Aroma associated with cardboard box

7

CHAPTER 7: REFERENCES

Addis, P. B. (1986). Occurrence of lipid oxidation products in foods. *Food Chemistry Toxicity*, 24(10/11), 1021–1030.

Alamed, J., Mcclements, D. J. and Decker, E. A. (2006). Influence of heat processing and calcium ions on the ability of EDTA to inhibit lipid oxidation in oil-in-water emulsions containing omega-3 fatty acids. *Food Chemistry*, *95*, 585–590.

Arancibia, C., Jublot, L., Costell, E. and Bayarri, S. (2011). Flavor release and sensory characteristics of o/w emulsions. Influences of composition, microstructure and rheological behaviour. *Food Research International*, *44*, 1632–1641.

Ares, G., Antunez, L., Bruzzone, F., Vidal, L., Gimenez, A., Pineau, B., Beresford, M. K., Jin, D., Paisley, A. G., Chheang, S. L., Roigard, C. M. and Jaeger, S. R. (2015). Comparison of sensory product profiles generated by trained assessors and consumers using CATA questions: Four case studies with complex and/or similar samples. *Food Quality and Preference*, *45*, 75–86.

Asnaashari, M., Farhoosh, R. and Sharif, A. (2014). Antioxidant activity of gallic acid and methyl gallate in triacylglycerols of Kilka fish oil and its oil-in-water emulsion. *Food Chemistry*. *159*, 439–444.

Barden, L and Decker, E. A. (2016). Lipid oxidation in low-moisture food: A review. *Critical Reviews in Food Science and Nutrition, 56, 2467-2482*

Baron, C. P. and Andersen, H. J. (2002). Myoglobin-induced lipid oxidation. A review. *Journal of Agricultural and Food Chemistry*, *50*, 3887–3897.

Berton, C., Ropers, M. H., Bertrand, D., Viau, M. and Genot, C. (2012). Oxidative stability of oil-in-water emulsions stabilised with protein or surfactant emulsifiers in various oxidation conditions. *Food Chemistry*, *131*, 1360–1369.

Boon, C. S., Xu, Z., Yue, X. and McClements, D. J. (2008). Factors affecting lycopene oxidation in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, *56*, 1408–1414.

Böttcher, S., Steinhäuser, U. and Drusch, S. (2015). Off-flavour masking of secondary lipid oxidation products by pea dextrin. *Food Chemistry*, *169*, 492–498.

Bou, R., Guardiola, F., Grau, A., Grimpa, S., Manich, A., Barroeta, A. and Codony, R. (2001). Influence of dietary fat sources, α-Tocopherol and ascorbic acid supplementation on sensory quality of dark chicken meat. *Poultry Science*, *80*, 800–807.

Bradley, D. G. and Min, D. B. (1992). Singlet oxygen oxidation of foods. *Critical Reviews in Food Science and Nutrition*, *31*(3), 211–236.

Branco, G. F., Rodrigues, M. I., Gioielli, L. A. and Castro, I. A. (2011). Effect of the simultaneous interaction among ascorbic acid, iron and pH on the oxidative stability of oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, *59*, 12183–12192.

Cardelli, C. and Labuza, T. P. (2001). Application of Weibull hazard analysis to the determination of the shelf-life of roasted and ground coffee. *Lebensmittel-Wissenschaft Technology*, *34*, 273–278.

Celus, M., Salvia-Trujillo, L., Kyomugasho, C., Maes, I., Van Loey, A. M., Grauwet, T. and Hendrickx, M. E. (2018). Structural modified pectin for targeted lipid antioxidant capacity in linseed/sunflower oil-in-water emulsions. *Food Chemistry, 241,* 86-96.

Cercaci, L., Rodriguez-Estrada, M. T., Lercker, G. and Decker, E. A. (2007). Phytosterol oxidation in oil-in-water emulsions and bulk oil. *Food Chemistry*, *102*, 161–167.

Chaiyasit, W., Elias, R. J., McClements, D. J. and Decker, E. A. (2007). Role of physical structures in bulk oils on lipid oxidation. *Critical Reviews in Food Science and Nutrition*, *47*, 299–317.

