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Advanced platform for shelf life extension in liquid foods

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Summary

The shelf life of lipid based foods is often determined by the development of rancid flavours attributed to lipid oxidation reactions. These reactions are highly complicated and readily change when the reaction system is altered. As a result, researchers have struggled to make significant advances in their understanding of the mechanisms and rates of lipid oxidation.

This thesis focuses on the generalised three step mechanism of lipid oxidation and develops understanding, through mathematical modelling exercises, about the factors that influence the rates of lipid oxidation. More specifically, this thesis focuses on bulk oils, bulk oils with added antioxidants, oil-in-water emulsions and the effects of oxygen supply and consumption rates in real food systems.

For this thesis, methods were developed to identify and validate findings that suggest that lipid hydroperoxides are the rate defining reactant in lipid oxidation reactions. These methods were then used to measure the solubility of oxygen in oil and to define the role oxygen plays in determining the rates of lipid oxidation in a range of systems.

The use of a newly developed batch oxidation apparatus led to the development and validation of models to predict the rates of oxygen consumption during oxidation. The model showed that the rates of oxygen consumption were half order with respect to the lipid hydroperoxide concentration. Through further validation experiments, it was shown that, during the initial stages of lipid oxidation before rancidity, each mole of lipid hydroperoxides formed required 5.04 moles of oxygen to be consumed when there was oxygen present.

The same model and methods were then used to predict the changes in rates of lipid oxidation triggered by changes in reaction temperature. From this work, it was found that the Arrhenius law was capable of predicting the rates of oxygen consumption.

The addition of butylated hydroxyanisole (BHA) to mixed fish oil samples brought with it a reduction in the rates of lipid oxidation, the magnitude of which was proportional to the concentration of BHA added. It was found that the inclusion of a modifier into the half order model was capable of predicting the rates of lipid oxidation when antioxidants were added. Methods to quantify the modifier were supplied for future use.

The dilution of bulk oils by the formation of oil-in-water emulsions was also studied. It was found that the rates of lipid oxidation were proportional to the concentration of

lipids in the emulsion. It was shown that the extent of oxidation during a batch oxidation was inversely proportional to the concentration of lipids in the emulsions as the aqueous phase acted as sump of oxygen for reaction in the oil droplets.

Through modelling and short validation exercises, it was shown that changes to the surface area to volume ratio of oil droplets in emulsions had no effect on the rates of oxygen supply/lipid oxidation and that any effects noted in literature are likely to be the result of other surface active compounds.

Finally, a modelling exercise showed that the rates of oxygen consumption via reaction were likely to be significantly faster than the rates of oxygen supply in unmixed systems in polymer packaging and, to some extent, open to the atmosphere. The diffusion of lipid hydroperoxides was shown to be important in bulk oils stored in polymer packaging as it allowed for a greater proportion of the oil to react with the oxygen transferred, thus reducing the potential for the oxygen supplied to take part in secondary and tertiary product formation. It was suggested that it is better, for a given quantity of oxygen supplied, for the entire oil product to react as it would result in fewer tertiary products being formed than if the oxygen were to be consumed at the surface of the oil only. Following this, it was suggested that an oil-in-water emulsion should be less stable than a bulk oil.

Short experimental work showed that storing bulk oils in the absence of oxygen brings with it a decrease in the rates of lipid oxidation caused by a decrease in the concentration of lipid hydroperoxides. This decrease, coupled with anecdotal evidence that products do become rancid over long periods of time, suggests that the radicals formed during lipid hydroperoxide breakdown can be used in two different sets of reactions. That is, the relative rates of reformation of lipid hydroperoxide via reaction with lipids and the formation of tertiary oxidation products will likely determine the rates of lipid hydroperoxide breakdown and rancidity in real food systems.

An indepth analysis of lipid hydroperoxide breakdown rates in the absence of oxygen as well as a set of validation experiments for the storage of bulk oils and oil-in-water emulsions in polymer films was suggested as being the final piece of information needed to complete a comprehensive model capable of quantitatively predicting the rates of lipid oxidation reactions and the shelf life of lipid oxidation prone foods.

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I was told that I should finish off with something profound or interesting. Unfortunately I am all out of profound. Instead I feel that one more plot in my thesis is needed.

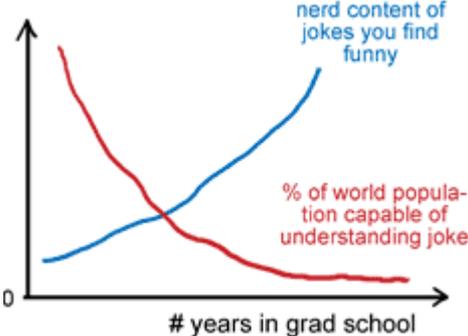


Figure XYZ: Ironically un-funny joke

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CHAPTER 1
INTRODUCTION

1.0 Introduction

Fats and oils are common ingredients used throughout the food industry. Fats and oils give foods important flavours and unique textural qualities that are difficult to mimic despite the best efforts of food chemists (Frankel, 2005). Recently there has been much media attention given to the health issues and benefits created by the consumption of different fats. As a result, consumers have begun to replace food products containing large amounts of saturated fatty acids with similar products containing the healthier polyunsaturated fatty acids.

In order to ensure good sales and competitive advantage, most food manufacturers have responded by producing new or substitute products that are now in high demand from health conscious consumers. Although healthy, polyunsaturated fats are prone to oxidation reactions which give rise to distinct rancid flavours and aromas. This rancidity can cause a significant reduction in shelf life compared with products containing saturated fatty acids (Jacobsen, 1999; van Ruth *et al.*, 1999; Richards *et al.*, 2005). Therein lies a new challenge that is yet to be fully tackled by food scientists and engineers.

For a number of decades, chemists have been investigating the reactions that describe lipid oxidation and explain the rancidity commonly associated with the products of lipid oxidation. The introduction of new chromatography techniques has allowed researchers to identify a vast number of stable oxidation products. Despite such technological advances, there is no way of fully describing every reaction taking place during lipid oxidation. The addition of complex systems, such as those found in emulsion systems, makes the probability of finding and describing all lipid oxidation reactions very small.

A complete description of all lipid oxidation reactions, including the effects external conditions have on the reactions, would be the ideal solution to the problems currently faced by food producers. Unfortunately, there are a vast number of lipid oxidation reactions that occur and are responsible for the development of rancidity in oils (Frankel, 1982). External factors such as temperature changes and the addition of

additives, such as antioxidants, bring with them a significant change in the number and type of reactions that take place and, therefore, a significant increase in the complexity of the reaction system. This has been a significant stumbling block for many years and has been, arguably, the greatest hurdle that has prevented researchers from making significant progress over the past few decades.

Although nowhere close to fully describing lipid oxidation, a simplified three stage mechanism has been successfully used to characterise lipid oxidation reactions (Labuza, 1971; Takahashi *et al.*, 2000). The three stage pathway, which includes initiation, propagation and termination reactions, provides a generalised framework for grouping reactions according to the extent of oxidation at which they appear. For example, the primary oxidation products, formed by initiation reactions, are broken down and further oxidised to create secondary oxidation products. The creation of tertiary oxidation products, aldehydes, ketones etc., from the subsequent oxidation of secondary oxidation products brings with it the development of rancid flavours and aromas. The development of rancidity, therefore, can be described by the passage of lipids through the three stage oxidation pathway. This simple pathway is, therefore, capable of removing the need to characterise individual reactions and, in doing so, reduces the complexity of reaction systems to more manageable proportions.

Researchers, including Labuza (1971) and Jacobsen (1999), have suggested that only very low concentrations (often measured in parts per billion) of tertiary oxidation products are required for an oil to be deemed rancid. As such, the three stage pathway could potentially be further reduced. Labuza (1971) suggested that the concentration of lipid hydroperoxides, the only semi-stable primary oxidation product, determine the rates of lipid oxidation. Others, including Takahashi *et al.* (2000), have suggested that the rates of lipid oxidation are also governed by the concentration of oxygen and lipids. Although it seems most authors agree that tertiary oxidation products are not rate controlling, there is still a lack of understanding of lipid oxidation in bulk oils, even when the highly simplified three stage pathway is applied.

Until recently, carrying out investigations to determine the effects of lipid, oxygen and lipid hydroperoxide concentrations has been difficult. There is some qualitative information showing the changes in lipid hydroperoxide concentrations with time in

various systems. Similarly, it is possible to find some evidence that lipid concentrations decrease as lipid oxidation occurs. Oxygen consumption rates are equally hard to come by in literature, probably because of the lack of a reliable method to measure dissolved oxygen concentrations.

Obviously there are significant gaps in our knowledge of lipid oxidation. In many cases the effects of reactants like lipid hydroperoxides and oxygen have been theorised using the three stage oxidation pathway. By using newly developed technology to test these theories and build on them, it should be possible to create a mathematical model to describe the rates of oxidation. Unlike most, this model would have a mechanistic basis and could be applied and modified according to the reaction system.

Perhaps the most complete models available are presented by Labuza (1971) and Takahashi *et al.* (2000). They both suggest that the rates of lipid oxidation are dependent on lipid hydroperoxide concentrations. Labuza (1971) suggested that the rate of oxygen consumption is half order with respect to the lipid hydroperoxide concentration. Takahashi *et al.* (2000) suggested that the reaction mechanism is far more complicated and developed a comparatively complicated model. The model proposed by Takahashi *et al.* (2000) suggested that lipids, lipid hydroperoxides and oxygen were responsible for determining the rates of lipid oxidation. Both models used curve fitting techniques for validating their models. Both the Labuza and Takashi models were shown to fit well with data from experiments conducted on pure oil samples. However, neither used their models to predict oxidation rates or conducted the kind of mechanistic investigations required for any real confidence in the models.

The foundation laid by Labuza (1971) and Takahashi *et al.* (2000) and the vast amount of qualitative lipid chemistry information available in literature, however, are not enough to understand lipid oxidation in bulk oils, let alone in more complex systems with additives or emulsions. A collation of the chemistry of lipid oxidation and quantitative and predictive models that describe lipid oxidation rates in bulk oils and oil-in-water emulsions is needed. New research is needed to provide comprehensive kinetic investigations to identify and quantitatively describe the effects of important internal and external factors that govern the time required for lipid oxidation to occur.

There is little quantitative information about the individual and combined effects of lipid, lipid hydroperoxide and oxygen concentrations on lipid oxidation rates. A purely experimental approach for investigating lipid oxidation rates is not necessarily the most suitable approach for this particular problem. Certainly, concentrating on individual reactions in the lipid oxidation pathway is not likely to yield significant information. A modeling approach, combined with experimental validation, is likely to be a better method of investigation for this problem. Using the chemistry already developed and available in literature, models can be developed to mechanistically describe lipid oxidation. In particular, the effects of oxygen, lipids and lipid hydroperoxides on the three stage reaction pathway are likely to be pivotal to quantifying the rate of lipid oxidation in bulk oil systems. By carefully forming and testing assumptions about the reaction system, the effects of each reactant on the system as a whole can be determined. This would move our knowledge about lipid oxidation to a level where the rates of lipid oxidation are quantifiable and, ultimately, predictable. The application of such a mechanistic model could then be expanded to include the effects of more complex reaction systems. Such systems could include those with antioxidants, pro-oxidants and oil-in-water emulsions where the lipids are dispersed as droplets of oil and physically separated from one another.

As Labuza (1971) has shown, models of lipid oxidation need not require intimate knowledge of the reaction system. That is, the net effects of a number of reactions can be grouped as long as the model predictors, the model inputs, are gained from careful experiments. This is key to making the models manageable as it is nearly impossible to determine the exact concentrations of every reactant in even the most refined oil.

Having developed an understanding about the rates of lipid oxidation in different mixed environments, the effect of holding a sample of oil or oil containing product in an oxygen-starved environment could be analysed. As yet, the effects of oxygen supply rates have been only briefly described in the literature. Surprisingly, the effects of lipid and lipid hydroperoxide diffusion and reaction in such systems have not received significant attention in the literature, despite such systems being common during the storage of foods in polymer packaging materials.

Through modelling exercises, this thesis will focus on developing understanding of the mechanisms of lipid oxidation rates in bulk oil systems as well as the effects of antioxidants and the dilution of lipids by the formation of oil-in-water emulsions. This will require new methods to be developed to track lipid oxidation rates in all of these systems. The knowledge gained from this analysis will then be applied to systems where the rates of oxygen supply are limited. From this analysis, recommendations will be made to help develop further understanding about lipid oxidation reactions that take place in oxygen-deprived environments.

The specific aims of this work were to:

- 1) Develop methods to control the supply of oxygen and measure lipid oxidation rates in bulk oils, oil-in-water emulsions, and other liquid food systems.
- 2) Develop a mathematical model, with a mechanistic basis, to predict the rates of lipid oxidation in bulk oils.
- 3) Provide a framework to determine the mechanism of hydrophobic antioxidant action and predict the rates of lipid oxidation in systems with added antioxidants.
- 4) Determine the effects of diluting lipids by the formation of oil-in-water emulsions and provide a mechanism that describes the rates of lipid oxidation in oil-in-water emulsions.
- 5) Investigate the effects of oxygen supply rates and lipid hydroperoxide diffusion rates in systems with limited oxygen supply.

CHAPTER 2
LITERATURE REVIEW

2.0 Introduction

Lipid oxidation has been studied for many years. There is a large amount of literature available to help provide the information required to develop the predictive models in this thesis. A thorough literature review will, therefore, help to identify:

- A consistent reaction mechanism.
- The range of potential oxidation scenarios and conditions that models could be developed for.
- The types of food components and physical structures that may alter lipid oxidation rates.
- The practical implications of lipid oxidation on foods, including the extents of lipid oxidation that are of interest to food technologists
- Methods that are commonly used to measure lipid oxidation rates.

By conducting a thorough literature review, it will be possible to identify the gaps in the literature that need to be investigated in order to complete the objectives of this thesis.

2.1 Introduction to lipids and their place in food systems

There are a number of different definitions of what a lipids are (Frankel, 2005; Angelo, 1996; Kates, 1986; Christie, 1982). Whether defined according to their solubility, function or chemical structure, the single fact is that lipids make up a group of complex molecules whose oxidation products are responsible for the development of rancidity in foods. So, for foods, lipids can be seen as molecules that, for the most part, come in the form of triacylglycerides created by the bonding of three fatty acids to a glycerol backbone and that the difference between triacylglycerides is in the type and combination of the constituent fatty acids.

In foods, lipids have a number of important functions. For example, lipids provide organoleptic characteristics which are often used to determine the quality of foods (Kinsella, 1988). Such characteristics include mouthfeel, flavour, aroma, colour and texture. The importance of lipids in foods, however, is not limited to their sensory functions. Lipids also play a key role in the utilisation of fat soluble vitamins (A, D, E, K) and provide a concentrated source of metabolic energy and essential fatty acids. Oxidation of lipids, however, significantly alters some of these functions. In particular, the development of rancid flavours and aromas is common in food products with lipids and is often shelf life determining.

The chemistry of the oxidation reactions that take place in bulk oil systems is reasonably well understood. That is, researchers have been able to characterise many of the products of lipid oxidation and have provided a reaction mechanism that, although not proven beyond any doubt, is capable of explaining many of the reactions that take place (Frankel, 1998; Walstra *et al.*, 2006).

The information that is available, however, has not led to the development long shelf life food products that are rich in lipids. Nor has it resulted in a universal method to predict the progression of lipid oxidation in bulk oils or more complex foods such as emulsions. Many food manufactures still rely on expensive long term shelf life testing.

This thesis, therefore, seeks to combine the considerable qualitative information that is available in literature with quantitative analysis and experiments to develop mechanistically based models capable of predicting rates of lipid oxidation and the effects of system changes. Mathematical models, based on and validated by experimental data, will be used as analysis tools to drive development of new knowledge.

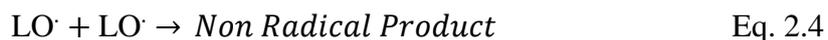
2.1 Lipid oxidation reactions

Lipids in foods, although essential for food function and nutrition, are not stable molecules. Lipid oxidation is a problem faced by food producers that is characterised by the formation of off flavours and aromas. Secondary oxidation products, as will be discussed in detail in further chapters, have been shown to be responsible for the rancid flavours and aromas that characterise the ‘going-off’ of products containing lipids (Jacobsen, 1999).

2.1.1 Lipid autoxidation reactions

According to Kubow (1992) the generally accepted process of lipid oxidation is that of a free radical mechanism termed lipid autoxidation. The mechanisms of lipid autoxidation can generally be broken into three stages (Coupland & McClements, 1996; Kubow, 1992). They are initiation (Eq. 2.1), propagation (Eq. 2.2 & 2.3) and termination (Eq. 2.4 to 2.7).





The reaction begins with an initiator molecule ($\text{X}\cdot$) reacting with a lipid molecule (LH). The initiator is said to abstract a hydrogen molecule from the methylene between a *cis* double bond pair of an unsaturated fatty acid molecule. The newly formed lipid radical is then free to react with molecular oxygen to form a peroxy radical ($\text{LOO}\cdot$). The peroxy radical then reacts with other lipid molecules to yield lipid hydroxides (LOOH) and more lipid radicals.

Interestingly, Kubow (1992) does not include a reaction depicting the breakdown of lipid hydroperoxides. According to the mechanism provided, lipid hydroperoxides are stable and unreactive. Furthermore, the reaction mechanism described in Eq. 2.1 to 2.7 suggests that only initiation and termination reactions occur. That is, radicals are formed by reaction of lipids with an initiator and removed by reaction with each other.

Most authors, including Frankel (2005) and Walstra *et al.* (2006), suggest that lipid hydroperoxides are only an intermediate in the reaction mechanism. In this case, it is suggested that lipid hydroperoxides are the first semi-stable product of lipid oxidation and that the lipid hydroperoxides do break down to form radical species. The newly formed radicals are said to react with lipid species to form more lipid hydroperoxide, or with other radicals to form stable non-reactive products. The cyclical formation, breakdown and re-formation of lipid hydroperoxides is characteristic of the propagation phase.

The third and final stage of lipid autoxidation is the termination stage in which free radicals are removed and, therefore, rendered unable to take part in any further lipid

oxidation reactions. The removal of free radicals can be achieved by either the combination of two radicals or by the donation of a hydrogen or electron by a suitable donor. It is these products that are usually responsible for the rancid flavours attributed to lipid oxidation (Frankel, 2005; Jacobsen, 1999).

2.1.2 Lipid autoxidation initiators

As already discussed, initiation is the first stage of lipid autoxidation. The general reaction is based on the formation of a free radical from an unsaturated lipid molecule by the action of an initiator (Angelo, 1996) as depicted in Eq 2.1.

Initiators are compounds that must be present in a system in order for lipid autoxidation to begin. Other factors that increase the rate of oxidation or are required for oxidation to propagate are not considered initiators of lipid autoxidation.

Proposed direct initiators include:

- 1) Transition metal ions
- 2) Photosensitisers and light

Metal catalysts

Much attention has been given to the overall effects of metal catalysts in the autoxidation of lipids. Interestingly, many of the discussions have centered on metal catalysts' ability to increase the rate of the propagation stage of lipid autoxidation (McClements & Decker, 2000; Osborn & Akoh, 2003). However, there have been suggestions that transition metals are capable of initiating lipid autoxidation.

Kubow (1992) describes how initiation can occur through the iron-mediated hemolytic cleavage of preformed hydroperoxides to form free radicals. The question that arises from the above theory is to how the preformed hydroperoxides are formed. Such hydroperoxides are likely to be the product of other lipid autoxidation reactions which were initiated by other initiators. If true, the reaction described by Kubow (1992) would be best described as a propagation reaction.

A review by Schaich (1992) suggested that high valence state transition metals can cause direct initiation of lipid autoxidation when reacted directly with lipids as shown in Equation 2.8.



This reaction, however, is taken from literature dating back to 1961 and is challenged by McClements & Decker (2000). It was concluded that the reaction given in Eq 2.8 occurs extremely slowly and is not believed to be an important initiator of lipid autoxidation. Although it seems unlikely that transition metals are responsible for the significant initiation of lipid autoxidation, their role in the propagation stage is quite clear and will be discussed in section 2.3.

Photosensitisers

Photoxidation occurs through one of two pathways (Kim *et al.*, 2006). The type I reaction involves the formation of superoxide anions and other radicals due to the transfer of hydrogen atoms or electrons by the interaction of a triplet sensitizer and molecular oxygen or other components. Type II reactions (as shown in Figure 2.1), which are also described by Kim *et al.* (2006), involve the generation of singlet oxygen by the transfer of energy from an excited triplet sensitizer to triplet oxygen.

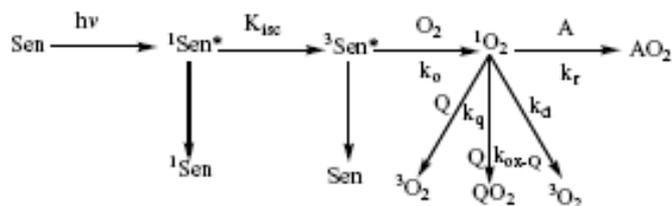


Figure 2.1: Figure, from Kim *et al.* (2006), shows the type II photoxidation reaction.

Photosensitised autoxidation does not require the formation of lipid radicals as singlet oxygen is able to react directly with double bonds of lipid molecules. It has been

shown that the reaction rate of singlet oxygen and linoleic acid is approximately 1450-1500 times greater than that of triplet oxygen and linoleic acid (Kim *et al.*, 2006; Angelo, 1996).

The presence of photosensitisers, such as chlorophyll, does not automatically mean that the substrate they are found in will oxidise. Experiments conducted by Kim *et al.* (2003) illustrated that chlorophyll containing products do not show any increase in peroxide values over time when stored in containers covered in foil to prevent light penetration. Infact, a paper published by Lanfer-Marquez *et al.* (2005) discussed the potential antioxidant effect of chlorophylls when products containing chlorophylls are stored in the absence of light.

It is evident that the combination of light and a photosensitiser will promote lipid oxidation in food products. The effects of different sources of light, however, are less well known. It is suggested that the wavelength, and therefore energy, of the light supplied to a product has a significant effect on the rate of lipid oxidation (Thron *et al.*, 2001; Boselli *et al.*, 2005). Studies on turkey meat have shown that the use of a light source rich in light from the blue end (wavelength of ~480nm) of the visible light spectrum results in oxidation rates greater than sources of light rich in light from the red end (wavelength ~680nm) of the visible light spectrum. This finding correlated with the maxima absorbance range of different oxidation states of haemoglobin which is between 410 and 588nm.

Similar findings have been noted for the wavelengths of light that promote lipid oxidation in chlorophyll containing products. A study by Thron *et al.* (2001) found that sunflower oil spiked with chlorophyll containing pigments was more reactive to light around 665nm than around 408nm, however, exposure to the full spectrum of light resulted in significantly greater oxidation rates than was obtained when using light filters.

Hansen & Skibsted (2000) found that the nature of the oil (purified to remove antioxidant tocopherol molecules), the wavelength of light and the presence or absence of β -carotene had significant effects on the rates of oxidation in rapeseed oils. They concluded that the wavelength of light was the most significant factor and that

exposure to 366nm wavelength light caused significantly greater rates of oxidation compared to oils exposed to 405 and 436nm wavelength light. Interestingly, they also concluded that the antioxidant capacity of β -carotene was due to its ability to filter light rather than react with radical species.

Light absorption

According to Hansen & Skibsted (2000), only light that is absorbed can initiate chemical reactions. The effects of photosensitisers have already been discussed but a discussion on the measures of light absorption is required.

The Beer-Lambert Law is commonly used to describe the transmittance of light through a dilute solution (Grum & Becherer, 1979). Originally the Beer-Lambert Law was described as

$$T = \frac{\phi}{\phi_0} = e^{(-k \cdot c' \cdot l)} \quad \text{Eq. 2.9}$$

where k is the molecular cross-section in square centimeters, c' is the number of molecules per cubic centimeter, l is the optical path length in centimeters and Φ_r the transmitted flux. Equation 2.9 can also be rewritten as:

$$A = \log\left(\frac{1}{T}\right) = a_c' \cdot c \cdot l \quad \text{Eq. 2.10}$$

where A is the absorbance, a_c' is the molar absorption coefficient and c is the molar concentration.

Hansen & Skibsted (2000) suggest that the proportionality factor between the light absorbed (I_{abs} in quanta per minute per milliliter) and the rate of resulting chemical reaction is called the quantum yield (Φ) which is defined in Equation 2.11

$$\phi = \frac{\text{molecules reacted}}{\text{photons absorbed by reacting compound}} = \frac{\Delta C_i}{Q_i} \quad \text{Eq. 2.11}$$

where ΔC_i is the change in concentration of the specified compound resulting from absorption of the number of photons by this compound, Q_i .

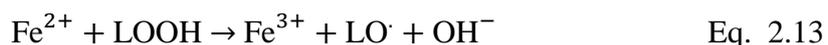
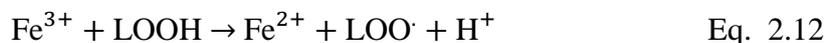
Despite there being literature discussing the effects of light on oxidation (Mortensen *et al.*, 2004), it is quite difficult to find molar absorbance coefficients for common foods. This is likely to be due to the vast number of different compounds that are present in even the most simple food products. However, during early investigations conducted by placing simple emulsions, in the form of common store bought mayonnaise, in a spectrophotometer and measuring the light that passes through the sample, it became quite obvious that light, irrespective of the wavelength, does not penetrate the product any significant distance. Certainly the investigations showed that no light passed through a 1cm thick sample of mayonnaise. For photosensitisers to have any significant impact on the rates of lipid oxidation, they must be present near the surface of the product.

The actual volume of the product that is exposed to light, is dependent on the shape of the product/package. For example, if a product were in a cylindrical glass bottle (assumed to provide no resistance to light) that was 10 cm tall and had a radius of 3 cm and light was able to penetrate 0.5cm into the bottle, approximately 31% of the bottle would be exposed to light (ignoring the ends). If, however, a 5cm cylinder with the same volume were used, 22% of the product would be exposed by light. The effects of supply of light and concentration of photosensitisers, therefore, should not be decoupled.

2.1.3 Free metal ions: catalysis of lipid autoxidation

Metal catalysts such as the ferrous and ferric forms of iron have been shown to significantly increase the rate of lipid oxidation in food products (Alamed *et al.*, 2006; Osborn *et al.*, 2003; Kubrow, 1992). As suggested above, metal catalysts have the largest effect during the propagation stage of lipid autoxidation. From a chemistry point of view, the exact reactions that take place during iron catalysed oxidation

reactions are being debated (Ahn & Kim, 1998; Minotti & Aust, 1992,). The generally accepted iron catalysed oxidation reactions (Carlsen *et al.*, 2005; McClements & Decker, 2000; Schaich, 1992; Sheldon & Kochi, 1981) are shown in Equations 2.12 and 2.13.



Equations 2.12 and 2.13 show the reduction and oxidation of iron species to form highly reactive peroxy (LOO·) or alkoxy (LO·) radicals (McClements & Decker, 2000). These radicals are then free to react with other lipid molecules during the propagation stage of lipid autoxidation. Examples of these reactions, shown below, are given by McClements & Decker (2000).



Although iron species are the only ones discussed above, they are not the only metal species that are capable of catalysing lipid autoxidation. Schaich (1992) described how other transition metals such as copper and nickel can catalyse lipid autoxidation reactions. The important factor to consider is the source of metals in foods. Iron is concentrated on as it is abundant in natural systems such as heme-iron and can be introduced through processing equipment which is predominantly constructed of iron based materials (Shahidi & Hong, 1991).

Heme-iron and lipid autoxidation

Many different mechanisms for the catalysis of lipid oxidation by heme-iron have been suggested. Interestingly, the extensive research conducted on this subject area has failed to fully describe the mechanisms and processes involved in heme-iron catalysis of lipid oxidation.

Much attention has been given to the oxidation state of the iron species in heme-iron proteins (hemoglobin, myoglobin). One mechanism often used to describe the pro-oxidant activity of heme-iron species is based on the oxidation/reduction of heme-iron by peroxides as shown in Figure 2.2

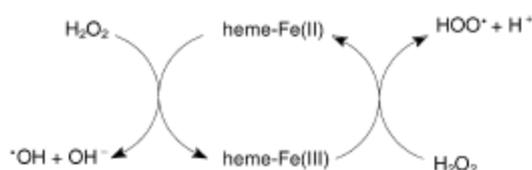


Figure 2.2: Figure from Carlsen *et al.* (2005). Figure describing Fenton-like oxidation/reduction of heme-iron by peroxides.

Oxidation of heme-iron to higher oxidation states (heme-Fe(IV)) has also been shown to occur with peroxides. In this reaction, the mechanism involves the transfer of one electron and an oxygen atom from the peroxide to the heme group (Carlsen *et al.*, 2005). The regeneration of heme-Fe(III) is possible as the heme-Fe(IV)=O species is capable of extracting hydrogen from a lipid hydroxide to form a lipid radical as shown in Figure 2.3

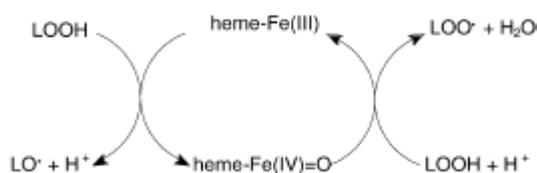


Figure 2.3: Figure from Carlsen *et al.* (2005). Figure describes the oxidation of heme-Fe(III) to higher oxidation states.

The complexity of heme-iron reactions in lipid oxidation is not realised by the reactions depicted in Figures 2.2 and 2.3. A more complex set of reactions, called the pseudoperoxidase mechanism, is proposed by Carlsen *et al.* (2005) that involves a transfer of two electrons from heme-Fe(III) and subsequent regeneration by the donation of electrons from suitable donors. These donors can either be lipids or proteins.

In the two electron transfer reaction, characteristic of the pseudoperoxidase mechanism, an oxygen atom from a peroxide binds to the heme-iron (Carlsen *et al.*, 2005). One electron is donated from the Fe(III) which is oxidised to Fe(IV) while the other comes from the porphyrin ring which is oxidised to a porphyrin radical (HemeFe(IV)=O). The porphyrin radical readily oxidises lipid molecules to form lipid radicals which, as already discussed, take part in other propagation reactions.

The best way to cement the ideas and the interaction of the three types of heme-iron oxidation of lipids is by the use of a diagram proposed by Carlsen *et al.* (2005) and reproduced below in Figure 2.4.

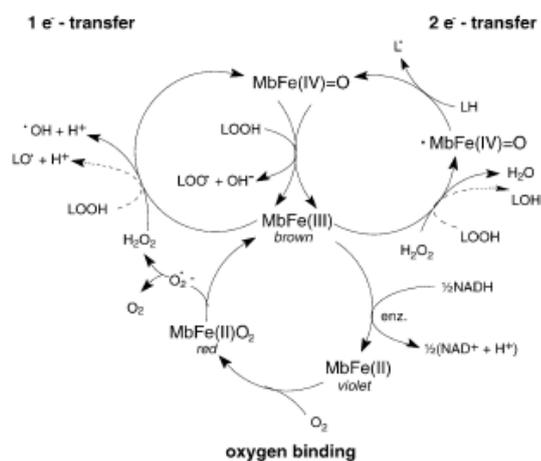


Figure 2.4: Figure from Carlsen *et al.* (2005). Figure shows the interaction of three suggested mechanisms of heme-iron catalysed lipid oxidation reactions.

Despite the above discussions, hemoglobin has been shown to have a strong pro-oxidant effect in oil-in-water emulsions, regardless of the presence of ascorbate which releases iron species from hemoglobin (Ahn & Kim, 1998). The same study concluded

that hemoglobin itself is a strong pro-oxidant in oil emulsions and that the iron released from the hemoglobin is less important than the effect of hemoglobin itself. Similar findings given by Shahidi & Hong (1991) who noted that the release of iron species from added iron-porphyrin (myoglobin, hemoglobin and hemin) may not be as important as the effects of iron-porphyrin on the rate of autoxidation of ground pork slurries.

Interestingly, the same study noted that the addition of chelating agents, such as Ethylenediaminetetraacetic acid disodium salt (EDTA), caused significant decreases in the rate of oxidation in the pork slurries, including those with minimal free iron species. The binding of the chelating agents to the free iron species was expected, but the inhibition of the pro-oxidant effect of the iron-porphyrin species was not.

The lipid to heme protein ratio of foods has been shown to have a strong effect on the ability of heme proteins to catalyse lipid oxidation reactions (Baron *et al.*, 2002). At high linoleate to heme protein ratios it has been shown that heme proteins possess strong pro-oxidant activity while at low ratios the pro-oxidant effect is heavily diminished. To introduce extra complication, a study by Richards & Dettmann (2003) have shown differences in the pro-oxidant effects of hemoglobin from different animal sources. The reasons for the difference in pro-oxidant effect were based on differences in heme structure and chemistry, but will not be explored in this review.

2.1.4 Secondary oxidation products by hydroperoxide breakdown

Discussions outlining the breakdown of hydroperoxides are given by Min & Lee (1999) and Frankel (1991 and 1982). Fat hydroperoxides are generally tasteless and odourless, however their decomposition products can be extremely potent and have a large impact on the flavour and aroma of foods (Frankel, 1982). Some volatile products such as aldehyde cleavage products are able to affect the flavour of products at levels lower than one part per million. It has been suggested that the breakdown of hydroperoxides is most likely to occur through the cleavage between two oxygen molecules rather than between hydrogen and oxygen molecules as shown below in Figure 2.5.

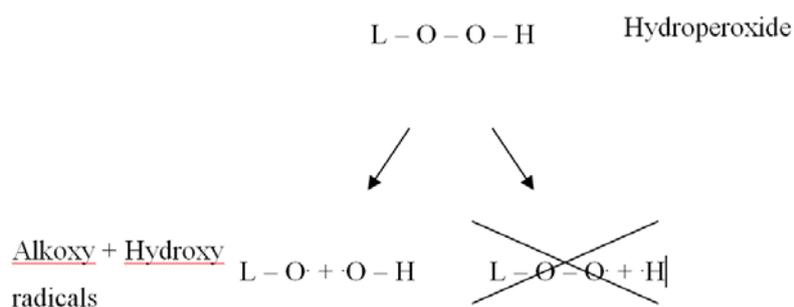


Figure 2.5: Figure shows the two potential breakdown products of lipid hydroperoxides. According to Min & Lee (1999), alkoxy and hydroxyl radical products are preferred.

The choice of one reaction pathway over another can be explained by the activation energies of each of the reactions. The activation energy required for splitting the component between the two oxygen molecules is 184 kJ/mol while 377 kJ/mol is required to break the compound between the oxygen and hydrogen molecules (Min & Lee, 1999).

The alkoxy radical is cleaved in one of two positions. β -scission of the carbon-carbon bond on the side of the oxygen containing carbon atom results in oxo-compounds and alkyl radicals. β -scission of the carbon-carbon bond between the double bond and oxygen containing carbon atom results in the production of olefin radicals and alkyl-compounds. These products are said to react with either $\cdot\text{OH}$ or $\cdot\text{H}$ (Frankel, 1991). The reaction with $\cdot\text{OH}$ produces vinyl alcohols which are unstable and tautomerise to yield saturated aldehydes. The reaction with $\cdot\text{H}$ yields either α -olefins or a short chain ester.

Frankel (1991) suggests that the β -scission reactions discussed above are able to explain the majority of volatile products from the thermal decomposition of hydroperoxides of oleate, linoleate and linolenate.

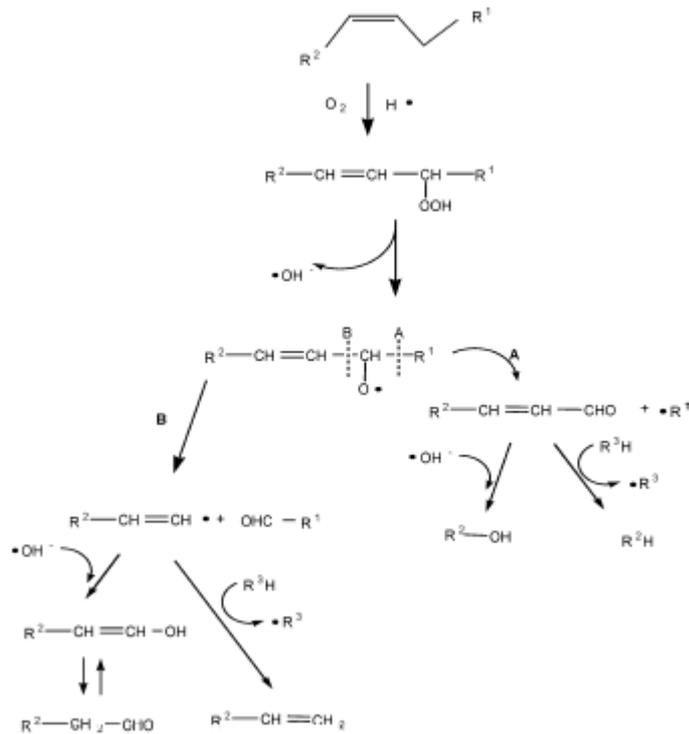


Figure 2.6: Decomposition of lipid-hydroperoxides to form secondary oxidation products. Figure from Choe & Min (2006).

The basic cleavage and breakdown reactions given above cannot explain all the volatile oxidation products observed in lipid oxidation reactions (Frankel, 1981). Unsaturated aldehydes and ketones formed from the primary decomposition of hydroperoxides are susceptible to further oxidation. This secondary oxidation results in low molecular weight compounds that are often volatile and contribute to the rancid flavour of oxidised fats and fat containing products.

2.2 Antioxidants

Antioxidants either delay the onset or diminish the rate of lipid oxidation in foods containing lipids (Reische *et al.*, 1998; Roginsky & Lissi, 2005). Antioxidants do not enhance but rather maintain the quality of foods over a period of time that is greater than if they were not present in the food. Many antioxidants occur naturally in foods while others are either concentrated from natural sources or created synthetically and added directly to foods. Antioxidants can be classed in a number of ways. Reische *et*

al. (1998) use two classes, primary and secondary, according to their mechanism of action.

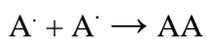
2.2.1 Primary antioxidants

Primary antioxidants are often referred to as chain breaking antioxidants (Reishe *et al.*, 1998). They are free radical acceptors that delay or inhibit initiation or hinder the propagation stages of lipid autoxidation. By donating hydrogen atoms to radicals, primary antioxidants are able to convert lipid and peroxy radicals into stable compounds which are unable to take part in further lipid oxidation reactions. Examples of such reactions are given by Reishe *et al.* (1998) and are shown below in Eq. 2.18 to 2.20



As can be seen in Eq. 2.18 to 2.20, the reactions involve the formation of antioxidant radicals, $\text{A}\cdot$. The antioxidant radical, however, has very low reactivity with lipids. The reaction of the antioxidant radical with lipids is therefore very slow which results in a greatly reduced rate of the propagation stage of lipid autoxidation (Reishe *et al.*, 1998). Note that primary antioxidants will not halt propagation reactions but will greatly reduce the rate of them. Antioxidant radicals have been shown to participate in termination reactions (Reishe *et al.*, 1998). The reaction of an antioxidant radical with either a peroxy or oxy radicals or itself results in a stable, non-radical product as shown in Eq. 2.21 to 2.23.





Eq. 2.23

Generalised kinetics of primary antioxidants

As described by Reische *et al.* (1998), the addition of primary antioxidants creates a lag phase in which free radicals are consumed by the primary antioxidants. Once consumed, the antioxidants are generally deemed unable to quench any newly created radicals. This is the point where the lag phase ends and autoxidation continues unhindered. Results from research by Huang *et al.* (1996a & 1996b), as shown below in Figure 2.7, shows the lag phases that can be introduced by the addition of primary antioxidants to foods containing lipids.

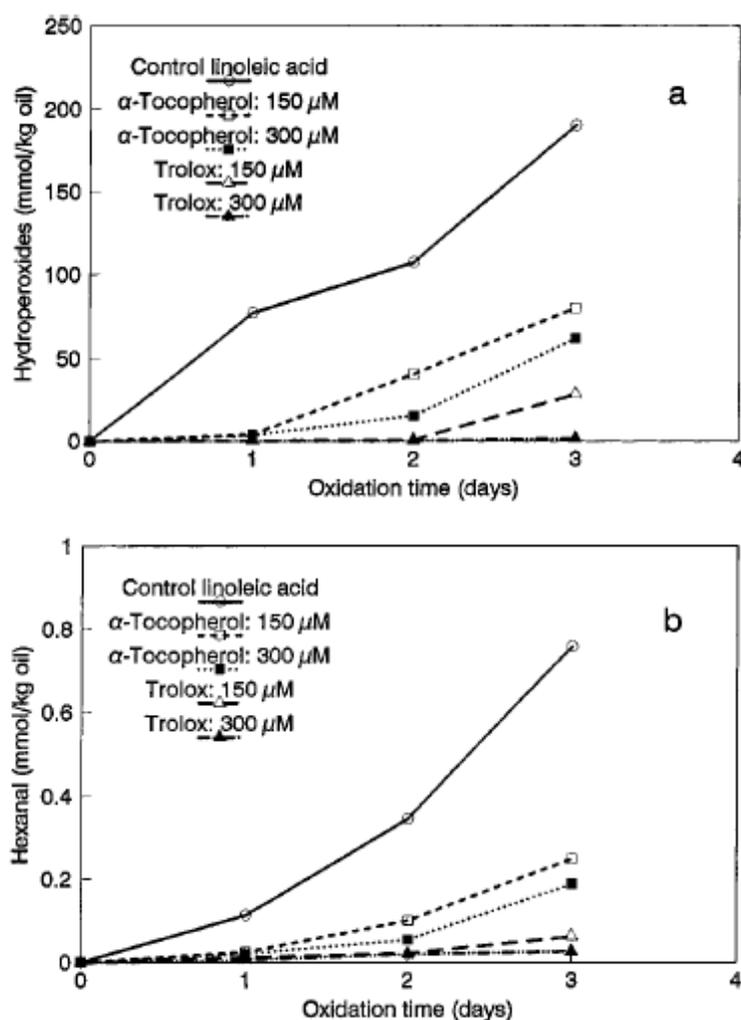


Figure 2.7: Figure from Huang *et al.* (1996a). Figure describes the reduction in the rate of lipid hydroperoxide and hexanal production from linoleic acid with different antioxidants added.

From the above discussion it should be clear that the addition of primary antioxidants before considerable oxidation has occurred hinders the creation of new radicals and causes a lag phase. If the primary antioxidant were added to a product already containing large amounts of radical species, the effect would likely depend on the concentration of radicals/lipid hydroperoxides relative to the antioxidants supplied. Although not shown in literature, it seems reasonable to expect that antioxidants will remove radicals no matter when added to an oil. The effect the antioxidants have on the rate of oxidation, however, will likely be quite different depending on the state of the oil and the concentration of antioxidants added.

2.2.2 Secondary antioxidants

Secondary antioxidants slow oxidation reactions but do not transform radicals to stable non-radical products (Reishe *et al.*, 1998). Examples of secondary antioxidant action include the ability to chelate metals, replenish primary antioxidants by donating hydrogen, deactivate singlet oxygen, absorb ultraviolet light or act as oxygen scavengers. Metal chelators and oxygen scavengers will be discussed in this section.

Metal chelators

As already discussed transition metals, such as iron and copper, are known to catalyse lipid oxidation reactions. Binding metals to other species that renders the metal unable to catalyse reactions would greatly reduce the rate of oxidation reactions. Such a process is called chelation. By binding metal ions, the chelator is able to reduce the redox potential and stabilise the oxidised form of the metal (Reishe *et al.*, 1998; Yoshida *et al.*, 1993).

Ethylenediaminetetraacetic acid disodium salt (EDTA) is an example of a compound that is commonly used at low concentrations in foods for its ability to chelate metal ions. Studies by Alamed *et al.* (2006) showed that a concentration of just 2.5µM of EDTA was able to almost completely inhibit oxidation reactions in freshly prepared samples of salmon oil.

Oxygen scavengers and reducing agents

Oxygen scavengers have been used throughout the food industry for more than 20 years (Tewari *et al.*, 2002). Commonly used oxygen scavengers include ascorbic acid, sodium erythorbate and erythorbic acid (Reishe *et al.*, 1998). The theory behind the use of oxygen scavengers is that the removal of oxygen to create a low oxygen environment will reduce the rate of oxygen concentration dependent lipid oxidation reactions.

In many circumstances, the rate of oxygen absorption is of great importance as only small amounts of initiation products need to be present before the reaction system becomes self-catalytic (Tewari *et al.* 2002). In terms of this thesis, there is little need to know the exact mechanisms of oxygen scavenging. Instead it is important to know whether or not scavengers are present (through deliberate addition or as a consequence of the ingredients used) in a system and that they will modify the atmosphere within a package enough to change the rates of lipid oxidation reactions.

2.3 Common methods of detecting & measuring lipid oxidation

There are a number of different methods of detecting and measuring lipid oxidation reactions. Each method has its positive and negative attributes which need to be considered when choosing the appropriate method. It is suggested that the methods of measuring lipid oxidation reactions can be separated into two groups according to the target compound to be measured:

- 1) Methods measuring universal lipid oxidation reactants (oxygen/lipids)
- 2) Methods targeting lipid oxidation products

2.3.1 Peroxide value

Perhaps the most well-known measure of lipid oxidation is the peroxide value (PV). As has been discussed in Section 2.1, lipid hydroperoxides are primarily formed by the reaction of a lipid peroxy radical (LO_2^\cdot) with a lipid molecule (LH) as shown in Eq. 2.3. Current methods of measuring the peroxide value of oils are based on the oxidation of potassium iodide (KI). The method assumes that the substances that cause the oxidation of KI are lipid hydroperoxides.

Typical PV curves described in literature begin with a gradual increase in concentration as lipid oxidation occurs. The increase reaches a peak at which the rate of lipid hydroperoxide formation is equal to the rate of lipid hydroperoxide destruction. After this point, the relative rates of lipid hydroperoxide formation and destruction shift and result in a decrease in the concentration of lipid hydroperoxides. A bell curve, as shown in Figure 2.8, is expected (Gutierrez & Fernandez, 2002).

Rather than getting the bell curve, most literature shows a linear increase in the lipid hydroperoxide concentration with time. Possible reasons for this include:

- 1) Depletion of reactants before the lipid hydroperoxide concentrations change significantly
- 2) Researchers ending their experiments before lipid hydroperoxide concentrations change significantly
- 3) The proposed bell curve is not true in some or all conditions.

The first two reasons for not seeing the bell shaped curve are easy to understand. The third, however, is not quite so straight forward to judge as it is quite difficult to find literature that actually shows the bell type curve.

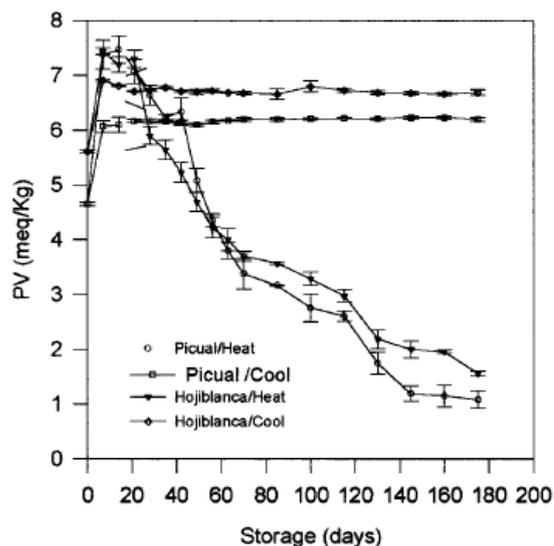


Figure 2.8: Figure from Gutierrez & Fernandez (2002). Figure shows the typical peaks formed when PV values are plotted against time.

The PV measure serves as an indicator of reactions that will continue to form products that are commonly related to rancidity (Irwin & Hedges, 2004). As PV is commonly measured using a simple and relatively quick titration method, it is commonly used to examine the shelf life of products over a period of time as shown by Figure 2.8 above. However, PV measures may not correlate well with sensory analysis. For example, olive oils may not be deemed rancid by sensory analysis until the PV reaches 20meq/kg while fish oils may develop rancidity at levels less than 1 meq/kg (Gordon, 2004). Furthermore, it is quite common to find that the product is deemed rancid long before the peak PV is reached (Labuza, 1971).

2.3.2 *Para*-anisidine value

According to Irwin & Hedges (2004), the *para*-anisidine value (AV) is a measure of the unsaturated aldehyde content in fats and oils. The measure is based on the reaction of unsaturated fats with *para*-anisidine in glacial acetic acid to form yellow compounds which can be measured according to their absorbance at 350nm.

Although the AV measure is only sensitive to unsaturated aldehydes, sensory analysis has also proven that the detectable levels of unsaturated aldehydes are much lower than that of saturated aldehydes (Gordon, 2004). The AV, therefore, can be

successfully used to monitor secondary oxidation products (albeit a small proportion of all oxidation products) over time as has been done in many studies including one by Osborn & Akoh (2004).

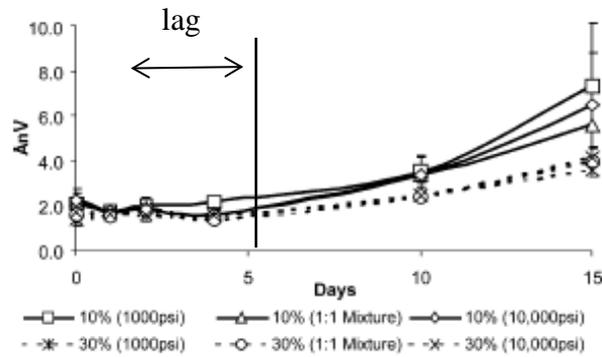


Figure 2.9: Figure from Osborn & Akoh (2004). Figure shows a typical graph obtained when AV is plotted against time.

A typical graph that results from the measure of AV over time is shown in Figure 2.9. The figure shows a lag phase before the production of unsaturated aldehydes. Using such a measure, it is possible to begin to try and understand the relationship between the formation of lipid hydroperoxides and secondary oxidation products. For example, during the lag phase (the first five days shown on Figure 2.9) it seems plausible that the concentration of lipid hydroperoxides is building up but is yet to reach a concentration where the breakdown of lipid hydroperoxides to form secondary products is significant. However, without having information regarding the concentration of other reactants such as oxygen, it is impossible to determine the exact relationship between the formation of lipid hydroperoxides and secondary oxidation products.

2.3.3 Thiobarbituric acid value

Originally, the thiobarbituric acid, used in the TBA methodology, was thought to principally react with malondialdehyde (Irwin & Hedges, 2004) a secondary product of lipid oxidation. More recently a number of articles have been published that suggest that TBA reacts with a series of compounds other than malondialdehyde, thus leading to the formation of a name for the measure called TBARS or thiobarbituric acid reactive substances. Examples of such are found in a review by Hoyland & Taylor

(1991) who suggested that a number of unsaturated aldehydes are capable of taking part in the TBA reaction while Guillén-Sans & Guzmán-Chozas (1998) suggest that, besides aldehydes, other substances such as ketones, ketosteroids, acids, esters, sugars, imides and amides (urea), amino acids, oxidised proteins, pyridines, and pyrimidines can react with TBA.

TBARS is commonly used as an indicator in meat products (Irwin & Hedges, 2004). Alamed *et al.* (2006) were successfully able to show the changes in lipid oxidation rates due to differences in concentrations of EDTA. Although none of the papers used in this review used the TBA measure on non-meat products, a review by Guillén-Sans & Guzmán-Chozas (1998) suggest that the TBA measure can be used to measure the levels of oxidation in vegetable oils as well as lard and cooking fats although the same authors state that, “the results obtained with the TBA assay are highly correlated with the oxidised flavour of animal foods, such as milk”.

2.3.4 Iodine value

The Iodine Value (IV) is an overall indication of the level of unsaturation of fats and oils (Hudson, 1989). Since research has proven that the rate of oxidation of oils in excess oxygen is increased by the level of unsaturation of the oils (Parker *et al.*, 2003), a high initial IV should indicate that the rate of oxidation will be high. As oxidation reactions take place, polyunsaturated fatty acids (PUFA) polymerise or breakdown into smaller molecules with fewer double bonds which should correlate with a reduction in the IV of the oils over time (Hudson, 1989).

Although introduced in 1884, the iodine value is still used by some researchers today. Naz *et al.* (2004) used the IV in combination with the PV and AV's. Although not explicitly stated, their data suggested that the PV and AV's were capable of tracking oxidation reactions while the IV was not sensitive enough to the chemical changes caused by the oxidation reactions. Furthermore, results from studies by Farag & El-Anany (2006) yielded significant decreases in TBA, PV and CD but not in IV, also suggesting the IV is not particularly suited for tracking oxidation reactions in oils.

2.3.5 Conjugated dienes

According to Shahidi & Wanasundara (1997), the oxidation of polyunsaturated fatty acids is accompanied by an increase in the ultraviolet absorption (234 and 236nm) of the product. This is due to the presence of ultraviolet light absorbing conjugated dienes (CD) in substances such as the hydroperoxides of linolenic and linoleic acids (Irwin & Hedges, 2004).

The CD test, when compared to that of the PV tests, has been described as being simpler, not dependent on colour development or chemical reactions and requires a much smaller sample for testing (Shahidi & Wanasundara, 1997). The same authors, however, suggest that the presence of double bonds in carotenoids may cause carotenoid rich products to have a naturally high absorbance at 234 and 236nm.

Early research described by Shahidi & Wanasundara (1997) indicated that an increase in absorption due to the formation of conjugated dienes is proportional to the uptake of oxygen and that CD measures can correlate well with other measures such as PV. Makhoul *et al.* (2006) used a combination of PV, CV, AV, IV, TBA and hexanal gases as indicators of rancidity of sunflower oils. They found a seemingly good correlation between PV and CD measures as shown in Figure 2.10 below.

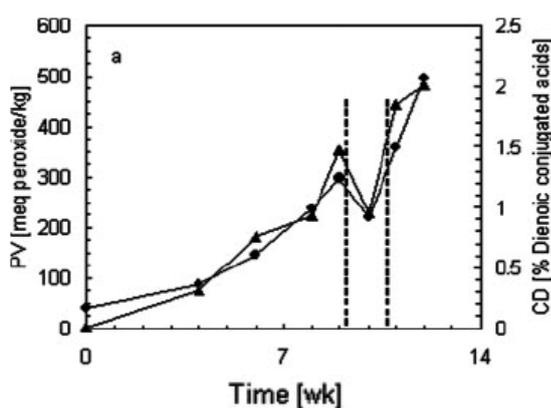


Figure 2.10: Figure from Makhoul *et al.* (2006). Figure shows the correlation between PV and CV for times less than that required to produce a peak concentration of hydroperoxides.

2.3.6 High performance liquid chromatography

High performance liquid chromatography (HPLC) can be used to identify and measure a number of different compounds either responsible for or products of lipid oxidation reactions. There are methods for determining the state of an oil and predicting its ability to resist lipid oxidation. The IV is one such method that has already been discussed. However, if more detailed information is required, it is possible to use HPLC (often in combination with mass spectroscopy).

Irwin & Hedges (2004) describe how different lipid classes can be identified using HPLC equipment. Such classes include free fatty acids (FFA) as well as mono- and diglycerides. The accurate determination of the fatty acid composition of an oil should provide insight into the oil's ability to resist oxidation. The disappearance of polyunsaturated fatty acids (PUFA) and/or creation of FFA can be used to monitor lipid oxidation reactions.

Instead of monitoring the breakdown of reactants, it is possible to measure the production of non-volatile oxidation products using HPLC techniques. Steenhorst-Slikkerveer *et al.* (2000) successfully used HPLC methods to determine the non-volatile oxidation products of vegetable oils. Unfortunately, due to the length of time required to process a sample, it seems unlikely that HPLC methods would be useful for processing samples of oils that are not fully oxidised. That is, the samples placed into the HPLC are likely to continue to oxidise during the time required to prepare and process the sample. HPLC also requires the use of expensive and often unavailable equipment and, therefore, are not particularly well suited for industrial situations.

2.3.7 Gas chromatography

As already discussed, the end products of lipid oxidation are a mixture of complex compounds many of which are volatile. Such volatile products include alcohols, aldehydes, ketones, alkanes and organic acids (Irwin & Hedges, 2004). Gas Chromatography (GC) methods have been used for a number of years and are becoming one of the most popular methods of measuring lipid oxidation reactions.

GC has proven to be sensitive enough to determine small changes in volatile concentrations and, when coupled with a mass spectrophotometer (MS), can also be used to identify a very large range of different volatile compounds. Richards *et al.* (2005) were able to use GC coupled with MS to identify and quantify the volatile oxidation products of canola oil. Figure 2.11 shows a partial GC-MS chromatogram that was produced from their experiments.

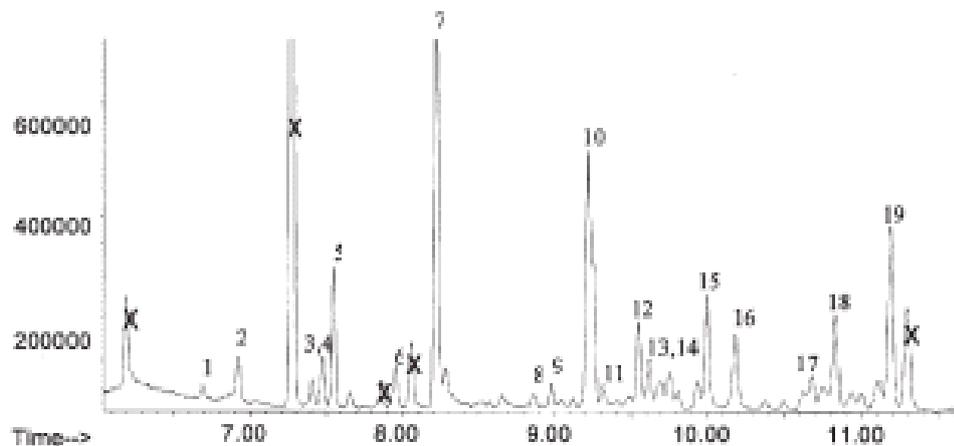


Figure 2.11: Figure from Richards *et al.* (2005). Figure shows a typical chromatogram in which each peak corresponds to a different compound and concentration. Major peaks include: (7) hexanal, (10) limonene, (19) *trans*-3,5-octadien-2-one/nonanal

GC can also be successfully used to monitor the oxidation process over a period of time. Andersson and Lingnert (1999) used GC to quantify the levels of 2-pentanal produced in the headspace of samples of rapeseed oil. Figure 2.12, from Andersson and Lingnert (1999), clearly shows the effects of changing the headspace oxygen concentration as measured by GC.

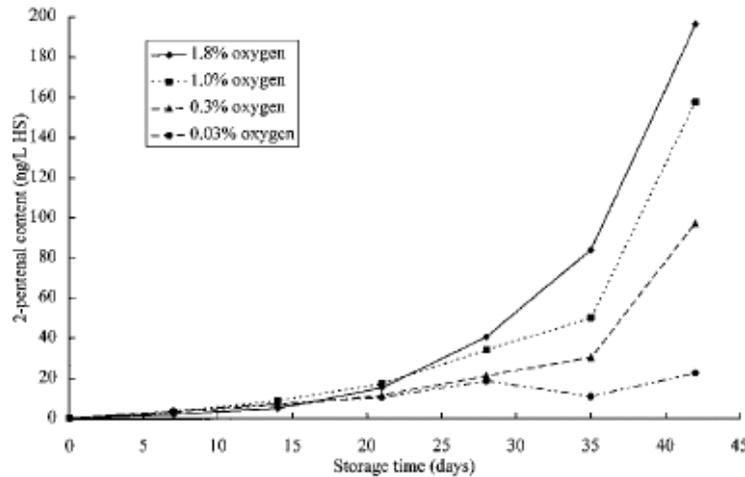


Figure 2.12: Figure from Andersson and Lingnert (1999). Figure shows how GC can be used to track changes of head spaces gases over time.

The choice of volatile to analyse depends on the lipid being oxidised. Silvestre *et al.* (2000) used the concentration of propanol in the headspace of samples of salmon oil as their measure of lipid oxidation reactions. Andersson and Lingnert (1999) chose to investigate a range of volatile gases by coupling GC and MS together. They found differences in the rates of changes of the different volatiles under different conditions. This suggests that the use of a coupled GC-MS would provide more detail and allow for a more thorough and detailed picture of the system being tested.

Like HPLC, the use of GC requires specialist equipment that is often not affordable or readily available for many food producers. The results need careful interpretation as changes to the oxidation system can bring with it a change in the products measured. A less specific measure would be suited if it were needed for application over a wide range of products/conditions.

2.3.8 Rancimat

Generally speaking, the rates of lipid oxidation in oils such as canola, sunflower and olive oils are quite slow. Storage of these oils under normal conditions (~25°C) results in a shelf life of the order of several months. To circumvent the time requirements for long term storage, it is common for researchers to conduct experiments at elevated

temperatures to increase the rates of reactions and to gain data in a much shorter period of time. The Rancimat system was designed with this in mind.

The Rancimat test is generally conducted at 98°C although some have chosen to use higher temperatures to further increase the rates of oxidation (Mateos *et al.*, 2006). In the Rancimat test, a sample of oil is heated to the selected temperature and air is bubbled through it. During this process, volatile oxidation products are formed, recovered and measured conductimetrically in distilled water. According to Mateos *et al.* (2006), the time required to produce a sudden increase in the conductivity determines the induction period, which is used as a measure of the stability of the oil.

Although the Rancimat test is a very fast way of gaining data, it comes at a cost. Firstly, the evolution of volatiles from non-oil sources (i.e. not caused by the development of rancidity) can render the results of the Rancimat test useless as they change the conductivity measurements significantly (Allen & Hamilton, 1994). Common antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) cannot be used during Rancimat tests for this reason. Secondly, the use of very high temperatures will almost definitely bring with it a change in reaction mechanism. This will undoubtedly cause skewed results that would not be gained had the same process been carried out at a lower temperature. As a result, the use of extreme temperatures during oxidation reactions can only be found sporadically throughout literature.

2.3.9 Oxidograph

The Oxidograph is similar to the Rancimat method in that high temperatures (~100°C) are employed to increase the rates of lipid oxidation and gain results quickly. The Oxidograph (an automated version of the Astell apparatus), however, monitors the pressure above a sample of oil without bubbling. In theory, the oxidation of the oil in the closed system will consume oxygen and therefore reduce the pressure in the system (Allen & Hamilton, 1994). This system, unlike the Rancimat, is less sensitive (but not unaffected) by additives like BHA and BHT.

The results gained from the Oxidograph, however, must be carefully analysed. Allen & Hamilton (1994) have shown that the effects of oxygen consumption (causing a pressure decrease) can be counteracted by the evolution of some volatiles (that increase the pressure). In some cases the oxygen absorption appears very nearly zero during the induction period. Only when the rate of oxygen consumption becomes much greater than the production of volatile products will the reading deviate significantly from the baseline.

It seems the Oxidograph, like the Rancimat, is unable to give fast results that can be confidently used to generate kinetic models for foods in normal storage conditions. The use of high temperatures is risky and the effects of volatiles can lead to erroneous results. Simply put, a direct measure of oxygen in the oil/headspace would be needed to avoid this.

2.4: Sensory impact of lipid oxidation

As already stated, lipid oxidation gives rise to compounds responsible for objectionable flavours (Jacobsen, 1999). It has been shown that the rancid flavours that characterise lipid oxidation reactions are due to the formation of secondary oxidation products such as aldehydes, ketones and alcohols (Frankel, 1991).

Although sensory evaluation ultimately decides the consumer acceptability of a product, it is often difficult to conduct sensory analysis for all experiments carried out. Additionally, the time and money required to train sensory panels to allow for pseudo-quantitative and repeatable data to be extracted is often not justifiable. It is often much more appropriate to use chemical tests to determine levels of oxidation in products.

2.4.1 Sensory and analytical method correlations

As one would expect, the degree of correlation between analytical tests and sensory analysis depends on the analytical test conducted. Different analytical tests target different compounds as their measure of oxidation. For example, the peroxide value measures initial products (hydroperoxides) of lipid oxidation while the anisidine value is assumed to be a measure of aldehydes produced by secondary reactions.

More generalised analytical techniques, which target a range of secondary oxidation products (i.e. AV or TBA), tend to provide good correlation with sensory analysis in most food products are available. Gas chromatography coupled with mass spectroscopy as well as electron spin techniques have been successfully used to measure lipid oxidation reactions and show good correlation with sensory analysis (Frankel, 2005).

Jacobsen (1999) used PV and AV's as well as sensory panels to track the progression of lipid oxidation and discussed the link between the analytical techniques and sensory analysis. Since the rancid flavours of lipid oxidation are secondary products it is not surprising that the correlation between peroxide values and sensory scores presented by Jacobsen (1999) and shown in Figure 2.13a was very poor. The anisidine value, however, measures aldehyde levels which are secondary products of lipid oxidation reactions. So, when the anisidine value was plotted against sensory scores as shown in Figure 2.13b, a good or better correlation was expected.

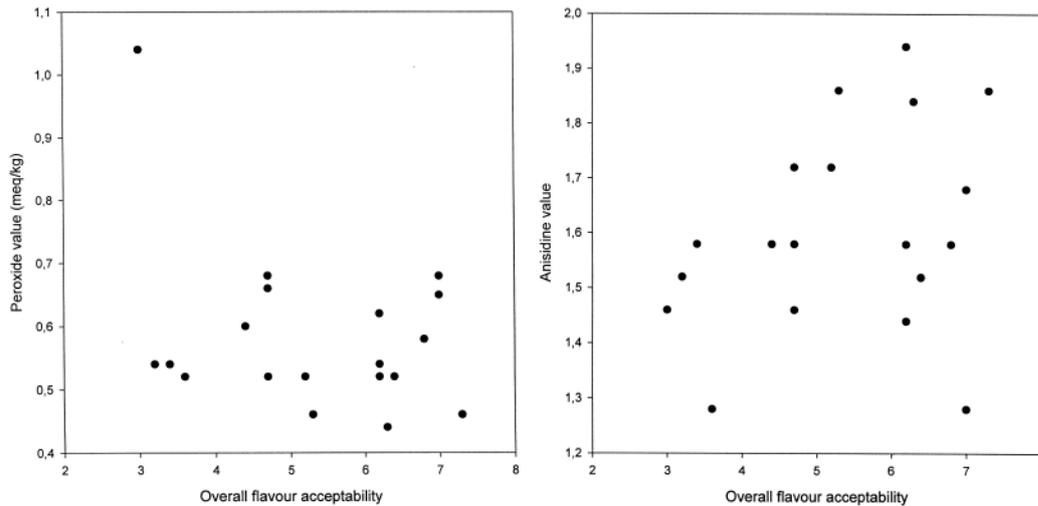


Figure 2.13: a (left) - from Jacobsen (1999). Relationship between the PV and acceptability for fish oil enriched spreads b (right) - from Jacobsen (1999). Relationship between the AV and acceptability for fish oil enriched spreads

In the case of Figure 2.13, Jacobsen (1999) suggested that the sensitivity and specificity of the anisidine test was too low and was unable to detect the changes in concentrations of volatile compounds responsible for off flavour formation in a fish oil enriched spread.

The lack of good correlation between anisidine value and sensory score, as shown in Figure 2.13 is a prime example of two key issues faced when conducting analytical tests without using sensory analysis:

- 1) When interpreting analytical test results, even for tests targeting secondary oxidation products, it is important to avoid the assumption that the results gained by the tests will correlate with sensory results.
- 2) If it is necessary to assume that the analytical test correlates with sensory results (i.e. for preliminary studies) an analytical test that has been shown to give reasonable correlation for the food being tested must be used.

It seems that there is no single analytical test that can be used to track changes that correlates well with sensory analysis in all food systems. To begin with, the initial reactants (fats) present will determine the products of oxidation. Furthermore, the influences of other food components such as proteins have significant effects on the sensory perception of the food. For example, Giroux *et al.* (2007) found that whey

proteins used as an emulsifier had a significant effect on the release of ethyl hexanoate from oil droplets.

To date, there is no replacement for trained sensory panels when sensory information is required. It is, however, perfectly justifiable to use measures such as PV to determine rates of oxidation and how different factors influence the rates of oxidation. By doing this the amount of sensory analysis can be minimised.

2.4.2 Partitioning of flavours and its effect on flavour retention

The reactions that take place in bulk oils are not necessarily the same as those that take place in oil droplets that are suspended in an aqueous phase. As such, it makes sense that the sensory effects observed in bulk oils are different to those of emulsions although the reasons may be more complicated than expected.

The sensory perception of volatile compounds differs according to the composition of the food being tested (Druaux & Voilley, 1997). One particular feature, which has been proven by studies by Overbosch *et al.* (1991), is the differences in volatile flavour release from bulk oils and oil-in-water emulsions. For example, the flavour threshold values of aldehydes are much lower when released from water than from an oil (Druaux & Voilley, 1997).

Interestingly the threshold values of compounds, such as aldehydes, in emulsions tend to lie in between that of bulk oil or water phases. Maier (1970) showed that the odour threshold of hexanal and heptanal in milk were 0.05 and 0.12 parts per billion respectively. This lay above the thresholds in water (0.016 and 0.03ppb) and below the thresholds in oil (0.19 and 0.75ppb).

Work by van Ruth *et al.* (2002) showed that the partitioning coefficients (ratio of the volatile concentration in the air phase relative to the concentration in the liquid phase) of volatile compounds in oil and water can be significantly different. Table 2.1 shows the partitioning coefficients for 20 compounds. One of the most commonly quoted volatile products of oxidation is hexanal which, according to Table 2.1, would be

present in much higher concentrations in the aqueous phase of an oil-in-water emulsion. Perhaps most interestingly, the partitioning coefficients for all 20 compounds in water are higher than in oil.

Table 2.1: Table from van Ruth *et al.* (2002). Table shows the partitioning coefficient (x1000) for 20 common volatile products in the oil and water phases of a 20%w/w sunflower in water made with and without Tween emulsifier.

	<i>Oil</i>		<i>Water</i>	
	<i>-Emulsifier</i>	<i>+ Emulsifier</i>	<i>-Emulsifier</i>	<i>+ Emulsifier</i>
Dimethyl sulphide	7.31 ^a	7.26 ^a	61.02 ^l	15.97 ^{ll}
1-Propanol	4.26 ^a	2.84 ^b	1.20 ^l	0.52 ^l
Diacetyl	2.74 ^a	2.63 ^b	1.85 ^l	1.72 ^l
2-Butanone	3.65 ^a	3.57 ^b	3.91 ^l	3.91 ^l
Ethyl acetate	3.58 ^a	3.51 ^b	9.94 ^l	9.85 ^l
1-Butanol	1.10 ^a	1.04 ^b	0.86 ^l	0.86 ^l
2-Pentanol	0.78 ^a	0.75 ^b	1.30 ^l	1.30 ^l
Propyl acetate	1.32 ^a	1.30 ^b	9.93 ^l	9.45 ^{ll}
3-Methyl-1-butanol	0.53 ^a	0.51 ^b	1.19 ^l	1.14 ^{ll}
Ethyl butyrate	0.58 ^a	0.58 ^b	18.11 ^l	15.97 ^{ll}
Hexanal	0.40 ^a	0.39 ^b	14.34 ^l	11.98 ^{ll}
Butyl acetate	0.46 ^a	0.46 ^b	13.99 ^l	12.16 ^{ll}
1-Hexanol	0.14 ^a	0.13 ^a	1.53 ^l	1.32 ^{ll}
2-Heptanone	0.19 ^a	0.19 ^a	10.05 ^l	8.19 ^{ll}
Heptanal	0.14 ^a	0.14 ^a	19.12 ^l	12.12 ^{ll}
α -Pinene	0.16 ^a	0.16 ^a	27.98 ^l	5.03 ^{ll}
2-Octanone	0.07 ^a	0.07 ^a	13.55 ^l	8.06 ^{ll}
Octanal	0.06 ^a	0.05 ^a	18.27 ^l	6.75 ^{ll}
2-Nonanol	0.02 ^a	0.02 ^a	5.96 ^l	1.66 ^{ll}
2-Decanone	0.01 ^a	0.01 ^a	21.73 ^l	3.29 ^{ll}
CV (%)	1.4	1.2	5.1	10.1

Combining the effects of flavour retention and partitioning, it seems quite likely that an oil-in-water emulsion would be deemed rancid at a lower extent of oxidation compared with its equivalent bulk oil. The actual effects, of course, will depend on the other components present in the oxidation system. For example, the inclusion of storing flavoured ingredients that mask rancid flavours would likely extend the apparent shelf life of an emulsion.

2.5 Emulsions

In most food products, oils do not exist alone and, in many cases, are in contact with an aqueous phase. If the two phases are to be mixed, an emulsion must be formed. According to Walstra (2005), an emulsion is a dispersion of drops in another liquid where the two liquids are not miscible. In most foods, emulsions exist as discrete oil droplets suspended in a continuous aqueous phase. This review will be focusing on emulsions where the drops (the dispersed phase) consist of oil while the continuous phase is primarily water (the aqueous phase).

Most emulsions require an emulsifier which is needed to reduce the interfacial tension between the dispersed and continuous phases (Walstra, 2005). All emulsifiers are surfactants that adsorb onto the surface of the dispersed phase. These emulsifiers generally contain polar and non-polar groups that associate themselves with their corresponding phase (dispersed or continuous).

2.5.1 Surfactants/emulsifiers

An emulsifier has two main functions: allowing emulsion formation and providing stability to the emulsion once it has been made (Walstra, 2005). According to McClements (1998) all lipid-based emulsifiers are amphiphilic molecules. Amphiphilic molecules contain polar and non-polar groups which will try to align themselves with their corresponding phase according to their polarity. For example, the non-polar group will align itself such that it is in contact with the oil phase.

It has been well documented that the surfactant or emulsifier is capable of lowering the surface tension and thereby stabilising the emulsion it is in (Walstra & Smulders, 1998). This however, is not the only way in which emulsifiers are able to stabilise emulsions. A review by Rousseau (2000), also noted that emulsifiers form mechanically, cohesive interfacial films around droplets that prevent coalescence.

The effectiveness of amphiphilic emulsifiers depends on a number of factors. The following factors were suggested by Walstra (2005) and were considered important to this thesis:

- 1) Emulsifier solubility
- 2) Hydrophile lipophile balance
- 3) Surfactant concentration and critical micelle concentration

Emulsifier solubility

According to Walstra & Smulders (1998), there is a distinct difference between insoluble and soluble emulsifier's relative abilities to enhance the stability of an emulsion. That is, during the formation of an emulsion, the emulsifier must be soluble.

The solubility of amphiphilic molecules is highly dependent on temperature (Walstra, 2005). Some amphiphilic molecules will form crystals below moderate temperatures, thus leaving only a very small concentration of molecules in solution. If this occurs before the formation of the emulsion, then a very limited number of emulsifier molecules will be available to stabilise the emulsion. The exact effect of crystallisation of emulsifiers after emulsion formation is complex and depends on the emulsifier in question. The knowledge of this phenomenon, and therefore the ability to avoid it, is more important than the exact mechanisms which causes it.

Hydrophile lipophile balance

Another important factor that determines the effectiveness of an emulsifier is the hydrophile lipophile balance (HLB). Walstra (2005) describes the HLB as being a measure of the predominance of the hydrophilic action over the hydrophobic action of a surfactant molecule. A surfactant with a low HLB value (2-6) will be more soluble in oil while a surfactant with a high HLB will have a much greater solubility in water.

Emulsifier concentration

The concentration of an emulsifier is of great importance. Below a certain concentration, emulsifiers are dispersed as monomers (McClements, 1998). The addition of more emulsifier will increase the concentration of these monomers until a critical emulsifier concentration is met. At this point, any additional emulsifiers form micelles and the monomeric concentration remains constant. The emulsifier concentration at this point is called the critical micelle concentration (CMC).

