Shelf life of Goat Infant Formula Powder

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Abstract

Oxidative rancidity was found to be a problem in goat milk infant formula powder. Oxidative rancidity results from the lipid oxidation processes, where oxygen reacts with unsaturated fatty acids from milk powder to produce lipid hydroperoxides and radicals, the primary oxidation products. These primary oxidation products are odourless; however, they are very reactive to breakdown into hydrocarbons, aldehydes and ketones. Aldehydes have low flavour threshold limits and are responsible for causing the rancid flavour in the milk powder.

Peroxide value (PV) is one of the most widely used tests for oxidative rancidity as it is a measure of the concentration of lipid hydroperoxides; however, it is difficult to provide a specific guideline relating PV to rancidity. A reliable test is needed to determine whether the goat milk infant formula powder is unacceptable due to oxidative rancidity to the consumer. It was found that oxygen was a useful parameter to monitor lipid oxidation. Oxygen is the main reactant in lipid oxidation, and the rate of oxygen consumption is a useful tool to track lipid oxidation. Hexanal was determined to be the main secondary oxidation product responsible for the off flavour of milk powder.

An experiment of accelerated storage trials for two infant formula products (Powder A and Powder B) was conducted by using a range of higher temperatures from 37°C to 57°C over a period of 12 to 24 weeks. Headspace oxygen and headspace hexanal of the milk powder in the glass vials were measured over the storage period. Sensory analysis was also conducted in parallel with the storage trial to provide a relationship between the sensory score and hexanal concentration, ultimately determining the unacceptable flavour threshold limit for hexanal concentration. The chemical kinetic constants were estimated by fitting a general n^{th} order reaction with an Arrhenius law model with the concentration of oxygen obtained experimentally. The model followed half order reaction for both products. The Arrhenius rate constant, k_0, and activation energy, E, were found to be 7.8×10^{9} $\%^{0.5}$ $week^{-1}$ and 62.0 kJ mol^{-1} for Powder A and 1.34×10^{7} $\%^{0.5}$ $week^{-1}$ and 45.60 kJ mol^{-1} for Powder B.

It was discovered that oxygen and hexanal were highly correlated with R^2 of 0.905 for Powder A and R^2 of 0.918 for Powder B when fitted exponentially. It was predicted that Powder A would be unacceptable after a storage time of 40 weeks, and 31 weeks for Powder B under 25°C storage temperature.

Data tables were generated to outline the different maximum storage times allowed with different storage temperatures and different initial storage oxygen concentration.

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And last but not least, I thank God for His love and bountiful grace that always strengthen me. I am always reminded by this verse: 1 Peter 5:7 “Casting all your anxiety on Him because it matters to Him concerning you”.

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Chapter 1 Introduction

1 Introduction

If the quality of foods and beverages decreases with time, there is generally a finite time before the product becomes unacceptable. The time from production to unacceptability is described as “shelf life”. The shelf life of the product is determined by its quality in terms of flavour, nutritional value or appearance. Therefore, determining the shelf life of the product is important.

One of the concerns in the dairy industry is the development of rancidity in milk powder with time. The milk powder will become unacceptable to the consumer if the flavour becomes rancid. Therefore, the degree of rancidity is one of the most important factors determining its shelf life. Although the quality of stored milk powder will not be the same as the fresh milk powder as it will constantly deteriorate, the differences should not be too great to influence the acceptability to the consumer within the projected shelf life.

Rancidity can be considered to be based on the subjective organoleptic appraisal of the off-flavour quality of food. It is associated with a characteristic, unpalatable flavour of fats and oils (Allen & Hamilton, 1994). Rancidity is mainly caused by “oxidative rancidity” where fats and lipids undergo oxidation causing deterioration of the quality of fat products. This rancidity can cause a significant reduction in shelf life. The off-flavours can also be produced by hydrolytic reactions which are catalysed by enzymes and are called lipolytic rancidity. However, lipotytic rancidity can be reduced greatly by handling the process well.

The milk powder industry has confronted problems from time to time where the milk powder shows lower flavor stability than expected (Nielsen, Stapelfeldt, & Skibsted, 1997). It is desired for the milk powder producers to be able to predict storage stability from measurements at an early stage after manufacture so that the projected shelf life can meet the consumer standards. Chemists and food technologists have been investigating the lipid oxidation reactions for a number of decades and have outlined the cause of the reactions and the products of lipid oxidation that are commonly associated with the flavouring. Many methods have been outlined in the literature to track the lipid oxidation reaction; however, there is no universal way to fully describe the overall reaction completely. Due to the complexity of the food system, with many different lipid oxidation reactions, the oxidative stability of each product is different. The external environment factors such as temperature changes, packaging and additional additives (formulation) will affect the overall reaction significantly.

The company, New Image Group was having difficulty in identifying the oxidative rancidity of the Infant formula of the goat milk powder. Normally the company would test the peroxide value (PV) of milk powder to determine the oxidation stage of the milk powder (The methods of lipid oxidation will be described fully in the literature review section). However, these results do not truly represent and correlate well with the development of rancidity in the milk powder. As a result, the current objective is to develop a method and a test that can predict the point where the milk powder will become rancid and unacceptable to the consumer.

Chapter 2 Literature Review

2.0 Introduction

The aim of the project is to develop a reliable test to measure whether goat milk based powder will deteriorate over time to give an off flavoured taste. It is not acceptable for the milk powder to develop a rancid flavour within the expiry date that is given by the supplier. Two types of rancidity may develop in the milk products to cause the rancid flavour; oxidative rancidity and lipolytic rancidity caused by enzyme hydrolysis. There is a large amount of literature available providing information on the two types of rancidity. A literature review has been conducted to identify:

- Oxidative rancidity of milk fat.
  - Reaction mechanism
  - Factors and conditions affecting the rate of oxidation
  - Common methods used to measure lipid oxidation rates
- Lipolytic rancidity of milk.
  - Causes of lipotyic rancidity
  - Identification of lipolytic rancidity
  - Determination of the lipolytic rancidity
- Methods approach to conduct the experiment

By conducting a thorough literature review, it will provide a direction for the current project and the tasks that need to be investigated. It is assumed that the oxidative rancidity is the major cause for the milk powder to go rancid rather than lipolytic rancidity. As a result, identification of the major cause of rancidity is necessary before further work can be done. Based on the literature review, a method will be developed to monitor the development of rancidity.

2.1 Physical form of milk fat

Milk fat is secreted in the form of globules surrounded by a milk fat globule membrane that maintains the integrity of the globules. The fat globule membrane helps to stabilise the fat globules in an emulsion within the aqueous environment of milk (Fox, 1995). The fat globules consist mostly of triglyceride and the membranes consist of complex lipids.

Triglycerides are the major lipid class for all the species of milks. It is reported that triglycerides contribute to 90% or more of the total lipids, with di-glycerides and monoglycerides accounting for the remaining fraction (Patton & Keenan, 1975).

The lipid content and composition of the milk of different species vary markedly with the stage of lactation, the age, breed and number of lactation (Christie, 1978). The fat content of the milk from different species is shown below in table Table 2.1-1

Table 2.1-1: Milk lipid content from some species (Christie, 1978).

Species	Fat Content (g/100 g of milk)
cattle	3.3-4.7
sheep (Domesticated)	4.0-9.9
Sheet (Bighorn)	7.8-16.1
Goat	4.1-4.5
Buffalo (Indian)	7.5
Reindeer	16.9-22.5
Human	3.8-4.3

2.1.1 Phospholipids and Fatty acids compositions of milk Fats

Phospholipids are a small fraction of the lipids of the milk and are associated with the milk fat globule membrane and some other membranous material of the skim milk phase. The composition of the phospholipids of milk from different species is shown in Table 2.1-2:

Table 2.1-2: Composition of phospholipids in milk from different species (Fox, 1995).

	Amount (mol% of the total lipid phosphorus)					
Species	Phosphatidyl-cholin	Phosphatidyl-ethanolamine	Phosphatidyl-serine	Phosphatidyl-inositol	Sphingomyelin	Lysophos-pholipids
Cow	34.5	31.8	3.1	4.7	25.2	0.8
Sheep	29.2	36	3.1	3.4	28.3	
Buffalo	27.8	29.6	3.9	4.2	32.1	2.4
Goat	25.7	33.2	6.9	5.6	27.9	0.5
Camel	24	35.9	4.9	5.9	28.3	1
Human	27.9	25.9	5.8	4.2	31.1	5.1

Fatty acids are the building blocks of lipids and play an important role on their physical-chemical and physiological properties. The fatty acids in milk are derived from two sources, the plasma lipids and synthesis *de novo* in the mammary gland (Fox, 1995). The composition of fatty acids of milk of non-ruminants are highly dependent on the dietary fatty acid profile, the concentration of milk fat would increase if there is an increase in the concentration of a particular fatty acid in the diet. However, there is not much difference at the effect on the composition of the milk fatty acids for ruminants due to the biohydrogenation that occurs in the rumen.

The fatty acids from synthesis *de novo* in the mammary gland are generally short to medium chain length constituents. The proportions are determined by the properties of acylthiol ester hydrolases associated with the fatty synthase of each species. The composition of the fatty acids in the milk of a number species is shown in the table below.

Table 2.1-3: Fatty acids in milk triglycerides or total lipids from various species (Fox, 1995)

	Fatty acid composition (%, w/w)				
Fatty acid	Cow	Sheep	Goat	Buffalo	Human
4;0	3.9	4	3.1	3.6	0.2
6;0	2.5	2.8	2.2	1.6	0.2
8;0	1.5	2.7	2.4	1.1	0.5
10;0	3.2	9	6.3	1.9	1
12;0	3.6	5.4	2.9	2	4.4
14;0	11.1	11.8	7.7	8.7	6.3
14;1	0.8				
15;0	1.2				0.4
16;0	27.9	25.4	22	30.4	22
16;1	1.5	3.4	1.9	3.4	3.7
18;0	12.2	9	10.6	10.1	8.1
18;1	21.1	20	23.7	28.7	34
18;2	1.4	2.1	2.7	2.5	10.9
18;3	1	1.4	1	2.5	0.3

2.1.2 Significance of Milk fat

Milk fat is the most important source of flavour and off-flavour compounds associated with milk powders. The complex molecules that make up the milk fats are responsible for the development of rancidity in milk powder. Milk fats have a number of important functions. The lipids play an important role in human nutrition and development. They provide a major energy source, they act as vehicles for absorption and transport of fat-soluble compounds such as vitamins and also provide organoleptic characteristics such as flavour and aroma (Kinsella, 1988). The essential fatty acids from the lipids are required for normal growth development of the central nervous system and the retina (Quan, Barness, & Uauy, 1990). Oxidation of lipids, however, will affect these functions and also alter the shelf life of the products.

2.2 Rancidity

The major concern for the company is the development of rancid flavour of milk powder that has been stored over a period of time. Rancidity is associated with a characteristic, unpleasant odour and flavor of the oils and fats. The rancidity can be caused by the changes that occur from reaction with atmospheric oxygen, oxidative rancidity. The off flavours can also be produced by hydrolytic reactions caused by enzymes, lipolytic rancidity in dairy products (Allen & Hamilton, 1994). The lipids act as reservoirs for the off flavours. The lipolytic rancidity can sometimes be minimised by handling the raw milk carefully, such as proper cold storage, good transportation, careful packaging and sterilisation. However, oxidative rancidity is difficult to prevent due to the lower activation energy of the chemical reactions involved. Therefore, two types of rancidity will be studied and a determination will be made as to which is the major cause of the rancid flavour in goat milk powder.

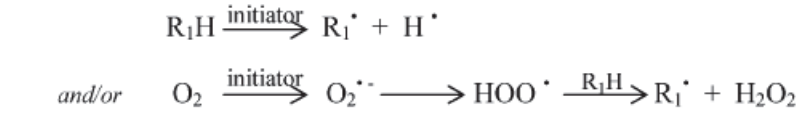
2.3 Lipid Oxidation

Lipid oxidation is a chemical reaction that results in quality deterioration in food. It is the process by which oxygen reacts with unsaturated lipids present in food. The oxidative changes caused food to produce off-flavours, loss of nutrients and bioactives. Lipids are susceptible to oxidative process in the presence of catalytic systems such as light, enzymes, heat and metals (Fereidoon Shahidi & Zhong, 2010). Lipid oxidation is essentially a free radical chain reaction involving initiation, propagation and termination stages. The unsaturated fatty acids in the milk powder are oxidised to form odourless, tasteless hydroperoxides, the products are unstable and can be degraded to form flavourful carbonyls and other compounds. Lipid may undergo autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation under different conditions (Fereidoon Shahidi & Zhong, 2005). Understanding the process of oxidation is a key factor in maintaining quality and extending shelf-life of the milk.

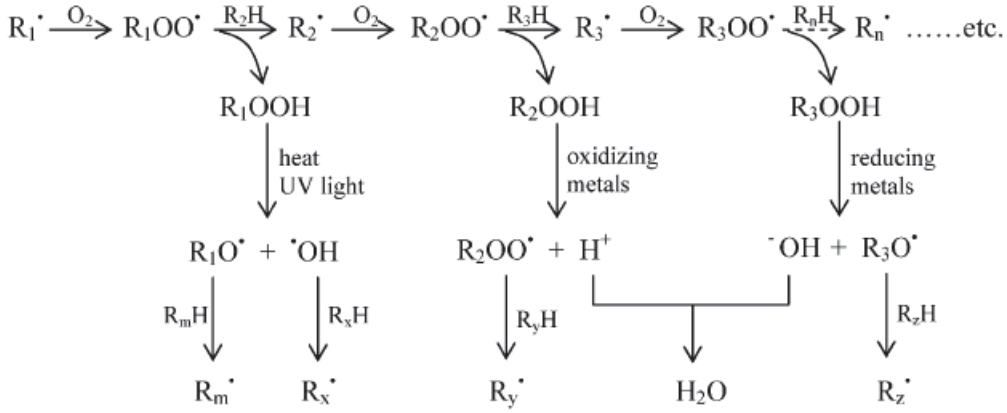
2.3.1 Mechanism of lipid autoxidation reactions

The most common oxidation process is autoxidation and is a spontaneous reaction of atmospheric oxygen with lipids. The process shown in Figure 2.3-1 involves propagation and termination reactions and has been used to explain the autoxidation of lipids:

Initiation:



Propagation:



Termination:

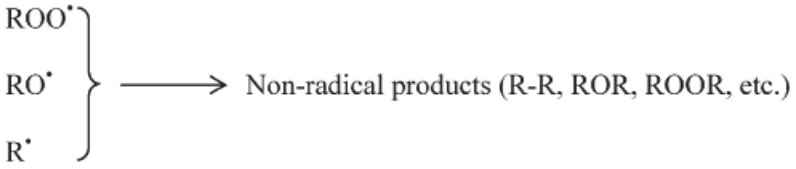


Figure 2.3-1: Autooxidation mechanisms involving initiation, propagation and termination.

The general pathway of lipid oxidation in the three stages:

Initiation:

$$RH \xrightarrow{Initiator} R\cdot + H\cdot \qquad \text{Eq. 2.3-1}$$

Propagation:

$$R\cdot + O_2 \rightarrow ROO\cdot \qquad \text{Eq. 2.3-2}$$

$$ROO\cdot + RH \rightarrow ROOH + R\cdot \qquad \text{Eq. 2.3-3}$$

Termination:

$$ROO\cdot + ROO\cdot \rightarrow non - radical\ products \qquad \text{Eq. 2.3-4}$$

The formation of the initial free radical at the initiation step may be due to the presence of initiators such as heat, light enzymes, metal complexes or active oxygen species. In the presence of initiators, unsaturated lipids lose hydrogen radical to form lipid free radicals. At the propagation step, the lipid radicals then react with ground state molecular oxygen to form peroxyl radicals. The peroxide free radical then reacts with another unsaturated molecule to continue the chain reaction and generate hydroperoxide. The propagation step can be repeated many times until no hydrogen source is present or the chain is interrupted by antioxidants. The hydroperoxides are unstable and can undergo secondary oxidation reactions to break into a wide range of products including ketones, aldehydes, alcohols, hydrocarbons and volatile organic acids. Among these, some, such as carbonyl compounds, produce undesirable odours with low threshold values.

In the termination step, two radicals are joined to form a non-reactive unit. In the condition of oxygen excess, peroxyl radicals will join to form a termination product. In conditions of low oxygen levels, the termination products are a result of interference between alkyl radicals resulting in fatty acid dimers (Semb, 2012).

Figure 2.3-2 below shows the general plot of the lipid oxidation over time. Depending on the type of food, the rate of oxidation can be different. There will be a lag phase where the rate of reaction is very slow initially while initiation reactions dominate. At some point, the reaction accelerates, as enough lipid hydroperoxides build up so that the rate of reaction increases exponentially.

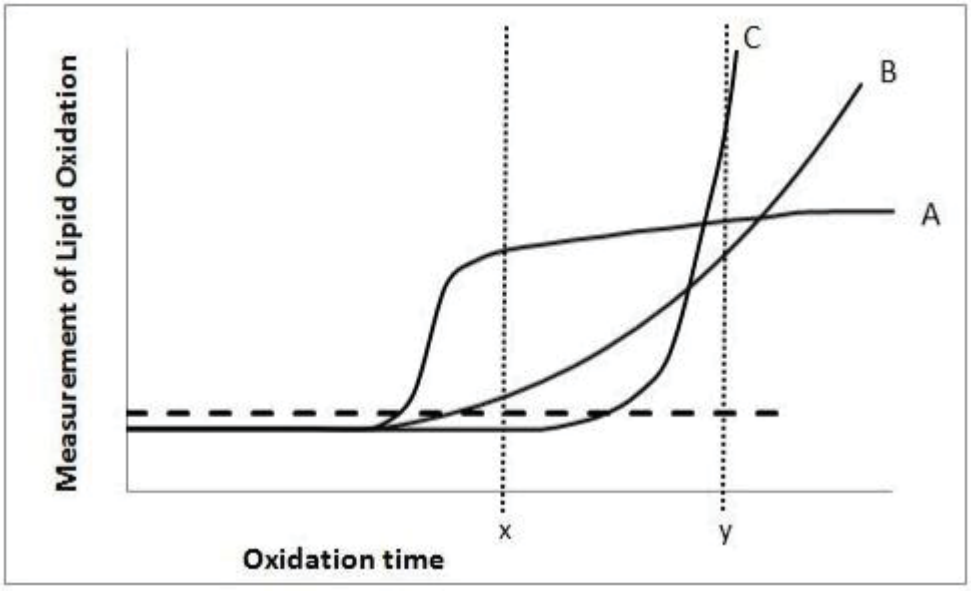


Figure 2.3-2: Oxidation curves for different types of food where the y-axis might represent a measurement of primary or secondary oxidation products. At values above the sensory cut-off line, trained panelists found the product unacceptably oxidised (Barden & Decker, 2013).

2.3.2 Primary importance of Fatty acids

It should be noted that fatty acids in the milk fat are the main compound that are susceptible to oxidation. Fatty acid can be classified into two categories, saturated or unsaturated, shown in Table 2.3-1.

Table 2.3-1: Major fatty acids found in foods.

Fatty Acids		
Saturated	Lauric	$CH_3(CH_2)_{10}COOH$
	Myristic	$CH_3(CH_2)_{12}COOH$
	Palmitic	$CH_3(CH_2)_{14}COOH$
	Stearic	$CH_3(CH_2)_{16}COOH$
	Arachidic	$CH_3(CH_2)_{18}COOH$
Unsaturated	Oliec	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
	Linoleic	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
	Linolenic	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$

Unsaturated fatty acids are more susceptible to oxidation since they are reactive to attack by oxygen. Unsaturated fatty acids are of primary importance to the rate of development of rancidity. It has been well established from literature that the unsaturated fatty acid caused the most the development of rancid flavour in foods. Unsaturated fatty acids such as oleate, linoleate, and linolenate have been reviewed extensively (E. N. Frankel, 1980) (Theodore P. Labuza & Dugan, 1971). Labuza (1971) reported that it is not the total fat content that is important, but the amount of unsaturated fatty acids moieties. Figure 2.3-3 below showed the typical mechanism of autoxidation of oleate forming hydroperoxides.