Chale-Rush, A., Burgess, J. R. and Mattes, R. D. (2007). Evidence for human orosensory (taste?) sensitivity to free fatty acids. *Chemical Senses Advance Access*, *32*(5), 423–431.

Chen, B., McClements, D. J. and Decker, E. A. (2011). Minor components in food oils: A critical

review of their roles on lipid oxidation chemistry in bulk oils and emulsions. *Critical Reviews in Food Science and Nutrition*, *51*(10), 901–916.

Choueiri, L., Chedea, V. S., Calokerinos, A. and Kefalas, P. (2012). Antioxidant/pro-oxidant properties of model phenolic compounds. Part II: Studies on mixtures of polyphenols at different molar ratios by chemiluminscene and LC-MS. *Food Chemistry*, *133*, 1039–1044.

Chung, C. and McClements, D. J. (2014). Structure-function relationships in food emulsions: Improving food quality and sensory perception. *Food Structure*, 1, 106–126.

Coppen, P. P. (1999). The use of antioxidants, in Allen, J. C. and Hamilton, R. J. (eds) *Rancidity in Foods*. 3rd edn.

Córdova, A., Quezada, C. and Saavedra, J. (2011). A MALST method comparison over univariate kinetic modeling for determination of shelf-life in cereal snack of dried apples. *Procedia Food Science*., *1*, 1045–1050.

Corradini, M. G. and Peleg, M. (2004). A model of non-isothermal degradation nutrients, pigments and enzymes. *Journal of the Science and Food Agriculture*, *84*(3), 217–226.

Corrigan, V., Hedderley, D. and Harvey, W. (2012). Modeling the shelf life of fruit-filled snack bars using survival analysis and sensory profiling techniques. *Journal of Sensory Studies*, *27*(6), 403–416.

Coupland, J. N. and McClements, D. J. (1996). Lipid oxidation in food emulsions. *Trends in Food Science and Technology*, *7*, 83–91.

Crapiste, G. H., Brevedan, M. I. V and Carelli, A. A. (1999). Oxidation of sunflower oil during storage. *Journal of the American Oil Chemists' Society*, *76*(12), 1437–14443.

Curia, A. V. and Hough, G. (2009). Selection of a sensory marker to predict the sensory life of a fluid human milk replacement formula. *Journal of Food Quality*, *32*, 793–809.

Decker, E. A. (2002). Antioxidant mechanisms. In. Akoh, C. C. and Min, D. B., Eds., Food Lipids: Chemistry, Nutrition and Biotechnology. Marcel Dekker, New York.

Depree, J. A. and Savage, G. P. (2001). Physical and flavour stability of mayonnaise. *Trends in Food Science and Technology*, *12*, 157–163.

Derossi, A., Mastrandrea, L., Amodio, M. L., de Chiara, M. L. V and Colelli, G. (2016). Application

of multivariate accelerated test for the shelf life estimation of fresh-cut lettuce. *Journal of Food Engineering*, *169*, 122–130.

Dixon, J. and Hewett, E. W. (2000). Factors affecting apple aroma/flavour volatile concentration: A review. *New Zealand Journal of Crop and Horticultural Science*, *28*(3), 155-173.

Dooley, L., Lee, Y. and Meullenet, J. (2010). The application of check-all-that-apply (CATA) consumer profiling to preference mapping of vanilla ice cream and its comparison to classical external preference mapping. *Food Quality and Preference*, *21*, 394–401.

Durand, E., Zhao, Y., Coupland, J. N. and Elias, R. J. (2015). Assessing intercations between lipophilic and hydrophilic antioxidants in food emulsions. *Journal of Agricultural and Food Chemistry*, *63*, 10655–10661.

Ebong, P. E., Owu, D. U. and Isong, E. U. (1999). Influence of palm oil (Elaesis guineensis) on health. *Plant Foods and Human Nutrition*, *53*, 209–222.

Elias, R. J., Kellerby, S. S. and Decker, E. A. (2008). Antioxidant activity of proteins and peptides. *Critical Reviews in Food Science and Nutrition*, *48*, 430–441.

Embuscado, M. E. (2015). Spices and herbs: Natural sources of antioxidants - a mini review. *Journal of Functional Foods*, *18*, 811–819.