The formation of micelles has dramatic consequences on the physical properties of the emulsifiers. McClements (1998) demonstrates that surfactant monomers are amphiphilic and have a high surface activity but the micelles formed have little surface activity because their surface is covered with the hydrophilic groups of the individual surfactant molecules. This means that the surface tension of an emulsion will continuously drop as emulsifier concentration is increased until the CMC is reached. When the CMC is reached, the addition of emulsifier should have little or no effect on the surface tension of the emulsion.

From the above discussion it seems feasible to add surfactants at a high concentration to ensure that all oil droplets are covered. However, the micelles formed in such a process are capable of solubilising components of the lipid droplets into the water phase (Richards *et al.*, 2002). In some cases, the micelles formed are able to solubilise triglycerides although this is not common as most surfactants form micelles with hydrophobic cores which are too small to fit a large triglyceride molecule. Richards *et al.* (2002), however, showed that phenolic antioxidants could be removed from emulsion droplets by surfactant solubilisation. It was also found that levels of solubilisation by surfactant micelles were dependent on the polarity of the antioxidant and the concentration of emulsifier used.

Other surface active molecules

Although not technically classed as a lipid-based emulsifier under the definition given by McClements (1998), some thickening agents are also surface active (Rousseau, 2000). Examples of such agents include gum arabic and gum tragacanth. Egger & McGrath (2006) give polymers, proteins and finely divided solids as other examples of emulsifiers that cannot be described as surfactants. According to Rousseau (2000), these agents are capable of influencing interfacial phenomena such as surface tension, surface viscosity and elasticity.

2.5.2 Main causes of emulsion instability

According to Walstra (2005), Binks (1998) and McClements (1998), there are four main forms of physical instability. They are:

- 1) Ostwald Ripening
- 2) Aggregation
- 3) Sedimentation/Creaming
- 4) Phase inversion

Ostwald ripening

Ostwald ripening describes the growth of large droplets at the expense of smaller ones (McClements, 1998). It has been shown that the solubility of a material in a spherical droplet increases with decreasing droplet size. McClements (1998) and Binks (1998) suggest that Eq. 2.24 can be used to show this relationship.

$$S(r) = S(\infty)e^{\left(\frac{2\gamma V_m}{RT r}\right)} \quad \text{Eq. 2.24}$$

In Eq. 2.24, V_m is the molar volume of the solute, γ is the interfacial tension, R is the gas constant, $S(\infty)$ is the solubility of the solute in the continuous phase for a droplet with infinite curvature and $S(r)$ is the solubility of the solute when contained in a spherical droplet of radius r .

According to McClements (1998), the greater solubility of the material around a small droplet means that there is a higher concentration of a solubilised material around a smaller droplet than a larger one. Thus the material moves towards a larger droplet because of the concentration gradients that exist.

Once at steady state, McClements (1998) and Binks (1998) suggest that the growth in droplet radius with time due to Ostwald ripening is given by Equation 2.25.

$$\frac{\partial r^3}{\partial t} = \frac{8\gamma V_m S(\infty) D}{9RT r} \quad \text{Eq. 2.25}$$

Note that in Eq. 2.25, D is the diffusion coefficient of the material through the continuous phase. This equation assumes that the emulsion is of low concentration and that the rate limiting step is the diffusion of the material through the continuous phase. Thus, the effects of interactions with neighboring droplets has been omitted from Eq. 2.25. McClements (1998) suggests that food emulsions are often much too concentrated for this equation to apply and that the effects of neighboring droplets must be considered.

A further factor governing the rates of ripening is the rate of droplet diffusion through the aqueous phase. In Ostwald ripening, however, the rate of diffusion of material through the continuous phase is generally so slow that the effects of Ostwald ripening can be deemed negligible. Only in emulsions that have high levels of water soluble lipids, such as flavour lipids, could Ostwald ripening occur at significant rates.

Walstra (2005) suggests that the point at which the above effects become significant can be predicted by the Peclet number as shown in Eq. 2.26 where m is the droplet mass, a is the droplet radius, T is temperature in Kelvin and k is the Boltzmann constant. Walstra (2005) suggests that a $Pe > 1$ indicates that Brownian motion will enhance Ostwald ripening.

$$Pe \approx \frac{a}{D} \left(\frac{3kT}{m} \right)^{0.5} \quad \text{Eq. 2.26}$$

When the distance between the drops is much larger than that of a droplet diameter, Eq. 2.26 holds true. Walstra (2005) suggests that this only holds true for emulsions with very low volume fractions (φ). In fact, experiments discussed by Walstra (2005) showed that Ostwald ripening rates increased by a factors of 1.4, 1.75 and 2.2 for $\varphi = 0.03, 0.1$ and 0.3 respectively.

Aggregation

Aggregation refers to the process that results when droplets stay together for an appreciable time after having come in contact with one another (Walstra, 2005). This contact may be the result of Brownian motion or due to the movement described by Stokes law (Eq. 2.31). The aggregates can grow to large volumes as a result of van de Waals interactions between droplets.

According to McClements (1998), there are two types of aggregation. These are flocculation and coalescence. Flocculation occurs when two or more droplets come together to form an aggregate, but maintain their individual integrity. Coalescence is the process when two or more droplets merge to form a single larger droplet.

The rate of aggregation of droplets is dependent on two factors: the collision frequency and the collision efficiency. The collision frequency is the number of collisions between droplets that occur in a given period of time and for a given volume (McClements, 1998). Obviously, the collision frequency will be different for a still and stirred system. McClement (1998) suggests Equation 2.27 and 2.28 can be used to determine the collision frequency for each system.

$$N = \frac{4kTn_0^2}{3\eta} \quad \text{Eq. 2.27}$$

$$N = \frac{16}{3} Gr^3 n_0^2 \quad \text{Eq. 2.28}$$

Eq. 2.27 shows the collision frequency (N) when the system is still, where η is the shear viscosity, k is the Boltzmann constant, T is the temperature in Kelvin and n_0 is the initial number of particles per unit volume. Eq. 2.28 shows the collision frequency when the system is stirred where r is the radius of the particles and G is the shear rate caused by stirring.

As already mentioned, collisions between two or more particles will not necessarily result in coalescence or flocculation. The collision efficiency (E) should be included as it represents the percentage of colliding particles that will coalesce or flocculate. Furthermore, the droplet-droplet interaction potential, as described by McClements (1998) can be included when calculating E . For example, Eq. 2.27 can be modified to include E as shown in Eq. 2.29.

$$N = \frac{4kTn_0^2}{3\eta} \cdot E \quad \text{Eq. 2.29}$$

where

$$E = \int \left(\frac{x^2}{e^{\left(\frac{\Delta G(x)}{kT}\right)}} \right) \quad \text{Eq. 2.30}$$

Note that x is the distance between the centres of the droplets and $\Delta G(x)$ is the droplet-droplet interaction potential as given by McClements (1998). Furthermore, the equations given above are only applicable for the initial stages of aggregation in dilute emulsions (McClements, 1998). In most food emulsions, the concentration of the dispersed phase is high and the interactions between flocs as well as individual droplets become important. Therefore, the above equations should be modified to include these interactions.

In many cases, the arrangement of a floc may differ depending on the attractive forces between droplets (McClements, 1998). If the attractive forces are large then the droplets tend to become 'locked' in place as soon as the floc is formed. If the

attractive forces are weak then the droplets tend to 'roll' around one another to create a denser floc.

Coalescence, however, leads to what is commonly known as 'oiling off' which is the formation of an oil layer at the surface the continuous phase. Basically, coalescence involves a decrease in the surface area to volume ratio by the formation of larger droplets. According to McClements (1998) and Binks (1998), coalescence occurs most rapidly when the droplets are not covered by a suitable emulsifier.

When droplets are stabilised by an emulsifier, the tendency of droplet-droplet interaction and the potential for film rupture governs the stability against coalescence. That is, if there is a strong repulsive force between droplets or if the film is resistant to rupture, then the emulsion should be relatively free from coalescence (McClements, 1998).

2.5.3 Creaming/sedimentation

Creaming is the separation of an emulsion into two distinct layers without a change in the droplet size distribution (Binks, 1998). In oil-in-water emulsions, the dispersed phase generally has a lower density than the continuous phase and, the dispersed phase moves through and forms a layer above the continuous phase. Sedimentation is the opposite of this process. The dispersed phase of water in oil emulsions tends to move to the base of the continuous phase due to its higher density. In this case, the water has sedimented.

Creaming occurs because of density differences between the dispersed and continuous phases (McClements, 1998). The rate at which creaming occurs is dependent on a number of factors including the densities of the continuous and dispersed phases, the viscosity of the continuous phase, and the size of the dispersed particles. Stokes law (Eq. 2.31) describes the rate of creaming (v) of a single spherical particle in a fluid.

$$v = \frac{2gr^2(\rho_2 - \rho_1)}{9\mu} \quad \text{Eq. 2.31}$$

As can be seen in Eq. 2.31, the rate of creaming of a single particle in an infinite fluid is proportional to the square of the radius of a particle (r), the difference in densities of the continuous and dispersed phases (ρ_s), and the acceleration due to gravity (g). The creaming rate is also inversely proportional to the Newtonian shear viscosity of the continuous phase of the emulsion (μ).

Flocculation and coagulation also have a strong influence on creaming rates. McClements (1998) suggests that at low droplet concentrations flocculation increases creaming rates as it increases the effective droplet size. However, at high concentrations flocculation can inhibit creaming as the droplets become trapped within a three-dimensional network of aggregate emulsion droplets.

Binks (1998) suggests that, up to volume fractions of ~ 0.05 , the average creaming rate (v_a) is less than that given by Stokes Law by an amount proportional to the volume fraction (φ). Furthermore Binks (1998) suggests that Batchelor's result (Eq. 2.32) can be used to account for the effects of container walls where the movement of a drop upwards must be equalled by the movements of the same volume of the continuous phase downwards.

$$\frac{v_a}{v} = 1 - 0.655\varphi \quad \text{Eq. 2.32}$$

Eq. 2.32, however, does not apply for concentrated emulsions. For more concentrated emulsions, Binks (1998) suggests that the semi-empirical Eq. 2.33 fits experimental data reasonably well.

$$v_a = \frac{v(1-\varphi)}{(1+\varphi)^3} \cdot e^{\left(\frac{5\varphi}{3(1-\varphi)}\right)} \quad \text{Eq. 2.33}$$

2.5.4 Phase inversion

Phase inversion is when a system changes from an oil-in-water emulsion to a water-in-oil emulsion (McClements, 1998). Phase inversion usually occurs because the composition of the system changes. In foods this may mean that the volume fraction of each phase changes or because a different emulsifier is added to the system.

Although phase inversion does occur in some systems, and being aware of the possibility of phase inversion is important, it is very unlikely that it will be found in any part of this research. By avoiding scenarios where the aqueous and oil concentrations become close, phase inversion can be avoided. Because this will be easy to achieve in future experimental designs, it shall not be discussed any further.

2.6 Lipid oxidation in emulsions

2.6.1 Influence of interfacial region

The interfacial region has already been mentioned in a number of the previous sections, however a more in depth look at the boundary that separates the continuous and dispersed phases is required.

According to Silverstre *et al.* (2000) there are three distinct regions within an emulsion. They are the continuous phase, the dispersed phase and the droplet interfacial membrane. This membrane has a considerable effect on the rates of lipid oxidation reactions in emulsions. There are a number of factors that enhance or hinder lipid oxidation reactions either by changing properties of the interfacial membrane or by being hindered by the presence of the interfacial membrane. The following factors, although not able to be completely separated from each other, have been identified and shall be briefly discussed in turn:

- 1) Membrane charge
- 2) Partitioning of antioxidants
- 3) pH
- 4) Droplet size

2.6.2 Membrane charge

Depending on the molecules present in and around the interfacial region, droplet membranes can, and often do, carry a charge. Intuitively, it is reasonable to assume that a positively charged interfacial membrane would attract negatively charged and repel positively charged particles within the continuous phase. Silvestre *et al.* (2000) and Mei *et al.* (1998b) suggest that negatively charged membranes attract neighboring metal ions to the surface of the membrane and thus give higher lipid oxidation reaction rates than those of a positively charged membrane.

There are a number of articles that support the idea of metal ion attraction by negatively charged interfacial membranes. For example, Donnelly *et al.* (1998) found that lipid oxidation rates were greatest when an emulsion was prepared with whey proteins at a pH less than the pI (isoelectric point) of the protein. Mancuso *et al.* (1999) found that lipid autoxidation reactions in cationic emulsions were not significantly enhanced by Fe^{3+} ions in the continuous phase while Mei *et al.* (1998) found that Fe^{3+} and Fe^{2+} were strongly bound to negatively charged SDS-stabilised emulsion droplets and not to positively charged droplets.

Like metal ions, charged antioxidants are attracted to and repelled from the surface of the interfacial membrane depending on their relative charges (Stockmann *et al.*, 2000). Huang *et al.* (1996a & 1996b) suggested that the degree to which antioxidants partition themselves changes according to their surface charge. Therefore the relative charges of the interfacial membrane and antioxidant must determine the affinity of the two.

2.6.3 Partitioning of antioxidants

In many cases, an apparent oil/water partitioning coefficient is used to describe the differences in concentrations of a substance in the oil or aqueous phases of an emulsion. The partitioning coefficient is defined as the concentration of a compound in the oil phase divided by the concentration of the same compound in the aqueous phase of an emulsion (Jacobsen, 1999).

Many compounds can partition themselves between the oil and water phases of an emulsion. For example, the partitioning of antioxidants into the oil phase of an emulsion has been shown to have a significant effect on the rates of lipid oxidation reactions (Huang *et al.*, 1996a & 1996b). Similarly the products of lipid oxidation partition themselves according to their affinity to polar or non-polar substances.

Care must be taken when describing partitioning effects and coefficients as a number of compounds will partition themselves within the oil/water interface. For example, antioxidants commonly aggregate at oil/water interfaces so that their non-polar tails are positioned within the oil droplet while their polar heads are positioned in the aqueous surrounding.

Partitioning effects are often used to explain why polar antioxidants are particularly good at preventing or hindering lipid oxidation reactions in bulk oil systems (Decker, 1998). Oxygen, which is required for lipid oxidation initiation reactions, is at its highest concentration at the surface of the oil. Since high rates of oxidation reactions are correlated with high concentrations of oxygen it makes sense that the rates of oxidation reactions in bulk oils are highest at the air/oil interface (McClemments & Decker, 2000). However in bulk oils polar antioxidants, such as Trolox® (Hoffman-LaRoche, Switzerland), tend to aggregate at the oil-air interface as this is the most polar part of the oil. The increased concentration of antioxidants at the air/oil interface means that the oxidation reactions are quenched at a greater rate than if non-polar antioxidants had been used since the non-polar antioxidants would distribute themselves evenly throughout the bulk oil.

The partitioning of antioxidants in oil-in-water emulsions is the almost opposite to that of bulk oils. In oil-in-water emulsions, non-polar antioxidants are more effective at hindering lipid oxidation reactions as they are mainly partitioned within the oil phase of emulsions and are therefore concentrated within the oxidation substrate (Decker, 1998). However, there is some evidence that amphiphilic antioxidants are capable of significantly reducing the rates of lipid oxidation reactions.

Amphiphilic antioxidants are able to partition themselves across the oil/water interface (McClemments & Decker, 2000). It is suggested that amphiphilic antioxidants should

be the most effective at retarding lipid oxidation reactions in emulsions as they are positioned at the point where the lipid oxidation reactions are promoted (Huang *et al.*, 1996a & 1996b).

There are a number of factors that have been suggested as being able to change the partitioning behavior of antioxidants. Stockmann *et al.* (2000) and Richards *et al.* (2002) suggested and proved that the solubilisation of antioxidants in emulsions is highly dependent on the type emulsifier. It was shown that emulsifiers that accumulate at the oil and water interface dominate the properties of the interface in terms of the solubilisation capacity for antioxidants. Furthermore, as already discussed, it has been shown that antioxidants may be solubilised by surfactant micelles (Richards *et al.*, 2002).

2.6.4 pH

The effects of pH have been touched on in previous sections. For example, it was noted that the pH of an emulsion can change the charge of the interfacial membrane as well as the charge of some antioxidants. The pH can therefore affect the partitioning ratio as described above. There are, however, a few articles that look directly at the effects of changing pH on lipid oxidation rates.

Osborn and Akoh (2003) conducted tests which showed that iron-catalysed reactions of oil-in-water emulsions at pH 3 were significantly greater than those at pH 7. This trend was also noted by Mei *et al.* (1998), although no mechanisms were proposed.

Jacobsen *et al.* (2001) investigated the effects of pH and iron on oxidation rates of fish oil enriched mayonnaise. They suggested that the iron which is associated with the interfacial membrane is 'covered' and therefore inaccessible to oxidation initiators at a pH of 6. When the pH is reduced, the molecules 'covering' the iron are less strongly bonded and allow initiator molecules to come in contact with iron. This however, is a theory that may only apply to emulsions containing proteins and other such molecules that may form the 'covering' described by Jacobsen *et al.* (2001).

Although it is not possible to discuss all the interactions between emulsifiers and antioxidants and how pH affects each, it is worth noting that emulsions stabilised by emulsifiers such as proteins can be significantly affected by changes in pH. For example Gu *et al.* (2005) looked at the influence of pH and carrageenan type on the stability of beta-lactoglobulin stabilised oil-in-water emulsions. They were able to show significant differences in emulsion stability at different pH levels.

2.6.5 Droplet size

Literature characterising the effects of droplet size on lipid oxidation reactions in emulsions is scarce. When available, the literature seems contradictory. The ideas surrounding the effects of droplet size are summarised by Lethuaut *et al.* (2002) and is included in the following paragraphs.

The first theory is that decreasing the size of an emulsion droplet will increase the rates of lipid oxidation. This is because decreasing the size of the droplet will increase the surface area to volume ratio of the droplet. This implies that there will be a greater potential for the contact of diffusing oxygen, water soluble free radicals, and antioxidants with the interfacial membrane. It also suggests that the ratio of oxidisable fats at the surface of the droplet compared to the hydrophobic core of the droplet will be large.

The second theory suggests that decreasing the size of an emulsion droplet will reduce the rate of oxidation reactions. This is because a reduction in the size of the droplet will decrease the amount of oxidisable substrate within the droplet relative the surface area of the droplet. It is suggested that the number of surface active agents that adhere to the surface of the droplet increases thus reducing the amount of contact of initiators with oxidisable substrate as well as hindering the diffusion of compounds such as oxygen.

There is evidence to support both theories. Lethuaut *et al.* (2002) describes how homogenisation is reported to protect milk fat from metal-catalysed oxidation by increasing the surface area over which casein, an efficient antioxidant milk protein,

can adsorb. Nakaya *et al.* (2005) also showed that decreasing the size of emulsion droplets increased the oxidative stability of the emulsion. Osborn & Akoh (2004) found that droplet size had no effect on the rates of lipid oxidation in a canola oil based emulsion while Lethuaut *et al.* (2002) found the rate of lipid oxidation increased with increasing interfacial surface area.

From the above it seems quite clear that the effect of interfacial surface area is not fully understood. It seems that the effects of emulsifiers and other ingredients used to create the emulsions as well as the methods used to create the emulsions have not been decoupled from the effects of interfacial surface area alone.

2.7 Packaging and its effects on lipid oxidation reactions

There is no doubt that oxidation rates are affected by the rate of oxygen supply. A real food product packed in a permeable package (i.e. for chilled soups) will allow some movement of oxygen into the food. The rate of oxygen transfer across the permeable packaging material will, along with the rate of oxygen consumption, determine the concentration of oxygen in the food.

The laws governing the migration of gases through packaging materials are given by Robertson (2006). It has been shown that the quantity of gas that has moved across a packaging layer of known surface area per unit time (J) is equal to the product of the gas concentration gradient across the packing layer ($\partial c/\partial x$) and a diffusion coefficient (D).

$$J = -D \frac{\partial c}{\partial x} \quad \text{Eq. 2.34}$$

Steady state mass transfer occurs when the concentration profile across the packaging layer reaches a constant value. When this occurs, Eq. 2.35 and 2.36 can be applied.

$$J = \frac{D(c_2 - c_1)}{x} \quad \text{Eq. 2.35}$$

$$Q = \frac{D(c_2 - c_1)At}{x} \quad \text{Eq. 2.36}$$

For Eq. 2.35 and 2.36, c_2 and c_1 are the concentrations of gas on the outer and inner surfaces of the packaging layer, x is the thickness of the packaging, A is the area of the packaging and Q is the total amount of gas that has moved across the packaging layer over a given time, t .

For gases, it is often more convenient to measure the partial pressure of the gas on each of the surfaces and relate it to the concentration of each surface by using Henry's law as shown in Eq. 2.37.

$$c = S \cdot p \quad \text{Eq. 2.37}$$

where S is the solubility coefficient of the permeate in the polymer layer and p is the partial pressure of the gas. The combination of Eq. 2.36 and 2.37 results in Eq. 2.38. Note that DS is often referred to as the permeability coefficient or permeability and is represented by the symbol P as shown in Eq. 2.39.

$$Q = \frac{DS(p_1 - p_2)At}{x} \quad \text{Eq. 2.38}$$

$$P = \frac{Qx}{At(p_1 - p_2)} \quad \text{Eq. 2.39}$$

Examples of permeability's for different film types are given by Frankel (2005) and shown in Table 2.2. The data shows that it is possible to manufacture films that span a very wide range of oxygen transfer rates. The ability to use composite films and multilayer films make it possible to tailor a film for almost any application.

Table 2.2: Permeability data for different polymer packaging materials as given in Frankel (2005); data gained from experiments at 23°C and 50% relative humidity.

Polymer	O ₂ Permeability cm ³ mm(m ² day.atm) ⁻¹
Polyethylene terephthalate (PET)	1-5
Polyvinyl chloride (PVC)	2-8
High density PE (HDPE)	5
Polypropylene (PP)	50-100
Polystyrene (PS)	100-150

Using this information, it is possible to limit the rates of oxygen transfer to an oil. An example of such work is that conducted by Cho *et al.* (2010) who created a soy protein film and a corn zein/soy protein film. Shelf life tests that monitored the formation of lipid hydroperoxides by measuring PVs showed that the low permeability films hindered the formation of lipid hydroperoxides to larger extent compared with the same oil packaged in a higher permeability film (nylon-metalocene catalysed low-density polyethylene or NY/mLLDPE). For this experiment, Cho *et al.* (2010) measured the oxygen permeability of each material and found that the corn zein/soy isolate and NY/mLLDPE had permeabilities of 7.10 and 30.72 cm³mm.m⁻²day⁻¹atm⁻¹.

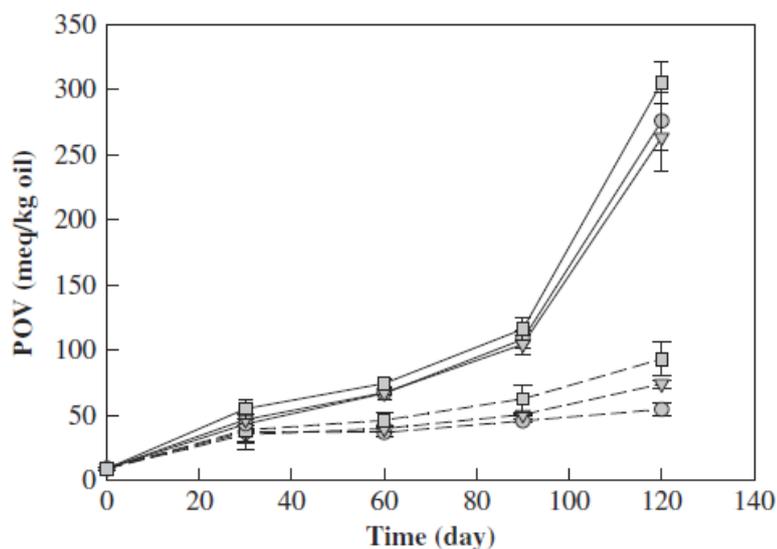


Figure 2.14: Figure taken from Cho *et al.* (2010). Peroxide values (POV) of olive oil stored in corn zein/soy protein isolate bilayer film pouches (dotted line) and in NY/mLLDPE pouches (solid line) at 50°C. Shape of points represents different storage relative humidities (-O- 30%, -□- 40%, -▽- 50%)

2.8 Research areas lacking significant investigation to date

Lipid oxidation has been studied for many decades and there is a considerable volume of literature available on the subject. Most of the research, however, has been carried out in simplified systems. The systems are generally well mixed and most investigations have been designed to help identify the many different reactions that take place. There have, however, been very few studies carried out on complex food systems which tend to be unmixed systems.

Since much of the research available in literature has been conducted on mixed systems, the effects of supply of oxygen have been largely neglected. The ideas of rates of supply and reaction form the basis for the following discussion.

2.8.1 Importance of oxygen as a measure of lipid oxidation

Food chemists have identified a number of different reactant species that take part in the complex lipid oxidation reactions. Despite considerable research, tracking lipid

oxidation reactions by measuring the time of onset or concentrations of these reactants and products of oxidation is difficult and error prone.

The main issues with using chemical reactant species to measure oxidation rates are caused by their relative instability and inconsistent production. For example, some products of lipid oxidation only occur under certain reaction conditions. As a result, measuring one particular species is unlikely to result in any useful information. Many researchers have chosen to utilise a range of different methods in order to ensure that they are capable of extracting statistically significant information.

Unlike other reactant species, oxygen is consumed in almost all of the lipid oxidation reactions. Until recently, however, researchers have not had the tools to measure dissolved oxygen concentrations with ease or certainty. Recent technological developments have made such measurements simple to carry out and, in doing so, have provided a new avenue for tracking lipid oxidation reactions and a way of gathering key kinetic information.

2.8.2 Use of oxygen to measure lipid oxidation reaction rates

Unlike traditional oxidation measures, measuring oxygen consumption should allow a researcher to track nearly all reactions throughout lipid oxidation with a single measure. Furthermore, by tracking the rates of oxidation, it should be possible to determine the extent of reaction and the stage of oxidation. It is, therefore, possible that dissolved oxygen measures could do away with the need for multiple and time consuming tests such as those required for peroxide, anisidine and TBA values.

There are two key pieces of information that can be gathered by monitoring the dissolved oxygen concentrations in lipid systems. They are:

- 1) The total amount of oxygen consumed.
- 2) The rates of oxygen consumption.

Total oxygen consumption

By measuring the oxygen consumption rates over time it should be possible to determine the total amount of oxygen consumed at any point in time. Since there is a finite amount of oxidisable product and a finite quantity of oxygen required to react, it would be possible to determine the extent of reaction in a simple system.

Although unlikely, there is a chance that the amounts of oxygen required for key quality changes will be similar for all lipid oxidation prone products. For example, the total oxygen required for peak lipid hydroperoxide concentration may be the same for all oxidation systems. If this is true, then the use of oxygen as a measure will be able to replace the multiple other measures currently in use.

The idea that oxygen may be able to either partially or completely replace traditional lipid oxidation tracking methods requires attention as any move away from the time consuming methods of today will greatly improve the ability of both industry and researchers to better understand the kinetics of lipid oxidation reactions.

Rates of oxygen consumption

There is a significant volume of literature, summarised by Fennema (1996) and Frankel (2005), that deals with the qualitative rates of each of the three stages of oxidation. In particular it has been suggested that the rates of initiation are slow and remain so until significant amounts of lipid hydroperoxides have been formed. Such information can be quickly, easily and continuously gathered when using oxygen as the measure of lipid oxidation rates.

Knowing the actual rates of oxidation throughout the entire process of lipid oxidation becomes incredibly valuable when the rates of oxygen consumption by reaction are less than the supply of oxygen through the packaging. The reaction rates of lipid based products are heavily dependent on the storage conditions they are held in. For example, chilling a product should significantly reduce the rates of reaction. If the reaction rates drop below the rates of oxygen supply, oxygen will be able to accumulate within a product.

If reaction rates drop below oxygen supply rates, a complex interaction between the oxygen concentration and extent of reaction is set up. Takahashi *et al.* (2000) have shown that the rates of lipid oxidation are dependent on oxygen concentration below approximately 5% and that the rates of oxidation are different at different stages of lipid oxidation (Labuza, 1971). If such a situation was created, knowing the reaction rates at different oxygen concentrations and extents of reaction becomes essential to be able to model lipid oxidation reactions.

2.8.3 Supply versus consumption of oxygen

Well mixed versus unmixed systems

Investigations that attempt to describe lipid oxidation reactions have typically been conducted in mixed systems where reactants and products are assumed to be in equal concentrations throughout the reaction system. The rates of oxidation in such systems are not dependent on the position within the system.

Real food products, however, are not well mixed systems. To be able to characterise such a system, it is important to investigate the effects of diffusion of reactant species through the system. By introducing the effects of diffusion it is possible to understand why many researchers have found it difficult to transfer the results of their controlled experiments to more complicated products.

As already discussed, oxygen is arguably the most important reactant species in all of the lipid oxidation reactions. It has been shown that, when using homogeneously reacting systems, the rates of oxidation depend on oxygen when the oxygen concentrations are low (Fennema, 1996). This situation is of particular importance as initial investigations have shown that the concentrations of oxygen in food packaged at 90°C are very nearly zero. Any oxygen that is present in the food at packaging is quickly used by oxidation reactions. Once the oxygen concentration in a food has dropped to zero, the only source of oxygen is the oxygen that diffuses through the

product. However, before the oxygen can diffuse through the product, it must first pass through the packaging.

The rate of oxygen transfer through packaging is dependent on the packaging material and thickness and varies from no transfer, as in metallic foils or glass jars, to a transfer with little resistance, as in cling films used to package meats in supermarket chillers. Lipid oxidation prone products tend to be packaged in barrier films with very low oxygen transfer rates. The rates of oxygen transfer through barrier films are usually many times lower than the rates of diffusion through the product contained in the packaging.

Diffusion vs. reaction

Assuming there are no oxygen consuming reactions occurring, there are two possibilities for the movement of oxygen into the product. They are:

- 1) The oxygen diffusion through the packaging is much slower than through the product.
- 2) The oxygen diffusion through the packaging is much faster than through the product.

In the first scenario, the movement of oxygen through the product will be so fast relative to the movement through the pouch that the oxygen concentration throughout the product will be uniform. In the second case, initially there will be a concentration gradient throughout the product until the steady state oxygen concentration is reached. It seems more likely that the former will be true for most lipid oxidation susceptible products. If it is not true, then a packaging change would be required.

Introducing oxygen consuming reactions, however, brings about an additional complication. Like the effects of relative diffusion rates, there are two possibilities that can occur due to the relative rates of diffusion and reaction. They are:

- 1) The rate of oxygen supply by diffusion is greater than the rate of oxygen consumption by reaction.
- 2) The rate of oxygen supply by diffusion is lower than the rate of oxygen consumption by reaction.

If the former is true, then a concentration gradient from the surface to the centre of the product will exist. In such cases, the rates of oxidation will vary depending on the position within the product. If, however, the latter of the suggested mechanisms is true, then the oxygen will move through the product as a front. Any oxygen that enters the product through the packaging will be consumed instantaneously. Only when the lipid oxidation reactions at the surface of the product have been completed can the oxygen front move further into the product.

The relationship between diffusion and reaction is such that the environment may cause a switch from a diffusion limited system to a reaction limited system. For example, at low temperatures the rates of lipid oxidation are significantly reduced while the diffusivity of oxygen remains relatively unchanged. Identifying such situations is critical to understanding the system under investigation.

Interestingly, the rates of oxygen supply and consumption are generally not taken into account by many researchers. In particular, it seems common practice to measure volatile oxidation products by storing oils in closed vessels with a headspace. Such methods were used by Wang *et al.* (2011), Beltran *et al.* (2010), Garcia-Martinez *et al.* (2009), Cercaci *et al.* (2007) and others. This method, however, may bring with it two problems. Firstly the use of an unmixed system may mean that the surface of the oil is the only point where oxygen is present and, the rate of oxidation is limited by the supply of oxygen. This may not be a problem if a careful control is used but makes comparing different literature sources very difficult. The second issue is that there is potential for the system to run out of oxygen. The reactions taking place without oxygen may be different to those when oxygen is available and may result in misleading conclusions.

Secondly, analytical methods such as the Oxidograph may be sensitive to oxygen supply limitations. In the case of the Oxidograph, the pressure above a sample of oil is measured without the oil being mixed or sparged with gas. The pressure change, aside from being sensitive to volatile production, is also likely to give different results depending on the relative rates of oxygen supply and consumption.

2.9 Conclusions

Lipid oxidation has been well researched over the last few decades. There is clear evidence that shows the negative effects lipid oxidation product has on foods. The production of rancid flavours and aromas clearly reduces the shelf life of lipid oxidation prone foods. Much attention has been given to methods to increase the shelf life of lipid oxidation prone foods. The focus, however, has been on qualitative investigations that, in most cases, only show trends of either shelf life extension or reduction.

Three key areas that have been well studied and are of particular interest for this thesis are:

- 1) The three stage mechanism of initiation, propagation and termination.
- 2) Methods of tracking the products of the three stages of lipid oxidation.
- 3) The qualitative effects of both internal and external factors on lipid oxidation rates.

Careful exploration of the above mechanisms makes it possible to force oxidation and track the subsequent reactions. Coupling the qualitative effects of the factors that cause changes in lipid oxidation rates with the three stage lipid oxidation mechanism will help form a reasonable conceptual model to base future investigations on.

Current research, although useful, has been targeted toward defining the mechanisms of oxidation in well mixed systems. Such investigations avoid the oxygen limiting situations that are likely to exist in real food products which are generally unmixed systems. The non-oxygen limiting situation does not supply the information required to make shelf life predictions.

Discussions have suggested that oxygen will be the most crucial reactant species and that the supply of oxygen is likely to have significant effects on lipid oxidation rates. After a significant review, it has become clear that the relationship between autoxidation reaction rates and oxygen diffusion rates will be critical and will require significant investigation.

Discussions have shown that there is a significant lack of literature investigating the kinetics of lipid oxidation under different storage conditions. Of the literature available, very few have conducted investigations on unmixed systems. To date, there is only a very limited amount of literature discussing or investigating the effects of storage conditions or emulsion makeup on lipid oxidation rates in unmixed (i.e. non homogenous oils and emulsions) systems has been discovered. Furthermore, there seems to be very little information or studies that use kinetic information to accurately and quantitatively predict the shelf life changes that come about from changes in either emulsion characteristics or storage conditions.

In order to predict the shelf life of lipid oxidation prone products, experiments to identify the effects of each factor (both internal and external) on the rates of lipid oxidation are required. To ensure the complexity is minimised a number of experiments would be required to screen each of the factors to determine whether their effects on the rates of oxidation are large enough to warrant their inclusion in any shelf life predicting models.

The collation of the results from a number of experiments would be required. To date, no literature that attempts to bring together the effects of a vast number of factors, both physical and chemical, to predict the changes in quality parameters has been identified. The collation of such information requires new methods.

It is suggested that mathematical modelling is one method that could utilise the results of simplified oxidation systems to describe the effects of different factors on lipid oxidation rates. The availability of powerful computers and modelling software seems likely to be the solution to overcoming the complexity required to predict shelf life changes in complex, unmixed, lipid oxidation systems.

CHAPTER 3

CONCEPTUAL MODEL DEVELOPMENT

3.0 Introduction

In order to identify the areas that require investigation and to help define future experiments, a conceptual model is needed. The results of a comprehensive review (Chapter 2) suggest that it would be difficult to predict the individual effects and interactions of all the ingredients in a food system. Therefore, the conceptual model must attempt to reduce the degree of complexity outlined in the literature review while providing enough information to allow for oxidation rate and shelf life predictions.

3.1 Brief description of oxidation system

This thesis is concerned with the prediction of lipid oxidation rates in bulk oils and oil-in-water emulsions. The system under investigation can be described by Figure 3.1.

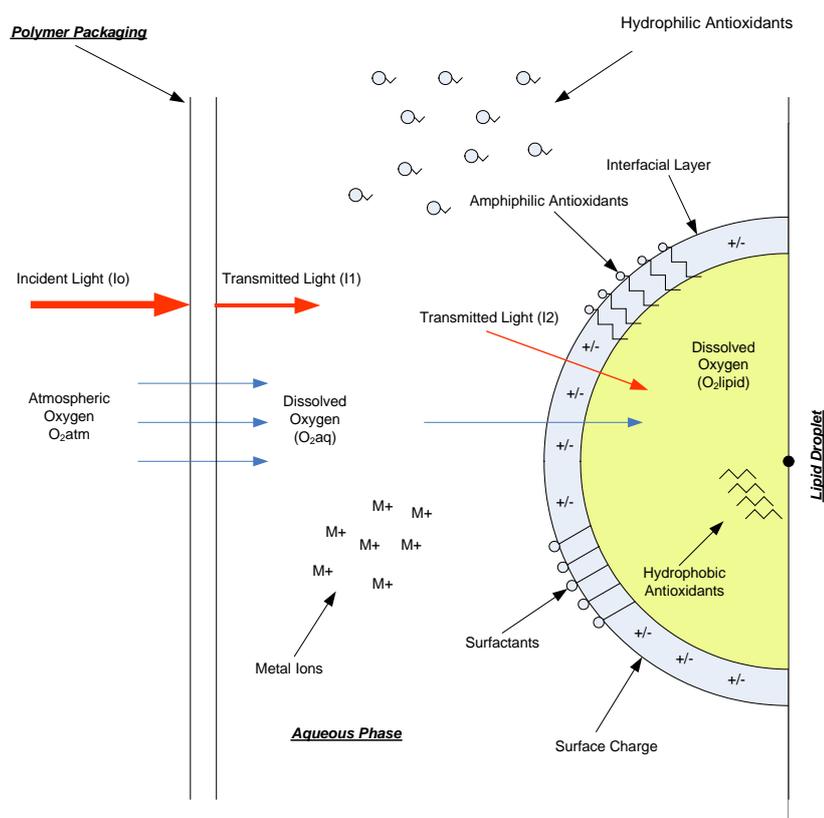


Figure 3.1: Depiction of the oxidation system containing oil droplets suspended in an aqueous phase and packaged in a polymer pouch.

As can be seen in Figure 3.1, the system essentially involves an unmixed oil-in-water emulsion contained in a polymer package. The system can be broken down into four distinct components:

- 1) Lipid phase
- 2) Aqueous phase
- 3) Interfacial layer
- 4) Packaging

The lipid phase exists as a distribution of small droplets dispersed evenly throughout the aqueous phase. Hydrophobic antioxidants exist within this phase and tend to situate themselves away from the surface of the droplets to ensure that they are away from the aqueous phase. The lipid droplets tend to remain stationary within a stable emulsion which is unmixed. When the droplets do move, they tend to aggregate or coalesce thus changing the droplet size distribution of the emulsion and increasing the probability of phase separation.

The aqueous phase makes up the largest proportion of an oil-in-water emulsion and contains all the hydrophilic reactants and catalysts including antioxidants, metal ions and chlorophyll. In the absence of oxygen consuming reactants, the aqueous phase provides a sump of oxygen for reaction with lipids. The aqueous phase also provides physical separation between oil droplets and the surface of the packaging.

The interfacial layer forms a physical barrier between the aqueous and lipid phases. The barrier consists of amphiphilic antioxidants and surfactants which can affect the surface charge of the droplet. The interfacial layer serves to reduce the surface tension between the two immiscible liquids and is responsible for the stability of the emulsion. Depending on the composition of this barrier, it is possible that it could govern the rates of oxygen transfer from the aqueous phase to the lipid droplet and, therefore, the rate at which oxygen can be consumed by reaction with lipids.

Finally, the packaging contains the emulsion and provides a barrier between the emulsion and the outside environment. The packaging, although permeable, limits the rate of oxygen supply to the emulsion. Some packaging, however, allows light to reach the product which can lead to oxidation when photosensitive reactants are

present. In many industrial applications, it is the packaging that allows the producer to optimise the shelf life and cost of a product by using cheaper materials which still give the product the required shelf life instead of using more expensive materials that would needlessly increase the cost of the product.

3.2 Defining the model required

As suggested in the literature review and briefly shown in Figure 3.1, the system includes a number of variables that require investigating. Although a model that uses an ingredient list to predict the exact shelf life of a product would be ideal, such a model is not able to be formulated given the volume of work required and the limited information available in literature. The individual effects and interactions between each of the variables would not only be prohibitively time consuming, but the resulting model would have limited application.

Rather than formulate an all-encompassing model, a model that has mechanistic basis will be formulated in such a way that simple and fast tests can be carried out on a product to define values within the model. The model will be able to:

- 1) Explain the rate of lipid oxidation using a mechanistic basis.
- 2) Quantify the relationship between temperature and lipid oxidation rates.
- 3) Define a relationship between the extent of lipid oxidation and lipid oxidation rates.
- 4) Describe the effects the addition of antioxidants has on lipid oxidation rates.
- 5) Describe the effects of oil concentration in an oil-in-water emulsion.
- 6) Explain and quantify the effects of limiting the oxygen supply using polymer packaging.

3.3 Adding detail to the conceptual model

Since the emulsion consists of two immiscible liquids divided by a well defined boundary, it seems reasonable to treat them as separate but interacting entities. Additionally, since common packaging materials have been thoroughly investigated by other researchers it is sensible to treat the packaging as a second level of complexity. For these reasons and as a method of breaking the system into manageable pieces, the system will be investigated as three separate systems of increasing complexity, which are:

- 1) Lipids/bulk oils.
- 2) Oil-in-water emulsions.
- 3) Packaged oil/emulsions.

Although the system will be broken down into manageable pieces, it is important not to lose sight of the role each piece plays on the system as a whole. Every experiment/investigation must be justified according to the expected effects on the whole system and not just the effects on the individual section being investigated. In order to achieve this, each section will be discussed and its likely effects on the entire system will be identified.

3.4 Lipids – investigations on bulk oils

As suggested earlier, the lipids exist as evenly distributed droplets within an emulsion and are surrounded by a well-defined boundary. The droplets can be thought of as packets of bulk oil contained in a permeable packaging whose properties are defined by the interfacial layer. The reactions in the droplets are, to some degree, independent of the composition of the aqueous phase and are only affected by the supply of oxygen from the aqueous phase and the reactions that occur at the boundary. This can be estimated as a net effect in an emulsion. The reactions that take place in the droplet can be investigated in a bulk oil system free from the complications that arise in emulsion systems.

An important question that must be asked about lipids in emulsions is, what causes the lipids to oxidise and how fast? A literature review identified the following factors according to their potential to significantly change the rates of lipid oxidation:

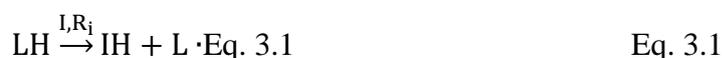
- Extent of reaction
- Degree of saturation of lipids
- Temperature
- Pro-oxidants
- Antioxidants

Before each of the factors listed above can be discussed, it is important to develop some background information about the way lipid oxidation occurs in the system under investigation and the way in which the reactions are tracked. These two areas will be considered alongside the factors listed above.

3.4.1 Reaction mechanism: three stage oxidation pathway

A literature review revealed a large number of reactions and products of lipid oxidation. Thankfully, the vast number of reactions can be summarised into a three stage oxidation pathway including initiation, propagation and termination reactions, all of which were discussed in the literature review but will be briefly described (in their simplest form) here as they form an important foundation of this thesis.

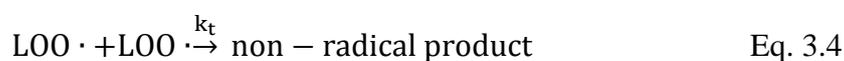
Initiation:



Propagation:



Termination:



As can be seen in Equations 3.1 to 3.4 (from Frankel, 2005), the three stage reaction pathway begins with the formation of radicals via the slow initiation reactions that usually involve the reaction of singlet oxygen with lipids. The radical species are highly unstable and react with nearby oxygen and/or lipids to form other radical species and lipid hydroperoxides which are semi-stable intermediate products and are in equilibrium with radical species.

Although not given above (as it was not presented by Frankel, 2005) lipid hydroperoxides, also called primary oxidation products, breakdown in the presence of oxygen and, to a limited degree, in the absence of oxygen to form more radical species which are free to react with oxygen and lipids. This recycling of radical species and consequential consumption of oxygen and lipids is characteristic of the propagation phase and explains the exponential phase often used to describe the increase in oxidation rate caused by the buildup of highly reactive species.

While propagation reactions are continually consuming lipids, termination reactions are removing radical species and lipid hydroperoxides from the system. The radicals are capable of reacting with each other to form stable non-reactive products. These products are often the cause of the rancid odours and flavours associated with oxidised fats. The rate of termination is much slower than the rate of propagation initially but as the rates of propagation slow, because of either the removal of oxygen or through the lack of other reactant species, the termination reactions become more prevalent.

Although the above descriptions are simplified, they can explain the relative rates of each process. For example, the initial rate of oxidation is very slow and correlates very well with the build-up of lipid hydroperoxides/radical species during initiation reactions. The increase in reaction rate and consequential move into the exponential phase correlates very well with the formation of significant quantities of lipid hydroperoxides, which are essentially a pool of potential radical species, which are formed by propagation reactions. Finally, the gradual development of rancidity is generated during the propagation phase as significant quantities of tertiary oxidation products are formed by termination reactions.

3.4.2: An alternative representation of lipid oxidation

The reaction mechanisms proposed in the literature and used to develop a revised reaction pathway (Figure 3.2) suggest that the rate of lipid oxidation is dependent on the concentration of lipid hydroperoxides. Certainly experiments by Takahashi *et al.* (2000) and Labuza (1971) suggest that there is a close relationship between lipid hydroperoxide concentrations and the rate of lipid oxidation.

After an in depth literature review, however, it seems that the rate of lipid oxidation is in fact dependent on the concentration of radical species rather than lipid hydroperoxides. Rather than lipid hydroperoxides themselves, it is the radicals that react with oxygen, lipids and other radicals to form oxidation products. This hypothesis, combined with results from experimental work reprinted in the literature, suggests that an equilibrium relationship exists between radical species and lipid hydroperoxides. This is depicted schematically in Figure 3.2

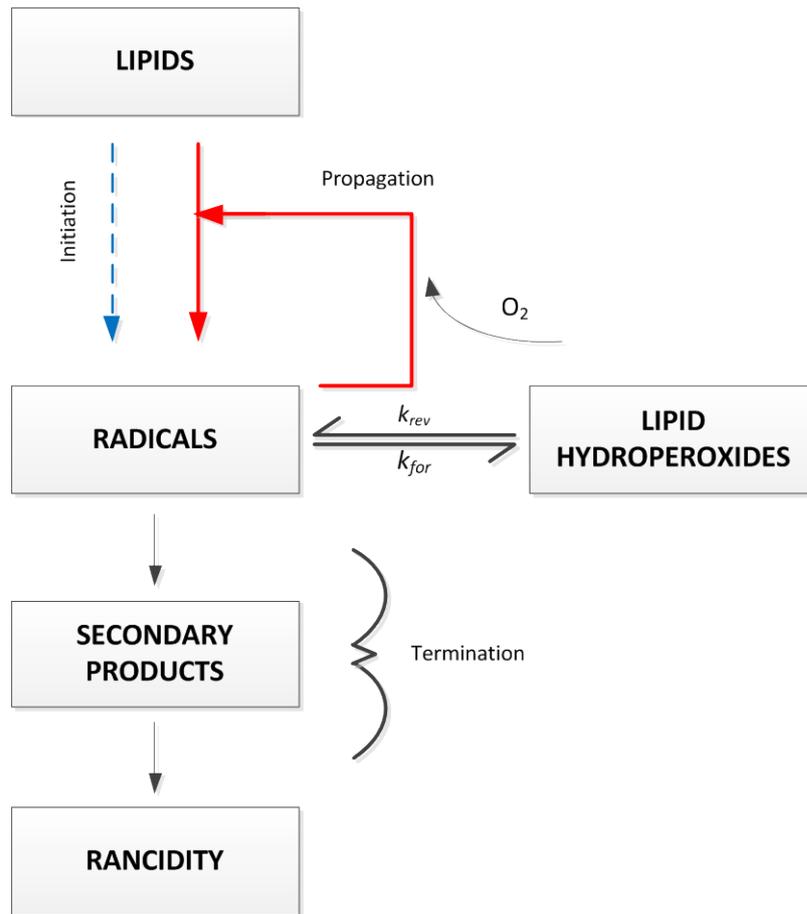


Figure 3.2: Revised lipid oxidation pathway including the equilibrium between radical species and lipid hydroperoxides. The thickness of the lines represents the relative amount of each of the reactions that takes place during the development of rancidity in oils.

This equilibrium based reaction mechanism suggests that the concentration of lipid hydroperoxides can still be used to predict lipid oxidation rates, because it can describe the concentration of radical species. That is, Equation 3.5 applies:

$$C_{RADICALS} \propto C_{LOOH} \quad \text{Eq. 3.5}$$

where $C_{RADICALS}$ is the concentration of radical species and C_{LOOH} is the concentration of lipid hydroperoxides

This way of presenting the mechanism highlights the significance of radical species rather than lipid hydroperoxides alone. It suggests that any system change that shifts the equilibrium balance toward the radical side of the reaction would increase the rate of lipid oxidation. Interestingly, a shift in the equilibrium position toward the radical

side would act to increase the rate of radical reaction with lipids and, in doing so, increase the concentration of lipid hydroperoxides.

This presentation does not introduce new chemical reactions. Instead it simplifies the mechanism in a way that redefines the importance of radical species and provides some relationship between lipid hydroperoxides and radical species. Unfortunately, directly measuring radical species concentrations is difficult as they exist for such a short period of time. Lipid hydroperoxides are used as an estimate of the concentrations of radicals and will be an important measure of lipid oxidation rates and act as a predictor for lipid oxidation models.

3.4.3 Summary hypothesis and mathematical expression

From the above discussions it should be clear that the rate of lipid oxidation is proportional to the availability of radicals to participate in reactions. Since the radicals are suggested to be proportional to the concentration of lipid hydroperoxides in the system, the rate of lipid oxidation (after the initial slow initiation reactions) should be proportional to the concentrations of lipid hydroperoxides. This is shown in Eq. 3.5 and 3.6 where the rate of oxygen consumption is used as the measure of the lipid oxidation rate.

$$\frac{\partial C_{O_2}}{\partial t} = f(C_{LOOH}) \quad \text{Eq. 3.6}$$

where C_{O_2} is the concentration of oxygen in oil

Although the exact form of Eq. 3.6 is yet to be determined, it is suggested that it will hold true until the extent of lipid oxidation reaches a point where the consumption of oxygen by secondary and tertiary reactions becomes significant. However, because the flavour thresholds for rancidity-causing oxidation products is often measured in parts per million (Jacobsen, 1999), most of the oxygen consumption is likely to occur during the formation of lipid hydroperoxides rather than tertiary products. Additionally, because secondary and tertiary reactions occur at a relatively slow rate as long as radicals are present, it is likely that the product will already be unacceptable

for consumption before oxygen consumption via tertiary reactions becomes significant (Takahashi *et al.*, 2000; Labuza, 1971).

Although not significant in terms of oxygen consumption, secondary and tertiary oxidation reactions determine the rate of rancidity development (Takahashi *et al.*, 2000; Labuza, 1971). These products are formed from radical species and can, therefore, be shown to be some function of the lipid hydroperoxide concentration (Equations 3.7 and 3.6).

$$\text{Rate of secondary and tertiary reaction} = \frac{\partial C_S}{\partial t} = f(C_{LOOH}) \quad \text{Eq. 3.7}$$

$$\text{Rate of rancidity} = \frac{\partial R}{\partial t} = f\left(\frac{\partial C_S}{\partial t}\right) \quad \text{Eq. 3.8}$$

where $f\left(\frac{\partial C_S}{\partial t}\right)$ is determined by the route taken to form rancid products from lipid hydroperoxides.

So, in its simplest form, the rate of rancidity development $\left(\frac{\partial R}{\partial t}\right)$ is proportional to the concentration of lipid hydroperoxides. Therefore, any measure of the lipid hydroperoxide concentration should provide an indication of the rate of rancidity development.

3.4.4 Measures of lipid oxidation: pathway and reaction rates

The rate of lipid oxidation is dependent on a number of different factors including the makeup of the emulsion/oil as well as the external conditions. What is often overlooked is the true meaning of the results gained from methods used to measure lipid oxidation rates. For example, because the rate of tertiary product formation is proportional to the lipid hydroperoxide concentration, a test that measures the formation of tertiary oxidation products will have a lag phase and can be misinterpreted as a low rate of oxidation. Conversely, a measure of a primary oxidation product will show very large changes in initial oxidation products, potentially before rancidity has occurred.

Although tests that focus on tertiary products correlate well with rancidity as measured by sensory analysis, they are not suitable for studying the initial lipid oxidation reactions which have been shown to be the most significant in terms of the proportion of time occupied during lipid oxidation. For this reason, it is best to measure oxidation in terms of a reactant that can be used to track the changes in the initiation and propagation rates (encompassing the initiation and exponential phases of lipid oxidation). Lipid hydroperoxides have proven to be a useful measure of lipid oxidation but on their own cannot be used to compare two different oils as the absolute results, peroxides value, tend to be different for different oils and cannot be applied to whole foods.

Unlike peroxide values, dissolved oxygen and individual lipid concentrations allow for direct comparison of oxidation rates and the extent of oxidation of different oils. While the methods for measuring lipids and fatty acid profiles can be difficult and requires expensive equipment, dissolved oxygen is relatively easy and inexpensive to measure. Since oxidation occurs quickly in the presence of oxygen and since oxygen is required during the initiation stage of lipid oxidation, the rate of oxygen consumption should prove to be useful tool to track lipid oxidation. Additionally, the movement of oxygen through permeable polymer packaging has been shown to cause changes to the shelf life of oxidation prone products, thus highlighting the importance of oxygen in lipid oxidation.

3.4.5 Extent of reaction: linking oxygen and lipid hydroperoxides

The way in which the rate of lipid oxidation changes with time has already been discussed. That is, the rate of reaction is very slow initially while initiation reactions dominate. When enough lipid hydroperoxides have built up, the rate of lipid oxidation increases exponentially. The rate of lipid oxidation is therefore, dependent on the extent of reaction.

The dependence of reaction rate on extent of lipid oxidation, however, needs to be determined in order to fulfill the requirements outlined above. Since the formation and destruction of lipid hydroperoxides in the presence of oxygen is significantly

greater than in scenarios where the oxygen has been depleted, it is reasonable to expect that the rate of oxygen consumption should give a direct measure of the rate of lipid oxidation (Takahashi *et al.*, 2000). It should be possible to determine the relationship between the rate of lipid oxidation and extent of lipid oxidation by relating the concentration of lipid hydroperoxides to the rates of oxygen consumption.

Having identified the relationship between oxidation rate and extent of oxidation, it should be possible to correlate the amount of oxygen required to cause a given change in lipid hydroperoxide concentration. For example, by integrating Eq. 3.6, it should be possible to determine the amount of oxygen required for a given change in lipid hydroperoxide concentration. Thus:

$$C_{O_2} - C_{O_{2i}} = \int f(C_{LOOH}) \partial t \quad \text{Eq. 3.9}$$

where $C_{O_{2i}}$ is the concentration of oxygen in oil initially.

Depending on the results of such a relationship, it may be possible to use the cumulative oxygen consumption as a measure of extent of reaction. Because of the relationships between radical species, lipid hydroperoxides and rancid products (shown in Eqs. 3.6 to 3.8) it may be possible to use the cumulative oxygen consumption to predict the rate of oxygen consumption and vice versa. This would be useful when trying to predict the extent of oxidation of a given oil or the remaining shelf life of a packaged product.

3.4.6 Saturation of lipids: stability against oxidation

The effect of lipid saturation has been the focus of a number of different studies and has been discussed in the literature review. A general rule of thumb is that the more saturated the lipid, the more stable it will be to lipid oxidation (Frankel, 2005). Oils rich in omega-3's tend to oxidise quickly and animal fats such as lard tend to be highly resistant to lipid oxidation. The ratio of oxygen consumed relative to a given change in lipid hydroperoxide concentration in the proposed mechanism should, however, remain unchanged if there are no other oxygen consuming reactions occurring.

Although the ‘rule of thumb’ described above stands true for the vast majority of oils, the exact relationship between the degree of unsaturation and consequential rate of lipid oxidation is not clear. Although a simple modification in rate constant is likely, the effects of degree of unsaturation cannot be easily studied independently of other factors such as the position of the double bonds on a fatty acid chain. The resulting complications make it much more practical to include the degree of unsaturation in the model without needing an explicit value that requires complicated experimental design.

Although the effects of degree of unsaturation will not be directly dealt with in the model required for this thesis, it will be useful to provide some evidence that shows how the degree of unsaturation changes the rates of lipid oxidation. Rather than test directly, a method that will yield the net effect of factors, including the degree of saturation, will allow shelf life predictions to be made.

3.4.7 Temperature: reaction rates and the Arrhenius law

The effect of temperature on lipid oxidation rates is less complicated than some of the other factors that have been discussed here and in the literature review. Despite this, very few researchers have described the effect of changing the reaction temperature on lipid oxidation rates.

For moderate changes in temperature (below ~60°C) the reaction mechanism is not expected to change. Unfortunately, many accelerated shelf life testing methods (Allen & Hamilton, 1994; Mateos *et al.*, 2006) use temperatures close to 100°C. By ensuring the reaction temperatures remain below 60°C, it should be possible to use an Arrhenius type approximation to model the effect temperature has on lipid oxidation rates. Depending on the sensitivity to temperature, the reaction rates could then be modified within the predicting model to simulate environments like storage and transport.

3.4.8 Antioxidants: delaying lipid oxidation

Like the degree of unsaturation, the effects of antioxidants on lipid oxidation are not as straight forward as some would suggest. As discussed in the literature review (Section 2.2), antioxidant action can differ for different antioxidants. For example, primary antioxidants remove radicals from the system while secondary antioxidants act to replenish primary antioxidants (Fennema, 1996). To make matters more complex, some antioxidants have been shown to act as pro-oxidants in some situations.

The ability for an antioxidant to retard lipid oxidation is also dependent on the overall system properties including the solubility of the antioxidant (be it lipid or water soluble) and the reaction or interaction with other species in the system. In the case of a whole food, there may be a series of naturally occurring antioxidants whose individual effects cannot be quantitatively determined.

Although the effects of antioxidants in whole food systems are difficult to quantify, the individual effects of added antioxidants can be measured. There is a fairly large quantity of literature that discusses how the addition of antioxidants affects the rates of lipid oxidation (examples include, Alamed *et al.*, 2006; Frankel 2005; Roginsky & Lissi, 2005; Reische *et al.*, 1998; Huang *et al.*, 1996b; Fennema, 1996; Yoshida *et al.*, 1993). Much of the information is, however, qualitative. Few actually attempt to quantify the effects of antioxidant addition and understand the mechanisms of antioxidant action (examples of those who do include Roginsky & Lissi, 2005; Kortenska *et al.*, 2002; Takahashi *et al.*, 2001).

The effect of antioxidant addition on the reaction scheme developed in this chapter is dependent on the type of antioxidant being added. Primary antioxidants act to remove radical species. Effectively they are proton donors and, through resonance structures, are relatively stable when oxidised. In the reaction scheme developed here, primary antioxidants would be removing radical species and in doing so, they would reduce the rate of lipid oxidation. The magnitude of the rate reduction is likely to be proportional to the antioxidant concentration. In this case Eq. 3.6 still applies. Instead, the rate of lipid hydroperoxide formation is reduced at a rate proportional to the antioxidant

concentration. That is, the rate of oxygen consumption is a function of the lipid hydroperoxide concentration and antioxidant action.

$$\frac{\partial C_{O_2}}{\partial t} = f(C_{LOOH}, C_A) \quad \text{Eq. 3.10}$$

where C_A is the antioxidant concentration

The effectiveness of the primary antioxidant (i.e. the relative reactive properties of the antioxidant) will likely lead to an effective antioxidant concentration. For example, if butylated hydroxyanisole (BHA) was used as a benchmark and had an effectiveness of one, and vitamin E was less reactive, then the effectiveness of vitamin E would be less than one. Multiplying the concentration and the effectiveness of the antioxidant would then yield an effective antioxidant concentration.

The effective concentration of a primary antioxidant can also be altered by the addition of a secondary antioxidant that acts to replenish the primary antioxidant. In this case, the net effect of the primary and secondary antioxidant would need to be tested. Furthermore, the effectiveness of other additives such as metal chelators are highly dependent on the properties of the system and should be tested within the whole food system and not individually.

In any case, the effects of antioxidants should be thought of as a net effect. In general terms, a combination of antioxidants will likely reduce the concentration of radical species and, as a result, the rate of lipid oxidation. Eq. 3.10 should hold true for any combination of antioxidants, including combinations that result in pro-oxidant activity.

3.4.9 Pro-oxidants: catalysing lipid oxidation

Common pro-oxidants like metal ions are suggested to assist the breakdown of lipid hydroperoxides to form radicals (Labuza, 1971). The result is a net increase in the rate of radical formation and/or a shift in the amount of radicals available for reaction at any point in time. According to the reaction scheme in Figure 3.2, the net result should be an increase in the rate of lipid oxidation.

Like the effects of antioxidants, the magnitude of the effects of pro-oxidant addition is likely to be proportional to the concentration of pro-oxidant. As such, Eq. 3.11 should apply. Much like antioxidants, the effects of pro-oxidants are not only going to depend on the type of pro-oxidant but also on the interactions between the pro-oxidants and the other species in the system.

$$\frac{\partial C_{O_2}}{\partial t} = f(C_{LOOH}, C_A, C_P) \quad \text{Eq. 3.11}$$

where C_P is the concentration of pro-oxidants

Extra complication is introduced when considering the ability, or lack thereof, to remove pro-oxidants from test samples, let alone from real food products. However, because pro-oxidants are likely to exist in all lipid oxidation systems (especially in food products), the addition of further pro-oxidants will accelerate lipid oxidation without changing the mechanism of lipid oxidation. Furthermore, it is quite possible that pro-oxidants continue to act while the effects of antioxidants are diminished over time.

Rather than attempt to determine a complex relationship between pro-oxidants and other chemicals in foods, it seems realistic to formulate a model, with a mechanistic basis, that can accommodate the net effect of pro-oxidants using simple, quick and cost effective methods. The model, although not capable of explaining all reactions, could be used to predict the lipid oxidation rates without needing to define all the complex reactions that take place in a whole food system.

3.5 Emulsions – effects of bulk oils in water

After characterising the reaction mechanism and consequential rate equation for lipid oxidation in bulk oils, it is important to determine how the reaction mechanism and rates are affected by placing the oils in an emulsion complete with all the hydrophilic

reactants, catalysts and antioxidants that exist in the aqueous phase. This will move the system one step closer to approximating a whole food.

Like bulk oils, a host of factors have been identified in the literature review as being present in the aqueous phase and as having an effect on the rates of lipid oxidation.

These factors include:

- Hydrophilic antioxidants
- Catalysts (i.e. metal ions, heme, chlorophyll)
- Other reactant species

3.5.1 The aqueous phase

Although there are a number of separate hydrophilic species that either increase or decrease lipid oxidation rates in emulsions, the fact remains that food producers have limited control over the species that are present in the aqueous phase of an emulsion. It is possible, through careful selection of machinery, to minimise the addition of metal ions, but the contents of the aqueous phase are usually determined by the foods used in the emulsion. For example, a soup containing emulsified oil will have an aqueous phase which is determined by the ingredients used. Removal of catalysts cannot be realistically done without removing ingredients or by using impractical extraction methods. Furthermore, foods rich in plant matter will undoubtedly contain chlorophyll and meat products will always contain heme, both of which have been shown to have a significant effect on the rates of lipid oxidation (Carlsen *et al.*, 2005; Chen & Liu, 1998).

As a result of the lack of control over the contents of the aqueous phase, it seems unrealistic to expect a model to be able to identify the effects of each reactant/catalyst/antioxidant that may or may not be present in the system. Like many other reactants, the effect of complex interactions with other reactants makes such an investigation purely academic with little chance of a practical application. Rather, the ability to test and predict the net effect of the constituents in the aqueous phase would

be useful in that the overall effects can be determined without needing to know the exact contents of the aqueous phase, thus eliminating complex investigations.

3.5.2 The interfacial layer

The interfacial layer provides researchers with a unique challenge. The layer forms the boundary between the lipid and aqueous phases and consists of surfactants (which make it possible to form emulsions) and amphiphilic antioxidants. Qualitatively, it is simple to conclude that the addition of amphiphilic antioxidants or any barrier molecule that can sit between the two phases will decrease the rate of oxygen supply or rate of oxidation at the surface of a lipid droplet. The quantitative effect, however, is somewhat more complex.

Although somewhat repetitive, the issue at hand is the complexity that is created by the vast number of different amphiphilic species that may present in an emulsion and the ability for each to behave differently in the company of other species and under different reaction conditions. Again, the solution is to measure the net effect of the collection of amphiphilic species without needing to quantify the concentrations of amphiphilic species present in the emulsion under investigation. The inclusion of amphiphilic species as a net effect would also include the effects of physical changes such as those seen by the formation of surfactant micelles.

3.5.3 Surface area to volume ratio

Exposure to oxygen is obviously a key factor that defines how a system will oxidise. Bulk oils and emulsions packaged in such a way as to maximise the surface area to volume ratios will obviously have higher oxygen transfer rates than those with lower surface area to volume ratios. Such products are much more likely to have a higher proportion of oil exposed to oxygen which are, therefore, able to react much more quickly than oils that have limited or no oxygen exposure.

A second aspect that applies only to emulsions is the surface area that is exposed by each droplet. The reasons for its importance are similar to those given above in that

the rates of lipid oxidation may be affected by the exposure to oxygen. The effects of individual droplet surface area to volume ratio are likely to be limited, however, as the droplet surface area will have little effect if the aqueous phase surrounding the droplet has no oxygen available for reaction. The effects of droplet surface area could be diluted by the effects of the overall product surface area to volume ratio and the proportion of the emulsion that contains oxygen. These are issues which must be investigated as they form a key part in determining how an unmixed product will oxidise.

3.6 Oxygen supply and the role of packaging

Having settled on a reaction mechanism and having quantified the effects of the important characteristics addressed above, it is imperative that the role which oxygen plays in the system be investigated. Literature has suggested that initiation reactions require oxygen and that propagation reactions occur much more quickly in the presence of oxygen than when oxygen is limited (Nobile *et al.*, 2003). Certainly oxygen barrier polymers have been effectively used to lower lipid oxidation rates and extend shelf life. This alone stands as evidence that oxygen plays a very significant role in determining lipid oxidation rates.

Up to this point the role of oxygen in the reaction system has only been briefly discussed and has not received the attention that is needed to explain the importance that oxygen plays in moderating the rates of lipid oxidation in bulk oils and oil-in-water emulsions. For clarity, the role of oxygen will be broken into two distinct areas:

- The effects of oxygen in bulk oils
- The effects of oxygen in oil-in-water emulsions

3.6.1 Bulk oils: supply and consumption of oxygen

To allow shelf life predictions to be made, a link must be drawn between the rates of lipid oxidation and the effects of oxygen and oxygen supply. The rates of lipid

oxidation in the presence of oxygen are significantly greater than when oxygen is limiting. The rates of oxygen supply to the reacting lipids must, therefore, be one of the defining characteristics of the oxidation rates. Furthermore, the balance between reaction rate and rate of oxygen supply will determine whether the system acts as a well mixed system where there is oxygen throughout or a system where there is a concentration gradient from the surface to the centre of the product. These ideas will be the focus of the following paragraphs.

3.6.2 Bulk oils: oxygen supply and consumption rates

At any point in the oxidation system, the rate of change in oxygen concentration will be governed by the rate of oxygen supply via diffusion (or mass transfer from the environment at the surface) and the rate of oxygen consumption via oxidation. This is shown in Eq. 3.12.

$$\frac{\partial C_{O_2}}{\partial t} = \text{Rate of } O_2 \text{ supply} - \text{Rate of } O_2 \text{ consumption} \quad \text{Eq. 3.12}$$

As suggested earlier, the formation of lipid hydroperoxides requires oxygen. Similarly, the formation of some secondary and tertiary oxidation products can require oxygen. The rate of oxygen consumption must, therefore, be proportional to the concentration of lipid hydroperoxides so Eq. 3.6 can be re-written as shown in Eq. 3.13.

$$\frac{\partial C_{O_2}}{\partial t} = \text{Rate of } O_2 \text{ supply} - f(C_{LOOH}) \quad \text{Eq. 3.13}$$

In a system that is well mixed through operations such as transporting via trucks, the oxygen concentration throughout the product will be the same. However, because the shelf life of such products is often measured in years, the few hours spent in these conditions would be unlikely to cause significant changes to the overall shelf life of the product. Instead stationary storage on pallets and shelving is likely to represent the vast majority of the product's lifetime.

Although not being mixed during storage, a product will begin its life with a uniform oxygen concentration throughout. During storage the oxygen initially dissolved in the product will be consumed by lipid oxidation and other oxygen consuming reactions. The only supply of oxygen will be through the packaging or directly from the air in the case of a product stored in the open air. Once lipid oxidation reactions have begun, the concentration of oxygen at any point in the oil will be determined by the rate of oxygen supply and the rate of oxygen consumption via reaction.

There are three scenarios that can be formed by the relationship between reaction rate and the rate of oxygen supply. They are:

- 1) Uniform oxygen concentration throughout a bulk oil
- 2) No oxygen at any point in the bulk oil
- 3) An oxygen concentration gradient is formed from the surface to the centre of the bulk oil

The first scenario, where the concentration of oxygen is uniform throughout a bulk oil, can only exist when the rates of oxygen supply are greater than the rates of oxygen consumption or if there is significant mixing occurring. Conversely the second scenario, where there is no oxygen anywhere in a bulk oil, can only exist when the potential rate of oxygen consumption is significantly greater than the rate at which it can be supplied. Finally, the third, scenario would exist if the rates of oxygen supply and consumption do not differ by orders of magnitude allowing a significant and measurable concentration gradient from the surface and into the centre of the bulk oil. Figure 3.3 depicts the different outcomes that may arise according to the relative rates of oxygen consumption and supply.

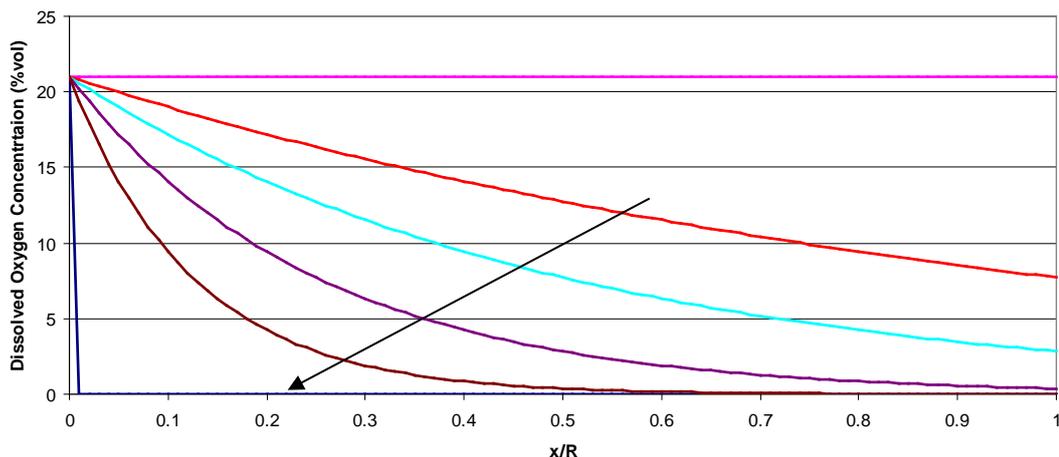


Figure 3.3: Schematic demonstration of the outcomes caused by altering the relative rates of oxygen supply and reaction.

As can be seen in Figure 3.3 it is theoretically possible to force a specific concentration gradient across a product by manipulating the rates of oxygen supply and consumption. Essentially the higher the rate of consumption relative to the rate of oxygen supply the steeper the resulting concentration gradient will be. Practically, there is a limit to how much the rates of consumption and supply can be changed. This, however, will form a key part of the investigation required for this thesis.

3.6.3 Diffusion versus reaction in emulsions

Although the physical system is different, the way in which a stable emulsion reacts should show a similar trend to those discussed above for bulk oils. That is, the relative rates of oxygen consumption and supply will determine the type of oxygen profile found throughout the emulsion system. In the case of emulsions, it is expected that the consumption rates in the individual droplets will be the same as those in bulk oils (although the droplets nearer the surface may oxidise faster than those not exposed to oxygen near the centre). The measured rate of oxygen consumption, however, is likely to be lower than those noted in bulk oils as the concentration of oil in emulsions is simply lower than that in bulk oils. The aqueous phase of an emulsion could act as a sump of oxygen that will be able to travel into the droplets of oil. The result is that, although the oils are oxidising at a rate similar to those in bulk oils, a batch reaction

where all oxygen must be removed from a saturated sample will take longer in an emulsion than in a bulk oil.

3.6.4 Diffusion of other reactant species

Up to this point most of the discussions have been focused on the supply and consumption of oxygen. As of yet, the movement of other reactant species has not been considered. It is, however, likely that the movement of reactant species (other than oxygen) will change the rates at which lipid oxidation can occur. These effects are likely to couple with the effects of localised oxygen consumption and the rates of oxygen supply.

If the movement of reactant species, other than oxygen, is significantly slower than the rates of oxygen supply and reaction, then it is logical to assume that oxidation will occur as a front moving from the surface to the centre of an oil as the reactants are consumed. This is almost certainly going to be the case in a solid system where the reactants are effectively frozen in place. If the rates of oxygen consumption and/or the rate at which oxygen can be supplied to the surface of a bulk oil are not significantly higher than the rate at which reactants can diffuse, then the movement of reactants will play a significant role in determining the rate of shelf life deterioration. In this case, oxidation will not move as a front but will instead form a concentration gradient from the surface towards the centre of the oil.

In emulsions, where the oil is distributed in small droplets, the diffusion of reactants within a droplet is unlikely to change the rates of lipid oxidation. The movement of individual droplets or the movement of reactants from one droplet to another may, however, change the oxidation characteristics of an emulsion. Furthermore, the distance between the droplets and the concentration gradient from the aqueous phase to the surface of the droplet may determine how much, if any, oxygen is able to diffuse past a droplet and move towards the centre of the emulsion. This effect is depicted in Figure 3.4.

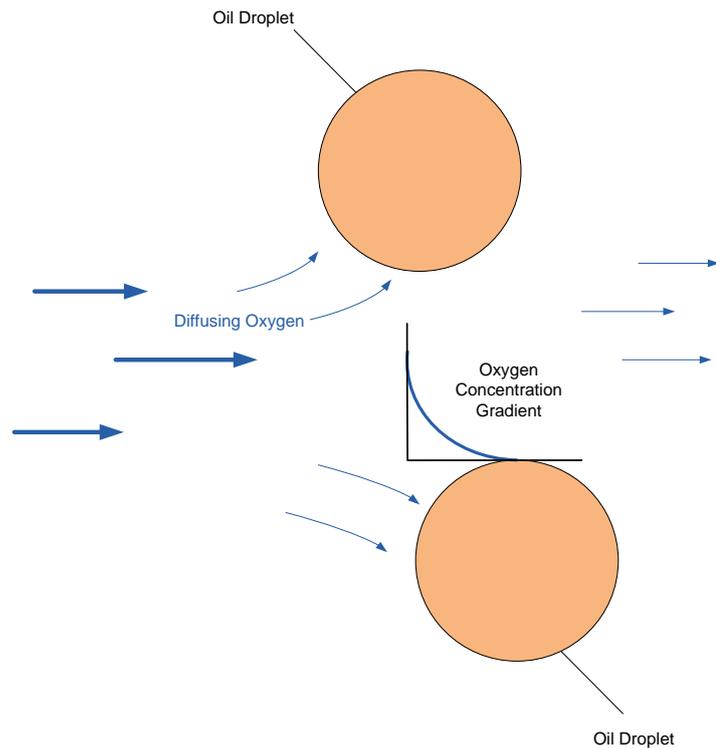


Figure 3.4: Figure depicting the movement of oxygen past oil droplets suspended in an unmixed emulsion.