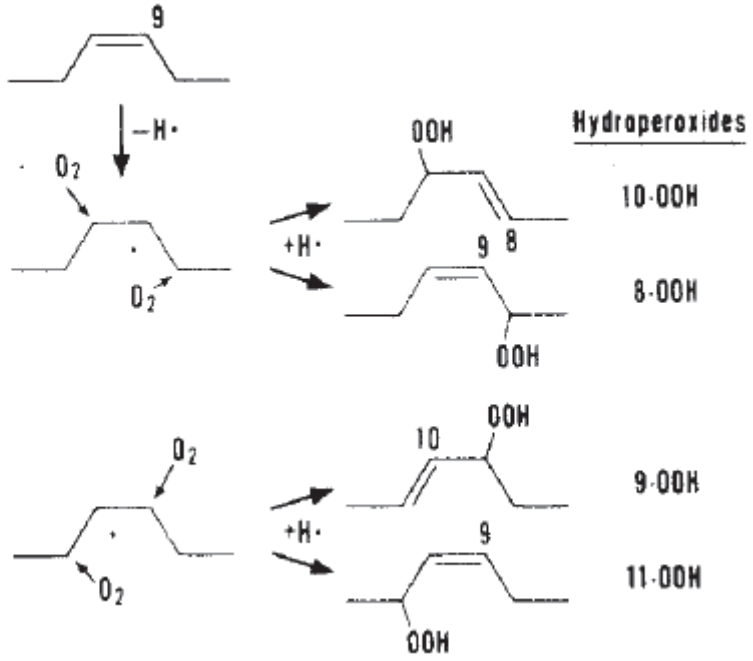


Figure 2.3-3: Mechanism of oleate autoxidation forming hydroperoxides Frankel *et al. (1980).*

2.3.3 Oxidation Products

Autoxidation of unsaturated fatty acids give rise to off flavours in dairy products due to unstable hydroperoxides decomposing to a wide range of carbonyl products such as saturated and unsaturated aldehydes, ketones, saturated and unsaturated hydrocarbons, semialdehydes and saturated and unsaturated alcohols (Fox, 1995). Other literature has also reported that the overall flavour produced during autoxidation is the combination of many flavours imparted by individual carbonyls present at minute concentrations. Weihrauch *et al.* (1988) had suggested that further oxidation of unsaturated aldehydes initially formed, migration of double bonds or isomerisation may occur during autoxidation (Weihrauch, 1988).

It is difficult to determine or correlate which of the specific carbonyl or carbonyl groups contribute to the specific off flavour taste in dairy products due to (Weihrauch, 1988):

- The multitude of compounds produced,
- Difficulties arising in qualitative analyses of oxidised dairy products,
- Differences in the threshold values of individual compounds,
- Smilarity of flavours imparted by individual compounds near the threshold,
- A possible additive or antagonistic effect,
- Existence compounds or groups of compounds not previously identified, and
- The difficulties involved in adding pure compounds to dairy products as a means of evaluating their flavour characteristics (Weihrauch, 1988).

Day *et al.* (1963) had investigated and defined the interaction threshold concentrations of carbonyl compounds to give a detectable flavour. Flavour threshold concentration of carbonyl may be influenced by factors such as the number of carbon atoms, degree and location of

unsaturated double bonds and additive or antagonistic effects of mixtures of carbonyl compounds. It is possible that certain carbonyl compounds have a greater significance than others. It had been concluded that the interaction of the identified carbonyl compounds in oxidised milk fat can alter the flavour (Day, Lillard, & Montgomery, 1963).

2.3.4 Factors affecting oxidation of lipids

There is a range of environmental and physical factors to which milk lipid is exposed during processing and storage conditions that may affect the lipid oxidation in milk and milk products. The factors such as oxygen content, light, metals, storage temperature and water activity.

2.3.4.1 Oxygen

The oxygen content is the key parameter in the oxidation of the milk lipid. The triplet-state oxygen ($^3O_2$) is less reactive compared to the singlet oxygen which restricts the oxidation of food lipids. Triplet oxygen can be activated to facilitate oxidative reactions. The activation of oxygen can be seen in the work of Fridovich (1977) outlying the principle processes involved in the activation of oxygen.

Singlet oxygen, on the other hand, is very reactive, as it is very electrophilic and readily reacts with unsaturated lipids forming hydroperoxides resulting in homolytic decomposition to initiate new free-radical chain reactions. Low levels of $^1O_2$ are sufficient to generate a large number of reaction chains from initiation of oxidation (Fox, 1995).

2.3.4.2 Temperature

The rate of oxidation reaction increases as the temperature increases. An increase in temperature under the storage condition results in a reduction in the length of the shelf life of the product. It was found that the rate of the reaction increases exponentially with the absolute temperature (Gómez-Alonso, Mancebo-Campos, Desamparados Salvador, & Fregapane, 2004). One of the authors conducted a storage test of milk powder at 25°C and 45°C; the sensory quality of the milk powder at 45°C had a lower score than at 25°C under the same water activity (Stapelfeldt, Nielsen, & Skibsted, 1997). It was also reported that the process of oxidative deterioration is accelerated by a factor of 10 for a 10°C increase in temperature.

2.3.4.3 Light

In addition to the ground state oxygen in autoxidation, photoxidation can also be encountered. Light is very effective in promoting off flavour development in milk and milk products. The extent of off flavour development is a function of the wavelength involved, and the intensity and duration of exposure (Fox, 1995). The light-induced flavour has two distinctive components a) sunlight flavour that is activated to the oxidation of the protein or amino acids, and b) oxidised flavour resulting from lipid oxidation (Shipe et al., 1978). It is suggested that the protein, riboflavin, present in milk, acts as a potent photosensitiser that induces the oxidation of ascorbic acid (or amino acids) resulting in the photoxidation of milk fat (Aurand, Singleton, & Noble, 1966). In photoxidation, the excited state singlet oxygen reacts with the double bond of the lipid through non-radical pathways. The photosensitiser absorbs energy from light and is activated to an excited state as Sen^* (shown below (Aurand et al., 1966)). The energy is then transferred from the photosensitiser to oxygen, activating

triplet oxygen to singlet oxygen. The singlet oxygen then reacts directly with the lipid to form hydroperoxides. The singlet oxygen is a major reactive oxygen species that participates in the oxidation process. Shahidi and Zhong (2010) reported that the rate of reaction of singlet oxygen is 1500 times faster than normal triplet oxygen.

$$Sen + hv \rightarrow Sen^{*}(excited\ molecule) \quad \text{Eq. 2.3-1}$$

$$Sen^{*} + O_2 \rightarrow Sen + O_2^{-} \quad \text{Eq. 2.3-2}$$

$$RH + O_2^{-} \leftrightarrow ROOH\ (Hydroperoxide\ formation) \quad \text{Eq. 2.3-3}$$

2.3.4.4 Metals (pro-oxidants)

Metals such as Cu and Fe are very effective pro-oxidants; they are effective at catalysing the decomposition of hydroperoxides by mechanisms involving one electron transfer and they have the largest effect during the propagation stage of the oxidation reaction (Steele, 2004):

$$M^{n+} + ROOH \rightarrow M^{(n+1)} + RO\cdot + OH^{-} \quad \text{Eq. 2.3-4}$$

$$M^{(n+1)} + ROOH \rightarrow M^{n+} + ROO\cdot + H^{+} \quad \text{Eq. 2.3-5}$$

Small quantities of metal ion can generate a large number of reaction chains in both oxidised and reduced forms interchangeably. In the study of the milk fortification, the milk fortified with the mix lacking iron did not develop an oxidised flavour; however, all the other mixes with iron component did cause oxidised flavour. This suggested that the iron component is responsible for causing the off flavour (Scanlan & Shipe, 1962).

2.3.4.5 Water activity

There is a complex relationship between water activity (A_w) and lipid oxidation. In the food industry, removal of the water by dehydration was the ideal method to prevent microbiological decay. However, it was reported that below a certain water activity and moisture content (2-3%), lipid oxidation was promoted. It was found that there was an optimum level of moisture content for dehydrated foods to minimise the rate of oxidation from which rancidity developed (Fisher, 1962). Labuza *et al.* (1971) observed that at an intermediate A_w (0.4) level the oxidation was at its minimum and an increased rate of oxidation occurs at either very high or low A_w. (Figure 2.3-4).

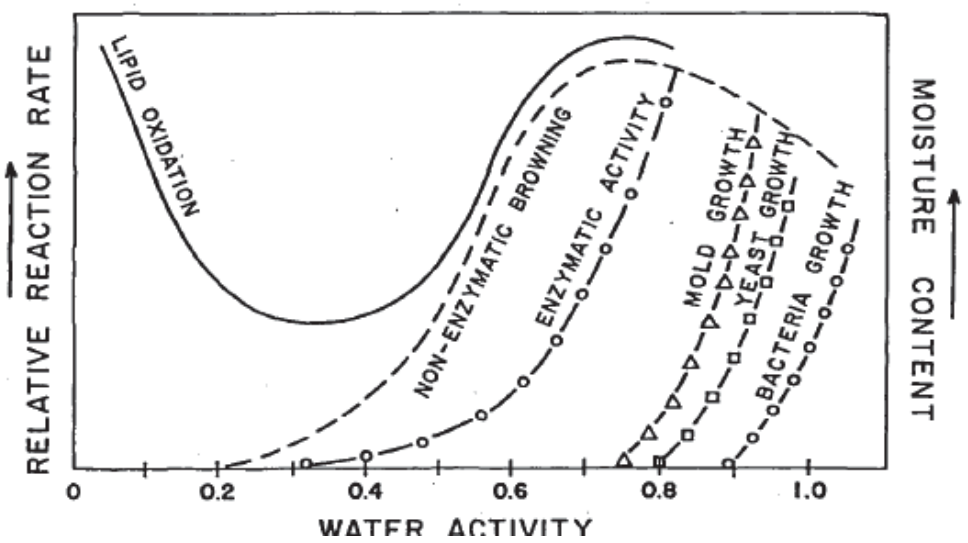


Figure 2.3-4: Stability of foods as a function of water activity (Theodore P. Labuza & Dugan, 1971).

The effect of water on lipid oxidation could be accounted for by two reasons (Theodore P. Labuza & Dugan, 1971):

1. Water interacts with metal catalysts making them less effective through changes in their coordination sphere.
2. Water hydrogen bonds with hydroperoxides so they are no longer available for decomposition through initiation reactions.

High A_w promotes lipid oxidation by mobilising pro-oxidants and facilitating their diffusion through the food. Whereas very low A_w also promotes lipid oxidation as the water monolayer is not available to retard the decomposition of hydroperoxides. However, very high A_w values slow down oxidation by diluting the reactants (Fox, 1995).

In one of the studies on the effect of water activity on the oxidative stability of milk powder, ranging from 0.11 to 0.33 A_w, the rate of autoxidation increased steadily with increasing water activity. The author reported the possible minimum of autoxidation corresponds to a water activity lower than 0.11. The author reported that the generalisation of Labuza (1971) regarding food does not seem to hold for milk powder, but the milk powder with an extremely low moisture content is susceptible to oxidation as the powder particles disintegrate, and as a result, increasing the amount of lipid exposed to air (Stapelfeldt et al., 1997).

2.3.4.6 Degree of Saturation of lipids

The degree of saturation of lipids affects the rate of oxidation. Unsaturated lipids are more susceptible to oxidation than saturated lipids. A general rule of thumb is that the more saturated the lipid, the more stable it will be to lipid oxidation (E Frankel, 2005). The milk powder for the current project only deals with the intermediates before formulation; therefore, it is assumed the degree of saturation of lipids is high. However, the relationship between the degree of unsaturation and the rate of lipid oxidation is not so clear. It is assumed the susceptibility of fatty acids to lipid oxidation increases with the degree of unsaturation due to increasingly lower bond dissociation energies of methylene-interrupted carbons (Damodaran & Parkin, 2008).

2.4 Methods for measuring lipid oxidation

The methods for measuring lipid oxidation in foods can be classified into five groups based on what they measure (Fereidoon Shahidi & Zhong, 2005):

- The absorption of oxygen,
- The loss of initial substrates,
- The formation of free radicals,
- The formation of primary oxidation products, and
- The formation of secondary oxidation products.

There are a number of different methods of measuring and detecting lipid oxidation reactions. Each method has its advantages and disadvantages; thorough consideration should be made to select the most appropriate method.

2.4.1 Peroxide Value (PV)

Hydroperoxides are formed as the primary oxidation product in lipid oxidation; they can be broken down further into a variety of non-volatile and volatile secondary products (Dobarganes & Velasco, 2002). During food storage at room temperature, the major reaction in oxidation is the formation of the hydroperoxides. Therefore, the peroxide value is an indicator of the initial stages of oxidative change (Fereidoon Shahidi & Zhong, 2005). It is generally shown in the literature that the concentration of PV increases gradually as lipid oxidation occurs. The increase of the PV reaches a peak where the rate of formation of hydroperoxide is equal to the rate of decomposition of hydroperoxide. After this point, the concentration of hydroperoxide decreases as the degradation of lipids take place. A typical PV curve is shown in Figure 2.4-1 below (Romeu-Nadal, Chávez-Servín, Castellote, Rivero, & López-Sabater, 2007):

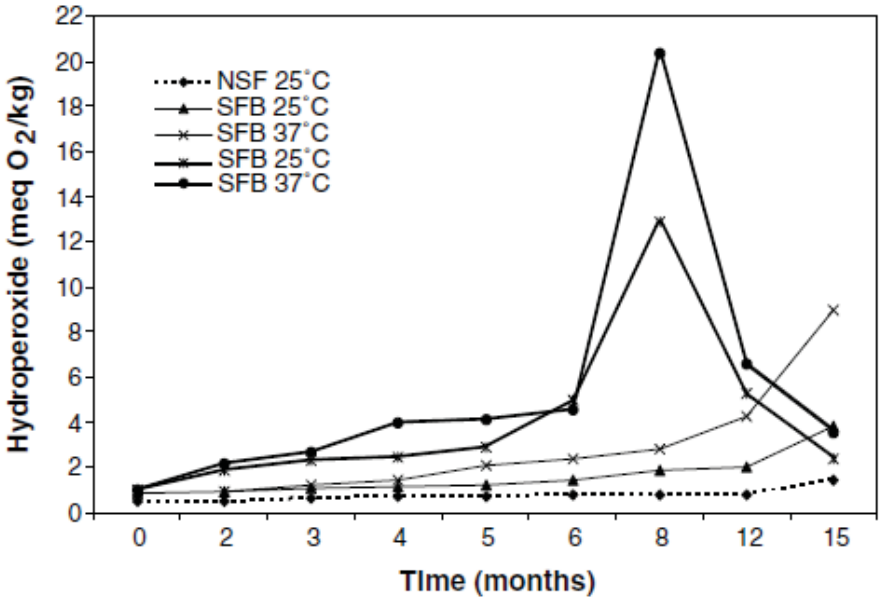


Figure 2.4-1: Concentration of PV in infant formula products over time in various temperatures (Romeu-Nadal et al., 2007).

The common methods of measuring the peroxide value are the iodometric titration or ferric ion complexes. Iodometric titration assay is based on the oxidation of the iodide ion (I^-) by

hydroperoxides (ROOH). In the Iodometric titration method a saturated solution of potassium iodide is used to react with hydroperoxides in the sample. The liberated iodine is then titrated with a standardised sodium thiosulfate solution with starch as an endpoint indicator.

$$ROOH + 2H^+ + 2KI \rightarrow I_2 + ROH + H_2O + 2K^+ \quad \text{Eq. 2.4-1}$$

$$I_2 + 2NaS_2O_3 \rightarrow Na_2S_2O_6 + 2NaI \quad \text{Eq. 2.4-2}$$

Ferric ion complexes are based on the oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) with hydroperoxides in an acidic medium. The method involves the formation of iron complexes which are complexed by either thiocyanate or xylenol orange (Eymard & Genot, 2003). The ferric ion forms chromophores, which when complexed to thiocyanate, give a red-violet complex and have a strong absorption at 500-510nm. The complexes can be measured by spectrophotometry to measure the ability of lipid hydroperoxides to oxidise ferrous ion to ferric ions. The method of determining PV by colorimetric detection of ferric thiocyanate was reported to be more sensitive than the standard iodometric assay, simple and reproducible (Dobarganes & Velasco, 2002) & (Fereidoon Shahidi & Zhong, 2005).

The measurement of PV may not correlate well with sensory analysis. Literature had reported that it is common the find the product has become rancid long before the peak PV is reached (Theodore P. Labuza & Dugan, 1971).

2.4.2 Para-anisidine value

Anisidine value (AV) is a measurement of secondary products of oxidation; it is mainly a measure of aldehydes. The method measures the content of aldehydes (principally 2-alkenals and 2,4-alkadienals) that are produced from the decomposition of the hydroperoxides (Fereidoon Shahidi & Zhong, 2005). The reaction between para-anisidine (reagent) and aldehydes in acidic condition produces a yellow-coloured compound with absorbance at 350nm (F. Shahidi & Wanasundara, 2002). The p-anisidine value is defined by Gordon *et al.* (2001) as "the absorbance of a solution resulting from the reaction of 1 g fat in isooctane solution (100ml) with p-anisidine (0.25% in glacial acetic acid" (M. Gordon 2001). It was reported that the coloured compound from the reaction with unsaturated aldehydes (2-alkenals) absorbs more strongly with the wavelength at 350nm; therefore, the test is more sensitive to unsaturated aldehydes than to saturated aldehydes. However, it has been shown that for the sensory analysis, the detectable levels of unsaturated aldehydes are much lower than saturated aldehydes; therefore, AV can be used to monitor secondary oxidation products (Gordon, 2004). The proposed reaction between p-anisidine reagent and malonaldehydes is shown in Figure 2.4-2:

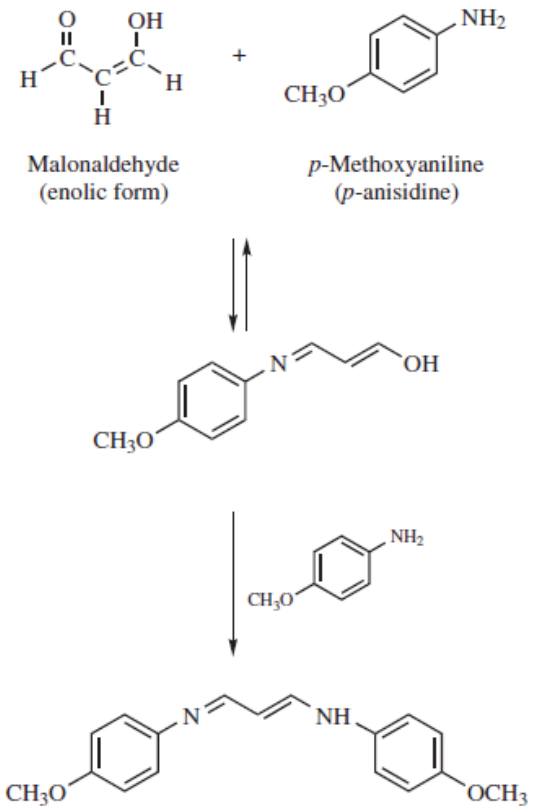


Figure 2.4-2: Reaction between p-anisidine reagent and malonaldehyde (F. Shahidi & Wanasundara, 2002).

2.4.3 Totox value

Experimental results of PV and AV from List *et al.* (1974) were shown to be highly correlated (List et al., 1974). Measurements of AV are commonly used together with PV measurement. Holm *et al.* (1972) have developed a concept that combined the expression of peroxides and secondary oxidation products called TOTOX value. The Totox value has an expression of:

$$Totox\ value = (2 \times PV) + AV \qquad \text{Eq. 2.4-3}$$

The TOTOX value is a measure of the total oxidation, including primary and secondary oxidation products and was shown that a unit peroxide value corresponds to an increase of 2 anisidine value units (Holm & Ekbom-Olsson, 1972). Totox value measures both hydroperoxides and their breakdown product, as it is observed during storage stability test of fat products that PV rises first, and then falls due to hydroperoxides decay (Stauffer, 1996). It was suggested to be a better measurement of the progressive oxidative degradation of the fat.

2.4.4 Thiobarbituric acid (TBA)

A test involving thiobarbituric acid as an analytical reagent is one of the most extensive methods used to detect oxidative deterioration in dairy products (Dunkley & Jennings, 1951). Cesa (2004) described the method as simple and fast for the detection of the oxidation level of the infant milk powders (Cesa, 2004). This method is based on the condensation of two molecules of TBA with one

molecule of the oxidation end product i.e. malonaldehyde (MA), resulting in the formation of a red pink complex with strong absorbance at 532-535 nm that can be measured spectrophotometrically (Figure 2.4-3). The extent of oxidation is reported as the TBA value and is expressed as milligrams of MA equivalents per kilogram sample. However, TBA reagent can also react with other secondary oxidation products such as alkenals or alkadienals; the reacting of secondary products are often referred to as TBA-reacting substance or TBARS in short (Fereidoon Shahidi & Zhong, 2005).

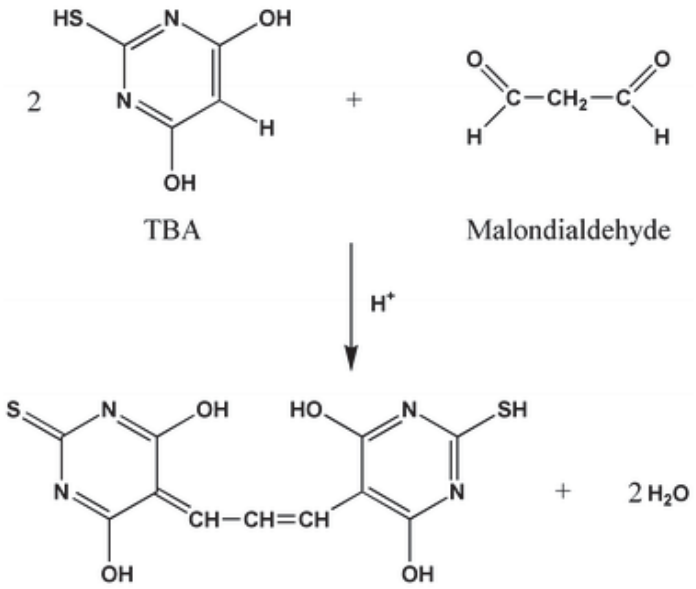


Figure 2.4-3: Reaction of 2 thiobarbituric acids (TBA) and malonaldehyde (MA) (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002).