Engelen, L., De Wijk, R. A., Prinz, J. F., Janssen, A. M., Weenen, H. and Bosman, F. (2003). The effect of oral and product temperature on the perception of flavor and texture attributes of semi-solids. *Appetite*, *41*, 273–281.

Erkan, N., Ayranci, G. and Ayranci, E. (2008). Antioxidant activities of rosemary (Rosemarinus Afficinalis L.) extract, blaskseed (Nigella Sativa L.) essential oil, carnosic acid, rosmarinic acid and sesamol. *Food Chemistry*, *110*, 76–82.

Esterbauer, H. (1993). Cytotoxicity and genotoxicity of lipid-oxidation products. *American Journal of Clinical Nutrition*, *57*(Suppl), 779S–786S.

Fang, X. and Wada, S. (1993). Enhancing the antioxidant effect of ar-tocopherol with rosemary in inhibiting catalyzed oxidation caused by -Fe^{2+} and hemoprotein. *Food Research International*, 26, 405–411.

Faraji, H., McClements, D. J. and Decker, E. A. (2004). Role of continuos phase protein on the

oxidative stability of fish oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, *52*, 4558–4564.

Feng, J. L., Wang, Z. W., Zhang, J., N, W. Z. and Liu, F. (2009). Study on food-grade vitamin E microemulsions based on nonionic emulsifiers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *339*, 1–6.

Fomuso, L. B., Corredig, M. and Akoh, C. C. (2002). Metal-catalyzed oxidation of a structured lipid model emulsion. *Journal of Agricultural and Food Chemistry*, *50*, 7114–7119.

Food Standards Agency (2015). Food additives legislation guidelines to compliance. www.food.gov.uk/sites/default/files/media/document/food-additives-legislation-guidance-to-compliance. Acessed: 3 February 2019.

Frankel, E. N. (1998). *Lipid Oxidation*, *The Oily Press*, Dundee, Scotland.

Frankel, E. N., Huang, S. W., Kanner, J. and German, J. B. (1994). Interfacial phenomena in the evaluation of antioxidants - bulk oil vs. emulsions. *Journal of Agricultural and Food Chemistry*, *42*, 1054–1059.

Furia, T. E. (1964). EDTA in foods - Technical review. *Food Technology*, 18, 50-58.

Gan, H. L., Man, Y. B. C., Tan, C. P., Norani, I. and Nazimah, S. A. H. (2005). Characterization of vegetable oils by surface acoustic wave sensing Electronic nose. *Food Chemistry*, *89*(4), 507–518.

Garitta, L., Langohr, K., Gomez, G., Hough, G. and Beeren, C. (2015). Sensory cut-off point obtained from survival analysis statistics. *Food Quality and Preference*, *43*, 135–140.

Garrido, M.D., Frias, I., Diaz, C. and Hardisson, A. (1994).Concentrations of metals in edible vegetables oils. *Food Chemistry, 50(3), 237-243.*

Gatti, E., Noe, D., Pazzucconi, F., Gianfranceschi, G., Porrini, M., Testolin, G. and Sirtori, C. R. (1992). Differential effect of unsaturated oils and butter on blood glucose and insulin response to carbohydrate in normal volunteers. *European Journal of Clinical Nutrition*, *46*(3), 161–166.

Gomes, A., Costa, A. L. R., de Assis Perrechil, F. and da Cunha, R. L. (2016). Role of phases composition on the incorporation of gallic acid in o/w and w/o emulsions. *Journal of Food Engineering*, *168*, 205–214.

Gorji, S. G., Smyth, H. E., Sharma, M. and Fitzgerald, M. (2016). Lipid oxidation in mayonnaise and the role of natural antioxidants: A review. *Trends in Food Science and Technology*, *56,* 88–102.

Gotoh, N., Watanbe, H., Osato, R., Inagaki, K., Iwasawa, A. and Wada, S. (2006). Novel approach on the risk assessment of oxidised fats and oils for perspectives of food safety and quality. I: Oxidised fats and oils induce neurotoxcity relating pica behaviour and hypoactivity. *Food and Chemical Toxicology*, *44*, 493–498.