3.7: Conclusions

There are a number of competing and interacting reactions that occur during lipid oxidation. The physical and chemical aspects of an oxidation system combine to create complicated effects that, if studied alone, could take many years to even partially define. Furthermore, the results of the studies required to create an all-encompassing model would be of little more use than the model proposed here. Instead a simplified model with mechanistic basis will be used. The model will use a series of simple experiments to provide values for model inputs and will be carried out in such a way as to define the net effects of antioxidants and pro-oxidants.

The effects of oxygen diffusion and reaction need to be extensively studied as they will likely determine whether the system will act as if well-mixed, i.e. no concentration gradients, or whether there will be significant oxygen concentration gradients throughout the product. Furthermore, the movement of reactants, other than oxygen, needs to be investigated. This coupled with the diffusion and reaction

models, will determine whether the reactions can occur solely at the surface of a product. Finally, the rate at which oxidation can occur in the absence of oxygen needs to be determined as this will help determine whether lipids can partially oxidise at the surface of a product and diffuse into the centre where they can continue to oxidise in the absence of oxygen.

CHAPTER 4
METHOD DEVELOPMENT

4.0 Introduction

From the literature review, it was found that the rate of oxygen consumption would be a useful measure to assess and characterise lipid oxidation rates. There are, however, some problems with current methods that can be used to measure oxygen consumption. For example, the Rancimat and Oxidograph tests require high temperatures, which may lead to changes in reaction mechanisms. This chapter outlines the development of a new method that attempts to overcome the problems identified with current methods.

There are three broad areas that require new methods of study. They are:

- 1) Lipid oxidation rates in bulk oils
- 2) Lipid oxidation rates in oil-in-water emulsions
- 3) Effects of oxygen supply on reaction rates in bulk oils and oil-in-water emulsions.

4.1 Lipid oxidation rates

4.1.1 Measuring lipid oxidation rates

The rate at which lipid oxidation occurs will define the time that a lipid oxidation prone product can be stored before it is no longer satisfactory for consumption. The focus of all experiments must be on the rates of lipid oxidation and how the rates of lipid oxidation change when changes are made to the oxidation system. It is, therefore, important to develop a method to track lipid oxidation reactions in such a way as to fully describe the oxidation rates in both bulk oils and oil-in-water emulsions.

4.1.2 Tracking initial versus final products of lipid oxidation

As has been discussed in the literature review, there are a number of different methods that can be used to describe the state of a lipid. Each method essentially measures the concentrations of reactants or products of lipid oxidation. Some methods track the initial products of lipid oxidation while others track the changes in secondary or final oxidation products such as ketones and aldehydes. The value of each of these measures depends on the stage of oxidation that is most important to the experiment being conducted. For example, gas chromatography (GC) analysis of volatiles such as hexanal tend to correlate well with sensory analysis which suggests that it would be a useful way of identifying when a product is rancid. Conversely, peroxide values (PV) are an indicative measure of the concentration of lipid hydroperoxides present in a sample.

As suggested in the literature review, lipid hydroperoxides are the only semi-stable product formed during the initial stages of lipid oxidation. PVs are, therefore, good at determining the initial rates of lipid oxidation which tend to account for most of the time required to produce a rancid product. Furthermore, lipid hydroperoxides are always formed in lipid oxidation while individual tertiary products (hexanal, propanal etc.) can be formed in different ratios according to the conditions in which oxidation is taking place.

PVs have been used for a number of years by a vast number of different researchers and industry groups. The methods have been well established and are published as official methods by the American Oil Chemists Society. Even though PVs are an indicative and empirical measure, they are capable of explaining changes in lipid hydroperoxide concentrations without the need for expensive and complicated high performance liquid chromatography (HPLC) techniques. Furthermore, their ability to track changes in primary oxidation products makes them ideal for explaining the effects of the slow initiation reactions and induction phase in the lipid oxidation pathway. Additionally, since the lipid oxidation pathway must include the formation of lipid hydroperoxides (unlike tertiary products which may or may not be formed depending on the reaction system), PVs should always give good estimates of lipid

oxidation rates irrespective of the oxidation system. For these reasons, PVs will be used extensively during future investigations.

4.1.3 Tracking common reactants versus reaction products

While PVs are a good universal measure of the net rates of lipid hydroperoxide formation, they are not capable of describing the entire lipid oxidation process. To do so using conventional techniques would require an array of measures targeting secondary and tertiary products so that any changes in reaction pathway and mechanism could be accounted for. The results of such analysis would be hard to understand and would yield questionable results purely because the researcher would have to make assumptions about the reaction pathway and know which reactions are or are not taking place. Such measures would not be practical as they would require highly skilled persons and an excessive amount of time. Furthermore, the exercise would have to be repeated every time a new product or lipid were being analysed.

Rather than measuring the array of potential secondary and tertiary products, some researchers have turned to measuring the concentrations of fatty acids and/or lipids in a sample. Since the lipids are the initial reactants, it makes sense that a drop in the number of double bonds (by oxidation of unsaturated fatty acids) would correlate with lipid oxidation rates. This is indeed the case during initial reactions but does not account for the reaction of radical species to form the tertiary products. Like the measures described earlier, measuring fatty acid concentrations requires extensive prior knowledge and expensive equipment that most industries would struggle to justify.

Unlike fatty acids and lipids, oxygen consumption rates can be easily measured and require far more cost effective equipment. Like fatty acids/lipids, oxygen is consumed during lipid oxidation. Unlike lipids, oxygen is consumed during the vast majority of the lipid oxidation pathway from the early initiation reactions to the tertiary reactions. Lipid oxidation, as has been discussed earlier, occurs far more slowly, if at all, in the absence of oxygen. Such dependence, coupled with the ease of measurement and low cost of equipment makes oxygen an excellent measure of lipid oxidation rates.

Coupled with PVs, oxygen consumption rates should be able to explain the changes in lipid oxidation rates in any system and under any conditions.

4.1.4 Method requirements

As discussed in Sections 2.3.8 and 2.3.9 of Chapter 2, both the Oxidograph and Rancimat methods require the use of high temperatures to accelerate the rates of lipid oxidation. Without the use of high temperature, both methods would likely require weeks or months to gain useful oxygen consumption data. Unfortunately, lipid oxidation is the combination of many reactions. It is, therefore, risky to assume that all the reactions would be affected by temperature changes equally. That is, the rates of all the lipid oxidation reactions occurring at 25°C are unlikely to be the same at 100°C. Furthermore, Rancimat and Oxidograph tests are reliant on pressure and conductivity measurements and, as a result, are sensitive to the addition of other compounds (i.e. BHT or BHA antioxidants) and the evolution of volatile products.

Due to their sensitivity to additives and volatile production as well as the need for high temperatures, the Rancimat and Oxidograph methods are unlikely to be able to measure oxidation rates in real food systems. To be able to measure oxygen consumption rates at lower temperatures (~35°C), without being sensitive to the effects of additives and within a reasonable timeframe a new method must be developed. The method must:

- 1) Accurately and reliably measure oxygen consumption rates.
- 2) Measure oxygen concentrations directly and not rely on pressure or conductivity measurement.
- 3) Be sensitive enough to describe the effects of system changes.
- 4) Yield useful information in a short period of time (<24hr).

4.2 Design – new method of measuring oxygen consumption

Using dissolved oxygen concentrations as a measure of lipid oxidation brings with it unique challenges. Aside from overcoming the shortfalls of the Rancimat and Oxidograph methods, the most important aspects of a new system are:

- 1) The ability to gain useful results in a short period of time.
- 2) Confidence that diffusion limitations are avoided
- 3) Strict control over oxygen supply

4.2.1 Rig design: Temperature versus time

There are a number of ways to gain useful oxygen consumption data in a short period of time. The Rancimat and Oxidograph methods do this by raising the temperature and, therefore, rates of reaction. This has been identified as a potential fault in the methods and, therefore, will need to be avoided in the new method. The new method will need to be sensitive enough to measure low rates of lipid oxidation.

One method of ensuring that the system is sensitive to changes in oxygen concentrations is to remove the sources of error that would otherwise cloud the results of experiments. The addition of oxygen during experimentation, especially low rates of addition to small samples of oil, is inherently difficult and, as a result, would likely be a large source of error. Since the rates of oxygen consumption during lipid oxidation are likely to be significantly smaller than the addition of oxygen through sparging mass transfer, a more complicated membrane system would likely be needed. Such a method would add further complexity and be hard to control. As such, a batch oxidation system is more likely to form the basis of a controllable and repeatable method.

4.2.2 Rig Design: Homogeneous Reaction

Another issue that occurs frequently during lipid oxidation experiments is the introduction of diffusion limited lipid oxidation. For example, in an unmixed system where oil is stored in a closed bottle with a headspace of air, as used by Shim & Lee (2011), the reaction at the surface of the oil/air interface will likely be faster than that further from the oxygen source. This is because it is likely that the rate of oxygen consumption will be greater than the rate at which oxygen can diffuse through the oil sample. The rate of reaction within the sample of oil would, in this case, be a function of the rate of oxygen supply, which can be difficult to predict. Although only a hypothesis at this stage, understanding and avoiding this type of system would help remove uncertainty from any experimental data.

Removing the headspace is obviously the first step in ensuring that the reaction system is homogeneous. A bulk oil that is in a sealed vessel without a headspace will react at the same rate throughout the sample as long as the sample is well mixed at the beginning of the experiment. During a batch oxidation, no physical mixing would be required. The same can be said for a real food product. As long as the food product is physically stable during the measurement period, the reaction system will be homogenous and no reactant concentration gradients will exist.

4.2.3 Rig design: oxygen supply

Having decided that a batch oxidation system is most suitable for low temperature lipid oxidation, careful control of the starting oxygen concentration becomes of utmost importance. Furthermore, because the rates of oxygen consumption are dependent on the reaction system it is important to have a method to supply oxygen to the system at rates that are orders of magnitude faster than the rates of oxygen consumption.

Perhaps the simplest method of supplying large quantities of oxygen to the system quickly is to sparge gas directly through the sample. Should different oxygen concentrations be required, then sparging oxygen and nitrogen gas mixtures can be

used. If the rate of oxygen addition is too low, then either increasing the rate of sparging and/or decreasing the size of the bubbles passing through the sample could be used to increase the rate of oxygen mass transfer to the system. Furthermore, as long as the rate of sparging is high enough to ensure mixing of the sample, no additional physical mixing would be required to create a homogenous sample.

4.2.4 Rig design: construction

The solution to the problems noted above is shown in Figures 4.1 and 4.2. The rig consists of three main pieces all of which are made of stainless steel; the main reaction chamber, the solid sparging base and the plunger.

The solid base was designed to allow the sparging air to enter in the centre of the main reaction chamber which helps ensure the sparging operation creates significant mixing of the sample. The base, shown in the bottom of Figure 4.2, includes a ball valve (1/4" stainless steel Swagelok) to isolate the reaction vessel from the atmosphere that is welded directly to the bottom of the main reaction chamber.

The main reaction chamber is a 25mm internal diameter hollow stainless steel tube. A septum sampling port is fitted halfway up the reaction chamber. The sampling port is a 1/4" Swagelok fitting as pictured in Figures 4.2 and 4.3. Before each experiment, a 1/2" septum (Grace David Discovery Sciences, Illinois, USA) is placed inside the Swagelok fitting to create an airtight seal. During an experiment, the dissolved oxygen probe can be placed through the septum and into the sample while still ensuring the sample is isolated from the outside environment.

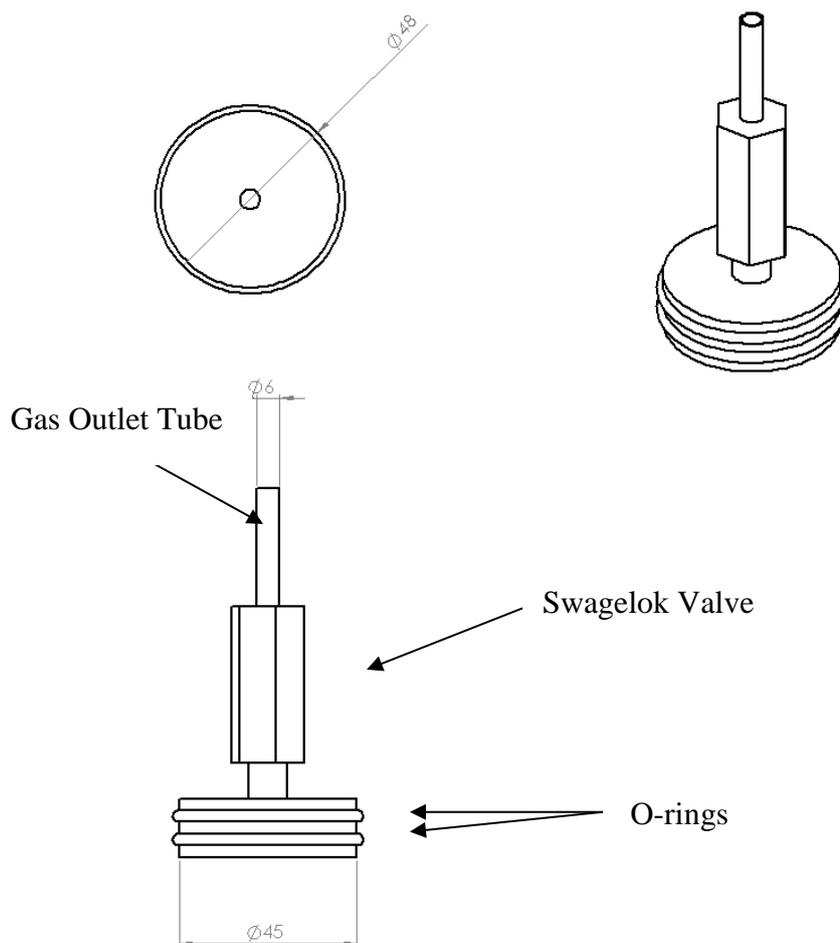


Figure 4.1: Schematic of the plunger part of the oxidation rig.

The plunger was designed to allow for the gas headspace that accumulates above the sample to be expelled from the main reaction chamber. During sparging, the plunger can be fitted loosely on top of the main reaction chamber. Once sparged, the plunger can be pushed down into the main reaction chamber to remove the air (which occurs when only sample is being expelled from the rig). An airtight seal is created by two o-rings that have an outer radius 1.5mm greater than the steel plunger (Figures 4.1 and 4.3). A ball valve (1/4" stainless steel Swagelok) on top of the plunger can then be closed to ensure the rig is airtight. To ensure the oxygen probe is not damaged by the plunger, the inside surface of the reaction rig has been machined to create a 1.5mm step above the septum sampling port and ensures that the plunger cannot be forced past that point. The machined step can be seen in Figure 4.3.

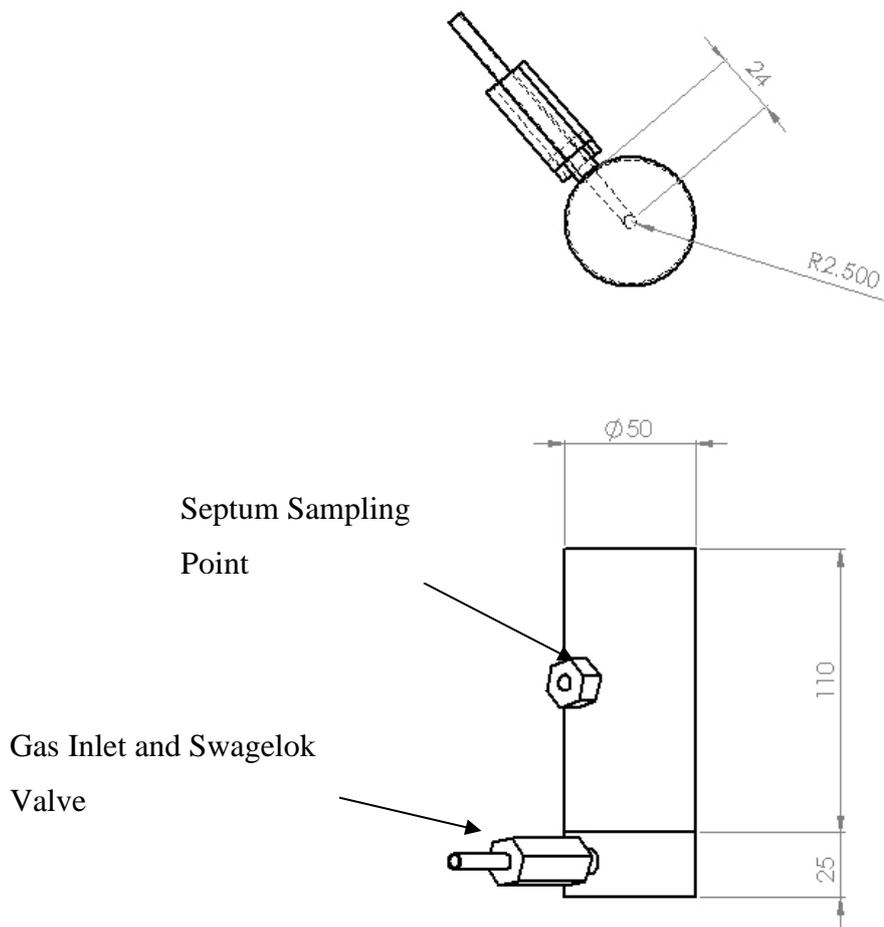


Figure 4.2: Schematic of the main reaction chamber and base.

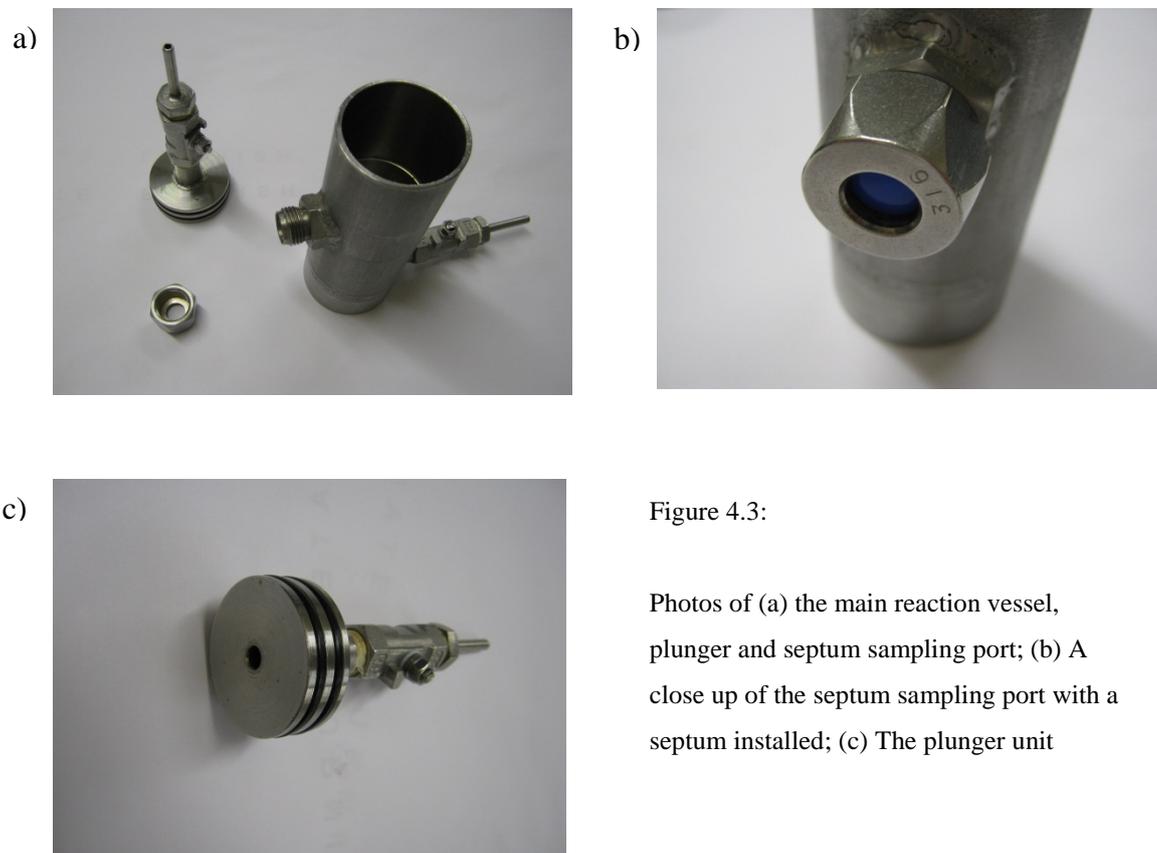


Figure 4.3:

Photos of (a) the main reaction vessel, plunger and septum sampling port; (b) A close up of the septum sampling port with a septum installed; (c) The plunger unit

4.2.5 Brief operation guide

A normal batch oxidation (depicted in Figure 4.4) begins with the sparging, using a mass flow controller (GFC17S, Aalborg, New York, USA), of the preheated sample of oil with air to ensure that the oil contains a predefined concentration of oxygen (usually saturated with air at atmospheric pressure). To do so the plunger must be loosely fitted to the top of the rig and air must be passed through the sample at a predefined rate. The rate of sparging is usually between $0.2\text{-}0.3\text{ L}\cdot\text{min}^{-1}$ but can vary from $0 - 0.5\text{ L}\cdot\text{min}^{-1}$ and is only limited by the volume of oil that is lost with the exiting sparging gas. When the required oxygen concentration is reached, which can be identified by either monitoring the oxygen concentration using the dissolved oxygen probe, or by sparging for a predefined length of time, the rig can be sealed by closing the bottom valve and pushing the plunger down until only sample free of gas

bubbles is leaving the reaction chamber. At this point there should be no headspace remaining and the top valve can be closed. The sample and reaction vessel can then be placed in a pre-heated incubator (WatVic Ltd) that is controlled externally using a PID controller (CAL3200, CAL Controls, Illinois, USA) to gain temperature control more sensitive than the incubators onboard temperature controller can achieve.

Once sparged with air (or any other gas mixture), the dissolved oxygen concentration can be continuously monitored and recorded. Once the reaction has finished, the sample can be disposed of by opening both valves and removing the plunger and DO probe. If a further batch oxidation of the same sample is required, the user can simply sparge air through the sample using the same method given above.

When using the reaction rig as a batch reactor, no mixing is required other than that created by sparging. Once sparged, the oxygen (as well as other reactant species) will be evenly distributed throughout the reaction rig. Because of the uniform distribution of reactants and the lack of oxygen concentration gradients produced by a headspace, lipid oxidation reactions will occur at the same rate throughout the sample.

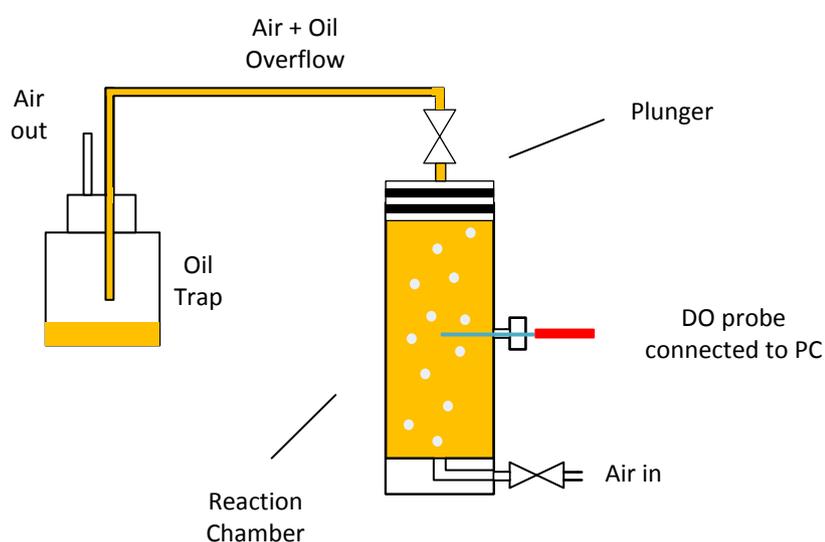


Figure 4.4: Schematic depicting the operation of the oxidation rig and dissolved oxygen probe.

The oxidation rig can also be used for continuous oxidation. To do this, the sample in the rig can be continuously sparged at a given rate while the dissolved oxygen concentration is measured. Care must be taken when using the vessel as a continuous

reactor as the sparging must not create oxygen concentration gradients within the sample. To avoid this, the sparging must create suitable mixing. For this method to yield measurable results, the rate of oxygen consumption and supply must be carefully balanced. If the rate of oxygen supply is greater than the rate of reaction, then the concentration of oxygen in the sample will always be at the saturation point. It is suggested that batch oxidations are likely to be far easier to control and analyse.

4.3 Dissolved oxygen and fluorescence probe

4.3.1 Dissolved oxygen probe operation

The ability to accurately and reliably measure dissolved oxygen concentrations is central to all the investigations required for this thesis. Recent advances in technology have made it possible to measure oxygen consumption in real time and to do so with confidence that the results will be repeatable.

The dissolved oxygen probe (FOXY-R-8cm, OceanOptics, Florida, USA) includes an immobilised dye at the end of a double fibre optic cable. The dye is excited by blue light which is supplied through one of fibre optic cables. Once excited, the dye fluoresces. The rate of decay of the fluorescence, displayed as fluorescence lifetime, is recorded by the spectrophotometer unit (MFPP100, OceanOptics, Florida, USA). The lifetime is displayed and recorded along with the sample temperature, time, and atmospheric pressure by the TauTheta (OceanOptics, Florida, USA) software supplied with the oxygen measuring hardware.

The relationship between lifetime and concentration is given by the Stern-Volmer equation (Equation 4.1) .

$$\frac{\tau_0}{\tau} = 1 + kC_v \quad \text{Eq. 4.1}$$

where:

τ = Lifetime at a known C_v

τ_0 = Lifetime at $C_v=0$

C_v = Volumetric concentration or partial pressure of oxygen

k = quenching rate coefficient

Calibration of the probe response requires collecting lifetime readings for at least two known oxygen concentrations. This is usually done by sparging an oil with air until it is saturated with 21.2% oxygen by volume, often displayed in partial pressure, and by allowing it to react (or by sparging with nitrogen) until there is no oxygen left. A plot of τ_0/τ versus C_v will have an intercept of 1 and a gradient equal to k .

4.3.2 Probe response to changes in temperature

As the method and hardware for measuring dissolved oxygen concentrations is relatively new, it was important to determine how sensitive the response from the probe is to temperature, as temperature is likely to be a factor in further investigations. To do this, a 200mL sample of mixed fish oil (Bakels Ltd, NZ) was heated to different temperatures (selected randomly) and sparged with air or nitrogen at 0.2 L.min⁻¹ to ensure that it was either saturated with oxygen or had no oxygen present. The steady state response from the probe (lifetime of fluorescence) was then measured at each temperature and oxygen concentration.

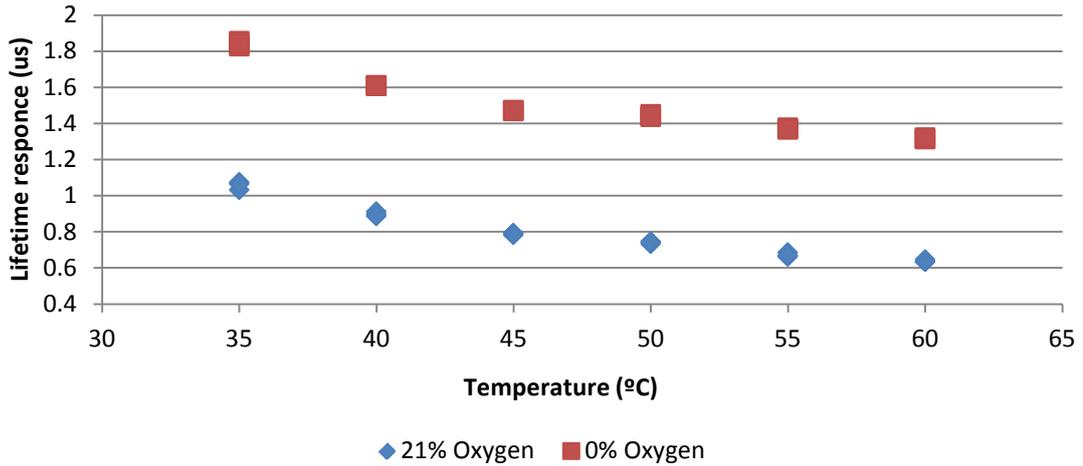


Figure 4.5: Plot showing the effects of temperature on the response from the dissolved oxygen probe. Note that the partial pressure of oxygen in solution was maintained by sparging with air or nitrogen at the given temperature.

As can be seen in Figure 4.5, the response from the probe changes with temperature even though the partial pressure of oxygen in solution does not. Intuitively, this makes sense as the response of the probe is dependent on the concentration of oxygen in the dye which in turn is dependent on the solubility of oxygen and mass transfer properties of the sample and dye. Not only will the solubility of oxygen in the sample change with temperature, but the solubility of oxygen in the dye will change with temperature as well. The probe response will likely change with temperature even through the partial pressure of oxygen in the sample does not. Regardless of the reasoning, this data suggests that the probe needs to be calibrated at each temperature and, to be sure of the results gained, before or after each batch oxidation.

4.3.3 Probe response to changes in media

During initial investigations, including those used to create Figure 4.5, it was noted that the response from the probe changed when moving from one medium to another. A short investigation was carried out to show the response that can be expected from the probe in different media. Since the probe measures partial pressure, the type of medium should have no effect on the response from the probe. Any changes in response would only occur if the partial pressure or temperature changes. The hypothesis is that only changes in solubility of oxygen in the dye would alter the

response given by the probe. It is unlikely that a change in sample would alter the response from the probe if the temperature and oxygen concentration were the same.

To test the above hypotheses, samples of mixed fish oil (Bakels Ltd, NZ), reverse osmosis water and air were heated to and held at 35°C using an incubator (WatVic Ltd). The oil and water samples were sparged with air for 45 minutes to ensure the samples were saturated with oxygen. The probe was then placed in each of the three samples in turn and the response recorded. Table 4.1 outlines the responses achieved.

Table 4.1: Probe response in different media saturated with 21% oxygen (from air).

Media	Average Lifetime (μs)	Standard Error ($\times 10^4$)
Air	2.155	0.653
Water	2.171	0.036
Oil	2.187	1.210

The results shown in Table 4.1 show that the average lifetime readings for the different media were significantly different ($P=0$, 95% CI). This difference, however, is unlikely to have a large impact on a batch oxidation where the difference between the lifetime responses at saturated and zero oxygen concentrations would be approximately one microsecond. For example, the difference between response in air and oil ($2.187-2.155=0.032\mu\text{s}$) is just 3.2% of the change during a batch oxidation. Furthermore, the difference seen could be due to minor differences in temperature.

Interestingly the results for mixed fish oil at 35°C are not the same as those shown in Figure 4.5. The experiments conducted to create Figure 4.5 and Table 4.1 were done using different oxygen probes. Although both probes are FOXY probes, the probe used in the experiment to give the results in Figure 4.5 is approximately 12 months older than the probe used to construct Table 4.1. This shift seems to be characteristic of the probes and according to the manufacture, and is expected as the probe ages. The results, however, reinforce the need for careful calibration.

Although the results suggest the response from the probe is nominally the same in different media, it is good practice to calibrate the probe in the medium that is being tested. The probe will deteriorate over time, so careful calibration will ensure that the effects of minor temperature changes, media effects and probe deterioration do not have a significant effect on the results gained during experiments.

4.4 Rig and method validation

Before continuing with experiments, the rig and method must be tested to prove that they function as required. To be sure of the results from the method developed are reliable, tests are required to show that:

- 1) The rig was free from 'leaks' allowing oxygen transfer
- 2) The temperature of the rig could be held constant
- 3) The oil in the rig could be suitably saturated with oxygen
- 4) The oxidation occurring between batch oxidations was small relative to a batch oxidation
- 5) The rig can ultimately produce batch oxidation curves repeatedly and reliably.

4.4.1 Oxidation rig – evidence of oxygen exclusion

Any oxygen that is supplied to an oil during lipid oxidation will increase the time that oxidation can continue and increase the extent of oxidation that can occur. If oxygen is able to enter the rig while a batch oxidation is occurring, and the rate of oxygen leakage is not accounted for, the results would suggest that the oil was in fact more stable than it actually is. Since no physical mixing is being used, oxygen leaks would potentially create an oxygen concentration gradient which would introduce extra complications to the system and make any analysis very difficult. It is, therefore, important to ensure that all rigs used are air tight and that the results gained are not skewed by oxygen migration.

To provide evidence that the rigs are air tight, the rigs were sterilised with ethanol, filled with reverse osmosis (RO) water at 35°C and placed in an incubator (WatVic) at the same temperature. The water was then sparged with nitrogen (pre-heated to 35°C) before being sealed within the rigs as described above. The oxygen concentration was then monitored for 4.5 days. The results of one such test are shown in Figure 4.6 below.

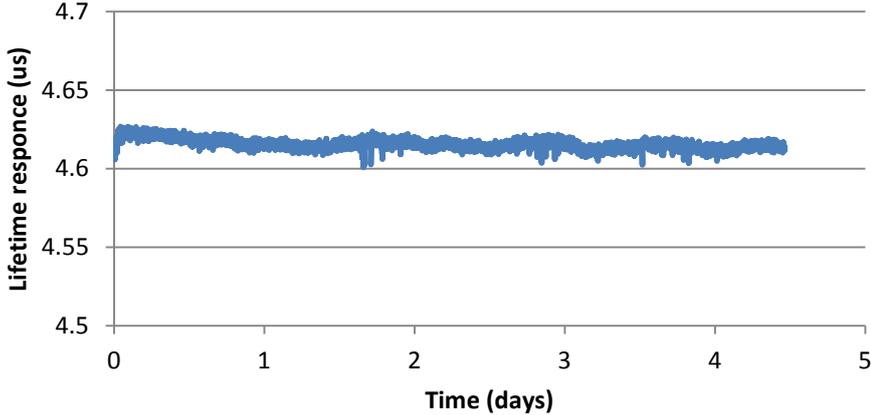


Figure 4.6: Results of a storage test to prove the rigs ability to exclude oxygen. Test carried out at 35°C.

The results shown in Figure 4.6 show that, over a period of 4.5 days, the lifetime response from the probe does not change. Infact the range of lifetime response is just 0.025µs which is equivalent to a change in oxygen concentration approximately 0.023% Furthermore, the change of 0.025 µs was not seen as a consistent trend over time, but instead simply the variation in response of the probe. At the end of the storage period, the rig was sparged with air to show that the lifetime measurement would have been different had the rig been leaking air. Sparging with air caused the lifetime measure to decrease to 2.32 µs which is the lifetime that can be expected if the water were saturated with oxygen from air at 35°C. These results suggest that there is no movement of oxygen into the rig and that the rig can be considered as air tight. All rigs used gave the same results and were shown to be air tight.

4.4.2 Temperature control

As has been discussed in the literature review and will be discussed in Chapter 6, rates of oxygen consumption via lipid oxidation are affected by changes in temperature. Furthermore, it has already been shown that the response of the fluorescence probe varies with temperature. It is, therefore, important that the temperature of the system is carefully controlled.

To achieve careful control of the temperature of the system, the entire system (air supply tubes and reaction vessel) was placed in an incubator (WatVic Ltd). The temperature of the incubator was controlled externally by a PID controller (CAL3200, CAL Controls, Illinois, USA) to ensure there is minimal deviation from the temperature setpoint. Figure 4.7 shows an example of the type of control that was gained from the externally controlled incubator. As can be seen in Figure 4.7, the temperature fluctuations are very small and well within the limits required for any analysis needed. In this case, the incubator was maintained at an average temperature of 35.36°C with a standard error of just 0.003°C. Note that there was an offset of 0.36°C. This offset is not considered important as a desired temperature of 35°C can be gained by using a setpoint of 34.64°C and because the ability to maintain a temperature is more important than being able to achieve a selected mean temperature with a large standard deviation.

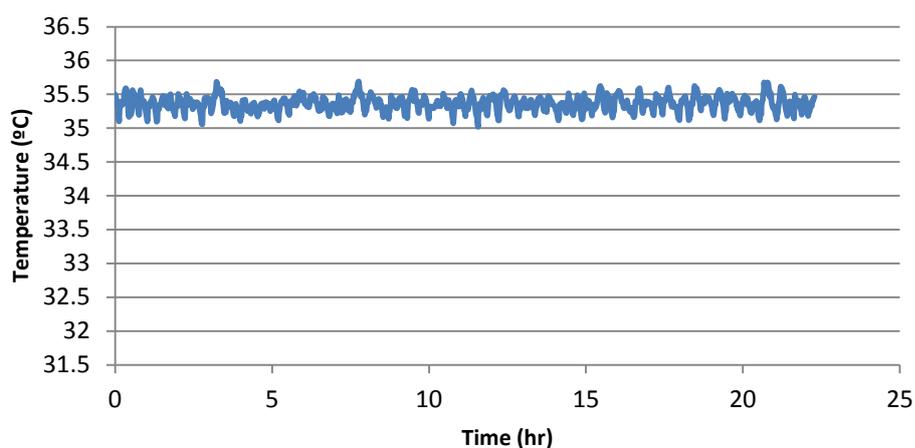


Figure 4.7: Temperature in the incubator with a set point of 35°C.

4.4.3 Saturation of oil with oxygen

Having shown that the rig is air tight and that the reaction system temperature is well controlled, it was important to develop a method to saturate the oil within the reaction rig with oxygen from the surrounding air or any other oxygen/nitrogen mixture. The ability to reliably saturate the oil with oxygen provides a useful method of defining the start point for any batch oxidation and provides the first calibration point for each batch oxidation.

To reach saturation, oxygen must be supplied at a rate much faster than it is removed by oxidation. In order to be sure that oxygen can be supplied at a rate fast enough to saturate the oil, a reaction system consisting of 90 mL of fish oil at 35°C was set up. Once preheated to the test temperature, 35°C, the oil was placed into a reaction vessel and sparged with nitrogen at a flowrate of 0.3 L.min⁻¹. The oxygen probe (FOXY-R-8cm, OceanOptics, Florida, USA) was placed in a plastic tube attached to the outlet valve of the reaction chamber (see Figure 4.3 and Figure 4.4) and the probe response was recorded every two seconds (TauTheta, OceanOptics, Florida). Once all oxygen had been removed, signaled by a steady state response from the probe during sparging with nitrogen, air was sparged through the sample at a rate of 0.3 L.min⁻¹. This sparging rate was chosen as this was deemed to be approximately the highest flowrate that could be used to sparge the sample without there being significant losses of sample with the exiting gas and because it provided significant mixing.

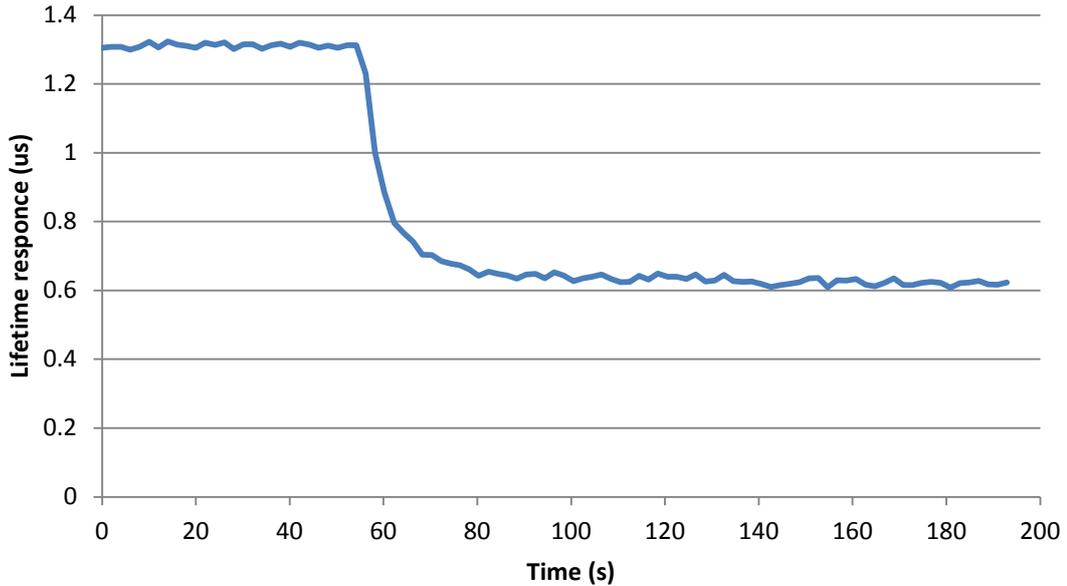


Figure 4.8: Oxygen probe response while in the exit gas stream above a sample of mixed fish oil at 35°C. Initial results are during sparging with nitrogen, followed by sparging with air.

As expected, the response from the probe (lifetime) initially began at its highest point when the sample of oil was sparged with nitrogen gas. Upon commencement of sparging, the response from the probe dropped quickly and reached a steady state value after approximately 180s. At the end of the experiment, the probe was removed from the exiting gas stream and placed in 35°C air at atmospheric pressure. The resulting probe response was equal to the steady state value given during the experiment and showed that the sample of oil must have been saturated with oxygen.

From the data used to create Figure 4.8 it is possible to determine the mass transfer coefficient, $k_L a$, for this system and shown in Equation 4.2. By estimating the magnitude of $k_L a$ it should be possible to understand how oxygen consumption rates alter the time needed for the oil to become saturated with oxygen.

$$\frac{\partial O_2}{\partial t} = k_L a (O_2^* - O_2) - r \quad \text{Eq. 4.2}$$

where O_2^* is the saturated oxygen concentration, O_2 is the oxygen concentration, and r is the oxygen consumption rate by oxidation reactions

As the dissolved oxygen probe gives a measure of the partial pressure of oxygen rather than the concentration in traditional concentration units, Eq. 4.2 cannot be used directly to estimate $k_L a$ without knowing the saturated oxygen concentration in oil. To estimate $k_L a$, we must first ensure that the rate of oxygen supply is significantly greater than the rate of oxygen consumption via reaction. Doing this allowed for the removal of the reaction term from Eq. 4.2. Secondly, using Henry's Law, which suggests a linear relationship between partial pressure and concentration, it is possible to convert the units of Eq. 4.2 from $\text{mol.m}^{-3}\text{s}^{-1}$ to Pa.s^{-1} as shown in Eq. 4.3.

$$\frac{\partial P_{O_2}}{\partial t} = k_L a (P_{O_2}^* - P_{O_2}) \quad \text{Eq. 4.3}$$

where $P_{O_2}^*$ is the saturated partial pressure of oxygen and P_{O_2} is the partial pressure of oxygen

Integrating Eq. 4.3 gives Eq. 4.4 which can then be used to create a plot to estimate $k_L a$.

$$\ln \left[\frac{P_{O_2}^* - P_{O_{2i}}}{P_{O_2}^* - P_{O_2}} \right] = k_L a (t - t_0) \quad \text{Eq. 4.4}$$

where $P_{O_{2i}}$ is the initial partial pressure of oxygen

By collecting data during sparging of oil and plotting the logarithmic term in Eq. 4.4 versus time, it was possible to estimate $k_L a$. The results, shown in Figure 4.9, gave a $k_L a$ of $8.5 \times 10^{-2} \text{ s}^{-1}$ for sparging of a sample of 90 mL of mixed fish oil (Bakels Ltd, NZ) at 35 °C with an air flowrate of 0.3 L.min^{-1} .

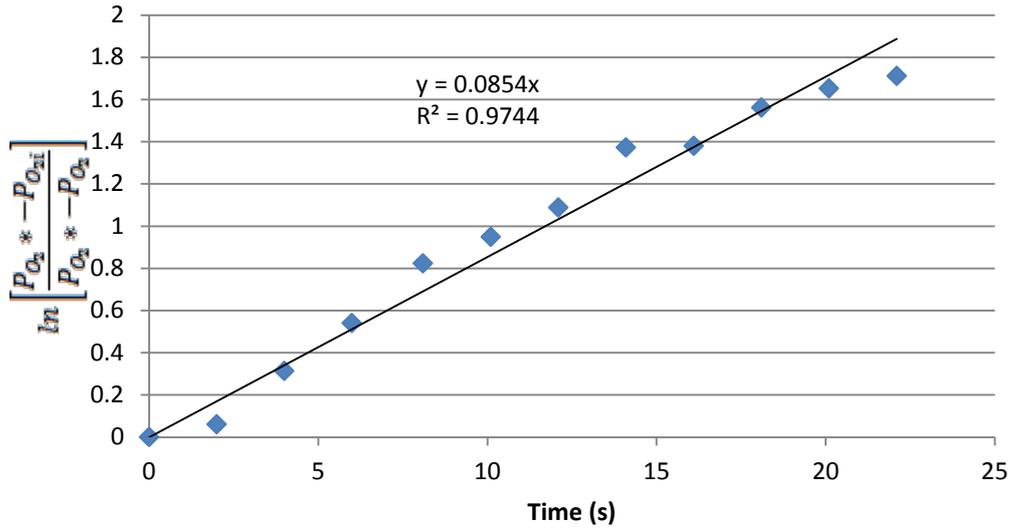


Figure 4.9: Plot to estimate the mass transfer coefficient kla for sparging of mixed fish oil with $0.2 \text{ L}\cdot\text{min}^{-1}$ air at $35 \text{ }^\circ\text{C}$

The above experiment suggests that it does not take long for a sample of oil to become saturated with oxygen. However, the results also show that the temperature of the sample does have an effect on the response from the probe. Allowing a preheated sample of oil to equilibrate with the incubator environment is likely to be a good practice. Therefore, a 30 minute sparging and equilibration time was used for all experiments from this point on.

4.4.4 Example of dissolved oxygen measurement

Early oxidations of fresh mixed fish oil were carried out in the rig using the methods described above in Section 4.2.2. For these tests, however, the same sample of mixed fish oil (Bakels Ltd, NZ) was put through three consecutive batch oxidations at $35 \text{ }^\circ\text{C}$. The results of one such test are shown in Figure 4.10.

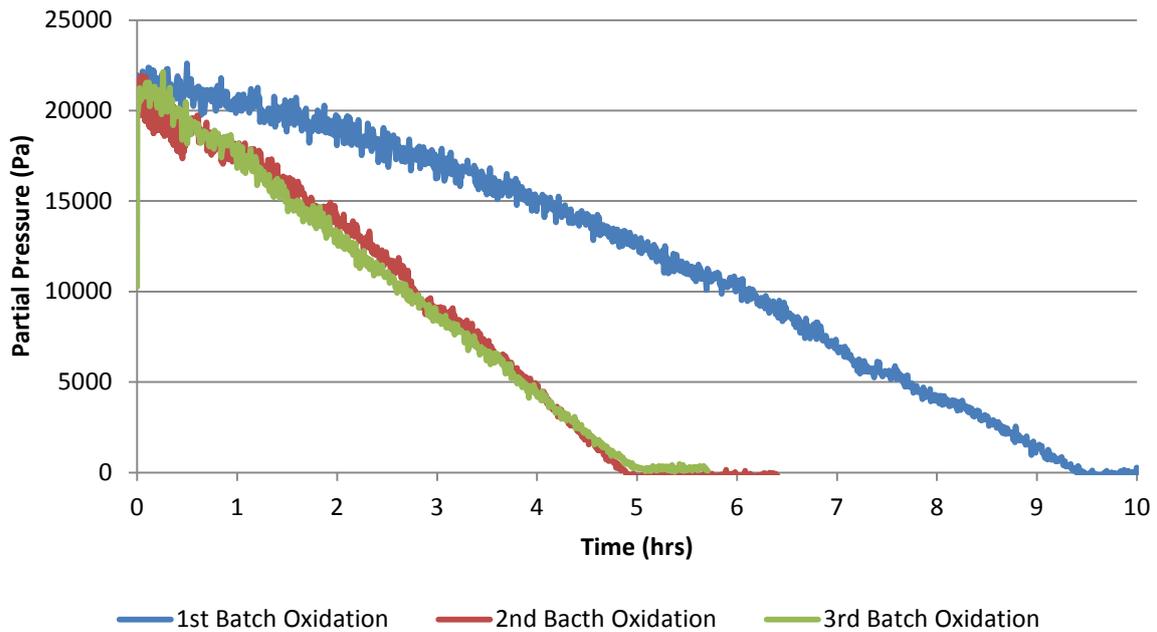


Figure 4.10: Dissolved oxygen profiles for mixed fish oil oxidised (batch) three times at 35 °C

The results in Figure 4.10 clearly show the increase in oxygen consumption rate caused by the successive batch oxidation of the same sample of oil. The results suggest a measureable difference with minimal scattering of the data. There is no lag phase or irregularities in the data shown in Figure 4.10 which suggests that the oil is free from bubbles that would rise in the rig and create concentration gradients. Furthermore, the response from the probe is smooth, suggesting that the minor temperature variations in the system are not causing shifting or fluctuating data.

4.4.5 Repeatability of the oxygen consumption method

Having shown that the rig is air tight and that the method is capable of producing oxygen consumption curves for bulk oils, it was important to show that the method can produce repeatable and reliable results. To do this, three separate batch oxidations were carried out at 35°C using three mixed fish oil samples (Bakels Ltd, NZ) from the same source. The results of this experiment are shown below in Figure 4.11.

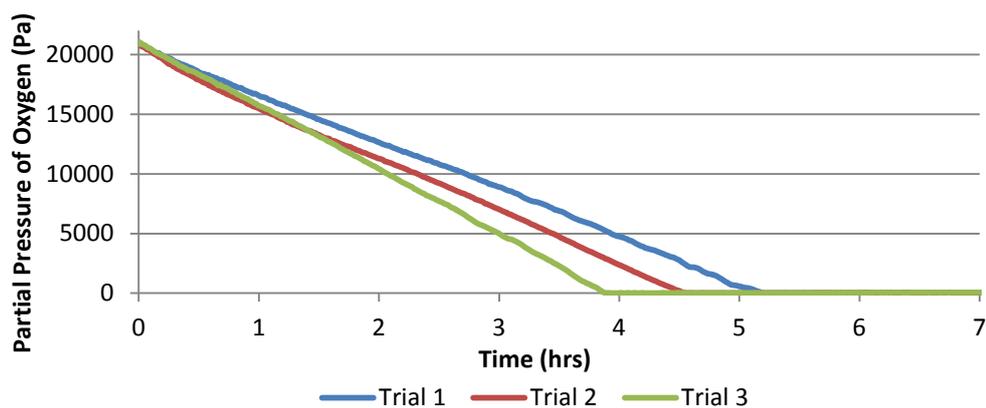


Figure 4.11: Results of three separate batch oxidations of fish oil.

As can be seen in Figure 4.11, the data gained from each individual batch oxidation (1st batch oxidation for three samples of the same oil) are not exactly the same. The time taken for the batch oxidations varies slightly. In this case, the average first batch oxidation time for fish oil at 35°C was 4.45 hrs with a standard error of 0.36 hrs. The error, however, is small compared to the changes in oxygen consumption rates between different oils as shown in Figure 4.10.

4.4.6 Effects of half hour sparging on reaction rate

Having decided upon a method of saturating oils with oxygen from air, it is important to determine what effects, if any, the half hour of sparging with air has on the oxygen consumption rates. This is because lipid oxidation will occur during sparging and will cause some change in the composition of the oil (i.e. lipid hydroperoxide concentration) and, therefore, the oxidation rates.

With fish oil at 35°C taking approximately five hours for its first batch oxidation, and approximately four hours for the second, the half hour of sparging represents approximately 0.1 to 0.125 of the time taken for a batch oxidation. This will have some effect on the concentration of lipid hydroperoxides within the sample. The magnitude of this effect, however, is not known.

From a qualitative viewpoint, if the change in lipid hydroperoxide concentration were large, then a significant change in the rate of oxygen consumption will occur as a result of the sparging period. A plot of the oxygen consumed versus the time of oxidation without including the half an hour for sparging would include step change between batch oxidations if the half hour of sparging were significant. This plot is shown in Figure 4.12 below.

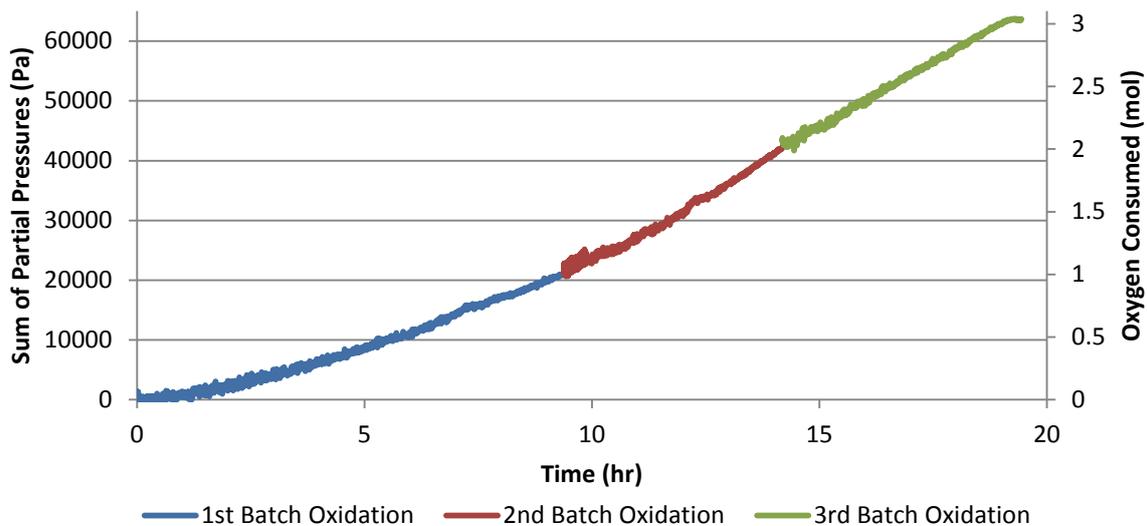


Figure 4.12: Oxygen consumed versus time for three successive batch oxidations of a sample of fish oil at 35°C. Note that the plot does not include the time taken for sparging between batch oxidations.

Figure 4.12, however does not suggest that a step change in oxygen consumption rate occurs between batch oxidations. This suggests that, although the lipid oxidation occurs during the sparging period, the effect on oxygen consumption rates is small. By assuming that the rate of oxygen consumption during sparging is constant and equal to the rate of oxygen consumption at the end of the previous batch oxidation, it is possible to estimate the amount of oxygen consumed during sparging. This, however, requires some knowledge of the relationship between the amount of lipid hydroperoxides formed per mole of oxygen consumed as well as better understanding of the solubility of oxygen in mixed fish oil. Both of these will be explored in detail in Chapter 6.

4.4.7 Batch oxidation of other oils and oil-in-water emulsions

So far there is significant evidence that the newly developed method is capable of measuring oxygen consumption rates in fish oils. Although there was no reason to believe that it would not be equally capable in other media, it was worth testing the method using different oils and oil-in-water emulsions to provide further proof that the method was suitable for the measurements required for this thesis.

For these tests, samples of fish oil, soya oil, canola oil, and flax oil were heated to the required reaction temperature before being put through a batch reaction as described in Section 4.2.2. Because of the large differences in reaction rates, different reaction temperatures were used to carry out the batch reactions.

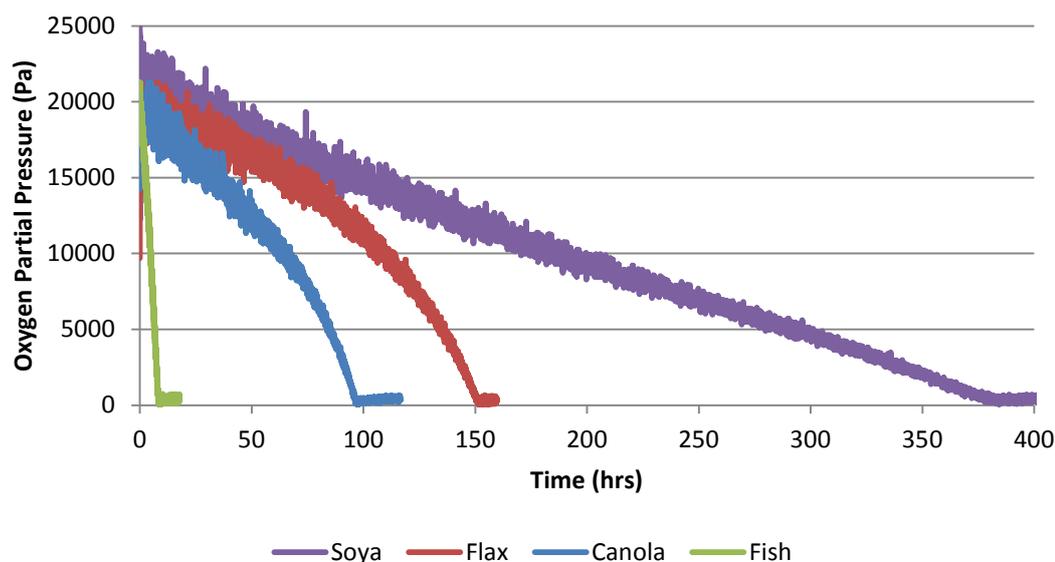


Figure 4.13: Results of batch oxidations of different oils and different temperatures. Temperatures were: Soya = 55°C, Flax = 35°C, Canola = 60°C, and Fish = 35°C.

There is clearly a large difference (see Figure 4.13) in the oxygen consumption rates for different oils at different temperatures. Fish oil, for example, had the highest rate of oxygen consumption despite being at a significantly lower temperature than the other oils. Although the initial state (i.e. extent of lipid oxidation) of the oils was not known, the curves presented on Figure 4.13 have the same shape which gives an early indication that the mechanisms of lipid oxidation are the same and that the newly

developed method can yield mechanistic information. Although it is too early to draw significant conclusions from these tests, it is possible to conclude that the new method is sensitive enough to see differences in oxygen consumption rates from different oils.

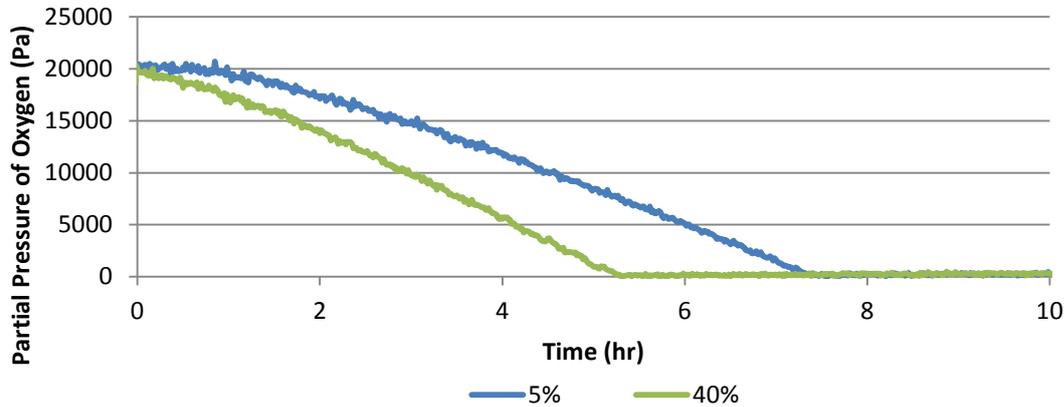


Figure 4.14: Results of batch oxidations of fish oil-in-water emulsions of different oil concentrations stabilised with 5% w/w lecithin.

To test the effects of diluting the oil by creating oil-in-water emulsions, two emulsions were constructed. The emulsions were created by mixing 40 °C fish oil (Bakels Ltd, NZ) at either 5% w/w and 40% w/w concentration with RO water at 40 °C using a Silverson mixer. 5% w/w lecithin (sourced from Heinz Wattie's Ltd) was used to stabilise the emulsions. Once formed, the emulsions were subjected to batch oxidations as described in Section 4.2.2. The results (Figure 4.13), suggest that the rate of oxygen consumption is proportional to the concentration of oil in the sample. This may seem obvious but it is useful to show that the method is still capable of showing the differences in oxygen consumption rates in oil-in-water emulsions; which is something that is difficult to achieve with standard analytical methods. Analytical methods like those used to measure the Peroxide Value (PV) would first require the oil to be separated from the water phase.

4.5 Conclusion

This work has produced a new method to measure oxygen consumption rates in oils and oil-in-water emulsions. By using carefully controlled temperature incubators, careful calibrations, an air tight batch lipid oxidation rig and a newly available dissolved oxygen probe, it is possible to reliably measure oxygen consumption rates in liquids and gasses. It was shown that it is possible to saturate an oil with oxygen from air and that the error in measurement is small compared to the differences in oxidation rates between different oils. It was shown that the new method appeared sensitive enough to see differences in oxygen consumption rates between different oils, oils at different temperatures and in oil-in-water emulsions. This new method can now be applied to investigate lipid oxidation rates in bulk oils as well as oil-in-water emulsions.

CHAPTER 5
BULK OILS - KINETICS

5.0 Introduction

In spite of the vast collection of literature describing the qualitative effects different factors have on the shelf life of lipid oxidation prone products, few (including Takahashi *et al.*, 2000; Yoshii *et al.*, 1999; Ozilgen & Ozilgen, 1990; Labuza, 1971) have been capable of transferring this knowledge into a predictive and accurate mechanistic model.

In Chapter 3, an overall but simplified conceptual model for characterising the reaction pathway and the factors influencing the rate of oxidation were outlined. In Chapter 4 a method to characterise lipid oxidation in oils and emulsions was developed. This chapter outlines the development of predictive rate equations to characterise how oxygen consumption and lipid hydroperoxide concentrations change in a system over time.

5.1 Empirical models in literature

Although there are models in literature that can be used to track lipid oxidation reactions, few have a mechanistic basis and even fewer are complete enough to be able to have application for food manufacturers. The issue in using a fully empirical model is that, by definition, empirical models are simply equations that are fitted to experimental data. These empirical models are usually combined with other pre-defined models, such as the Arrhenius law, to explain systems changes like reaction temperature. One such model is presented by Ozilgen and Ozilgen (1990) and modified by Yoshii *et al.* (1999). These models do not attempt to include any reference to the oxidation system but instead fit a logistic equation such as the one shown in Equation 5.1.

$$\frac{\partial C}{\partial t} = kC \left[1 - \frac{C}{C_{max}} \right] \quad \text{Eq. 5.1}$$

where k is a rate constant, C is any measure of lipid oxidation and C_{max} is the maximum value of C

This equation suggests that in early stages of oxidation when $C \ll C_{\max}$, the rate of oxidation is first order. At later stages when the reaction rate decreases, the C/C_{\max} term becomes important and eventually ensures that the reaction rate reaches zero when the $C=C_{\max}$. Ozilgen & Ozilgen (1990) found this model to be an excellent approximation ($R^2 > 0.95$) for a number of meats and egg being oxidised under different conditions and tracked using TBA values. Yoshii *et al.* (1999) found similarly impressive agreement between a modified logistic equation model, shown in Equations 5.2 and 5.3, and their experimental data. In this case, the measure of oxidation was the concentration of oxidisable media.

$$\frac{\partial Y}{\partial t} = -k_a Y(1 - Y) \quad \text{Eq. 5.2}$$

$$\ln \left[\frac{(1-Y)}{Y} \right] = k_a t + \ln \left[\frac{(1-Y_0)}{Y_0} \right] \quad \text{Eq. 5.3}$$

where Y is the extent of oxidation according to any measure of lipid oxidation, t is time and k_a is the appropriate rate constant.

Using curve fitting techniques (i.e. Langmuir type equations) it was possible to predict the changes to k_a caused by changes to reaction system. Unfortunately this method was usually limited to simple systems such as bulk oils and systems in which temperature is the only variable that changes. Such an empirical model requires a host of time consuming experiments that need to be repeated every time the reaction system changes. Furthermore, extrapolating the results from accelerated shelf life tests (which would be needed in order to meet time constraints) is risky and can be erroneous, especially when the temperature range for extrapolation is large (Gomez-Alonso *et al.*, 2004).

Another empirical model that fitted well with experimental data was presented by Shim & Lee (2011). This model used simple reaction kinetics to explain the rates of change in Peroxide Values (PV) in samples of perilla oil. The model was based on results before and after the end of the induction period. Before and after the induction period, the reaction was shown to follow pseudo-zero-order kinetics. This suggests

that the results can be broken down into two distinct linear regions as shown in Figure 5.1 below.

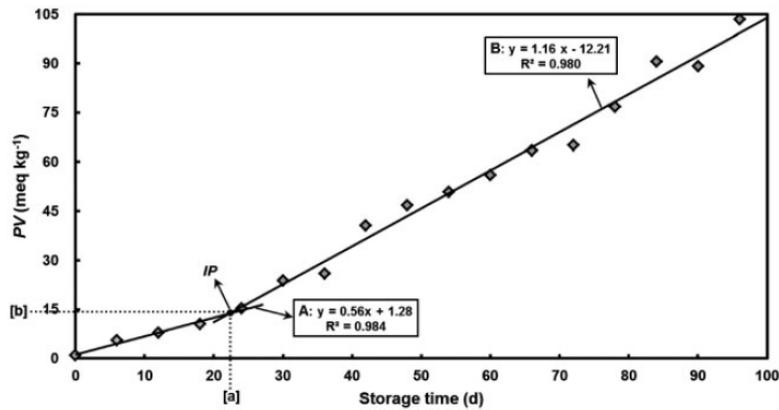


Figure 5.1: Plot from Shim and Lee (2011). Results of fitting zero order kinetics to the PVs within and after the induction period.

The results suggest that the PV can be simply predicted using Equation 5.4. Furthermore, coupling the model with the Arrhenius law, the rate constants k_1 and k_2 were successfully modified to predict the effects of changes in reaction temperature. Like the model presented by Ozilgen & Ozilgen (1990), this model does nothing to explain the mechanisms of lipid oxidation. This type of model is heavily dependent on experiments to characterise the system accurately. The models were, therefore, good at fitting experimental data but are unlikely to be predictive models.

$$PV = k_1IP + k_2(t - IP) \quad \text{Eq. 5.4}$$

where k_1 and k_2 are rate constants during and after the induction period, t is the time and IP is the induction period (time).

5.2 Mechanistic models in literature

Unlike empirical models, mechanistic models are derived from a known set of chemical reactions that, at least partially, describe the process of oxidation. A full mechanistic model will have the ability to predict the changes that occur through changes in both the environment and oxidation system. This makes mechanistic models particularly powerful predictive tools.

The downside of such a model is the inherent difficulty involved in acquiring enough information and knowledge to produce it. As yet, no one has been able to provide an all-encompassing model. Furthermore, the sheer number of experiments that would be required to define reaction constants would render the model impractical for any industrial application. There needs to be a balance between the model's ability to predict accurately the progression of lipid oxidation and the time and effort required to provide system inputs to the model. The solution lies with simple models that use a small number of the possible oxidation reactions in a way that ensures both simplicity and accuracy. To date there are four models that have been constructed on this principle and provide considerable insight.

5.2.1 The Labuza model

The first model that can be described as being mechanistic and capable of predictions was presented by Labuza (1971). Labuza began by making a series of key assumptions based on previous work. The first is the role that lipid hydroperoxides play in determining the rates of lipid oxidation. Labuza states that the extent of oxidation required before a product is deemed rancid is very low. This is almost certainly due to the low threshold concentrations of tertiary oxidation products (often measured in parts per billion). Labuza managed to reduce the complexity of the reaction mechanism, for the purpose of predicting lipid oxidation rates up to the point where it can no longer be consumed, by eliminating the formation of secondary and tertiary products from the problem.

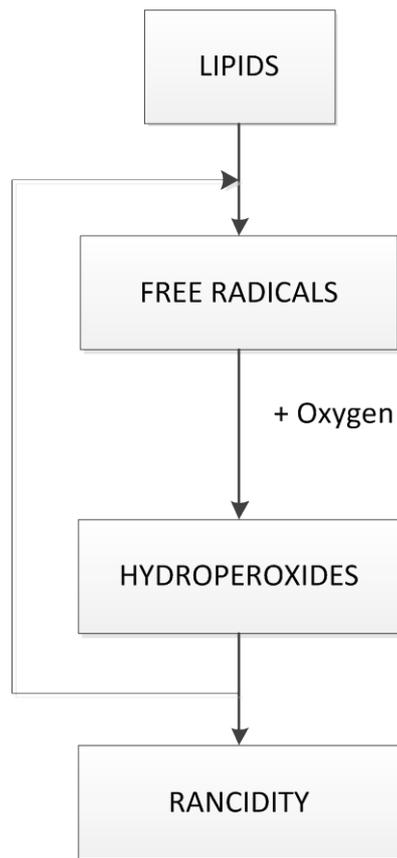


Figure 5.2: A simplified representation of the reaction scheme being modelled.

Labuza also defined the way in which lipid hydroperoxides were formed and broken down. From the outset, lipid hydroperoxides are formed through initiation reactions which occur at a very low rate. Once formed, lipid hydroperoxides breakdown and react with lipids to form more lipid hydroperoxides as well as radicals capable of reacting further. While this is occurring, lipid hydroperoxides are removed through reactions that form secondary and tertiary products, some of which are responsible for the rancidity of fats. This reaction scheme is shown in Figure 5.2. The rates at which lipid hydroperoxide concentrations change, assuming that there are enough lipid hydroperoxides present to react, are governed by the rate of hydroperoxide breakdown and subsequent reaction.

Initiation:



Propagation:



Monomolecular lipid hydroperoxide decomposition:



Bimolecular lipid hydroperoxide decomposition:



Termination:



where LH denotes a lipid, O is oxygen and H is hydroperoxide. For example, LOOH is a lipid hydroperoxide and L· is a lipid radical. *NRP* denotes a non-radical product.

As has been shown in Equations 5.8 to 5.10, there are two ways in which lipid hydroperoxide breakdown occurs – monomolecular and bimolecular breakdown. Monomolecular breakdown occurs when the concentration of lipid hydroperoxides is relatively low, while bimolecular breakdown occurs when the lipid hydroperoxide concentration has reached a point where contact between lipid hydroperoxides is more

likely (Labuza, 1971). The result is a distinct change in reaction rate as the system moves from monomolecular to bimolecular breakdown as shown in Figure 5.3.

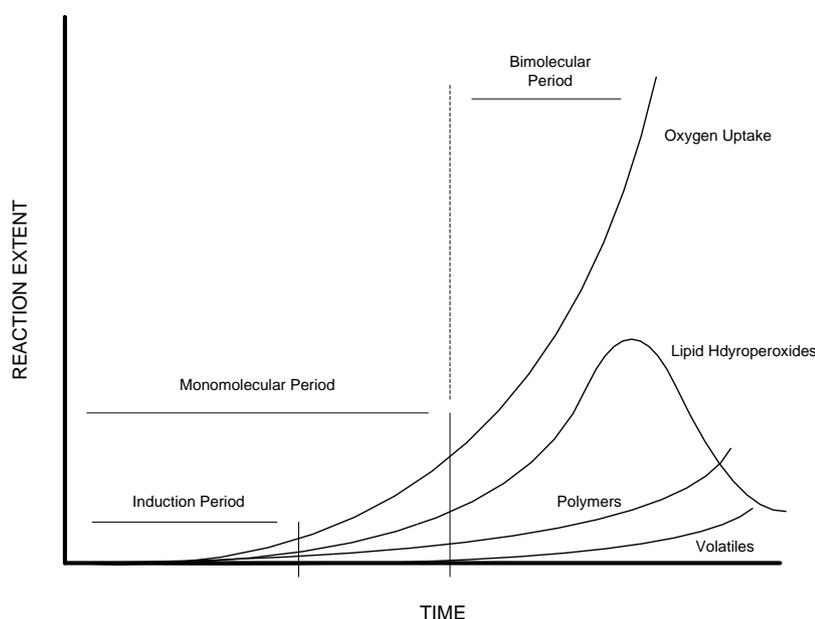


Figure 5.3: Figure reproduced from Labuza (1971). Figure shows the change in different oxidation products as a function of time and extent of reaction.

The shift in the way lipid hydroperoxides are broken down suggests that any model would need to include two types of breakdown reactions. Labuza (1971), however, suggested that a product would be deemed rancid at a lipid hydroperoxide concentration less than that required for bimolecular breakdown to be significant. As such, only monomolecular breakdown of lipid hydroperoxides need be considered when attempting to predict the shelf life of a product susceptible to lipid oxidation.

$$\frac{\partial C_{O_2}}{\partial t} = -k(C_{LOOH})^{1/2} \quad \text{Eq. 5.14}$$

Using the assumptions and reaction mechanism noted above, Labuza (1971) gave a simplified overall rate equation. The rate equation, shown in Equation 5.14, suggests that the relationship between lipid oxidation rate (both in terms of oxygen consumption and change in lipid hydroperoxide concentration) and lipid hydroperoxide concentration is half order and that a plot of the rate of oxidation versus the square root of the extent of oxidation should yield a straight line. Validation

exercises carried out by Labuza (1971) showed this model to be a good fit with experimental data.

5.2.2 The Del Nobile model:

Del Nobile *et al.* (2003a, 2003b) presented an investigation to determine the effects of storing olive oils in polymer packaging. The oxidation model was constructed such that the rate of oxidation was dependant on the concentration of lipid hydroperoxides in the system. The rate of change in the lipid hydroperoxide concentration is given in Equation 5.15.

$$\frac{\partial C_{LOOH}}{\partial t} = R_F - R_D \quad \text{Eq. 5.15}$$

where R_F and R_D are the rates of lipid hydroperoxide formation and decomposition respectively.

The model follows what would be expected. That is, the rate of lipid hydroperoxide formation would initially be greater than the decomposition of lipid hydroperoxides. Once the lipid hydroperoxide concentration becomes high, the rate of decomposition increases. Depending on the change in the relative rates of lipid hydroperoxide formation and decomposition, it is possible that there would be a net decrease in the concentration of lipid hydroperoxides.

To describe the rate of lipid hydroperoxide formation, Del Nobile *et al.* (2003a, 2003b) used a simplified version of a relationship first given by Quast *et al.* (1972). Quast *et al.* (1972) used regression techniques to fit a range of models until they found agreement between experimental and model data. In the original paper, Quast *et al.* (1972) presented data from experiments where potato chips were oxidised under different oxygen concentrations, relative humidity and extents of oxidation. The extent of oxidation was determined by the amount of oxygen consumed while the rate of oxidation was the rate at which oxygen was consumed.

The resulting fits between the experimental data and the simplest models given by Quast *et al.* (1972) were poor in many cases. Once additional fitting parameters were added, the model given in Equation 5.16 was found to be a good predictor of lipid oxidation rates.

$$\frac{\partial C_{O_2}}{\partial t} = \left[EXT + \frac{K_1}{RH^{1/2}} + \frac{K_2 EXT}{RH^{1/2}} \right] \cdot \frac{P_{O_2}}{(K_3 + K_4 P_{O_2})} \quad \text{Eq. 5.16}$$

where C_{O_2} is the concentration of oxygen, P_{O_2} is the partial pressure of oxygen, EXT is the extent of reaction measured as the amount of oxygen consumed, RH is the relative humidity, and K_1 , K_2 , K_3 and K_4 are constants.