The TBARS method is a good indicator for measuring the formation of secondary lipid oxidation products. The secondary oxidation products such as aldehydes have a profound impact on both sensory and functional properties, whereas the primary oxidation product, e.g. hydroperoxides, have practically no impact on the sensory properties (Stapelfeldt et al., 1997).

2.4.5 Gas chromatography

The end products of lipid oxidation are a mixture of complex compounds of secondary products (alkanes, alkenes, aldehydes, ketones). GC has become a common method in analysis of volatiles in food products such as oil, fish and milk. Literature reported using a gas chromatographic (GC) method for determining volatile compounds in infant formula (Romeu-Nadal, Castellote, & López-Sabater, 2004) & (García-Llatas, Lagarda, Romero, Abellán, & Farré, 2007). GC is proven to be sensitive in detecting the small changes in volatiles concentrations. GC can also be coupled with mass spectrophotometer to identify a large range of different volatile compounds.

In the study of oxidative stability of infant formula, Romeu-Nadal *et al.* (2004) used GC to quantify the level of volatile compounds (propnal, pentanal and hexnal) over the period of 4 weeks (Figure 2.4-4); the choice of volatile to analyse depends on the lipid being oxidised

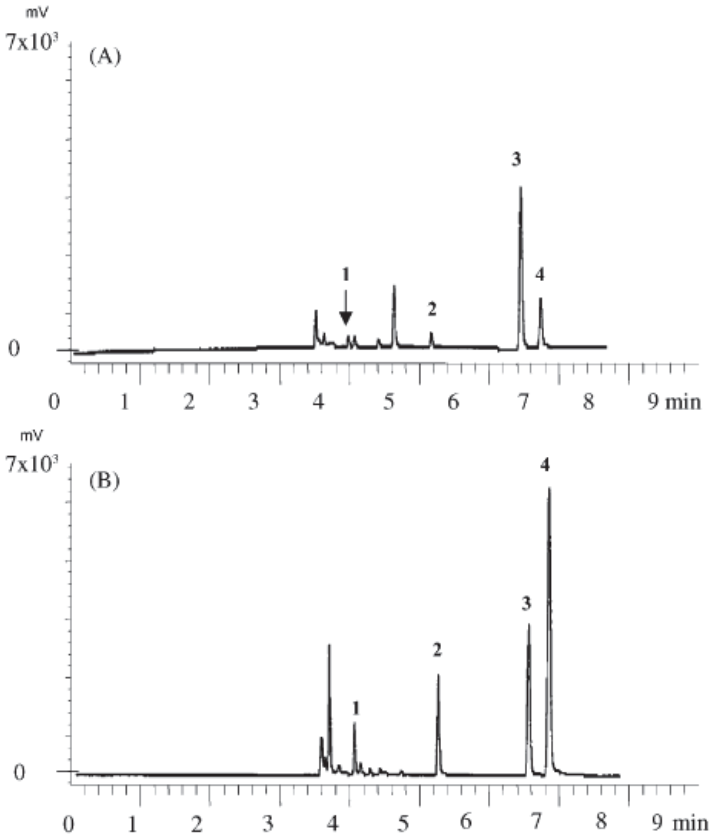


Figure 2.4-4: Chromatograms of volatile compounds in infant formula at 0 weeks (A) and after 4 weeks (B). Peaks numbered from 1 to 4 correspond to 1, propanal; 2, pentanal; 3, butyl acetate; 4, hexanal (Romeu-Nadal et al., 2004).

However, GC is an expensive specialised piece of equipment that is not available to some companies. The results need careful interpretation as changes to the oxidation system can bring with it a change in the products measured.

2.4.6 Rancimat

There are a few studies that used the Rancimat test for shelf life prediction of infant formula and vegetable oil (de la Presa-Owens, Lopez-Sabater, & Rivero-Urgell, 1995; Farhoosh, 2007). The Rancimat method is a form of accelerated test that is carried out at high temperature to measure its oxidative stability. This method shortens the long storage period in predicting the shelf life. Rancimat is similar to the active oxygen method (AOM), which was based on PV measurement, but in an automated version. It was found that AOM correlates very well with the Rancimat method (Läubli & Bruttel, 1986). The Rancimat apparatus maintains the sample at an elevated temperature (usually 70°C to 120°C) and provides a flow of air as a source of oxygen, therefore shortening the reaction time for analysis. The volatiles from the reaction are collected in a reservoir of distilled water to be measured by conductivity (Irwin, Hedges, & Sharnbrook, 2004). The induction period which is used as a measure of the stability of the oil can be determined as a sudden increase in the conductivity (Mateos, Uceda, Aguilera, Escuderos, & Beltrán Maza, 2006). The predicted shelf life can be calculated from the extrapolation of the temperature used from the test.

It is reported by Allen and Hamilton (1994) that Rancimat can overestimate the resistance of an oil/fat to rancidity, as the test is based on the evolution of volatile secondary products (fatty acids) which take place after oxidation. Some volatiles may not be caused by the development of rancidity which can alter the result (Allen & Hamilton, 1994). Also, use of high temperature in the Rancimat method can change the reaction mechanism causing skewed results that will not be so if carried out at a lower temperature.

2.5 Lipid oxidation versus sensory

As discussed above, lipid oxidation causes the formation of objectionable off flavours (Jacobsen, 1999). It is suggested that only secondary oxidation products such as aldehydes and ketones are responsible for the changes in the aroma and flavour properties. Secondary oxidation products such as hydrocarbons have high flavour thresholds; therefore, they are not considered to be significant (Edwin N. Frankel, 1991).

The shelf life of milk powder is often limited by the development of the off flavours. The flavouring affects the consumer acceptability of a product. Sensory analysis is closely associated with the quality of the food lipids as it measures and analyses the characteristics of food lipids evoked by the sense of taste and smell. It is often expensive and difficult to conduct sensory tests for all the experiments. Sensory panels need to be trained and calibration is needed to obtain a reliable result. Therefore, it is ideal to use chemical tests to determine the acceptable sensory shelf life. However sensory analysis provides useful information on the flavour defects that cannot be detected by chemical or instrumental analyses. For example, specific flavour defects characterised as “fishy” or “grassy” in vegetable oils occur at low levels of oxidation that can only be detected by sensory analysis (E Frankel, 2005).

Studies have been done in comparing the chemical test with the oxidised flavour in milk (Jacobsen, 1999; King, 1962; Kliman, Tamsma, & Pallansch, 1962). Different analytical tests target different compounds as their measure of oxidation. For example, PV measures the primary oxidation product hydroperoxides whereas AV measures the secondary oxidation product which is responsible for the off flavours. Kliman *et al.* (1962) concluded that the peroxide value of the milk powder could not be used to establish flavour quality of the sample. Jacobsen (1999) produced a result of correlation between the PV and AV with the sensory analysis (Figure 2.4-3).

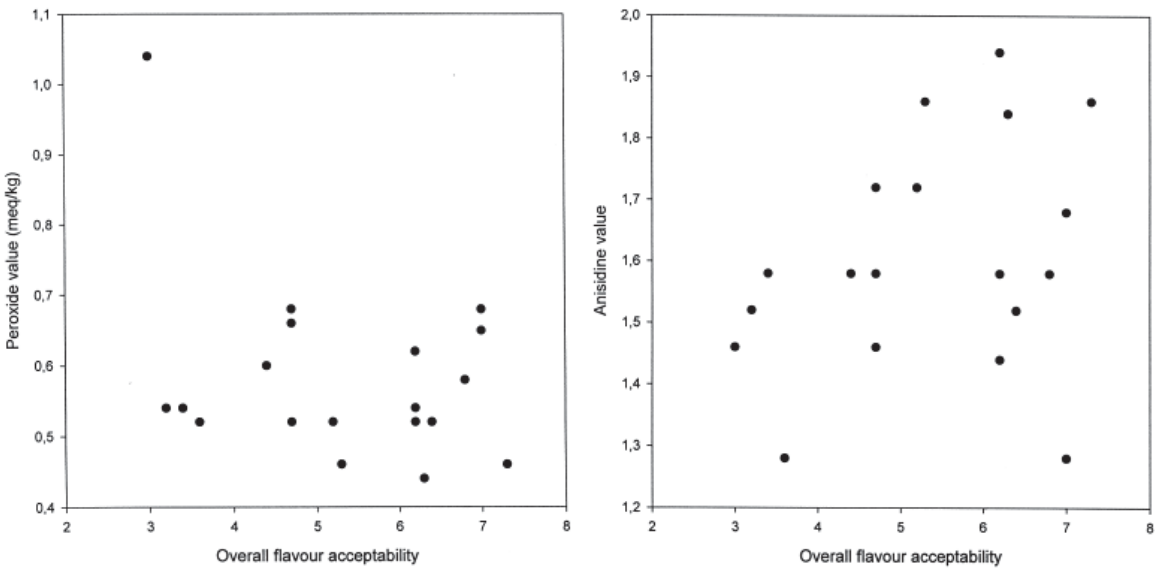


Figure 2.5-1: left: Relationship between PV and flavour acceptability of fish oil. Right: Relationship between the AV and acceptability of fish oil, where score of 1 is totally unacceptable (Jacobsen, 1999) .

From the figure above, peroxide value has a poor correlation with the flavour score whereas anisidine value also has a poor correlation. It is surprising that AV has a poor correlation as it is a measuring of the secondary oxidation products. The author concluded that AV test was not able to detect the changes in concentration of volatile compounds responsible for off-flavour formation in the fish oil due to low sensitivity and specificity.

Other analytical techniques that target the secondary oxidation products such as TBA and GC coupled with mass spectroscopy were reported to provide better correlation with sensory analysis in food products (E Frankel, 2005).

Lillard and Day (1960) did the investigation of the volatile monocarbonyls which were believed to be responsible for the off flavouring in oxidising milk fats. The author used TBA and the volatile monocarbonyls as the chemical test to compare with flavour threshold values of different degrees of oxidised milks. It was suggested that the TBA and total carbonyl test are largely indirect measures of lipid hydroperoxides, and like the peroxide determination, are measuring precursors or indicators of oxidised flavour. It was concluded that there is an exponential relationship between the chemical tests for oxidised flavour intensity and the absolute flavour thresholds of fats (Lillard & Day, 1961). The table below showed the correlation coefficients between the reciprocals of the flavour threshold values with various chemical tests for measuring the intensity of oxidised flavour.

Table 2.5-1: Correlation coefficient between 1/FTV and all chemical tests for measuring the intensity of oxidised flavour (Lillard & Day, 1961).

Chemical test	Correlation coefficient[a]
TBA No.	.887*
Peroxide value	.798*
Total unsaturated carbonyls	.666**
Total saturated carbonyls	.683**
Total carbonyls	.714**
Volatile unsaturated carbonyls	.996*
Volatile saturated carbonyls	.896*
Total volatile carbonyls	.887*
Sum of individual volatile unsaturated carbonyls	.948*

2.5.1 Hydroperoxides concentration

Although the PV test does not truly reflect the rancidity of the product, it does however provide information on the rate of lipid oxidation. As the literature stated, the concentration of PV increases then decreases over the time as the rate of depletion of hydroperoxides becomes greater than the rate of formation of hydroperoxides (Romeu-Nadal et al., 2007) (Theodore P. Labuza & Dugan, 1971). Therefore, the concentration of hydroperoxide may alter depending on which stage of lipid oxidation is being measured and therefore does not correlate well with the sensory test, as the PV value can be similar at the initial stage and also at the end of the oxidation reaction. As discussed before, the rancid flavour is caused by the secondary oxidation products; these products are formed from radical species. The radical species are the function of the lipid hydroperoxide concentration (Brown, 2012). The author suggested that the rate of rancidity development is proportional to the concentration of hydroperoxides; as a result, PV value should provide some information regarding the rate of rancidity.

2.5.2 Relationship between sensory test and chemical test

There is very little literature that quantifies the chemical test with the sensory evaluation of oxidised milk. Most of the studies have been done on the relationship between the chemical and sensory methods. There is no clear indication of the acceptable limit of the chemical test to the degree of rancidity. King (1962) observed a direct relationship between sensory evaluation of the intensity of an oxidised off-flavour and optical density readings of milk filtrates (King, 1962). The author used the TBA reaction as a measure of oxidised flavour in milk and found that the organoleptic values correlate well with the TBA values. The figure below showed the samples of oxidised milk diluted with different amounts of non-oxidised milk and was evaluated with the TBA test. The optical density increases as the concentration of oxidised milk increases, giving a good correlation.

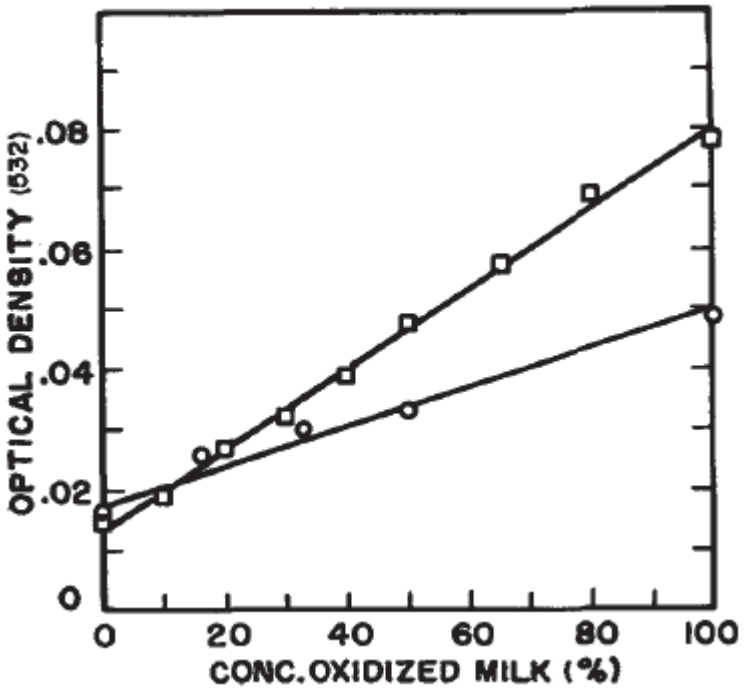


Figure 2.5-2: Concentration of oxidised milk versus the optical density of the TBA test from two types of milk (King, 1962).

Table 2.5-2 below showed the flavour score and the corresponding optical density at 532 mμ of the milk that was measured spectrophotometically. However, there is not much information relating the organoleptic values with the chemical test.

Table 2.5-2: Relationship between sensory evaluation and intensity of oxidised off-flavour and optical density readings of milk (Clark & Bodyfelt, 2009).

Flavour Score	Flavour description	Range of optical density (532mμ)
0	No oxidised off flavour	0.01-0.023
1	Questionable to slight oxidised	0.024-0.029
2	Slight but consistently detectable	0.03-0.04
3	Distinct or strong oxidised	0.041-0.055
4	Very strong oxidised	≥0.056

Labuza and Dugan (1971) outlined some threshold values of various compounds responsible for rancid off flavours, Table 2.5-3, for example, reported that hexanal as a major breakdown off flavour product in various foods. In milk hexanal can be detected at 50ppb (parts per billion). The author suggested that a very low concentration (0.00002%) of the fat has to be oxidised in order to form an objectionable off flavour (Theodore P. Labuza & Dugan, 1971). Thus, sometimes measuring the secondary oxidation products may be too late as the rancid flavour may already have occurred. As a

result, other methods may be better to monitor the lipid oxidation such as looking at the kinetics of the reaction, as the kinetics of this early reaction are important.

Table 2.5-3: Olfactory Perception Thresholds of various flavour compounds isolated from dairy foods (ppm) (Theodore P. Labuza & Dugan, 1971)

	Water	Milk	Oil
Compound (ppm)			
Butyric	6.8		0.6
Hexanoic	5.4		2.5
Decanoic	3.5	7	200
Dodecanoic		8.5	700
Ketones			
2-butanone		79.5	
2-pentanone		8.4	
2-hexanone		0.4	
2-heptanone		0.7	
Aldehydes			
Ethanal		1.2	
Propanal		0.43	1.6
Butanal		0.19	0.02
Pentanal		0.13	0.15
Hexanal		0.05	0.15

Figure 2.5-3 displays the overall reaction graphically. It shows the extent of reaction as a function of time. From the figure, the rancidity is observed before a measurable increase in peroxide value.

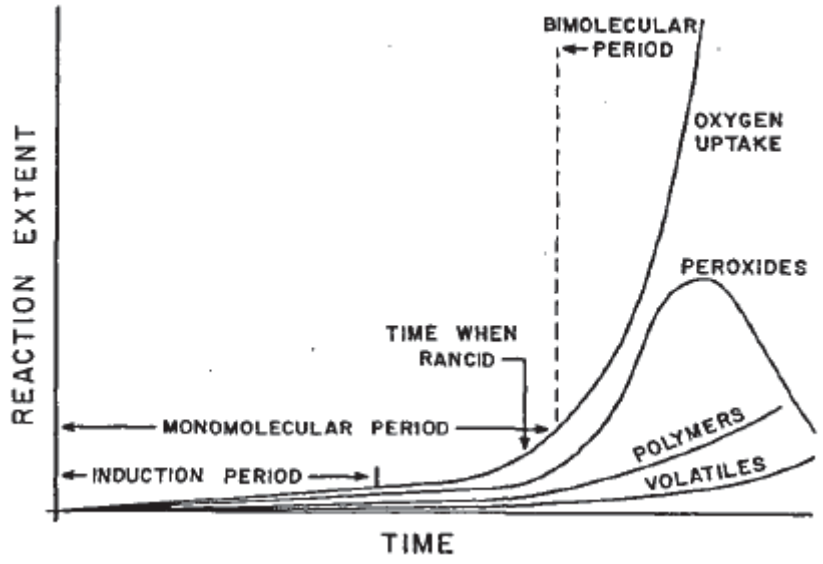


Figure 2.5-3: Extent of reaction as a function of time (Theodore P. Labuza & Dugan, 1971)

2.6 Lipolytic Rancidity

After milk is withdrawn from the udder of the animal, many chemical and physical changes occur (Jensen, 1964). The lipolytic rancidity in milk results from the hydrolytic degradation of milk lipids through the process called Lipolysis. Lipolysis can be defined as enzyme-catalysed hydrolytic cleavage of milk-lipids (glycerides and fats) producing free fatty acids (FFAs) (Ray, Chatterjee, Chakraborty, & Ghatak, 2013). Hydrolysis is catalysed by enzymes called lipases. Free fatty acids (C4 to C10) are associated with the flavours and have low flavour thresholds, causing unpleasant flavours in milk and milk products. It is often described as giving a soapy taste. It should be noted that this is hydrolytic rancidity rather than oxidative rancidity. Released unsaturated fatty acids are susceptible to oxidation to carbonyl compounds such as aldehydes and ketones which also give rise to off flavours. In goat milk, short-chain FFA could play a positive role which contributes to the characteristic flavour of goat milk products (Skjevdal, 1979). Interestingly, Delacroix-Buchet *et al. (*2003) pointed out that goat flavour, which is regarded as a positive, is sometimes seen as a negative feature in milk and cheese, and appears at lipolysis levels much lower than those responsible for the rancid-butyric flavour (Delacroix-Buchet, Degas, Lamberet, & Vassal, 1996).

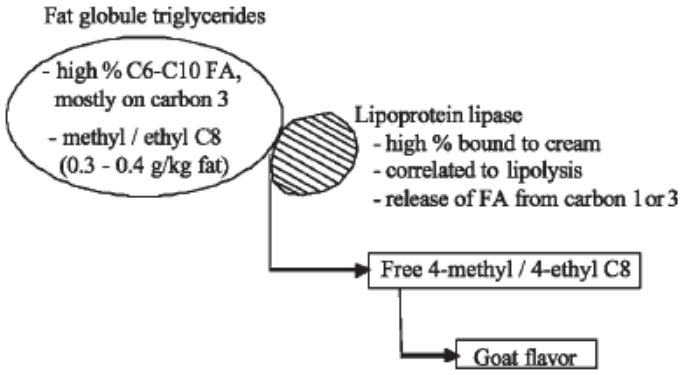


Figure 2.6-1: Fatty acids, lipolysis, and goat flavour (Chilliard, Ferlay, Rouel, & Lamberet, 2003).

2.6.1 Enzyme

The enzyme lipase can be classified into two types: The native enzymes which are the endogenous milk enzymes and the bacterial enzymes which come from microbial origin.

2.6.2 Lipoprotein Lipase

The literature suggested that lipoprotein lipase (LPL) is the major lipase in bovine milk and it is generally accepted that the milk enzyme can be classified as a LPL (Fox, 1995). Chilliard *et al.* (1984) had also reported that LPL is the only significant indigenous milk lipase present in the goat milk (Chilliard, Selselet-Attou, Bas, & Morand-Fehr, 1984).