Gupta, M. K. (2014). Sunflower oil: History, application and trends. *Lipid Technology*, *26,* 260–263.

Haizman, A. A., Razali, I. and Ainie, K. (2016). Effect of frying on the palm oil quality attribute - A review. *Journal of Palm Oil Research, 28,* 143-153

Hamilton, R. J. (1999). The chemistry of rancidity in foods. in Allen, J. C. and Hamilton, R. J. (eds) *Rancidity in foods*. 3rd edn. Gaitersburg, MD, USA: Aspen Publishers.

Hartvigsen, K., Lund, P., Hansen, L. F. and Holmer, C. (2000). Dynamic headspace gas chromatography/mass spectrometry characterization of volatiles produced in fish oil enriched mayonnaise during storage. *Journal of Agricultural and Food Chemistry*, *48*, 4858–4867.

Haumann, B. F. (1993). Health implications of lipid oxidation. *Inform*, *4*(7), 800-818.

Hedegaard, R. V., Kristensen, D., Nielsen J. H., Frøst, M. B., Østdal, H. J., Hermansen, E., Kröger-Ohlsen, M. and Skibsted, L. H. (2006). Comparison of descriptive sensory analysis and chemical analysis for oxidative changes in milk. Journal of Dairy Science, 89:495-504

Heiniö, R. L., Lehtinen, P., Oksman-Caldentey, K. M. and Poutanen, K. (2002). Differences between sensory profiles and development of rancidity during long-term storage of native and processed oats. *Cereal Chemistry*, *79*(3), 367–375.

Heynderickx, P.M., Španèl, P. and Van Langenhove, H. (2014). Quantification of octanol-water partition coefficients of several aldehydes in a bubble column using selected ion flow tube mass spectrometry. *Fluid Phase Equilibria, 367*, 22-28.

Hinnenberg, I., Dorman, H. J. D. and Hiltunen, R. (2006). Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry*, *97*, 122–129.

Hough, G., Garitta, L. and Gomez, G. (2006). Sensory shelf-life predictions by survival analysis accelerated storage models. *Food Quality and Preference*, *17,* 468–473.

Hough, G., Langhour, K., Gomez, G. and Curia, A. (2003). Survival analysis applied to sensory shelf-life of foods. *Journal of Food Science*, *68,* 359–362.

Hsieh, Y. and Regenstein, J. M. (1991). Factors affecting quality of fish oil mayonnaise. *Journal of Food Science*, *56*(5), 1298–1301.

Huang, S. W. and Frankel, E. N. (1997). Antioxidant activity of tea catechins in different lipid systems. *Journal of Agricultural and Food Chemistry*, *45*(8), 3033–3038.

Hudson, B. J. F. and Gordon, M. H. (1999). Evaluation of oxidative rancidity techniques. in Allen, J. C. and Hamilton, R. J. (eds) *Rancidity in foods*. 3rd edn. Gaitersburg, MD, USA: Aspen Publishers.

Hung, S. S. O. and Slinger, S. J. (1981). Studies of chemical methods for assessing oxidative quality and storage stability of feeding oils. *Journal of the American Oil Chemists' Society*, 58, 785-792.

Israelachvili, J. (1994). The science and application of emulsions - an overview. *Colloids and Surfaces A: Physiochemical and Engineering Aspects*, *91*, 1–8.

Jacobsen, C. (1999). Sensory impact of lipid oxidation in complex food systems. *European Journal of Lipid Science and Technology*, *101*(12), 484–492.

Jacobsen, C., Hartvigsen, K., Lund, P., Thomsen, M. K., Skibsted, L., Adler-Nissen, J., Holmer, C. and Meyer, A. S. (2000). Oxidation of fish oil enriched mayonnaise 3: Assessment of the influence of the emulsion structure on oxidation by discriminant partial least squares regression analysis. *European Food Research and Technology*, *211*, 86–98.

Jacobsen, C., Let, M. B., Nielsen, N. S. and Meyer, A. S. (2008). Antioxidant strategies for preventing oxidative flavour deterioration of foods enriched with n-3 poly-unsaturated lipids: A comparative evaluation. *Trends in Food Science and Technology*, *19*(2), 76–93.