Because the relative humidity in the experiments used by Del Nobile *et al.* (2003a) did not change, Equation 5.16 was simplified. As shown in Equation 5.17, the simplified model given by Del Nobile *et al.* (2003a) uses the concentration of lipid hydroperoxides as the extent of oxidation rather than the oxygen consumed as used by the original author. Doing so may be reasonable if the relationship between the oxygen consumed and lipid hydroperoxides formed were constant. If this were the case, then the constant K_2' , would account for this ratio.

$$R_F = (K_1' + K_2' C_{LOOH}) \left(\frac{P_{O_2}}{K_3' + K_4' P_{O_2}} \right) \quad \text{Eq. 5.17}$$

For the “sake of simplicity”, Del Nobile *et al.* (2003a) chose to model the decomposition of lipid hydroperoxides, R_D , as a first order reaction as given in Equation 5.18.

$$R_D = K_5 C_{LOOH} \quad \text{Eq. 5.18}$$

To determine the values of the constants, K_1' , K_2' , K_3' , K_4' , and K_5 , Del Nobile conducted long term shelflife experiments where olive oil was packaged glass bottles. The peroxide value (PV) of the oil samples were measured in regular intervals. The resulting constants are given in Table 5.1.

Table 5.1: Rate constants developed for the Del Nobile model.

Constant	Unit	Value
K ₁	cm ³ cm ⁻³ s ⁻¹	8.52 x 10 ⁻⁹
K ₂	-	2.47 x 10 ⁻¹⁰
K ₃	atm	7.32 x 10 ⁻³
K ₄	-	1.02
K ₅	s ⁻¹	3.28 x 10 ⁻⁸

In the experiment carried out by Del Nobile *et al.* (2003a), the glass bottles used to store the oils ensured that oxygen was not able to enter the reaction system. The headspace above the oil and the oxygen present in the oil at the beginning of the experiment would, therefore, be the only source of oxygen for reaction. Interestingly, Del Nobile *et al.* (2003a) did not show any oxygen measurements or discuss the presence of a headspace. This means that, although the fitted curve worked well for the current experiment, applying the findings to other experimental systems is not likely to be accurate.

The PV's of the oil samples found throughout the study were in the range of 0.07-0.12 cm³cm⁻³. Using this range of PV and three oxygen pressure levels (high, medium and low), the rates of lipid hydroperoxide formation and decomposition were estimated. The results, shown in Table 5.2, suggest that the rate of lipid hydroperoxide formation is greater than the rate of decomposition as long as there is some oxygen present. Although not shown in Table 5.2, the same analysis showed that the rate of lipid hydroperoxide formation is still greater than the rate of lipid hydroperoxide decomposition when the partial pressure of oxygen is very low (0.01 atm) and the concentration of lipid hydroperoxides is high. This suggests that the rate of lipid hydroperoxide decomposition does not need to be accounted for directly in models as long as oxygen is present.

Table 5.2: Rates of lipid hydroperoxide formation and decomposition for different combinations of oxygen and lipid hydroperoxide concentrations as predicted by the Del Nobile model

Oxygen Partial Pressure (atm)	Lipid Hydroperoxide Concentration (cm ³ cm ⁻³)	$R_F \times 10^9$ (cm ³ cm ⁻³ s ⁻¹)	$R_D \times 10^9$ (cm ³ cm ⁻³ s ⁻¹)
0	0.07	0.00	2.30
0	0.09	0.00	2.95
0	0.12	0.00	3.94
0.105	0.07	7.83	2.30
0.105	0.09	7.84	2.95
0.105	0.12	7.85	3.94
0.21	0.07	8.09	2.30
0.21	0.09	8.10	2.95
0.21	0.12	8.11	3.94

The model presented by Del Nobile *et al.* (2003a) also suggest that the rate of change in lipid hydroperoxide concentration is first order with respect to oxygen when the concentration of lipid hydroperoxides is high and zero order when the concentration of oxygen is low. Equation 5.17 is, therefore, similar to the Monod equation used to describe bacterial growth as a function of substrate concentration.

The Del Nobile model uses five constants which need to be defined using carefully controlled experiments. It is difficult to justify the use of these constants as they seem to have little mechanistic relevance aside from the additional tuneable parameters that are available for curve fitting. The first order rate equation used for describing lipid hydroperoxide decomposition, Equation 5.18, was not validated directly which makes its use difficult to justify wider application of it.

5.2.3 The Coutelieris and Kanavouras model

In a series of three papers (Kanavouras *et al.*, 2004; Coutelieris & Kanavouras, 2006; Kanavouras & Coutelieris, 2006) Coutelieris and Kanavouras modelled the rates of lipid oxidation in olive oils in different packaging systems. The models were based on the very simple reaction mechanism shown in Equations 5.19 to 5.21.



where k_a , k_b , and k_c are constants, hv is light energy.

According to Kanavouras & Coutelieris (2006), Equations 5.19 and 5.21 only apply when light is present. If light is not available, the reaction mechanism used to describe lipid oxidation is that given in Equation 5.22.

$$\frac{\partial C_{O_2}}{\partial t} = -k_c C_{O_2} (C_{O_2}^{\infty, in} - C_{O_2} - C_{hexanal}) \quad \text{Eq. 5.22}$$

where $C_{O_2}^{\infty, in}$ is the initial concentration of oxygen at the inner surface of the packaging material, and $C_{hexanal}$ is the concentration of hexanal

The formation of hexanal in a well-mixed system was given as:

$$\frac{\partial C_{hexanal}}{\partial t} = -\frac{\partial C_{O_2}}{\partial t} \quad \text{Eq. 5.23}$$

At the beginning of a batch reaction without hexanal present, which would be the ideal starting point for oil when it is first packaged, the model suggests that no reaction would occur without light. Once some initiation has occurred and a small amount of

hexanal has formed, the reaction rate would be at its peak according to Equation 5.22. As oxygen is consumed and hexanal is formed, the reaction rate would decrease. This suggests that the rate of oxygen consumption is a function of the oxygen concentration in the system which follows the ideas given by Fennema (1996). To illustrate these ideas, Figure 5.4 was created using initial oxygen and hexanal concentrations of 1 mol.m^{-3} . The relative rate of oxygen consumption was defined according to Equation 5.24.

$$\frac{R_{t=t}}{R_{t=0}} = \frac{C_{O_2 t=t} (C_{O_2}^{\infty, in} - C_{O_2 t=t} - C_{hexanal t=t})}{C_{O_2 t=0} (C_{O_2}^{\infty, in} - C_{O_2 t=0} - C_{hexanal t=0})} \quad \text{Eq. 5.24}$$

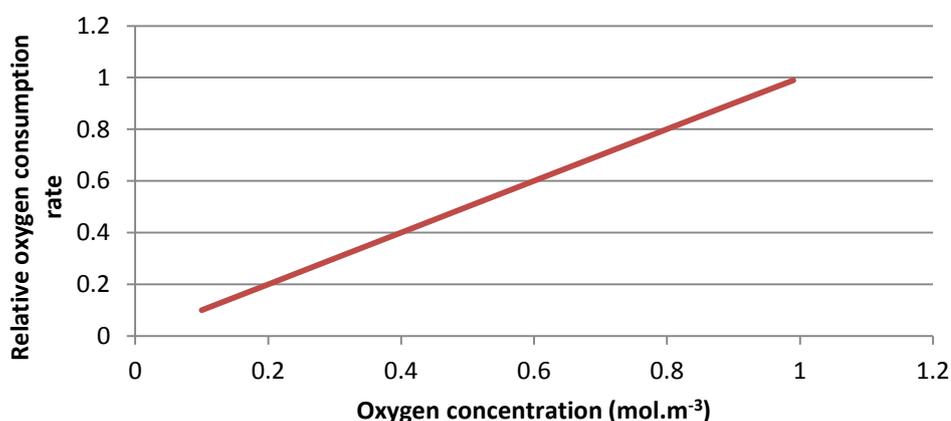


Figure 5.4: Relative oxygen consumption rate as a function of oxygen concentration as predicted by the Kanavouras & Coutelieris model.

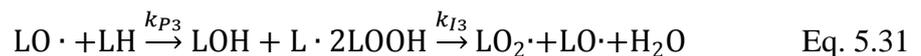
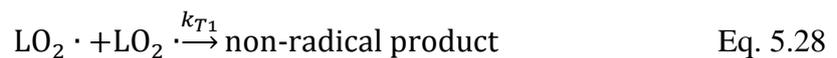
Although the choice of oxygen and hexanal concentrations was arbitrary, the trend of decreasing rate of oxygen consumption holds true for whatever values are chosen. The slope of the line given in Figure 5.4, however, is partially governed by Equation 5.23 which suggests that each mole of oxygen consumed results in the formation of one mole of hexanal. Furthermore, the authors assumed that lipid hydroperoxides ultimately decompose to give hexanal. These assumptions were not directly justified or validated.

Like Del Nobile *et al.* (2003a), the models presented by Kanavouras *et al.* (2004) were fit to data from long term shelflife experiments to determine the values of the relevant

constants. The methods used to fit the constants, however, were only vaguely described by Coutelieris & Kanavouras (2006). It is, therefore, impossible to determine if the constants were fit from one set of experimental data and validated with another, or if the model was fit once and incorrectly labelled a prediction.

5.2.4 The Takahashi model

Like Labuza (1971), Takahashi *et al.* (2000) developed a model describing lipid oxidation rates, in a mechanistic way, for a simple system. Takahashi *et al.* (2000), however, began without making the assumptions that were described earlier. Instead, a full reaction mechanism including both the monomolecular and bimolecular breakdown and subsequent tertiary reactions were described.



Takahashi *et al.* (2000) developed a set of differential equations that, based on Equations 5.25 to 5.32, were used to predict the rate of lipid oxidation. The equations,

shown in Equations 5.33 to 5.38, include a number of reactants and rate constants which needed defining in order for the model to be applied. To do so, the lipid, lipid hydroperoxide and oxygen concentrations were measured either continuously or periodically during experiments carried out.

$$\frac{\partial C_{LH}}{\partial t} = -k_{I1}C_{LH}C_{O_2} - k_{I3}C_{LOOH}^2 - aC_{LH}w_i^{1/2} \quad \text{Eq. 5.33}$$

$$\frac{\partial C_{LOOH}}{\partial t} = aC_{LH}w_i^{1/2} - k_{I2}C_{LOOH}C_{O_2} - 2k_{I3}C_{LOOH}^2 \quad \text{Eq. 5.34}$$

$$\frac{\partial C_{O_2}}{\partial t} = k_{la}(C_{O_2}^* - C_{O_2}) - r \quad \text{Eq. 5.35}$$

$$r = 2k_{I1}C_{LH}C_{O_2} + aC_{LH}w_i^{1/2} + k_{I2}C_{LOOH}C_{O_2} + k_{I3}C_{LOOH}^2 + k_{P4}C_sC_{O_2} \quad \text{Eq. 5.36}$$

$$w_i = k_{I1}C_{LH}C_{O_2} + k_{I2}C_{LOOH}C_{O_2} + 2k_{I3}C_{LOOH}^2 \quad \text{Eq. 5.37}$$

$$a = k_{P2}(2k_{T1})^{-1/2} \quad \text{Eq. 5.38}$$

where C_{LH} is the concentration of lipids, C_{LOOH} is the concentration of lipid hydroperoxides, C_{O_2} is the concentration of oxygen, k_{la} is the mass transfer coefficient, $C_{O_2}^*$ is the saturated oxygen concentration and k_x denotes a rate constant of the corresponding reaction (Equations 5.25 to 5.32)

Takahashi *et al.* (2000) constructed an experimental rig that allowed for a sample of oleic acid to be held at a constant temperature while being continuously sparged with oxygen gas which ensured the system remained well mixed. The experimental design included continual monitoring of the oxygen content of the headspace gases and dissolved in the oil sample.

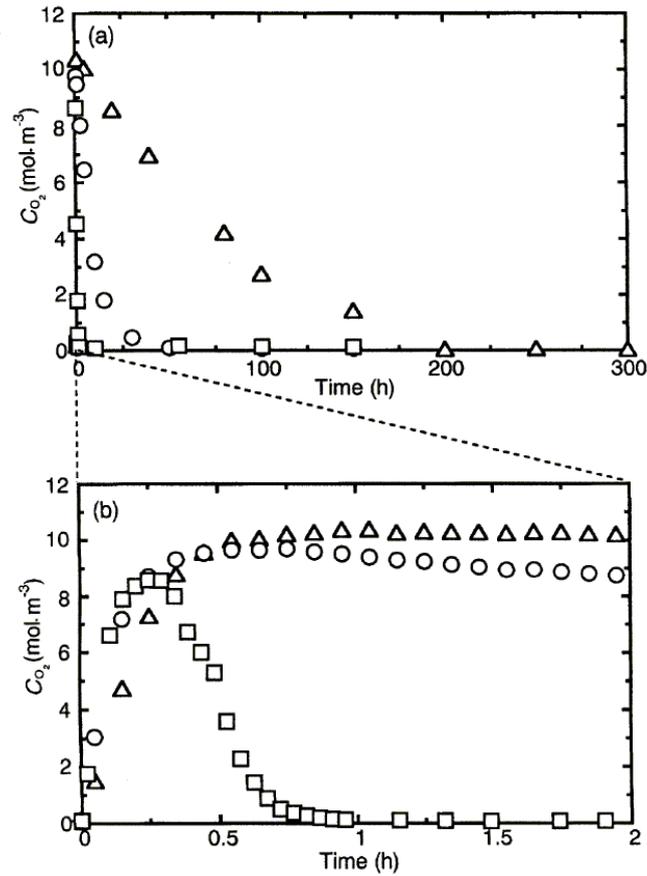


Figure 5.5a&b: Figure from Takahashi *et al.* (2000) showing the experimental results taken from the continual oxidation of oleic acid at 333K (triangle), 348K (circle), and 363K (square).

The results (Figure 5.5) show that the rate of oxygen consumption was initially lower than the rate of oxygen supply. As the extent of lipid oxidation increased, the rate of oxygen consumption increased until the rate of oxygen supply was not able to match the rate of consumption. The results also show that the rate of lipid oxidation is highly dependent on temperature.

Table 5.3: Arrhenius constants and activation energies used to estimate the rate constants required in the Takahashi model. Values are taken from Takahashi *et al.* (2000). These represent model inputs that were used to derive the predictions shown in Figure 5.6.

Rate constant	Pre-exponential factor	Units	Activation energy (J.mol ⁻¹)
k_{I1}	2.63×10^{-2}	$\text{m}^3\text{mol}^{-1}\text{s}^{-1}$	1.34×10^5
α	1.13	$\text{m}^{3/2}\text{mol}^{-1/2}\text{s}^{-1/2}$	3.01×10^4
k_{I2}	2.67×10^{10}	$\text{m}^3\text{mol}^{-1}\text{s}^{-1}$	1.02×10^5
k_{I3}	2.67×10^{11}	$\text{m}^3\text{mol}^{-1}\text{s}^{-1}$	1.28×10^5
k_{P4}	7.13×10^6	$\text{m}^3\text{mol}^{-1}\text{s}^{-1}$	6.74×10^4

Using their experimental data, including that shown in Figure 5.5, Takahashi *et al.* (2000) were able to fit their models (Equations 5.33 to 5.38) and estimate the corresponding rate constants, shown in Table 5.3. By applying Arrhenius approximations, Takahashi *et al.* (2000) were able to predict the changes in rate constants brought about by changes in temperature. An example of the model fit is shown in Figure 5.6 using inputs shown in Table 5.3 and 5.4.

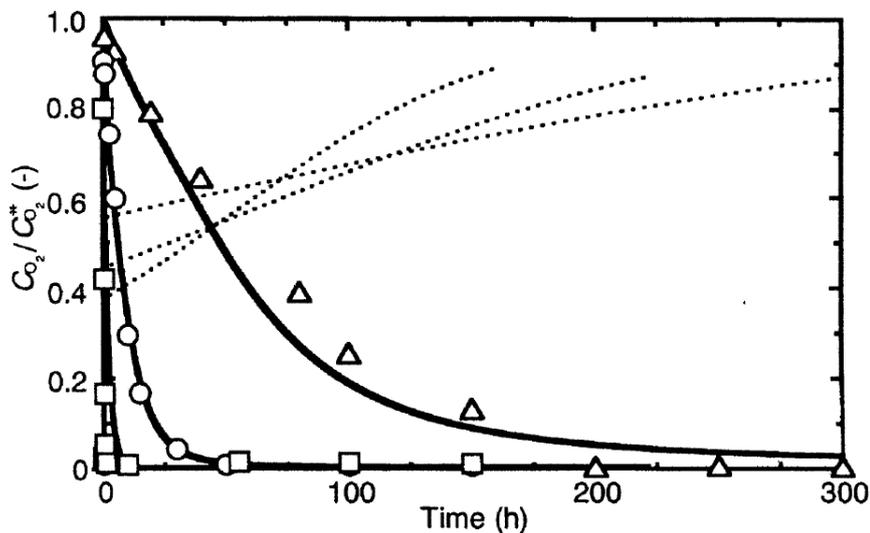
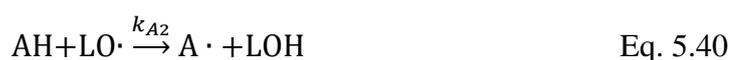


Figure 5.6: Results of applying the Takahashi model at three different temperatures. The solid lines represent the predictions given by the model while the points represent the experimental data (333K -triangle, 348K -circle, and 363K -square). Note that the dotted lines represent a separate model fitted by Takahashi *et al.* (2000) and have no relevance to this discussion.

Table 5.4: Table showing the different mass transport coefficients and saturated oxygen concentrations at the three temperatures used by Takahashi *et al.* (2000).

Temperature (K)	$k\alpha$ (s ⁻¹)	Co_2^* (mol.m ⁻³)
333	1.47 x 10 ⁻³	10.8
348	2.28 x 10 ⁻³	10.7
363	2.55 x 10 ⁻³	10.6

While the model developed by Takahashi *et al.* (2000) was successful in predicting the rates of lipid oxidation in a pure oleic acid, it did so with the use of a variety of complex experiments in order to define rate constants for each reaction. As can be seen in Equations 5.33 to 5.38, this is no small task. For a simple, pure and constant system, this may be a feasible method to predict changes in shelf life. A more complex system, such as those in real food systems, would likely increase the complexity of the required model to such an extent that the model would have little chance of being adopted by industrial manufacturers. For example, the inclusion of a primary antioxidant may add the following reactions into the mechanism:



where AH is an antioxidant and A· is antioxidant radical.

Takahashi *et al.* (2001) did managed to achieve a model that included the effects of β-carotene, but in doing so created a highly complicated model using many more reactions than shown in Equations 5.39 to 5.41. Furthermore, the model relied on more data fitting of rate constants from extra experiments. If a similar model were to include the effects of other factors such as catalysts, surfactants, light and secondary antioxidants, the required experimentation and complexity of the model would be too

great to handle. The number of constants requiring fitting would be so large that, even with careful experimentation, it would be difficult to determine whether the model fit is due to sound reasoning or the model's ability to curve fit. The conclusions taken from such a model would be of questionable value.

It is clear that the model proposed by Takahashi *et al.* (2000), although being a very interesting and insightful piece of work, does not fit the criteria of being able to predict changes in lipid oxidation rates in real food systems, with a mechanistic basis, and without the need for copious and complex experimentation. However, it is possible that the Takahashi model could be reduced to more manageable proportions. This will be the focus of the following discussions.

5.3 Investigating the Takahashi model – application in a batch system

The analysis carried out by Takahashi *et al.* (2000) was based on a continuous oxidation system where oxygen is continuously supplied to the reaction oil. The advantage of this system is its ability to allow the oil to oxidise quickly and, in doing so, cover a large change in the extent of lipid oxidation. The disadvantage is that continuous oxidation is hard to control. The rates of oxygen supply and consumption must be carefully matched in order to see a reduction in the dissolved oxygen concentration. Takahashi *et al.* (2000) achieved this by having a low oxygen sparging rate and a highly reactive oil at high temperature. The same method, however, could not be applied for a less reactive system at lower temperatures. To analyse such a system, batch oxidation should be used.

Since batch oxidations are likely to be better suited to analysis of less reactive oils/foods, it is important to discuss the Takahashi model in terms of its ability to predict oxidation rates in a system where oxygen is not continually supplied. By simply removing the first term in Equation 5.35, it is possible to use the Takahashi model to predict the rates of lipid oxidation in a batch system such as that developed in Chapter 4. The modification moves the result from the one shown in Figure 5.6 to that shown in Figure 5.7. Unless otherwise stated, these reactions were made by numerically solving Equations 5.33 to 5.38 using the 'ode45' solver in MATLAB

(R2011b) (with $k_{la}=0$ for a batch system) and the input parameters shown in Tables 5.3 and 5.4.

5.3.1 Takahashi model – batch oxidation sensitivity analysis

Although the Takahashi model fits well with experimental data, it is possible that some of the reaction pathway included in the model has minimal impact on the rates of lipid oxidation. A sensitivity analysis can uncover which reactions are significant to the model outcome. It may be possible to reduce a full model, to one which is based on one or two reactions whilst still being capable of accurately predicting the rates of lipid oxidation. Such simplification would not remove the mechanistic basis but serve to reduce the complexity of the model while retaining the underlying reaction mechanism. Furthermore, it would provide insight into the reactions that would be best to target when designing new products or antioxidants.

For this sensitivity analysis, the point of interest is which reactions are more important under both low and high lipid hydroperoxide concentrations. Two separate simulations are required, one at low lipid hydroperoxide concentrations and one at higher lipid hydroperoxide concentrations. For each set, the reaction constants are varied (in this case the rate constants, shown in Equation 5.25 and Table 5.3, were multiplied by 1.5) separately and the result plotted. The first simulation used an initial lipid hydroperoxide concentration of 0 mol.m^{-3} while the second began with an initial lipid hydroperoxide concentration of 25 mol.m^{-3} . Both simulations used a reaction temperature of 333K and the corresponding reaction constants given in Tables 5.3 and 5.2. The MATLAB code used to simulate both sensitivity analyses are given in the Appendix (see folder Chapter 5, ‘Sensitivity_Analysis.m’ and ‘Sensitivity_Analysis_2.m’).

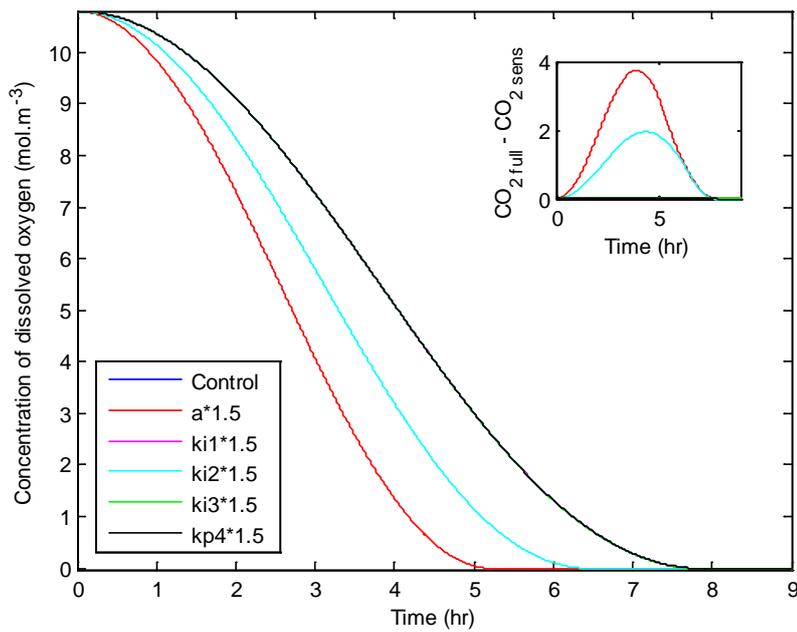


Figure 5.7: Figure shows the results of a sensitivity analysis carried out, using the data presented by Takahashi *et al.* (2000) and reproduced in Tables 5.3 and 5.4. The initial lipid hydroperoxide concentration was set to 0 mol.m⁻³. Note that only the ki2*1.5 and a*1.5 lines differ. All the other lines lie on top of each other.

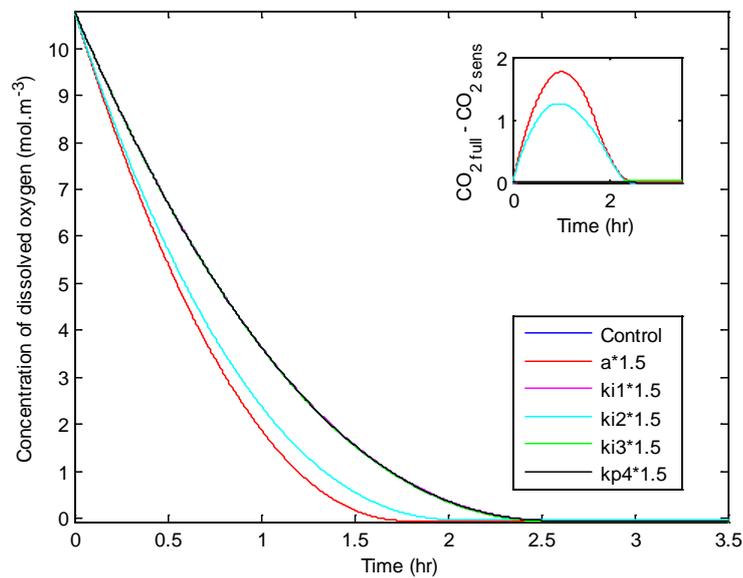


Figure 5.8: Figure shows the results of a sensitivity analysis carried out, using the data presented by Takahashi *et al.* (2000) and reproduced in Tables 5.3 and 5.4. The initial lipid hydroperoxide concentration was set to 25 mol.m⁻³. Note that only the ki2*1.5 and a*1.5 lines differ. All the other lines lie on top of each other.

As can be seen in Figures 5.7 and 5.8, the model proposed by Takahashi *et al.* (2000) is driven by Equations 5.27 and 5.29 once there are lipid hydroperoxides available for reaction. This result suggests that the bimolecular breakdown of lipid hydroperoxides is insignificant and supports the assumptions given by Labuza (1971). Increasing the lipid hydroperoxide concentration in the simulations above seems to have little effect on the significance of bimolecular breakdown. This suggests that the concentration of lipid hydroperoxides required to create a system where bimolecular breakdown overshadows monomolecular breakdown is unlikely to be seen in a real system or product that is still acceptable for consumption.

Not only can the Takahashi model be used to confirm suggestions given by Labuza (1971), it can also be reduced to a more practical level. Equation 5.35 can be significantly simplified by removing the terms that were shown to be insignificant by the sensitivity analysis. The results of the simplification and substitution for the change in oxygen concentration in a batch reactor are shown in Equation 5.42.

$$\begin{aligned}\frac{\partial C_{O_2}}{\partial t} = -r &= \left[aC_{LH}(k_{I_2}C_{LOOH}C_{O_2})^{1/2} + k_{I_2}C_{LOOH}C_{O_2} \right] \\ &= - \left[b(C_{LOOH}C_{O_2})^{1/2} + k_{I_2}C_{LOOH}C_{O_2} \right]\end{aligned}\quad \text{Eq. 5.42}$$

where

$$b = ak_{I_2}^{1/2}C_{LH}\quad \text{Eq. 5.43}$$

Since the change in concentration of lipids in the sample changes very little compared to the oxygen and lipid hydroperoxides, it can be treated as constant.

Further reductions could be made through a more indepth look at the two remaining terms. It is possible, using values from the paper given by Takahashi *et al.* (2000), to determine which of the remaining is more significant and whether they differ by orders of magnitude. Table 5.5 gives some brief results of a further sensitivity analysis.

Table 5.5: Results gained from a sensitivity analysis concentrating on the remaining terms in Equation 5.42. Note that the value for k_{12} was taken directly from Takahashi *et al.* (2000) for a temperature of 333K.

C_{LOOH} (mol.m ⁻³)	C_{O_2} (mol.m ⁻³)	$b(C_{LOOH}, C_{O_2})^{1/2}$	$k_{12} \cdot C_{LOOH} \cdot C_{O_2}$
1	10.8	3.65E-04	2.88E-05
1	1	1.11E-04	2.67E-06
10	10.8	1.15E-03	2.88E-04
10	1	3.51E-04	2.67E-05
20	10.8	1.63E-03	5.76E-04
20	1	4.96E-04	5.34E-05
50	10.8	2.58E-03	1.44E-03
50	1	7.85E-04	1.33E-04

The results clearly show that a change in the first term has a much larger impact in the rate of oxygen consumption than the second. This continues until the concentration of lipid hydroperoxides becomes very high. By removing the less significant term, Equation 5.44 is formed. Interestingly, Equation 5.44 does not take the same form as that given by Labuza (1971). Although still a half order reaction, the process describes one which is half order with respect to both lipid hydroperoxide concentration and oxygen concentration. Only when the lipid hydroperoxide concentration becomes significantly larger than the oxygen concentration, can we see some agreement between the simplified Takahashi and Labuza models.

$$\frac{\partial c_{O_2}}{\partial t} = -b(C_{LOOH}C_{O_2})^{1/2} \quad \text{Eq. 5.44}$$

A comparison of both the full and reduced versions of the model given by Takahashi *et al.* (2000) was achieved by conducting simulations of batch oxidations using both models. The simulation began with a lipid hydroperoxide concentration of 0.01 mol.m⁻³ as the reduced model does not allow for a prediction beginning at 0 mol.m⁻³ of lipid hydroperoxides. This is realistic as it is unlikely that any sample of oil would be

completely free of lipid hydroperoxides. The simulation began with an initial oxygen concentration of 10.8 mol.m^{-3} and was run at 333K using the rate constants given by Takahashi *et al.* (2000). The MATLAB code used for this simulation is given in the Appendix; see folder ‘Chapter 5’, ‘Comparing_Reduced_Models.m’. The results of the simulations, shown in Figure 5.9, suggest that the reduction has little impact on the rates of oxygen consumption during a batch oxidation.

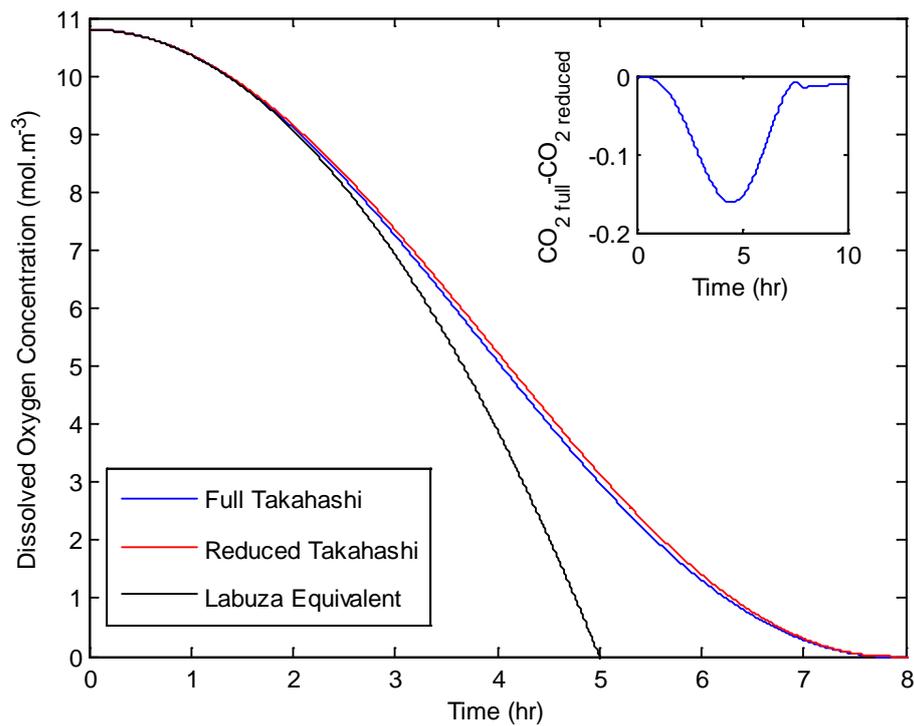


Figure 5.9: Note the full Takahashi model has been modified to model a batch system. The models were run at 333K using the rate constants and other system inputs given by Takahashi *et al.* (2000). Insert shows the difference in the results of the full and reduced Takahashi model.

To compare the Labuza model with the reduced Takahashi model, the rate constant ‘ k ’ in Equation 5.14 was set to equal to the rate constant ‘ b ’ multiplied by $C_{O_2}^{1/2}$ (shown in Equation 5.44). The formation of lipid hydroperoxides (which alters the rate of oxygen consumption) was simulated by assuming the rate of lipid hydroperoxide formation was equal to the rate of oxygen consumption (Labuza, 1971). A simulation of a batch oxidation was carried out, beginning with 10.8 mol.m^{-3} of

oxygen, 0.01 mol.m^{-3} of lipid hydroperoxides and a rate constant 'k' equal to $bC_{O_2}^{1/2}$ as used previously.

The results of the simulation, shown in Figure 5.9, show that the Labuza model follows both the full and reduced Takahashi models during the initial stages of the batch oxidation but begins to diverge after about 2.5 hours. Most importantly, the half order model suggests that the rate of oxygen consumption does not slow as the oxygen concentration approaches zero. This is a trend that was found with experimental work and is discussed later.

5.3.2 Batch oxidation and initial lipid hydroperoxide concentrations

During a batch oxidation, the concentration of lipid hydroperoxides in a sample of oil will increase. Because both the Labuza and reduced Takahashi models suggest that the rate of lipid oxidation is dependent on the concentrations of lipid hydroperoxides in the sample, both models will predict an increase in oxidation rate during a batch oxidation. Furthermore, the increase in the lipid hydroperoxide concentration during a batch oxidation (when the system is closed and initially saturated with oxygen) is dependent on the concentration of oxygen that is available for reaction. That is, there is a finite amount of oxygen that is available to take part in lipid oxidation reactions.

The significance of the increase in lipid hydroperoxides during a batch oxidation is, however, dependent on the concentration of lipid hydroperoxides at the start of the batch oxidation. For example, if the initial hydroperoxide concentration were low, then the increase in lipid hydroperoxide concentration during the batch oxidation will cause a significant change in the rate of lipid oxidation according to both the Labuza and reduced Takahashi models. Conversely, if the initial lipid hydroperoxide concentration were large, then the increase in lipid hydroperoxide concentration during batch oxidation would be proportionally small and would have minimal impact on the rate of lipid oxidation.

By assuming that every mole of oxygen consumed forms one mole of lipid hydroperoxides (Labuza, 1971), it is possible to quantify the significance of increases

in lipid hydroperoxide concentration during a batch oxidation. In the case of the reduced Takahashi model, Equation 5.45 should apply for a single batch oxidation.

$$\frac{\text{Rate at 90\% completion}}{\text{Rate at beginning}} = \frac{R_{0.1}}{R_1} = \frac{([C_{LOOH_i} + (C_{O_{2i}} - C_{O_2})]C_{O_2})^{1/2}}{(C_{LOOH_i}C_{O_{2i}})^{1/2}} \quad \text{Eq. 5.45}$$

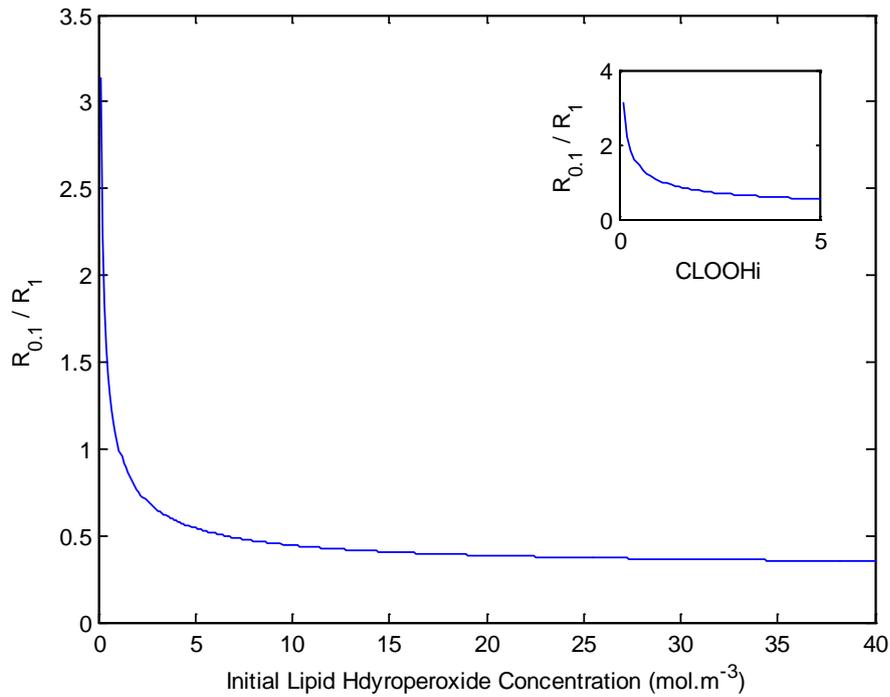


Figure 5.10: Figure shows the effect of the initial lipid hydroperoxide concentration on the difference between the rate at the beginning and the end of a batch oxidation.

Applying Equation 5.45 to a range of initial lipid hydroperoxide concentrations yields the data shown in Figure 5.10. In the case of the reduced Takahashi model, a significant increase in the rate of lipid oxidation can be expected during a single batch oxidation when the initial lipid hydroperoxide concentration is low. As the initial lipid hydroperoxide concentration increases, the significance of the increase in lipid hydroperoxide concentration during a single batch oxidation becomes smaller and smaller until it becomes negligible. The effect is equally pronounced when using the same method to test the Labuza model (Equation 5.46).

$$\frac{\text{Rate at 90\% completion}}{\text{Rate at beginning}} = \frac{R_{0.1}}{R_1} = \frac{(C_{LOOH_i} + (C_{O_{2i}} - C_{O_2}))^{1/2}}{(C_{LOOH_i})^{1/2}} \quad \text{Eq. 5.46}$$

These results suggest that the rate of lipid oxidation would increase significantly during a batch oxidation if the sample is fresh and free from significant quantities of lipid hydroperoxides. The change in rate of lipid oxidation during a batch oxidation would become less significant as the number of batch oxidations increases. This would continue until the change in lipid hydroperoxide concentration during a batch oxidation has no noticeable effect on the rate of lipid oxidation.

5.4 Initial oxygen consumption investigations

Through different investigations, Labuza (1971) and Takahashi *et al.* (2000) proposed different models for predicting the rates of lipid oxidation in terms of oxygen consumption. Careful reduction of the complicated model proposed by Takahashi *et al.* (2000) validated key assumptions that were proposed by Labuza (1971). This shows that the rate of oxygen consumption through lipid oxidation is governed by lipid hydroperoxides and that the bimolecular breakdown of lipid hydroperoxides is unlikely to be significant in determining the shelf life of a food product. The discrepancy, and therefore issue, is that the two models differ in the way they describe the role of oxygen in the system.

To attempt to determine the effect, if any, of oxygen on the rates of lipid oxidation in terms of oxygen consumption a series of initial investigations were carried out in which oils were oxidised and the dissolved oxygen concentrations measured. These investigations used mixed fish oils that were sourced from Bakels Edible Oils Ltd (Auckland, NZ) and stored in a freezer at or below -18°C. The oils were heated to the required temperature and placed into the oxidation rig as stated in Chapter 4.

The results of the initial investigations suggest that the rates of lipid oxidation are not affected by the oxygen concentration as long as there is some oxygen present in the system to react. Figure 4.10 in Chapter 4 clearly shows that the rate of oxygen consumption is not altered significantly when the oxygen concentration is reduced. This finding supports the model suggested by Labuza (1971).

The initial batch oxidation of fish oil yields a curve that, at first glance, seems to suggest a reaction order less than one. The existence of curvature from the beginning to the end of the batch oxidation suggests that the reaction order is greater zero. This supports the model suggested by Labuza (1971).

5.4.1 Fitting the Labuza model to the initial investigations

Since investigations and results of both Labuza (1971) and Takahashi *et al.* (2000) suggest that the tertiary oxidation reactions and products do not have a significant effect on the rates of lipid oxidation during the development of rancidity, it can be concluded that the oxygen consumed is used to create lipid hydroperoxides. This was also done by Labuza (1971) and is shown in Equation 5.47.

$$C_{LOOH} - C_{LOOH_i} = C_{O_{2i}} - C_{O_2} \quad \text{Eq. 5.47}$$

By rearranging for the lipid hydroperoxide concentration, and substituting into Equation 5.14, it is possible to characterise the rate of oxygen consumption without needing to explicitly know the lipid hydroperoxide concentration at every point in time. By rearranging and integrating the resulting equation, it is possible to predict the oxygen concentration at any time.

$$\frac{\partial C_{O_2}}{\partial t} = -kC_{LOOH}^{1/2} = -k(C_{LOOH_i} + C_{O_{2i}} - C_{O_2})^{1/2} \quad \text{Eq. 5.48}$$

$$C_{O_2} = C_{LOOH_i} + C_{O_{2i}} - \left[\frac{k}{2}(t - t_0) - C_{LOOH_i}^{1/2} \right]^2 \quad \text{Eq. 5.49}$$

Applying Equation 5.49 to the experimental data used to produce Figure 4.10 results in a good fit. The fit, conducted using the SOLVER function in Microsoft EXCEL and shown in Figure 5.11, results in rate constants and initial lipid hydroperoxide concentrations of 5.22×10^{-5} , 6.27×10^{-5} , $6.11 \times 10^{-5} \text{ mol}^{1/2} \text{ m}^{-3/2} \text{ s}^{-1}$ and 10.46, 13.20, 22.35 $\text{mol} \cdot \text{m}^{-3}$ respectively (when fitted separately) for subsequent batch oxidations of the same sample of oil using an oxygen solubility of $10.8 \text{ mol} \cdot \text{m}^{-3}$ as suggested by Takahashi *et al.* (2000). Certainly these initial fits imply that the half order relationship suggested by Labuza (1971) could be capable of predicting the rates of

oxygen consumption due to lipid oxidation. Furthermore, there is no obvious evidence of any oxygen dependence suggested by Takahashi *et al.* (2000).

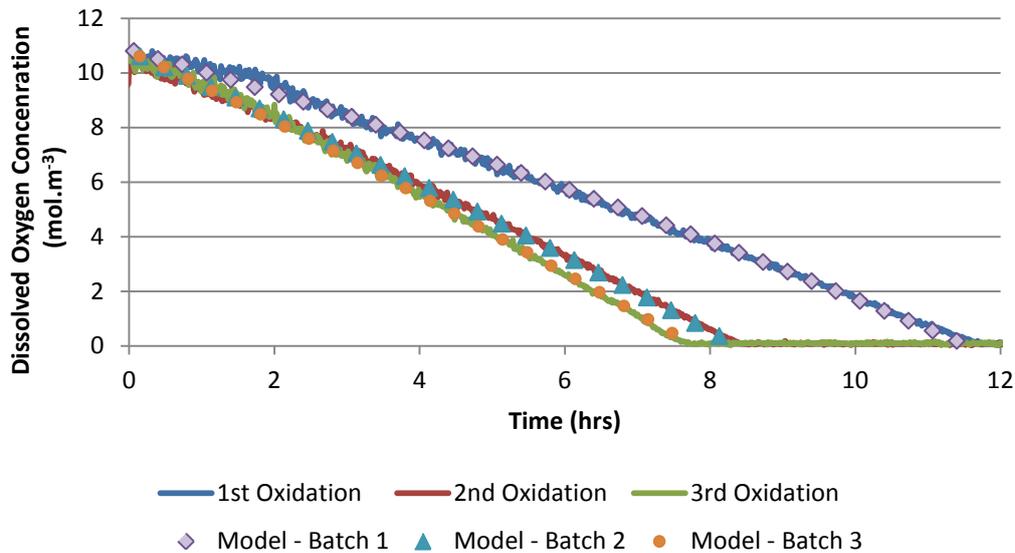


Figure 5.11: Plot showing the ability of a half order model to fit three successive batch oxidation of mixed fish oil at 40°C.

5.5: Conclusions

Models with a mechanistic basis capable of predicting the rates of lipid oxidation are scarce. Of the mechanistic models available, two models were selected. These models, either studied explicitly or determined by careful manipulation, included three key assumptions:

- 1) The rate of oxygen consumption is dependent on the concentration of lipid hydroperoxides.
- 2) The oxygen consumed by secondary and tertiary reactions during the development of rancidity is not significant in terms of the effect on the rates of oxygen consumption and can therefore be excluded from investigation.
- 3) The bimolecular breakdown of lipid hydroperoxides does not occur at significant rates compared with monomolecular breakdown during the development of rancidity.

The application of these assumptions coupled with an analysis of initial results suggest that the relationship between oxygen consumption rates and lipid hydroperoxides is half order and that it is possible, through application of the second assumption, to predict the oxygen concentration and oxygen consumption rate without explicitly knowing the lipid hydroperoxide concentration at every point during the development of rancidity.

CHAPTER 6
OXIDATION IN BULK OILS

6.0 Introduction

From an extensive literature review and some initial work done in Chapter 5, it was shown that there is a strong relationship between the concentration of lipid hydroperoxides and the rates of lipid oxidation. Furthermore, there is some evidence to suggest that oxygen consumption and lipid hydroperoxide formation rates can be predicted if the concentration of lipid hydroperoxides is known.

So far, there is some evidence to suggest that the rate of oxygen consumption by lipid oxidation is half order with respect to the concentration of lipid hydroperoxides. This relationship was constructed after an indepth analysis into works by Labuza (1971) and Takahashi *et al.* (2000) and was tested against batch oxidation data. Although the relationship between lipid hydroperoxide concentrations and oxygen consumption rates seems to be well explained by the half order model (the half order reaction mechanism fits the data presented earlier) the relevance of the fitting method and corresponding results of the model have not been established. These matters will be the focus of this chapter.

6.1 Oxygen solubility in oil

The data presented in the previous chapter was generalised and not intended to give quantitative information. Oxygen partial pressures were used to describe oxygen concentrations for the majority of the work done up to this point. Where concentration units (mol.m^{-3}) were used the solubility of oxygen was assumed to be that given by Takahashi *et al.* (2000). It was acceptable to use the oxygen solubility given by a single author as the actual oxygen solubility in oil has no effect on the trends discussed in the last chapter. For a quantitative assessment, however, the ability to know exactly how much oxygen is present in the oil for reaction, is highly important.

6.1.1 Literature values for oxygen solubility in oil

Despite lipid oxidation being a relatively well published subject, there are relatively few papers that include any estimate of the solubility of oxygen in oils. Even when oxygen is measured, it is often measured as a fraction of the headspace gasses above a sample, which does not provide any indication of the solubility of oxygen in the oil itself. Of those that do give some solubility estimates, the methods used to develop the estimates are often not given. Furthermore, when estimates are available, they are often quite different from one another. This is best shown in Table 6.1 below.

Table 6.1: Oxygen solubilities in literature for a range of oils. The data presented has been manipulated in order to present them in the same units. Superscripts describe sources of information: ^a Takahashi *et al.* (2000), ^b Lango *et al.* (1996), ^c Ke & Ackman (1973), ^d Del Nobile *et al.* (2003).

Temperature (°C)	Solubility (mol.m ⁻³) (100% O ₂ at 1atm)	Lipid Source
25	10.60 ^a	Oleic Acid
25	5.81	Olive Oil
15	5.14 ^b	Cod Liver oil
23-26	5.45 ^b	Corn oil
23-26	5.41 ^b	Cottonseed oil
20	0.69 ^c	Red fish oil
20	1.07 ^c	Herring oil
20	0.84 ^c	Harp seal oil
20	0.70 ^c	Flounder oil
20	1.15 ^c	Cod liver oil
20	1.02 ^c	Olive oil
25	6.70 ^d	Olive oil

The data presented in Table 6.1 shows the differences in solubility values that can be gained from literature. Although some of the variability could be due to the difference between the oil samples themselves, the majority is likely to be due to the differences in methods used to determine the solubility of oxygen in oils. Unfortunately, it is

difficult to determine the exact methods used to gain these values let alone the magnitude of error that came along with them. Although, by very careful analysis it may be possible to qualitatively determine the likely effects of each method (i.e. sources of error in the final solubility reported), a more certain number for solubility must be gained for this thesis as the solubility is likely to have a large impact on any future investigation.

Although small compared to the differences between literature sources, the solubility of oxygen in oils does seem to change with temperature. An example of the magnitude of change in oxygen solubility is given by Ke and Ackman (1973) and is reproduced in Figure 6.1 below.

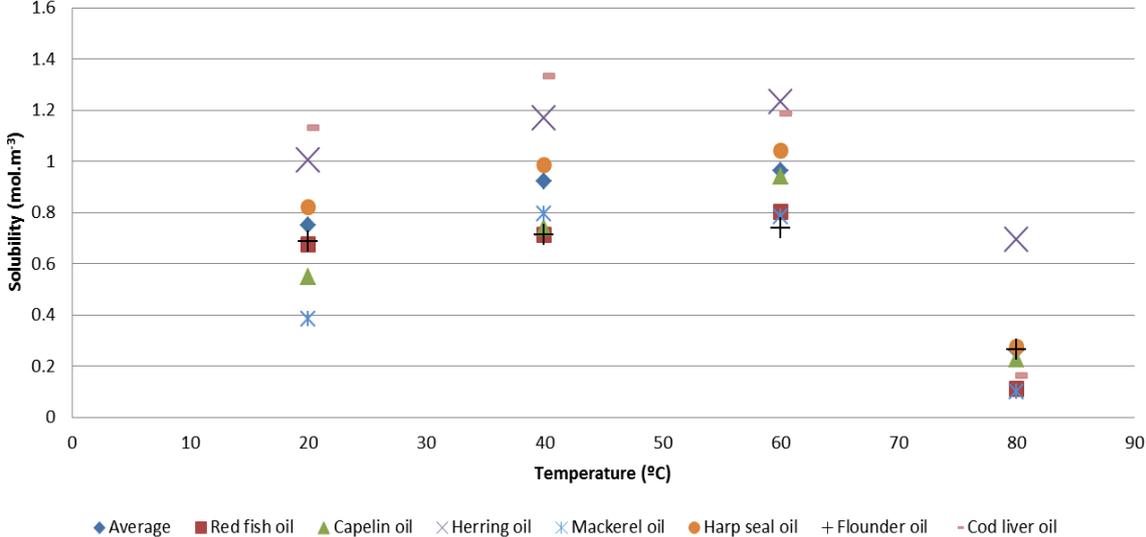


Figure 6.1: Figure shows the relationship between the temperature and oxygen solubility's for different fish oils. Figure created from data presented by Ke & Ackman (1973).

As can be seen in Figure 6.1, the relationship between the oxygen solubility in oil and the temperature is not linear. Instead there seems to be gradual and small increase in the solubility to a maximum at around 40°C followed by a slow decrease as the samples are heated further. The changes in solubility over the range from 20 to 60°C, however, will likely have little effect especially when compared with the variation in solubility values for different oils as shown in Table 6.1.

According to the solubility data presented above, there is little point in attempting to vary the solubility coefficients used in future experiments if the experiments are kept within the range of 20 to 60°C. The data, however, also shows that estimating the solubility of oxygen is either inherently erroneous or is naturally quite different for different oil samples. In any case, before continuing with new investigations, it is important to determine the solubility of oxygen in the oils being used and at the temperature that reactions are being conducted.

6.1.2 Measuring the solubility of oxygen in oil

During sparging of oil with air, as described in Chapter 4, oxygen moves from the air bubbles to the oil. This is generally approximated as a first order reaction (Equation 6.1).

$$\frac{\partial C_{O_2}}{\partial t} = kla(C_{O_2}^* - C_{O_2}) \quad \text{Eq. 6.1}$$

where $C_{O_2}^*$ is the saturated oxygen concentration, C_{O_2} is the oxygen concentration and kla is the mass transfer coefficient.

If there is no oxygen in the oil, the driving force for mass transfer is high. As the concentration of oxygen in the oil increases the rate of oxygen transfer decreases. This continues until the oil becomes saturated i.e. the concentration of oxygen in the oil is equal to the saturated oxygen concentration.

By carrying out a mass balance over the system (Equation 6.2) it is possible to show that the rate of oxygen being added to the oil during sparging is equal to the rate of oxygen entering the vessel minus the rate of oxygen leaving the vessel, minus the rate at which oxygen is being consumed by reaction.

$$v_{oil} \frac{\partial C_{O_2}}{\partial t} = F(C_{O_{2in}} - C_{O_{2out}}) - r \quad \text{Eq. 6.2}$$

where v_{oil} is the volume of oil, F is the flowrate of air passing through the sample, $C_{O_{2in}}$ is the concentration of oxygen in the air entering the vessel, $C_{O_{2out}}$ is the concentration of oxygen in the air leaving the vessel and r is the rate of oxygen consumption by reaction.

By modifying the method described in Chapter 4 it is possible to monitor the partial pressure of oxygen within the exiting gas. For this experiment, the dissolved oxygen probe was placed into the gas stream exiting the reaction rig. Using the ideal gas law, the partial pressure can be converted to a concentration. Doing so allows Equation 6.2 to be rewritten in units that can be measured using the oxygen probe.

$$v_{oil} \frac{\partial C_{O_2}}{\partial t} = \frac{F}{RT} (P_{O_{2in}} - P_{O_{2out}}) - r \quad \text{Eq. 6.3}$$

where R is the ideal gas constant, T is the temperature in kelvin, $P_{O_{2in}}$ is the partial pressure of oxygen in the air entering the vessel and $P_{O_{2out}}$ is the partial pressure of oxygen in the air leaving the vessel.

By starting with a known volume of oxygen free oil, sparging the oil with air at a known rate, and continuously measuring the partial pressure of oxygen leaving the vessel, it is possible to determine the amount of oxygen that is added to the oil. If the rate of oxygen addition is fast relative to the rate of reaction then the rate of reaction, r , can be assumed to be negligible. The amount of oxygen added to the sample during sparging up to the point where the partial pressure of oxygen in the gases entering and exiting the rig are the same, is then equal to the solubility of oxygen in the sample. This can be calculated by integrating Equation 6.4.

$$C_{O_2} - C_{O_{2i}} = \frac{F}{v_{oil}RT} \int_{t_0}^t (P_{O_{2in}} - P_{O_{2out}}) \partial t \quad \text{Eq. 6.4}$$

The integral is the area under a plot of the difference in partial pressure in and out of the vessel with time. An example of one such plot is shown in Figure 6.2. The results of three identical experiments suggest that the solubility of oxygen in mixed fish oil at 35°C was $4.39 \pm 0.22 \text{ mol.m}^{-3}$ when exposed to air at atmospheric pressure.

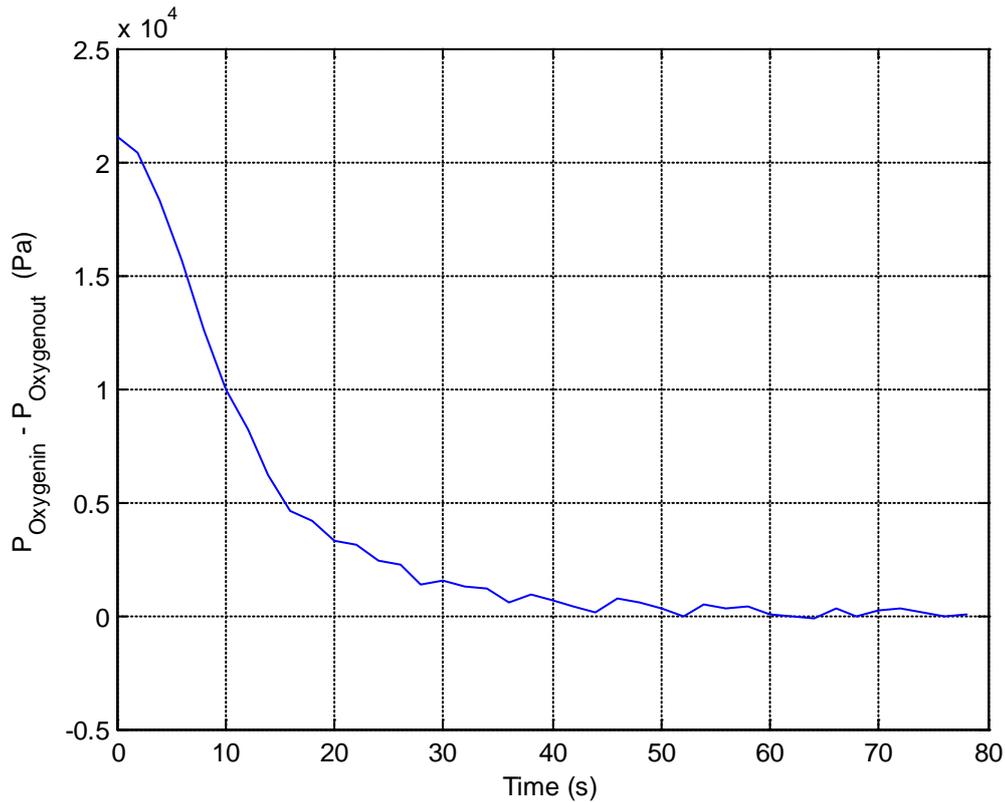


Figure 6.2: Example plot of the results gained by measuring the partial pressure of oxygen in the air exiting a sample of oil, initially free of oxygen, being sparged with air (atmospheric pressure) at 35°C.

The oxygen solubility gained from the above experiments are higher than those presented in Table 6.1. Assuming Henry's law applies, it is reasonable to expect that the oxygen solubilities presented in Table 6.1 to be approximately one fifth as they were exposed to oxygen from air (21% oxygen at 1atm). This, however, is not the case.

The results above, although repeatable, do not take into account the time required for the probe to respond. As discussed in Chapter 4, the probe measures the fluorescence lifetime of a dye. The concentration of oxygen within the dye changes the fluorescence time. The probe, however, does not respond instantly to changes in oxygen concentrations in the sample surrounding the dye. Instead, a second first order mass transfer process occurs as shown in Equation 6.5.

$$\frac{\partial P_p}{\partial t} = -k_p(P_p - P_{O_{2out}}) \quad \text{Eq. 6.5}$$

where P_p is the partial pressure of oxygen in the probe and k_p is the first order mass transfer coefficient.

By rearranging Equation 6.5, the partial pressure of oxygen in the oil around the probe is:

$$P_{O_{2out}} = P_p - \frac{1}{k_p} \cdot \frac{\partial P_p}{\partial t} \quad \text{Eq. 6.6}$$

The probe constant, k_p , was measured from the response of the probe when transferred from nitrogen gas to air at 25°C. The value of k_p was $0.33 \pm 0.012 \text{ s}^{-1}$ as calculated from six experiments (data not shown).

Combining Equation 6.4 and Equation 6.6 allows for a solubility calculation free from the effects of a delay in response for the probe. An example of the type of effect is shown below in Figure 6.3.

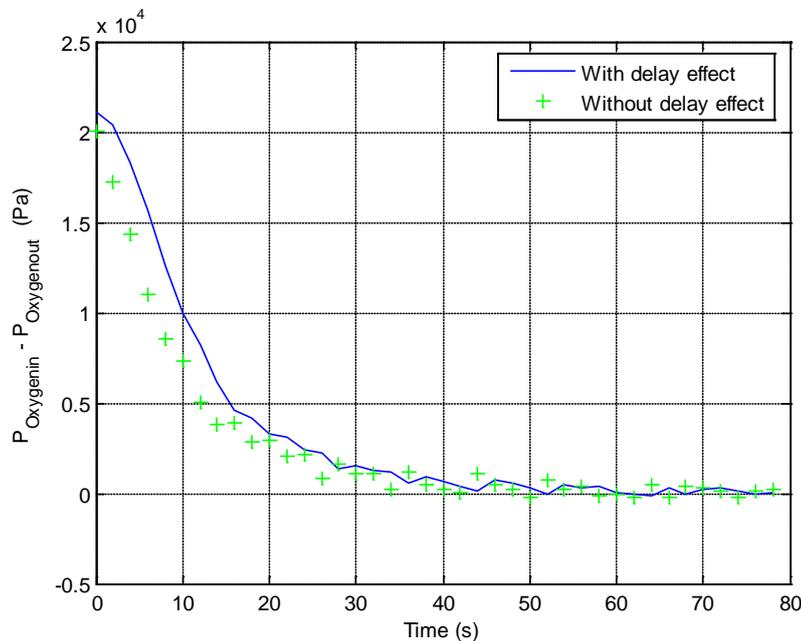


Figure 6.3: Example plot illustrating the difference in results gained by allowing for the time taken for the oxygen probe to respond to changes in oxygen in the oil. Data gained from sparging a sample of oil, initially free of oxygen, with air at atmospheric pressure at 35°C.

Table 6.2 shows the change in calculated oxygen solubility before and after the response of the probe had been accounted for. The results suggest that the probe does introduce a significant error when dealing with measurements over a short period of time. The solubility of oxygen at 35°C is, therefore, $3.35 \pm 0.19 \text{ mol.m}^{-3}$.

Table 6.2: Calculated oxygen solubilities from replicate experiments using mixed fish oil at 35°C before and after accounting for the response time of the oxygen probe.

Calculated Solubility (mol.m⁻³) at 35°C	
With Delay Effect	Without Delay Effect
4.39	3.35
4.76	3.67
4.00	3.01

6.1.3 Oxygen solubility and temperature

According to literature, the solubility of oxygen in oil is dependent on the temperature of the oil (Ke and Ackman, 1973). However, like the measures of solubility at a single temperature, it is hard to be sure how reliable the literature information really is. The data shown in Figure 6.1 certainly suggests that, although relatively small, the solubility of oxygen in oil may have a significant effect on the rates of lipid oxidation measured during a batch oxidation.

By using the same method as that used to determine the solubility of oxygen at 35°C (results shown in Figure 6.3 and Table 6.2) in oil samples at different temperatures, the change in oxygen solubility brought about by changes in temperature was measured. The results of these measurements are shown in Figure 6.4 and summarised in Table 6.3.

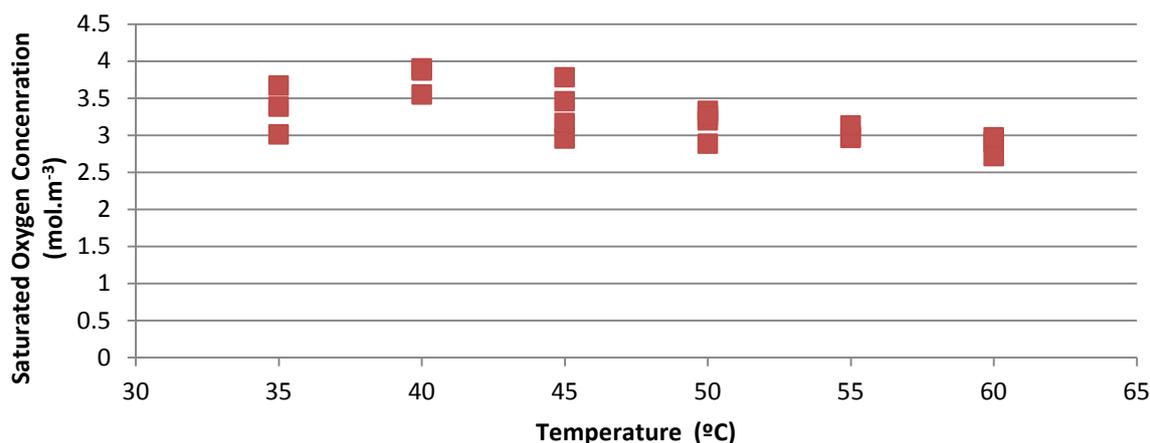


Figure 6.4: Change in oxygen solubility with temperature for sample of mixed fish oil (Bakels Ltd, NZ)

Table 6.3: Oxygen solubility in mixed fish oil (Bakels Ltd, NZ) at different temperatures

Temperature (°C)	Average Calculated Oxygen Solubility (mol.m ⁻³)	Standard Error
35	3.35	0.19
40	3.77	0.11
45	3.34	0.18
50	3.17	0.10
55	3.03	0.05
60	2.87	0.08

The results suggest that the oxygen concentration is slightly dependent on the temperature of the sample. Interestingly, the results seem to have a similar trend as those presented in Figure 6.1. That is, there seems to be a rise in solubility with temperature to a peak at around 40°C followed by a fall in oxygen solubility as the temperature rises. This trend, however, is not statistically significant. Infact, when using a two-tailed t-test at 95% confidence, the only difference that is significant is between the solubility at 40°C and 50°C and between 40°C and 60°C. There was no significant difference between any other points. The average solubility across all the temperatures was $3.25 \pm 0.08 \text{ mol.m}^{-3}$. Because of the lack of a significant difference in the solubility at different temperatures, the average will be used as the solubility at all temperatures between 35°C and 60°C.

6.14 Oxygen concentration dependence

As this study developed it became clear that all models, using a mechanistic basis, should include lipid hydroperoxide concentrations and have some way of relating them to oxygen concentrations. The role that lipid hydroperoxides play in the oxidation pathway has been well established both in literature and in this thesis. Lipid hydroperoxides are the single semi-stable primary product of lipid oxidation of which all other oxidation breakdown products come from. The concentration of lipid hydroperoxides, during the period of oxidation before peak hydroperoxide concentrations, is directly related to the rate of lipid oxidation.

Unlike lipid hydroperoxides, the role oxygen plays in lipid oxidation is not quite so clear. There is no doubt that oxygen is consumed during lipid oxidation reactions. Certainly the reaction mechanisms given in literature, including those used in the model proposed by Takahashi *et al.* (2000), suggest a strong dependence on oxygen. This dependence has been described by Anderson and Lingnert (1999) amongst others, who suggest that the rate of lipid oxidation is constant until the oxygen concentration drops to a level of approximately 1%. This, however, is not what is seen in the investigations in Chapter 4.

To provide further evidence to investigate whether the rate of oxygen consumption is dependent on the concentration of oxygen in the system, a sample of mixed fish oil (Bakels Ltd, NZ) was heated to 45°C using a hot plate. The sample was then transferred to a batch oxidation vessel, placed in a 45°C incubator (see Chapter 4) and sparged with air at 0.3 L/min for 30 minutes before being sealed in the batch oxidation rig and a batch oxidation carried out according to the methods developed in Chapter 4. The results of this experiment are shown in Figure 6.5.

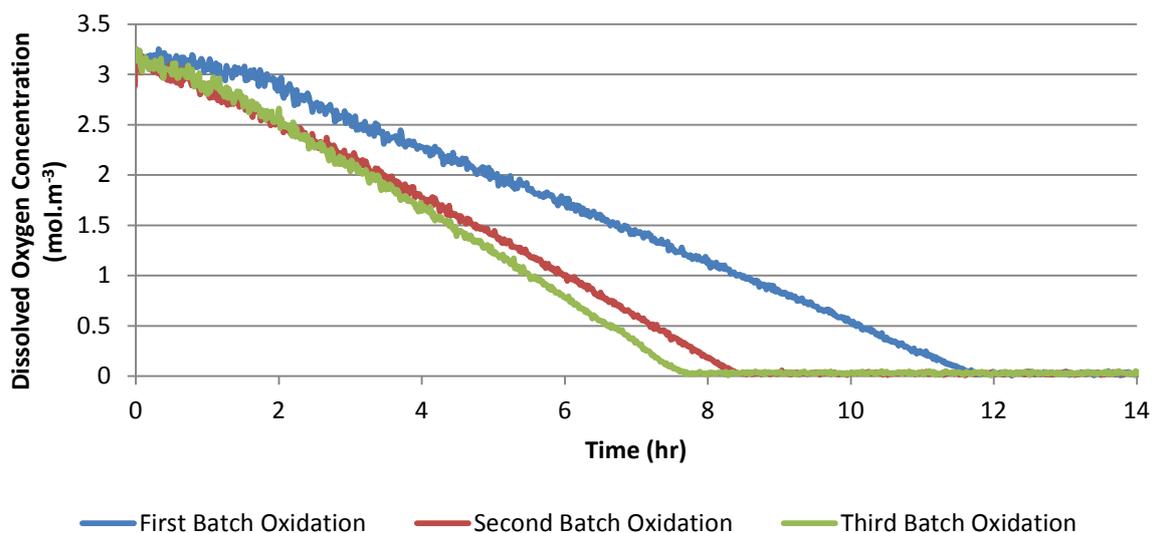


Figure 6.5: Results of three successive batch oxidations of a single sample of mixed fish oil at 45°C.

As can be seen in Figure 6.5, there is no significant change in the rate of oxygen consumption as the concentration of oxygen approaches zero, for the batch oxidation of mixed fish oil. Instead, the reaction rate continues at a seemingly constant rate until there is no oxygen present in the oil sample. Only subsequent batch oxidations make it possible to see that the reaction order is greater than zero. Furthermore, the effect cannot be attributed to the sample of oil used as the same results can be seen when using different samples of oil. For example, Figure 6.6 shows the results of a single batch oxidation of a sample of Flaxseed oil at 45°C. These findings suggest that the rate of oxygen consumption by lipid oxidation is not oxygen limited until there is no oxygen left for reaction.

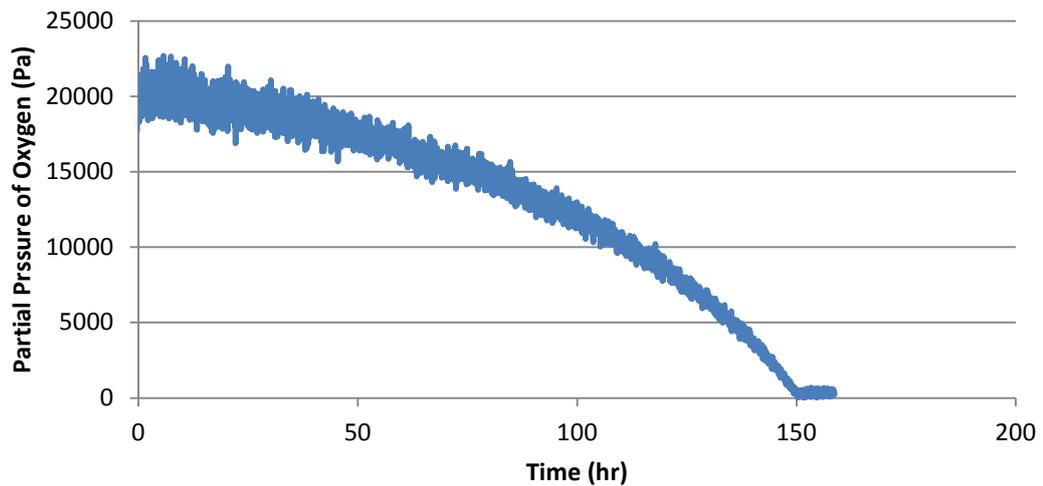


Figure 6.6: Results of a single batch oxidation of flax seed oil at 45°C

6.2 Applying the half order model

Having effectively removed oxygen dependence (other than when completely depleted) as a factor for any model, the half order model presented by Labuza (1971) becomes the only remaining model capable of explaining, with a mechanistic basis, the rates of lipid oxidation in a bulk oil. The application of this model, however, can only give the rate of oxygen consumption at a given lipid hydroperoxide concentration. Continual measurement of the lipid hydroperoxide concentration, using complicated and expensive HPLC techniques or time consuming titrations, is required. Furthermore, any predictions rely on the researchers' ability to predict the change in lipid hydroperoxide concentrations under different conditions. As it is, the model presented by Labuza (1971) is of limited usefulness.

A method of estimating the rates of lipid hydroperoxide formation is required so that the need to continuously monitor lipid hydroperoxide production directly can be avoided. Some relationships between oxygen consumption and lipid hydroperoxide formation have been discussed. For example, Labuza (1971) suggested that there is a 1:1 ratio of lipid hydroperoxides formed to oxygen consumed (Eq. 6.7). Re-arranging the relationship in Eq. 6.7 and substituting into Eq. 6.8, it is possible to predict the rate of oxygen consumption without needing to measure the lipid hydroperoxide concentration continuously. The rate of oxygen consumption then becomes a function

of the initial lipid hydroperoxide at the beginning of a batch experiment, initial oxygen and current oxygen concentrations.

$$C_{LOOH} - C_{LOOH_i} = C_{O_{2i}} - C_{O_2} \quad \text{Eq. 6.7}$$

$$\frac{\partial C_{O_2}}{\partial t} = -kC_{LOOH}^{1/2} = -k(C_{LOOH_i} + C_{O_{2i}} - C_{O_2})^{1/2} \quad \text{Eq. 6.8}$$

$$C_{O_2} = C_{LOOH_i} + C_{O_{2i}} - \left[\frac{k}{2}(t - t_0) + C_{LOOH_i}^{1/2} \right]^2 \quad \text{Eq. 6.9}$$

The relationship given by Labuza (1971) and shown in Eq. 6.7, however, was not validated. Because the relationship between the oxygen consumed and lipid hydroperoxides formed is crucial to accurately predicting the rates of lipid oxidation, an investigation to validate this finding is required.

There are two ways to validate Labuza's suggestion that the amount of hydroperoxides formed is equal to the amount of oxygen consumed. They are:

- 1) Comparison with other literature data/models
- 2) Comparison with experimental data

6.2.1 Takahashi - lipid hydroperoxides and oxygen consumed

As described in Chapter 5, Takahashi *et al.* (2000) presented a model that accurately predicted the rates of lipid consumption, oxygen consumption and lipid hydroperoxide formation in oleic acid. The model was validated for a well-mixed system where oxygen was continuously supplied. Although the model is too complicated to be applied to a real food system, replicating the model allows for the amount of lipid hydroperoxides formed to the amount of oxygen consumed to be calculated. By using the Modified Takahashi model, presented in Chapter 5, the rates of lipid hydroperoxide formation and oxygen consumption can be plotted against each other.

Using MATLAB (R2011b), a simulation of a batch oxidation of oleic acid at 60°C was run beginning with an oxygen concentration of 10.8 mol.m⁻³, the value given for oleic acid by the author, and a lipid hydroperoxide concentration of 5 mol.m⁻³. The rate constants used are presented in Tables 5.3 and 5.4 in Chapter 5. The MATLAB code for this simulation is given in the Appendix; see folder 'Chapter 6', file 'Batch_Oxidation_Takahashi.m'. Figure 6.7 shows the formation of lipid hydroperoxides and the consumption of oxygen during the batch oxidation.

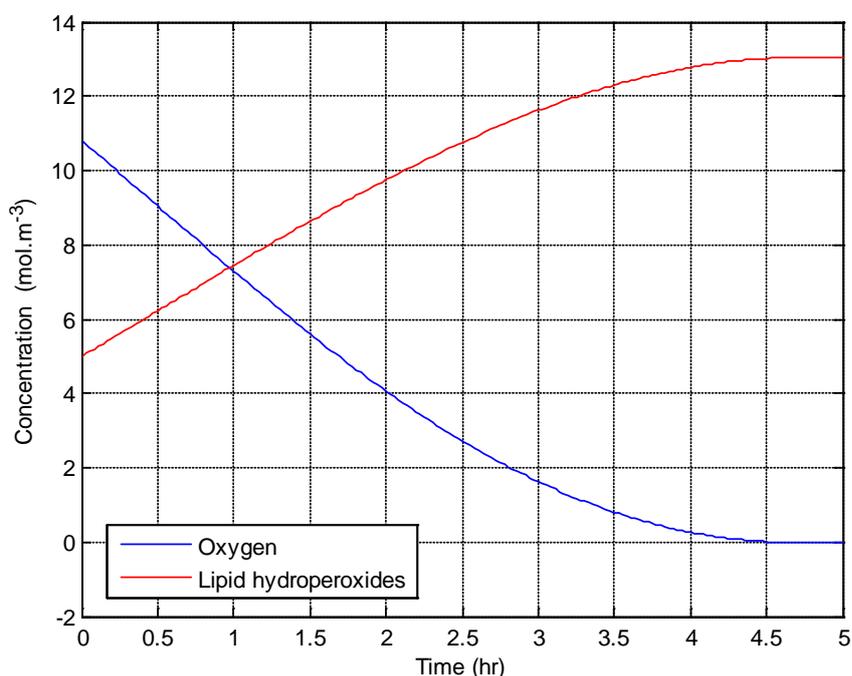


Figure 6.7: Results of a simulation of a batch oxidation of oleic acid at 60°C. Model is a modification of the model presented by Takahashi *et al.* (2000)

As expected, the results shown in Figure 6.7, suggest that the formation of lipid hydroperoxides is dependent on the oxygen concentration. Interestingly, however, the change in lipid hydroperoxide concentration during a batch oxidation is approximately 8 mol.m⁻³ while the change in oxygen concentration is 10.8 mol.m⁻³. This is not the 1:1 relationship suggested by Labuza (1971). Plotting the rate of change in concentration, oxygen and lipid hydroperoxides, as a function of time during a batch oxidation (Figure 6.8) shows this effect even more clearly. Note the MATLAB code used to create Figure 6.8 is given in the Appendix (see folder 'Chapter 6', 'Rate_of_LOOH_O2_formation.m').