LPL is synthesised in the mammary gland secretory cells and is transported to the capillary endothelium where it hydrolyses triglycerides in plasma lipoproteins to FFA and 2-monoglycerides. The products are absorbed by the mammary gland and used for milk fat synthesis (Fox, 1995). LPL in goat milk is distributed in the cream and serum phases equally and a small amount is also attached

to casein. Chilliard *et al.* (1984) stated that the total activity of the LPL in goat milk is much lower than in cow's milk.

2.6.3 Lipases of psychrotrophic bacteria

Pre or post-manufacture contamination by micro-organisms can also cause the lipolytic rancidity in the production of milk and milk products. The extracellular lipases are produced by psychrotrophic bactiera such as pseudomonads (*Pseudomonas fluorescens)* and P. *fragi*, Enterobacteriaceae (*Serratia & Acinetobacter spp.)* (Stewart, Murray, & Neill, 1975). The psychrotrophic bacteria have arisen in raw milk as the dominant organisms due to the introduction of a bulk cold storage system (Jeon, Thomas, & Reineccius, 1978). The storage system on farms and in the factory creates an extended period of time before processing, allowing sufficient growth for spoilage and lipolysis from the bacteria.

One of the significant properties of bacterial lipases is their heat stability. Some are sufficiently stable to retain at least some activity after pasteurisation. It was reported that it is common to cause microbial lipolysis in milk products in post-pasteurisation (Ray et al., 2013). It was suggested that the development of flavour defects in pasteurised milk and cream is associated with the presence of high numbers of psychrotrophs. This can be improved by better processing methods and hygiene.

2.6.4 Causes of lipolytic rancidity in milk and milk products

Fox (1995) stated that raw bovine milk contains a large amount of lipase activity, but seldom lipolysis sufficientto acquire an off-flavour. The fresh raw milk from an udder generally has ≤0.5mmol FFA ml^{-1} (Brathen, 1980). The FFA results from incomplete synthesis rather than lipolysis. Under normal storage conditions with proper handling of the raw milk, there will only be a small increase in the level of FFA, but in some cases there is a substantial increase. This can be caused either by 'spontaneous' or 'induced' lipolysis. In general, lipolysis caused by indigenous milk lipase accounts for most of the rancidity. Lipolysis by microbial lipases is of greater significant in stored milk products (Fox, 1995).

2.6.4.1 Induced lipolysis

Induced lipolysis is defined as when the milk lipase system is activated externally by physical, thermal or chemical means. Lipolysis can be induced by agitation, foaming, temperature changes or homogenisation. In processing and also transportation of milk, for example by a pump or in a tanker, can cause induced lipolysis. All the processes causing induced lipolysis are known to damage the fat globule membrane. It was reported that around 50% of the FFA present in pasteurised milk is generated by the time it is pumped into the bulk tank. As a result, keeping pipeline lengths down and ensuring the exclusion of entrapped air can minimise induced lipolysis (Allen & Hamilton, 1994).

2.6.4.2 Spontaneous Lipolysis

Spontaneous lipolysis is described as 'naturally active', 'susceptible 'or 'spontaneous lipolytic' in contrast to 'normal' milk where no lipolysis occurs. Spontaneous lipolysis was defined by Tarassuk and Smith (1940) as the lipolysis which occurs in some individual milks when cooled to below about 15°C soon after milking (Tarassuk & Smith, 1940). Jellema *et al.* (1991) reported that a significant increase of the fat acidity may be observed in correctly handled fresh farm milk after cooling and subsequent storage of the samples (Jellema et al., 1991).

2.6.5 Effects of lipolysis in milk

Many studies had been done to examine the relationship between the flavour of milk and its FFA content. The rancid flavour is caused mainly by the fatty acids of chain lengths C_4 to C_8 (Scanlan, Sather, & Day, 1965). Tallamy & Randolph *et al.* (1969) had generated a reasonable and acceptable threshold FFA levels for the detection of rancid flavour, i.e. 1.2-1.5 mmol of fatty acids per 100g of fat for the trained expert and 2.0-2.2 meq per 100g fat for the average consumer (Tallamy & Randolph, 1969). Also it was reported that milk with concentration of >2µmol FFA ml^{-1} has a rancid flavour (Lombard & Bester, 1979). Table 2.6-1 below shows the theoretical concentrations of the C_4 to C_{12} in rancid milk (Fox, 1995).

Table 2.6-1: Concentrations of C4 to C¬12 free fatty acids (FFA) in rancid milk (Fox, 1995).

	FFA level ($mgkg^{-1}$)		
Fatty Acid	Theoretical for milk with 2meq l^{-1}	Found for range of rancid milks	Threshold levels of added acids
C4	26.6	27.5-85	46.1
C6	15.1	16-48.7	30.4
C8	9.1	8.3-27.9	22.5
C10	17.6	27.6-78.6	28.1
C12	17.6	26.7-63.3	29.7

2.6.6 Determination of Free Fatty Acids in milk products

It was reported that the freshly secreted milk will always contain a small quantity of free fatty acids and is not necessarily the result of lipolysis. Needs *et al*. (1986) suggested that fresh milk had about 0.2 mmol of fatty acids per 100 g of fat (Needs, Anderson, & Morant, 1986). The development of lipolysis can be assessed by the determination of the overall quantity of free fatty acids of the isolated fat, or the determination of the individual fatty acids. Although the later method gives more information, it is more difficult and time consuming to carry out. It was reported that if only the extent of lipolysis is to be measured, a determination of the global free fatty acids content is sufficient (Jellema et al., 1991).

The determination of global acidity can be done by titrimetry which is easy to perform and readily available. Fat is first extracted from the milk; the isolated fat can then be titrated to determine the global acidity. The acidity of the fat is expressed in mmol per 100 g of fat. By determining the global acidity of fat, it is possible to assess the risk of the appearance of an organoleptic defect. Anderson (1983) suggested that lipolysed flavour will be detected at threshold values of 2.2 mmol/100g fat (Anderson, 1983).

It is interesting to note that literature had suggested that in concentrated milk such as evaporated milk and in milk powder, some of the volatile short chain free fatty acids will be removed during evaporation and drying; therefore, lipolysis is not a major problem in these products (Jellema et al., 1991).

2.7 Oxidative Rancidity versus Lipolytic Rancidity

As discussed in early sections above, two types of rancidity can cause the dairy products to go rancid. It is necessary to find out whether the milk powder is subject to oxidative rancidity or the lipotlytic rancidity, as it will alter the approach to the current project. If lipolytic rancidity is the major cause, then the project may need to focus on the supply line and the production line rather than the post production as for oxidative rancidity. However, lipolytic rancidity in dairy products is rare if the milk from which they were made was properly pasteurised or dried. Also, as mentioned before, dried dairy products are less susceptible to hydrolysis but are more susceptible to oxidation in the presence of air. It is assumed that oxidative rancidity is responsible for deterioration of the milk powder, causing off flavours.

An experiment can be conducted out to rule out the possibility of lipolytic rancidity. By determining the fat acidity of the milk powder, a conclusion can be made to decide the significance of lipolysis. As a result, oxidative rancidity will be the main focus of this project once the result of the fat acidity is found, and it is confirmed that lipolysis is not the major cause of rancidity.

2.8 Effects of packaging in the lipid oxidation

The rate of oxidation is affected by the packaging which is influenced by the transmission of oxygen through the packaging. One of the most effective methods of extending the shelf life of milk powder is to package it in a high oxygen barrier package slowing the rate of oxidation. It is also reported that the moisture content corresponding to the water activity that gives the minimum oxidation rate will extend the shelf life of the milk powder (Robertson, 2013). Therefore, a package bag that has both low oxygen and water vapour permeability are desirable. The expression for the steady state permeation of a gas or vapour through the packaging material can be written as (Robertson, 1999):

$$\frac{\delta w}{\delta t} = \frac{P}{X} \times A \times (p_1 - p_2) \qquad \text{Eq. 2.8-1}$$

Where P/X is the permeance (the permeability constant P divided by the thickness of the film X in m), A is the surface area of the package in m^2, p_1 and p_2 are the partial pressures of water vapour outside, and inside of the package in Pa, $\frac{\delta w}{\delta t}$ is the rate of gas or vapour transport across the film.

A generalised scheme of degree of protection required is shown in Table 2.8-1 below. The key requirements for the package such as maximum moisture gain or oxygen uptake can be used to determine whether the packaging material would provide the necessary barrier to give the desired shelf life.

Table 2.8-1: Degree of protection required by different foods and beverages (assuming 1 year shelf life at 25°C) (Salame, 1974).

Food/ Beverage	Maximum Amount of O_2 Gain (ppm)	Other Gas Protection Needed	Maximum Water Gain or Loss	Requires High Oil Resistance	Requires Good Barrier to Volatile Organics
Canned milk and meats	1–5	No	3% Loss	Yes	No
Baby foods	1–5	No	3% Loss	Yes	Yes
Beers and wine	1–5	< 20% CO_2 Loss	3% Loss	No	Yes
Instant coffee	1–5	No	2% Gain	Yes	Yes
Canned soups, vegetables, and sauces	1–5	No	3% Loss	No	No
Canned fruits	5–15	No	3% Loss	No	Yes
Nuts, snacks	5–15	No	5% Gain	Yes	No
Dried foods	5–15	No	1% Gain	No	No
Fruit juices and drinks	10–40	No	3% Loss	No	Yes
Carbonated soft drinks	10–40	< 20% CO_2 Loss	3% Loss	No	Yes
Oils and shortenings	50–200	No	10% Gain	Yes	No
Salad dressings	50–200	No	10% Gain	Yes	Yes
Jams, jellies, syrups, pickles, olives, vinegars	50–200	No	3% Loss	No	Yes
Liquors	50–200	No	3% Loss	No	Yes
Condiments	50–200	No	1% Gain	No	Yes
Peanut butter	50–200	No	10% gain	Yes	No

2.8.1 Bulk Density

Bulk density depends on the product. Different bulk densities have different free space volumes for packages of similar shape. The free space volume of a package (V) is related to the bulk density, ρ_b and the true density ρ_p (Robertson, 2013):

$$V = V_t - V_p = \frac{W}{\rho_b} - \frac{W}{\rho_p} \qquad \text{Eq. 2.8-2}$$

Where V_t is the total volume of the package in m^3, V_p is the volume of the product m^3 and W is the weight of the product in kg.

The free space volume has an important influence on the rate of oxidation. A large free space volume is undesirable as it constitutes a large oxygen reservoir. It is reported that a large package surface area and a low food bulk density result in greater oxygen transmission (Robertson, 1999).

2.9 Oxygen as an important parameter in lipid oxidation

Brown (2012) suggests that oxygen can be used to measure lipid oxidation reaction rates effectively. Although there are many methods that measure the lipid oxidation as discussed earlier, they are often inconsistent and not stable in producing a reliable result for comparison. As some products produced from lipid oxidation only occur under certain reaction conditions, it is often difficult to generate useful information. The author discussed that oxygen is consumed in most of the lipid oxidation and is easy and simple to carry out the measurement of oxygen throughout the reaction. By knowing the oxygen concentration in the reaction, key kinetic information of lipid oxidation can be tracked (Brown, 2012). By tracking the rates of oxidation, the stage and extent of reaction can be determined.

There is information that can be obtained by monitoring the oxygen concentrations in lipid system:

- Total amount of oxygen consumed
- The rates of oxygen consumption

By measuring the total oxygen used in a simple system, the extent of reaction can be obtained as there is a set amount of oxidisable product and a finite amount of oxygen required to react. Wewala (1997) stated that oxygen is a preliminary step in the lipid oxidation reaction pathway and has demonstrated that the oxidised flavour intensity increases with an increasing rate of oxygen consumption on dried milk powder (Wewala, 1997).

The rates of oxygen consumption will also be a significant parameter to measure the extent of oxidation reaction. Labuza and Dugan (1971) stated that rates of oxidation are different at different stages of lipid oxidation. For example, it was reported that the rates of initiation are slow until there are significant hydroperoxides built up. By monitoring the consumption of oxygen, the reaction rate can be understood. It was also reported that the rates of lipid oxidation are dependent on oxygen concentration (Takahashi, Kitakawa, & Yonemoto, 2000). As a result, by knowing the rates of oxygen consumption in each stage of lipid oxidation, the lipid oxidation reaction can be modelled.

Also, most oxygen consumption is likely to occur during the formation of lipid hydroperoxides; the oxidation rate is slow in secondary reactions. As concentration of lipid hydroperoxides builds up, the rate of oxidation increases exponentially. Correlation can be made between the oxygen consumption and the concentration of lipid hydroperoxide.

2.10 Storage Trial

Food quality changes over time, usually deteriorating; an understanding of and the ability to predict the process are important. The most common and direct way of determining shelf life is to carry out the storage trial of the product experimentally under similar conditions that is likely to be encountered during storage and consumer use.(Steele, 2004). Studies have been made to evaluate the influence of the storage time and the storage temperature on the shelf life of milk powder (Lloyd, Hess, & Drake, 2009; Romeu-Nadal et al., 2007). Storage trials of milk powder have frequently been monitored by measuring the concentration of hydroperoxide and the volatile compounds, such as hexanal, produced from lipid oxidation over time. Sensory analysis has also been done to correlate with the chemical analysis.

One of the problems of the storage test is the time needed to conduct the test. Some food products may have a very long shelf life. The process of lipid oxidation, for example, has a slow reaction under normal conditions. As a result, accelerated storage has been a widely used method to assess the shelf life of foods. Accelerated shelf testing assumes that the principles of chemical kinetics can be applied to quantify the effects which extrinsic factors such as temperature have on the rate of deteriorative reactions. By subjecting the food products to a controlled environment in which one or more of the extrinsic factors is maintained at a higher than normal level, the rates of reaction will be accelerated and this shortens the time for a storage test (Robertson, 1999). An accelerated test is a practical way to conduct the storage test of the food.

Oxidation stability of the lipid fraction in milk powder can be monitored by an accelerated storage test. Romeu-Nadal *et al.* (2007) conducted the storage test of the milk powder at 25°C and 37°C to predict the shelf life of distinct milk powder formulas by measuring hydroperoxides, headspace volatile compounds and sensory quality. It was reported that the powder samples stored at 37°C were less stable than the same samples stored at 25°C, indicating that storage temperature is important in lipid oxidation of milk powder formulas.

2.10.1 Methods combination

As discussed earlier, monitoring the oxygen consumption rate is an effective tool to obtain the information for the lipid oxidation of the milk powder. Storage trial of the milk powder can be done to obtain the information relating to the rate of the formation of hydroperoxides and the secondary oxidation product such as hexanal. Sensory tests throughout the storage trial can also be conducted to correlate with the chemical analysis. The oxygen consumption rate of the milk powder at different storage stages can be measured, followed by the measurement of hydroperoxides and hexanal. Combinations of all the information gathered from the storage trial can generate a very useful result to predict and monitor the lipid oxidation of the milk powder.

2.10.2 Sensory Test

The main interest for the current project is to detect the level of the rancidity of the milk powder due to lipid oxidation. Sensory tests can be coupled with the storage tests to provide a detailed analysis. The development of off-flavours is particularly important as an index of deterioration of the milk powder. By conducting a sensory test, correlation can be made between the chemical test and the sensory data. For example, it is possible to determine the threshold limit of the concentration of hexanal that will give the rancid flavours of the particular milk powder by conducting a sensory test. However, sensory analysis is often difficult to conduct as it requires a trained panel which is time consuming to train.

2.11 Conclusion

The shelf life of milk powder of baby formula is based on maintenance of the nutritional value and the taste. The development of rancidity in the milk powder reduces the shelf life of the product and is not acceptable to the consumer. From the literature study, development of off flavors of the milk powder can be caused by oxidative and lipolytic rancidity. It is assumed that oxidative rancidity is the major problem that is responsible for deterioration of the milk powder causing rancid flavor in the product. Dried dairy products such as milk powder, are less susceptible to hydrolysis, and improving the processing of the milk powder greatly reduces the chance of lipolytic rancidity. An experiment

determining the fat acidity of the milk powder is necessary to confirm the above assumption. As a result, current work will be focused on the lipid oxidation of the milk powder

Lipid oxidation has been well studied over many years; the key areas related to the current project are the reaction of lipid oxidation, methods of tracking the oxidation products, the factors that affect the reaction such as temperature and oxygen concentration, and the analytical method to correlate with the flavouring of the milk powder.

By understanding the rate of lipid oxidation of the milk powder, it is possible to model the lipid oxidation of the milk powder. Relationship can be made between the stages of lipid oxidation and the development of rancid flavour under certain storage conditions.

Some of the methods at measuring lipid oxidation from the literature study will be used to compare the model of lipid oxidation. As discussed earlier, both PV and AV have poor correlation with sensory tests, therefore measuring only PV and AV will not provide the necessary information on the degree of lipid oxidation and the development of rancid flavour. However, from the literature review, oxygen is an important parameter to model the lipid oxidation. The oxygen concentration and the rate of oxygen consumption can be determined to provide the information of the lipid oxidation of the milk powder. There is very little information that utilises kinetic information to quantitatively predict the shelf life changes under certain storage conditions. In order to predict the shelf life of the milk powder, the rates of lipid oxidation must be identified. Although PV does not reflect the rancidity of the product well, as the concentration of hydropeoxides increase then decrease when the rate of formation of hydroperoxide is smaller than the rate of depletion; however, the concentration of lipid hydroperoxide influences the extend of the lipid oxidation. As mentioned earlier, correlation can be made to determine the oxidation reaction between the oxygen consumption and the concentration of lipid hydroperoxide. By coupling the measurement of oxygen consumption and the concentration of lipid hydroperoxides from the lipid oxidation, it will provide greater detail on the rate of lipid oxidation. As development of off-flavours in milk powder is caused by the secondary oxidation products, it will be ideal if the secondary oxidation volatiles can be quantified. Therefore, the rate of rancidity development can be compared with the rate of lipid oxidation, by measuring both the primary and secondary oxidation products.

Storage trials of the milk powder will be conducted to determine the key parameters over the storage period such as PV, the rate of oxygen consumption, and the volatile compounds such as hexanal that caused the off flavours. Sensory analysis can be done parallel with the storage trial to correlate the sensory score with the concentration of hexanal volatiles.

It is suggested that a simple mathematical model can be generated to model the lipid oxidation of the milk powder. The objectives of the current project will be:

- A model to explain the rate of lipid oxidation.
- Quantifying the relationship between temperature and lipid oxidation.
- A simple test method to quantify the rancid flavour of milk powder.
- To determine a point of lipid oxidation that is not acceptable due to rancidity.

Chapter 3 Preliminary Experiment

3 Lipolytic Rancidity

3.1 Introduction

Before focusing on the oxidative rancidity, it is necessary to determine whether lipolytic rancidity has an effect on the rancidity of the milk powder. As discussed in the literature review section, lipolytic rancidity in dairy products is rare if the milk from which they were made was properly pasteurised or dried (Allen & Hamilton, 1994). The occurrence of lipolytic off-flavours in dairy products only increases when pipeline milking and prolonged cold storage of milk at the farm were introduced (Jellema et al., 1991).

The degree of lipolysis can be determined by measuring the total acidity of the milk fat from the powder. As the lipolytic off flavors are mainly due to the increase in the concentration of short chain (C_4 –C_{12}) free fatty acids in milks, several methods have been developed to determine the concentration of free fatty acids in dairy products, and are able to detect changes of the acidity of the fat which give a good indication of the degree of lipolysis.

The development of the lipolysis may be assessed by determining the overall quantity of free fatty acids, or the acidity of the isolated fat or by the quantitative determination of the various individual acids. If the aim is to obtain the information on the organoleptic defects due to lipolysis, then a precise determination of the individual free fatty acids is necessary. If only the extent of lipolysis is to be measured then a determination of the global free fatty acids content is adequate. Determination of overall quantity of free fatty acids is simple and easy to carry out by a titration method and has proved to be sufficient to determine the degree of lipolysis (Jellema et al., 1991).

3.2 Method

The goat milk powder was provided by New Image Group. Two product samples were used to determine the degree of lipolysis, the powder A and the powder B sample. Stale samples for both products were also provided. The fat content of the goat milk power was 24% for both products. To determine the degree of lipolysis, the milk fat needed to be extracted then dissolved in a neutralised solvent and then a titration carried out.

3.2.1 Fat Extraction

Fat is extracted by the Soxhlet extraction method. The Soxhlet extractor is a specialised piece of glassware consisting of a condenser, porous container and a distilling pot. The reflexing solvent repeatedly washes the solid (milk powder), extracting the milk fat into the flask. Soxhlet extraction was chosen because it is simple and easy to perform, yielding high quality extraction with desired high yields. The equipment was already available at Massey University food laboratory. Figure 3.2-1 below shows the experimental setup.

Figure 3.2-1: Experimental set up for Soxhlet extraction.

In Soxhlet extraction, the milk powder is placed in a porous thimble which is washed with organic solvent, in this case diethyl ether, repeatedly. The extraction is refluxed for 6 hours to extract the fat from the milk powder. The detailed procedure of Soxhlet extraction methods can be obtained from Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1990).