Jacobsen, C., Meyer, A. S. and Adler-Nissen, J. (1999). Oxidation mechanisms in real food emulsions : oil-water partition coefficients of selected volatile off-flavor compounds in mayonnaise. *Z Lebensm Unters Forsch A*, *208*, 317–327.

Jayasinghe, C., Gotoh, N., Aoki, N. and Wada, S. (2003). Phenolics composition and antioxidant activity of sweet basil (Ocimum Basilicum L.). *Food Chemistry*, *51*, 4443–4449.

Jayasinghe, C., Gotoh, N. and Wada, S. (2013). Pro-oxidant / antioxidant behaviours of ascorbic acid, tocopherol, and plant extracts in n-3 highly unsaturated fatty acid rich oil-in-water emulsions. *Food Chemistry*, *141*(3), 3077–3084.

Kellerby, S.S., McClements, D.J. and Decker, E.A. (2006). Role of proteins in oil-in-water emulsions on the stability of lipid hydroperoxides. *Journal of Agricultural and Food Chemistry, 54,* 7879-7884.

Kiosseoglou, V. D. and Sherman, P. (1983). The influence of egg yolk lipoproteins on the rheology and stability of O/W emulsions and mayonnaise. *Colloid and Polymer Science*, *261*, 520–526.

Kirchner, S. and Gyarfas, E. C. (1957). Barium (ethylenediaminetetracetato) cobalt(III)4-hydrate. *Inorganic Syntheses*, *5*, 186–188.

Labuza, T. P. (1982). *Shelf Life Dating of Foods*. Westport, Connecticut, USA: Food and Nutrition Press. Inc.

Labuza, T.P., and Dugan, Jr., L.R. (1971). Kinetics of lipid oxidation in foods. *Critical Review of Food Science and Nutrition, 2*, 355-405.

Labuza, T. P. and Schmidl, M. K. (1985). Accelerated shelf-life testing of foods. *Food Technology*, *39*, 57–64.

Lawless, H. T., and Heymann, H. (2010). Sensory evaluation of food: Principles and practices. 2nd edition. Springer, New York

Lennersten, M. and Lingnert, H. (2000). Influence of wavelength and packaging material on lipid oxidation and colour changes in low fat mayonnaise. *Food Science and Technology*, 33(4), 253–260.

Lerma-García, M. J., Cerretani, L., Cevoli, C., Simó-Alfonso, E. F., Bendini, A. and Toschi, T. G. (2010). Sensors and Actuators B : Chemical use of Electronic nose to determine defect percentage in oils. Comparison with sensory panel results. *Sensors and Actuators B: Chemical*, 147, 283–289.

Lett, A. M., Yeomans, M. R., Norton, I. T. and Norton, J. E. (2016). Enhancing expected food

intake behaviour, hedonics and sensory characteristics of oil-in-water emulsion systems through microstructural properties, oil droplet size and flavour. *Food Quality and Preference, 47,* 148-155.

Let, M. B., Jacobsen, C. and Meyer, A.S. (2007). Ascorbyl Palmitate, δ-Tocopherol, and EDTA affect lipid oxidation in fish oil enriched salad dressing differently. *Journal of Agricultural and Food Chemistry, 55*, 2369-2375

Lewis, M. J. and Heppel, N. (2000). *Continues thermal processing of foods, pasteurization and UHT sterilization.* Gaitersburg, MD, USA: Aspen Publishers.

Li, C. Y., Kim, H. W., Li, H., Lee, D. C. and Rhee, H. L. (2014). Antioxidative effect of purple corn extracts during storage of mayonnaise. *Food Chemistry*, *152,* 592–596.

Liang, Y., Matia-Merino, L., Gillies, G., Patel, H., Ye, A. and Golding, M. (2017). The heat stability of milk protein-stabilized oil-in-water emulsions: A review. *Current Opinion in Colloid & Interface Science, 28, 63-73.*

Liu, X., Dong, M., Chen, X., Jiang, M., Jiang, L., Jiang, X. and Yan, G. (2007). Antioxidant activity and phenolics of an endophytic Xylaria sp. from Ginko biloba. *Food Chemistry*, *105*, 548–554.