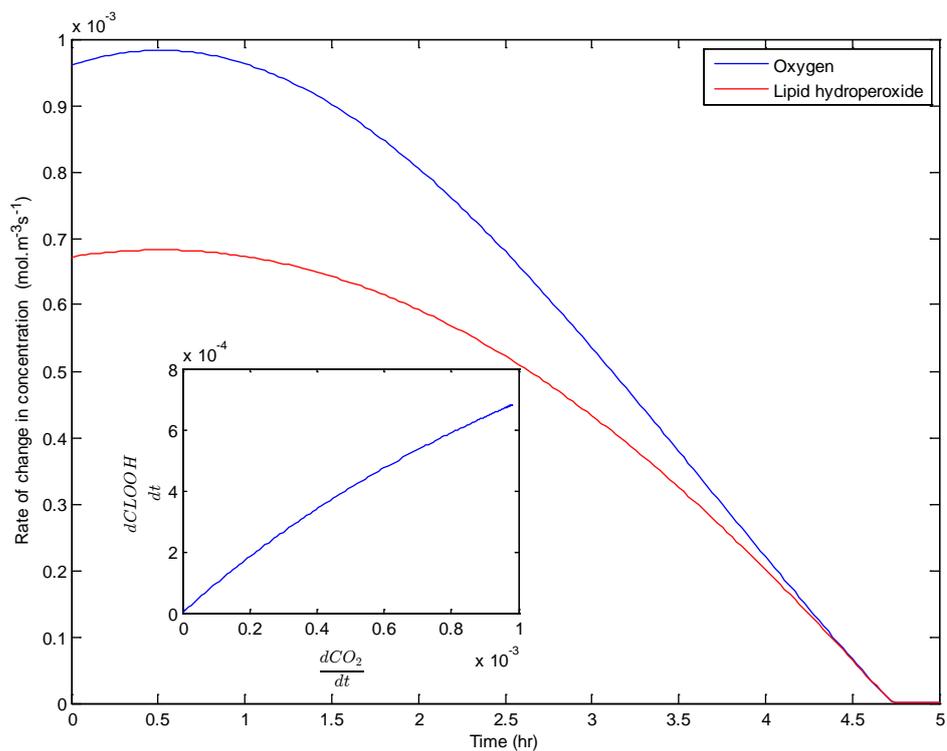


Figure 6.8: Results of a simulation of a batch oxidation of oleic acid at 60°C. Model is a modification of the model presented by Takahashi *et al.* (2000). Insert: Plot of rate of change in lipid hydroperoxide concentration versus the rate of change oxygen concentration.

The results shown in Figure 6.8 suggest that the rate of oxygen consumption is not equal to the rate of lipid hydroperoxide formation or that the relationship between the two is perfectly linear over a batch oxidation. The results suggest that the rate of oxygen consumption is between one and two times faster than the rate that lipid hydroperoxides are formed.

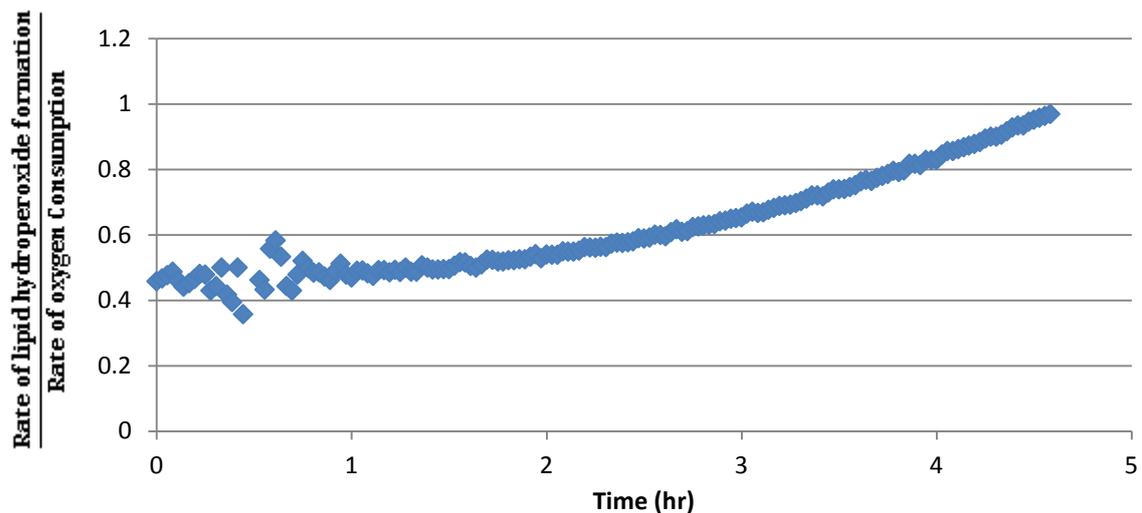


Figure 6.9: Results of a simulation of a batch oxidation of oleic acid at 60°C. Model is a modification of the model presented by Takahashi *et al.* (2000).

The results from further analysis of the Takahashi model seem to suggest that the ratio of hydroperoxides formed to the rate of oxygen consumption does not stay the same throughout a batch oxidation. This result, however, is likely to be due to the model's dependence on oxygen concentration. That is, the Takahashi model suggests that the rate of oxygen consumption decreases at a rate proportional to the concentration of oxygen in the sample. This is not what was observed during batch oxidation of mixed fish oil. The model formulated by Takahashi was not validated in a batch system where oxygen dependence becomes magnified during experiments. The results, although only indicative, suggest a relationship between oxygen consumption and lipid hydroperoxide formation that is quite different to that suggested by Labuza (1971).

6.2.2 Lipid hydroperoxide formation versus oxygen consumption

There are clear differences between the suggestions made by Labuza (1971) and the results of Takahashi's model. As such, an experiment was carried out to determine the relationship between the oxygen consumed and lipid hydroperoxides formed in a controlled system.

Methodology

Two kilograms of mixed fish oil (Bakels Ltd, NZ), previously stored at -30°C, was heated to 35°C in a conical flask using a hot plate with a magnetic stirrer. Once at temperature, the oil was transferred to a 35°C incubator (WatVic Ltd). Air was sparged through the sample at 0.4 L.min⁻¹ using a mass flow controller (GFC17S, Aalborg, New York, USA). To ensure that the oil contained oxygen, the dissolved oxygen probe was used to test the concentration of oxygen leaving the vessel. At all times, the concentration of oxygen was greater than 18% (or 18.2 kPa). Samples were removed from the flask for batch oxidation.

Peroxide value (PV) analysis was conducted using the American Oil Chemists Society (AOCS) official method Cd 8b-90 (AOCS, 2009). Briefly, the method involved dissolving an oil sample in a 60:40 mixture of glacial acetic acid and iso-octane. Potassium iodide was added and left to react for one minute. The reaction was quenched with distilled water and a small amount of sodium dodecylsulphate (SDS) was added to help suspend the oil in the solution. Finally, the liberated iodine was measured by titrating with a sodium thiosulphate solution. For exact volumes and procedures the reader should refer to the AOCS official method.

The PV determined by titration was then converted to lipid hydroperoxide concentrations using the relationship given by Frankel (2005).

$$PV \text{ in } \frac{meq}{kg \text{ oil}} = 2 \times PV \text{ in } \frac{mmol}{kg} \quad \text{Eq. 6.10}$$

Results and discussion

As expected, the rate of oxygen consumption increased with the time spent exposed to oxygen via sparging (Figure 6.10). The results of batch oxidations had the same, almost linear, trends and none showed any decrease in oxygen consumption rate as the oxygen concentration approached zero. This is consistent with earlier investigations.

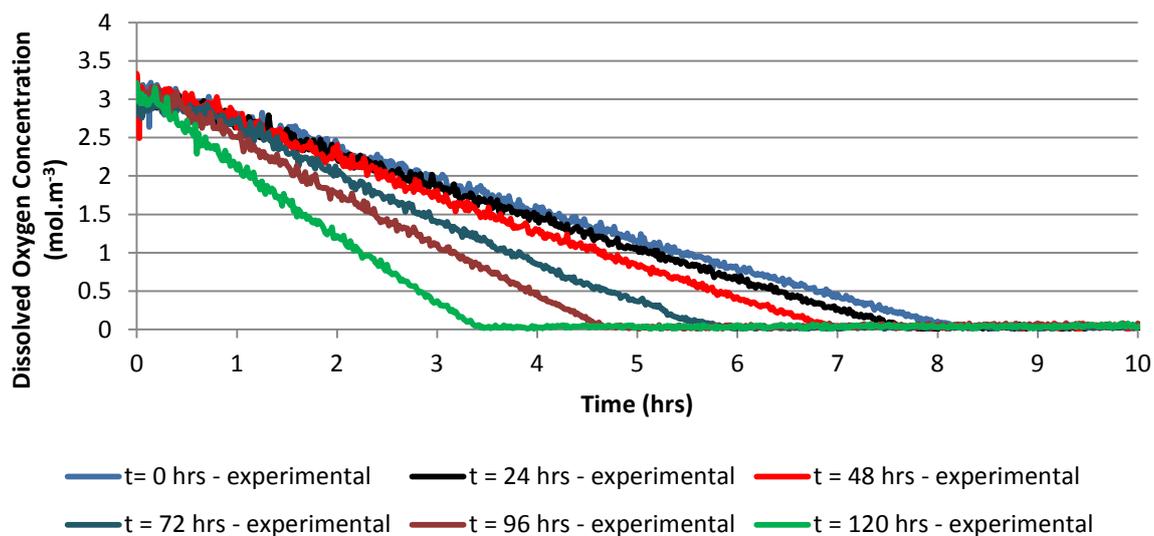


Figure 6.10: Oxygen consumption profile for samples of mixed fish oil (Bakels Ltd, NZ) at 35°C sparged continuously sparged with air for varying lengths of time.

The PV and, therefore, lipid hydroperoxide concentration, increased as the time of sparging increased. The increase in lipid hydroperoxide concentration brought with it an increase in the rate of oxygen consumption; Figure 6.11.

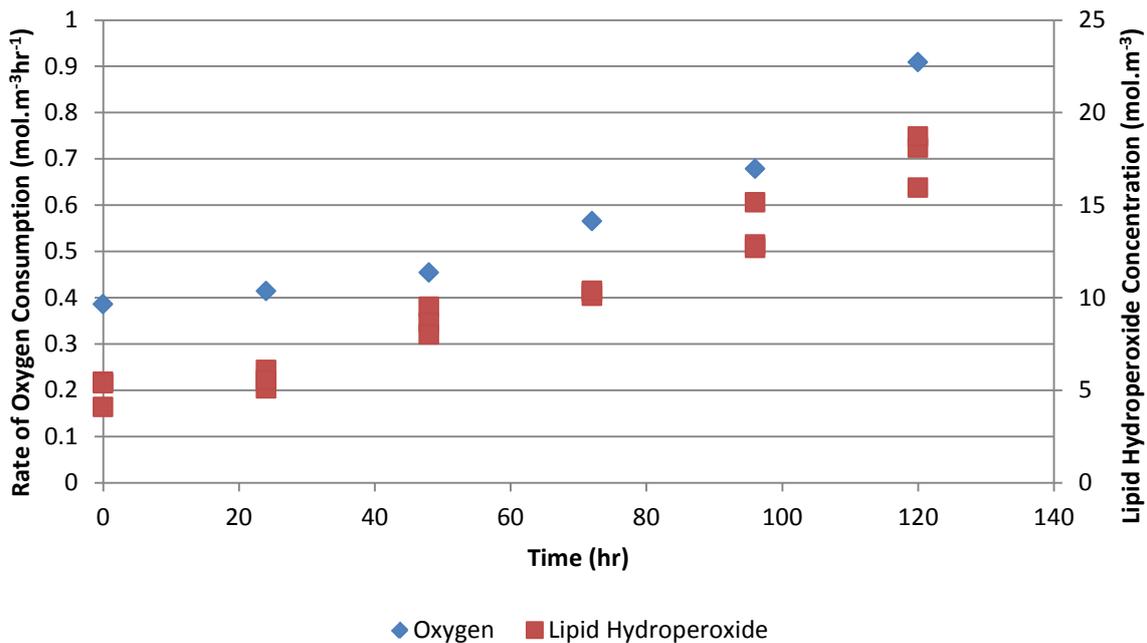


Figure 6.11: Peroxide values and lipid hydroperoxide concentrations for samples of mixed fish oil (Bakels Ltd, NZ) at 35°C sparged continuously sparged with air for varying lengths of time.

By numerically integrating the oxygen consumption rate versus time plot, Figure 6.10, using the ‘cumtrapz’ function in MATLAB (R2011b), the oxygen consumed during sparging was calculated. The results of this integration, shown in Figure 6.12, show that over 120 hours of sparging, 67.9 mol.m⁻³ of oxygen was consumed by reaction. Over the same period, the lipid hydroperoxide concentration changed by approximately 12.6 mol.m⁻³ which suggests that the ratio of oxygen consumed to lipid hydroperoxides was approximately 5.4.

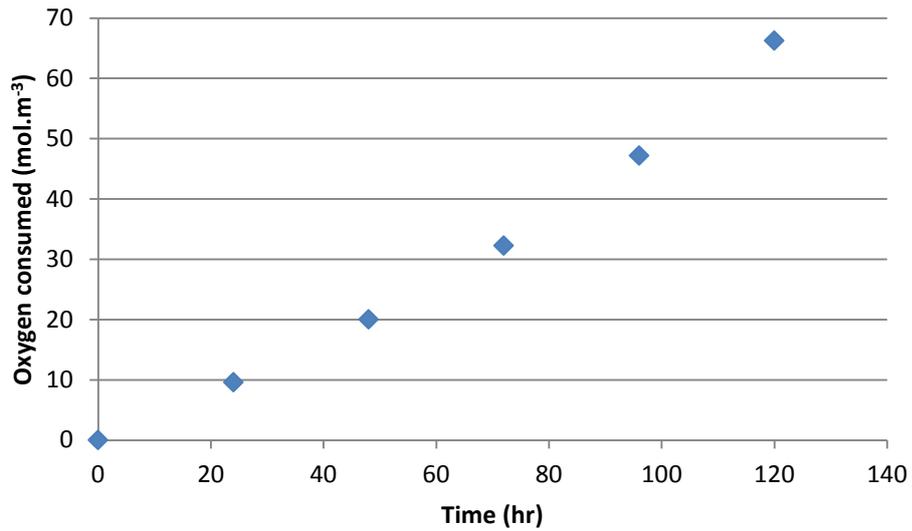


Figure 6.12: Oxygen consumed during sparging of mixed fish oil (Bakels Ltd, NZ) at 35°C

By plotting the oxygen consumed against the lipid hydroperoxide concentration (Figure 6.13) it was shown that 5.04 mol of oxygen is required to form 1 mol of lipid hydroperoxides. This is significantly greater than that presented by Labuza (1971) and estimated from the model presented by Takahashi et al (2000).

Like Labuza (1971), these results suggest that the amount of oxygen required to form lipid hydroperoxides does not change as the lipid hydroperoxide concentration changes. This suggests that the model presented by Takahashi *et al.* (2000) is not applicable for a system where oxygen supply becomes limited.

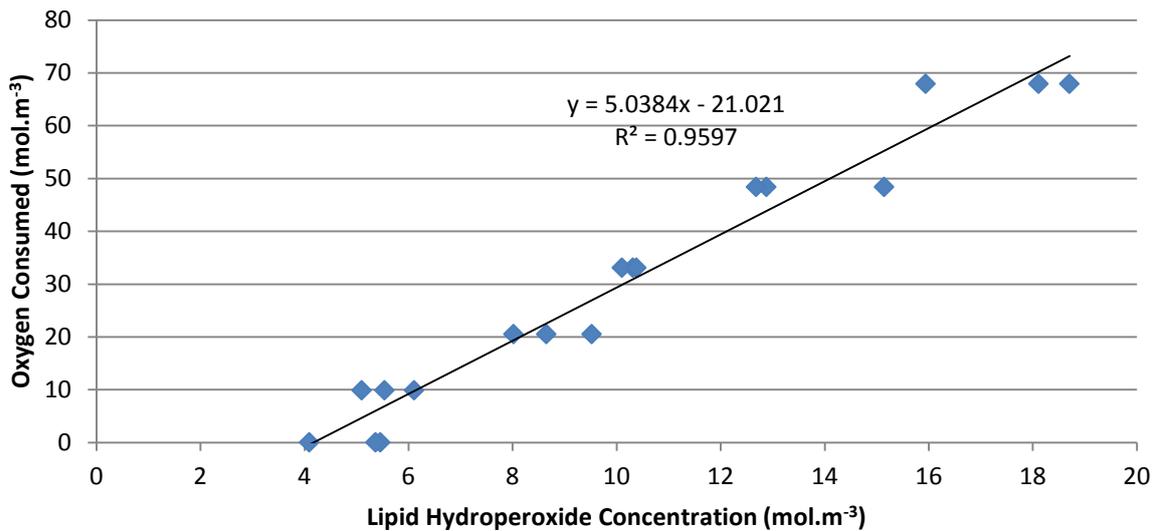


Figure 6.13: Oxygen consumed versus lipid hydroperoxide concentration for continuously sparged mixed fish oil (Bakels Ltd, NZ) at 35°C

Explaining why the ratio of oxygen consumed to lipid hydroperoxides formed is greater than one is not entirely straight forward as there are a number of factors which may impact the ratio. The consumption of oxygen to form non-lipid hydroperoxide products would account for some of the oxygen consumed. Reactions that form secondary and tertiary oxidation products will consume some oxygen. This analysis, like those carried out by Labuza (1971), assumed that the oxygen consumed by these reactions was small during the initial stages of lipid oxidation and that the oil would be rancid, and therefore inedible, before these reactions have a large impact on oxygen consumption rates. Other oxygen consuming reactions such as those with antioxidants would also increase the ratio of oxygen consumed to lipid hydroperoxides formed. Finally, there will have been some error in the measurement of the lipid hydroperoxide concentrations and oxygen consumption rates. A firm reason for this ratio, however, is not known.

6.2.3 Expected errors in analysis

Aside from the effects of other oxygen consuming reactions, there are two sources of experimental error:

- 1) Error in measuring the oxygen solubility in oils
- 2) Error in measuring the lipid hydroperoxide concentration

It was shown that the solubility of oxygen in a sample of mixed fish oil (Bakels Ltd, NZ) at temperatures between 35°C and 60°C was $3.25 \pm 0.08 \text{ mol.m}^{-3}$. By running the same analysis as that done to create Figure 6.13, using a value of oxygen solubility that is one standard error less than the mean value calculated, the ratio of oxygen consumed to hydroperoxides formed became 4.99, a change of just 0.05 or 1%. Infact, using the lowest, and therefore the worst case, value of solubility calculated in section 6.13 (2.82 mol.m^{-3}) a ratio of 4.2 or 15.6% would be gained (see Figure 6.13). Although significantly lower than the ratio gained from the mean oxygen solubility value, this change in not enough to explain the difference in results between this investigation and that by Labuza (1971).

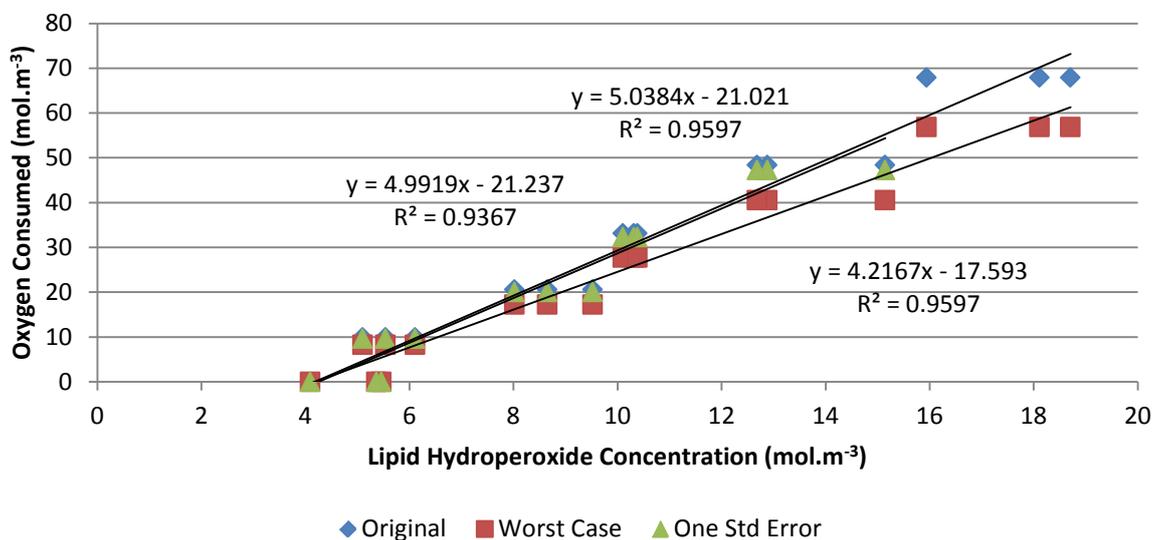


Figure 6.14: Resulting oxygen consumed versus lipid hydroperoxide concentration plots for continuously sparged mixed fish oil (Bakels Ltd, NZ) at 35°C when different oxygen solubility values are used. Original solubility: 3.25 mol.m^{-3} . Worst case solubility: 2.82 mol.m^{-3} . One standard error solubility: 3.17 mol.m^{-3} .

A second, and potentially more significant, error is caused by the measurement of lipid hydroperoxide concentrations. The determination of lipid hydroperoxides in this investigation was based on the conversion from PVs which are, although commonly used and widely accepted, somewhat empirical in nature.

To put the error in perspective, a sensitivity analysis was conducted, focusing solely on the possible error in measuring the PV using the common titration method given by the American Oil Chemists Society Cd8b-90. In particular, the concentration of the standard solution and the ability to accurately recognise the end point of the titration were identified as the most likely sources of error. A summary of a sensitivity analysis looking at errors in these two areas is shown in Figure 6.15.

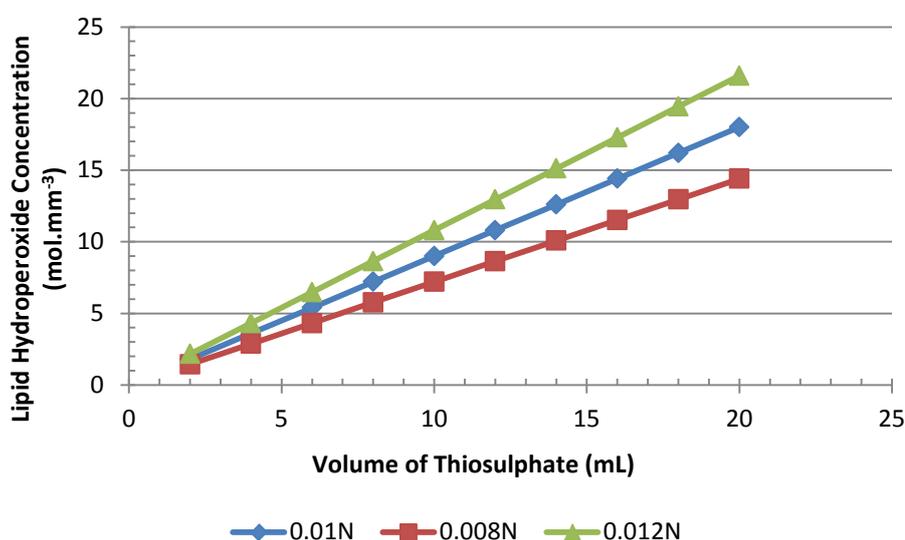


Figure 6.15: Plot depicting the errors in PV determination caused by both the end point detection and standardisation of the sodium thiosulphate solution.

Perhaps the most common error to note is the poorly defined titration endpoint. The endpoint is characterised by a change from a dark blue/black solution to a clear solution. This colour change, however, is not immediate or exact and relies on a skilled technician to continually pick the same endpoint. It is easy to miss the endpoint of such a titration and, as a result, get an erroneous PV.

The effects of failing to accurately detect the end point of the titration can be seen in Figure 6.15. For example, using 10mL of a standardised solution of 0.01 N

thiosulphate and exactly 5g of oil, the resulting lipid hydroperoxide concentration would be 9 mol.m^{-3} ; it is assumed that the oil has a density of 900 kg.m^{-3} . If, however, the end point is missed and an extra milliliter of solution is used, the resulting lipid hydroperoxide concentration would be calculated to be 9.9 mol.m^{-3} . If three samples are done for each point during the experiment it is possible to get an error of $\pm 1 \text{ mol.m}^{-3}$ or more.

The errors incurred during titration are compounded by similar errors that are noted during standardising procedures. The standardisation procedure is a titration and is, therefore, susceptible to the same errors as the original PV analysis. The effects of wrongly standardising a sodium thiosulphate solution, unlike the errors in detecting the end point during PV analysis, become more significant as the volume of standard solution is used during a titration increase. This effect can be clearly seen in Figure 6.15.

Because the error in the PV measurement is dependent on the amount of sodium thiosulphate added, an analysis was carried out to determine the effect an error in the PV measurement has on the ratio of oxygen consumed to lipid hydroperoxide formed. This analysis represents the effects that would have occurred if the actual PVs were 10% higher than the values measured. Although the 10% error is larger than the error between replicate PV measurements during this investigation, it does represent the type of systematic error that could occur. That is, the end point of the titration is hard to determine and it is possible that the same endpoint chosen, in terms of colour change, may be consistently over-estimated. Under-estimating is unlikely as the colour change would not have occurred.

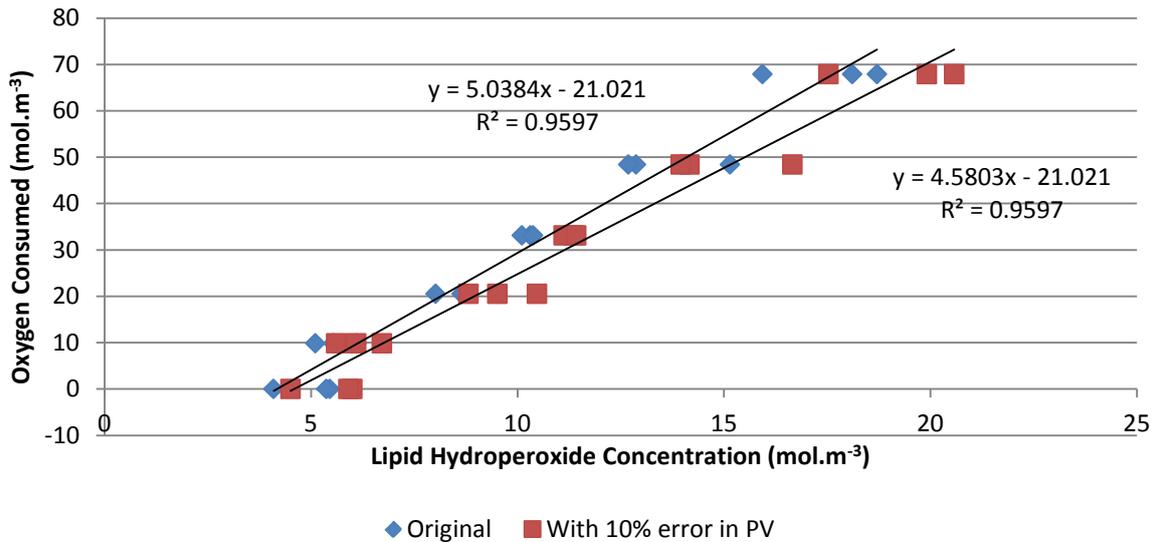


Figure 6.16: Plot showing the effects of a 10% error in the PV measured during experimentation.

The resulting ratio of oxygen consumed to lipid hydroperoxides formed when a 10% PV overestimation is included is 4.58. In this case, the error in PV measurement and calculation moves the results closer to those suggested by Labuza (1971), but does not have anywhere near the effect required to create a 1:1 ratio of oxygen consumed to lipid hydroperoxides formed.

With all the expected errors, the ratio of oxygen consumed to lipid hydroperoxides formed calculated in this analysis was not the same as that suggested by Labuza (1971) or gained through an analysis of the model proposed by Takahashi *et al.* (2000).

6.2.4 Exploring the ratio of oxygen consumed to lipid hydroperoxides formed

Although there is error in the analysis, the ratio of oxygen consumed to lipid hydroperoxides formed was not one as suggested by Labuza (1971). The inclusion of other oxygen consuming reactions may have played a part in moving that away from a ratio of one, but is unlikely to account for it being 5.04. Furthermore, the extra reactions that are included would be present in a real food system and would alter the shelf life anyway. That is, extra oxygen would be required if antioxidants were

present and the total oxygen consumption required to reach the end of its shelf life, and alter the lipid hydroperoxide concentration, would be higher. The use of the 5.04 ratio is exactly what is required to create a shelf life predicting model for a system consisting of the mixed fish oil sample.

These results, although not changing the half order reaction mechanism described in Chapter 5, does mean that Equation 6.7 does not apply. To fit with the results presented above, Equation 6.7 must include the 5.04 ratio. However, because the ratio of lipid hydroperoxides formed could be different for different oils and/or products, the ratio should be redefined as a yield factor (Y). For mixed fish oil, Y will be equal to 5.04, for other samples, the value of Y will need to be measured using the same methods as used in this investigation.

In a batch experiment the change in lipid hydroperoxide concentration can be related to the change in oxygen levels (Equation 6.11)

$$C_{LOOH} - C_{LOOH_i} = \frac{(C_{O_{2i}} - C_{O_2})}{Y} \quad \text{Eq. 6.11}$$

Rearranging:

$$C_{LOOH} = \frac{(C_{O_{2i}} - C_{O_2})}{Y} + C_{LOOH_i} \quad \text{Eq. 6.12}$$

The resulting rate equation in a batch system, therefore, becomes:

$$\begin{aligned} \frac{\partial C_{O_2}}{\partial t} &= -k(C_{LOOH})^{1/2} = -k \left(\frac{(C_{O_{2i}} - C_{O_2})}{Y} + C_{LOOH_i} \right)^{1/2} \\ &= -\frac{k}{\sqrt{Y}} (C_{O_{2i}} - C_{O_2} + Y C_{LOOH_i})^{1/2} \end{aligned} \quad \text{Eq. 6.13}$$

Integrating:

$$C_{O_2} = C_{O_{2i}} + Y C_{LOOH_i} - \left[\frac{k(t-t_0)}{2\sqrt{Y}} + (Y C_{LOOH_i})^{1/2} \right]^2 \quad \text{Eq. 6.14}$$

Using a yield factor, Y , of 5.04, and initial oxygen concentration of 3.25mol.m^{-3} the half order model (Equation 6.14), was fitted to data collected from three successive batch oxidations of mixed fish oil (Bakels Ltd, NZ) at 45°C . Using the SOLVER function in Microsoft EXCEL, the lipid hydroperoxide concentration and rate constant, k , for each batch oxidation was estimated by allowing the SOLVER function to minimise the difference, sum of squares, between the resulting model and experimental data.

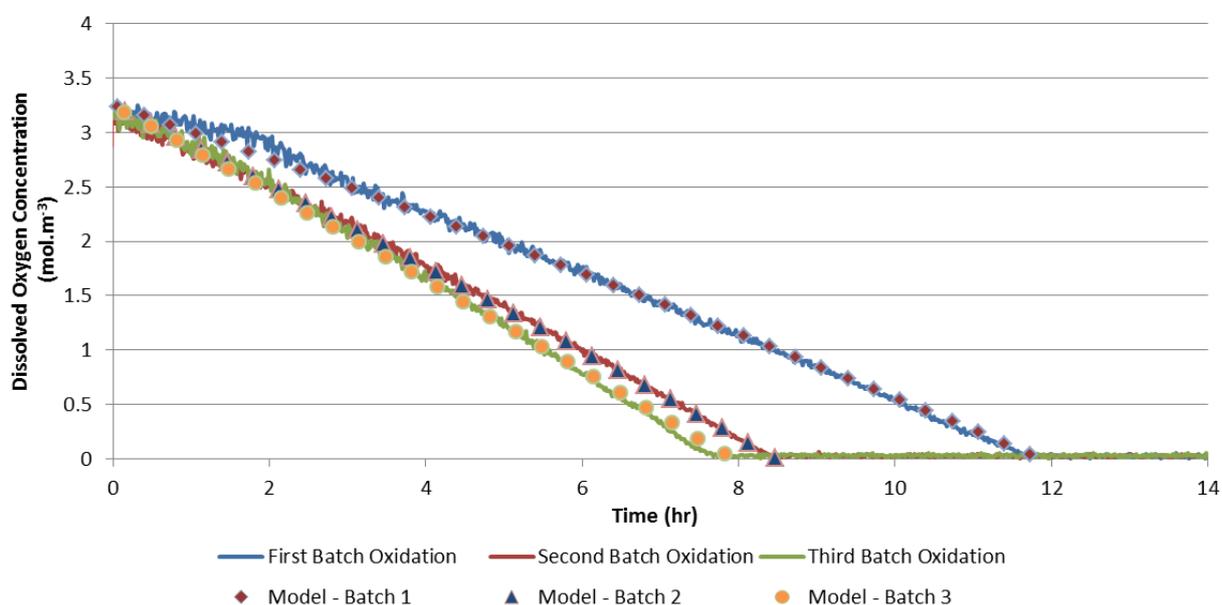


Figure 6.17: Plot showing the results of individually fitting the half order model to three successive batch oxidations of mixed fish oil (Bakels Ltd, NZ) at 45°C .

As can be seen in Figure 6.17, the model fits the experimental data very well. During oxidation experiments, Takahashi *et al.* (2000) measured lipid hydroperoxide concentration from close to zero for a fresh oil to approximately 300mol.m^{-3} for a highly oxidised oil. As such, the fitted initial lipid hydroperoxide concentration in the order of $1\text{-}3\text{mol.m}^{-3}$ is reasonable. Furthermore, the initial lipid hydroperoxide concentration for each batch increases as the number of successive batch oxidation, and therefore extent of oxidation, increases. Perhaps most interesting is that the rate constants (k), presented in Table 6.4, are quite similar for all batch oxidations even though the half order model was fitted to each batch oxidation separately. This result

was expected, as a change in the rate constant would only come about if the progression of lipid oxidation brought with it a change in the reaction mechanism. As the degree of oxidation that occurred over the three successive batch oxidation was very small, i.e. a change of less than 1 mol.m^{-2} per batch oxidation according to the model, no change in reaction mechanism should have occurred.

Table 6.4: Results of individually fitting the half order model to three successive batch oxidations of mixed fish oil (Bakels Ltd, NZ) at 45°C.

Batch Oxidation	Initial Lipid Hydroperoxide Concentration (C_{LOOH_i})	Rate Constant (k) $\times 10^5$
1	0.99	6.70
2	2.03	6.95
3	2.83	6.43

Because there is no reason to expect a mechanism change, a single rate constant (k) was fitted to the same data used to construct Figure 6.18. As expected, the use of a single rate constant for all three successive batch oxidations still resulted in a good fit between the half order model and the experimental data (Figure 6.18). Furthermore, the resulting initial lipid hydroperoxide concentrations predicted by the model were only altered slightly (Table 6.5) and still showed an increase that was expected from the increase in the extent of oxidation.

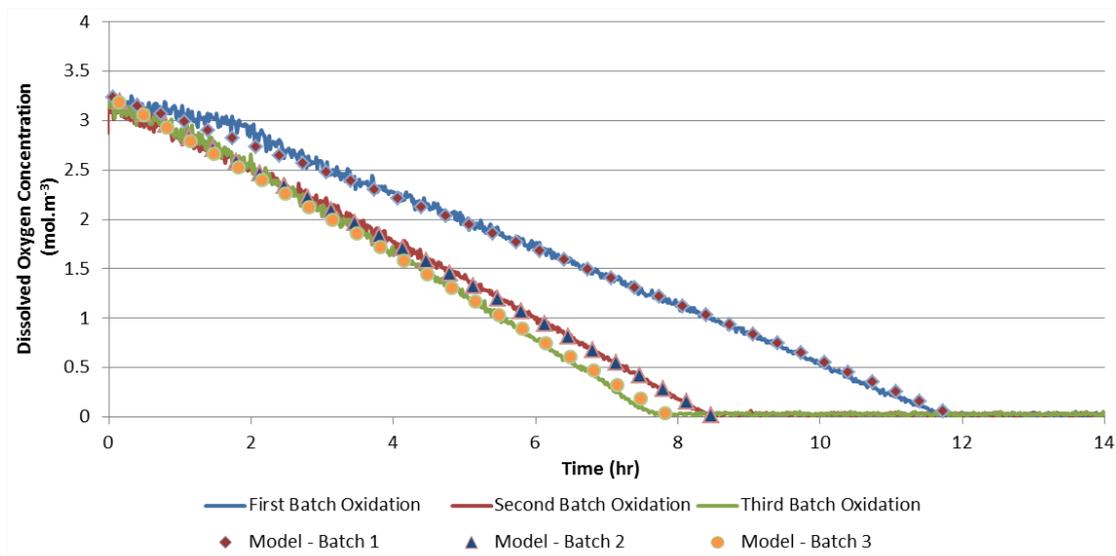


Figure 6.18: Plot showing the results of fitting the half order model with a single rate constant (k) to three successive batch oxidations of mixed fish oil (Bakels Ltd, NZ) at 45°C.

Table 6.5: Results of fitting the half order model with a single rate constant (k) to three successive batch oxidations of mixed fish oil at 45°C.

Batch Oxidation	Initial Lipid Hydroperoxide Concentration (C_{LOOH_i})	Rate Constant (k) $\times 10^5$
1	1.25	6.06
2	2.74	6.06
3	3.22	6.06

According to the yield analysis conducted in Section 6.2.3, the amount of lipid hydroperoxides formed during a batch oxidation of oil should be equal to the amount of oxygen consumed divided by the yield factor, Y . This is shown in Equation 6.11. For a batch oxidation of mixed fish oil at 45°C, starting after being started with air, it was shown that 3.25 mol.m⁻³ of oxygen would be consumed (Section 6.2.3). Because the yield factor for mixed fish oil was shown to be 5.04, the change in lipid hydroperoxide concentration expected during a batch oxidation would be 0.64 mol.m⁻³. The initial lipid hydroperoxides shown in Table 6.5 do not follow this trend. Instead the difference between the initial lipid hydroperoxide concentrations between samples is greater than 0.64 mol.

Before attempting to fit the model, the formation of lipid hydroperoxides during sparging should be accounted for. Because each batch oxidation begins with 30 mins of sparging with air, and the batch oxidation takes approximately 10 hrs, the lipid hydroperoxides formed during sparging is likely to be significant, especially when investigating successive batch oxidations of the same sample of oil. By measuring the rate of oxygen consumption at the end of the previous batch oxidation and by assuming that the rate of oxygen consumption is constant during the sparging period, the amount of lipid hydroperoxides formed during sparging was estimated. The results show that 0.15 and 0.20 mol.m⁻³ of oxygen was consumed during sparging before the start of the second and third batch oxidations. Applying the yield factor, it was found that 0.030 and 0.039 mol.m⁻³ of lipid hydroperoxides were formed during the sparging periods before the second and third batch oxidations.

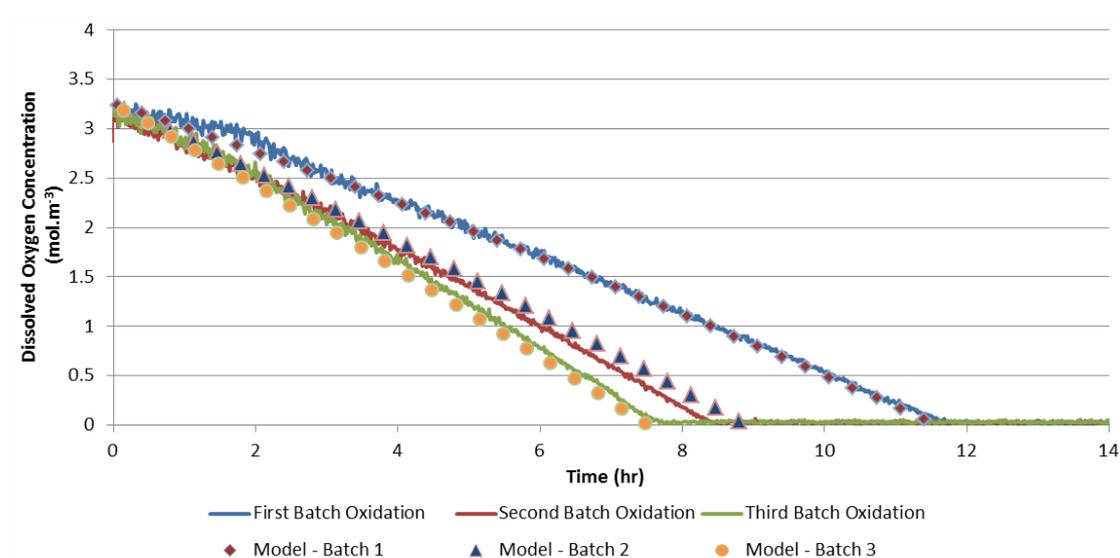


Figure 6.19: Plot of results of fitting the half order model with a single rate constant (k) and accounting for the oxygen consumed during sparging and batch oxidation, of three successive batch oxidations of mixed fish oil at 45°C.

Again, using the SOLVER function in Microsoft EXCEL, the half order model was fitted to experimental data from three successive batch oxidations of mixed fish oil at 45°C. The fit was based on a single rate constant and was run such that the initial lipid hydroperoxide concentrations of the second and third batch oxidation included the lipid hydroperoxides formed during sparging and limited the formation of lipid hydroperoxides to 0.64mol.m⁻³ during batch oxidation. The resulting fit, shown in

Figure 6.19, was still very good. The fitted model under predicted the rate of oxygen consumption in the second batch oxidation but fitted the first and third batch oxidations extremely well. According to an error analysis carried out in Chapter 4, the standard error in the time required to complete a batch oxidation is 0.36 hrs. The difference in time required to complete the second batch oxidation and the time predicted by the model was 0.42 hrs. Without additional data for statistical analysis, the difference in the model and experimental data can be, at least partially, explained by the error in the data collected. This analysis offers good degree of confidence that the half order kinetic model provides a good description of the oxidation process.

Table 6.6: Results of fitting the half order model with a single rate constant, k , and accounting for the oxygen consumed during sparging and batch oxidation, of three successive batch oxidations of mixed fish oil at 45°C.

Batch Oxidation	Initial Lipid Hydroperoxide Concentration (C_{LOOH_i}) mol.m ⁻³	Rate Constant (k) x10 ⁵ mol ^{1/2} m ^{-3/2} s
1	0.71	7.77
2	1.38	7.77
3	2.06	7.77

6.3 Fitting the half order model – further validation

The results of fitting the half order model to three successive batch oxidations of mixed fish oil at 45°C showed that the model was able to describe the rates of lipid oxidation over a limited range of extent of reaction. The data, however, only included small changes in the extent of lipid oxidation and so, a longer test was needed to validate the model's ability to predict the rates of lipid oxidation over a larger range of lipid hydroperoxide concentrations.

Method

A 3.5 L sample of mixed fish oil (PV=11.05 meq.kg⁻¹) was placed in two, 2 L flasks and heated to 35°C using a hotplate and magnetic stirrer. Once heated, the flasks were transferred to a preheated incubator at the same temperature. The oil samples were

then continuously sparged with air at $0.4 \text{ L}\cdot\text{min}^{-1}$ to ensure they remained saturated with oxygen and were well mixed to avoid any oxygen concentration gradients existing in the samples. The concentration of oxygen in the air exiting the flasks was periodically monitored to ensure that oil samples were saturated with oxygen.

At 24 hr periods, an 80 mL sample of oil was removed from the vessel and placed into the batch oxidation vessel. A batch oxidation was carried out on the sample according to the method give in Chapter 4. At the same time, 20 mL of oil was removed from the sparging vessel and the PV of the sample was measured using the American Oil Chemists Society Cd8b-90 method.

Results and discussion

The oxygen consumption profiles for each of the batch oxidations is presented in Figure 6.20. The results clearly show that the rates of oxygen consumption were a function of the sparging time. The corresponding lipid hydroperoxide concentrations (estimated from PV analysis) are presented in Figure 6.21. These results suggest that the rates of oxygen consumption and lipid hydroperoxide formation were both a function of the sparging time and that the rates of oxygen consumption were dependent on the concentration of lipid hydroperoxides in the oil samples, both of which are the basis for the half order model presented in Equation 6.14.

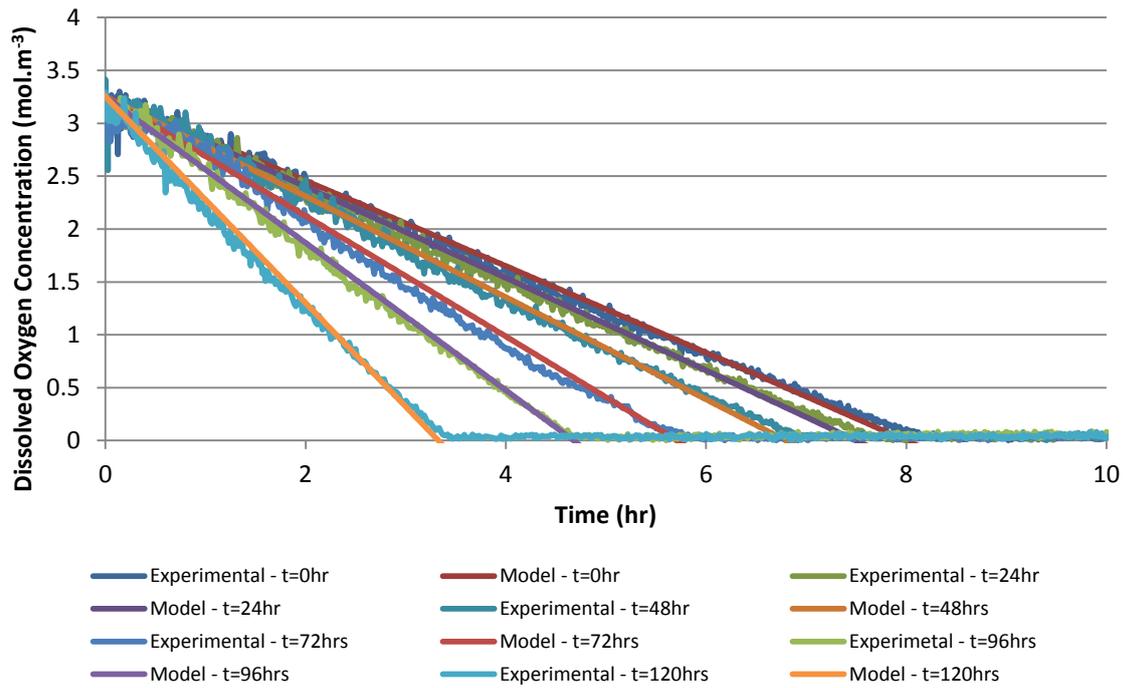


Figure 6.20: Plot shows the raw experimental results from batch oxidations of samples of mixed fish oil (Bakels Ltd, NZ) at 35°C sparged for different lengths of time. Smooth lines are the fit produced by fitting the half order model to the raw data.

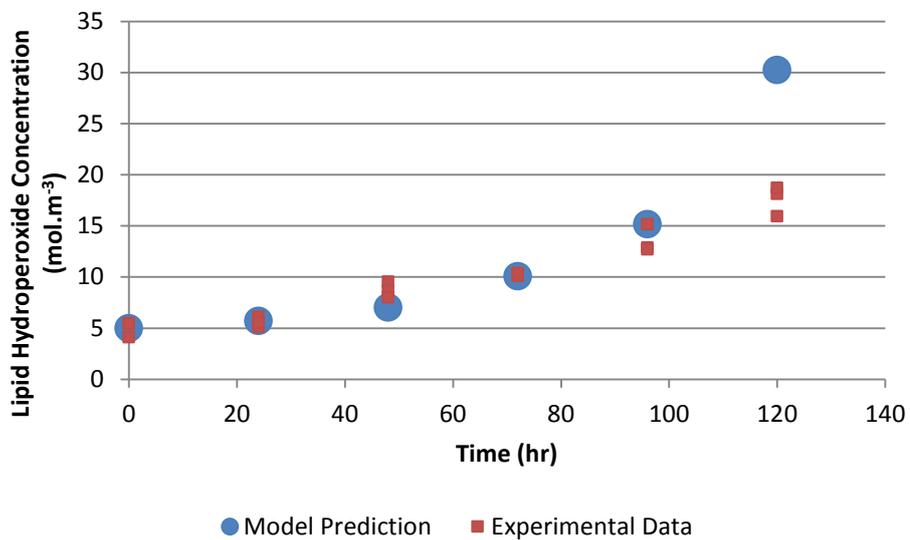


Figure 6.21: Figure shows the results of experimental and predicted lipid hydroperoxide concentrations for mixed fish oil (Bakels Ltd, NZ) at 35°C that has been sparged with air for different lengths of time.

Using the SOLVER function in Microsoft EXCEL, the half order model presented in Equation 6.14 was fitted to the experimental data. The fitting process required that the concentration of lipid hydroperoxides in the oil at the beginning of the experiment was 4.97 mol.m^{-3} (estimated from an initial PV of $11.05 \text{ meq.kg}^{-1}$ and an oil density of 900 kg.m^{-3}) and that the rate constant, k , was constant for all batch oxidations. The resulting model was very good at predicting the rates of oxygen consumption, Figure 6.20. Furthermore, the resulting rate constant was estimated to be $4.91 \times 10^{-5} \text{ mol}^{1/2} \text{ m}^{-3/2} \text{ s}^{-1}$ which is very close to the rate constant calculated for the sample of mixed fish oil used in Section 6.2.4. Because the oil samples were from different batches, there will have been a difference in the fatty acid profiles between the samples, probably because the samples would have been made from different fish sources. The small difference in the rate constants, therefore, is not unexpected.

Fitting the half order model to oxygen consumption data from experiments resulted in impressively accurate predictions of the lipid hydroperoxide concentrations. Table 6.7 shows how well the model is able to predict the concentrations of lipid hydroperoxides from oxygen consumption data. Aside from the final data point, the predictions are well within the error, see Section 6.2.3, in measured lipid hydroperoxide concentrations.

Table 6.7: Comparison between measured and predicted lipid hydroperoxide concentrations for mixed fish oil (Bakels Ltd, NZ) at 35°C that has been sparged with air for different lengths of time.

Time (hrs)	Lipid Hydroperoxide Concentration (mol.m^{-3})	
	Predicted	Measured
0	4.97	4.97
24	5.69	5.58
48	7.00	8.73
72	10.05	10.27
96	15.14	13.57
120	30.24	17.58

A PV in the region of 40 meq.kg^{-1} is very high by any standards. It is difficult to compare PVs gained for other systems with these results and it is even more difficult to find a PV value that can be used as a marker for the point where the product is no longer fit for consumption. With that in mind it is often quite appropriate to use a sensory type approach for determining the quality of the oil. In the case of the oil bubbled for 120 hours (with a PV of approximately 40 meq.kg^{-1}), it is safe to conclude that the oil is well beyond the point where any sensory panel (let alone consumer) would be willing taste the oil. The divergence of the lipid hydroperoxide concentration at high PV is less of a concern especially since the model's ability to predict oxygen consumption rates is still extremely good.

This study shows that, although a key feature in the rate equation (Eq. 6.14), only an estimation of the initial lipid hydroperoxide concentration is needed for the model to be capable of predicting the rates of oxygen consumption in oils of varying oil quality. This not only reduces the time required for experimentation, but increases the accuracy of the model by removing the variable results that would be gained from titrations. Furthermore, it will ultimately lead to the ability to predict oxygen consumption rates in more complex foods where the oil cannot be easily removed or stripped from the food for testing.

6.4 Reaction rate and temperature changes

The rates of lipid oxidation, like most chemical reaction systems, tend to increase as the temperature of the reaction system is increased. Depending on the literature source, the Q_{10} (rate increase for a 10°C increase in reaction temperature) for lipid oxidation rates ranges from 1.6 to 3.3 (Evranuz, 1993; Tazi *et al.*, 2009). The differences in Q_{10} quoted by different authors could be explained by the methods used to measure lipid oxidation and the system being oxidised as well as the differences in the oxidation systems themselves. For example, it is quite possible that the reaction mechanism, will be different when using high temperatures ($>60^\circ\text{C}$) compared with lower temperatures and for systems that contain different additives such as antioxidants.

The complexity of the oxidation systems makes it difficult to determine the effects of temperature on the rates of all the competing oxidation reactions that take place in bulk oils. To make matters worse, the complexity increases when dealing with emulsions and real food systems. No attempt should be made to completely characterise the effects of all these reactions. Instead the net effect of temperature on oxidation rates should be determined. This would allow for the information required for shelf life predictions to be gathered, while being achievable in a reasonable length of time.

To determine the net effect of temperature changes on oxygen consumption rates, a series of batch oxidation reactions was carried out using mixed fish oil (Bakels Ltd, NZ), according to the methods outlined in Chapter 4, for temperatures between 35 and 60°C. The results of the batch oxidations, shown in Figure 6.22, illustrate that a significant change in oxygen consumption rate is brought about by small changes in reaction temperature.

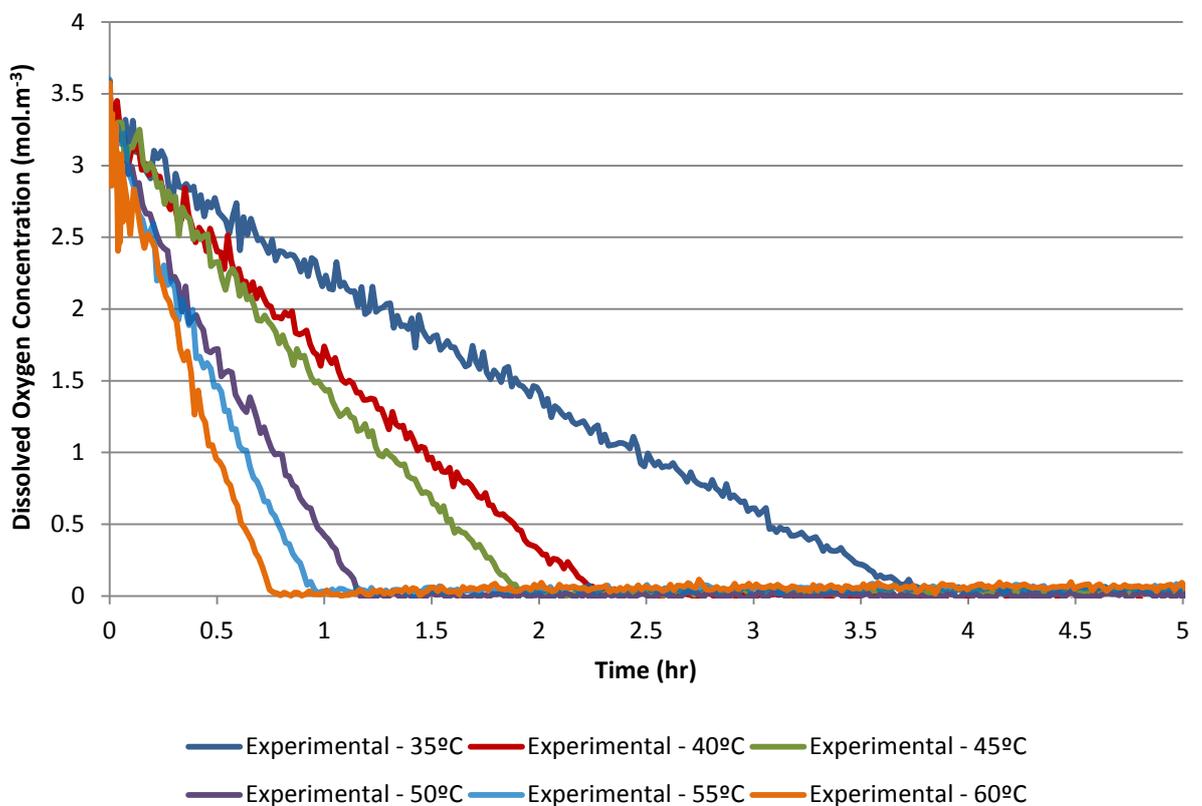


Figure 6.22: Oxygen consumption profiles for batch oxidations of mixed fish oil (Bakels Ltd, NZ) at different temperatures.

The Arrhenius law, given in Eq. 6.15, is often used to quantitatively describe the effects of temperature changes on reaction rates. Using the SOLVER function in Microsoft EXCEL, the half order rate equation (Equation 6.14) was fitted to the experimental data. The fit included an initial lipid hydroperoxide concentration of 4.97 mol.m⁻³ which was measured in a previous experiment. The resulting rate constants were then used to draw an Arrhenius plot, Figure 6.23, using Equation 6.15.

$$k = Ae^{-E_a/RT} \quad \text{Eq. 6.15}$$

The straight line shown on the Arrhenius plot, suggests that the Arrhenius approximation is good at explaining the changes in oxygen consumption rate for temperatures between 35 and 60°C. The resulting straight line was used to estimate the activation energy, E_a , and Arrhenius constant which were $5.74 \times 10^4 \text{ J.mol}^{-1}\text{K}^{-1}$ and $666192 \text{ mol}^{1/2}\text{m}^{-3/2}\text{s}^{-1}$ respectively. This result is similar to that gained by Lopez-Duarte & Vidal-Quintanar (2009) who found an activation energy of $4.266 \times 10^4 \text{ J.mol}^{-1}\text{K}^{-1}$ for the change in PV (note the correlation between oxygen consumption rate and PV developed earlier) in corn flour samples.

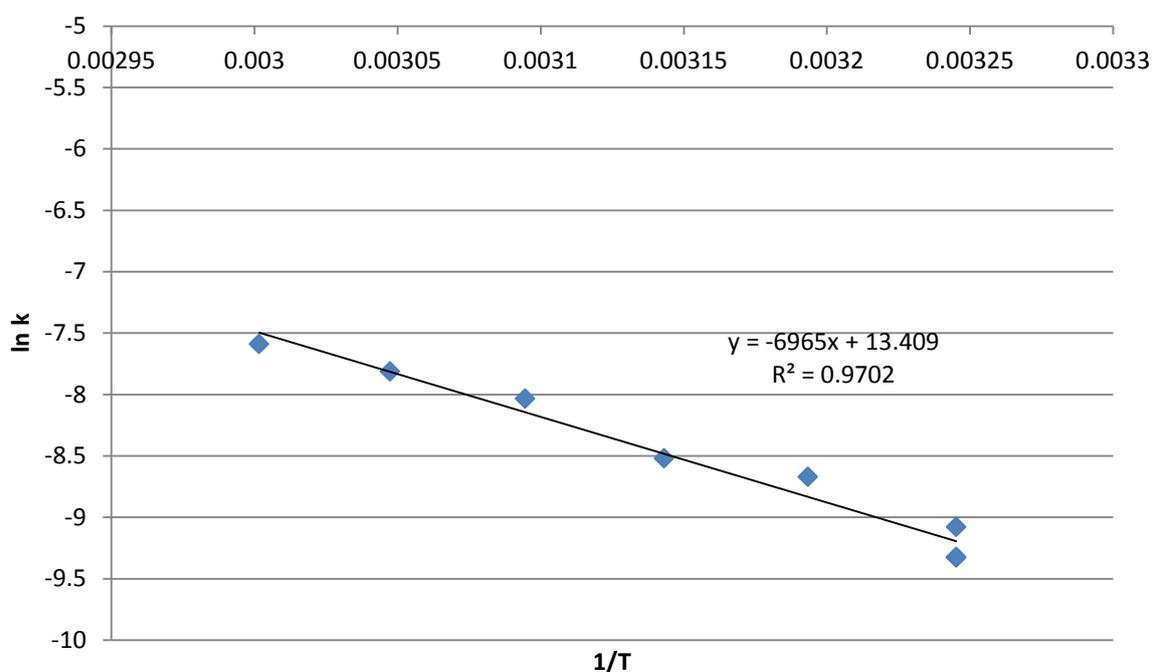


Figure 6.23: Arrhenius plot derived from the data shown in Figure 6.21.

Using Equation 6.16, the activation energy calculated above can then be used to calculate the magnitude of the increase in rate brought about by a 10°C increase in the reaction temperature, Q_{10} . For the temperature range used in the experiments described above, it was shown that the Q_{10} ranged from 2.03 to 1.84 (Figure 6.24). This lies within the range given in literature .

$$Q_{10} = \exp \left[\frac{10E_a}{RT(T+10)} \right] \quad \text{Eq. 6.16}$$

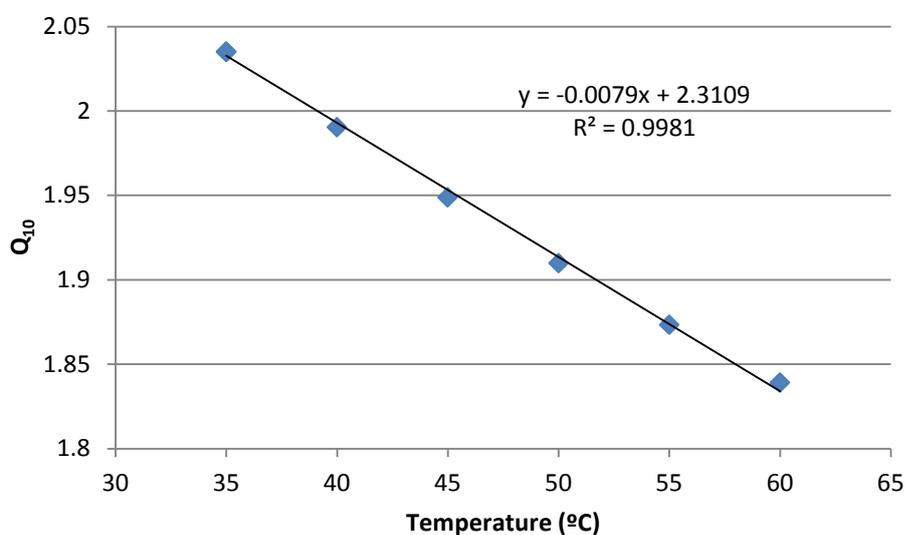


Figure 6.24: Plot shows the Q_{10} as a function of time for mixed fish oil (Bakels Ltd, NZ) at reaction temperatures from 35°C to 60°C.

6.6 Conclusions

Investigations in this chapter have shown that the half order model proposed in the previous chapter fits well with experimental data when an additional yield factor is introduced. In particular the investigations in this chapter show:

- 1) The rate of oxygen consumption is not a function of oxygen concentration as long as oxygen is present
- 2) The solubility of oxygen in air (21% oxygen) is 3.25 mol.m^{-3} for mixed fish oil and does not change significantly between 35 and 60°C.
- 3) The ratio of oxygen consumed to lipid hydroperoxides formed is 5.04:1 for mixed fish oil and not 1:1 as suggested by some authors
- 4) The rate of oxygen consumption can be predicted if the lipid hydroperoxide concentration is known
- 5) The rate of oxygen consumption can be used as an estimate of the lipid hydroperoxide concentration
- 6) Only the initial lipid hydroperoxide concentration is required to make oxidation rate predictions
- 7) No noticeable changes in reaction mechanism were noted as the reaction temperature changed meaning the Arrhenius law can be used to predict the effects changes in temperature have on the rates of oxygen consumption

CHAPTER 7
ANTIOXIDANTS AND LIPID
OXIDATION

7.0 Introduction

Antioxidants have been used successfully by industrial food manufacturers for many years. They have a proven record of retarding lipid oxidation and extending the shelf life of lipid oxidation prone foods. Despite being in use for decades, the ability to predict the change in shelf life brought about by addition of antioxidants is still very difficult. As such, this chapter will focus on extending the kinetic models developed in Chapters 5 and 6. The focus will, therefore, be on the development of mechanisms of lipid oxidation in the presence of primary antioxidants.

7.1 Generalised chemistry of antioxidants in bulk oils

As suggested in the literature review, there are two types of antioxidants. They are;

- 1) Primary Antioxidants
- 2) Secondary Antioxidants

Briefly, primary antioxidants are free radical quenchers or proton donors while secondary antioxidants, generally, act to replenish or aid the action of primary antioxidants. For a more indepth discussion refer to the literature review, Section 2.2 of Chapter 2. The focus of this chapter will be on primary antioxidants.

According to many authors including Frankel (2005), Takahashi *et al.* (2000) and Walstra (2006), the oxidation pathway can be described by the three stage pathway introduced earlier and reproduced below. The progression of lipid oxidation is dependent on the formation and subsequent reaction of radicals with other molecules including lipids, lipid hydroperoxides and, if present, antioxidants.

Primary antioxidants, being proton donors, are able to react with radicals formed during lipid oxidation. The summary of reactions shown in Eq. 5.5 to 5.13 below provides some examples of how and when radical species are formed. The reduction of any of the radical species, however, involves the formation of an antioxidant radical. Despite being radicals these reduced antioxidants are many times less reactive than the original radicals as they are stabilised through resonance. This is why antioxidants tend to include benzene ring structures (like butylated hydroxyanisole or BHA shown in Figure 7.1) and include many double bonds between molecules.

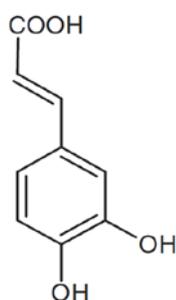


Figure 7.1: The chemical structure of butylated hydroxyanisole (BHA). Figure from Michotte *et al.* (2011).

Initiation:



Propagation:



Monomolecular lipid hydroperoxide decomposition:



Bimolecular lipid hydroperoxide decomposition:



Termination:



From Eq. 5.5 to 5.13 it should be clear that, by removing radical species, antioxidants can alter the rates of radical reaction with other species in different stages of the oxidation pathway. That is, the antioxidants can act in the primary, secondary and tertiary stages of the lipid oxidation pathway. The part of the pathway the antioxidants react with (determined by the extent of reaction) is likely to determine the significance of the effect the antioxidants have on the system.

At this point it should be clear that the reaction of antioxidants with radicals from the tertiary stage of lipid oxidation would have limited if any effect on the rate of lipid oxidation. The rates that tertiary oxidation products are formed would be slightly altered, however, due the small concentrations of tertiary products required to make a product rancid, it is unlikely to have a significant effect on the shelf life of the product. That is, adding antioxidants to a partially oxidised product is likely to be ineffective at significantly increasing the shelf life of a product.

The addition of antioxidants to a completely unoxidised product, however, is likely to have a much larger effect on the shelf life of a product. It has been well established in this thesis that initiation reactions occur at a very low rate. Removal of any radicals formed during initiation will, therefore, only act to slow down initiation even more. This could occur by reaction with lipid radicals or singlet oxygen and would likely continue until the antioxidant concentration is depleted. In practical applications in food processing, however, it is unlikely that lipid hydroperoxides are not already present.

Because it is likely that oils used in industry have some degree of oxidation, it is reasonable to expect that added antioxidants are mostly likely to alter the rates of propagation type reactions. That is, the formation and decomposition of lipid

hydroperoxides are likely to be retarded by the addition of antioxidants. This should continue until the antioxidants have been depleted.

7.2 Kinetics of antioxidant action

The descriptions above can only give qualitative information and cannot be used to predict the changes in lipid oxidation rates. To lift these to a quantitative level, a more formal look at the effects on the models developed in the previous chapters is required. As outlined above, there is little point in looking at the effects of antioxidants on tertiary reactions as the oil/product is likely to be rancid at that point. Instead focus is kept on the reactions that lead up to rancidity. These include initiation and propagation reactions i.e. the reactions that include the formation and breakdown of lipid hydroperoxides.

7.2.1 Antioxidants – mechanism of action

To model lipid oxidation in the presence of antioxidants, the mechanism of antioxidant action must be determined. Despite there being quite a large amount of literature on antioxidants, there is still a general lack of understanding around the actual mechanism of lipid oxidation and antioxidants. Most of the literature available focuses on the relative effects of the addition of different antioxidants on lipid oxidation. Examples of such work include that done by Ozcan & Arslan (2011), Thiyam *et al.* (2006) and Athukorala *et al.* (2007) all of which tested the antioxidant effects of extracts on lipid oxidation rates. Examples of their results are shown in Figures 7.3 and 7.4.

Perhaps the most common description of primary antioxidant action is that of proton donation and radical quenching (Frankel 2005). In itself, this is a good starting point for understanding how primary antioxidants will function within the lipid oxidation pathway. The issue is when and how the antioxidants react with radical species.

By looking carefully at the lipid oxidation pathway (Equations 5.5 to 5.13 for example), it is possible to identify the reactions that a primary antioxidant may take part in. Because the flavour threshold of tertiary oxidation products is so low (see Chapter 2, Section 2.4), it is reasonable to remove tertiary oxidation reactions from the reactions to investigate as they are likely to only account for a tiny proportion of reactions that occur. Having done this, there are only two sets of reactions remaining.

- 1) Initiation reactions
- 2) Propagation reactions

7.2.2 Initiation versus propagation reactions

The line defining the switch from initiation reaction limited oxidation to one where propagation reactions dominate is somewhat blurred. Investigations in Chapter 5, however, suggested that the initiation reactions are only important when there are no lipid hydroperoxides present in the oil. This, however, is nearly impossible to achieve and so, in an industrial oil, it is quite likely that the initial formation of radicals from an initiator (Equation 5.5) would have limited or no impact. The concentration of lipid hydroperoxides relative to the amount of antioxidant added is likely to have a much greater impact.

From the investigations in Chapters 5 and 6, it was shown that the rate of oxygen consumption by lipid oxidation is proportional to the lipid hydroperoxide concentration. It follows that any additive that stops the formation of lipid hydroperoxides would also stop the consumption of oxygen as long as the additive itself does not consume oxygen. If antioxidants were added to a fresh oil sample, with a very low concentration of lipid hydroperoxides, then a lag phase in the formation of lipid hydroperoxides and consumption of oxygen would occur. An example of the type of effect that would be gained from a batch oxidation of this type is depicted in Figure 7.2.

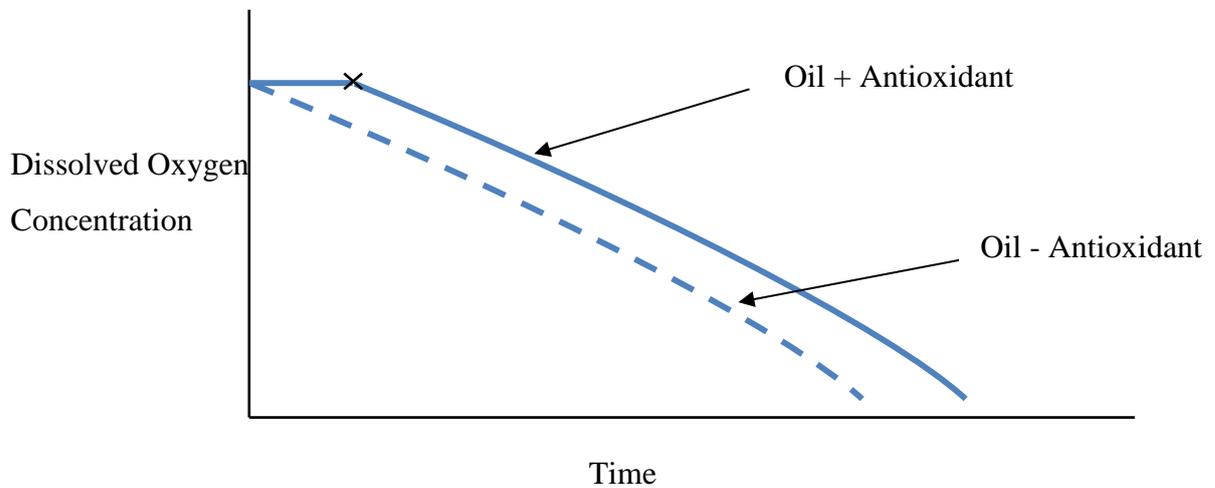


Figure 7.2: Possible effect of adding primary antioxidants to bulk oil in a batch oxidation experiment. Action likely if the initiation reactions are a significant source of radical species.

Because it is suggested that the lipid hydroperoxides and radical species are in equilibrium with each other (See Chapter 5), the antioxidants added to the oil would remove lipid hydroperoxides at the same time. The concentration of lipid hydroperoxides could be decreased by the addition of primary antioxidants. According to the theory developed in Chapters 5 and 6, to achieve a complete halt in the consumption of oxygen, the antioxidants added would need to remove all lipid hydroperoxides to reach a system that is initiation reaction limited.

If the antioxidants' action was based on the removal of lipid hydroperoxides from the system then, depending on the relative concentrations of lipid hydroperoxides and antioxidants added, two scenarios may occur. Firstly, if the amount of antioxidants was not enough to deplete all lipid hydroperoxides in the oil, then the lipid hydroperoxide concentration would decrease but the oil will continue to oxidise as if beginning from a lower lipid hydroperoxide concentration (without being further hindered by antioxidants). Secondly, if the concentration of antioxidants is greater than that required to reduce the lipid hydroperoxide concentration to zero, then lipid oxidation would halt for the period required to completely deplete the antioxidants in the system.

Work by Ozcan & Arslan (2011) and Athukorala *et al.* (2004), shown in Figures 7.3 and 7.4, suggests that there is no measureable drop in the lipid hydroperoxide concentration upon the addition of antioxidants and that the rates of lipid hydroperoxide formation are reduced throughout the experiment (not simply a function of the initial lipid hydroperoxide concentration).

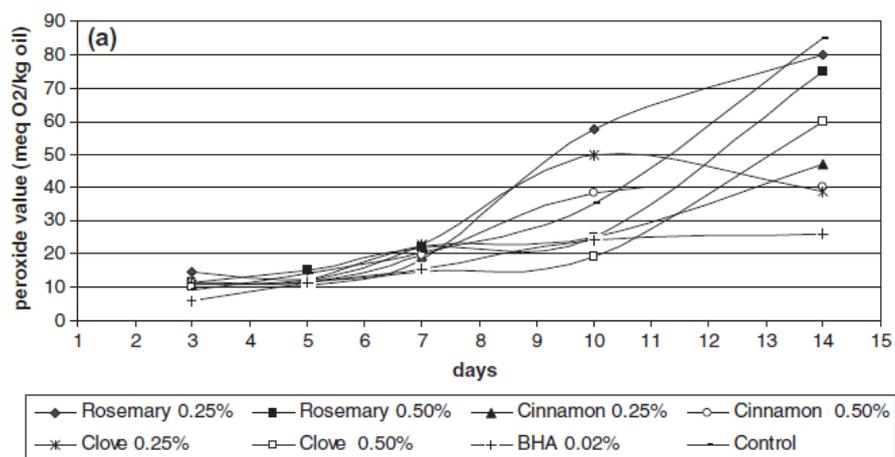


Figure 7.3: Effect of rosemary, cinnamon and clove extracts on the Peroxide Value of hazelnut oil. Figure from Ozcan & Arslan (2011).