3.2.2 Determination of fat acidity

The method to determine the fat acidity of the milk powder follows the standard set by International Organisation for Standardisation (IOS) of International Dairy Federation, IDF (*Milkfat products and butter : determination of fat acidity (Reference method)*, 2004). A titration method is used to determine the acidity of the milk fat and is defined as the amount of substance of alkali required to neutralise the free fatty acids contained per mass of fat present in the test sample. The acidity of the fat is expressed in mmol per 100 g of fat. The detailed procedure can be seen at the IDF standard method.

Figure 3.2-2: Fat acidity titration set up.

3.3 Result

The table below shows the fat acidity of the milk fat of both Powder A and Powder B of the goat milk powder. The fat acidity of the Powder A samples ranged from 0.542 to 0.598 mmol per 100g of fat, whereas the Powder B samples have a larger range of fat acidity between 0.531 to 0.619 mmol per 100g. The stale Powder A powder and stale Powder B had ranges between 0.688-0.712 and 0.732 - 0.846 mmol per 100g of fat respectively.

Table 3.3-1: Fat acidity of the goat milk powder (New Image Group Ltd) obtained from titration.

Powder A sample	Fat acidity (mmol/100g fat)	Powder A Stale sample	Fat acidity (mmol/100g fat)
1	0.552	**1**	0.688
2	0.578	**2**	0.695
3	0.542	**3**	0.702
4	0.598	**4**	0.690
5	0.574	**5**	0.712
6	0.562	**6**	0.708
Powder B sample		**Powder B Stale Sample**	
1	0.534	**1**	0.846
5	0.531	**2**	0.829
3	0.545	**3**	0.732
4	0.619	**4**	0.8
5	0.574	**5**	0.846
6	0.571	**6**	0.829

3.4 Discussion

From the literature review, it was reported that the degree of lipolysis would be significant if the fat acidity of the milk fat reached the value of 2.2 mmol per 100g of fat and above (Tallamy & Randolph, 1969). The result therefore suggested that the extent of lipolysis of the milk powder is not a main concern. The fat acidity of both samples is much lower than the threshold value of 2.2 mmol per 100g of fat. Although the fat acidity of the stale samples was higher than the fresh samples, it was not close to the threshold limit. This suggested that the degree of lipolysis at the end point of shelf life of the powder would not be significant. Freshly secreted milk will always contain a small quantity of free fatty acids regardless of the proper handling of the milk, and is not necessary for the result of lipolysis. From the result, there is a trace amount of fat acidity in the milk fat; however, the amount is not significant.

3.5 Conclusion

There are two types of rancidity that can cause dairy products to go rancid, lipolytic and oxidative rancidity. From the results above, it can be concluded that lipolysis is not the main cause of rancidity in milk powder for Powder A and Powder B products. Literature had already suggested that dried dairy products are less susceptible to hydrolysis but are more susceptible to oxidation in the presence of air. Lipolysis can be greatly reduced as long as the processing methods and hygiene are emphasised. In the conclusion, the lipolytic rancidity was deemed insignificant and the current work would thus focus on the oxidative rancidity, the oxidation of the milk fat.

Chapter 4 Model Development

4 Introduction

A simple model is needed to generate useful information on the lipid oxidation of milk powder. As discussed from the literature review, lipid oxidation is a very complex system involving many factors, complicated interactions, and is difficult to predict the individual effects and interactions of all the food system. The model should be simple and target specific milk powder products, and must reduce the degree of complexity outlined from the literature review. The model should provide adequate and well represented information on the oxidation reaction and the shelf life predictions.

A simple model will be able to:

- Explain the rate of lipid oxidation.
- Quantify the relationship between temperature and lipid oxidation.
- To find out the shelf life of the milk powder under normal storage conditions, for a given level of lipid oxidation.

4.1 Lipid Oxidation Reaction

From the literature review, the oxidation reaction can be broken into three simple reaction pathways including initiation, propagation and termination reactions (See Chapter 2 Eq. 2.3-1 to 2.3-4).

Radicals are formed via slow initiation reactions in the presence of initiators, usually involving the singlet oxygen with lipids. The radical species are unstable and react further with oxygen and lipids to form peroxyl radicals and lipid hydroperoxides. The primary oxidation product, lipid hydroperoxides are semi-stable intermediates and are in equilibrium with radical species. Lipid hydroperoxides break down in the presence of oxygen and form other radical species which react with oxygen and lipids. As Brown (2012) stated, the recycling of radical species and consequential consumption of oxygen and lipids is characteristic of the propagation phase and explains the exponential phase of the consumption of oxygen rate caused by the build-up of highly reactive species. The reaction shown in eq. 4.1-1 demonstrates the decrease in the oxygen concentration in the beginning of the oxidation when lipid hydroperoxides reacts with oxygen and decomposes to free radicals (Takahashi et al., 2000).

$$ROOH + O_2 \rightarrow RO_2 \cdot + H \qquad \text{Eq. 4.1-1}$$

At the termination stage, radical species and lipid hydroperoxides are removed from the system to form non-radical products. These non-radical products are the secondary oxidation products and are volatile aromatic compounds, which are perceived as off flavours associated with oxidised fats. Secondary oxidation products, such as aldehyde formed from hydroperoxides decomposition, can be further oxidised to organic acids and other tertiary oxidation products. Initially, the rate of termination is much slower than the rate of propagation, as the concentration of the oxygen decreases in the system, and the termination reactions become more dominant.

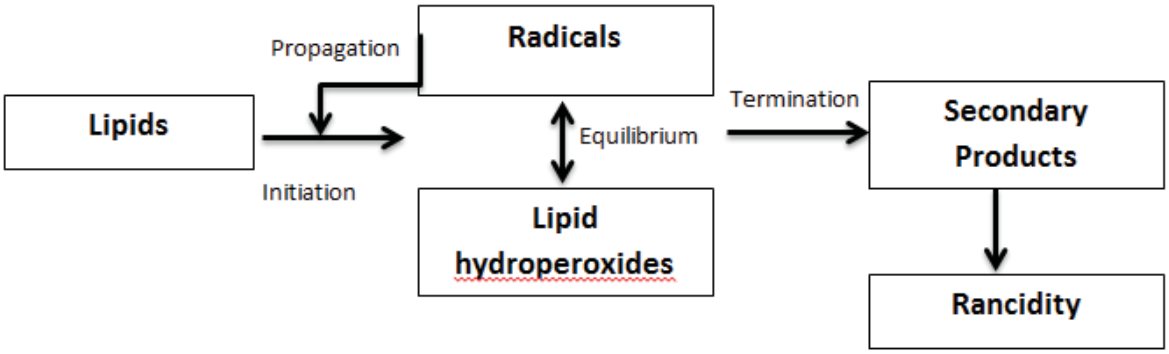


Figure 4.1-1: Lipid oxidation pathway of the lipids.

4.2 Rancidity development

4.2.1 Peroxide value

It is assumed that a small amount of oxidised fat can readily develop rancid flavour in a food product (Theodore P. Labuza & Dugan, 1971). Brown (2012) suggested that the rate of rancidity is proportional to the concentration of lipid hydroperoxides, and the measurement of the concentration of hydroperoxide should provide the rate of rancidity development. As discussed in the literature review, hydroperoxides are formed as the primary oxidation product in lipid oxidation. However, measuring the primary oxidation product such as peroxide value may not be a good representation of the level of rancidity. Measuring a primary oxidation product will show large changes in initial oxidation products, potentially before rancidity has occurred. And also, lipid hydroperoxides cannot be used to compare two different products as an absolute result, because the peroxide values are different for different products. The flavour threshold point for rancidity occurs when the peroxide volatile breakdown products reach a level in the headspace to cause an off flavour, and this is usually prior to the maximum peroxide value (Nawar, 1985).

New Image Group had found the results of peroxide value inconsistent with the rancidity development from their products and concluded that measuring the peroxide value of the milk powder is not a good indicator of the level of rancidity. However, the peroxide value can be an indicator of the initial stage of oxidative change on the fat products.

4.2.2 Oxygen Consumption

As discussed in the literature review, oxygen can be a useful parameter to monitor the lipid oxidation. Unlike peroxide values, the changes in oxygen concentration due to lipid oxidation can be compared directly with different products. Oxygen is required during the initiation stage of the lipid oxidation; the rate of oxygen consumption can provide useful information to monitor the reaction. In the measurement, the concentration of oxygen decreases as it is consumed by the substrate; oxygen is considered to be consumed all through the oxidation not only by the reaction with fat substrate but also by other reactions.

The initiation reaction of the hydroperoxide with oxygen is taken into account. Hydroperoxide reacted with oxygen to decompose. It was reported that this reaction is responsible for the drastic decrease in the concentration of oxygen at the beginning of the oxidation (Takahashi et al., 2000):

$$ROOH + O_2 \rightarrow ROO\cdot + HOO\cdot$$ Eq. 4.2-1

The lipid hydroperoxide can be also decomposed through bimolecular reaction through another initiation producing RO· ,the alkoxyl radical:

$$2ROOH + O_2 \rightarrow ROO\cdot + RO\cdot + H_2O$$ Eq. 4.2-2

The alkoxyl radicals react with fat to generate alcohol, ROH (Maccoll, 1985):

$$RO\cdot + RH \rightarrow ROH + R\cdot$$ Eq. 4.2-3

The secondary product such as alcohol are then oxidised further to generate aldehyde and carboxylic acid. These reactions are also known to occur consecutively (Maccoll, 1985). The overall reaction of the secondary oxidised product is as follows (Takahashi et al., 2000):

$$Secondary\ product + O_2 \rightarrow secondary\ product'$$ Eq. 4.2-4

4.2.3 Hexanal Formation

Many studies have suggested that only secondary oxidation products are responsible for the changes in flavour properties of food caused by lipid oxidation (Edwin N. Frankel, 1991). It has been reported that the development of off-flavours in milk is strongly dependent on the volatile compounds such as hexanal and pentanal (Chávez-Servín, Castellote, & López-Sabater, 2008; Ulberth & Roubicek, 1995). Buttery *et al.* (1988) suggested that hexanal content is directly related to oxidative off-flavours due to its low flavour thresholds in oils and fats (Buttery, Turnbaugh, & Ling, 1988). Li *et al.* (2012) reported that hexanal had the largest increase among the volatile compounds for the newly produced milk powders, and may be the main indicator of oxidised flavour in milk powder (Li, Zhang, & Wang, 2012). As a result, the headspace volatiles, especially hexanal, have been commonly used to monitor the extent of lipid oxidation (Koelsch, Downes, & Labuza, 1991).

The reason for aldehydes to be a dominant secondary oxidation compound is due to the breakdown of fatty acids such as oleate, linoleate and linolenate from oxidation. Unsaturated fatty acids are more susceptible to oxidation because its double bonds are attacked by atmospheric oxygen. Due to the chemical structure of unsaturated fatty acids and the position of the double bond, aldehydes are one of the main compounds formed in secondary oxidation products followed by hydrocarbon and alcohol.

Table 4.2-1 listed some typical breakdown products found in rancid foods and the lipid hydroperoxide from which they are derived.

Table 4.2-1: The specific breakdown of unsaturated fatty acids to primary oxidation product hydroperoxides and secondary oxidation product aldehydes.

Fatty acid	Hydroperoxide	Aldehyde Formed
Oleate	C9	2-decenal
	C10	nonanal
	C11	ocatanal
Linoleate	C9	2,4-decadienal
	C13	n-hexanal
Linoleanate	C12	2,4-heptadienal
	C13	3-hexanal
	C16	propanal

4.2.4 Measurement of oxidation reaction and rancidity

As discussed above, the consumption of oxygen and hexanal formation through oxidation reaction would be important parameters to measure the extent of the lipid oxidation and rancidity development. Although peroxide value may not be a good indication of the rancidity development, it does provide information regarding the oxidation reaction. Therefore, these parameters will be measured to monitor the lipid oxidation and rancidity development.

4.3 Sensory Analysis

The shelf life of milk powder is limited by the development of rancidity, as the flavouring affects the consumer acceptability of the product. The objective of the sensory analysis was to determine the recognition threshold of rancid defect. Rancid flavours are chemically very complex as it depends on the substrate and can be oxidised through several different mechanisms.

As discussed previously, lipid oxidation frequently contributes to flavour changes during storage of food. There are many measurements for lipid oxidation, such as peroxide determinations, anisidine value, and the thiobarbituric acid method. However, most of these measurements show little correlation with the sensory score (Jacobsen, 1999); they are either difficult to apply as they are indirect, or are not sensitive enough (Fritsch & Gale, 1977). GC has become a more common method to measure the flavour defect due to direct measurement of individual compounds responsible for rancid flavours. As hexanal has a low flavour threshold and is responsible for the rancid flavour, it means that small amounts of residual would cause the off-flavour to the milk powder. Therefore, hexanal will be measured to correlate with the sensory score. A simple relationship between the concentration of hexanal and sensory evaluation can be established.

4.4 Reaction Kinetics

A simple mathematical model can be used to derive basic kinetic information for a system in order to predict changes during the storage of the milk powder. The majority of food reactions (e.g. food oxidation, microbial growth, Maillard browning) that have been studied have been characterised as between pseudo zero or pseudo first order reaction (T. P. Labuza, 1984). The kinetic model would be able to predict the changes under the normal storage condition from the accelerated storage test.

As discussed above, oxygen depletion is a useful parameter to predict the changes due to lipid oxidation; the depletion of oxygen over the period of time can be mathematically modelled using the experimental data. For the rate of change of oxygen, the model can be derived using the general rate law as below:

$$\frac{dO_2}{dt} = -k{O_2}^n \qquad \text{Eq. 4.4-1}$$

Where k is a rate constant per unit time, oxygen concentration in % and n is the order of the reaction, this can be integrated to give an equation for O_2 as a function of time.

$$\frac{dO_2}{{O_2}^n} = -kdt \qquad \text{Eq. 4.4-2}$$

$$\int_{O_{2i}}^{O_2} {O_2}^{-n}\, dO_2 = -kt \qquad \text{Eq. 4.4-3}$$

$$\frac{{O_2}^{1-n}}{1-n} - \frac{{O_{2i}}^{1-n}}{1-n} = -kt \qquad \text{Eq. 4.4-4}$$

Where O_{2i} is the initial oxygen concentration (at roughly 20.9%) at t=0. Equation 4.4-4 can be rearranged and expressed as:

$${O_2}^{1-n} = {O_{2i}}^{1-n} - kt(1-n) \qquad \text{Eq. 4.4-5}$$

$$O_2 = ({O_{2i}}^{1-n} - kt(1-n))^{\frac{1}{1-n}} \qquad \text{Eq. 4.4-6}$$

The relationship of the oxidation reaction rate constant (k) to temperature can be quantified by the Arrhenius equation (equation 4.4-7):

$$k = k_0 \exp\left(\frac{-E}{R\square}\right) \qquad \text{Eq. 4.4-7}$$

Where k_0 is the Arrhenius constant, E is the activation energy (kJmol^{-1}), R is the universal gas constant (8.3145 Jmol^{-1}K^{-1} and T is absolute temperature (K). Therefore, k can be substituted from equation 4.4-7 and the concentration of oxygen at a given time can be predicted by equation 4.4-8:

$$O_2 = ({O_{2i}}^{1-n} - k_0 \exp\left(\frac{-E}{RT}\right) t(1-n))^{\frac{1}{1-n}} \qquad \text{Eq. 4.4-8}$$

The parameters O_2, n, E and k_0 from equation 4.4-8 were fitted to the experimental data of the concentration of oxygen simultaneously by minimising the residual sum of squared errors by non-linear regression with a Levenberg-Marquardt algorithm function *lsqcurvefit*, using MATLAB® (R2013, The Mathworks Inc, Natick, Mass. U.S.A). The MATLAB® code can be seen in Appendix 9.2.

Once the equations have been fitted with the experimental data, the rate constant and the activation energy of each product can be estimated by Matlab. Prediction on the rate of consumption of oxygen can then be made under different storage temperatures.

4.4.1 Reaction order

As mentioned before, most of the food reactions followed elementary reactions either zero or first order reaction. However, lipid oxidation is a complex reaction and the order of reaction can lie between zero to one. The rate of consumption of oxygen in lipid oxidation can be related to the form of Michaelis-Menten kinetics known as enzymatic reactions (McGoron, Nair, & Schubert, 1997):

$$-\frac{dO_2}{dt} = \frac{O_2 \times V_{O_2\,max}}{O_{2m}+O_2} \qquad \text{Eq. 4.4-9}$$

Where $-\frac{dO_2}{dt}$ is the rate of oxygen uptake, $V_{O_2\,max}$ is the maximum rate of oxygen consumption in % per week, O_{2m} is the oxygen concentration (%) at which $\frac{dO_2}{dt}$ is $\frac{1}{2}V_{O_2\,max}$ and O_2 is oxygen concentration (%)

From this model, it can be conceptualised as the smooth transition from first order to zero order kinetics. For low oxygen concentration, the rate of oxygen consumption is proportional to O_2 and for high oxygen concentration the rate is a constant and is independent of O_2.

Labuza (1971) also suggested an equation on the rate of consumption of oxygen similar to McGoron's (1997) equation:

$$-\frac{dO_2}{dt} = kR_i^{\frac{1}{2}}(RH)\frac{O_2}{O_2+k'(RH)} \qquad \text{Eq. 4.4-10}$$

Where R_i rate of initiation in lipid oxidation, k and k' are rate constants and RH is substrate concentration (fat). It is noted that the whole term k (RH) is essentially a constant assuming there is excess amount of fat.

Labuza (1971) proposed a free radical pathway, which is described by dividing the reaction into a typical three parts lipid oxidation reaction including initiation, propagation and termination (Theodore P. Labuza & Dugan, 1971):

Initiation

$$initator \xrightarrow{k_i} Free\ radicals \quad Rate = Ri \qquad \text{Eq. 4.4-11}$$

Propagation

$$R^{\cdot} + O_2 \overset{k_0}{\rightarrow} ROO^{\cdot} \quad \text{Eq. 4.4-12}$$

$$ROO^{\cdot} + RH \overset{k_p}{\rightarrow} ROOH + R^{\cdot} \quad \text{Eq. 4.4-13}$$

Termination

$$ROO^{\cdot} + ROO^{\cdot} \overset{k_t}{\rightarrow} Non\ Radical\ products \quad \text{Eq. 4.4-14}$$

$$ROO^{\cdot} + R^{\cdot} \overset{k_{t'}}{\longrightarrow} Non\ Radical\ products \quad \text{Eq. 4.4-15}$$

$$R^{\cdot} + R^{\cdot} \overset{k_{t''}}{\longrightarrow} Non\ Radical\ pr\square\square ucts \quad \text{Eq. 4.4-16}$$

Where k_i initiation rate constant, k_o oxygen step rate constant, k_p propagation step rate constant, k_t $k_{t'}$ and $k_{t''}$ are termination rate constants.

According to Labuza's work, under conditions where oxygen is not limiting, the assumption can be made that all the oxygen reacted is in the form of peroxides:

$$-\frac{dO_2}{dt} = \frac{k_p R_i^{\frac{1}{2}}}{2k_t}(RH) \quad \text{Eq. 4.4-17}$$

When the oxygen is limiting, the rate equation becomes more complex and has this expression:

$$-\frac{dO_2}{dt} = \frac{k_p R_i^{\frac{1}{2}}}{2k_t}(RH) \times \frac{O_2}{O_2 + \frac{k_{t'}^{0.5}}{k_t^{0.5}}\frac{k_p}{k_0}(RH)} \quad \text{Eq. 4.4-18}$$

The above equation can be simplified; if the initiation rate is constant and the amount of substrate (milk fat) is assumed to be in excess, then it can be written as:

$$-\frac{dO_2}{d\square} = A\left[\frac{O_2}{O_2 + B}\right] \quad \text{Eq. 4.4-19}$$

Therefore, the rate of oxygen consumption is affected by the available oxygen concentration in the system. When the system has high oxygen concentration, then it has order of reaction close to 0;

however, when oxygen becomes limited, the order of reaction is close to 1. As a result, it is expected that the order of reaction is between 0 and 1.

4.5 Conclusion

Lipid oxidation is a complicated reaction; the physical and chemical aspects of an oxidation system can combine to produce very complicated effects. It would be difficult and requires many years of research to create a model that is all encompassing. Based on the discussion above, a simple mathematical model can be generated to monitor the consumption of the oxygen from the goat powder. Oxygen is the primary reactant for lipid oxidation; by monitoring the oxygen concentration, the extent of reaction can be determined. The model can be coupled with other measurements such as hexanal analysis and sensory analysis to obtain a full analysis on the shelf life of the powder. Once the model has been developed, a method can be deduced to carry out the analysis.

Chapter 5 Method Development

5 Introduction

This chapter outlines the development of the method to obtain the result from lipid oxidation of the goat milk powder. An accelerated storage test was conducted to monitor lipid oxidation. Peroxide value, headspace concentration of hexanal and oxygen of the stored milk powder were measured over the storage period. Sensory analysis was also conducted to determine the relationship between hexanal concentration and the sensory score. Based on these requirements, methods were developed to achieve these analyses.

5.1 Storage Test

In order to determine the extent of lipid oxidation of the goat milk powder, a storage test was necessary to provide the information regarding the degree of rancidity development. Experimentally the storage test should ideally mimic those that are likely to be encountered during actual storage and consumer use; however, it is rarely possible to replicate the actual storage conditions and it is difficult to conduct a fully comprehensive shelf-life trial. As a result, a storage test is usually conducted under fixed storage conditions which do not fully simulate and cover all the aspects of storage found in practice.