MacPhail, A. P., Patel, R. C., Bothwell, T. H. and Lamparelli, R. D. (1994). EDTA and the absorption of iron from food. *American Journal of Clinical Nutrition*, 59, 644–648.

Malcolmson, L. J., Vaisey-genser, M., Przybylski, R., Ryland, D., Eskin, N. A. M. and Armstrong, L. (1996). Characterization of stored regular and low-linolenic canola oils at different levels of consumer acceptance. *Journal of the American Oil Chemists' Society*, *73*(9), 1153–1160.

Manzini, R., Accorsi, R., Piana, F. and Regattieri, A. (2017). Accelerated life testing for packaging decisions in the edible oils distribution. *Food packaging and shelf life, 12*, 114-127.

Mariod, A. A., Ibrahim, R. M., Ismail, M. and Ismail, N. (2010). Antioxidant activity of phenolic leaf extracts from Monechma ciliatum in stabilization of corn oil. *Journal of the American Oil Chemists' Society*, *87*, 35–43.

Martinez, C., Mucci, A., Hough, G. and Sanchez, R. (1998). Influence of temperature, fat content and package material on the sensory shelf life of a commercial mayonnaise. *Journal of Sensory Studies*, *13*(3), 331–346.

McBurney, D. H., Collings, V. B. and Glanz, L. M. (1973). Temperature dependence of human

taste responses. *Physiology and Behavior*, *11*, 89–94.

McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid and Interface Science*, *9*(5), 305–313.

Meeker, W. Q. and Escobar, L. A. (1998). *Statistical methods for reliability data.* New York: John Wiley & Sons.

Meilgaard, M., Civille, G. V and Carr, B. T. (2007). *Sensory evaluation techniques*. New York, NY, USA: CRC Press.

Mendez, E., Sanhueza, J., Speisky, H. and Valenzuela, A. (1996). Validation of the rancimat test for the assessment of the relative stability of fish oils. *Journal of the American Oil Chemists' Society*, *73*, 1033–1037.

Merril, L. I., Pike, O. A., Ogden, L. V and Dunn, M. L. (2008). Oxidative stability of conventional and high-oleic vegetable oils with added antioxidants. *Journal of the American Oil Chemists' Society*, *85*, 771–776.

Meyners, M., Castura, J. C. and Carr, B. T. (2013). Existing and new approaches for the analysis of CATA data. *Food Quality and Preference*, *30*, 309–319.

Min, D. B. and Boff, J. M. (2002). Lipid oxidation in edible oil. in Akoh, C. C. and Min, D. B. (eds) *Food lipids: Chemistry, Nutrition and Biotechnology*. New York: Marcel Dekker.

Morales-Aizpurua, I. C. and Tenuta-Filho, A. (2005). Oxidaton of cholesterol in mayonnaise during storage. *Food Chemistry*, *89*(4), 611–615.

Moskowitz, H. R. (1973). Effects of solution temperature on taste intensity in humans. *Physiology and Behavior*, *10*, 289–292.

Nakatani, N., Inatani, R., Ohta, H. and Nishioka, A. (1986). Chemical constituents of peppers (Piper spp.) and application to food preservation: Naturally occurring antioxidative compounds. *Environmental Health Perspectives, 67*, 135–142.

Nelson, W. (1990). *Accelerated testing. Statistical models, test plans and data analyses.* New York: John Wiley & Sons.

Official Methods of Analysis (1990) 15th Ed., AOAC, Arlington, VA, sec. 965.33

Pangborn, R. M., Chrisp, R. B. and Bertolero, L. L. (1970). Gustatory, salivary, and oral thermal responses to solutions of sodium chloride at four temperatures. *Perception and Psychophysics*, 8, 69–75.

Payne, R. W. (2012). *Introduction to GenStat for Windows*, *15th Edition*. Available at: http://www.genstat.co.uk (Accessed: 1 January 2012).

Pearson, D. (1970). *The chemical analysis of foods*. 6th edn. London: Churchill.

Pedro, A. M. K. and Ferreira, M. M. C. (2006). Multivariate accelerated shelf-life testing: a novel approach for determining the shelf-life of foods. *Journal of Chemometrics*, *20*, 76–83.