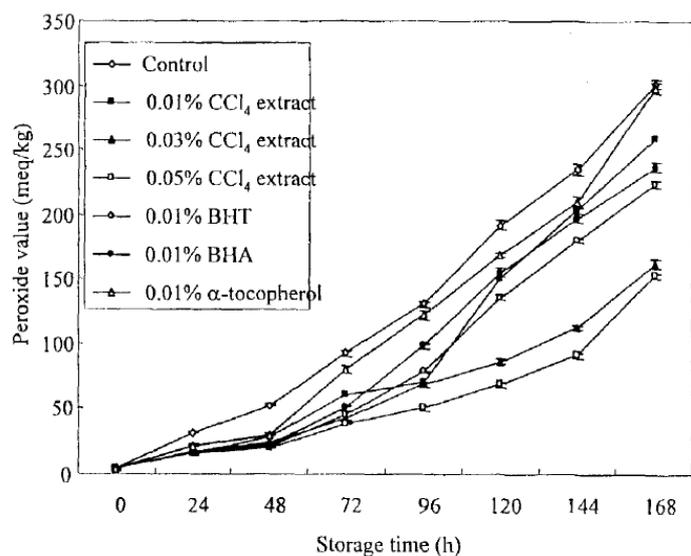


Figure 7.4: Effect of *Grateloupia Filicina* extract and commercial antioxidants on peroxide values of linoleic acid stored at 65°C. Figure from Athukorala *et al.* (2004).

The results shown in Figures 7.3 and 7.4 are typical of those found in literature. They suggest that antioxidant action, for the most part, is not based on the removal of large quantities of lipid hydroperoxides upon addition. Instead it seems more likely that the addition of antioxidants hinders the formation of new lipid hydroperoxides/radicals and, in doing so, reduces the rate of oxidation. A batch oxidation of an oil with antioxidants should result in an oxygen consumption profile with the same shape as that of an oil without antioxidants. The only difference should be the rate of oxygen consumption. This is depicted in Figure 7.5. It is this theory that will be carried forward and investigated in the following discussions.

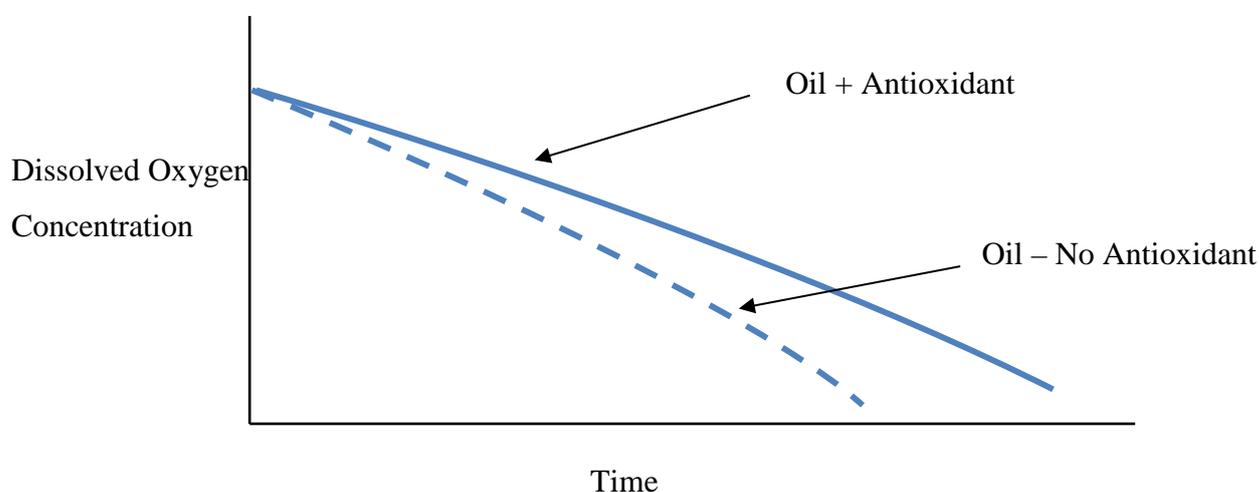


Figure 7.5: Possible effect of adding primary antioxidants to bulk oil. Action likely if the initiation reactions are not a significant source of radical species.

7.3 Initial batch oxidations

To validate the ideas presented in Figure 7.5, an initial investigation was carried out to look at the effects that antioxidant addition had on oxygen consumption rates. To do this, two 100 g samples of mixed fish oil (Bakels Ltd, NZ) were preheated to 35°C. Once heated to 35°C, 10g of oil was removed from one sample and heated to 50°C and butylated hydroxyanisole (BHA) antioxidant was added and dissolved. The 10 g sample was then returned to the main sample and cooled to 35°C in an ice bath. This helped ensure that the antioxidant was dissolved in the oil. Both samples were put through a batch oxidation according to the method given in Chapter 4. The results of the batch oxidations are shown in Figure 7.6 below.

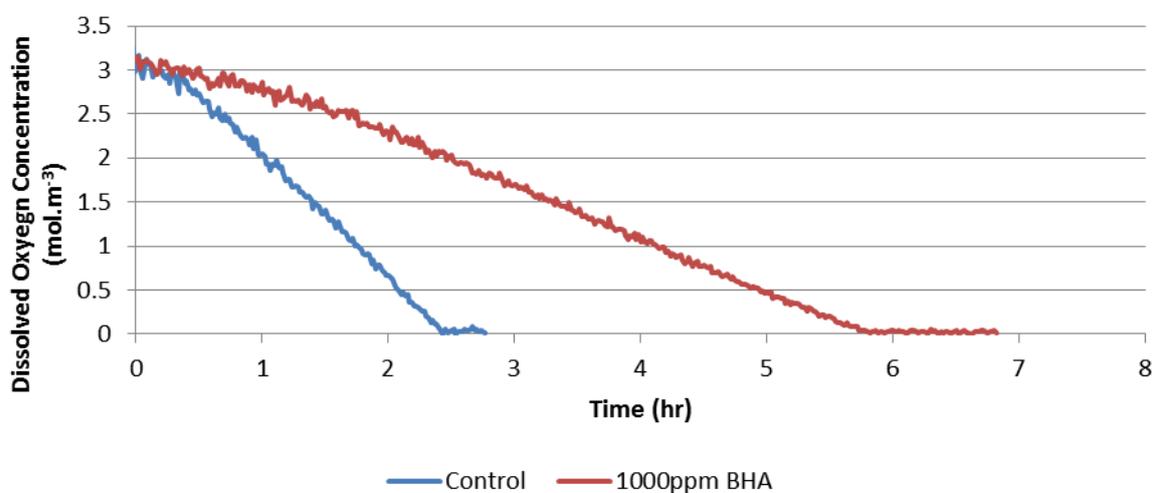


Figure 7.6: Results of initial batch oxidations of mixed fish oil samples with and without BHA antioxidant added.

The results of the initial batch oxidations show the trends depicted in Figure 7.5. That is, the oxygen consumption profiles for both the control and oil with antioxidant had the same shape. Additionally, there was no lag where oxygen was not being consumed in the sample with antioxidants. These results suggest that the addition of antioxidants follows the trends expected for a mechanism involving the reduction in the rate of new lipid hydroperoxide formation.

7.4 Modelling antioxidant activity

From the previous discussion and a literature search, there is evidence that the addition of antioxidants reduces the rate of lipid oxidation by hindering the formation of new lipid hydroperoxides/radicals. The shape of the oxygen consumption profiles in Figure 7.6, suggest that there is no mechanism change brought about by the addition of antioxidants. The slowing of the rate of oxygen consumption should be equal to that in Equation 7.1. That is, the rate constant ' k ' for a sample with added antioxidants, should be equal to the rate constant without antioxidant, k_o , minus the effect of the antioxidant, C_{AX} .

$$\frac{\partial C_{O_2}}{\partial t} = -(k_o - C_A X) C_{LOOH}^{1/2} \quad \text{Eq. 7.1}$$

where C_A is the concentration of antioxidant and X is the effectiveness of the antioxidant

The inclusion of an effectiveness factor, X , is based on the idea that different antioxidants are more or less effective at removing radical species than others. The effectiveness is a function of a number of factors within the oxidation system. Firstly, it is quite possible that an antioxidant may react with more than one radical species. This phenomenon, often referred to as radical scavenging activity, is discussed by researchers such as Mohdaly *et al.* (2011). The ability of an antioxidant molecule to remove more than one radical species would bring with it a significant change in the effective concentration of that antioxidant. For example, if two different antioxidants were available, one of which can remove two radical species per molecule of antioxidant while the other can only remove one, then the effectiveness of the two antioxidants would be quite different. The actual ratio of effective antioxidant concentration would, of course, be dependent on the structure of the antioxidants.

It is difficult to determine the effectiveness of an antioxidant without experimentation. Furthermore, as the system moves away from a simple bulk oil and into a whole food system, the complexity of the reactions that occur increases significantly. Therefore, a simple method to calculate the effectiveness of an antioxidant is required.

By including the modifier, $-C_A X$, into a batch oxidation model the rate of oxygen consumption as a function of the oxygen consumed can be calculated. This is shown in Equation 7.2. This equation includes both the yield of lipid hydroperoxide for the oxygen consumed, Y , as well as the effectiveness of the antioxidant, X .

$$\frac{\partial C_{O_2}}{\partial t} = \frac{-(k_o - C_A X)}{\sqrt{Y}} (C_{O_{2i}} - C_{O_2} + Y C_{LOOH})^{1/2} \quad \text{Eq. 7.2}$$

integrating:

$$C_{O_2} = C_{O_{2i}} + Y C_{LOOH} - \left[\frac{(k_o - C_A X) \Delta t}{2\sqrt{Y}} + (Y C_{LOOH})^{1/2} \right]^2 \quad \text{Eq. 7.3}$$

For Equation 7.3 to be validated using experimental data, the yield factor, Y , must be accurately calculated using methods described in Chapter 6. For mixed fish oil (Bakels Ltd, NZ) the yield factor was shown to be $5.04 \text{ mol.mol}^{-1}$.

7.5 Experimentation to validate models developed

To validate this model, varying amounts of BHA were added to mixed fish oil (Bakels Ltd, NZ) samples and batch oxidation reactions carried out at 35°C using the method outlined in Chapter 4. The methods for these batch oxidations are described above in Section 7.3. The resulting oxygen concentration profiles for these experiments were then compared with a control oil containing no BHA. The results of this experiment are shown in Figure 7.7.

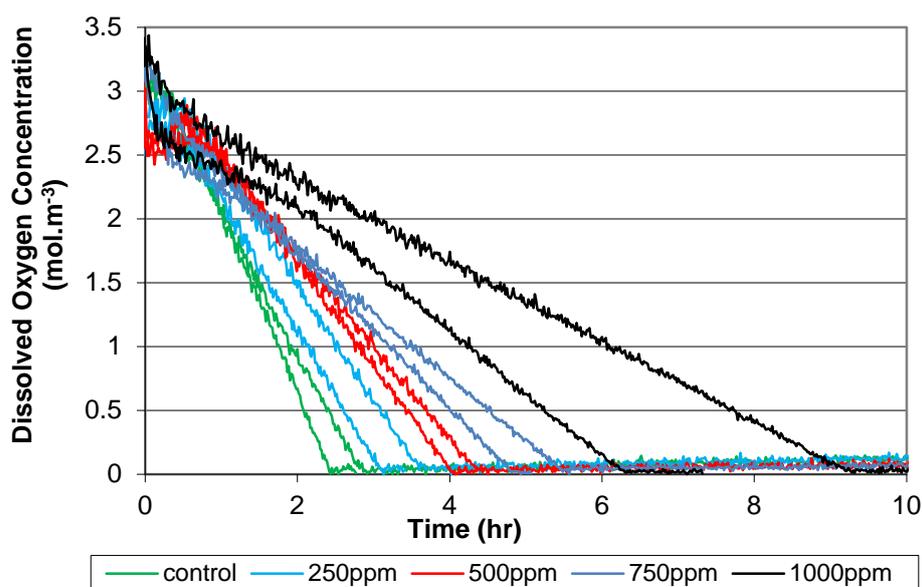


Figure 7.7: Results of batch oxidations of mixed fish oil at 35°C with varying levels of BHA.

The results gained from the antioxidant experiment suggest that the rates of oxygen consumption, and therefore lipid oxidation, are significantly reduced by the addition of BHA. There is no evidence of a lag phase where no oxidation occurs as depicted in Figure 7.2. The data does, however, show some acceleration in oxidation rate during the first half hour of each batch oxidation. These results were unexpected but could be due to small differences between the initial temperature of the oil samples and

incubator temperature. The effects of temperature on probe response was described in Section 4.3.2 of Chapter 4. Had the initial acceleration been caused by the addition of antioxidant, i.e. had there been a lag phase, the magnitude of the acceleration would likely have been proportional to the concentration of antioxidant added. This was not seen.

It must be noted that there is some variation in the data acquired and presented in Figure 7.7. The difference between the times for the batch oxidation of the two samples of fish oil with 1000 ppm BHA is quite large. This is not ideal, but does not significantly alter the analysis that is conducted next.

By fitting Equation 7.3 to the experimental data shown in Figure 7.7 using SOLVER function to minimise the sum of the square differences between model and experimental data in Microsoft EXCEL, the rate constant , k , was estimated for each batch oxidation. An example of such a fit is shown below in Figure 7.8.

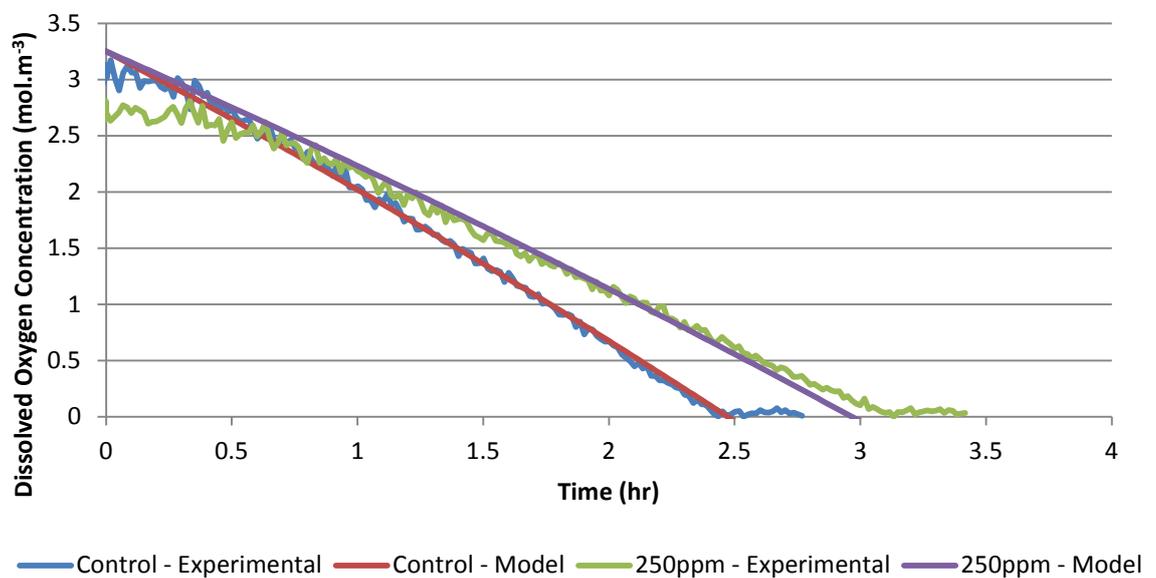


Figure 7.8: Example results of fitting the half order model to data obtained from the batch oxidation of mixed fish oil with 500ppm BHA. Figure

Using the same fitting technique, the half order model (Equation 7.3) was fitted to the remaining experimental data. The rate constants for each batch oxidation are summarised in Table 7.1 below.

Table 7.1: The rate constants resulting from fitting the half order model to batch oxidations of oil with different concentrations of BHA antioxidant

Concentration of BHA (ppm)	Apparent rate constant, $k \times 10^4$ ($\text{mol}^{1/2} \text{m}^{-3/2} \text{s}^{-1}$)
0	1.33
0	1.21
250	1.11
250	0.93
500	0.86
500	0.81
750	0.77
750	0.73
1000	0.60
1000	0.47

Because the same batch of mixed fish oil was used for all the batch oxidations (i.e. small samples were taken from a large batch of mixed fish oil), the initial lipid hydroperoxide concentration in each sample would have been the same. The following assumptions were made:

- 1) The change in antioxidant concentration during a batch oxidation is small
- 2) The solubility of oxygen in the oil is unaffected by the addition of small amounts of antioxidants

With these assumptions, a plot of the rate constant (k) versus the antioxidant concentration, C_A , was produced. The plot resulted in a straight line with a slope of $-X$ and an intercept of k_o , this plot is shown in Figure 7.9.

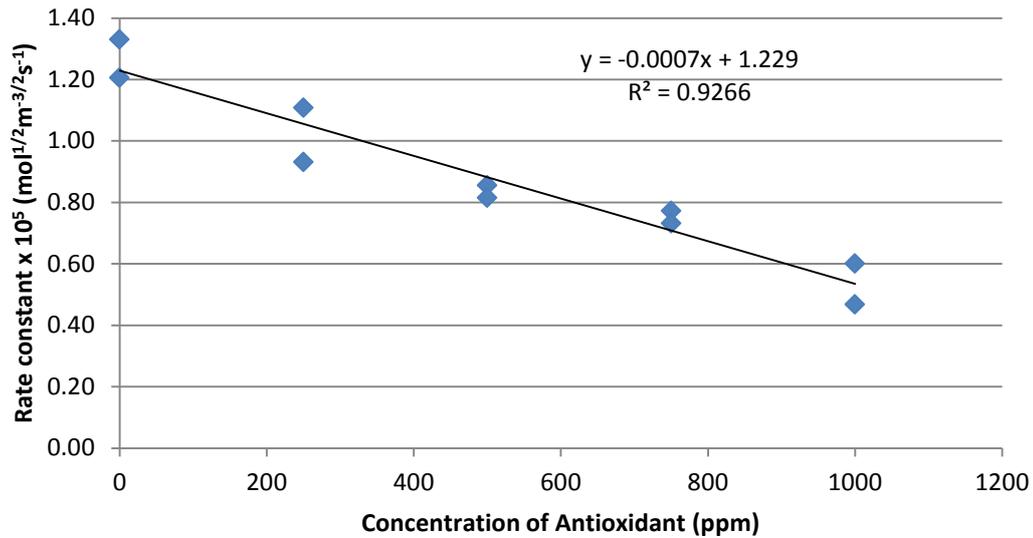


Figure 7.9: Results showing the rate constant, k , from batch oxidations of mixed fish oil at 35° with different concentrations of BHA antioxidant.

Figure 7.9 shows a linear relationship between the rate constant, k , and the concentration of antioxidants (C_A) added to the oil. For the case of mixed fish oil with BHA antioxidant, the rate constant would be $1.229 \times 10^{-5} \text{ mol}^{1/2} \text{ m}^{-3/2} \text{ s}^{-1}$ without added antioxidant and would be reduced by $6.94 \times 10^{-9} \text{ mol}^{1/2} \text{ m}^{-3/2} \text{ s}^{-1}$ for every ppm of BHA added. The effectiveness factor (X) for BHA in mixed fish oil is, therefore, 6.94×10^{-9} . These results demonstrate that inclusion of antioxidants into the kinetics model can be done by modifying the rate constant as expressed in Equations 7.2 and 7.3.

7.6 Modelling antioxidant consumption

In order to carry out the above analysis and to calculate the effectiveness of BHA in mixed fish oil, it was assumed that the concentration of antioxidants did not change significantly over the course of a single batch oxidation. Intuitively this makes sense as a batch oxidation is merely a snapshot of a long lipid oxidation process. If the concentration of antioxidants were to be significantly depleted after a single batch oxidation, then they would have little impact on the rate of lipid oxidation as a whole or on the shelf life of a real food product.

To put this into perspective, and to make the model applicable to long term storage scenarios, a model describing the depletion of antioxidants was formed. To do this it was assumed that the rate of antioxidant removal was equal to the difference in lipid hydroperoxide formation in samples with and without added antioxidant. In other words, the rate at which we can reduce the formation of lipid hydroperoxides must be proportional to the change in antioxidant concentration. This is shown in Equation 7.4.

$$\frac{\partial C_A}{\partial t} = Y' \left[\frac{\partial C_{LOOH_{no\ anti}}}{\partial t} - \frac{\partial C_{LOOH_{anti}}}{\partial t} \right] \quad \text{Eq. 7.4}$$

where Y' is the yield factor describing the amount of antioxidants used to prevent lipid hydroperoxides being formed.

By substituting the half order models for lipid hydroperoxide formation with and without added antioxidants, Equation 7.5 is obtained.

$$\frac{\partial C_A}{\partial t} = Y' \left[-kC_{LOOH}^{1/2} + (k - XC_A)C_{LOOH}^{1/2} \right] = -Y'XC_A C_{LOOH}^{1/2} \quad \text{Eq. 7.5}$$

In order to gain a full picture of a batch oxidation, including the formation of lipid hydroperoxides, Equations 6.12 and 6.13 (from discussions in Chapter 6) were combined with Equation 7.5 above. Using the ODE solver in MATLAB (R2011b) a batch oxidation of mixed fish oil at 35°C was conducted using initial oxygen and lipid hydroperoxide concentrations of 3.25 mol.m⁻³ and 1 mol.m⁻³, a yield factor, Y , of 5.04 mol.mol⁻¹ and five initial antioxidant concentrations (0, 250, 500, 750 and 1000ppm BHA) with an effectiveness factor, X , of 6.94x10⁻⁹ mol^{1/2}.m^{-3/2}.s⁻¹.ppm⁻¹. For this analysis it was assumed that each mole of antioxidant could remove one mole of lipid hydroperoxide forming substances. The MATLAB code for this analysis is give in the Appendix (see folder ‘Chapter 7’, ‘Oxygen_consumption_BHA_added.m’)

$$\frac{\partial C_{LOOH}}{\partial t} = \frac{k}{Y} C_{LOOH}^{1/2} \quad \text{Eq. 7.6}$$

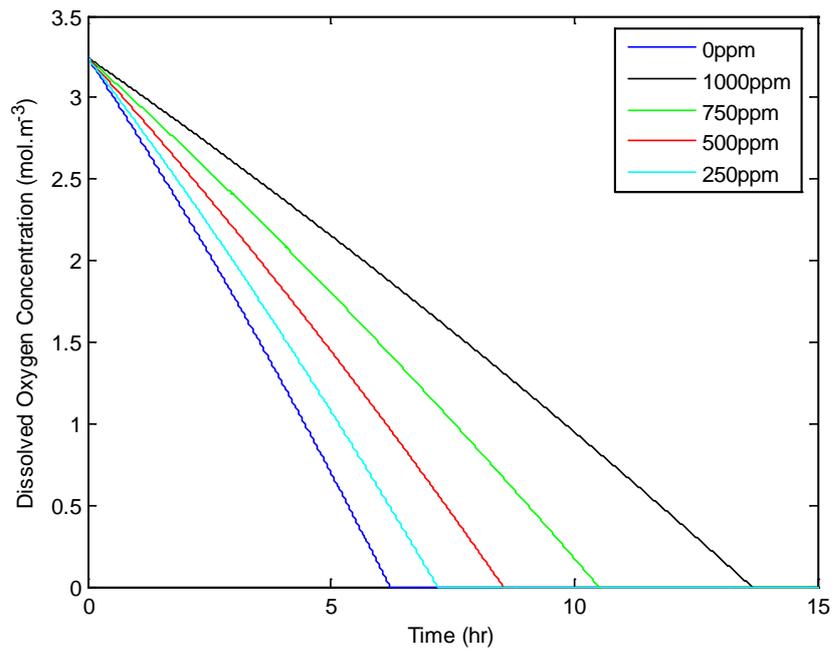


Figure 7.10: Model results for batch oxidations of mixed fish oil with different concentrations of BHA antioxidant.

As can be seen in Figure 7.10, the amount of oxygen consumed during a batch oxidation is not changed by the addition of antioxidants to the system. The extent of oxidation in all samples would be the same after a batch oxidation. As expected, the rate of oxygen consumption and lipid hydroperoxide formation (see Figures 7.10 and 7.11) is reduced by an amount proportional to the concentration of antioxidants added.

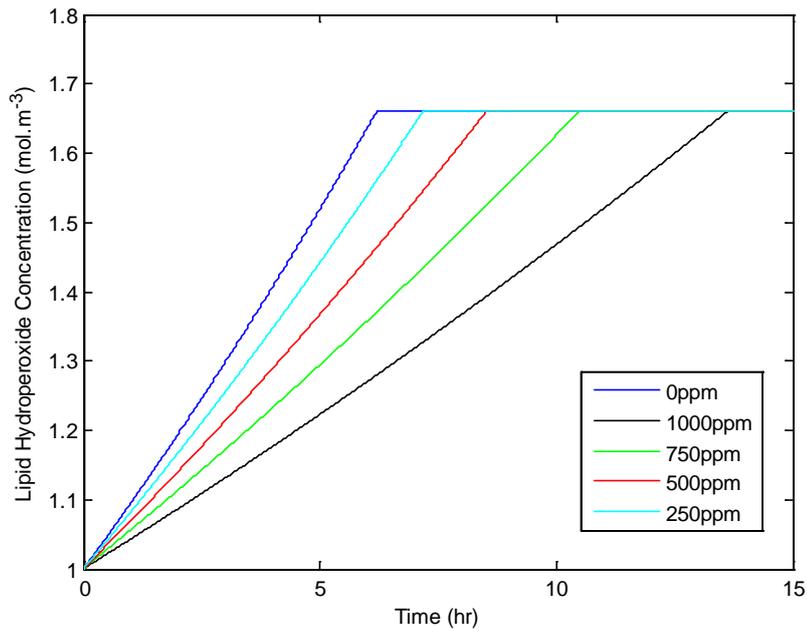


Figure 7.11: Model results for batch oxidations of mixed fish oil with different concentrations of BHA antioxidant.

More importantly, the model helps verify the assumption that the concentration of the antioxidants is not significantly changed during the course of a batch oxidation. Figure 7.12 shows that the antioxidant concentration drops by less than 2ppm during a batch oxidation of oil with 500ppm of BHA initially present. The drop in the concentration of antioxidant during a batch oxidation is, however, dependent on the initial antioxidant concentration. The additional decrease in the reaction rate (during batch oxidation) that is brought about by additional antioxidants does so with an increase in the consumption of antioxidants as shown in Figure 7.12.

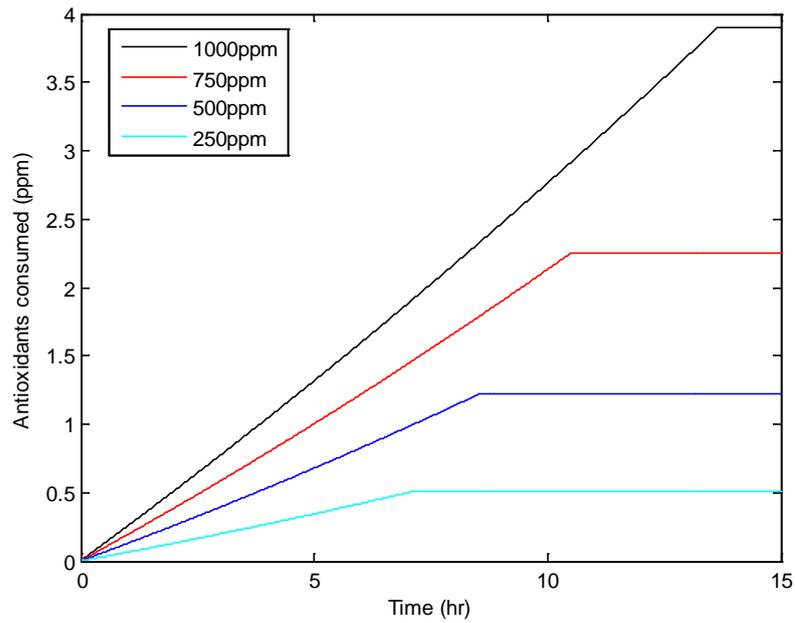


Figure 7.12: Model results for batch oxidations of mixed fish oil with different concentrations of BHA antioxidant.

If, however, the oil samples were supplied with excess oxygen rather than a set amount supplied for batch oxidation, the consumption of antioxidants would become significant. By modifying the model described above to ensure that oxygen is always present for reaction, the rate of lipid hydroperoxide formation and antioxidant consumption can be estimated. MATLAB code for this analysis is give in the Appendix

(see folder 'Chapter 7', 'Oxygen_consumption_BHA_added_no_oxygen_limits.m')

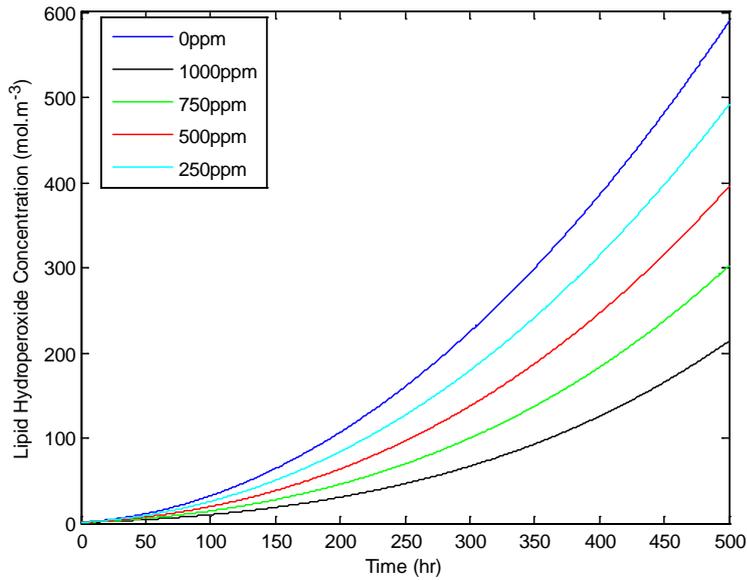


Figure 7.13: Model results for the continuous oxidation of mixed fish oil with different concentrations of BHA antioxidant.

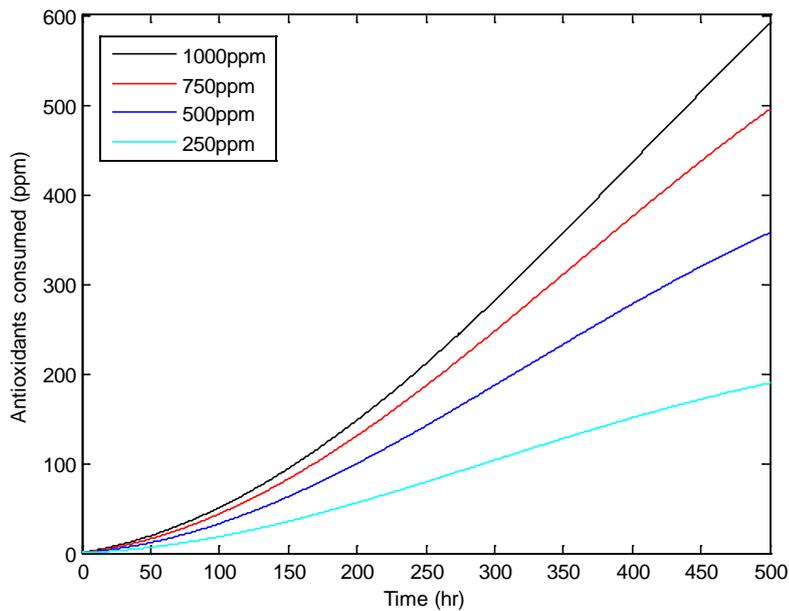


Figure 7.14: Model results for the continuous oxidation of mixed fish oil with different concentrations of BHA antioxidant.

As shown in Figures 7.13 and 7.14, there is a significant reduction in the concentration of antioxidants as lipid oxidation progresses. Furthermore, the effect of increasing the antioxidant concentration, in terms of the concentration of lipid hydroperoxides formed, becomes more significant as the lipid hydroperoxide concentration increases.

7.7 **Conclusions**

The analysis discussions in this chapter have shown that:

- 1) The addition of antioxidants to oils does not change the mechanism of lipid oxidation and the half order model still applies. No halt in oxygen consumption was caused by the addition of primary antioxidants.
- 2) The effects of initiation type reactions seem to be insignificant in that the use of the half order model, developed in Chapters 5 and 6, fitted experimental data well.
- 3) The addition of primary antioxidants decreases the rate of lipid oxidation and can be modelled with a modifier term, $-C_A X$, for the rate constant, that includes the concentration of antioxidant and the effectiveness of the antioxidant
- 4) The change in antioxidant concentration during a batch oxidation is not significant but the change over the course of a products life can be modelled if the yield factor, Y' , is known.

CHAPTER 8
OIL-IN-WATER EMULSIONS

8.0 Introduction

The kinetic descriptions developed in this work so far can only be applied to bulk oils. In many food systems, however, the oil phase is dispersed in an aqueous phase in the form of an emulsion. Because of the importance of this system in the food industry, lipid oxidation in oil-in-water emulsions has received considerable attention. Most of that attention has been focused around the qualitative type of effects of physical properties such as added antioxidants and surfactants have on shelf life (e.g. Faraji & Lindsay, 2004; Osborn & Akoh, 2004; Djordjevic *et al.*, 2004; Osborn & Akoh, 2003; Lethuant *et al.*, 2002; Silvestre *et al.*, 2000; Chaiyasit *et al.*, 2000; Mancuso *et al.*, 1999; Huang *et al.*, 1996).

Despite the amount of literature on oil-in-water emulsions, there are no articles that quantify even simple effects such as changing oil concentrations on oxidation rates. These gaps in literature make it difficult to predict rates of lipid oxidation and, hence, shelf life in food products.

Although not their direct focus, there are a handful of papers that show the effects, on lipid oxidation, of moving from a bulk oil to an oil-in-water emulsion. These papers generally focus on the effect of adding antioxidants to bulk oils and emulsions but in doing so show controls for each group which can be compared with each other. Some, like Lethuat *et al.* (2002) and Medina *et al.* (2009), show results and give discussions which suggest that the formation of an emulsion brings with it an increase in the rate of lipid oxidation. Others, such as Zhao & Hall (2007) and Paraskevopoulou *et al.* (2007), have shown that the rates of lipid oxidation in emulsions are in fact slower than in bulk oils.

The conflicting conclusions are not entirely surprising. To begin with, these papers were not written to show the difference in oxidation rates between bulk oils and oil-in-water emulsions. Secondly, they are different oxidation systems using different oils, oil concentrations, oil droplet sizes and surfactants.

The emulsion system introduces a number of additional considerations initially discussed in Chapter 3. There is a reduction in oil concentration, a potential influence of droplet size, as well as the partitioning of antioxidants and/or pro-oxidants in each phase or the interfacial layer. This chapter will focus on the effects of diluting oils in an aqueous phase and the effects of droplet size.

8.1 The emulsion system

Oil-in-water emulsions, in their simplest form, are small droplets of oil distributed throughout a continuous aqueous phase. Conceptually, this system can be considered as being made up of small, uniformly sized, packets of bulk oil that are distributed evenly in water. If the surfactants that aid emulsion stability are ignored, then the system (depicted in Figure 8.1) will be either well mixed or reacting equally throughout (as a batch system).

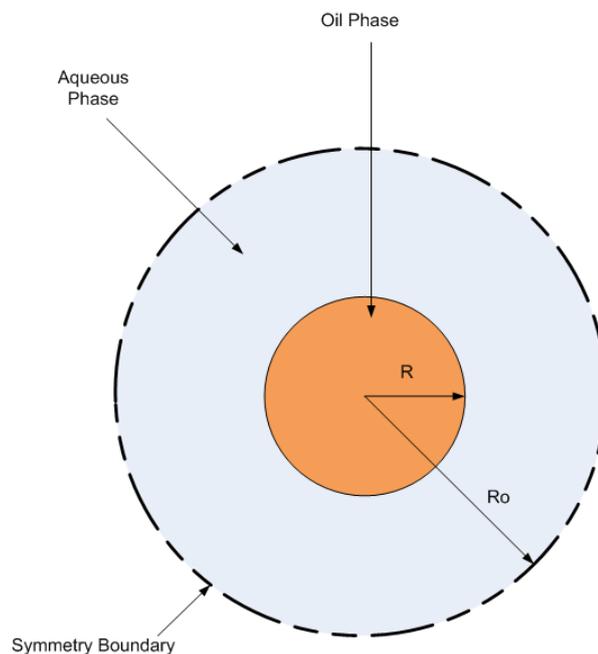


Figure 8.1: Schematic of a spherical droplet of oil surrounded by a sphere of water. Note R_o is half the centre to centre distance between adjacent oil droplets.

The concentration of oil in the emulsion and the droplet diameter will determine the volume of water that can be associated with each droplet of oil. The amount of

oxygen available for reaction can then be determined by the summing the amount of oxygen dissolved in each of the phases.

From geometric basis, the outer radius (R_o) can be calculated from the oil droplet radius (R) for a give oil concentration, C_{oil} in $\text{m}^3_{oil} \cdot \text{m}^{-3}_{emulsion}$.

$$R_o = RC_{oil}^{1/3} \quad \text{Eq. 8.1}$$

The system shown in Figure 8.1 suggests that there is no contact between droplets and that each droplet will react as if it were a bulk oil. The water surrounding the droplet is a sump of oxygen that can be consumed within the droplet. A batch oxidation of an emulsion, therefore, should follow the same trend as bulk oils until the oxygen in the oil has been consumed.

The effect of the oxygen in the water will be determined by the relative rates of oxygen diffusion to and within the oil droplet, and consumption in the droplet via oxidation. If the supply of oxygen from the water to the centre of the droplet is faster than the rate of removal, then it can be assumed that the system would act as if it were well mixed and that there would be no significant concentration gradients. If, however, the supply of oxygen is slow, concentration gradients would be formed. This is analogous to having a bulk oil in a polymer package, but on a much smaller scale.

If, however, the rate of oxygen supply from the water to the surface of the droplet was different to the rate of diffusion of oxygen through the droplet, then a range of different results could be gained. For example, if the rate of oxygen supply to the surface of the droplet is slower than the rate of diffusion within a droplet and the rate of oxygen reaction, then the system will oxidise as a bulk oil until the oxygen in the droplet is completely consumed. From that point on, oxidation will occur at the droplet surface.

These ideas form a hypothesis that is worth exploring. The key to understanding lipid oxidation in emulsions is in the ability to characterise the rates of oxygen supply and

reaction. To try to better understand the mechanism of lipid oxidation in emulsions, an indepth analysis of the relative rates of oxygen supply and reaction is required.

8.2 Limiting factors in oxidation in oil-in-water emulsions

There are a number of factors that change the relative rates of oxygen supply and consumption in emulsions. In the simplified system containing only droplets of oil-in-water, it is easy to see that the diffusivity of oxygen in water and oil as well as the rate of reaction will determine how the system acts. These factors, however, will also determine how the system responds to changes in other physical characteristics such as droplet surface area to volume ratio, SA/V, and the concentration of oil in the emulsion.

Using Thiele's modulus and corresponding effectiveness (Levenspiel, 1999) it is possible to determine the effect of diffusion of reactants on the rate of reaction in an oil droplet. The effectiveness is a factor placed in the rate equation that describes the decrease in reaction rate due to diffusion limitations. If there are no limitations, the effectiveness has a value of unity and shows that there will be no concentration gradients within the oil droplet.

$$(-r) = \frac{\partial C}{\partial t} = kC^n \varepsilon \quad \text{Eq. 8.2}$$

where ε is the effectiveness as defined in Eq. 8.3 and n is the reaction order (1/2 as determined in this work)

$$\varepsilon = \frac{\text{Actual mean reaction rate within a droplet}}{\text{Reaction rate when not slowed by reactant diffusion}} \quad \text{Eq. 8.3}$$

$$\varepsilon = \frac{1}{M_T} \left(\frac{1}{\tanh(3M_T)} - \frac{1}{3M_T} \right) \quad \text{Eq. 8.4}$$

where M_T is the Thiele modulus which can be described as shown in Equation 8.5

$$M_T = L \sqrt{\frac{(n+1)kC^{n-1}}{2De}} \quad \text{Eq. 8.5}$$

For a sphere, the characteristic length, L , is one third the radius of the sphere as shown in Equation 8.6

$$L = \frac{R}{3} \quad \text{Eq. 8.6}$$

By assuming a zero order reaction with respect to lipid hydroperoxide concentration it is possible to significantly reduce the complexity of the analysis. The effects of increasing lipid hydroperoxides with time can be shown by carrying out the analysis for a range of reaction rates. The Thiele modulus was calculated for a typical batch oxidation using a diffusivity of oxygen in oil of $2.72 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ (Del Nobile *et al.*, 2003) a large droplet radius of $50 \text{ }\mu\text{m}$, and a reaction rate equivalent to a six hour batch oxidation (a time typical for a batch oxidation of partially oxidised mixed fish oil at 35°C). The resulting Thiele modulus was 5.9×10^{-3} . Using Equation 8.3, it was shown that the effectiveness was equal to one.

The above analysis shows the result that would be obtained under worst case conditions where the droplet is large and the reaction rate is high. Under less extreme conditions, especially when using less reactive oils such as canola oils, the Thiele modulus would be even smaller. In any case, the actual reaction rate approximates the rate when not slowed by diffusion. The Thiele modulus, however, cannot explain the effects of diffusing reactants through the aqueous phase surrounding the droplet, to the surface of the droplet. To do this, a mathematical model was developed.

8.3 Modelling reaction and diffusion in oil-in-water emulsions

The Thiele analysis suggested that it is unlikely that lipid oxidation in oil droplets would be limited by the supply of oxygen by diffusion from the droplet surface. This approach did not include the effects of oxygen diffusion to the droplet surface or the effects that lipid hydroperoxide diffusion may have on lipid oxidation rates. To better understand these factors, a mathematical model was developed.

As suggested earlier in Section 8.2 and depicted in Figure 8.1, the mathematical model must include two phases.

- 1) The droplet of oil
- 2) The aqueous phase surrounding the droplet

Lipid oxidation in the oil droplet was assumed to occur in the same way that it occurs in a bulk oil. It was also assumed that the interfacial boundary between the oil and aqueous phases had no effect on the rates of oxygen diffusion or reaction and that there was no oxygen consuming components in the aqueous phase. The resulting model, therefore, is shown in Equations 8.7 to 8.16.

For the dissolved oxygen in the oil droplet:

$$\frac{\partial C_{O_{2o}}}{\partial t} = D_o \frac{\partial^2 C_{O_{2o}}}{\partial r^2} + \frac{2D_o}{r} \frac{\partial C_{O_{2o}}}{\partial r} - kC_{LOOH}^{1/2} \quad \text{Eq. 8.7}$$

for $0 < r < R$ and $t > 0$

where r is the radius, D_o is the diffusivity of oxygen in oil, $C_{O_{2o}}$ is the concentration of oxygen in oil, k is the rate constant and C_{LOOH} is the concentration of lipid hydroperoxides.

For lipid hydroperoxides in the oil droplet:

$$\frac{\partial C_{LOOH}}{\partial t} = D_l \frac{\partial^2 C_{LOOH}}{\partial r^2} + \frac{2D_l}{r} \frac{\partial C_{LOOH}}{\partial r} + \frac{k}{Y} C_{LOOH}^{1/2} \quad \text{Eq. 8.8}$$

for $0 < r < R$ and $t > 0$

where D_l is the diffusivity of lipid hydroperoxides in oil and Y is the yield factor to correct for the ratio of oxygen consumed to lipid hydroperoxides formed.

For dissolved oxygen in the water phase:

$$\frac{\partial C_{O_{2w}}}{\partial t} = D_w \frac{\partial^2 C_{O_{2w}}}{\partial r^2} + \frac{2D_w}{r} \frac{\partial C_{O_{2w}}}{\partial r} \quad \text{Eq. 8.9}$$

for $R < r < R_o$ and $t > 0$

where $C_{O_{2w}}$ is the concentration of oxygen in the aqueous phase and D_w is the diffusivity of oxygen in water.

The boundary conditions at the centre of the oil droplet are

$$\frac{\partial C_{O_{2o}}}{\partial r} = \frac{\partial C_{LOOH}}{\partial r} = 0 \quad \text{Eq. 8.10}$$

for $r=0$ and $t > 0$

The boundary condition at the edge of the oil droplet for lipid hydroperoxides assuming no lipid hydroperoxides diffuse into the water phase and can be expressed as:

$$\frac{\partial C_{LOOH}}{\partial t} = 0 \quad \text{Eq. 8.11}$$

for $r=R$ and $t > 0$

An equilibrium exists for oxygen concentrations between the two phases at the oil droplet surface

$$C_{O_{2o}} = N C_{O_{2w}} \quad \text{Eq. 8.12}$$

for $r=R$ and $t > 0$

where N is the factor used to account for the difference in solubility of oxygen in water and oil.

At the outer boundary of the water phase, a symmetry boundary condition was used as oxygen at this point would be equally likely to diffuse in the direction of the oil droplet considered in the model or another droplet adjacent to it.

$$D \frac{\partial C_{O_2w}}{\partial r} = 0 \quad \text{Eq. 8.13}$$

at $r=R_o$ and $t>0$

Initially the oxygen in the water and oil phases were assumed to be uniformly distributed and in equilibrium with each other and the lipid hydroperoxide concentration in the oil was uniform.

$$C_{O_2} = C_{O_{2i}} \quad \text{Eq. 8.14}$$

at $0 \leq r \leq R$ and $t=0$

$$C_{O_2} = N C_{O_{2i}} \quad \text{Eq. 8.15}$$

at $R \leq r \leq R_o$ and $t=0$

$$C_{LOOH} = C_{LOOH_i} \quad \text{Eq. 8.16}$$

at $0 \leq r \leq R$ and $t=0$

Finite difference approximations using the ode23 solver function in MATLAB (R2011b) were used to solve all models shown in the following discussions.

8.3.1 Concentration gradients – an investigation

From the Thiele analysis it was shown that the rates of lipid oxidation are not likely to be subject to oxygen diffusion limitations. The current model, however, includes three new aspects that the Thieles analysis was not able to account for. They are:

- 1) An aqueous phase around the oil droplet
- 2) Surface area to volume ratios (SA/V) of the oil droplet
- 2) Lipid hydroperoxide diffusion

The inclusion of an aqueous phase into the analysis is obviously quite important to any involving an emulsion. The aqueous phase, however, is not likely to have a large impact on the rate of oxygen supply because the diffusivity of oxygen in water is approximately an order of magnitude greater than that in oil.

To quantify the effect the aqueous phase has on the rates of oxygen supply and, therefore, lipid oxidation, a simulation of a batch oxidation of a fish oil-in-water emulsion was run using the model described in Section 8.3. By setting an oil concentration of 20% w/w and a droplet diameter of 20 μm the radius ' R_o ' (Figure 8.1) of water around the droplet was calculated to be 17.1 μm using Equation 8.1. A half order reaction with respect to lipid hydroperoxide concentration (as in Equation 8.6) was used to model the rate of lipid oxidation in the oil droplet. An initial lipid hydroperoxide concentration of $4.97\text{mol}\cdot\text{m}^{-3}$ and rate constant of $4.91\times 10^{-5}\text{mol}^{1/2}\cdot\text{m}^{-3/2}\cdot\text{s}^{-1}$ was estimated by fitting the half order model to batch oxidation results for bulk fish oil (see results in Chapter 6). The diffusivities of oxygen in oil and water were 2.72×10^{-10} and $2.41\times 10^{-9}\text{m}^2\cdot\text{s}^{-1}$ respectively (Del Nobile *et al.*, 2002; Geankoplis, 1993) while the oxygen solubilities of oxygen in oil and water were 3.25 (from this work) and $0.27\text{mol}\cdot\text{m}^{-3}$ (Geankoplis, 1993) respectively. The MATLAB code for this analysis is given in the Appendix (see folder 'Chapter 8', 'twenty_percent_emulsion_investigation.m')

The results of the batch oxidation simulation are shown in Figure 8.2. To avoid the complications that arise as a result of differences in oxygen solubilities in oil and water, the results are presented as oxygen partial pressure. The results suggest that, for any given time during a batch oxidation, there were no significant concentration gradients within the aqueous or oil phases.

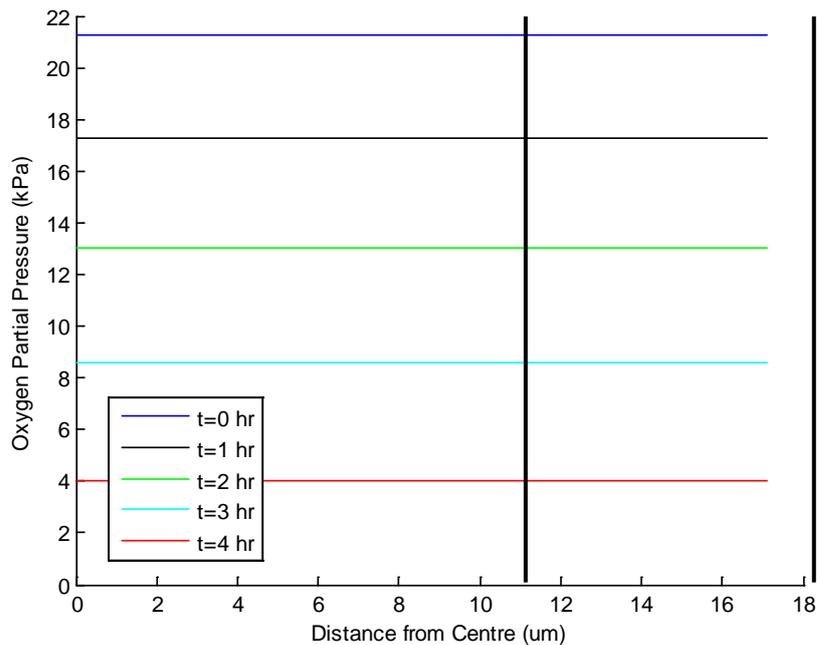


Figure 8.2: Results showing the concentration of oxygen as a function of the distance from the centre of the oil droplet at different time intervals during a batch oxidation of a 20%w/w fish oil in water emulsion. Solid lines represent the droplet surface and outer edge of aqueous phase.

The lack of oxygen concentration gradients suggests that the system is acting uniformly (i.e. diffusion rates are not limiting the overall reaction rate). This trend continues even as the rate of lipid oxidation is increased by a factor of 100 (this would be a reaction rate 100 times that of fish oil – an unrealistically unstable oil).

Since the ability to create oxygen concentration gradients within the system is dependent on the relative rates of oxygen supply and consumption it is worth looking at the other factors that could impact the rates of oxygen supply. The surface area to volume ratios (SA/V) of oil droplets in oil-in-water emulsions have been identified (Section 2.6.5 in Chapter 2) as being capable of altering the rates of oxygen supply (Lethuaut *et al.*, 2002). Conceptually it makes sense that the SA/V will impact the rate at which oxygen can be supplied to the droplet. However, because the rates of oxygen supply are so much higher than consumption rates, the effects of changing the surface area to volume ratio (SA/V) should be negligible.

To investigate the effect SA/V has on lipid oxidation, the model described above was run for 20%w/w fish oil-in-water emulsions using droplet diameters of 1, 5, 10 and 50 μm . The MATLAB code used for this simulation is given in the Appendix (see folder ‘Chapter 8’, ‘twenty_percent_emulsion_droplet_sizes.m’) The results, shown in Figure 8.3, show that there are no significant oxygen concentration gradients in or around the oil droplet even when the SA/V are changed. The results in Figure 8.3 suggest that, when the SA/V ratio is small (i.e. with 50 μm droplets in a 20%w/w oil-in-water emulsion), the concentration of oxygen at the surface is, at most, $1 \times 10^{-4} \text{ mol.m}^{-3}$ greater than that in the centre of the droplet. This difference is just 0.0031% of the initial oxygen concentration (3.25 mol.m^{-3}).

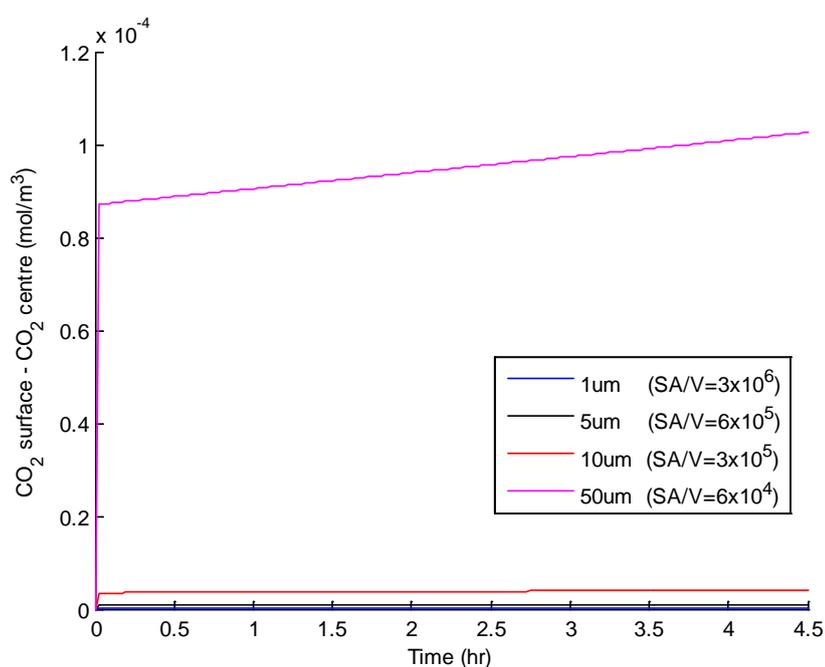


Figure 8.3: Model results showing the difference in oxygen concentration between the centre and surface of the oil droplet during a batch oxidation of 20%w/w mixed fish oil emulsions with different droplet sizes. Note: SA/V for each emulsion is shown in brackets in the key of the plot.

Another way to show the effects of the SA/V ratio on the concentration of oxygen in the system is to focus on the rates of oxygen supply to the droplet of oil. That is, once oxygen is being consumed within the droplet, the oxygen in the aqueous phase moves into the droplet. Any change in the oxygen profile in the oil droplet would alter the rate at which oxygen moves into the droplet from the aqueous phase.

Using the same model used to create Figure 8.3 above, the oxygen flux across the oil droplet surface was calculated. The MATLAB code used in this simulation is given in the Appendix (see folder ‘Chapter 8’, ‘twenty_percent_emulsion_SA_V_ratio.m’). The results, shown in Figure 8.4, show that the SA/V does not have a significant effect on the rates of oxygen movement into the oil droplet from the aqueous phase.

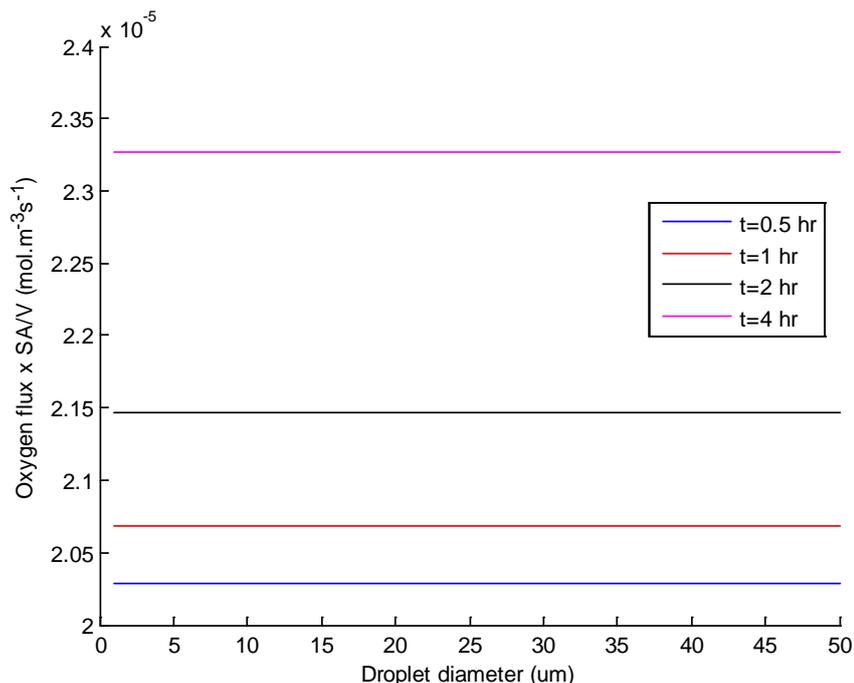


Figure 8.4: Model results showing the amount of oxygen transferred across the droplet surface during a batch oxidation of 20%w/w fish oil-in-water emulsions with different sized droplets (and SA/V's).

The above discussions have shown that it is unlikely that the rates of lipid oxidation in oil-in-water emulsions during a batch oxidation are limited by the supply of oxygen by diffusion or significantly altered by the change in SA/V brought about by changes in the oil droplet sizes. To complete this analysis, an investigation into the potential effects that lipid hydroperoxide diffusion may have on the rates of lipid oxidation is required.

As shown in Chapters 5 and 6, the rates of lipid oxidation are dependent on the concentration of lipid hydroperoxides when oxygen is present. If there were oxygen

concentration gradients within an oil droplet, then the ability for lipid hydroperoxides to migrate to an oxygen rich area and react may have a significant effect on the rates of lipid oxidation. In a 20% w/w fish oil-in-water emulsion, however, it was shown that there were no oxygen concentration gradients. The rates of lipid oxidation would be the same throughout the oil droplet. Furthermore, investigations in Chapters 5 and 6 showed that the rate of lipid oxidation was not affected by the concentration of oxygen as long as there was some oxygen present.

To confirm these ideas, the model presented in Equations 8.7 to 8.16 was used to simulate the effects of lipid hydroperoxide diffusion. The diffusivity of lipid hydroperoxides (D_{LOOH}) in oil was assumed to be the same as that for the self-diffusion of lipids (Metais & Mariette 2003) as no lipid hydroperoxide in oil diffusion coefficients could be found in literature. The model was run twice, once with a D_{LOOH} of zero and once with a D_{LOOH} of $9.7 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$. As before, the model simulated a batch oxidation of a 20% w/w fish oil-in-water emulsion containing $10 \text{ }\mu\text{m}$ droplets of oil. Again, an initial lipid hydroperoxide concentration of $4.97 \text{ mol} \cdot \text{m}^{-3}$ and rate constant of $4.91 \times 10^{-5} \text{ mol}^{1/2} \cdot \text{m}^{-3/2} \cdot \text{s}^{-1}$ was estimated by fitting the half order model to batch oxidation results for bulk fish oil (see results in Chapter 6). The diffusivities of oxygen in oil and water were 2.72×10^{-10} and $2.41 \times 10^{-9} \text{ mol} \cdot \text{m}^{-2}$ respectively (Del Nobile *et al.*, 2002; Geankoplis, 1993) while the oxygen solubility's of oxygen in oil and water were 3.25 and $0.27 \text{ mol} \cdot \text{m}^{-3}$ respectively. The MATLAB code used for this simulation is given in the Appendix (see folder 'Chapter 8', 'twenty_percent_emulsion_LOOH_diffusion.m')

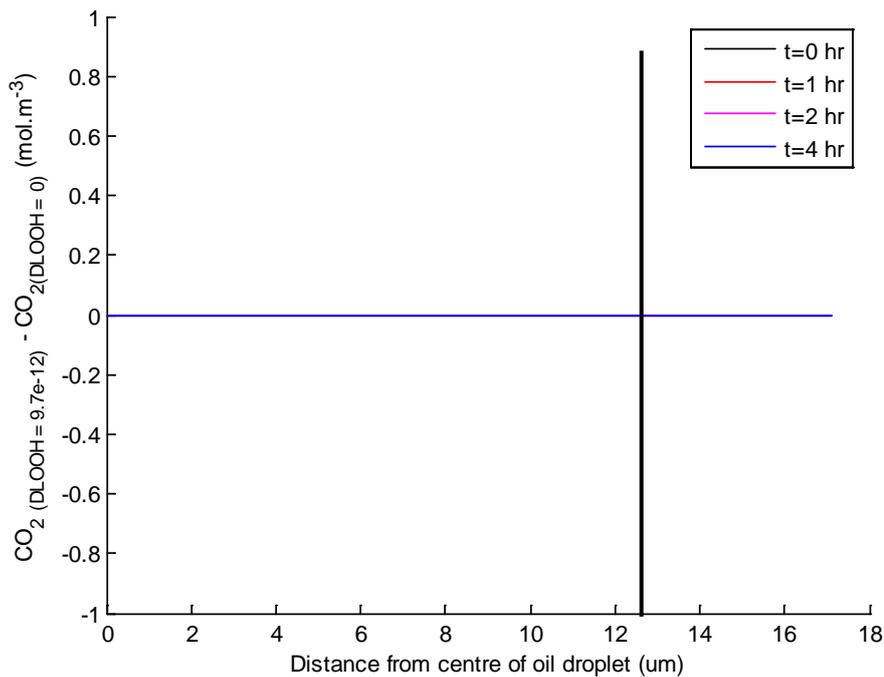


Figure 8.5: Model results showing the difference in oxygen concentrations as a function of the distance from the centre of the droplet for 20%w/w fish oil-in-water emulsions made from different droplet sizes. Note: all lines lay on top of one another. Solid lines represent the droplet surface and outer edge of aqueous phase.

As expected, the simulation of a batch oxidation with and without accounting for lipid hydroperoxide diffusion shows that lipid hydroperoxide diffusion has no effect on the rates of oxygen consumption. Combining the above results it was shown that:

- 1) In an oil-in-water emulsion, locally the supply of oxygen from the surrounding water and within the droplet via diffusion is faster than the consumption of oxygen.
- 2) In a simple system, changes in the SA/V of droplets in the emulsion do not alter the rates of oxygen supply enough to cause oxygen concentration gradients to form within the oxidation system. SA/V may, however, have an impact when surface active additives like antioxidants are present.
- 3) Lipid hydroperoxide diffusion is not important when considering the batch oxidation of oils in oil-in-water emulsions.

8.4 Analytical model of batch oxidation system

Having shown that there are no significant oxygen concentration gradients within an emulsion (uniformly reacting system) and that the SA/V of the droplet has negligible effect on the system, it becomes possible to develop an analytical model to show the effects of changing the oil concentration within the emulsion on the rates of lipid oxidation in a batch oxidation experiment. This model is shown below in Eq. 8.17 to 8.22.

In an emulsion, the rate of oxygen consumption in each phase is given by:

$$\left(V_w \frac{\partial C_{O_{2w}}}{\partial t} + V_{oil} \frac{\partial C_{O_{2oil}}}{\partial t} \right) = -k C_{LOOH}^{1/2} V_{oil} \quad \text{Eq. 8.17}$$

where V_{oil} is the volume of oil, V_w is the volume of water, $C_{O_{2w}}$ is the concentration of oxygen in the water phase and $C_{O_{2oil}}$ is the concentration of oxygen in the oil phase.

Because the system has two phases with differing oxygen solubilities, it is convenient express the concentration in terms of oxygen partial pressure. This can be done using Henry's Law.

$$C_{O_2} = \frac{P_{O_2}}{H} \quad \text{Eq. 8.18}$$

where P_{O_2} is the partial pressure of oxygen and H is the Henry's law coefficient.

In a batch system, the change in lipid hydroperoxide concentration is the result of oxygen consumption and therefore:

$$\frac{C_{LOOH} - C_{LOOH_i}}{Y} = C_{O_{2i}} - C_{O_2} \quad \text{Eq. 8.19}$$

where the subscript i denotes 'initial'

Substituting this into Eq. 8.19 above, and defining \emptyset as the ratio of the volume of oil to the volume of water and defining the Henry's law coefficient for oxygen in water (H_w) and oil (H_{oil}) gives:

$$V_{oil} \left[\frac{1}{H_w \emptyset} + \frac{1}{H_{oil}} \right] \frac{\partial P_{O_2}}{\partial t} = - \frac{k}{\sqrt{Y}} \left[Y C_{LOOH_i} + (P_{O_{2i}} - P_{O_2}) \left[\frac{1}{H_w \emptyset} + \frac{1}{H_{oil}} \right] \right]^{1/2} V_{oil} \quad \text{Eq. 8.20}$$

Which after integration gives:

$$P_{O_2} = P_{O_{2i}} + \frac{Y C_{LOOH_i}}{B} - \frac{\left[\frac{kt}{2\sqrt{Y}} + (Y C_{LOOH_i})^{1/2} \right]^2}{B} \quad \text{Eq. 8.21}$$

Where

$$B = \left[\frac{1}{H_w \emptyset} + \frac{1}{H_{oil}} \right] \quad \text{Eq. 8.22}$$

Using the new analytical solution, it is possible to investigate how oxygen consumption rates are affected by changing the concentration of oil in the emulsions during a batch oxidation. The following analytical solutions presented were compiled in MATLAB and can be viewed in the Appendix (see folder 'Chapter 8', files 'Analytical_consumption_rate_vs_LOOH.m', 'Analytical_consumption_rate_vs_LOOH_2.m', 'Analytical_LOOH_vs_time.m', and 'Analytical_oxygen_consumption.m')

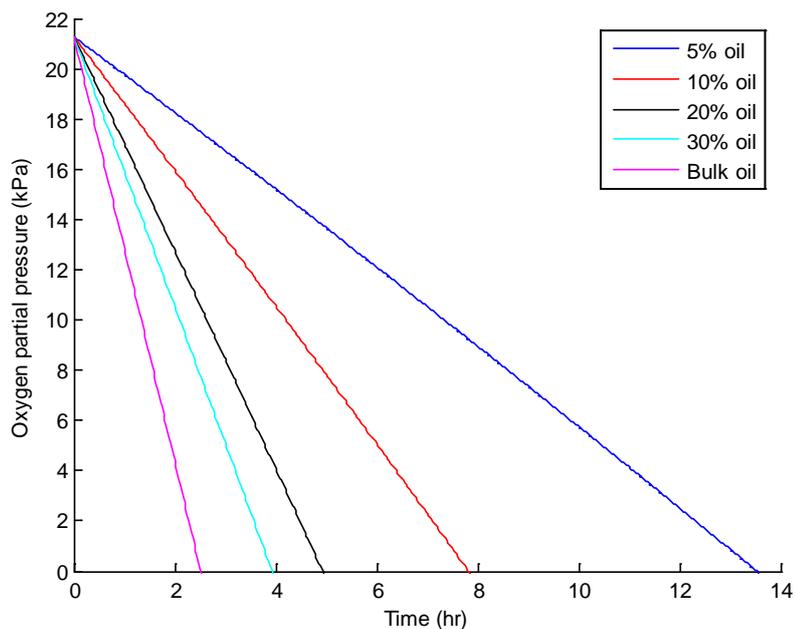


Figure 8.6: Results of simulations of oil-in-water emulsions made from the same oil (i.e. with the same reactivity) but with different oil concentrations.

Figure 8.6 above shows the results of altering the concentration of oil in the oil-in-water emulsion. The results show that the rate of oxygen consumption is inversely proportional to the concentration of oil in the emulsion.

The results shown in Figure 8.6 follow what is expected in that the oxygen in the water phase (even though oxygen is less soluble in water than oil) is a sump that can be consumed by lipid oxidation reactions. The effective oxygen concentration per unit of oil is higher in dilute emulsions than in more concentrated emulsions. The extent of reaction (i.e. the increase in lipid hydroperoxide concentration) of oils in the dilute emulsions will, therefore, be higher than that of more concentrated emulsions, for a batch oxidation.

The extent of reaction is also responsible for the increase in curvature shown in Figure 8.6 (difficult to see but more evident in lower concentrations). Dilute emulsions experience a greater change in oxygen consumption rate than those more concentrated. This is best shown in Table 8.1. Since there is a higher effective oxygen concentration in dilute emulsions, the increase in lipid hydroperoxides will be greater than in a

concentrated emulsion (for a batch oxidation) and hence the increase in rate of reaction during a batch reaction will be greater.

Table 8.1: Model results showing the change in lipid hydroperoxide concentration that can be expected after a batch oxidation of a fish oil-in-water emulsions containing different concentrations of fish oil.

Concentration of oil in emulsion (%w/w)	Moles of oxygen in oil (m^{-3} total vol)	Moles of oxygen in water (m^{-3} total vol)	Final lipid hydroperoxide concentration in emulsion (mol.m^{-3})	Final lipid hydroperoxide concentration in oil fraction (mol.m^{-3})
Bulk Oil	3.25	0.00	0.64	0.64
5	0.16	0.26	0.08	1.66
10	0.33	0.24	0.11	1.13
20	0.65	0.22	0.17	0.86
30	0.98	0.19	0.23	0.77
40	1.30	0.16	0.29	0.73

Less obvious (than the data shown in Table 8.1) is the effects of change in lipid hydroperoxides for each droplet of oil in emulsions with different oil concentrations. As the amount of oil in an emulsion decreases, the ratio of oxygen to oil increases significantly. A batch oxidation of a dilute emulsion should result in a greater increase in lipid hydroperoxide concentration compared with a batch oxidation of a concentrated emulsion. This effect is best shown in Figure 8.7.

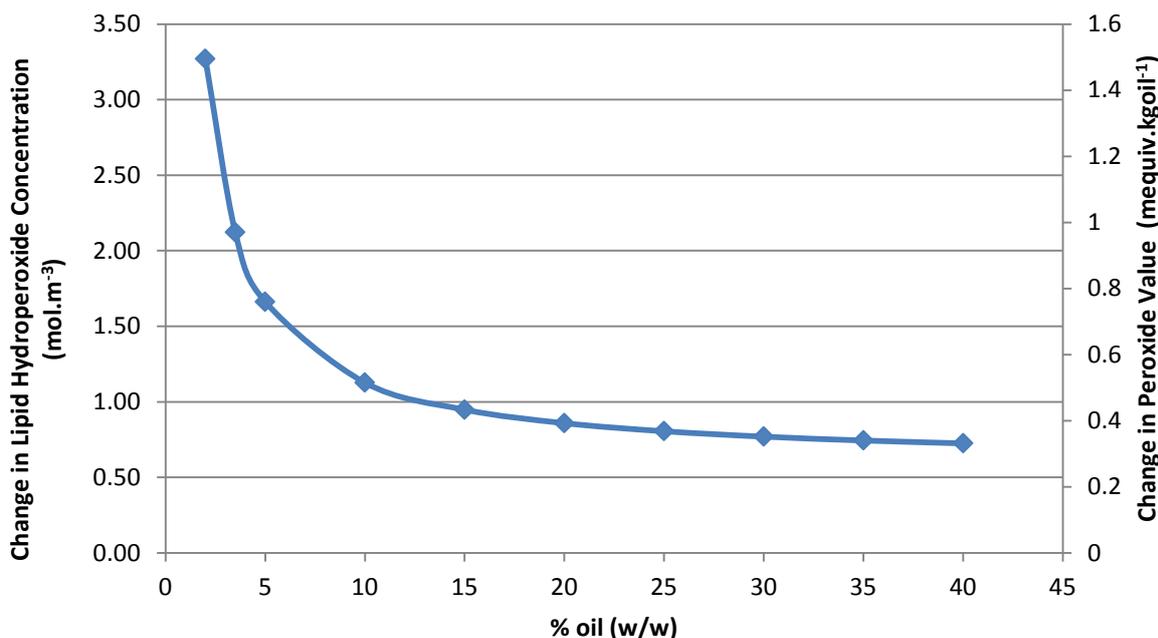


Figure 8.7: Figure shows the relationship between the change in lipid hydroperoxide concentration and oil concentration that can be expected for a single batch oxidation for oil-in-water emulsions.

The results in Figure 8.7 above also highlight what would be expected when oil is diluted by the formation of emulsions in real food products. That is, a greater rate of lipid hydroperoxide accumulation would occur for an emulsion with a lower oil content. The question is whether the increase in lipid hydroperoxide concentration would be great enough to bring with it a significant change in the flavour/aroma of the product. This will form part of the discussions in the next chapter.

While Figure 8.7 shows that the increase in lipid hydroperoxides expected for a single batch oxidation is greater in lower oil content emulsions, it does not show how the overall oxygen consumption rate is affected by the lipid hydroperoxide concentration. Figure 8.8 shows the relationship between the lipid hydroperoxide concentration and oxygen consumption rate in the system used to create in Figure 8.7.

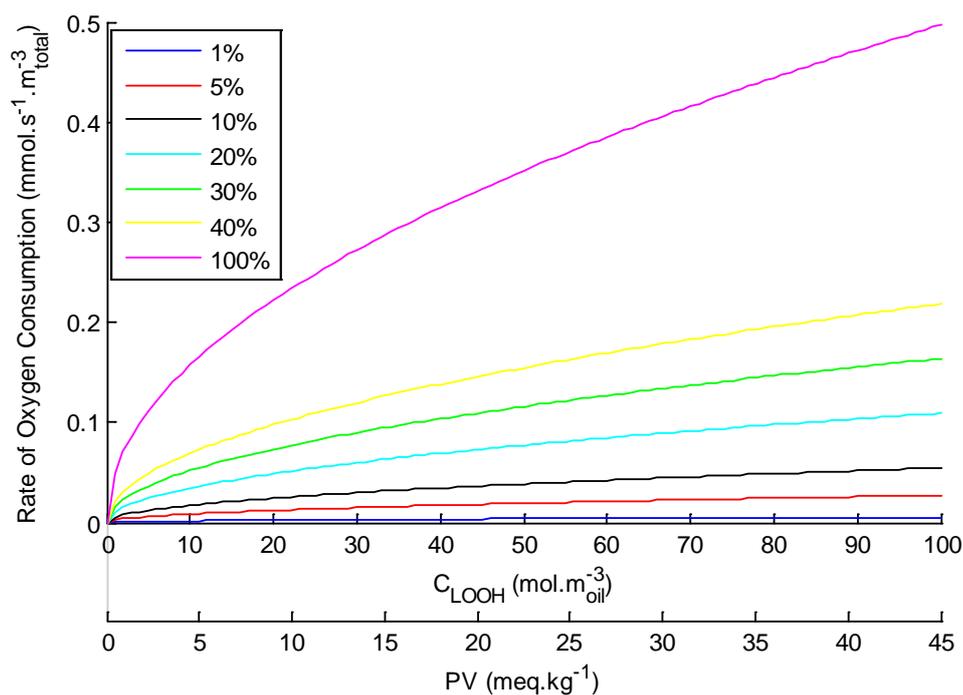


Figure 8.8: Figure shows the rate of oxygen consumption that can be expected as a function of the lipid hydroperoxide concentration in the oil/emulsion for oil-in-water emulsions containing different concentrations of oils.

Here it is quite easy to see how reducing the concentration of oil in an emulsion results in a lower overall oxygen consumption rate when dealing with the entire emulsion system (not oxidation limited). The oil in each of the emulsions, however, are oxidising at the same rate. That is, the oxygen consumption rate and, therefore, rate of lipid hydroperoxide formation, is still governed by the half order model and lie on a continuum given in Figure 8.9. By extrapolating these ideas it is possible to show that a well-mixed system, where oxygen is readily available, will oxidise at a rate independent of the oil content. That is, the rate of oxygen consumption by the oil is dependent on the lipid hydroperoxide concentration and not the concentration of oil.