The storage test will be able to provide the information regarding the kinetics of the lipid oxidation of the milk powder, the development of the rancidity over time, and the correlation between the sensory and the chemical analysis. Two products were investigated. Powder A and Powder B goat milk powder from New Image Group were used in the storage test.

5.1.1 Storage Conditions

The storage trial used a controlled experiment and could not be designed to cover all conditions. The fresh milk powder provided from New Image Group was supplied in the can without nitrogen flushing at the start of the experiment. The milk powder was transferred into the 125 ml glass vial and then stored under different temperature conditions. Each glass vial contained 40 g of powder. The glass vial with a rubber septum cap was assumed to be fully sealed. The headspace gas over the powder in the glass vial was analysed by gas chromatography.

The process of lipid oxidation is fairly slow under normal conditions such as at room temperature. In order to monitor the rate of oxidation of goat milk powder within a time constraint, accelerated methods were employed to estimate the oxidative stability of the milk powder in a relatively short period of time. There are several parameters both physically and chemically that can be used to increase the rate of oxidation, such as the temperature, metal catalysis, partial pressure of oxygen, and the intensity of the light. These factors alter the rate of reaction and consequently affect the development of the rancidity (EN Frankel, 1993). It was reported that the rate of the reaction increases exponentially with the absolute temperature and hence that was chosen as the single parameter to accelerate the oxidation process (Gómez-Alonso et al., 2004), (Mancebo-Campos, Fregapane, & Desamparados Salvador, 2008). Ragnarsson and Labuza (1977) also reported that increased temperature is the most common and effective means of accelerating the oxidation and

that the shelf-life should decrease logarithmically with increasing temperature (Ragnarsson & Labuza, 1977). Therefore, storage temperatures will be used to accelerate the rate of reaction. Elevated temperature such as above 60°C can alter the reaction mechanism causing skewed results. It is also reported that temperatures above 60°C rapidly produced caramel-like flavours due to the Maillard reaction (Lea, Moran, & Smith, 1943). Three accelerated temperatures, 37°C, 47°C and 57°C, were chosen for conducting the storage trial. Temperature at 37°C is considered safe for relatively long storage periods, and 57°C which has a faster rate of reaction may ultimately introduce undesirable high temperature effects towards the end of the storage. A temperature at 25°C was not chosen due to the slow rate of lipid oxidation reaction which cannot produce meaningful and useful results in a limited time; this will also reduce the experimental cost. All glass vials were stored in the incubator under different temperatures without light.

Arrhenius model (eq. 4.4-7) mentioned in a previous section can be used for an accelerated storage trial. For an n^{th} order system, the reaction rate constant is a function of temperature:

The value of E is a measure of the temperature sensitivity of the reaction; the reaction will go faster if the temperature is raised. In practice, there is substantial experimental error involved in the determination of the values of k and E from only two points (two temperatures for the experiment). Generally, if values of k are available at different temperatures and ln k is plotted against the reciprocal absolute temperature, 1/T, a straight line is obtained with a slope of –E/R. Therefore, the reaction rate is determined at three or more temperatures to reduce experimental error. Although it would be ideal to have more available accelerated temperatures for the storage trial, due to limited resources and a time constraint, three accelerated temperatures were chosen to determine the value of rate constant and activation energy (Steele, 2004).

Figure 5.1-1: Milk powder stored in the glass vial with rubber septum and fully sealed aluminium cap.

5.2 Headspace analysis

Recently, headspace gas chromatography (HSGC) became a popular and useful technique to quantify the volatiles produced by lipid oxidation in the storage of the product. Many literatures have reported using HSGC to monitor the concentration of volatiles of the milk powder from lipid oxidation (Chávez-Servín et al., 2008), (Romeu-Nadal et al., 2004), (Ulberth & Roubicek, 1995).

Both the Powder A and Powder B products of goat milk infant formula (New Image Group, NZ) were stored in the glass vial for headspace analysis. The glass vial consists of the rubber septum from an ultra-pure bromobutyl which has extreme low gas permeability and an aluminium cap to seal the septum (Wheaton®, US). Headspace hexanal and headspace oxygen were both measured using gas chromatography. Hexanal measurement was measured by Shimadzu GCMS-QP2010 model and oxygen measurement using Shimadzu GC 2014 model.

Oxygen and hexanal were measured separately in single individual glass vials. Each glass vial was discarded for each single measurement. The samples were measured in triplicate. The samples stored in a 37°C incubator were sampled fortnightly, whereas the samples stored at 47°C and 57°C were sampled weekly due to the faster reaction taking place at higher temperatures.

5.2.1 Headspace Gas Chromatography

The term 'headspace' denotes the vapour phase within a sealed container containing lipid or gas. In a typical headspace extraction, the liquid or solid is placed in a sealed vial with a specific conditions until equilibrium is reached, providing a constant composition mixture of gases in the vapour phase. The aliquot of this vapour phase is withdrawn from the vial and transferred to a gas chromatography for chemical analysis (Poole, 2012).

There are two methods in HSGC to collect the vapour gas from the sample and transfer to GC, static headspace and dynamic headspace. In static headspace, the volatile vapours are transferred using a gas tight syringe or a capillary (vapour trapped on the sorbent) to the GC. In dynamic headspace, a gas is passed through the lipid phase, evaporating analytes, which are then collected on a sorbent and transferred to GC; the dynamic headspace is similar to a purge and trap technique (Poole, 2012).

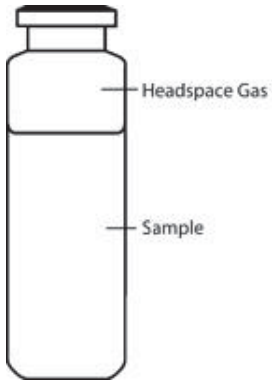


Figure 5.2-1: Diagram of the headspace analysis.

5.2.1.1 Static Headspace vs. Dynamic Headspace

Static headspace is a simple and fast technique that requires no sample preparation; it requires minimal sample treatment reducing artificial volatile compound formation. The headspace of the gas phase can be withdrawn manually by the air tight syringe and injected into the injection port of the

GC. Although it is reported that static headspace is less sensitive than dynamic headspace, for some sample types, as long as the amount of sample available is adequate, then it can provide adequate sensitivity (Pfannkoch & Whitecavage, 2000). With the dynamic headspace technique, the sample is warmed and the headspace atmosphere is constantly purged out of the sample vessel and through a trap. It would be difficult to perform the dynamic headspace method on the storage of the milk powder as the sample is required to be stored in the incubator and is measured over a period of time. Therefore, the static headspace technique was chosen to measure the hexanal concentration of the milk powder due to oxidation over a period of time. The aliquot of the sample is withdrawn from the glass vial by using a gas tight syringe manually.

5.2.2 Selection of the vial and sample volume

In manual operation, any small vessel can be utilised. The volume used is depended on the sample. A 125 mL glass vial was chosen as the storage vessel to conduct the measurement of the hexanal. The large vial volume provides a sufficient sample volume to conduct the analysis and to obtain a representative pattern. As literature had reported that the flavour thresholds for rancidity-causing oxidation products is often measured in parts per million (Jacobsen, 1999), a large sample volume is required to measure the small trace of hexanal in the milk powder. 40g of milk powder was stored in each glass vial for the storage test. The sample size enabled the analysis to be sensitive and also allowed a sufficient aliquot to be withdrawn from the vial for injection into the GC.

Hexanal concentrations in the headspace were measured by taking 2.2 ml samples using a gas tight syringe (Hamilton Gastight®, Hamilton Co.,US). Initially, 1 mL of aliquot was used to test its sensitivity; however, the result was not satisfactory as the peak was smaller than expected and also the shape of the peak was not symmetrical. As a result, 2.2 mL of aliquot was used and generated a satisfactory result. Oxygen concentrations in the headspace were measured by taking 5 ml samples from the glass vial instead of 2.2 ml. This is because oxygen concentrations were measured using a different GC and the length of GC column used for oxygen is longer than the hexanal column; therefore, a larger aliquot is required to fill the column.

5.2.3 GC operational conditions

It should be noted that if a powder sample is stored in a closed bottle for a long time, it must be mixed by shaking it before an aliquot is taken out for analysis. The reason for this is that a concentration gradient may have formed during storage, since the upper layers of the powder release the volatiles faster in the closed bottle into the headspace.

For hexanal measurement, the headspace gas was measured by taking 2.2 ml samples using a gas-tight syringe and injected through a port fitted with a Teflon-lined septum at the outlet of the exposure chamber of a gas chromatograph mass spectrometer (GCMS-QP 2010 Series, Shinadzu Corporation, Japan). The GC-MS was equipped with a 30m×0.25mm (I.D), 0.25µm film thickness, TG-5MS GC column (Thermo Scientific Inc). Operational conditions were: Inlet temperature, 200°C, the column oven temperature was maintained at 70°C for 1 minute and then programmed to increase at 6°C min^{-1} to 100°C. Helium gas was used as a carrier gas at a flowrate of 1ml min^{-1}. The optimal split flow ratio of 1:10 was used. Peak areas and retention times were recorded on an integrator.

Before withdrawing the headspace gas for analysis, the glass vial was heated in a water bath at 55°C for 20 minutes to achieve equilibrium so that the flavour analyte could be fully released to the

headspace and prevent variations in the conditions of the assay related to changes in the temperature of the incubator and laboratory.

For oxygen measurement, the headspace gas was measured by taking 5 ml samples using a gas-tight syringe through a metal tube connected to the exposed chamber. The samples were injected onto a gas chromatograph (GC 2014 series, Shinadzu Corporation, Japan) equipped with a 2m × 4.6 mm , Alltech®CTR I packed column (Alltech®). Operation conditions were: inlet temperature, 45°C, the column oven temperature was maintained at 35°C. Oxygen-free nitrogen (B.O.C Gases New Zealand Ltd) was used as a carrier gas at a flowrate of 25ml/min. Peak areas and retention times were recorded on an integrator.

5.2.3.1 Standard calibration for GC

A hexanal calibration curve was prepared according to the method of Ng *et al.* (2007) with some modification. The calibration curve was prepared using stock solution containing 100µl of hexanal (Sigma, St. Louis, MO, USA) in 100ml of ethanol. Hexanal standard solutions were made by adding 0.5, 1, 2.5, 5 and 10 ml of the stock solution to 19.5, 19, 17.5, 15 and 10 ml of ethanol, respectively, into 1L volumetric flasks. The flasks were filled to volume with Milli-Q water (Ng, Anderson, Coker, & Ondrus, 2007). The hexanal standards were prepared by placing 80ml of standard solution into the glass vial with the septum cap. The vial was then placed in a 55°C water bath for at least 15 minutes. A 2.2 ml headspace sample was manually injected into the gas chromatograph to obtain the standard curve for hexanal.

For oxygen standard curve, standards that contained various concentrations of oxygen in nitrogen were prepared from gravimetrically certified gas cylinders (B.O.C Gases New Zealand Ltd). The concentrations were 0, 5, 10 and 15 % of oxygen in nitrogen and 20.9% as air. 5ml of gas was withdrawn from the gas cylinder and injected into the gas chromatograph to obtain the standard curve of oxygen.

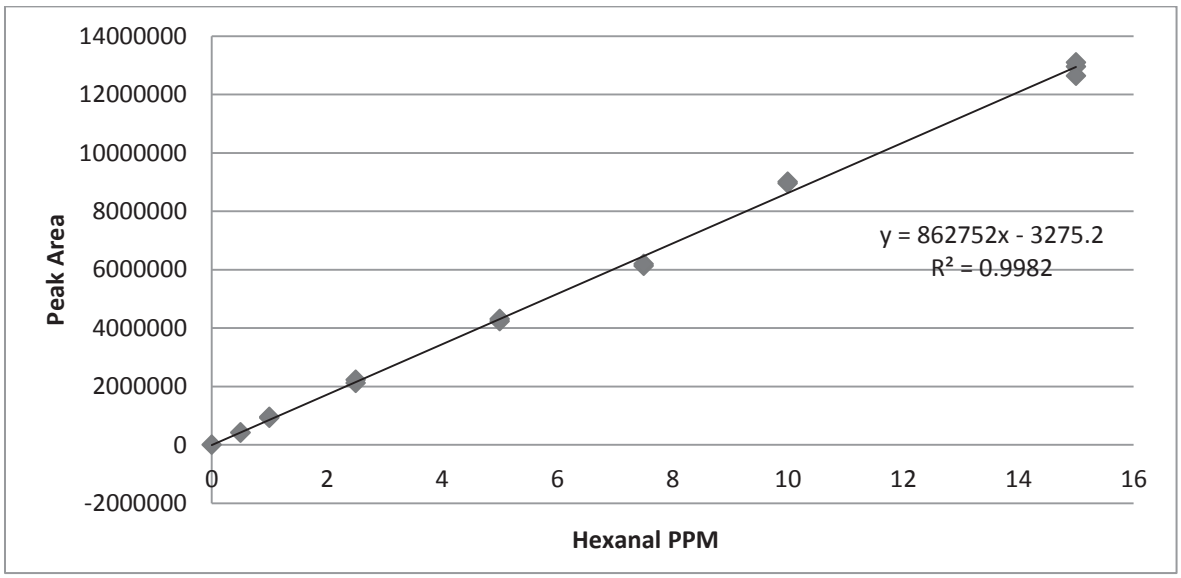


Figure 5.2-2: Hexanal standard curve.

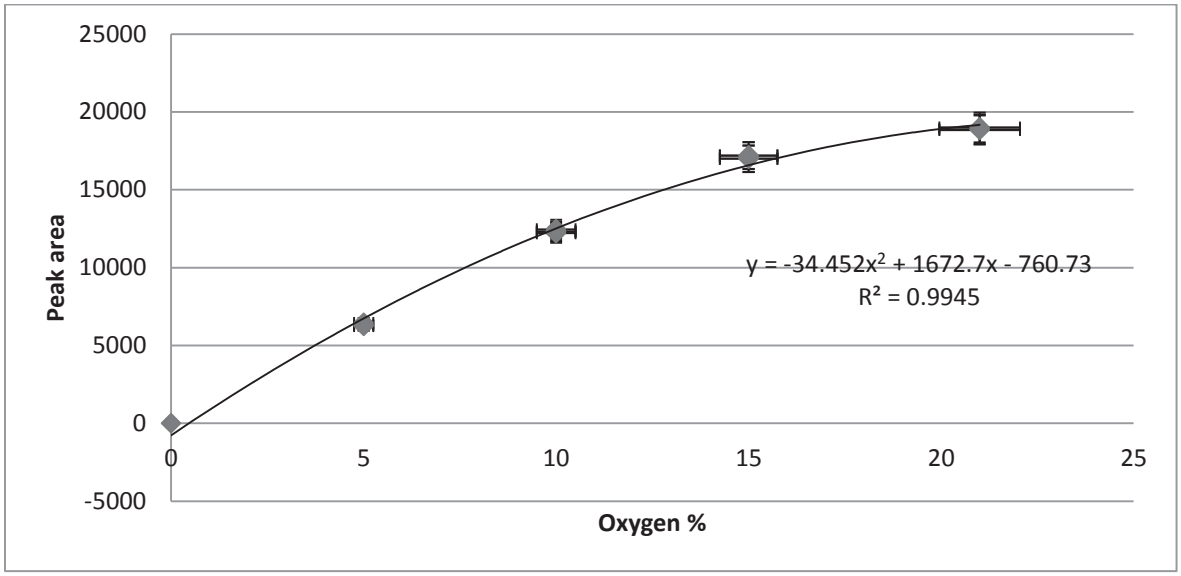


Figure 5.2-3: Oxygen Standard curve.

5.2.3.2 Gas Chromatography

As described in the preceding section, two different GC models were used for the oxygen and hexanal measurement. The oxygen measurement was done using the GC-2014 with a thermal conductivity detector. The column for GC 2014 model is specifically for the measurement of air such as O_2, N_2, CH_4, CO and CO_2 at ambient conditions.

The retention time for oxygen was 3.7±0.03 min. The figure below showed the typical oxygen peak:

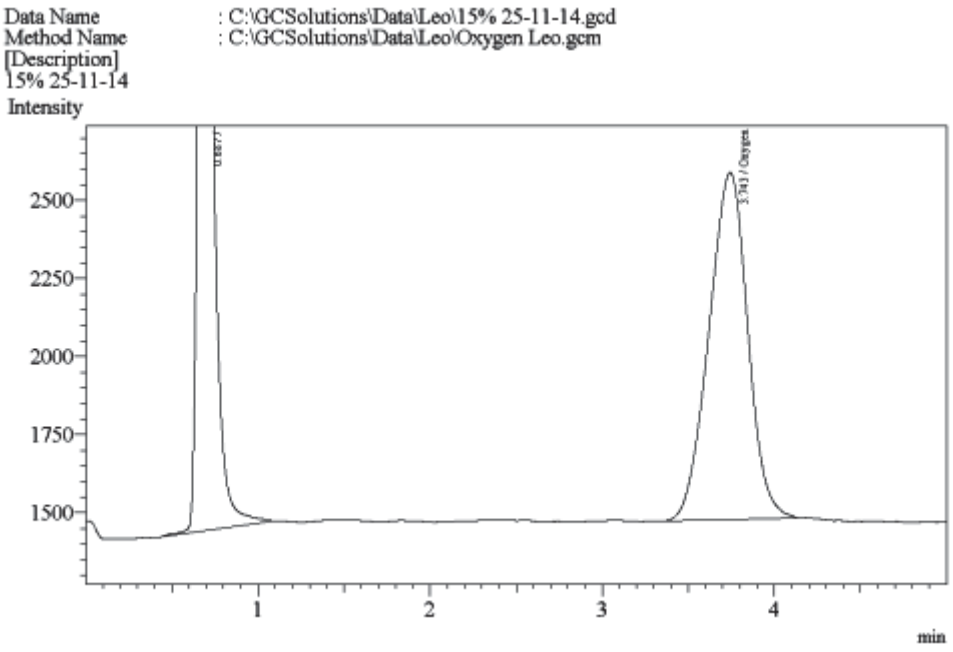


Figure 5.2-4: Chromatogram of oxygen peak (right) and air peak (left).

The unit of oxygen was presented as % of oxygen. With initial (maximum) oxygen concentration of roughly 20.9%, this is the oxygen concentration in the air.

The hexanal measurement used the GC-MS 2010 (Gas Chromatography-Mass Spectrophotometer). GC-MS combines the feature of gas chromatography and mass spectrometry. Although hexanal is the main compound for quantification, mass spectrophotometer can be used to identify unknown compounds that may be present in the sample. The retention time of hexanal was 2.60±0.03 min.

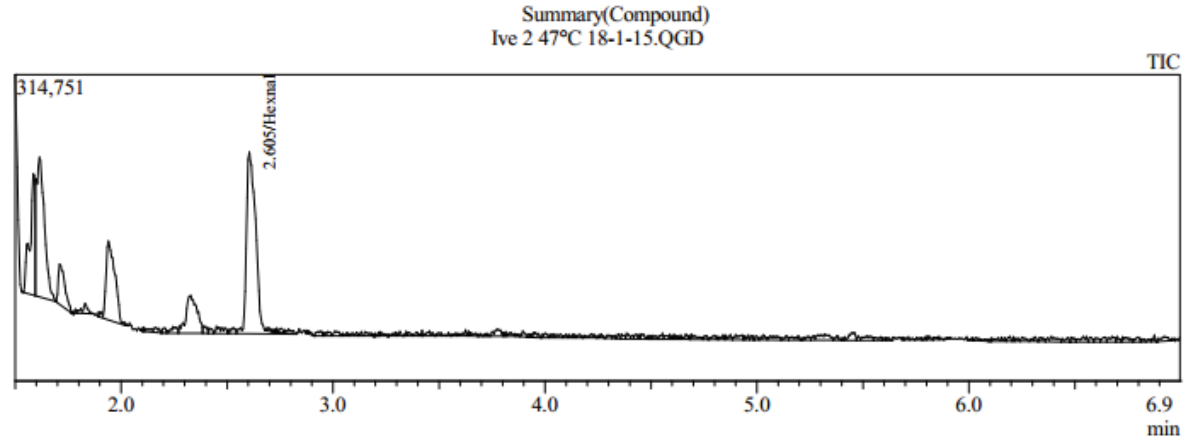


Figure 5.2-5: Chromatogram of hexanal peak and unknown peaks developed through the storage period.

The unit of hexanal concentration was measured in PPM from GC due to the calibration but was then converted into a unit of mg hexanal/kg powder.

5.3 Peroxide Value

The peroxide value was obtained by sending the sample to a laboratory for analysis (AsureQuality limited, Auckland, IANZ accredited Laboratory). The method reference is according to Loftus and Thiel (1946) (Loftus Hills & Thiel, 1946). The powder was sent to the laboratory on the same day as the headspace analysis, and similar time period for each week, giving consistency to reduce uncertainty.

5.4 Sensory Analysis

The sensory panel was selected from New Image Group internally. A screening process was done to screen out those who were not sensitive to the flavours. “Difference from control test” was used as the method for the sensory analysis. The objective for this method is to determine if a difference exists between one or more samples and a control sample, and to determine the size of the difference between the samples and the control.