Penumetcha, M., Khan, N and Partasarathy, S. (2000). Doetary oxidized fatty acids: an atherogenic risk? *Journal of Lipid Research, 41(9)*, 1473-80

Ragnarsson, J. O. and Labuza, T. P. (1977). Accelerated shelf-life testing for oxidative rancidity in foods - A review. *Food Chemistry*, *2*, 291–308.

Rehman, S., Banks, J. M., Brechany, E. Y., Muir, D. D., McSweeney, P. L. H. and Fox, P. F. (2000). Influence of ripening temperature on the volatiles profile and flavour of Chedder cheese made from raw or pasteurised milk. *International Dairy Journal*, *10*(1), 55–56.

Reinbach, H. C., Giacalone, D., Ribeiro, L. M., Bredie, W. L. P. and Frost, M. B. (2014). Comparison of three sensory profiling methods based on consumer perception: CATA, CATA with intensity and Napping. *Food Quality and Preference*, *32*, 160–166.

Rice-Evans, C. A., Miller, N. J. and Paganga, G. (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, *20*(7), 933–956.

Richards, A. and Golding, M. (2011). The influence of secondary emulsifiers on lipid oxidation within sodium caseinate-stabilized oil-in-water emulsions. *Journal of American Oil Chemists' Society*, *88*, 65-73.

Richards, M., De Kock, H. L. and Buys, E. M. (2014). Multivariate accelerated shelf life test of low fat UHT milk. *International Dairy Journal*, *36*, 38–45.

Robards, K., Kerr, A. F. and Patsalides, E. (1988). Rancidity and its Measurement in Edible Oils and Snack Foods. *Analyst*, *113*, 213–224.

Rodríguez-Rojo, S., Visentin, A., Maestri, D. and Cocero, M. J. (2012). Assisted extraction of

rosemary antioxidants with green solvents. *Journal of Food Engineering*, *109*, 98–103.

Rossel, J. B. (1999). Measurement of rancidity. in Allen, J. and Hamilton, R. J. (eds) *Rancidity in foods*. 3rd edn. Gaitersburg, MD, USA: Aspen Publisher.

Saavedra, J., Cordova, A., Galvez, L., Quezada, C. and Navarro, R. (2013). Principal component analysis as an exploration tool for kinetic modelling of food quality: a case study of dried apple cluster snacks. *Journal of Food Engineering*, *119*, 229–235.

Sanders, T. A. B. (1999). Nutritional aspects of rancidity. in Allen, J. C. and Hamilton, R. J. (eds) *Rancidity in Foods*. 3rd edn. Gaitersburg, MD, USA: Aspen Publisher.

Schwarz, J., Huang, S. W., German, J. B., Tiersc, B., Hartmann, J. and Frankel, E. N. (2000). Activities of antioxidants are affected by colloidal properties of oil-in-water and water-in-oil emulsions and bulk oils. *Journal of Agricultural and Food Chemistry*, *48,* 1874–1552.

Serfert, Y., Drusch, S. and Schwarz, K. (2010). Sensory odour profiling and lipid oxidation status of fish oil and microencapsulated fish oil. *Food Chemistry*, *123*(4), 968–975.

Shanta, N. C. and Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of the Association of Official Analytical Chemists International*, *77*(2), 421–424.

Shen, N., Moizuddin, S., Wilson, L., Duvick, S., White, P. and Pollak, L. (2011). Relationship of electronic nose analyses and sensory evaluation of vegetable oils during storage. *Journal of the American Oil Chemists' Society*, *79*(9), 937–940.

Sherwin, E. R. (1968). Methods for stability and antioxidant measurements. *Journal of American Oil Chemists' Society*, *45*, 632–649.

Sonntag, N. O. V (1979). Reactions of fats and fatty acids. in Swern, D. (ed.) *Bailey's Industrial oil and fat products*. 4th edn. New York: John Wiley & Sons, 99–175.

Sullivan, J.C., Budge, S.M. and St-Onge, M. (2011). Modeling the primary oxidation in commercial fish oil preparation. *Lipids, 46,* 87-93.

Sun, Y., Wang, W., Chen, H and Li, C. (2011). Autoxidation of unsaturated lipids in food emulsion. *Critical Review in Food Science and Nutrition, 51*, 453-466.