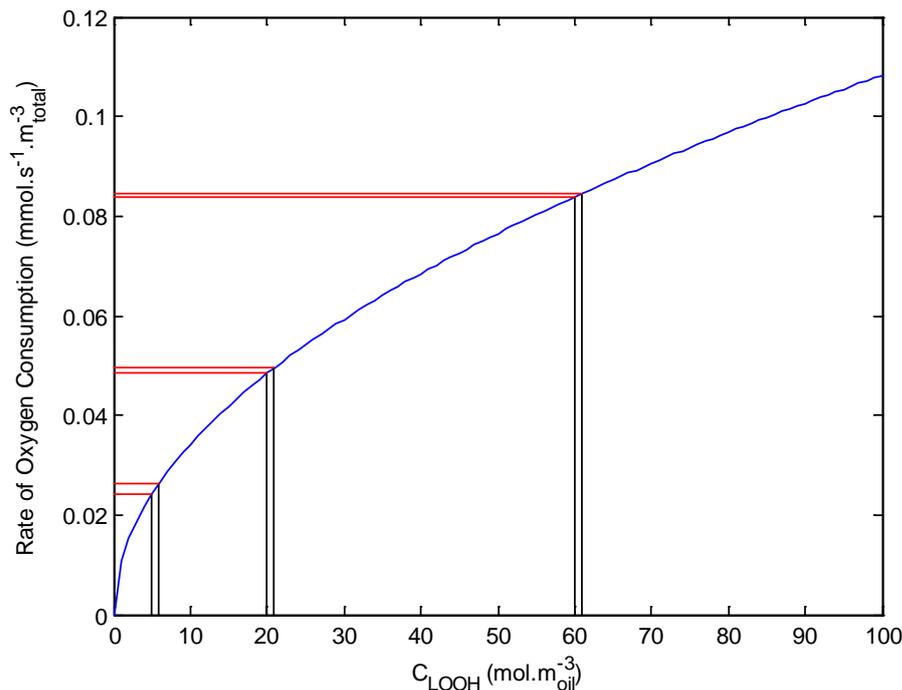


Figure 8.9: Figure shows the oxidation continuum for a 20% w/w fish oil-in-water emulsion. That is, the rate of oxygen consumption during a batch oxidation lies on the continuum and its position is described by the initial lipid hydroperoxide concentration.

The continuum described above can also be used to describe the expected increase in lipid hydroperoxide concentration for a batch oxidation. By using the analytical model given in Eq. 8.21, the continuum for oxygen consumption in a 20% w/w oil-in-water emulsion was drawn. The increase in lipid hydroperoxide concentration over a single batch oxidation, pairs of vertical lines in Figure 8.9, was calculated by applying the yield factor (Y) from Chapter 6 to account for the conversion of oxygen in the system to lipid hydroperoxides. From this, the resulting increase in oxidation rate was estimated. The results of this estimation, as depicted in Figure 8.9, show that there is a larger increase in oxidation rate during a batch oxidation when the initial lipid hydroperoxide concentration is low compared to when it is high.

Figure 8.11 clearly shows that the magnitude of the increase in oxygen consumption rate, when looking at the system as a whole, decreases as the extent of reaction increases. This suggests that the initial stages of oxidation, when the rate of oxidation is low, brings with it the greatest increase in lipid oxidation rate over a single batch oxidation. The increase in reaction rate described in Figure 8.9 is based on an oxygen

consumed basis. If, however, the analysis is based on the increase in rate over a given time, different conclusions would be drawn.

Initially, the rate of oxygen consumption is low. It takes some time for the increase in lipid hydroperoxide concentration, and corresponding increase in reaction rate, to occur. Conversely, when the extent of oxidation is greater, the rate of oxygen consumption is higher and the time required to achieve the same increase in lipid hydroperoxide concentration is much lower. To show this, a plot was constructed by simulating the production of lipid hydroperoxides in a 20% w/w oil-in-water emulsion when oxygen is not a limiting factor, available in excess at all times. The simulation was carried out using Equation 8.21. The inputs for this simulation included a rate constant, k , of $4.91 \times 10^{-5} \text{ mol}^{1/2} \text{ m}^{-3/2} \text{ s}^{-1}$, a yield factor, Y , of 5.04 (both calculated from mixed fish oil experiments in Chapter 6), an oxygen solubility of $3.25 \text{ mol} \cdot \text{m}^{-3}$ for oil and an oxygen solubility of $0.27 \text{ mol} \cdot \text{m}^{-3}$ for water. The results of this simulation are shown in Figure 8.10.

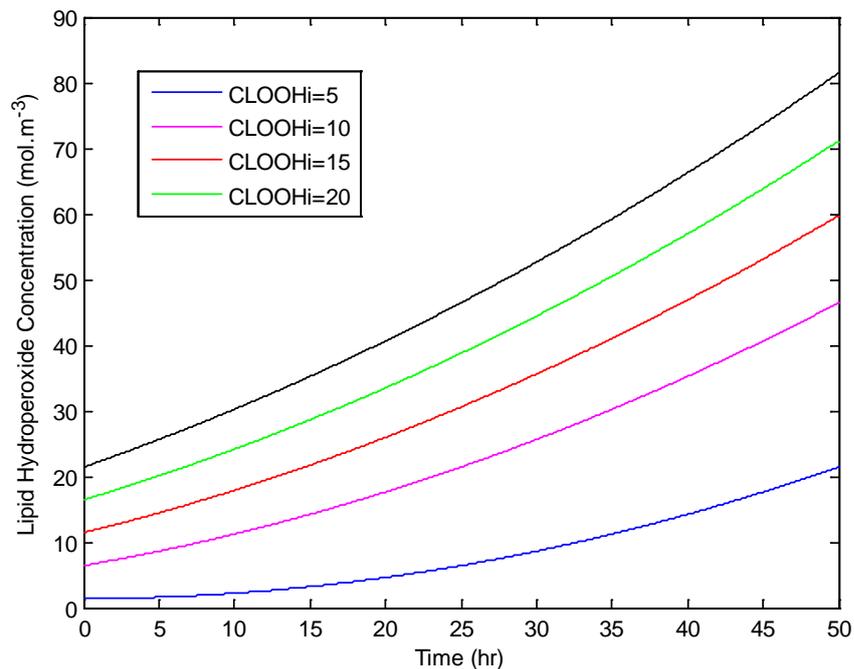


Figure 8.10: Oxidation continuum for 20% w/w oil-in-water emulsions with different initial lipid hydroperoxide concentrations with excess oxygen available.

Figure 8.10 shows how the rate of reaction increases with time when oxygen is not limiting. The slope of any of the lines at any point in time is the rate of lipid

hydroperoxide formation and/or the inverse of the rate of oxygen consumption (after accounting for the yield factor). The curvature of the lines indicates that the rate of change in reaction rate is not constant. By picking two lipid hydroperoxide concentrations and looking at the time required to move between them it is possible to understand the relationship between Figures 8.9 and 8.10. For example, the time required to move from 10 to 20 mol.m⁻³ lipid hydroperoxides, is greater than that required to go from 20 to 30 mol.m⁻³ no matter what starting lipid hydroperoxide concentration.

8.5 Comparisons and validation against experimental data

So far the analysis has been focused on bulk oil batch oxidation models, modified to simulate oxidation oil in water emulsions. The assumptions made to create the emulsion models need some validation. Unfortunately, not all of the discussions above can be directly validated. For example, the analysis suggesting the concentration of oxygen is the same throughout a droplet cannot be verified by measuring oxygen concentrations in oil droplets because of the size of the droplet relative to the dissolved oxygen probe used in experiments. Furthermore, the flux estimates given above cannot be measured for the same reasons. Due to the oxidation rig design (Chapter 4) it is possible, however, to validate the overall effect of oil concentration in an emulsion on oxygen consumption rates.

8.5.1 Dilution of bulk oils in emulsions

The theory developed earlier suggests that the dilution of a bulk oil by the formation of an oil-in-water emulsion should bring with it an increase in the time required to complete a batch oxidation. It was suggested that the quantity of oxygen available per mole of oil is greater for an oil-in-water emulsion than in a bulk oil.

To begin the validation process, batch oxidations of mixed fish oil-in-water emulsions were created. Firstly, the mixed fish oil samples (Bakels Ltd, NZ) were heated to 50°C. The required volume of reverse osmosis water was heated to 50°C before

2% w/w lecithin (Heinz Wattie's Ltd, NZ) was slowly mixed into the water using a kitchen stick blender. The oil and water/lecithin samples were then added together in the appropriate quantities and blended together using a stick blender to create a crude emulsion. The crude emulsions were then homogenised using one of two methods. The first method involved passing the crude emulsion through a two stage/valve high pressure homogeniser. Both stages were set to 200 kPa. The second method involved the use of a Microfluidiser (Microfluidics Ltd, Massachusetts, USA) operating at 1.4 MPa. Unless otherwise stated, emulsions were either passed through the two stage homogeniser once or through the Microfluidiser three times. Once the emulsions were created, they were either heated or cooled to the required oxidation temperature before a batch oxidation was conducted according to the methods described in Chapter 4.

As expected, the results of initial 45°C batch oxidations of mixed fish oil-in-water emulsions created using a two stage homogeniser (Figure 8.11) showed the same trends as those given in Figure 8.6. That is, the rate of oxygen consumption was a function of the oil concentration. The results in Figure 8.11 also suggest that the oxidation rig is suitable for use with oil-in-water emulsions and that the data gained from batch oxidations are reliable and repeatable as duplicate experiments gave very similar oxygen consumption profiles despite the droplet size distribution of each emulsion not being controlled or measured.

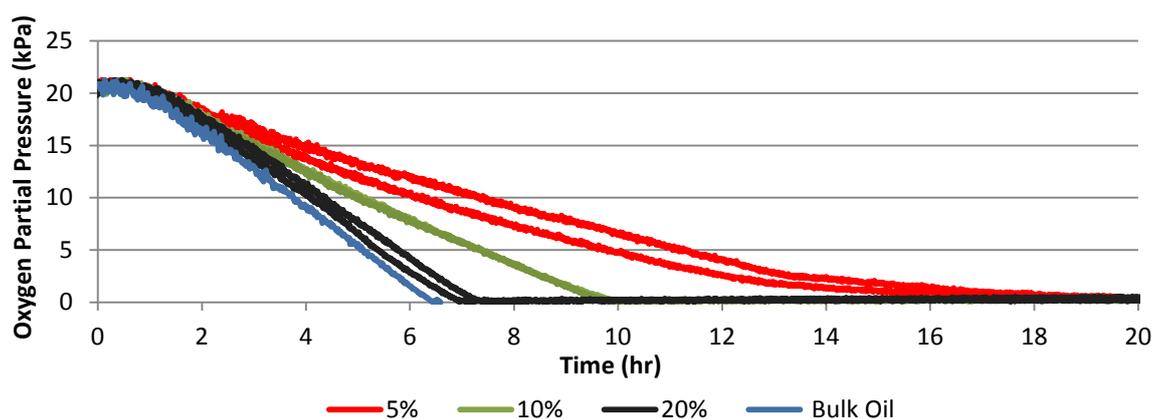


Figure 8.11: Results of conducting batch oxidation experiments at 45°C on fish oil-in-water emulsions with different oil concentrations. Duplicate of the 5 and 20% emulsions were conducted.

The samples in this experiment, however, do not clearly show the effects of the extent of reaction that is achieved during a single batch oxidation of fish oil emulsions. That is, the increase in extent of reaction per batch oxidation should be greater for more dilute emulsions than for bulk oils as the amount of oxygen available for reaction per mole of oils is greater.

To gain a closer look at the change in reaction rate during a batch oxidation, two mixed fish oil (Bakels Ltd, NZ) in water emulsions were oxidised at a lower temperature of 35 °C. In this case, the two emulsions (5 and 40%w/w) were created by passing a crude emulsion through a Microfluidiser. By using a lower reaction temperature, it was much easier to see the change in reaction rate from the beginning to the end of a batch oxidation. The results of the two batch oxidations are shown below in Figure 8.12.

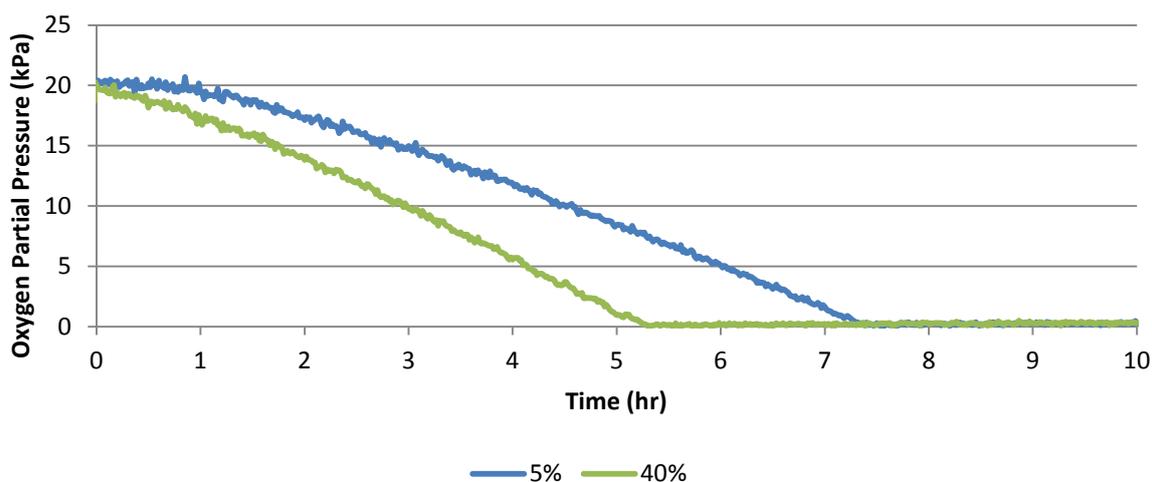


Figure 8.12: Results of batch oxidations at 35°C showing the differences in oxygen consumption rates of two oil in water emulsions with different oil concentrations.

From Figure 8.12 it can be seen that increase in oxygen consumption rate (slope of lines in Figure 8.12) from the beginning to the end of the batch oxidation for the 5% emulsion is much greater than for the 40% emulsion. Since the rate of oxygen consumption has been shown to be proportional to the concentration of lipid hydroperoxides, it makes sense that the change in lipid hydroperoxide concentration for one batch oxidation is greater for the 5% emulsion than for the 40% emulsion.

Furthermore, the amount of oxygen available for reaction per mole of lipid is greater for the emulsion with the lower oil content.

As shown in Equation 8.17, the rate of oxygen consumption is a function of the lipid hydroperoxide concentration. The increase in lipid hydroperoxide concentration should, therefore, be a function of the change in rate of oxygen consumption. This means that Equation 8.23 should apply.

$$\left[\frac{1}{H_w \emptyset} + \frac{1}{H_{oil}} \right] \frac{\partial P_{O_2}}{\partial t} = -k C_{LOOH}^{1/2} \quad \text{Eq. 8.23}$$

$$\frac{\frac{\partial P_{O_2}}{\partial t} \big|_{t=0}}{\frac{\partial P_{O_2}}{\partial t} \big|_{t=t}} = \frac{C_{LOOH_{t=0}}^{1/2}}{C_{LOOH_{t=t}}^{1/2}} \quad \text{Eq. 8.24}$$

where $t=0$ and $t=t$ indicate the beginning and end of a batch oxidation

The lipid hydroperoxide concentration at the end of the batch oxidation has been shown to be a function of the oxygen consumed. The amount of oxygen available for reaction and the change in lipid hydroperoxide concentration expected for a given emulsion during a batch oxidation is shown in Table 8.1. Applying the information in Table 8.1, it can be shown that Equation 8.25 applies.

$$C_{LOOH_{t=t}} = C_{LOOH_{t=0}} + \frac{(P_{O_2_{t=0}} - P_{O_2_{t=t}}) \left[\frac{1}{H_w \emptyset} + \frac{1}{H_{oil}} \right]}{Y} = C_{LOOH_{t=0}} + \frac{E}{Y} \quad \text{Eq. 8.25}$$

where E is the oxygen consumed during a batch oxidation.

Combining Equations 8.24 and 8.25:

$$\frac{\frac{\partial P_{O_2}}{\partial t} \big|_{t=0}}{\frac{\partial P_{O_2}}{\partial t} \big|_{t=t}} = \frac{C_{LOOH_{t=0}}^{1/2}}{\left(C_{LOOH_{t=0}} + \frac{E}{Y} \right)^{1/2}} \quad \text{Eq. 8.26}$$

For 5 and 40% w/w oil-in-water emulsions the change in lipid hydroperoxide concentration during a batch oxidation was shown to be 1.66 and 0.73 mol.m⁻³

respectively (Table 8.1). By calculating the initial and final rates of oxygen consumption for the experiments shown in Figure 8.12, the initial lipid hydroperoxide concentrations for each emulsion was calculated using Equation 8.26.

Table 8.2: Results of measuring the initial and final oxygen consumption rates from data shown in Figure 8.13 as well as the calculated initial lipid hydroperoxide concentrations.

Concentration of oil (%w/w)	Rate ($\text{Pa}\cdot\text{s}^{-1} \times 10^4$)		Initial Lipid Hydroperoxide Concentration ($\text{mol}\cdot\text{m}^{-3}$)
	Initial (t=0)	Final (t=t)	
5	3.66	9.89	0.26
40	5.79	12.33	0.21

The initial lipid hydroperoxide concentrations were then used as inputs to fit the half order model shown in Equation 8.21. The fit was carried out using an oil fraction of 0.05 and 0.4 (for each emulsion) and a Henry's law coefficient of 78788 and 6545.5 $\text{Pa}\cdot\text{m}^3\text{mol}^{-1}$ for oxygen in water and oil respectively. The SOLVER function in Microsoft EXCEL was used to minimise the square differences between the model and experimental data by changing the rate constant (which was the same for both emulsions). The resulting fit is shown in Figure 8.13.

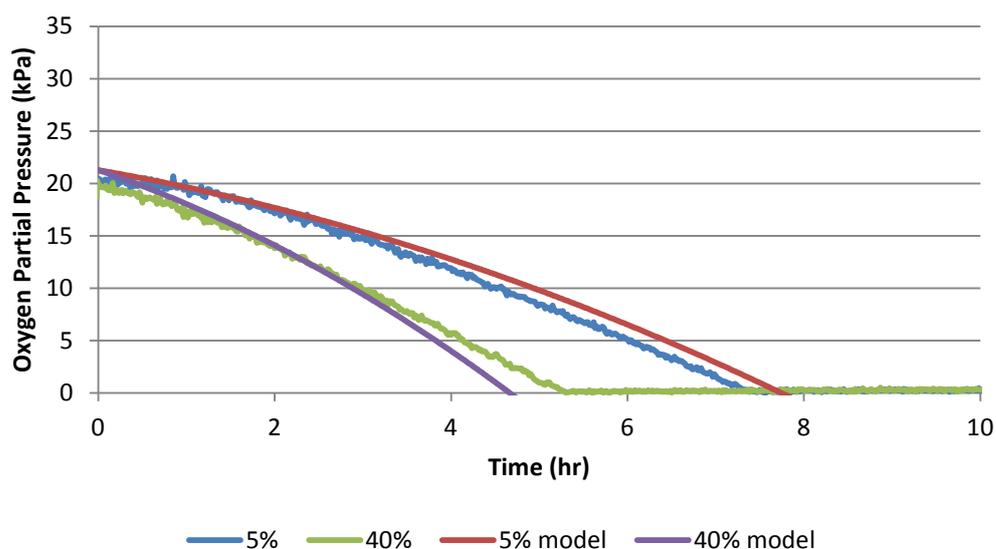


Figure 8.13: Figure shows the fit gained by applying the half order model (Equation 8.16) to experimental batch oxidation data for a 5% and a 40% w/w fish oil-in-water emulsions at 35°C.

According to the results shown in Figure 8.13, the half order model is able to explain the results gained from batch oxidations of mixed fish oil.

8.5.2 Droplet surface area to volume ratios

According to the modelling above, the changes to the droplet surface area to volume ratio should not change the rate of oxygen consumption in an emulsion. Simply put, the rates of oxygen supply from the surface to the centre of the droplet are likely to always be greater than the rate of oxygen consumption. This was assumed when fitting the half order model to experimental data in the previous section. The resulting fit was good despite there being no allowance for any differences in the droplet SA/V for the different emulsions.

For completeness, however, it was decided that carrying out a test to explicitly show that the droplet SA/V has no impact was needed. To show this, two emulsions with different droplet size distributions were created by first making a large crude emulsion (method given in Section 8.4.1) before passing the samples through a Microfluidiser at different pressures and a different number of times. The first emulsion was created by passing the a sample of crude emulsion through the Microfluidiser once at a pressure of 690 bar while the second emulsion was created by passing another crude emulsion sample through the Microfluidiser five times at a pressure 1380 bar. Both samples were then cooled/heated to the correct temperature (35°C) before a batch oxidation was conducted (see Chapter 4).

The droplet size distributions were measured using a Mastersizer (Malvern Instruments, Worcestershire, UK). The results of this measurement are shown in Figure 8.14 below. Using the Sauter mean diameters for the distributions, the SA/V for each of the distributions were calculated and compared with each other. It was found that the SA/V of the emulsion with larger particles was 1.68 times greater than the emulsion with smaller particles.

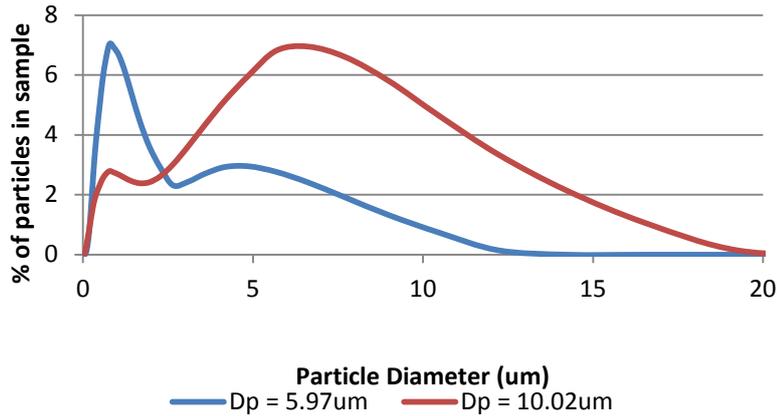


Figure 8.14: Droplet size distributions of two 20%w/w oil-in-water emulsions (1% lecithin) as measured by the Malvern Mastersizer. The blue line represents an emulsion created by one pass through a Microfluidiser at 690bar while the red line represents the emulsion created by three passes through the Microfluidiser at 1380bar.

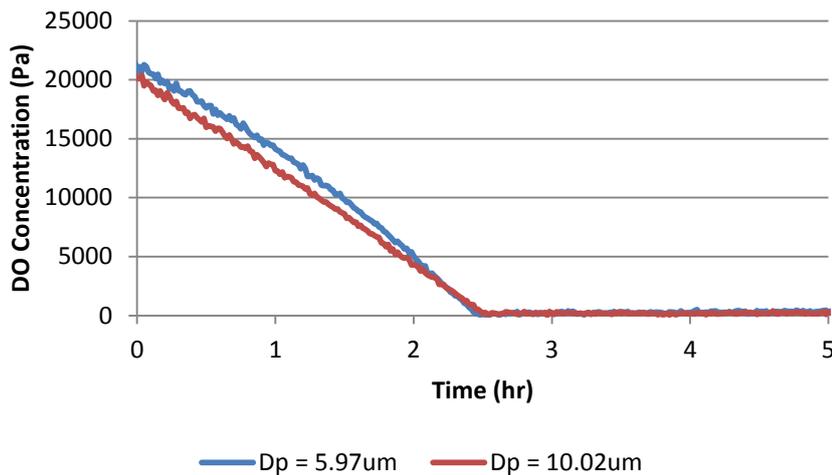


Figure 8.15: Results of batch oxidations of the two 20%w/w fish oil-in-water emulsions with different droplet size distributions (shown in Figure 8.14)

The results from batch oxidations of the two emulsions, shown in Figure 8.15, suggest that the difference in drop size distributions, average droplet diameters and SA/V's have no impact on the rates of oxygen consumption. This supports the findings of earlier modelling based investigations.

8.6 Conclusions

The above modelling provides information that cannot be found in literature. It shows that:

- 1) Oxygen concentration gradients within and around the oil droplets do not exist as the rate of oxygen supply was greater than the rates of oxygen consumption for fish oil-in-water emulsions. The reaction system, therefore, reacted as if it were uniform.
- 2) The increase in the rate of lipid oxidation for a given change in lipid hydroperoxide concentration is most significant when the lipid hydroperoxide concentration is low as the aqueous phase essentially acted as a sump of oxygen.
- 3) The SA/V of an oil droplet is unlikely to have any significant effect on the rates of lipid oxidation
- 4) The half order model with respect to lipid hydroperoxide concentration applies to emulsion systems when the extra sump of oxygen in the aqueous phase is accounted for.

CHAPTER 9
RATES OF OXYGEN SUPPLY AND
REACTION

9.0 Introduction

The work presented in previous chapters showed the development and validation of models that predict the rates of lipid oxidation in homogeneously reacting lipid oxidation systems when oxygen supply is not limiting. The next step in the process of characterising lipid oxidation rates is to extend the previous models to systems where homogeneity may not exist due to oxygen or lipid hydroperoxide gradients within oxidation systems. Systems where the supply of oxygen is limited are common within the food industry and are likely to behave differently to the systems analysed in the previous chapters.

This chapter begins with an analysis of two long-term shelf life experiments and models reported in the literature. The discussions highlight the shortfalls of the experimental approach that is commonly used and shows the need for a more in-depth analysis of the rate limiting steps in such oxidation system before designing experiments. In this chapter, a modelling approach was taken to explore the likely effects of competing rates of oxygen supply and consumption as well as the effect of lipid hydroperoxide diffusion. The aim of this chapter was to identify interactions and oxidation behaviours for experimental validation in future work. This chapter is, therefore, the pre-requisite analysis needed before future experimental work can be conducted.

9.1 Existing models of coupled reaction and diffusion

Of the models available in literature, the Del Nobile and Kanavouras & Coutelieris models were identified as being closest to those required for this thesis. The models focussed on predicting rates of lipid oxidation during long term storage of olive oils within different packaging systems. The reaction schemes that formed the basis of the models were partially presented in Sections 5.2.2 and 5.2.3 of Chapter 5. The application of the models to long term storage of olive oils, however, was not discussed. This will be the focus of this section.

According to both the Del Nobile and Kanavouras & Coutelieris models (Coutelieris & Kanavouras, 2006; Kanavouras & Coutelieris, 2006; Kanavouras *et al.*, 2004; Del Nobile *et al.*, 2003a; Del Nobile *et al.*, 2003b), the rate of oxidation is a function of both the extent of oxidation and the oxygen concentration. Del Nobile *et al.* (2003a) chose to model the extent of oxidation using the lipid hydroperoxide concentration while Kanavouras & Coutelieris (2005, 2006) chose to model it using hexanal concentration under the assumption that hexanal was formed from lipid hydroperoxides directly. Simplified versions of the Del Nobile and Kanavouras & Coutelieris models were presented in Sections 5.2.2 and 5.2.3 of Chapter 5. The full models as given by Del Nobile *et al.* (2003a, 2003b) and Kanavouras & Coutelieris (2005) are:

Del Nobile Model:

$$\frac{\partial C_{LOOH}}{\partial t} = R_F - R_D \quad \text{Eq. 9.1}$$

$$R_F = (K_1' + K_2' C_{LOOH}) \left(\frac{P_{O_2}}{K_3' + K_4' P_{O_2}} \right) \quad \text{Eq. 9.2}$$

$$R_D = K_5 C_{LOOH} \quad \text{Eq. 9.3}$$

$$\frac{\partial C_{O_2}}{\partial t} = \gamma \left[\frac{D_{O_2}}{r} \cdot \frac{\partial}{\partial r} \cdot \left(r \frac{\partial C_{O_2}}{\partial r} \right) \right] - R_F \quad \text{Eq. 9.4}$$

where C_{LOOH} is the concentration of lipid hydroperoxides, P_{O_2} is the partial pressure of oxygen, K_1' , K_2' , K_3' , K_4' and K_5 are model constants, C_{O_2} is the concentration of oxygen, D_{O_2} is the diffusivity of oxygen in olive oil, r is the distance from the centre of the package, and γ is a factor to turn the oxygen supply term on and off depending on the packaging type ($\gamma = 0$ for glass and $\gamma = 1$ for polymer packaging)

Kanavouras & Coutelieris Model:

$$\frac{\partial C_{O_2}}{\partial t} = D_{O_2} \frac{\partial^2 C_{O_2}}{\partial x^2} - k_a C_{O_2} - k_c C_{O_2} \left(C_{O_2}^{\infty, in} - C_{O_2} - C_{hexanal} \right) \quad \text{Eq. 9.5}$$

Eq. 9.6

$$\frac{\partial C_{hexanal}}{\partial t} = D_{hexanal} \frac{\partial^2 C_{hexanal}}{\partial x^2} + k_a C_{O_2} + k_c C_{O_2} (C_{O_2}^{\infty, in} - C_{O_2} - C_{hexanal})$$

where x is the distance from the packaging surface, $C_{O_2}^{\infty, in}$ is the initial oxygen concentration in the oil sample, $C_{hexanal}$ is the hexanal concentration, and k_a and k_c are model constants

A fundamental difference between the Del Nobile and Kanavouras & Coutelieris models is the inclusion of lipid oxidation product diffusion in the Kanavouras & Coutelieris model. The inclusion of hexanal diffusion, however, was likely to have been driven by the effects of hexanal loss to the environment rather than its effect as a reactant in lipid oxidation. This is fair as hexanal is not likely to take part in lipid oxidation reactions to a significant degree but, as hexanal was chosen as a direct substitute for lipid hydroperoxides which are important reactants, the effect of lipid oxidation product diffusion is likely to be far more significant than is presented by Kanavouras & Coutelieris (2006, 2005).

The supply of oxygen by diffusion through the packaging was modelled in both studies and is presented in Equations 9.7 and 9.9 for the Del Nobile and Kanavouras & Coutelieris models respectively. In the case of the Del Nobile model, lipid hydroperoxide transport across the packaging was, quite rightly, not allowed. Hexanal absorption and transport across the packaging material in the Kanavouras & Coutelieris model is shown in Equation 9.10.

Del Nobile Model:

$$\frac{\partial C_{O_2}^{poly}}{\partial t} = \frac{D_{O_2}^{poly}}{r} \cdot \frac{\partial}{\partial r} \left(r \cdot \frac{C_{O_2}^{poly}}{dr} \right) \quad \text{Eq. 9.7}$$

$$\frac{\partial C_{LOOH}}{\partial t} = 0 \quad \text{Eq. 9.8}$$

where $C_{O_2}^{poly}$ is the concentration of oxygen in the polymer packaging at position r and $D_{O_2}^{poly}$ is the diffusivity of oxygen in the polymer packaging.

Kanavouras & Coutelieris Model:

$$\frac{\partial C_{O_2}}{\partial t} = D_{O_2}^{poly} \frac{\partial^2 C_{O_2}}{\partial x^2} \quad \text{Eq. 9.9}$$

$$\frac{\partial C_{hexanal}}{\partial t} = D_{hexanal} \frac{\partial^2 C_{hexanal}}{\partial x^2} \quad \text{Eq. 9.10}$$

where $C_{hexanal}$ is the concentration of hexanal in the packaging material and $D_{hexanal}$ is the diffusivity of hexanal in the packaging.

To determine the values of the appropriate model constants, K_1' , K_2' , K_3' , K_4' , and K_5 , both Del Nobile *et al.* (2003a) and Kanavouras & Coutelieris (2006, 2005) conducted long term shelf life experiments. The experimental designs used for the long term shelf life experiments by Kanavouras & Coutelieris (2005) and Del Nobile *et al.* (2003a) were very similar. Both used glass bottles as the control system for testing olive oil oxidation rates without oxygen transfer to the system and both chose polymer bottles to analyse the effects of oxygen supply on the rates of lipid oxidation. Kanavouras & Coutelieris (2006, 2005) used both light and dark storage at three different temperatures while Del Nobile *et al.* (2003a) studied oxidation under light and a single temperature only.

Examples of the experimental results gained by Del Nobile *et al.* (2003a) and Kanavouras & Coutelieris (2005, 2006) are given in Figures 9.1 and 9.2 respectively. Both studies gave average concentrations of oxidation products and, despite oxygen being an integral part of the models presented in both studies, oxygen concentration was never measured. Instead, the concentrations of oxygen in each study were predicted and not validated.

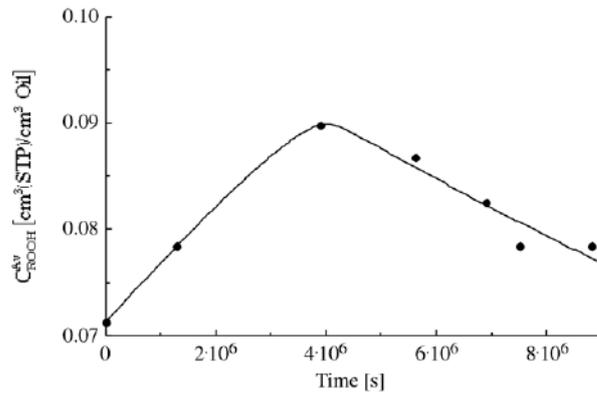


Figure 9.1: Figure from Del Nobile *et al.* (2003a) showing the model fit to experimental results from long term shelf life experiments of olive oil at 40°C in glass bottles.

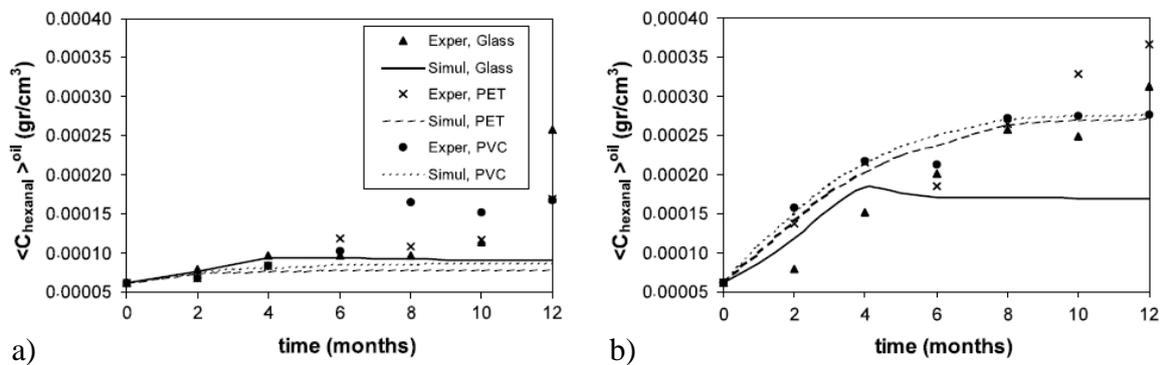


Figure 9.2: Figures from Kanavouras & Coutelieri (2005). a) hexanal concentration as a function of time for olive oil stored at 40°C in the dark for olive oil in different packaging materials. b) hexanal concentration as a function of time for olive oil stored at 40°C in light for olive oil in different packaging materials.

Del Nobile *et al.* (2003a) fitted the model constants (K_1' , K_2' , K_3' , K_4' and K_5) using the data presented in Figure 9.1. Although the model fits the data well, the number of tuneable parameters in the model means that it is difficult to determine if the fit is due to it being developed with a good mechanistic basis. Never-the-less, the model was relatively successful at predicting the average concentration of lipid hydroperoxides in samples of olive oil stored in different packaging materials (Figure 9.3).

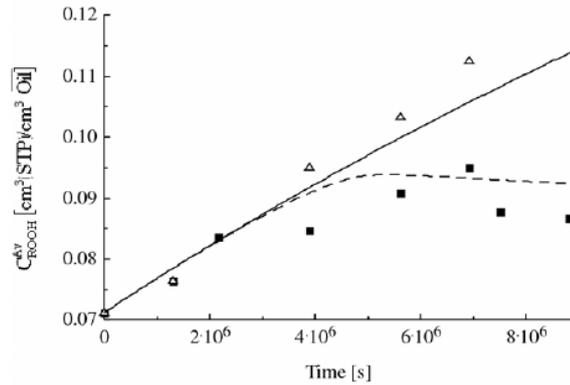


Figure 9.3: Figure from Del Nobile *et al.* (2003a) model predictions and experimental data for the oxidation of olive oil in starch-polymer blend bottles (triangles) and PET bottles (squares) at 40°C.

Kanavouras & Coutelieris (2006, 2005), on the other hand, did not make it clear whether the model constants (k_a, k_c) were fit once and used to predict hexanal production in future experiments or whether they were fit separately for each set of experimental data. In any case, the model used to describe lipid oxidation in the absence of light fits the experimental data poorly (Figure 9.2).

The models presented by Del Nobile *et al.* (2003a, 2003b) and Kanavouras & Coutelieris (2006, 2005) fail to accurately characterise the oxidation systems they are modelling. Aside from the deficiencies outlined above, neither model was presented in such a way as to describe the rate of oxidation as a function of time and position within a product despite the model being formulated to do just that. Without this analysis, the effects of changes in the relative rates of oxygen supply and consumption and the effects of lipid hydroperoxide diffusion cannot be determined. The analysis, therefore, does not go far enough and does not capture the complexity of lipid oxidation when the supply of oxygen is limited.

9.2 Oxygen supply vs. reaction

Many food products are packaged in permeable polymer packaging materials. There is some movement of oxygen from the atmosphere into the product. Products packaged in glass bottles, such as many cooking oils, also have some exposure to

oxygen although generally not through the packaging. These bottles, once opened, will have a headspace of air that is available for reaction. In any case, the new supply of oxygen will force some changes to the oxidation system. It may be as simple as causing an increase in the extent of reaction that can occur in a given time or be as complicated as creating significant oxygen concentration gradients through the product.

The effect of having oxygen supply is entirely dependent on the relative rates of oxygen supply and reaction. That is, is the possible rate of oxygen consumption greater than the rate at which it can be supplied to the product, bearing in mind that the concentration of oxygen cannot be negative? This question has already been half answered. The previous chapters have given some indication of the rates of lipid oxidation. What is needed now is some way of determining the rates of oxygen supply and a way to connect the two factors together.

9.2.1 Conceptual model development

To be able to draw conclusions about the oxidation system, a model is needed to simulate the effects of having bulk oil contained in a permeable material, and to predict shelf life. This was achieved by using the simple system depicted in Figure 9.4. It was assumed that the product was square and was infinitely long, thus allowing for end effects to be removed and for a one dimensional model to be used.

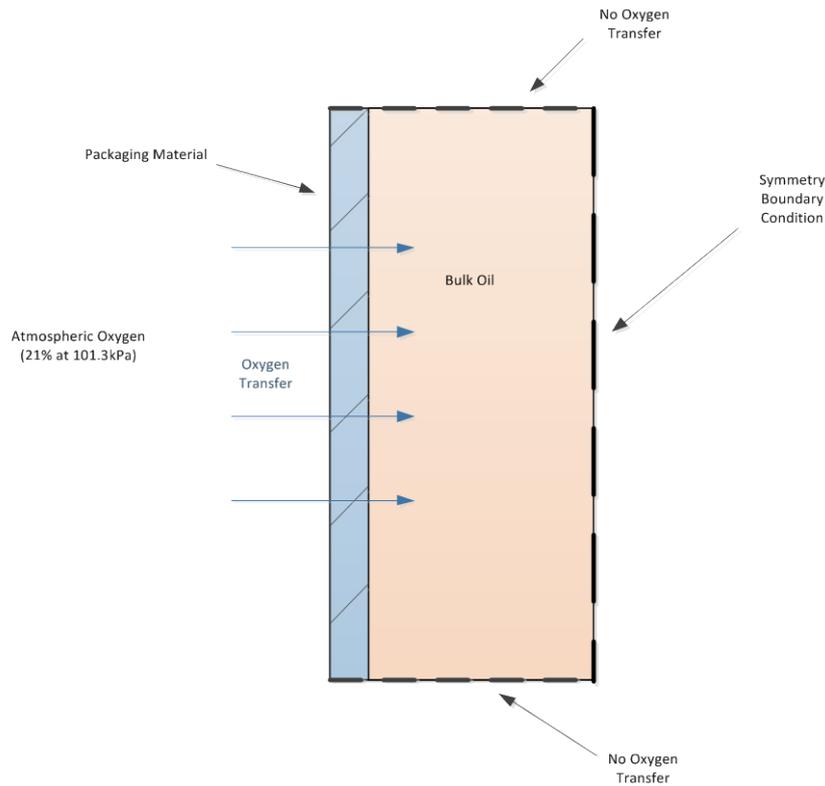


Figure 9.4: Figure depicting a bulk oil in a polymer package. The package has a length that is many times greater than its width which allows for the assumption that the end effects are negligible.

In the system depicted in Figure 9.4, there is one dimensional transfer of oxygen through the packaging to the surface of the product. Once on the surface of the oil, the oxygen will diffuse through and react with the oil. The relative rates of oxygen reaction and diffusion will determine the size of the oxygen concentration gradient setup across the product. If the rates of supply are lower than the rate at which oxygen can be reacted, there will be no oxygen concentration gradients within the system.

9.2.2 Analytical solution – initial investigations

To begin this analysis, an analytical model was formulated to investigate the worst case scenario of having an oil exposed to air without any barrier polymer. This model is developed in Equations 9.11 to 9.24 below. The steady state model began by setting the rate of oxygen supply via diffusion through the oil equal to the rate of oxygen consumption via reaction (Equation 9.11). At the surface of the oil, the flux of

which was initially given the notation ‘ q ’ and is expressed according to Equation 9.12. At some distance ‘ L ’ away from the surface of the oil, the concentration of oxygen in the oil would be zero , Equation 9.13.

$$\frac{\partial C_{O_2}}{\partial t} = D \frac{\partial^2 C_{O_2}}{\partial x^2} - r \quad \text{Eq: 9.11}$$

for $t > 0$ and $0 < x < L$

$$-D \frac{\partial C_{O_2}}{\partial x} = -q \quad \text{Eq: 9.12}$$

at $x=0$

$$C_{O_2} = 0 \quad \text{Eq: 9.13}$$

at $x=L$

By assuming a constant reaction rate, i.e. there is no formation of lipid hydroperoxides, then it is possible to use the following equations to develop the steady state model.

$$D \frac{\partial^2 C_{O_2}}{\partial x^2} = r \quad \text{Eq: 9.14}$$

integrating once:

$$D \frac{\partial C_{O_2}}{\partial x} = rx + k_1 \quad \text{Eq: 9.15}$$

integrating again:

$$DC_{O_2} = \frac{rx^2}{2} + k_1x + k_2 \quad \text{Eq: 9.16}$$

applying the boundary condition at $x=0$ in Equation 9.16:

$$k_1 = -q \quad \text{Eq: 9.17}$$

applying the boundary condition at $x=L$ in Equation 9.16:

$$k_2 = qL - \frac{rL^2}{2} \quad \text{Eq: 9.18}$$

therefore:

$$DC_{O_2} = q(L - x) - \frac{r}{2}(L^2 - x^2) \quad \text{Eq: 9.19}$$

As shown in Equation 9.19 above, the ability to predict the concentration of oxygen at any point in the oil is dependent on knowing the distance from the surface that the concentration of oxygen in the oil reaches zero, L .

At steady state the rate of oxygen entering the oil must equal the rate at which oxygen is being consumed. As such, Equation 9.20 should apply.

$$rA \int_{x=0}^{x=L} \partial x = qA \quad \text{Eq: 9.20}$$

The integral of ∂x in this case, is the distance from the surface where the concentration of oxygen first reaches zero. This distance is, therefore, L .

$$L = \frac{q}{r} \quad \text{Eq: 9.21}$$

Using Equation 9.19 and applying the surface boundary conditions it was shown that Equation 9.22 applies.

$$C_{O_{2surf}} = \frac{q^2}{2rD} \quad \text{Eq: 9.22}$$

Because the flux across the surface is defined by Equation 9.23 below, the flux ' q ' could be calculated using the quadratic rule to solve Equation 9.24.

$$q = k_g (C_{O_{2sat}} - C_{O_{2surf}}) \quad \text{Eq: 9.23}$$

$$0 = \frac{q^2}{2rD} + \frac{q}{k_g} - C_{O_{2sat}} \quad \text{Eq: 9.24}$$

Using Equations 9.23 and 9.24, the oxygen flux at the surface of the oil air interface was calculated, used to calculate L and to predict the oxygen concentration gradients that would occur in the system described above.

By using dimensionless number correlations including Sherwood, Schmidt and Reynolds numbers (Bailey & Ollis, 1986) the mass transfer coefficient for the transfer of oxygen in the air into an unmixed bulk oil was estimated for a range of air velocities. The constants used in to calculate the mass transfer coefficient k_g were: $D_a = 2.03 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$; $\mu = 2.40 \times 10^{-5} \text{ Pa.s}$; $\rho = 1.18 \text{ kg.m}^{-3}$; $L_c = 0.1 \text{ m}$.

$$Sc = \frac{\mu}{\rho D_a} \quad \text{Eq: 9.25}$$

$$Re = \frac{\rho u L_c}{\mu} \quad \text{Eq: 9.26}$$

$$Sh = 2 + 0.644 Sc^{1/3} Re^{1/2} \quad \text{Eq: 9.27}$$

To begin the analysis, the effect air velocity had on the system was calculated. Using the constants given above and Equations 9.25 to 9.27, the mass transfer coefficient, k_g , and distance from the surface where there is no oxygen, L , were calculated. A slow reaction rate of oxygen consumption, equivalent to a 60 hours batch oxidation expected from a sample of canola oil, was used to maximise the oxygen concentration gradients and, therefore, the chances of seeing differences in the concentration gradients from the following simulations.

Table 9.1: Results showing the mass transfer coefficient and distance at which no oxygen is present, L , for different air velocities

Air velocity (m.s^{-1})	Mass Transfer Coefficient (m.s^{-1})	Distance from surface where the oxygen concentration is zero (mm)
10	0.030	10.84
5	0.022	10.84
1	0.010	10.84
0	0.000	10.84

As can be seen in Table 9.1, the rate of mass transfer to the surface of the oil is not limiting, even when the velocity of air passing over the sample is zero. The distance from the surface of the oil where the concentration of oxygen in the oil reaches zero is unaffected by the velocity of air moving over the surface of the oil.

Unlike the effects of air velocity, the distance L is affected by the rate of oxygen consumption in the oil. This is shown in Figure 9.5 where the analytical solution was run for a range of different oxygen consumption rates. For example, for an oil which would take 60 hours to complete a batch oxidation (see Chapter 4), it can be shown that oxygen would be present in the outer 10 mm of the product. An example of such an oil would be a canola oil. If the reaction rate were increased to that of a fish oil, 5 hours to complete a batch oxidation at $\sim 40^\circ\text{C}$, oxygen would be present only in the outer 2.8 mm.

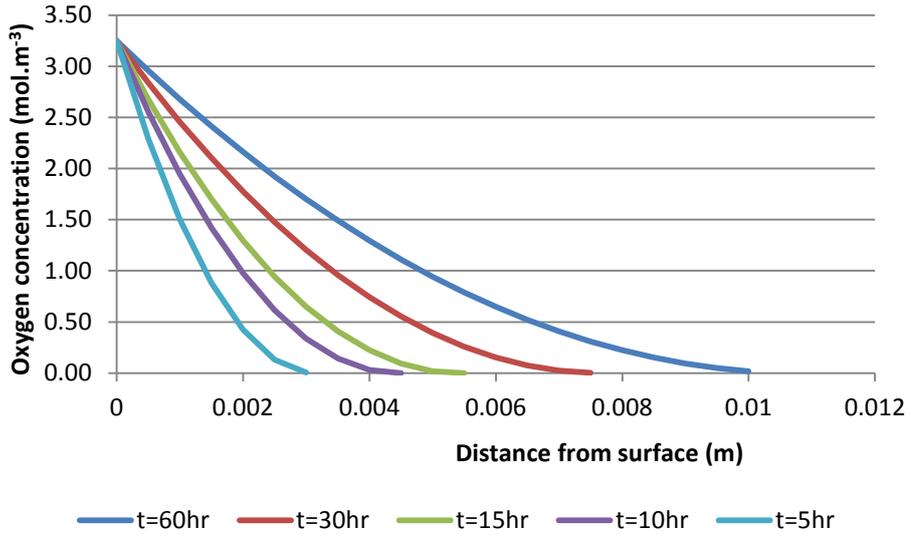


Figure 9.5: Steady state analysis of oxygen concentration gradients within oil that is open to the atmosphere with potential oil reaction rates. Reaction rates are depicted as the time taken to complete a batch oxidation.

The results shown in Figure 9.5 suggest that only the oil near the surface would have oxygen present and that the amount of oil that has oxygen is reduced when the rate of oxygen consumption increases. This effect is shown in Figure 9.6. The results also suggest that it is possible for a significant quantity of oil to be reacting in a bulk oil.

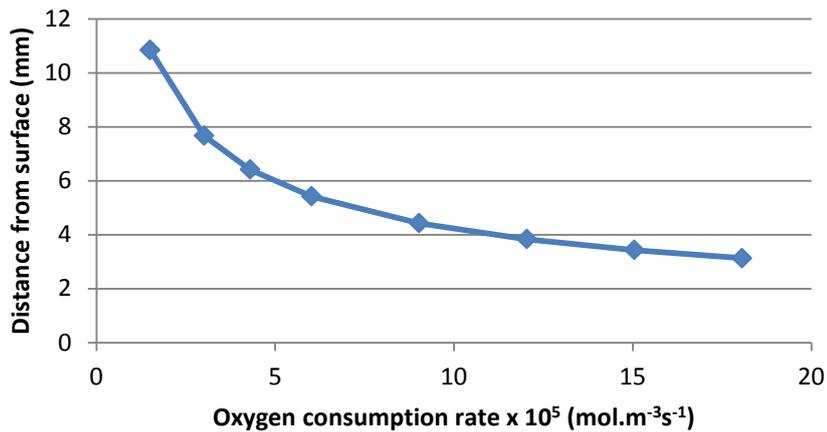


Figure 9.6: Resulting distance from the surface (L) where no oxygen is present as a function of the potential oxygen consumption rate

If a reduction in the rate of oxygen consumption brings with it an increase in the distance from the surface that oxygen is present, then it stands to reason that the reduction in the rate of oxygen consumption by the dilution of oils in oil-in-water emulsions would also increase the amount of a product that would be exposed to oxygen.

For example, Figure 8.13 in Chapter 8 showed batch oxidations of 5 and 40% w/w fish oil-in-water emulsions. Because the solubilities of oxygen in the oil and water phases are different, the saturated oxygen concentration was shown to be 1.46 and 0.42 mol.m⁻³ for the 40% and 5%w/w oil-in-water emulsions respectively. Assuming a zero order reaction and using the times for each batch oxidation (7.5 and 5.3 hours for the 5 and 40% oil-in-water emulsions respectively), the rates of oxygen consumption were shown to be 5.42x10⁻⁵ and 2.20x10⁻⁵ mol.m⁻³s⁻¹ for the 40 and 5%w/w oil-in-water emulsions respectively. Using Figure 9.6, it can be shown that the distances from the surface of the oil-in-water emulsions that contain oxygen are approximately 5.5 and 9 mm for the 40 and 5%w/w respectively. Again, the reduction in reaction rate brought about by dilution of of the oil should bring with it an increase in the amount of emulsion that contains oxygen and is, therefore, reacting.

9.2.3 Packaging – reducing oxygen supply rates

The above results show that, if a constant reaction rate were maintained, the size of the oxygen concentration gradients is defined by the rate of oxygen consumption when the system is exposed to air. Food products, however, are generally not exposed directly to air. Instead, an additional resistance to mass transfer is included as a method of not only physically containing the product but also to reduce the rates of oxygen transfer. Although some packaging materials, like glass, do not allow oxygen transfer, there are many polymer based packaging materials that are used for food products.

The rates of oxygen transmission through polymer packaging are generally well defined. Most packaging suppliers will provide their customers with some estimate of the rate of oxygen supply under a standard set of experimental conditions. Although the actual rate of oxygen transmission will vary, the use of standardised conditions

allows manufacturers to determine the relative rates of oxygen transfer for different polymer materials. It also provides us with a good estimate for the rates at which oxygen could be supplied to a product without needing to measure the exact rates directly.

Typical oxygen permeabilities can be gained from a range of texts. For the purposes of this investigation, the range of permeabilities of common polymer packaging was taken from Frankel (2005) and is reproduced in Table 9.2 below.

Table 9.2: Permeability data for different polymer packaging materials as given in Frankel (2005) and converted to SI units. Data gained from experiments at 23°C and 50% relative humidity.

Polymer	O ₂ Permeability	
	cm ³ mm/(m ² day.atm)	mol.m/(m ² s.Pa) x10 ¹⁸
Polyethylene terephthalate (PET)	1-5	4.21-21.1
Polyvinyl chloride (PVC)	2-8	8.43-33.7
High density PE (HDPE)	5	21.1
Polypropylene (PP)	50-100	211-421
Polystyrene (PS)	100-150	421-632

In order to apply Equation 9.19, the permeability data presented in Table 9.2 and the film thickness, x_{film} , must be used in place of the mass transfer equation presented in Equation 9.20. To do this, Equation 9.28 below was used to estimate the flux, q .

$$q = \frac{P}{x_{film}} (P_{outside} - P_{surf}) = \frac{P}{x_{film}} (P_{outside} - KC_{O_{2surf}}) \quad \text{Eq: 9.28}$$

rearranging for $C_{O_{2surf}}$ and substituting into Equation 9.22:

$$0 = \frac{Kq^2}{2rD} + \frac{qx_{film}}{P} - P_{outside} \quad \text{Eq: 9.29}$$

Using the quadratic rule to find the roots of Equation 9.29 the flux, q , was calculated for different packaging types (see Table 9.2) and for different oxygen consumption rates as in the earlier analysis. For this analysis the following constants were used: $K=6545.54 \text{ Pa}\cdot\text{m}^{-3}\text{mol}^{-1}$; $P_{outside}=21273 \text{ Pa}$; $D=2.72\times 10^{-10}\text{m}^2\text{s}^{-1}$.

For the steady state simulations conducted for oils in polymer packaging, the rates of oxygen supply to the surface of the oil were significantly lower. The reduction in the supply rates were enough to have an impact on the oxygen concentration gradients within the oil samples simulated. Figure 9.7 below shows how a reduction in the permeance of the polymer packaging brings with it a reduction in the distance that oxygen can penetrate an oil.

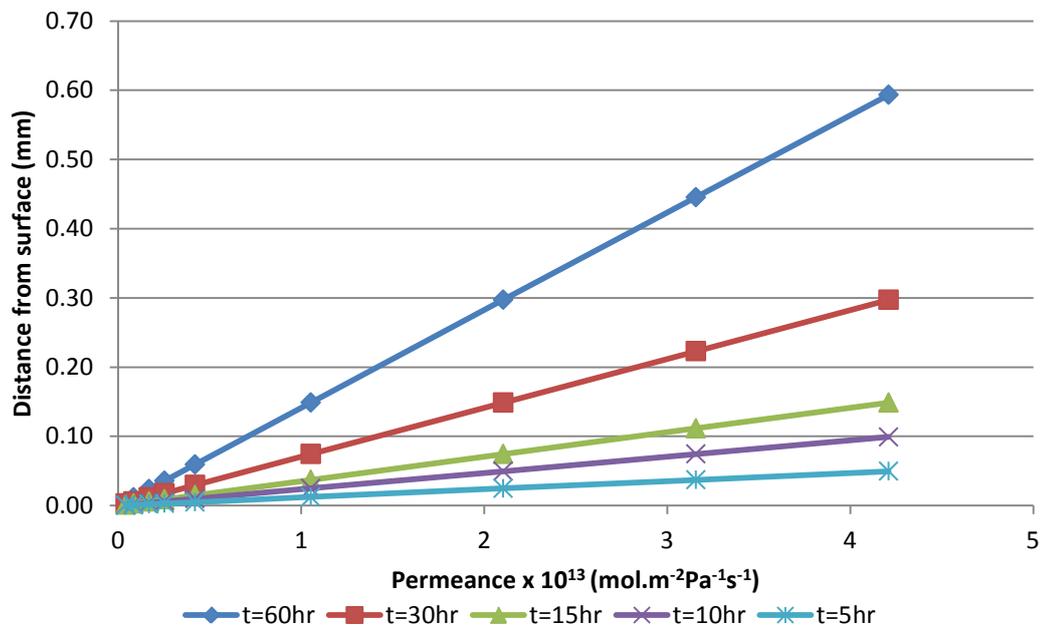


Figure 9.7: Distance from the surface where oxygen is no longer present (L) as a function of the packaging permeability and the potential rate of oxygen consumption. Note the rate of oxygen consumption is expressed as the time (t) for a batch oxidation.

The results in Figure 9.7 represent steady state simulations of various oils of different reactivities packaged in polymer films with different oxygen transfer rates. The plot covers the range of films presented in Table 9.2 and assumes a film thickness of 0.25mm. As expected, the results suggest that either an increase in the oxygen transfer

rate and/or decrease in the oxygen consumption rate bring with it an increase in the amount of oil that is exposed to oxygen.

The above analysis has shown that, when bulk oils or oil-in-water emulsions are packaged in a polymer film, the amount of product that contains oxygen at steady state is lower than if the oil were open to the atmosphere. The analysis also shows that the rate of oxygen consumption will, in part, determine the amount of oil or emulsion that is exposed to oxygen. The analysis, however, does not include the effects of lipid hydroperoxide formation and the rise in reaction rate that comes with it, nor does it include any effects of lipid hydroperoxide diffusion which may have some impact on the shelf life of an oil or emulsion. To conduct this analysis, a different model was required.

9.3 Reactant diffusion –full model analysis:

It has been shown that there will be oxygen present in a bulk oil or emulsion which is packaged in a polymer film. The amount of oxygen and, therefore, the amount of product exposed to oxygen is dependent on the rate of oxygen supply and consumption. When oxygen is not present, it is assumed that no reaction occurs and, in the above steady state analysis, that no lipid hydroperoxide formation or diffusion takes place. This, however, may not be a good assumption, and requires some investigation.

In a reaction system where the supply of oxygen is sufficient to allow the entire oil to react at the same rate, then the movement of lipid hydroperoxides would likely have no effect. However, in a system where oxygen supply is limited by diffusion through packaging (as depicted in Figure 9.7) and oxygen is only present in a small proportion of the oil (at the surface), the ability for lipid hydroperoxides to form in an oxygen rich environment and diffuse away may have a significant impact on the rates of oxygen consumption and lipid hydroperoxide formation.

Because the effect of lipid hydroperoxides is likely to be dependent on the relative rates of oxygen supply and reaction as well as the rate of lipid hydroperoxide diffusion, it makes sense that the system would act in one of three different ways:

- 1) If the diffusion of lipid hydroperoxides is slow, then oxidation at the surface would need to go to completion before oxygen could penetrate any further in the product. i.e. the surface would become fully oxidised before there is oxidation reactions/products at any other point in the product/oil.
- 2) If the diffusion of reactants is fast, then the system may act as if well mixed. In this case there would be continual supply of fresh reactants to the surface of the packaging and the extent of reaction throughout the product/oil would be the same.
- 3) If the diffusion of reactants is significant but not fast, then the system would contain oxidation product concentration gradients. Oxygen would still only be found near the surface of the packaging, but lipid hydroperoxides would be found at their highest concentrations at the surface and decrease as the distance from the surface increases.

Method

In order to determine which of the three scenarios is true for food systems; two finite difference models (compiled and executed in MATLAB R2011b) were created to include the diffusion of lipid hydroperoxides in oil directly exposed to air and oil packaged in a polymer film. The models, described in Equations 9.30 to 9.36, were based on the system given in Figure 9.4 with an exposed surface area of 0.1x0.1 m. When packaging was simulated, a packaging permeability of $1 \text{ cm}^3 \text{ mm} \cdot \text{m}^{-2} \text{ day}^{-1} \text{ atm}^{-1}$, PET film from Table 9.2, and a packaging thickness of 0.25mm was used. When simulating an oil without packaging, a mass transfer coefficient, k_g , of $4 \times 10^{-4} \text{ m} \cdot \text{s}^{-1}$ was used according to the calculations given in section 9.2.2. The diffusivity of oxygen in oil for this analysis was $2.72 \times 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$ (Del Nobile *et al.*, 2003a). Since there is no literature for the rates of diffusion of lipid hydroperoxides, the diffusivity was taken to be that of lipid self-diffusion which was measured by Metais & Mariette (2003) at $9.7 \times 10^{-12} \text{ m}^2 \cdot \text{s}^{-1}$. Finally, the initial lipid hydroperoxide concentration was set at $4.97 \text{ mol} \cdot \text{m}^{-3}$ and the half order rate constant of $5.0 \times 10^{-5} \text{ mol}^{1/2} \cdot \text{m}^{-3/2} \cdot \text{s}^{-1}$ was estimated using the activation energy and Arrhenius constant derived in section 6.5 of chapter 6.

Unless otherwise stated, the simulations were carried out at 23°C with a relative humidity of 50%. These conditions are the standard conditions used to evaluate packaging permeability on the basis that they represent reasonable storage conditions of many shelf-stable food products.

$$\frac{\partial C_{O_2}}{\partial t} = D \frac{\partial^2 C_{O_2}}{\partial x^2} - r \quad \text{Eq: 9.30}$$

for $t > 0$ and $0 < x < L$

$$\frac{\partial C_{LOOH}}{\partial t} = D \frac{\partial^2 C_{LOOH}}{\partial x^2} + \frac{r}{Y} \quad \text{Eq: 9.31}$$

for $t > 0$ and $0 < x < L$

where

$$r = k C_{LOOH}^{1/2} \quad \text{Eq: 9.32}$$

for $C_{O_2} > 0$

and

$$r = 0 \quad \text{Eq: 9.33}$$

for $C_{O_2} = 0$

$$D \frac{\partial C_{O_2}}{\partial x} = P (P_{outside} - K C_{O_{2surf}}) \quad \text{or} \quad D \frac{\partial C_{O_2}}{\partial x} = k_g (C_{O_{2sat}} - C_{O_{2surf}}) \quad \text{Eq: 9.34}$$

at $x=0$

$$D \frac{\partial C_{LOOH}}{\partial x} = 0 \quad \text{Eq: 9.35}$$

at $x=0$ and $x=L$

$$D \frac{\partial C_{O_2}}{\partial x} = 0 \quad \text{Eq: 9.36}$$

at $x=L$

9.3.1 Modelling results – open system

Finite difference approximations were used to solve Equations 9.30 to 9.36 in MATLAB (R2011b) using the ode45 solver. The MATLAB code used for the following simulations are given in the Appendix (see folder ‘Chpater 9’).

The results suggest that, although oxygen is only present in the oil up to 4mm from the surface (Figure 9.9), there are lipid hydroperoxides present up to 10mm from the surface (Figure 9.8). This suggests that, although the rate of lipid hydroperoxide diffusion is slow, the lipid hydroperoxides formed within the oil containing oxygen will move away from the surface.

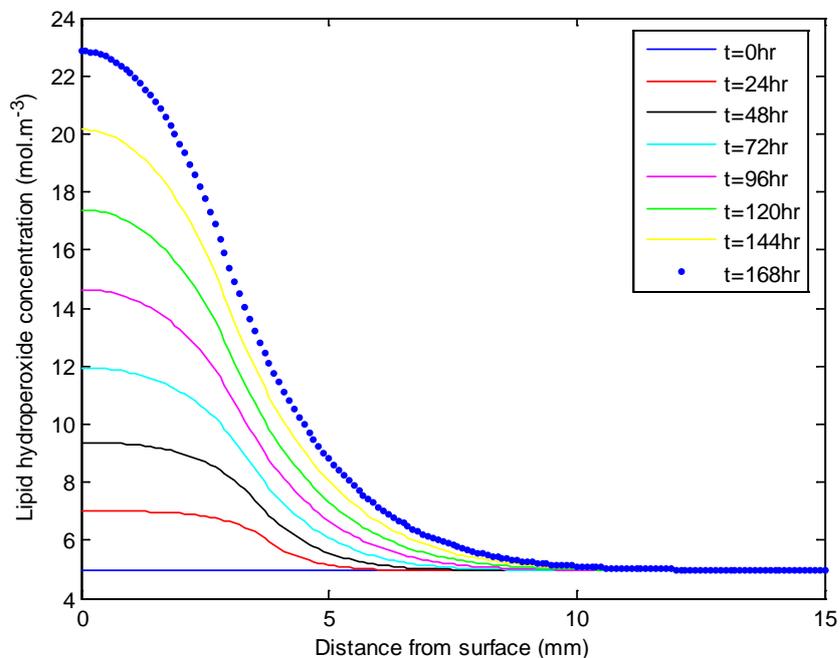


Figure 9.8: Results from a simulation of a sample of fish oil stored open to the air including the effects of lipid hydroperoxide diffusion. Results show the concentration of lipid hydroperoxides as a function of the distance from the surface of the packaging and for a range of storage times.

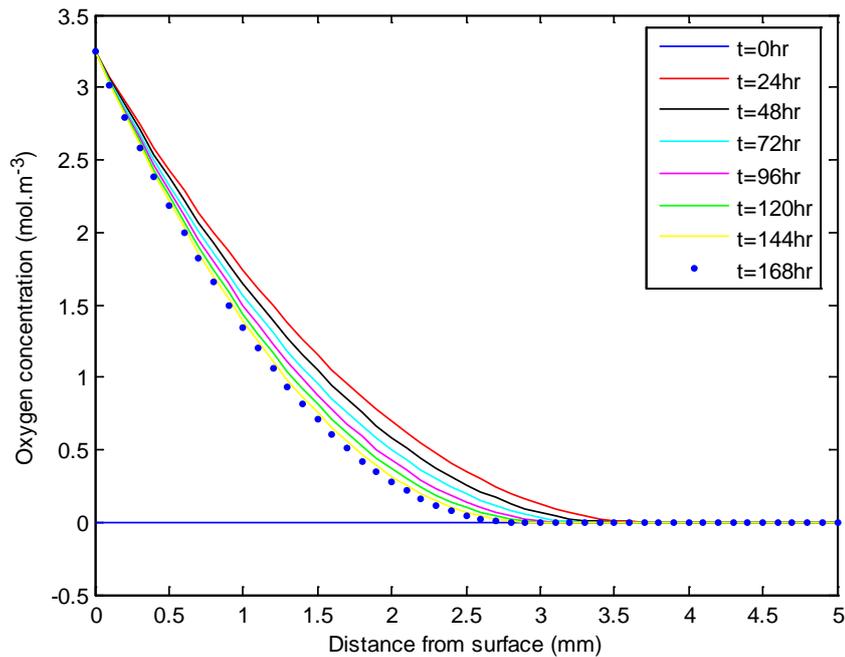


Figure 9.9: Results from a simulation of a sample of fish oil stored open to the air including the effects of lipid hydroperoxide diffusion. Results show the concentration of oxygen as a function of the distance from the surface of the packaging and for a range of storage times.

To show the magnitude of the effect that lipid hydroperoxide diffusion has, the model was modified to remove lipid hydroperoxide diffusion. Using the same system inputs and finite difference approximations, it was shown that the distribution of lipid hydroperoxides during oxidation was significantly different to that when lipid hydroperoxides are allowed to diffuse. In this case, the lipid hydroperoxide concentration was the same for all points where oxygen is present (see Figure 9.10). The decrease in lipid hydroperoxide concentration shown in Figure 9.10 only occurs because the rate of oxygen consumption increases as lipid hydroperoxides formed. As the rate of oxygen consumption increases, the distance from the surface of the oil that oxygen was present decreased which leaves some of the oil partially oxidised and unable to oxidise further.

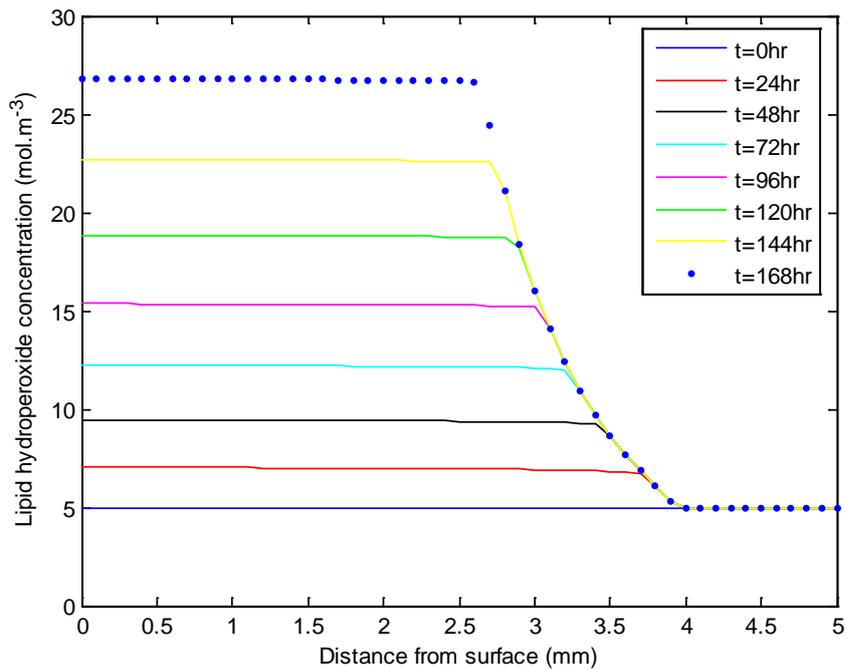


Figure 9.10: Results from a simulation of a sample of fish oil stored open to the air without accounting for lipid hydroperoxide diffusion. Results show the concentration of lipid hydroperoxides as a function of the distance from the surface of the packaging and for a range of storage times.

As can be seen in Figure 9.11, the oxygen concentration gradients that come about by modelling the system without lipid hydroperoxide diffusion seem to be the same as those resulting from the model with lipid hydroperoxide diffusion. This is because the rate of oxygen transferred is governed by the surface boundary condition shown in Equation 9.34.

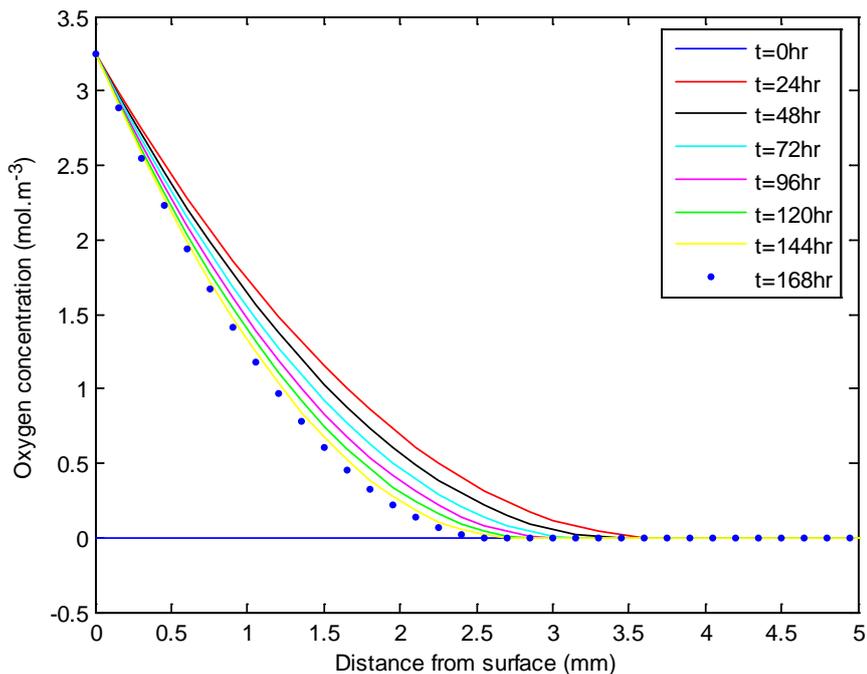


Figure 9.11: Results from a simulation of a sample of fish oil stored open to the air without accounting for lipid hydroperoxide diffusion. Results show the concentration of oxygen as a function of the distance from the surface of the packaging and for a range of storage times.

This analysis suggests that lipid hydroperoxide diffusion is likely to have a significant impact on the lipid hydroperoxide concentration gradient through the oil. This suggests that lipid hydroperoxides can be formed near the surface of the oil where oxygen is present and diffuse away. This would likely reduce the rate of formation of secondary oxidation products by the breakdown of lipid hydroperoxides as it is expected that the rate of lipid hydroperoxide breakdown is likely to be proportional to the concentration of lipid hydroperoxides. This, however, will be discussed at a later stage.

9.3.2 Modelling results – polymer packaging

Because the rates of oxygen supply and reaction determine the oxygen and lipid hydroperoxide concentrations in bulk oils, it makes sense that a reduction in the rate of oxygen supply by enclosing an oil in a polymer film would serve to reduce the rate of

lipid hydroperoxide formation and decrease the lipid hydroperoxide gradient through the oil. That is, while the diffusivity of lipid hydroperoxides remains constant, the rate of lipid hydroperoxide formation would be significantly reduced by the introduction of a oxygen barrier.

To test the effects of oxygen supply on a bulk oil system with polymer packaging, the models outlined in Equations 9.30 to 9.36 were simulated using finite difference approximations and solved using the ode23s solver in MATLAB (R2011b). The system inputs for this analysis are the same as outlined in the methods section above. The MATLAB code used to run the models and construct the following figures can be seen in the Appendix (see folder 'Chapter 9').

As expected, the results, shown in Figures 9.12 and 9.13 show that the rate of lipid hydroperoxide formation is significantly reduced by the addition of a barrier polymer (0.25mm thick PET). More interestingly, the lipid hydroperoxide concentration gradient is significantly smaller than that shown in Figure 9.8 and suggests that the lower the rate of oxygen supply, the greater the effect of lipid hydroperoxide diffusion on the system.

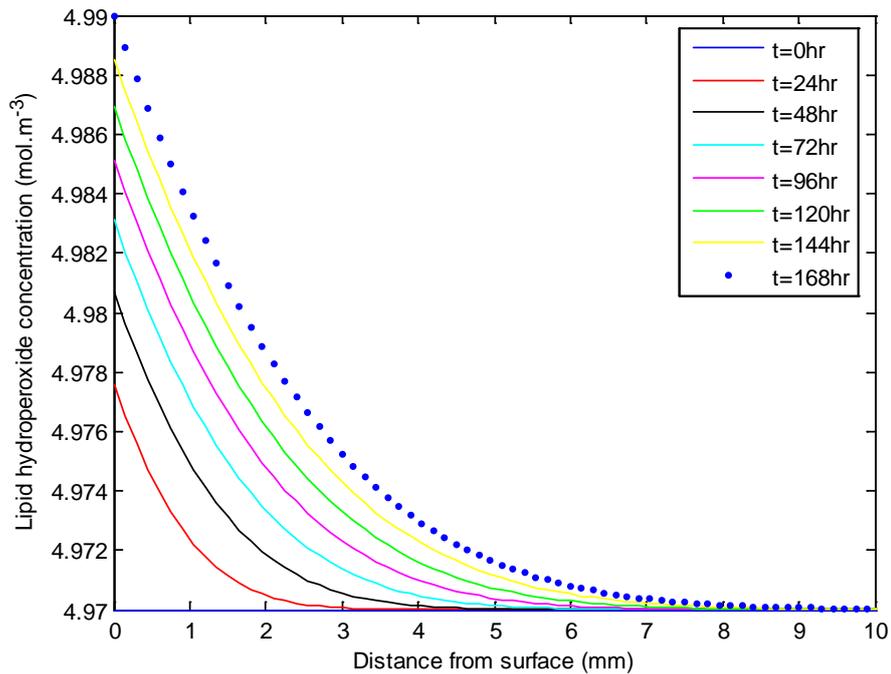


Figure 9.12: Results from a simulation of a sample of fish oil stored in 0.25mm thick PET. Results show the concentration of lipid hydroperoxides as a function of the distance from the surface of the packaging and for a range of storage times.

Unlike the models for oil exposed to air, the results (Figure 9.9) of the packaging based model showed that the concentration of oxygen in the system is zero throughout and that the potential rate of oxygen consumption is greater than the supply.