5.4.1 Experimental Design

The sensory powder was stored in the cans in the same storage condition as the powder for analysis. The powder was sent to New Image Group office for sensory analysis.

An off-flavour goat milk powder was reconstituted and presented to the panellists to taste. Comparison was made between the fresh milk powder and the off-flavour milk powder. Those who can pick up the differences were selected to be a sensory panellist.

Panellists were trained to this sensory analysis. Sensory training included:

- How to taste samples and manipulate them in the mouth to get a maximal sensory response and avoid fatigue
- What tastes are associated with fat rancidity in an infant formula product and how to rate the intensity response
- How to conduct a difference from a control test

Assessors are presented with the control sample (milk made up from fresh powder) and the test sample (milk made up from powder under different storage conditions). Samples were prepared using the preparation instructions as specified by the manufacturer. All samples were hydrated using a magnetic stirrer for a period of four minutes or until solids were completely dissolved. Water used for hydration was held constant at 20°C ± 1°C. Samples were poured into disposable cups marked with a unique three digit code specific to each product sample. Control samples were prepared under the same conditions and poured into a disposable cup labelled with a star. Samples were kept from UV light to avoid deteriorative reactions and sensory test was conducted in low UV light conditions. Samples were prepared and served to panellists as soon as possible. Sensory testing by panellists was conducted independently and the environment was managed by the panel leader

The assessors were asked to determine if a difference existed between the sample and the control. The sensory score was provided to record the magnitude of the difference. A 5 point ordinal scale was used to rate the difference from the control. The table below shows the sensory score corresponds to the level of the difference with control. The higher the score, the more different the sample is to the control.

Table 5.4-1: The sensory score for comparison between the control and the sample.

Taste	Score
No Diiference	1
Slight difference	2
Moderate Difference	3
Very different	4
Extremely Different	5

Rinsing water was provided at room temperature to cleanse the pallet between samples and spittoons to spit samples when necessary. To avoid fatigue, a maximum of six samples were presented at any one time. Panel results were collected and the associated difference rating for a given sample was paired with a given sample. The average difference was calculated to obtain the average sensory score. On average, there were 7 panellists conducting the sensory test.

5.5 Conclusion

The methods developed enable the results to be analysed correctly. Three storage temperatures at 37°C, 47°C and 57°C were used to accelerate the storage time to monitor lipid oxidation. The powders were stored in glass vials where they were fully sealed to prevent diffusion of oxygen and moisture into the system. Headspace analysis is a useful and easy way to measure oxygen concentration and hexanal concentration in a closed system. It should be noted that the unit of oxygen concentration for gas chromatography was measured in %, with the initial (maximum) oxygen concentration of roughly 20.9 % (available oxygen in air). Hexanal concentration has a unit of mg hexanal/kg powder. The sensory trial ran in parallel with the storage trial. The sensory panel was selected internally with a screening process being used to select the most appropriate panellists. Measurements were done weekly for samples stored at 47°C and 57°C and fortnightly for samples stored at 37°C.

Chapter 6 Results and Discussion

6 Introduction

The full analysis was conducted to obtain useful results for the determination of the oxidation reaction in goat milk infant formula powder. As discussed from chapter 4 and 5, an accelerated storage trial was conducted at 3 temperatures (37°C, 47° & 57°C). PV, hexanal and oxygen from the milk powder stored in the glass vials were measured over the storage period. Sensory analysis was done internally by the panel from New Image Group. This chapter covers the results from the experiment, interpretation and discussion that were made to outline the solutions to the problem, and how the objectives were matched.

6.1 Storage Trial

Two infant formula products were under investigation in this project, Powder A and Powder B. Powder A and Powder B have different formulations; therefore, the degree of oxidation can be very different. Two sets of storage trial experiments were conducted in the given timeframe. For the second storage trial, only two storage temperatures, 47°C and 57°C, were used due to time constraints and limited resources. The starting powders for the first trial and second trial were slightly different. The manufacture date for both Powder A baby formula powder product and Powder B baby formula powder product for the first trial was 28th of October 2014, whereas the manufacture date for Powder A for the second trial was 26th of September 2014 and 13th of March 2014 for Powder B. The date of manufacturing for Powder A between first and second trials was closer in date than between first trial and second trial Powder B. For convenience, Powder A trial 1= A1, Powder A trial 2= A2, Powder B trial 1= B1 and Powder B trial 2= B2.

The second trial was not a true replicate to the first trial but acted as a confirmation run to test the reliability of the experimental method. It was predicted that the data (reaction rates) between A1 and A2 would be more similar than the data between B1 and B2. The fat content for both A1 and A2 was found to be around 26%, whereas the fat content for B1 and B2 was found to be 26% and 28% respectively.

The storage trial for trial 1 was 26 weeks due to a lower storage temperature of 37°C which takes longer, whereas the storage time for trial 2 was 16 weeks.

6.1.1 Particle Size of the Powder

The size of the milk powder was analysed by a Mastersizer 3000 laser diffraction particle size analyser. Table 6.1-1 below shows the particle size distribution of D10, D50 and D90 for both trials of Powder A and Powder B.

Table 6.1-1: Size distribution of the milk powder for each product.

	Dv10 (µm)	Dv50 (µm)	Dv90 (µm)
Powder A1	33.8	95.4	191
Powder A2	30	86.8	179
Powder B1	47.5	134	260
Powder B2	72.5	197	392

The size distribution of Powder A powder between the two trials is closer when compared with the Powder B powder. Looking at the D50 size distribution, the difference between the first and second trial of Powder A powder is 8.6µm, whereas the second trial of Powder B powder is 63µm bigger than the first trial of Powder B powder. The size of the powder can play an important role on lipid oxidation, as the size of the powder correlates inversely with the surface area of the powder. The size of the powder can affect the rate of lipid oxidation due to availability of the fat exposed to the air (oxygen). Further research can be done to look at the relationship between the sizes of the powder with the rate of lipid oxidation; however, this is beyond the scope of the current objective.

6.1.2 Fatty Acid Profile of the powder

The fatty acid profile for each product (two trials) was analysed by the accredited laboratory (Nutrition Laboratory, Massey Institute of Food science and Technology, Palmerston North). The fatty acid profile for both trials of Powder A and Powder B is shown in Table 6.1-1

Table 6.1-2: Fatty acid profile analysis of the powder g/100g powder.

Fatty Acids	B1	B2	A1	A2
	g/100g powder	g/100g Powder	g/100g powder	g/100g powder
C6:0 Caproic	0.40	0.39	0.20	0.19
C8:0 Caprylic	0.50	0.49	0.36	0.35
C10:0 Capric	1.20	1.16	0.69	0.68
C11:0 Undecanoic	ND	ND	ND	ND
C12:0 Lauric	1.77	1.63	1.50	1.47
C13:0 Tridecanoic	ND	ND	ND	ND
C14:0 Myristic	1.72	1.67	1.20	1.19
C14:1n5 - cis-9-Myristoleic	0.02	0.01	0.07	0.05
C15:1n5 - cis-10-Pentadecenoic	ND	ND	ND	ND
C16:0 Palmitic	3.62	4.25	5.05	4.96
C16:1n7 - cis-9-Palmitoleic	0.11	0.13	0.08	0.08
C17:0 Margaric	ND	ND	ND	ND
C17:1n7 - cis-10-Heptadecenoic	0.04	0.04	0.02	0.02
C18:0 Stearic	1.20	1.33	1.15	1.14
C18:1n9t Elaidic	0.03	0.04	0.03	0.02
C18:1n7t Vaccenic	0.09	0.11	0.07	0.07
C18:1n9c Oleic	7.81	7.86	8.26	8.11
C18:1n7c Vaccenic	0.17	0.17	0.18	0.19
C18:2n6t Linolelaidic	ND	ND	ND	ND
C18:2n6c Linoleic	3.45	3.26	4.52	4.38
C20:0 Arachidic	0.05	0.05	0.06	0.06
C18:3n6 - cis-6,9,12-Gamma linolenic	0.01	0.01	0.03	0.03
C20:1n9 - cis-11-Eicosenoic	0.03	0.03	0.04	0.04
C18:3n3 - cis-9,12,15-Alpha linolenic	0.34	0.32	0.48	0.47
C21:0 Heneicosanoic	0.02	0.02	0.01	0.01
C20:2n6 - cis-11,14-Eicosadienoic	ND	ND	ND	ND
C22:0 Behenic	0.05	0.05	0.05	0.05
C20:3n6 - cis-8,11,14-Eicosatrienoic	ND	ND	ND	ND
C22:1n9 - cis-13-Erucic	ND	ND	ND	ND
C20:3n3 - cis-11,14,17-Eicosatrienoic	ND	ND	ND	ND
C20:4n6 - cis-5,8,11,14-Arachidonic	0.01	0.01	<0.01	ND
C23:0 Tricosanoic	0.01	0.01	0.01	0.01
C22:2n6 - cis-13,16-Docosadienoic	ND	ND	ND	ND
C24:0 Lignoceric	0.02	0.02	0.02	0.02
C20:5n3 - cis-5,8,11,14,17-Epa	ND	ND	ND	ND
C24:1n9 - cis-15- Nervonic	ND	<0.01	ND	ND

The fatty acid profile between the two products is different. Between the two trials, the fatty acid profiles are fairly similar. It can be noted that an amount of unsaturated fatty acid such as Oleic acid and linoleic acid presented in the powder which is susceptible to lipid oxidation. It has been reported from studies that fatty acids are of primary importance to the rate of development of rancidity, since they are susceptible to oxygen attack. As discussed in the literature review section, the amount of unsaturated fatty acids such as oleate, linoleate and linolenate, play an important role in lipid oxidation but not the total fat content. These unsaturated fatty acids were present in both of the products; as a result, lipid oxidation was expected.

6.2 Peroxide value

Peroxide value (PV) is widely used as an indicator for oxidative rancidity. It has been discussed that it is difficult to provide useful guidelines relating PV to rancidity. High peroxides values are definite indications of rancid fat, but, moderate values may be the result of depletion of peroxides after reaching a peak. PV was measured throughout the storage trial, regardless of the usefulness of the PV for the rancidity test, as the PV may provide some information regarding the reaction of lipid oxidation. Figure 6.2-1 below shows the PV of A1 and B1 baby formula powder at three storage temperatures (37°C, 47°C & 57°C).

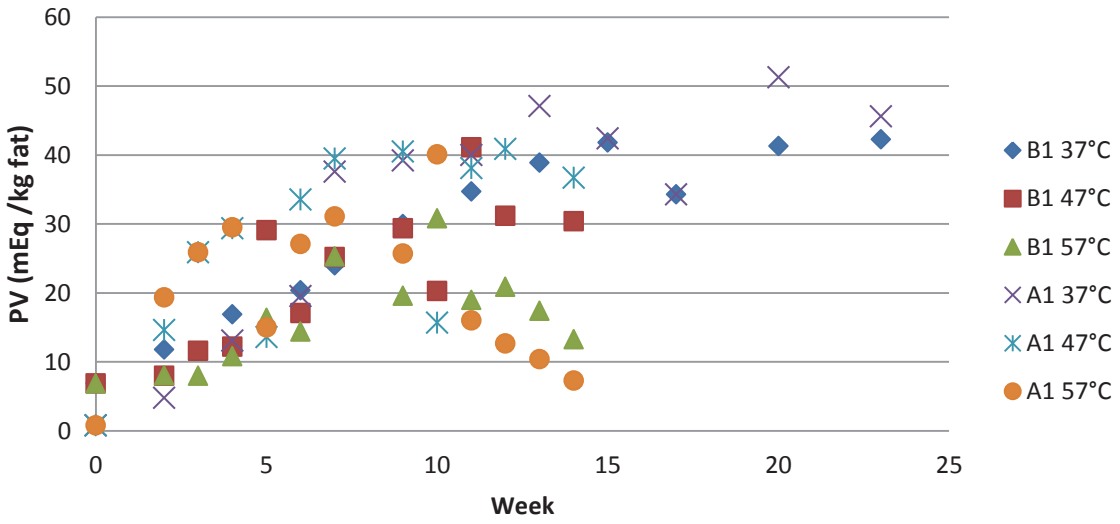


Figure 6.2-1: First trial of the PV result of Powder A and Powder B (A1 and B1).

Looking at Figure 6.2-1, there is a general trend where peroxide concentration increases to a maximum point then decreases for all three storage temperatures. This agrees with the literature which reported that the concentration of PV increases gradually as lipid oxidation occurs; the increase of the PV reaches a peak where the rate of formation of hydroperoxide is equal to the rate of decomposition of hydroperoxide. The concentration began to fall due to the depletion of hydroperoxides to form secondary oxidation products (Romeu-Nadal et al., 2007). Although the general curve pattern was shown in the figures, there were however, points that did not fit into this trend. The PV for Powder A at 47°C and 57°C and also Powder B at 47°C showed some fluctuations going up and down over the storage period. This may be due to experimental error or to the handling of the sample, as peroxide values are not static. The measurement can be very sensitive. The PV measurement was done by sending the sample to an accredited laboratory for analysis. There is the possibility of inconsistency in handling the sample by different technicians. It seems that the PV at week 5 and week 10 may be outliers for Powder A at 47°C and 57°C, and similar for Powder B at 47°C.

Figure 6.2-2 and Figure 6.2-3 below shows the PV of A2 and B2 in two storage temperatures (47° & 57°C). The PV for the second trial of Powder A showed a clear pattern of formation of hydroperoxides and depletion of hydroperoxide at 57°C. The PV at 47°C was steady after week 6 and did not show an obvious depletion as expected. In trial 2, the PV fluctuated less compared to the

trial 1 PV. However for Powder B, the starting powder had a PV of 25 mEq /kg fat, suggesting that the lipid oxidation had already occurred and is an indication that the powder was not fresh. This result was not surprising as the date of manufacture for B2 was quite old (13th of March 2014) as compared to the DOM for B1 and A1. Therefore, it can be concluded that the properties of Powder B product between first and second trial would be different and cannot act as a replicate. Further discussion will be made regarding the differences between first and second trial of Powder B from other results. It should be noted that the maximum PV was found to be around 40 meq /kg fat over the storage temperatures with a few exceptionally high at 50 meq /kg fat. The powder with the PV that close to this range (35-50 meq /kg fat) may have already become rancid.

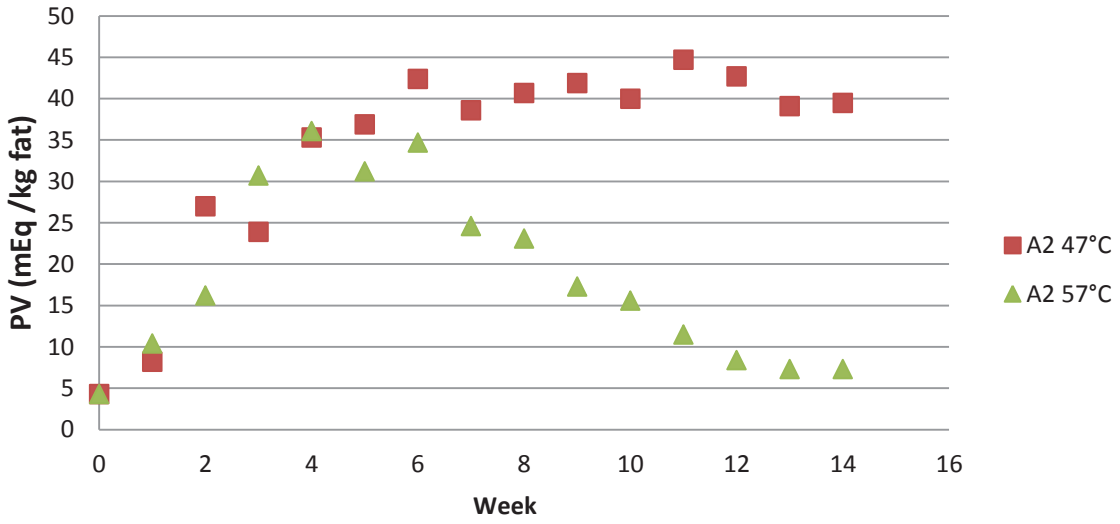


Figure 6.2-2: Peroxide value of A2.

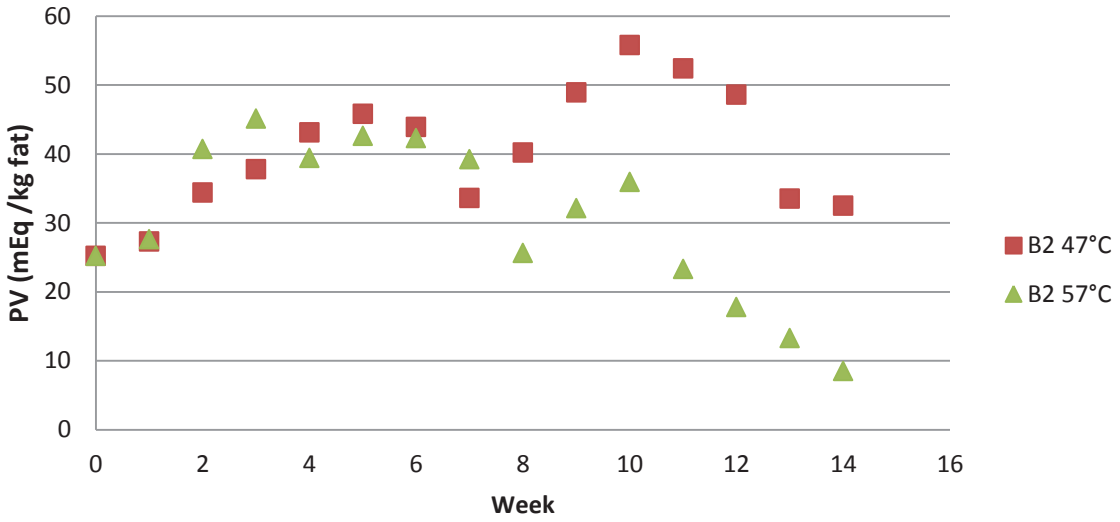


Figure 6.2-3: Peroxide value of B2.

6.3 Oxygen Consumption

Figure 6.3-1 shows the first trial of the headspace of oxygen concentration over the storage period for both Powder A and Powder B. The initial oxygen concentration in all the storage vessels was around 20.7% (equivalent to oxygen concentration in the air). The consumption rate of oxygen increases as the temperature increases. As the temperature increases, the rate of reaction increases following Arrhenius law; it only took the milk powder stored at 57°C 7 weeks to consume all the oxygen for Powder A and 8 weeks for Powder B. The powder stored at 37°C took between 20 to 24 weeks to use all the oxygen for Powder B and Powder A respectively. The result of the oxygen concentration from Figure 6.3-1 is expected; the higher the storage temperature, the faster the consumption of oxygen. Powder A and Powder B have different oxygen consumption rates. This is also expected as these two products have different fat profiles.

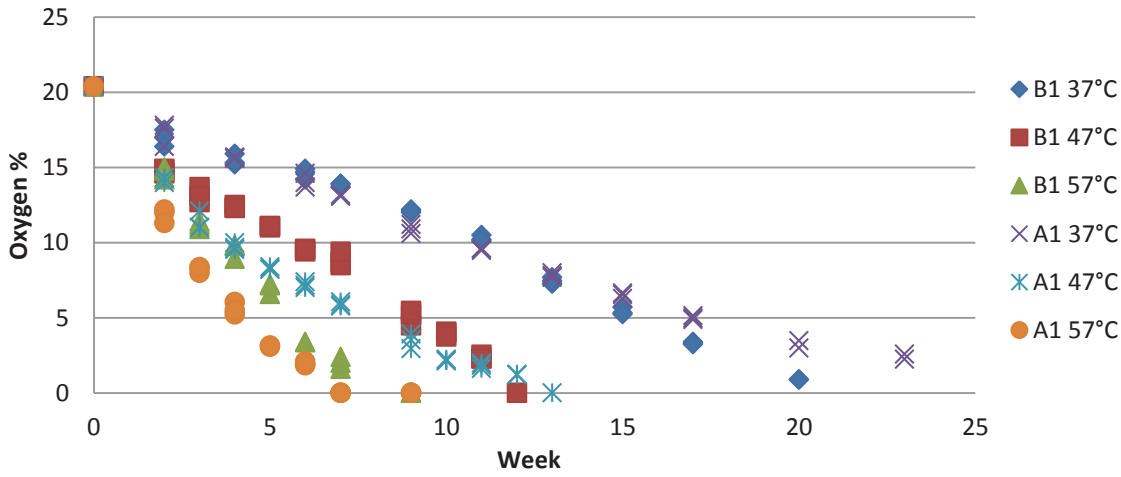


Figure 6.3-1: Headspace oxygen concentration throughout the storage period of the first trial result.

From previous discussion, it should be clear that the rate of lipid oxidation should be proportional to the concentration of lipid hydroperoxides and the concentration of oxygen is a function of concentration of lipid hydroperoxides. The depletion of lipid hydroperoxides is also related to the formation of secondary oxidation products.

$$\frac{\partial O_2}{\partial t} = f(ROOH) = Rate\ of\ secondary\ reaction \qquad \text{Eq 6.3-1}$$

Oxygen is required during the initiation stage of lipid oxidation to form peroxyl radicals and lipid hydroperoxides, and also oxygen is consumed during the vast majority of the lipid oxidation pathway from the early initiation reactions to the secondary reactions (See chapter 4 Eq. 4.1-1 to Eq.4.2-4).