Sun, X., Wang, Z., Kadouh, H. and Zhou, K. (2014). The antimicrobial, mechanical, physical and

structural properties of chitosan-gallic films. *Food Science and Technology*, *57*, 83–89.

Takai, J, I., Endo, Y., Okuzaki, M. and Fujimoto, K. (2003). Autoxidation of fish oil in mayonnaise-like O/W type emulsion. *Food Science and Technology Research*, *9*(4), 383–386.

Tamaru, S., Igura, N. and Shimoda, M. (2018). Effectiveness of water-air and octanol-air partition coefficients to predict lipophillic flavor release behavior from O/W emulsions. *Food Chemistry*, *239*, 712–717.

Tasan, M. and Demirci, M. (2005). Total and individual tocopherol contents of sunflower oil at different steps of refining. *European Food Research and Technology*, 220(3–4), 251–254.

Thomsen, M. K., Jacobsen, C. and Skibsted, L. (2000). Mechanisms of initiation of oxidation in mayonaise enriched with fish oil as studied by electron spin resonance spectroscopy. *European Food Research and Technology*, 211–381.

Vaisey-Genser, M., Malcolmson, L. J., Ryland, D., Przybylski, R., Eskin, N. A. M. and Armstrong, L. (1994). Consumer acceptance of canola oils during temperature accelerated storage. *Food Quality and Preference*, *5*, 237–243.

Van der Merwe, G. H., Du Plessis, L. M. and Taylor, J. R. N. (2003). Changes in chemical quality indices during long-term storage of palm-olein oil under heated storage and transport-type conditions. *Journal of Food and Agriculture*, *84*, 52–58.

Waraho, T., McClements, D. J. and Decker, E. A. (2011). Mechanisms of lipid oxidation in food dispersions. *Trends in Food Science and Technology*, *22*, 3–13.

Warner, K., Frankel, E. N., Snyder, J. M. and Porter, W. L. (1986). Storage stability of soybean oil-based salad dressings: effects of antioxidants and hydrogenation. *Journal of Food Science*, *51*(3), 703–708.

Wills, R. B. H. and Cheong, C. L. (1979). Use of peroxide value and carbonyl value to determine the onset of rancidity in mayonnaise. *Food Chemistry*, 4(4), 259–261.

White, P. (1995). Conjugated diene, anisidine value, and carbonyl value analyses. In: Methods to assess quality and stability of oils and fat-containing foods (edited by K. Warner & M. Eskin). 159–178

Yamamoto, Y., Sogo, N., Iwao, R. and Miyamoto, T. (1990). Antioxidant effect of egg yolk on linoleate in emulsions. *Agricultural and Biological Chemistry*, *54*(12), 3099–3104.

Yanishlieva, N. V., Marinova, E. and Pokorny, J. (2006). Natural antioxidants from herbs and spices. *European Journal of Lipid Science and Technology*, *108*(9), 776–793.

Yoon, S. H., Kim, S. K., Shin, M. G. and Kim, K. H. (1985). Comparative Study of Physical Methods for Lipid Oxidation Measurement in Oils. *Journal of the American Oil Chemists' Society*, *62*(10), 1487–1489.

Zhang, B., Xi, W., Wei, W., Shen, J., Ferguson, I. and Chen, K. (2011). Changes in aroma-related volatiles and gene expression during low temperature storage and subsequent shelf life of peach fruit. *Postharvest Biology and Technology*, *60*(1), 7–16.

Zhong, Y. and Shahidi, F. (2011). Lipophilized epigallocatechin (EGCG) derivatives as novel antioxidants. *Journal of Agricultural and Food Chemistry*, *59*(12), 6526–6533.

Zhou, L. and Elias, R. J. (2012). Factors influencing the antioxidant and pro-oxidant activity of polyphenols in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, *60*(11), 2906–2915.

Zidkova, J., Sajdok, J., Kontrova, K., Kortbova-Kozak, A., Hanis, T., Zidek, V. and Fucikova, A. (2004). Effects of oxidised dietary co liver oil on the reproductive functions of Wistar rats. *Journal of Food Science*, *22*, 108–120.