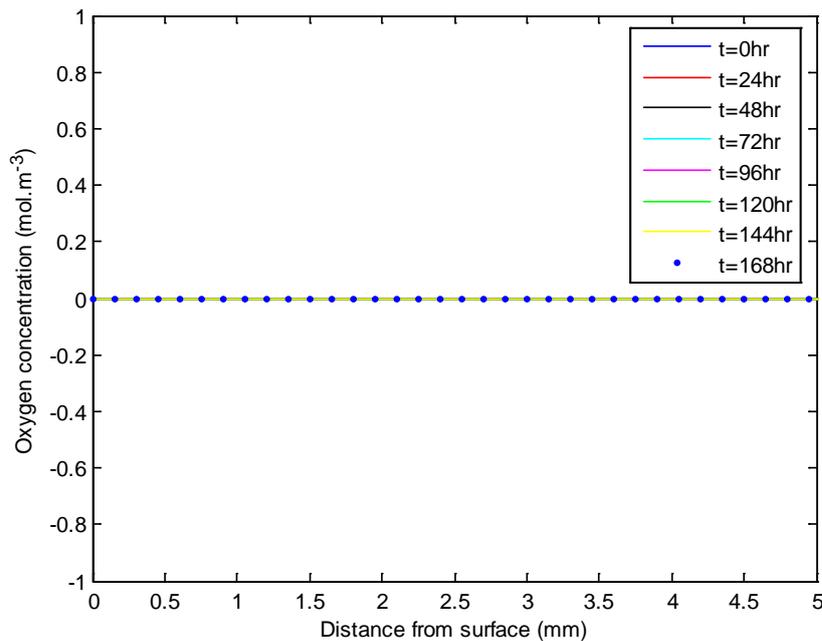


Figure 9.13: Results from a simulation of a sample of fish oil stored in 0.25mm thick PET. Results show the concentration of oxygen as a function of the distance from the surface of the packaging and for a range of storage times.

The results of this modelling exercise show two important pieces of information:

- 1) Oxygen concentration gradients can be present if the system is setup such that the rates of oxygen supply are larger than that of reaction at the surface.
- 2) The rate of lipid hydroperoxide diffusion is enough to setup lipid hydroperoxide concentration gradients.

While the above analyses were useful in providing a picture of the differences that could be expected from extreme systems (open to the atmosphere and packaged in a polymer that has excellent oxygen barrier properties), they do not provide enough quantitative information that could be used for shelf life predictions.

9.4 Modelling – relative rates of oxygen supply and consumption

The above modelling analysis showed that a shift to an oxygen supply limited system not only slowed the rate of lipid hydroperoxide formation, but also reduced the amount of oil that was oxidised. Figure 9.12 clearly shows a band of oil that contains significant amounts of lipid hydroperoxides while the rest of the oil contains no change in lipid hydroperoxides at all. So it seems there are two important aspects that must be considered when looking to predict the shelf life of oils where oxygen supply is through polymer packaging:

- 1) The amount of oxygen that is transferred and, therefore, the amount of lipid hydroperoxides formed
- 2) The amount of oil that is actually being oxidised.

9.4.1 Rates of oxygen transfer and consumption

Because the current oxidation system suggests that oxidation only occurs in the presence of oxygen, the amount of lipid hydroperoxides formed must be proportional to the amount of oxygen transferred across the packaging. To show this, the model shown in Equations 9.30 to 9.36 was used to simulate seven days of storage of mixed fish oil at 35°C in various packaging materials. Aside from the packaging permeability, the system inputs for the model were the same as described in Section 9.3. The permeability of the packaging materials was varied from 4.2×10^{-18} mol.m.Pa⁻¹s⁻¹ (equivalent to PET film shown in Table 9.2) to 210×10^{-18} mol.m.Pa⁻¹s⁻¹ (equivalent to a film 50 times more permeable than PET). The MATALAB code used in the following analyses can be found in the Appendix (see folder ‘Chapter 9’).

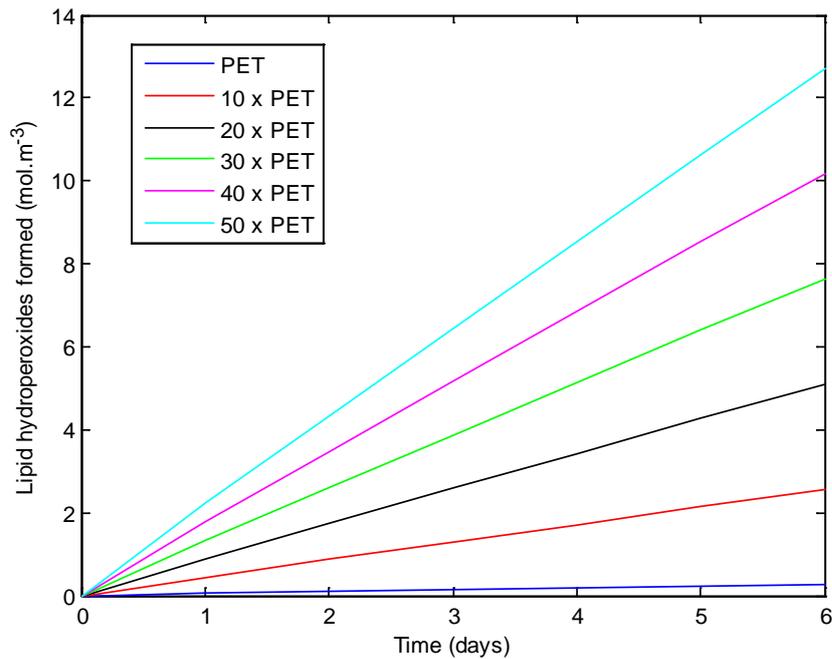


Figure 9.14: Results of a simulation of fish oil packaged in 0.25mm thick films with different oxygen permeability's. Permeability is presented as a multiple of that for PET (see Table 9.2).

The results, shown in Figure 9.14, illustrate the relationship between the supply of oxygen and the accumulation of lipid hydroperoxides within the oil sample. The model results show that the change in lipid hydroperoxide concentration is significantly increased by the additional oxygen supplied to the system.

In the above simulation, the reaction rate is high enough to ensure that oxygen is only present at or near the surface. The formation of lipid hydroperoxides would have occurred at the surface. The results shown in Figure 9.14 do not fully describe the system as the lipid hydroperoxide concentration is not the same throughout the oil sample. Infact what the model shows is that the vast majority of lipid hydroperoxide are present near the surface and that the presence of lipid hydroperoxides away from the surface is only due to lipid hydroperoxide diffusion. This is, of course, exactly what earlier predictions showed.

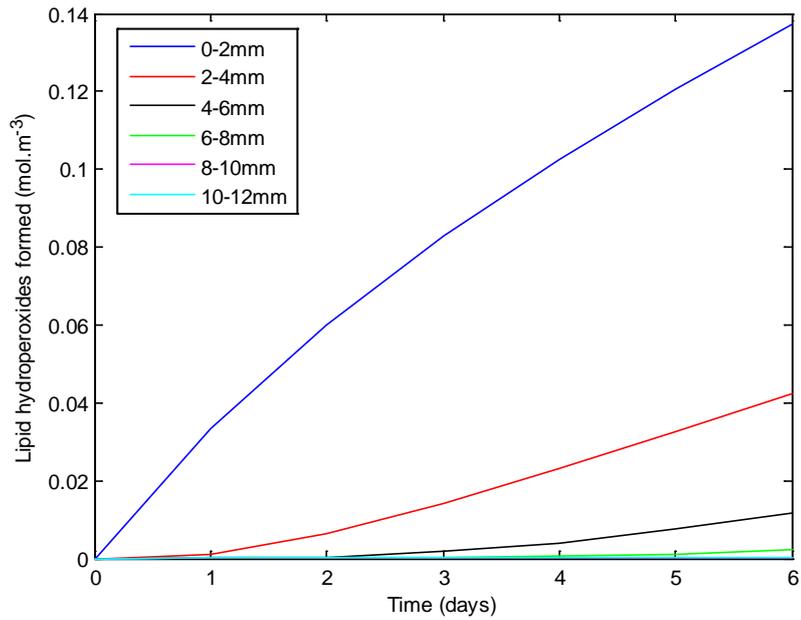


Figure 9.15: Results of a simulation of fish oil packaged in 0.25mm thick PET. Results show the concentration of lipid hydroperoxides as if the oil were cut into 2mm thick sections beginning at the oil surface.

What is more interesting is the impact of increasing the rates of oxygen supply relative to lipid oxidation rates. For example, if the same simulation is carried out with packaging 20 times more permeable, the distance oxygen travels into the oil does not increase 20 times. As before, lipid hydroperoxides are only present in the 6-8mm from the surface. They are, however, present in much higher concentrations.

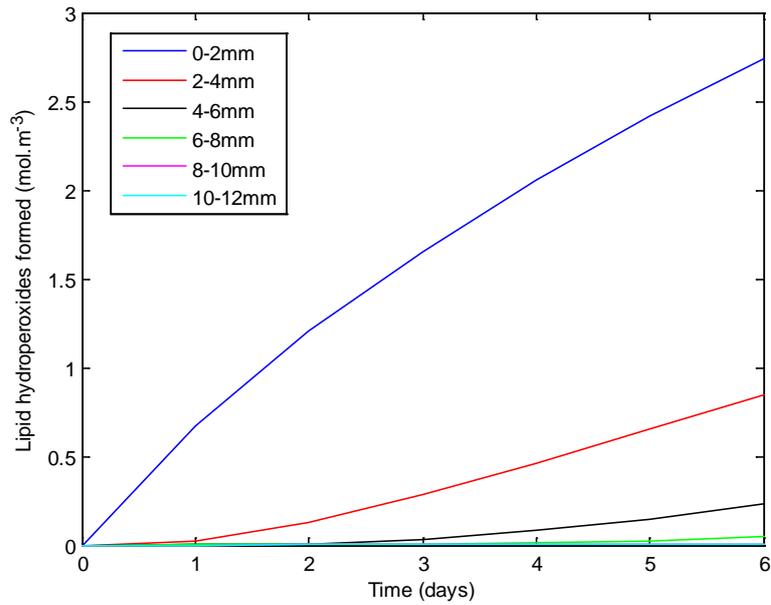


Figure 9.16: Results of a simulation of fish oil packaged in a 0.25mm thick with a permeability 20x that of PET. Results show the concentration of lipid hydroperoxides as if the oil were cut into 2mm thick sections beginning at the oil surface.

9.4.2 Amount of oil being oxidised

For an oil that reacts quickly in the presence of oxygen (i.e. fish oil), oxygen is only present at or near the surface of the packaging. For example, in the model simulation used to create Figure 9.15, it can be shown that the vast majority of the lipid hydroperoxides formed exist in the oil within 2mm of the surface of the polymer film. For a simulation of fish oil in 0.25mm thick PET polymer, it was shown that, after a week, approximately 75% of all lipid hydroperoxides exist within 2mm of the packaging surface. Furthermore, because the rate of lipid hydroperoxide formation is limited by the supply of oxygen, a 50 times increase (i.e. 50 times more permeable than PET) in the rate of oxygen supply has no impact on the proportion of lipid hydroperoxides in each of the sections given in Figure 9.17.

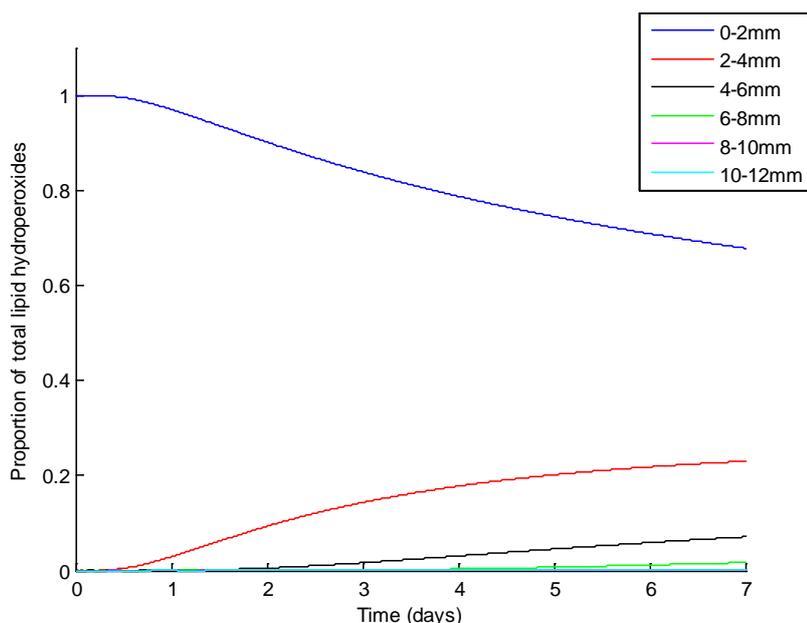


Figure 9.17: Results of a simulation of fish oil packaged in 0.25mm thick PET. Results show the proportion of the total lipid hydroperoxide formed in 2mm thick sections beginning at the oil surface.

According to the model, the only way that a larger lipid hydroperoxide concentration gradient could be seen is if the oil is very stable and the packaging very permeable. To explore this, the model was run with a reaction rate equivalent to that of canola oil, $k_{canola} \approx k_{fish} \times 0.1$, in a 0.25mm thick polypropylene (PP) film, $P_{PP} = 100 \times P_{PET}$. The results show that, in the case of canola oil in PP film, the amount of oxygen is above zero as far as 2.5mm from the surface of the packaging. This is shown in Figure 9.18 below.

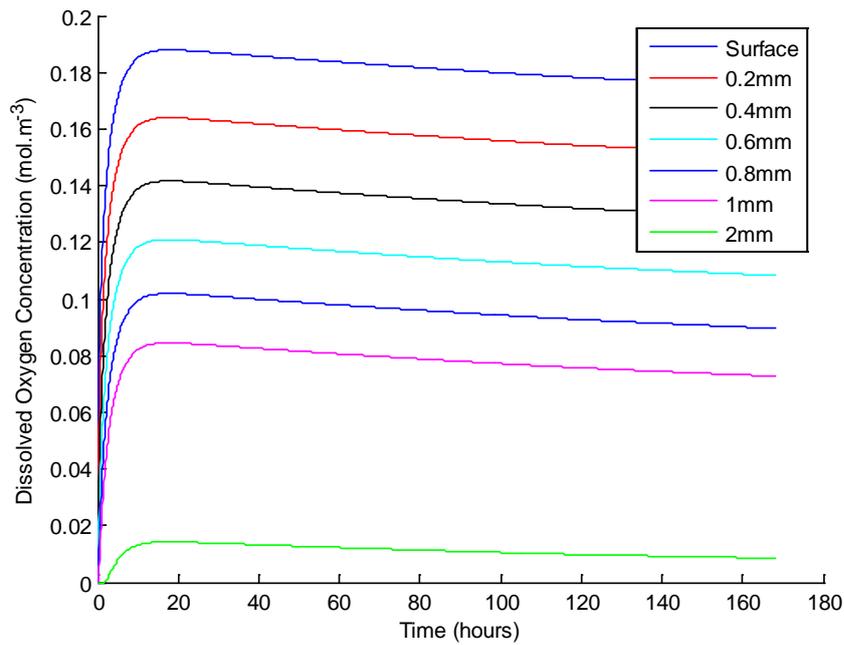


Figure 9.18: Results of a simulation of canola oil packaged in a 0.25mm thick film that has permeability 100x that of PET. Results show the oxygen concentration as a function of time for a range of distances from the surface of the oil.

This analysis shows that, in the worst case scenario of an oil with lower reactivity and a highly permeable packaging film, only the first 2-3mm of the product will be reacting. In this scenario, the concentration of lipid hydroperoxides in the first 2-3mm of the product still makes up the vast majority of the lipid hydroperoxides in the entire product (Figure 9.19).

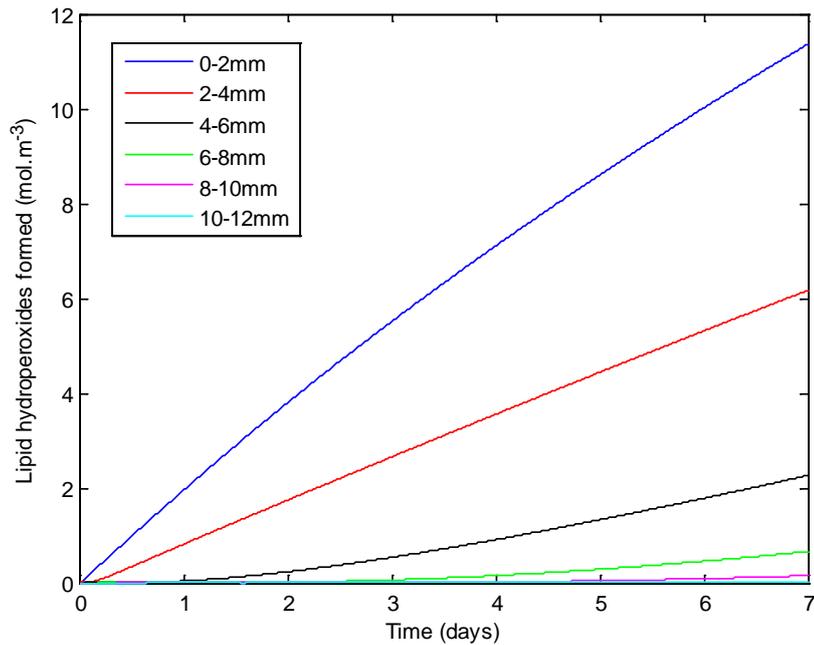


Figure 9.19: Results of a simulation of canola oil packaged in a 0.25mm thick film that has permeability 100x that of PET. Results show the lipid hydroperoxide concentration as a function of time for a range of distances from the surface of the oil.

9.4.3 1D modelling – effects of product surface area to volume ratio

Aside from the packaging permeance, the surface area of the packaging influences the rate of oxygen transfer in the system used above. The volume of the product, however, determines the quantity of reactants available for reaction. Having a product with a very small surface area to volume ratio should minimise the amount of oxygen available for reaction with a given quantity of reactants. This is best explained by Figure 9.20 which shows the amount of oxygen transferred to a 500 mL product stored in a cylindrical package as a function of the products surface area where the volume was maintained by changing the radius and height. End effects were included.

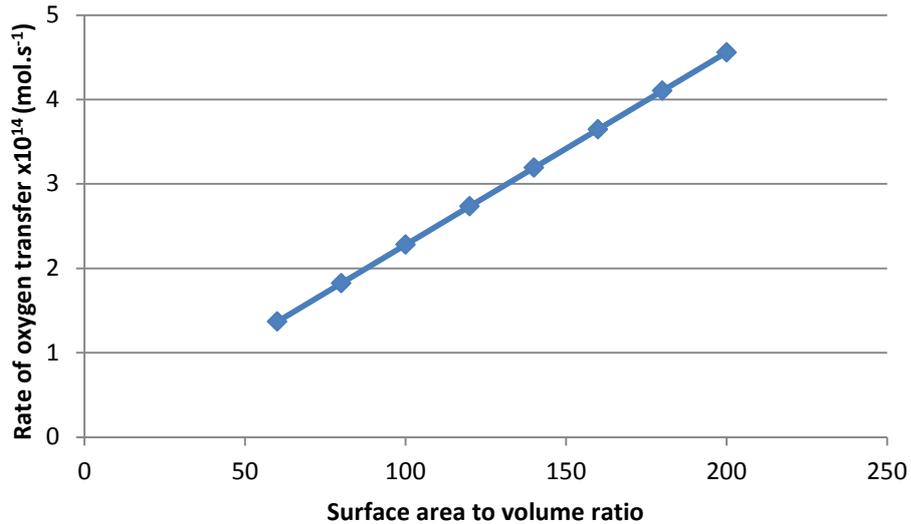


Figure 9.20: Rate of oxygen supplied through packaging for a 500 mL sample of oil in a cylindrical package. The oxygen permeance was $4.5 \times 10^{-18} \text{ mol.m}^{-2}\text{Pa}^{-1}\text{s}^{-1}$.

The results shown in Figure 9.20 suggest that doubling the SA/V will cause a doubling in the rate of oxygen transferred per unit of oil. Since the rate of reaction is limited by the rate of oxygen supply, then it would be reasonable to expect a significant reduction in the rate of oxidation to be caused by a reduction in the SA/V. Therefore, manufacturers should be looking to minimise the SA/V ratio for products that are susceptible to reaction with oxygen. Since the volume of most products is set, altering the shape of the package can be combined with the use of oxygen barrier polymers to reduce the rates of oxygen transfer.

The effects of packaging shape can be quite significant. For example, for a cylindrical product it can be shown that the surface area of the product is given by Equation 9.37 when end effects are included. For a given volume, V , the surface area of the product can vary. This is shown in Figure 9.21. If the rate of rancidity development is proportional to the amount of oxygen transferred, then a product with a low SA/V will have a longer shelf life than one with a high SA/V. This result suggests that significant cost savings can be made by not only choosing the correct packaging material, but by considering the package shape. Not only will the product last longer, the amount of packaging required can be significantly reduced.

$$SA = 2\pi r^2 + \frac{2V}{r} \quad \text{Eq. 9.37}$$

where SA is the surface area, r is the radius and V is the volume

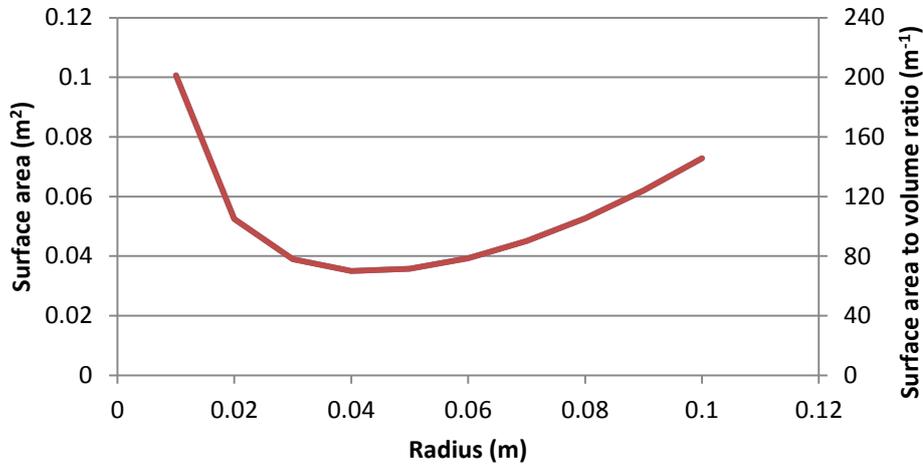


Figure 9.21: Relationship between the surface area and surface area to volume ratio with radius for a cylindrical product with a volume of 500 mL.

The line on Figure 9.21 has an inflection point. By differentiating the line $\left(\frac{\partial SA}{\partial r}\right)$ and determining the point where the differential is zero, the radius that results in the lowest surface area to volume ratio can be found. For this example, this is given by Equation 9.38.

$$r = \sqrt[3]{\frac{V}{2\pi}} \quad \text{Eq. 9.38}$$

9.4.4 Combining SA/V and oxygen concentration gradients

It is clear that the rate of oxygen supply (relative to the rate of oxygen consumption) is important in defining the amount of lipid oxidation that can occur. The depth that oxygen is present in a bulk oil packaged in a polymer film is key in defining the proportion of the oil that is oxidising at any point in time.

Previous modelling showed that the distance that oxygen is present in an oil packaged in a polymer film is between 0 and approximately 3 mm from the packaging surface.

The proportion of the oil that is reacting, therefore, must be dependent on the SA/V of the product. Figure 9.22 illustrates the influence that the SA/V ratio can have on the proportion of oil being oxidised (i.e. has oxygen present) for three different depths (L) in a cylindrical package.

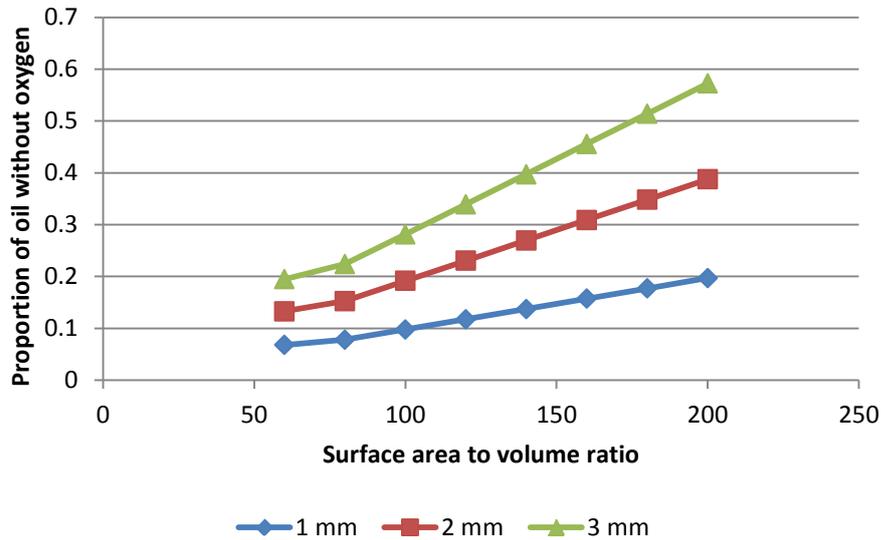


Figure 9.22: Proportion of oil with oxygen present as a function of the SA/V for a 500 mL sample of oil in a cylindrical package. The oxygen permeance was $4.5 \times 10^{-18} \text{ mol.m}^{-2}\text{Pa}^{-1}\text{s}^{-1}$ (23°C , 50%RH). End effects were included.

The results suggest that the use of a small SA/V would significantly reduce the proportion of oil that would be oxidising. This analysis is, however, misleading in that the rate of oxygen supply must be coupled with a low SA/V for the oxidation rate to be minimised. For example, if the SA were set and the SA/V minimised, then then all the oxygen that is supplied would be used to oxidise a very small proportion of the oil. Conversely, if the SA were minimised and the SA/V maximized, then the oxygen that is transferred would be smaller per unit volume of oil and would be used to oxidise a larger amount of oil.

The importance of this phenomenon is interesting as it suggests that it is better to supply a given quantity of oxygen to the entire oil sample than to supply the same amount of oxygen to a fraction of the oil sample. Even though the mass average lipid hydroperoxide formation would be the same for both scenarios the second, where oxygen is supplied to a fraction of the oil, would likely lead to higher levels of lipid

hydroperoxide breakdown products. It is these products which have been linked to rancidity (see Section 2.4 in Chapter 2) and it has been shown that only very low levels of these products are required before an oil is deemed rancid by sensory analysis (Jacobsen 1999).

9.5 Emulsions and packaging

From an oxidation point of view, the difference between a bulk oil and a simple oil-in-water emulsion (as used in Chapter 8) is simply the dilution of the oil in an aqueous phase and the removal of lipid hydroperoxide diffusion between droplets. This means that, when oxygen is supplied to an unmixed system, the oxygen must be consumed by the oil droplets at the surface before being able to move through further into the emulsion. If the ability to ensure that the oxygen transferred to an oil is used to form lipid hydroperoxides rather than rancid products is key to maximizing the shelf life in a given set of conditions, then it makes sense that oil-in-water emulsions would be less stable than their original bulk oils.

To explore this idea, a model was formulated using a mass average rate of reaction (see Chapter 8). For this analysis it was assumed that the rate constant 'k' was proportional to the concentration of oil in the emulsion (w/w). It was assumed that oxygen moved through the oil-in-water emulsion at a rate equal to that of the diffusion through the aqueous phase. Lipid hydroperoxide diffusion was not included in this model as it was assumed that lipid hydroperoxide diffusion between droplets (through the aqueous phase) is unlikely and assumed that the emulsion was physically stable (i.e. the droplets were suspended and unable to move). The model is shown in Equations 9.39 to 9.43.

$$\frac{\partial C_{O_2}}{\partial t} = D \frac{\partial^2 C_{O_2}}{\partial x^2} - r \quad \text{Eq: 9.39}$$

for $t > 0$ and $0 < x < L$

$$\frac{\partial C_{LOOH}}{\partial t} = + \frac{r}{Y} \quad \text{Eq: 9.40}$$

for $t > 0$ and $0 < x < L$

$$D \frac{\partial C_{O_2}}{\partial x} = \frac{P}{x_{film}} (P_{outside} - K C_{O_{2surf}}) \quad \text{Eq: 9.41}$$

at $x=0$

$$D_{LOOH} \frac{\partial C_{LOOH}}{\partial x} = 0 \quad \text{Eq: 9.42}$$

at $x=0$ and $x=L$

$$D \frac{\partial C_{O_2}}{\partial x} = 0 \quad \text{Eq: 9.43}$$

at $x=L$

The inputs for this model were: $k=4.91 \times 10^{-5} \text{ mol}^{1/2} \text{ m}^{-3/2} \text{ s}^{-1}$, $C_{LOOH_i}=4.97 \text{ mol} \cdot \text{m}^{-3}$, $D=2.4 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$, $A=0.01 \text{ m}^2$. The permeance data used for the flowing simulations is given in Table 9.2 and the reaction rates are taken from experimental data from Chapters 4, 5 and 6 and are explained during each simulation. Unless otherwise stated, the model was solved using finite difference approximations in MATLAB (R2011b). The MATLAB code used for these simulations is given in the Appendix (see folder ‘Chapter 9’)

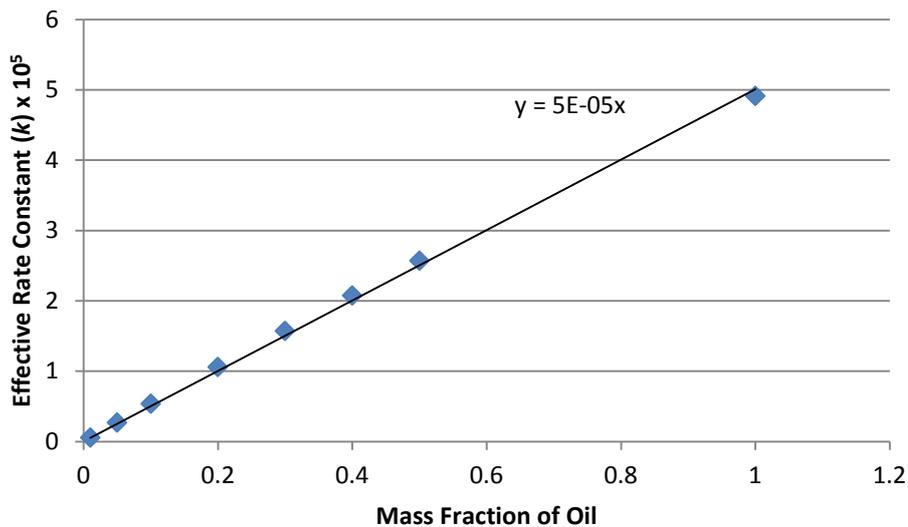


Figure 9.23: Figure shows the effective rate constant for a fish oil-in-water emulsion as a function of the mass fraction of oil in the emulsion.

As shown in Figure 9.23, the effective rate constant was calculated according to the mass fraction of oil. For this analysis the density of oil was taken as $910\text{kg}\cdot\text{m}^{-3}$. As expected, the results of simulations of different oil-in-water emulsions show that the rate of oxygen supply through the packaging must be greater than the rates of oxygen consumption for there to be reaction at any point other than the packaging surface. However, because the rate of oxygen consumption is significantly reduced by the dilution of oil in an emulsion, the rate of oxygen supply required to achieve an oxygen concentration gradient is much lower.

A worst case scenario of a 10% w/w canola oil-in-water emulsion packaged in 0.25 mm thick PP was simulated to show the maximum oxygen gradient that could be expected. The results, shown in Figure 9.24, demonstrate that it is possible for the system to contain oxygen at a depth of more than 25mm from the surface.

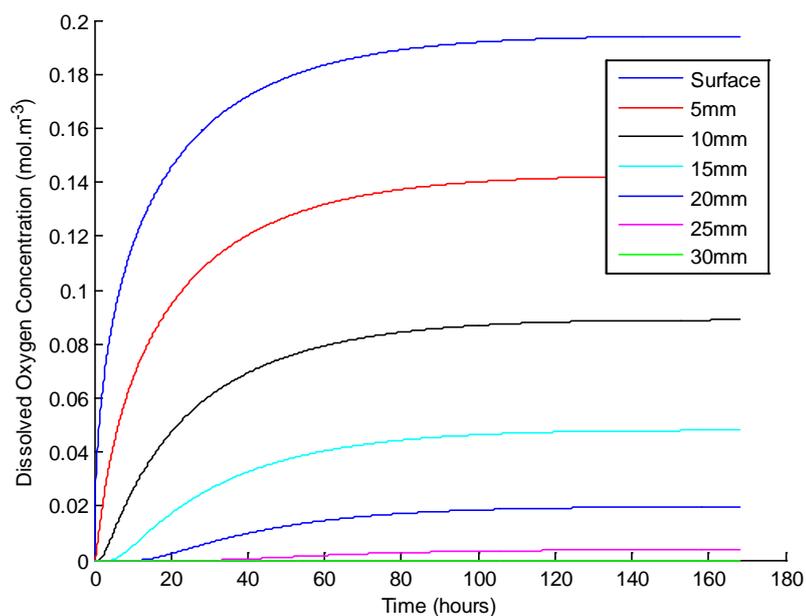


Figure 9.24: Figure showing the oxygen concentration as a function of time and position for a simulation of a 10% w/w canola oil-in-water emulsion packaged in PP.

Although the results suggest that the rate of oxidation is slow and the rate of lipid hydroperoxide formation is small, the rate of oxidation in the droplets of oil remains high. That is, the droplets are reacting as if they were small independent pockets of bulk oil. The aqueous phase is merely acting as an oxygen sump and carrier and is

preventing the lipid hydroperoxide formed from diffusing. The net result is that a small amount of the product will be oxidising and accumulating lipid hydroperoxides at an unhindered rate. Furthermore, the potential for secondary and tertiary product formation is greatly increased in an emulsion system of this type.

To illustrate the effect of immobilizing the oil in an oil-in-water emulsion, oxidation of a 20%w/w fish oil-in-water emulsion packaged in PET was simulated. The results, shown in Figure 9.25, suggest that the small volume of oil present at the surface of the product would oxidise very quickly while the rest of the emulsion would be unoxidised. Infact, compared to a bulk fish oil (using system inputs described earlier), any dilution by the formation of an oil-in-water emulsion would actually accumulate more lipid hydroperoxides per volume oil.

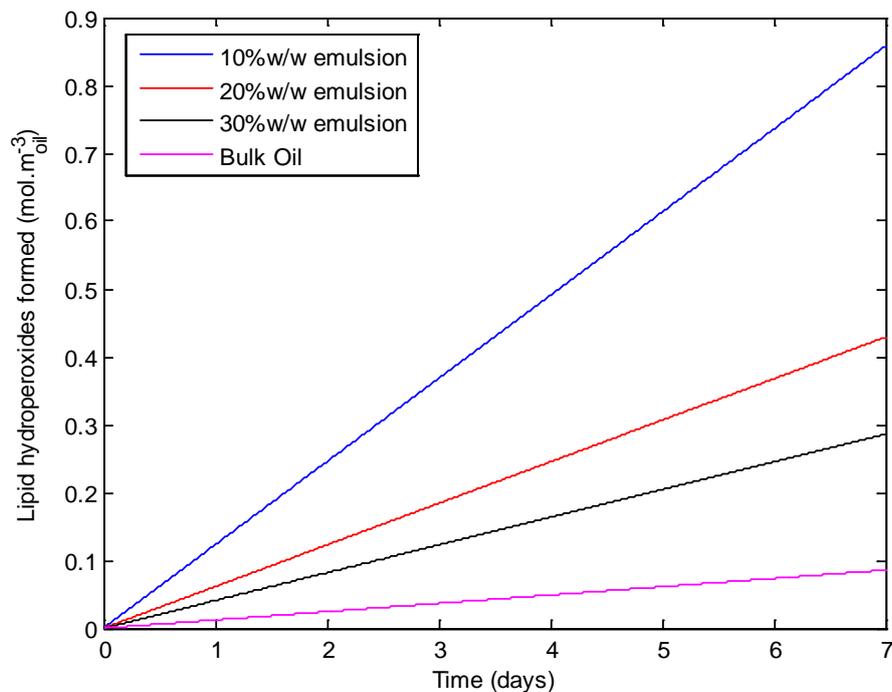


Figure 9.25: Figure shows the relationship between the lipid hydroperoxide formed per unit of oil and the concentration of oil in the emulsion.

9.6 Lipid hydroperoxide breakdown

Until now, the attention has been focused on rates of lipid hydroperoxide formation. Little discussion has been focused on the rates of lipid hydroperoxide breakdown as

the systems dealt with so far have had oxygen present for reaction. In these systems, the rate of lipid hydroperoxide formation has been assumed to be much greater than the rate of lipid hydroperoxide breakdown. In reality, the rate of change in the concentration of lipid hydroperoxides is equal to the sum of lipid hydroperoxide formation and breakdown rates. It is possible that, in the absence of oxygen, the rates of formation and consumption are not that dissimilar.

Lipid hydroperoxides breakdown to form radical species. How quickly lipid hydroperoxides breakdown and what becomes of the resulting radical species is very important. Figure 3.2 gives one such mechanism.

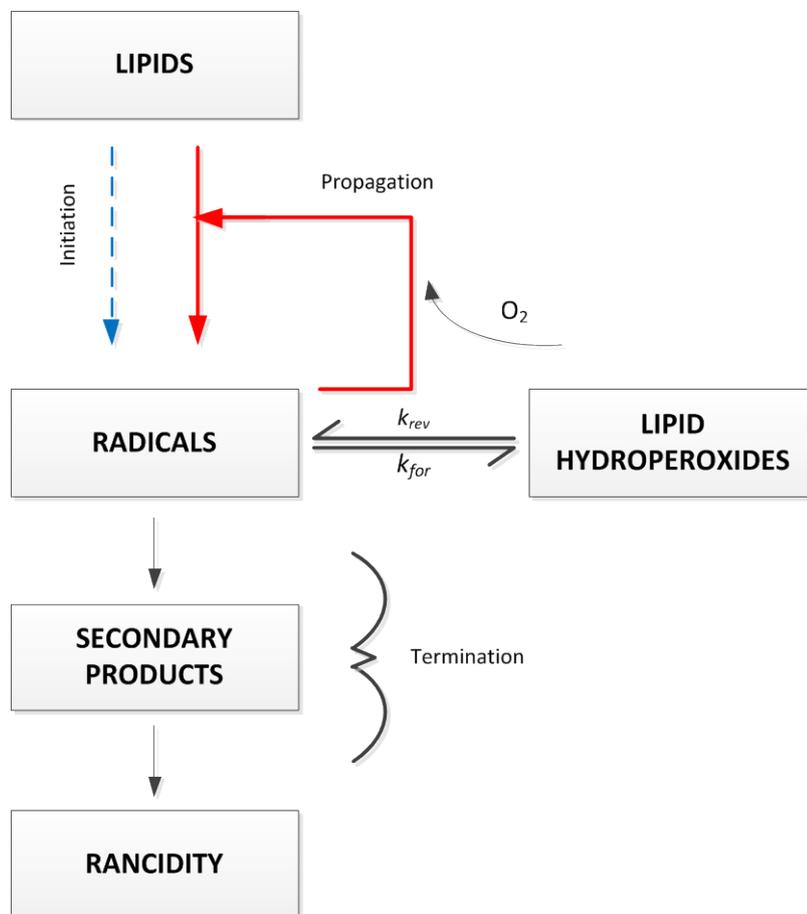
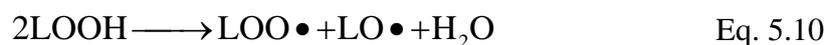


Figure 3.2: A simplified representation of the reaction scheme being modeled. Note the thickness of the lines connection the reactant/product species represents the proportion of the total reactions in each reaction stage during the early stages of lipid oxidation.

It seems that there are two ways that lipid hydroperoxides can breakdown. These are shown in Eq.'s 5.8 to 5.10. Essentially lipid hydroperoxides will either go through monomolecular breakdown or bimolecular breakdown to form radical species. According to current theories, monomolecular breakdown occurs during the initial stages of lipid oxidation while bimolecular breakdown occurs later in the oxidation process. The need for oxygen, however, is significantly reduced. That is, the breakdown and reaction of lipid hydroperoxides will continue without oxygen being present in the sample.



According to the Eq. 5.8 to 5.10, the rate of lipid hydroperoxide breakdown is proportional to the concentration of lipid hydroperoxides. Furthermore, Labuza (1971) suggests that bimolecular breakdown becomes significant only once the oil is significantly oxidised and has passed the point where the product is suitable for consumption.

According to Figure 3.2, there are two routes that lead to the consumption of radicals formed from the breakdown of lipid hydroperoxides. The radicals can be used to form new lipid hydroperoxides or they can be used to form non-radical species either directly or indirectly through a series of reactions. This is depicted below in Figure 9.26.

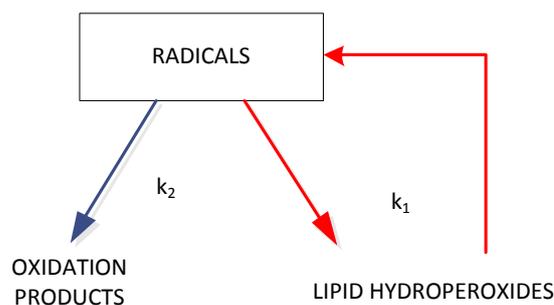


Figure 9.26: Figure depicts the reactions that compete for the radicals formed from the breakdown of lipid hydroperoxides.

The relative rates of formation of new lipid hydroperoxides and non-radical products will determine the rate at which the concentration of lipid hydroperoxides changes when the system is starved of oxygen. As there is going to be a change in the lipid hydroperoxide concentration during storage in the absence of oxygen, there should be a proportional change in the rate of lipid oxidation. This suggests that the rate of oxidation should actually slow during storage. To test this hypothesis, an experiment was setup.

Method

A 1kg sample of mixed fish oil (Bakels Ltd, NZ) was removed from storage, in a -30°C freezer, and heated to 37°C. Once at temperature, the sample was sparged with nitrogen at a flowrate of 0.5 L.min⁻¹ for five minutes. The oxygen concentration in the oil was measured and confirmed to be zero. The sample was then split into six equal parts and used to fill six separate reaction batch reaction vessels (see Chapter 4 for construction and operation). The batch reaction rigs, previously shown to be airtight, were sealed and placed in a 37°C incubator. Periodically, a batch reaction vessel was removed from the incubator and a batch oxidation carried out at 37°C according to the methods outlined in Chapter 4.

Results

The results of the batch oxidation (shown in Figure 9.27) show that the rate of oxygen consumption decreases as storage time increases. Because the rate of oxygen

consumption has been shown to be a function of the lipid hydroperoxide concentration, these results suggest that the lipid hydroperoxide concentration does decrease in the absence of oxygen.

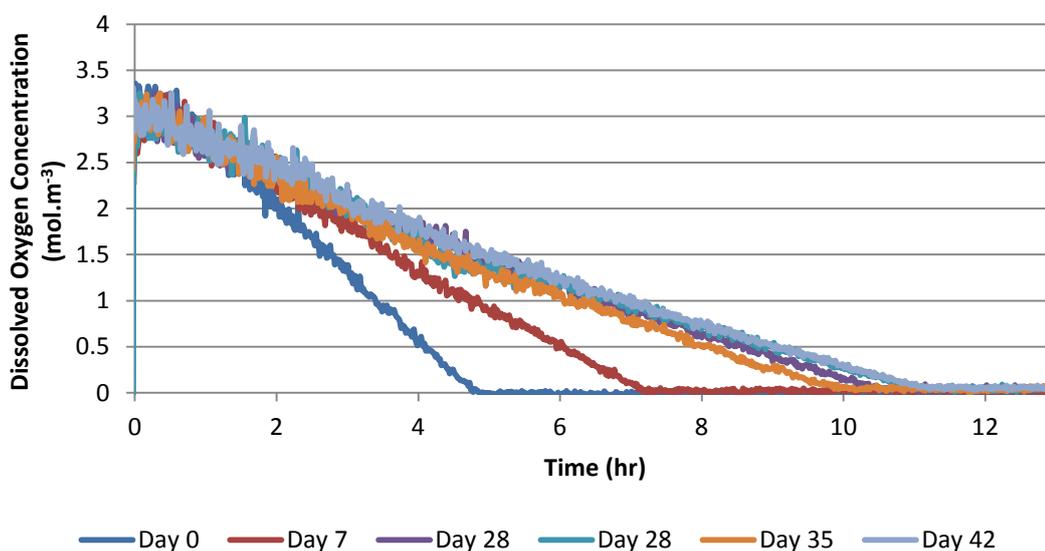


Figure 9.27 Results of batch oxidations of fish oil stored at 37°C in the absence of oxygen.

Interestingly, the decrease in oxygen consumption rates with storage time, shown in Figure 9.27, seems to reach steady state before 28 days of storage. Storing the oil for times longer than 28 days does not cause any further decreases in the rates of oxygen consumption. This suggests that a minimum or steady state lipid hydroperoxide concentration is reached. This suggests that the reformation of lipid hydroperoxides and the formation of non-radical products from the breakdown of lipid hydroperoxides follow different reaction kinetics. In this case, it seems that the reformulation of lipid hydroperoxides is less affected by the concentration of lipid hydroperoxide than the formation of non-radical products.

Using the results presented in Figure 9.27, the change in lipid hydroperoxide concentration can be estimated. To do this, the half order rate constant and initial lipid hydroperoxide concentration was fitted using the SOLVER function in Microsoft EXCEL to the data from a batch oxidation of the oil at the beginning of the experiment. The same rate constant was then used to fit the half order model to the data gained from a batch oxidation of 28 day old oil. The results of the fitting process are shown in Figure 9.28 below.

Applying the half order model to a batch oxidation of the oil before storage gives an initial lipid hydroperoxide concentration of 11.26 mol.m^{-3} and a rate constant of $2.55 \times 10^{-5} \text{ mol}^{1/2} \text{ m}^{-3/2} \text{ s}^{-1}$. An initial lipid hydroperoxide concentration of 2.49 mol.m^{-3} was calculated by applying the same model and rate constant to the batch oxidation of oil stored in the absence of oxygen for 28 days. These model fits are shown in Figure 9.28.

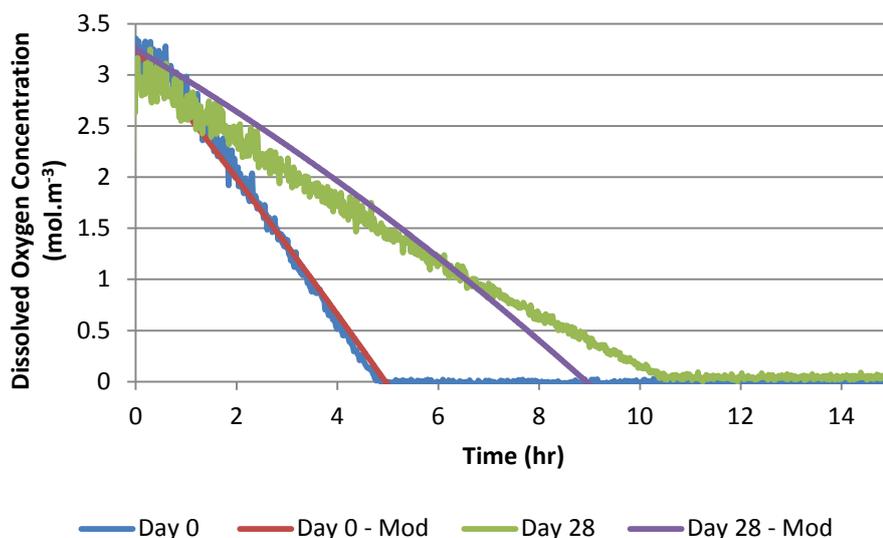


Figure 9.28: Results comparing the batch oxidation curves of oil stored at 37°C for 28 days and a control oil before storage.

The data gained after 28 days of storage does not result in a close fit to the half order model. The shape of the plot suggests that there may be a slight change in the reaction mechanism during the storage period. This may signify a shift from the consumption of oxygen by formation of lipid hydroperoxides to the consumption of oxygen by the oxidation of secondary and tertiary oxidation products.

9.6.1 Implications of lipid hydroperoxide decrease

The above results suggest that, over time, the concentration of lipid hydroperoxides will tend toward a limit of zero. This seems quite plausible. The extent of oxidation that occurs during the period of lipid hydroperoxide decline is, however, likely to be

quite significant and entirely dependent on the hydroperoxide concentration at the point where oxygen is removed/depleted in the oxidation system. Unfortunately, there is little information available in the literature that can be used to describe oxygen starved systems.

The methods used by most researchers have been in systems where the effects of oxygen supply rates have been overlooked. For example, work by Garcia-Martinez *et al.* (2009) carried out lipid oxidation in closed 20mL vials containing 14 g of samples. Depending on the oxygen consumption rates, there is likely to be no oxygen present in the system other than at the oil/air interface. The complexity that arises through the creation of oxygen and lipid hydroperoxide concentration gradients makes any thorough analysis impossible. The ability to determine whether the system is in an oxygen rich or anoxic environment is key to determining the reaction mechanism that is occurring. Unfortunately this has been overlooked by the majority of researchers.

Despite the methods used by most researchers, the qualitative effects of oxygen limited systems can be seen literature. For example, Alamed *et al.* (2009) formed corn oil-in-water emulsions and stored 1 g samples (unmixed) in 10 mL vials. In this case it is safe to assume that the oxygen in the headspace would be enough to sustain oxidation for the experiment. The results gained from this experiment, shown in Figures 9.28a and 9.28b, suggest that the formation of lipid hydroperoxides coincides with the formation of tertiary oxidation products (hexanal).

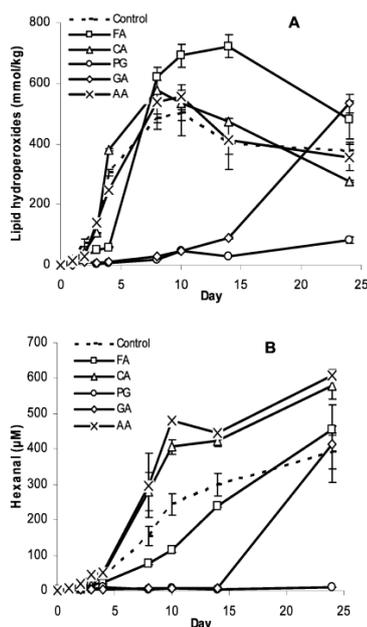


Figure 9.29 a&b: Results gained from work by Alamed *et al.* (2009) shown the evolution of hexanal and the formation of lipid hydroperoxides from corn oil-in-water emulsions.

These results suggest that hexanal is formed at the same time as lipid hydroperoxides. According to Labuza (1971), there should be lag between the formation of lipid hydroperoxides and the formation of tertiary products as the tertiary products are formed by the breakdown of lipid hydroperoxides. Initially, the oxygen in the system is predominantly used to form lipid hydroperoxides. In this system, however, the oxygen in the oil is used up very quickly leaving the headspace as the only source of oxygen. Lipid oxidation using oxygen occurs at the liquid/gas interface. Assuming the rate of oxygen consumption is greater than the rate of oxygen diffusion through the product, the bulk of the emulsion will be without oxygen. Because the system is an emulsion, the droplets of oil at the surface of the product will oxidise before the droplets further from the surface. In this case, the lipid hydroperoxides formed at the surface cannot readily diffuse away from the surface and, as a result, are further oxidised to tertiary products. This explains the early detection of hexanal in the headspace of the product.

If the system were a bulk oil rather than an emulsion, the lipid hydroperoxides would be more readily able to move away from the oil/air interface. In this case, it is more likely that more lipid hydroperoxide are built up before tertiary products can be

detected in the headspace. If hexanal were measured in this system, it is likely that the time for hexanal detection would be greater than the emulsion system. This is, of course, assuming that the effects of diluting the oil by the formation of an emulsion is not significant – something that has been proven to not be the case.

9.7 Conclusions

The results of modelling and experiments have shown that:

- 1) The relative rates of oxygen supply and consumption are likely to determine whether oxygen is present away from the packaging surface
- 2) Lipid hydroperoxide diffusion is significant and has an impact on the lipid hydroperoxide concentration gradients within a sample of oil
- 3) The majority of lipid hydroperoxides are formed at or near the surface of an oil packaged in a polymer film and that the extent of oxidation is likely to be much larger for the oil close to the surface compared to that further away from the surface.
- 4) The diffusion of lipid hydroperoxides is likely to aid the stability of an oil as the supply of oxygen will be used to create new lipid hydroperoxides rather than secondary oxidation products.
- 5) Oil-in-water emulsions are likely to be significantly less stable (oxidative) than their bulk oils are the oxygen transferred per volume oil is greater than that of a bulk oil and because lipid hydroperoxide diffusion is either zero or much lower than that in a bulk oil.

The effects of storing bulk oils in oxygen free environments requires some additional attention and is recommended as the next step required to move closer to understanding and developing a fully mechanistic model to predict lipid oxidation rates in oxygen limiting environments. Alongside this investigation, a set of experiments is required to validate the models developed above. This is outside the scope of this project due to the long timeframes required for experiments to achieve measurable changes in PVs or oxidation rate. These experiments, however, are needed before applying these models in shelf life predictions.

CHAPTER 10
GENERAL DISCUSSION AND
CONCLUSIONS

10.0 Introduction

This thesis has focused on what is an extremely complex topic. Despite being under the academic microscope for decades, the mechanisms of lipid oxidation are not understood to the point where accurate shelf life predictions can be made for any system. Much of the research carried out to date has been focused on the chemistry of lipid oxidation in simplified systems and, in many cases, is limited to the relative and qualitative effects of additives such as antioxidants. There are still gaps in the pool knowledge required that would allow for quantitative shelf life predictions.

This chapter will focus on bringing the contributions made in this work together and the application of these ideas to industrial processes. During the discussions, the remaining information not already available in this thesis or in literature will be alluded to in the hopes of creating a structure for future research that will fulfill the requirements needed to predict the shelf life of lipid oxidation prone foods.

10.1 Key outputs from this thesis

This thesis, through a series of modelling and experimental exercises has provided the following key outputs:

- 1) A new method of measuring lipid oxidation rates
- 2) A new representation of the lipid oxidation pathway that includes an equilibrium relationship
- 3) A simple but mechanistically based model that explains the rates of lipid oxidation in bulk oils
- 4) A mechanistic way of showing the effects, on lipid oxidation rates, of primary antioxidant addition
- 5) A mechanistic way of quantifying the effects of diluting bulk oils by the formation of oil-in-water emulsions
- 6) Evidence as to the effects of storing oils or oil-in-water emulsions in oxygen starved environments
- 7) Some initial indication as to the importance of lipid hydroperoxide breakdown in the absence of oxygen.

10.1.1 New method of measuring lipid oxidation rates

Through an extensive literature review, it was found that many of the methods used for measuring lipid oxidation rates are either time consuming, highly sensitive to external conditions or sample makeup, require expensive equipment or require significant knowledge of the reaction mechanism. It was found that many industrial users choose to use a simple but empirical measures such as the Peroxide Value (PV) or accelerated shelf life tests such as the Rancimat.

Although useful, measures like the PV were shown to be difficult to link to the shelf life of an oil and cannot be used for fast measurement of oxidation rates. Furthermore, measuring a PV of an oil-in-water emulsion or any other product that is not a bulk oil requires the oil to be extracted first. This, obviously, makes a one-off measurement of a reaction rate nearly impossible.

The Rancimat, however, does give rate information in a short period of time. To do so, however, it relies on high reaction temperatures (~100°) which is a risky option as there are a number of reactions that make up lipid oxidation and it is not safe to assume that each reaction will react the same to a change in temperature. Furthermore, methods like the Rancimat test have been shown to be sensitive to volatile products which are given off during lipid oxidation.

To avoid the significant complications found in current measures, a new method of measuring lipid oxidation rates by the batch oxidation of bulk oils and oil-in-water emulsions was created and validated against the commonly used Peroxide Value measurement technique. Briefly, the method (described in full in Chapter 4) was based on the measurement of oxygen consumption rates during a batch oxidation. The method was shown to be repeatable, fast and able to give rate data with a single measurement. This method was validated using a number of different oils, oils with antioxidants, oil-in-water emulsions and even red wine (wine not explained in this thesis but an interesting note).

10.1.2 New mechanistic model and application with bulk oils

An exploration into the applicability of literature models was conducted using the newly formed methodology. Of the two mechanistic models available, only one was found to be applicable and followed trends gained from validation experiments. The same analysis found that more complex models utilizing a vast array of chemical reactions and reaction constants are inherently erroneous due to the lack of indepth knowledge of the chemical reaction system and the complexity caused by the multitude of competing and complimentary reactions that take place.

The half order model developed during this thesis was validated against batch oxidations of mixed fish oils. The model was then modified to relate the consumption of oxygen with the formation of lipid hydroperoxides. The modified model was then used to predict the rates of lipid oxidation of mixed fish oil samples of varying degrees of oxidation and was shown to be an excellent fit. Furthermore, it was shown that the Arrhenius law could be applied to predict the changes in reaction rates caused by reaction temperature changes.

10.1.3 Model and modifier for primary antioxidant addition

The effects of adding BHA antioxidant to samples of bulk mixed fish oil were analysed. It was hypothesised that the addition of the antioxidant would either yield a lag phase in which no oxygen would be consumed while the antioxidant quenched radical species or reduce the overall reaction rate without the formation of a lag phase. It was thought that the introduction of a lag phase would only occur if the production of radicals was extremely slow, a condition that would only occur in a very 'clean' sample of oil. Rather, it was suggested the reaction rate would be reduced by an amount proportional to the concentration and effectiveness of the antioxidant. This is indeed what was found during experiments.

The addition of BHA antioxidant brought with it a reduction in the rate of oxygen consumption in batch lipid oxidations. It was found that the use of a modifier for the rate constant within the half order model could successfully predict the effects of

adding varying amounts of antioxidant to bulk oils. It was also found that the magnitude of the modifier was linearly proportional to the concentration of the antioxidant. The magnitude of the modifier would be antioxidant type and system specific and should be measured through batchwise oxidation experiments.

10.1.4 Model for oil-in-water emulsions

The half order model used in bulk oil systems was then applied to oil-in-water emulsion systems. It was found that the half order model was still applicable and that the magnitude of the rate constant was inversely proportional to the concentration of oil in the emulsion. It was also shown that the amount of lipid oxidation that could occur during a single batch oxidation was inversely proportional to the concentration of oil in the emulsion as the aqueous phase effectively acts as a sump of oxygen for reaction. This increases the amount of oxygen that is available for reaction by a given quantity of oil. Finally, it was shown, through modeling exercises, that changes to the surface area to volume ratio (SA/V) of oil droplets does not have a significant impact on the rates of lipid oxidation. The effects of changes to the SA/V, however, may be different when surface active compounds such as amphiphilic antioxidants are added to the reaction system.

10.1.5 Lipid oxidation in systems with low oxygen supply rates

Finally, it was shown that the possible rates of oxygen consumption via lipid oxidation tend to be greater than the rates of oxygen supply from the atmosphere when a bulk oil is stored open to the air. It was shown that, apart from the very surface of the oil, there is no oxygen present in the system at any time for most bulk oils packaged in polymer films. The ability for lipid hydroperoxides to diffuse was shown to likely have a significant impact on the rate of rancidity development. It was hypothesised that the ability for lipid hydroperoxides to form at the surface of an oil and diffuse away rather than continue to react and form secondary products is likely to result in a greater shelf life (from a sensory perspective) compared to a system where the lipid hydroperoxides aren't free to diffuse.

It was also shown that oil-in-water emulsions are likely to develop rancidity faster than bulk oils as lipid hydroperoxide diffusion is unlikely (would require movement through the aqueous phase) and because the depth oxygen can travel into the emulsion before it is completely consumed.

10.1.6 Lipid hydroperoxide breakdown – initial investigations

Through the application of the half order model without the inclusion of a term accounting for the breakdown of lipid hydroperoxides, it was shown that the rate of lipid hydroperoxide breakdown was significantly slower than the rate of formation when oxygen was present in the sample. The breakdown of lipid hydroperoxides in the absence of oxygen was suggested as being capable of slowing the rates of lipid oxidation in systems starved of oxygen. It was shown, through long term storage tests, that the rates of oxygen consumption during batch oxidations slowed significantly as bulk oils are stored in the absence of oxygen. This suggested that there was a net reduction in the lipid hydroperoxide concentration.

The competition for radical species formed by the breakdown of lipid hydroperoxides was suggested as a method of explaining the development of rancidity when a product is stored in an oxygen free environment. The rates of reformation of lipid hydroperoxides by reaction of radicals with other lipids was thought to be significantly faster than the rates of the removal of radicals by tertiary product formation. Should this be true, there would be a gradual increase in the concentration of rancid flavor forming tertiary products. This issue, however, requires significant attention as it is likely to be the shelf life determining factor. As the rates of lipid oxidation in the presence of oxygen are significantly faster than without oxygen, most lipid oxidation prone products are packaged in oxygen barrier polymers, bottles or cans. The simple fact that these products still develop some degree of rancidity suggests that rancidity development in low oxygen environments should be studied further.

10.2 Industrial applications of work so far

Although there are still some areas that require investigation, the work done to date does have some immediate applications in both industry and research. The following discussions are aimed at providing a glimpse at what the work in this thesis can be used for.

10.2.1 Measuring oxygen consumption rates – a quality control tool

Perhaps the most obvious application of the work done for this thesis is the application of the oxygen consumption measurement method as a way of determining the quality of oil to be used in products. Currently, it is common practice for industry processors to rely on chemical methods of determining the quality of oils. Most use a peroxide value (PV) measurement much like the one used in this thesis. This, however, can give misleading results.

The PV measurement, as discussed earlier, is an empirical measure that gives an estimate of the lipid hydroperoxide concentration in oil. The estimate is dependent on a skilled technician to be able to accurately determine the end point of the titration. As such, results can vary if multiple technicians carry out the work. Furthermore, the lipid hydroperoxide concentration will peak and fall again which means that a low PV measurement could mean the oil is either fresh and unoxidised or moving rapidly towards rancidity.

The variable results and the difficulty interpreting PVs can make analysing oxidation systems difficult, but do not make the PV measure redundant and in need of replacement. The inability to determine the rate of change in the lipid hydroperoxide concentration with a single measurement and the inability to do so in a sample that is anything other than a bulk oil, however, are serious limitations. Unlike the PV titration method, the batch oxidation method developed in this thesis can be used for these measurements.

By using the batch oxidation methods outlined in the Chapter 4, it is possible to determine the rate of oxygen consumption. Using the half order model developed

during this thesis, it is then possible to estimate the initial lipid hydroperoxide concentration and the rate constant. If more accuracy is needed, this can be repeated for multiple consecutive batch oxidations. This will determine the difference between a fresh oil and one which has passed the peak lipid hydroperoxide concentration (i.e. determine which side of the PV curve the oil is on).

Furthermore, the same tests can be applied to other ingredients such as oil blends, or any other oxidation prone ingredients. If other ingredients are to be used, care must be taken to ensure the oxidation mechanism can be described with a half order model. If this model does not fit, then some additional experimentation would be needed.

This type of method can be used alongside or as a replacement for the PV measurements used currently. This method may also mean that extra methods (iodine, anisidine, TBARs values) often employed to make up for the shortcomings of the PV measurement may no longer need to be conducted. This would save both time and money as the need for expensive chemicals and the time spent by laboratory technicians would be significantly reduced.

10.2.2 Process and machinery design and ingredient selection

The ability to predict the rates of lipid oxidation that would occur after the oil has been processed, however, would be useful in process and machinery design. Through the application of the half order model, it is possible to determine the amount of lipid hydroperoxides formed during processing. This allows manufacturers to qualitatively determine the effects of exposing an oil to oxygen during manufacture, without the need to make time consuming measurements.

By setting the maximum initial lipid hydroperoxide concentration a product may have at the point of packaging and by measuring the concentration of lipid hydroperoxides in the ingredient oils, it is possible to determine if the oil is fit for production or if the production is suitable for the oils available. That is, the difference between the lipid hydroperoxide concentration of the oil ingredient and that of the oil at the point of packaging is equal to the maximum amount of lipid hydroperoxides that can be

formed during production. Applying this principle can help avoid products being sent to the market place only to be recalled because of rancidity development.

10.2.3 Effectiveness of added antioxidants and other reactants

There is a significant amount of literature broadly foccued on the effects of antioxidants on lipid oxidation. To date, much of the literature does not contain quantitative information that can be safely applied by food manufacturers. The sheer number of different antioxidants available coupled with the way the antioxidants function in a food system make the quantitative prediction of the antioxidant's effect without experimentation very difficult.

Currently, most researchers and industrial manufacturers use a range of chemical and sensory measures that rely on accelerated shelf life tests. Although generally successful, accelerated shelf life tests cannot guarantee a reliable answer as they suffer from two problems.

- 1) The tests are carried out at high temperatures to speed up reactions and in doing so promote reactions that do not normally occur at normal storage temperatures. This can lead to a host of product changes that may not have existed at lower temperatures.
- 2) Accelerated shelf life tests still require a number of days to complete. They are not useful for giving quick results and cannot be used as quality control tools.

Although the oxygen consumption methods cannot be used to determine the exact extension of the shelf life caused by the addition of an antioxidant, without extra study, they can be used to quantify the relative effectiveness of two or more antioxidants. For example, if a comparison between alpha toccopherol (Toc) and butylated hydroxyanisole (BHA) was needed, then batch oxidations of oils with added BHA and Toc could be carried out and compared with one another. If the BHA brought a greater decrease in the rate constant compared with Toc, then the effectiveness of the BHA would be greater than the Toc. This, of course, could be

coupled with the models presented in Chapter 7 and can be used to quantify the effectiveness.

Using the same methods, it is possible to determine the effectiveness of a single antioxidant by comparing batch oxidations of bulk oil with and without the antioxidant. Again, the half order model can be used to determine the rate constants which can be compared with each other. For example, if the addition of 250ppm of BHA to an oil resulted in a 20% reduction in the rate constant compared with a control sample, then the shelf life could be expected to be approximately 20% longer. If work is done to determine the rates of lipid hydroperoxides breakdown in the absence of oxygen, then the effectiveness of the antioxidant in an oxygen-free system can be determined. This work would be highly beneficial to which ever manufacturer were to apply it.

10.2.4 Effects and application in manufacturing and storage

The methods and results gained from modelling during the development of this thesis have, aside from showing the effects, or lack thereof, of changing the droplet size distributions and oil concentrations, allowed for real time testing of the rates of lipid oxidation and the quality of the oils within the emulsion without the need to separate the oil and water phases of the emulsion.

It is common for researchers to create emulsions to conduct their oxidative studies only to have to break the emulsion to determine the amount of oxidation that has occurred. In studies used for this thesis it was found that breaking emulsions required either harsh freeze thaw processes or chemical methods, both of which take time and will undoubtedly have an effect on the oil being tested. Simply centrifuging the oils was found to be an ineffective method separating the oil from the aqueous phase. In any case, the reliability of such emulsion breaking methods is questionable. By using the methods developed in this thesis to test the state of an oil-in-water emulsion without needing to break the emulsion, the introduction of such errors can be avoided.

The ability to test the state of an oil without the need to first break the emulsion is not limited to simple oil-in-water emulsion systems. The addition of antioxidants, emulsifiers and other potentially oxidation affecting agents can be analysed and their effects quantified while still reacting in the system. Assuming the emulsion remains physically stable, it is even possible to determine the rates of oxygen consumption in products that are exposed to large quantities of oxygen by continuously sparging oxygen (or a mixture of oxygen and nitrogen) through the sample in the rig.

10.2.5 Oxidation rate determination – a whole food approach

Lipid oxidation is a complex phenomenon that, thanks to its chameleon-like ability to change in different environmental conditions, cannot be analysed in a single system with the expectation that the results can be extrapolated to all other systems. In fact the only way to gain a true reflection of the rates of lipid oxidation in a given system is to measure the lipid oxidation rates in that system. The methods developed for this thesis do just that.

From a shelf life prediction point of view, measuring the net effect of all the constituents of a real food product is more useful than attempting to characterise the individual and combined effects of all the ingredients in the food. Simply put, the system is far too complicated to do so without significant advances in the knowledge that is currently available. By applying this net effect method, it is also possible to quantify the effects, on oxygen consumption rates, of changes to the food product. Such effects will undoubtedly differ from the effects that would be expected had the same change been made to a simplified model system.

The ability to test in real food systems is not limited to changes in product formulation. The effects, on oxygen consumption rates, of processing and storage conditions can also be tested in real food systems. The effects of changes in temperature, for example, are nearly impossible to predict in real food systems due to the complex relationship between all the ingredients in a food product. The net effects can, however, be measured and used to predict the effects of changes to processing conditions. This allows for optimisation calculations to be carried out. For example,

it is possible to find the best conditions to achieve a required level of microbial death while minimising the degree of oxidation caused by the process.

10.3 Conclusions and recommendations

This thesis, through a series of modelling exercises, has provided a series of new and exciting ideas that not only increase our understanding of lipid oxidation systems but also moves toward a practical prediction tool that could be applied in industry. The development of a fast, accurate and reliable method of measuring lipid oxidation rates was key to the development and validation of mechanistically based mathematical models that explain the lipid oxidation process in the presence of oxygen and antioxidants as well as the effects of diluting oils by forming oil-in-water emulsions.

The processes used in this thesis led to the ability to present the three stage lipid oxidation system in a slightly different way that takes into account the equilibrium relationship between the semi-stable lipid hydroperoxides and highly reactive radical species. This understanding, coupled with the models constructed, allowed for the development of fast and reliable methods of characterising oils and oil-in-water emulsions that can be used to make quantitative predictions about the rates of lipid oxidation.

Finally, this thesis has outlined the work required to extend this research. Models developed in Chapter 9 provided an insight into the potential effects of limiting the oxygen supply to a lipid oxidation system. The models, however, require validation which would require long-term shelf life tests, something which was outside the scope of this thesis. Alongside this validation exercise, an investigation to quantitatively describe the rates of lipid hydroperoxide breakdown and subsequent termination product formation is needed to be able to complete a full shelf life predicting model.

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APPENDIX

The following MATLAB code has been included on the attached CD. The list below outlines the Function and Script files used in simulations throughout this thesis. The order presented below is based on separating the Function and Script files and does not represent the order that they were used and appear in this thesis.

Folder – Chapter 5

Function File: Takahashi.m

- Function file used to simulate the Takahashi model. Function file used by script files, 'Sensitivity_Analysis.m', 'Sensitivity_Analysis_2.m', and 'Comparing_Reduced_Models.m'

Reduced_Takahashi.m

- Function file used to simulate the reduced Takahashi model. Function file used by script file 'Comparing_Reduced_Models.m'.

Labuza_Equivalent.m

- Function file used to simulate the Labuza model using the inputs given in the Takahashi model. Function file used by script file 'Comparing_Reduced_Models.m'

Script File: Sensitivity_Analysis.m

- Used to conduct a sensitivity analysis of the Takahashi model beginning with an initial lipid hydroperoxide concentration of 0 mol.m^{-3} .

Sensitivity_Analysis_2.m

- Used to conduct a sensitivity analysis of the Takahashi model beginning with an initial lipid hydroperoxide concentration of 25 mol.m^{-3} .

Comparing_Reduced_Models.m

- Used to compare the full Takahashi, reduced Takahashi and Labuza models.

Folder – Chapter 6

Script File: Example_Saturation_Calculator.m

- File is used to calculate the solubility of oxygen in oil from the experimental data presented in the EXCELL spreadsheet also shown in this folder.

Function File: Takahashi.m

- Function file used to simulate the Takahashi model. This file is used by the script files, 'Batch_Oxidation_Takahashi.' and 'Rate_of_LOOH_O2_formation.m'

Script File: Batch_Oxidation_Takahashi.m

- Script file used to simulate a batch oxidation according to the Takahashi model.

Script File: Rate_of_LOOH_O2_formation.m

- Script file used to simulated a batch oxidation according to the Takahashi mode and present the rate of change in lipid hydroperoxide and oxygen concentrations.

Folder – Chapter 7

Function File: function_chpt7.m

- Function file used to simulate the consumption of oxygen and formation of lipid hydroperoxides using the half order relationship developed in Chapter 6

Function File: function_chpt7_2.m

- Function file used to simulate the consumption of oxygen, formation of lipid hydroperoxides and consumption of antioxidants when antioxidants are included.

Function File: function_chpt7_no_oxygen_limits.m

- Function file used to simulate the formation of lipid hydroperoxides using the half order relationship developed in Chapter 6 when oxygen is not limiting.

Function File: function_chpt7_2_no_oxyegn_limits.m

- Function file used to simulate the formation of lipid hydroperoxides using the half order relationship developed in Chapter 6 when oxygen is not limiting and antioxidants are present

Script File : Oxygen_consumption_BHA_added.m

- Script used to simulate a batch oxidation including the consumption of oxygen, and antioxidants as well as the formation of lipid hydroperoxides.

Script File : Oxygen_consumption_BHA_added_no_oxygen_limits.m

- Script used to simulate a batch oxidation including the consumption of oxygen, and antioxidants as well as the formation of lipid hydroperoxides when oxygen is not limiting

Folder – Chapter 8

Analytical Solutions:

The following files were used to solve the analytical solutions constructed for Chapter 8:

- Analytical_consumption_rate_vs_LOOH.m
- Analytical_consumption_rate_vs_LOOH_2.m
- Analytical_LOOH_vs_time.m
- Analytical_oxygen_consumption.m

The name of each of the files for the analytical solutions outline the output from the solution.

Function File: function_emulsion.m

- Function file includes the finite difference approximations for the one dimensional model developed in Chapter 8. This file is used to simulate various types of oil-in-water emulsions.

Script File: twenty_percent_emulsion_droplet_sizes.m

- File used to simulate the effect of changing the droplet size has on the rates of lipid oxidation

Script File: twenty_percent_emulsion_investigations.m

- Script file is used for initial investigations and to simulate a simple 20%w/w oil-in-water emulsion

Script File: twenty_percent_emulsion_LOOH_diffusion.m

- Script file used to show the effects that diffusion of lipid hydroperoxides in oil droplets has on lipid oxidation rates.

Script File: twenty_percent_emulsion_SA_to_V_ratio.m

- Script file used to simulate lipid oxidation in emulsions with droplets with different SA/V.

Folder – Chapter 9

Function File: Function_one_dimensional_bulk_oil.m

- Function file used to simulate lipid oxidation in an unmixed system where oil is exposed to the air.

Function File: Function_one_dimensional_bulk_oil_packaging.m

- Function file used to simulate lipid oxidation in an unmixed oil where the oil is packaged in a polymer film.

Script File: One_dimension_bulk_oil_in_highly_permeable_film.m

- Script file used to simulate the rates of lipid oxidation in a bulk oil that is packaged in a highly permeable polymer film

Script File: One_dimension_bulk_oil_open_air.m

- Script file used to simulate the rates of lipid oxidation in a bulk oil that is exposed to air without packaging

Script File: One_dimension_bulk_oil_open_air_no_DLOOH.m

- Script file used to simulate the rates of lipid oxidation that occur when a sample of bulk oil is exposed to air without packaging assuming that lipid hydroperoxide are not able to diffuse.

Script

File:Once_dimensional_bulk_oil_packaging_LOOH_proportional_analysis.m

- Script file used to simulate the rates of lipid oxidation when a bulk oil is packaged in a polymer film. Simulation output is the concentration of lipid hydroperoxide in segments of the oil.

Script File: One_dimensional_oxygen_supply_rate.m

- Scriptfile used to simulate the effect that oxygen supply rate has on the rates of lipid oxidation in a bulk oil packaged in a polymer film.

Script File: Ten_percent_canola_oil_emulsion_in_PP.m

- Script file used to simulate the oxidation of a 10% w/w canola oil-in-water emulsion that is packaged in a 0.25mm thick polypropylene film.

Script File: Twenty_percent_fish_oil_emulsion_in_PET.m

- Script file used to simulate a 20% w/w fish oil-in-water emulsion packaged in 0.25mm thick PET.