Comparing at the PV from Figure 6.2-1 with the oxygen concentration from Figure 6.3-1, a relationship can be identified between the two results. Ignoring the outliers from the PV (at week 5 and 10), the concentration of lipid hydroperoxides was at its highest when the concentration of oxygen was at its lowest (0% or close to 0%). The rancidity may already have occurred before the PV reaches the maximum value. As the flavour thresholds for rancidity-causing oxidation products are often measured in parts per million (Jacobsen, 1999), Brown (2012) suggested that most of the oxygen consumption is likely to occur during the formation of lipid hydroperoxides. There is a delay at the formation of secondary oxidation products, and these occur at a relatively slow rate as long as radicals are present. As a result, rancidity may already become significant before secondary reaction becomes significant (Theodore P. Labuza & Dugan, 1971) (Takahashi et al., 2000). The results of the concentration of lipid hydroperoxides and the oxygen concentration agree with the result reported from literature.

It is more difficult to extract the information from the PV than to monitor the lipid oxidation. It is less obvious to identify which storage condition has faster lipid oxidation reaction by looking at the PV results. Although a storage 37°C temperature for both Powder A and Powder B was shown to have slower oxidation reactions, as it took a longer time to reach the peak of the PV curve, it is more complicated to pin out the differences between the reaction at storage temperatures of 47°C and 57°C for both of the products. Only through careful observation can the difference be identified. However, it is much quicker and easier to identify the differences in the reaction rate by looking at the oxygen concentration versus storage period. The oxygen result has also less variance as compared to the PV result, making it a more useful tool.

Figure 6.3-2 showed the second trial of the headspace oxygen concentration over the storage periods.

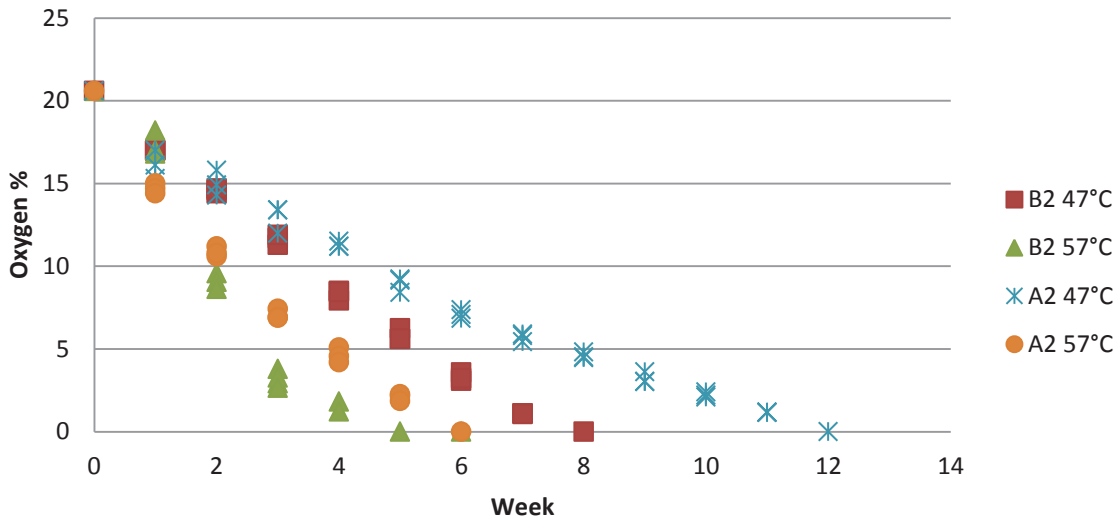


Figure 6.3-2: Headspace oxygen concentration throughout the storage period of the second trial result.

It can be noted that both Powder B at storage temperatures of 47°C and 57°C had a faster reaction than the first trial, whereas Powder A had a very similar reaction rate between the two trials (Figure 6.3-3 & Figure 6.3-4). This result is not surprising as it has been pointed out that the Powder B milk powder between the first trial and the second trial was quite different in their date of manufacture. The oxygen results were sensitive enough to pick it up, indicating that it is a useful tool to monitor the lipid oxidation for different products.

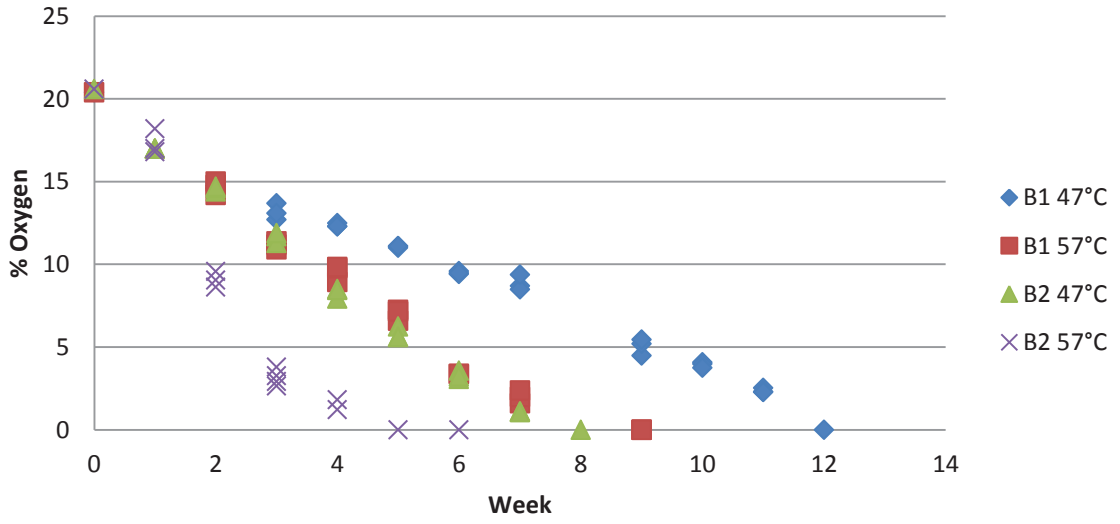


Figure 6.3-3: Comparison of the oxygen concentration over the storage period between first and second trials of Powder B.

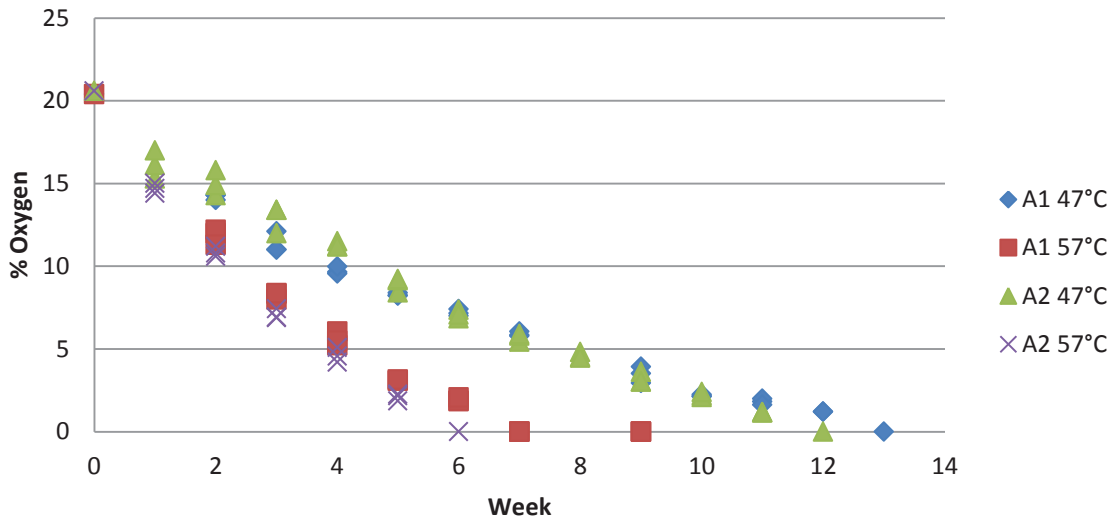


Figure 6.3-4: Comparison of the oxygen concentration over the storage period between first and second trials of Powder A.

6.4 Hexanal

As mentioned in previous chapters (chapter 2 & 4), development of off flavours in milk very much depends on the volatile compounds such as aldehydes and especially hexanal. Hexanal has been commonly used to monitor the extent of lipid oxidation due to its low threshold value for odours. Based on the literature study, hexanal was chosen to be the main focus for the secondary oxidation product. The rancidity of the milk powder is hugely influenced by the concentration of hexanal (Buttery et al., 1988) (Li et al., 2012).

Validation was made to confirm whether hexanal can be detected from Powder A and Powder B milk powder and to determine whether GC-MS can actually pick up the hexanal peak. Before the actual storage trial, milk powder stored in the glass vials was placed in the incubator at 57°C for 1.5 weeks. The retention time of the hexanal peak was found by preparing a stock solution (further dilution was made). The headspace was then withdrawn from the milk powder that had been stored for two weeks. Figure 6.4-1 below shows a tiny peak of hexanal from the headspace of the milk powder at the retention time of 2.6 min. The huge peak at the retention time of 1.4 min was air; no other major peaks were identified. This suggested that hexanal was the dominant volatile compound in the milk powder, and GC-MS is sensitive enough to measure hexanal. Therefore, it could be confirmed that hexanal would be a suitable volatile compound to measure and to quantify with the sensory result.

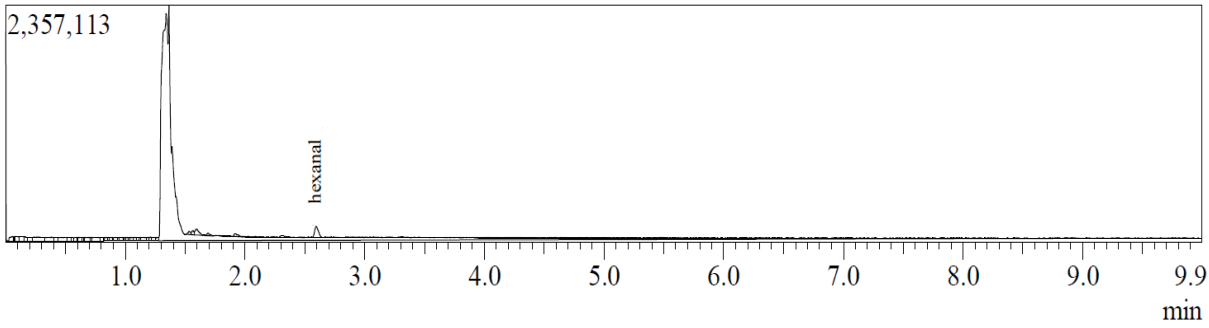


Figure 6.4-1: Chromatogram of the hexanal peak obtained from GC-MS.

Figure 6.4-2 shows the development of volatile hexanal over the storage period for each storage temperature from A1 and B2 milk powder. Powder A1 at 57°C showed the fastest reaction in formation of hexanal. The concentration reached to a maximum of 11 mg/kg of powder at week 11 then decreased afterwards. Overall, formation of hexanal is lower for Powder B1 than Powder A1; however, the hexanal concentration became higher for Powder B1 at the storage temperature of 37°C from week 24. The trend for the formation of hexanal is similar to the formation of lipid hydroperoxides. Over the storage period, hexanal concentration reached a maximum, and to a point where the depletion of hexanal dominates the formation of hexanal, the concentration of hexanal began to decrease. The measurement stopped at week 17 for powder stored at 47°C and 57°C for both the products due to limited resources i.e. glass vials. The experimental results were sufficient enough to draw the conclusion that it is not significant to continue the measurement from this point. Powder stored at 37°C were measured continually after week 17 because the measurement was done fortnightly as opposed to weekly for powders stored at 47°C and 57°C due to the slower reactions at 37°C.

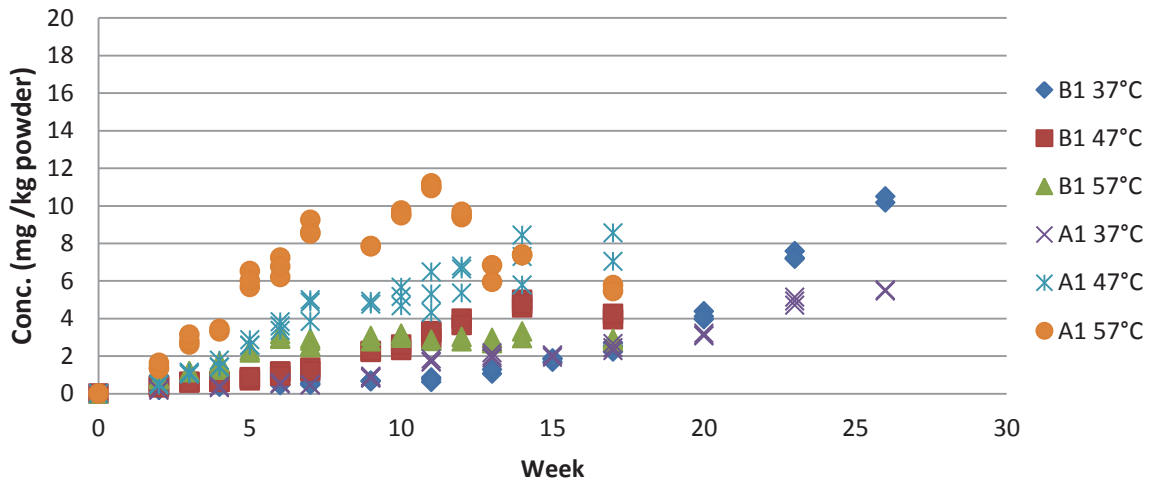


Figure 6.4-2: Experimental results of the development of hexanal over the storage period for the first trial of Powder A and Powder B (A1 and B1).

Figure 6.4-3 shows the development of the hexanal over the storage period for storage temperature at 47°C and 57°C for A2 and B2 milk powder. Comparing Figure 6.4-2 and Figure 6.4-3, it is obvious

that the results are remarkably different for Powder B2. Powder B2 at 57°C from the second trial produced the most volatile hexanal of all the powders. Also Powder B at 47°C had a higher concentration of hexanal than both trials of Powder A2 at 57°C. In week 10, the Powder B2 at a storage temperature of 57°C, reached a peak at the range from 26-29 mg of hexanal/kg powder, as opposed to the maximum hexanal concentration of 3.5 mg of hexanal/kg powder for Powder B1 at 57°C. The results between the two trials of Powder A were relatively similar. The maximum hexanal concentration for Powder A1 at 57°C was around 9 to 10 mg of hexanal/kg powder in week 10, as opposed to 11 mg of hexanal/kg powder in week 11 for Powder A2. The concentrations between Powder A1 at 47°C for both trials also agree with each other (within the range); the differences were small.

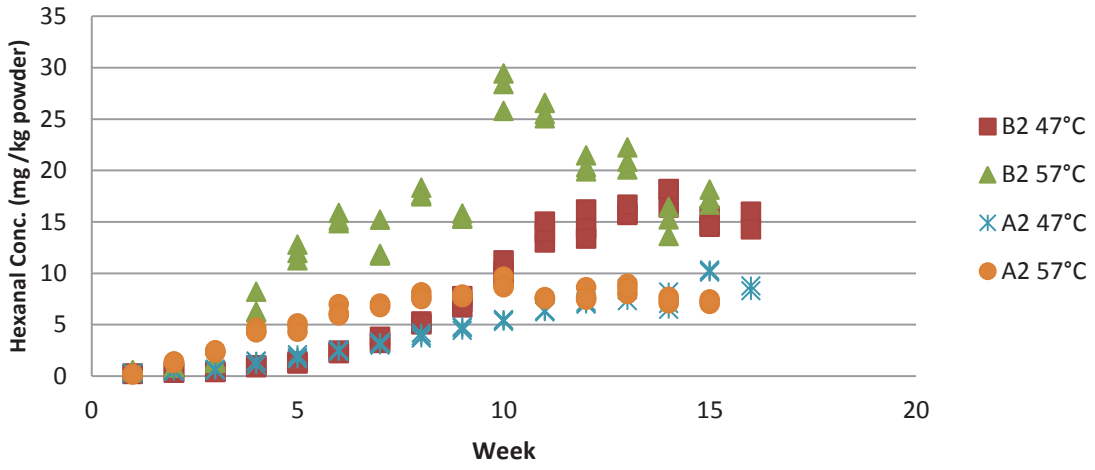


Figure 6.4-3: Experimental results of the development of hexanal over the storage period for the second trial of Powder A and Powder B (A2 and b2).

The hexanal measurement is sensitive and reproducible. The method was able to pick up the difference between the two products, and was also able to pick up the difference between the trials. It was expected Powder B1 and B2 may have slightly different results due to the differences in its manufacture date. The results from both oxygen and hexanal analysis have confirmed this difference. Also, the experimental method is reproducible as the results from both oxygen and hexanal analysis of Powder A are fairly close.

6.4.1 Hexanal Degradation

Unsaturated fatty acids in the lipid are susceptible to oxidation to form odourless lipid hydroperoxides. The unstable lipid hydropeoxides then undergo secondary oxidation reactions to form secondary oxidation products, such as volatile aldehydes, causing rancid flavour or odourless compounds such as hydrocarbons, acids and epoxides (Theodore P. Labuza & Dugan, 1971).

Both Powder A and Powder B powder contained unsaturated fatty acids, such as Oleic acid and linoleic acid (Table 6.1-2), that decomposed into lipid hydroperoxides. For example, the typical breakdown pathway for oxidation of linoleic acid to form linoleate hydroperoxides is shown below:

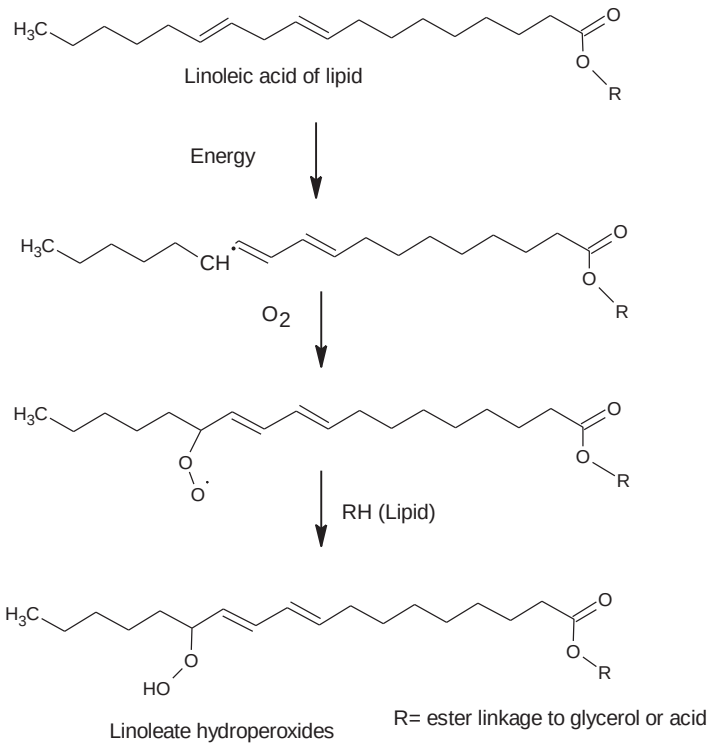


Figure 6.4-4: The formation of hydroperoxides from linoleic acid oxidation.

Linoleate hydroperoxides can then be broken down further to form hexanal, hexanol and pentane.

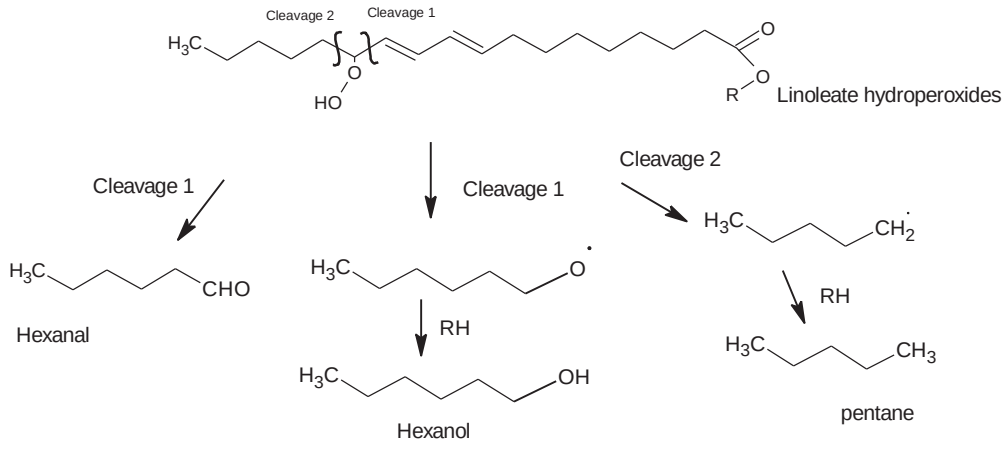


Figure 6.4-5: Formation of hexanal from the degradation of lipid hydroperoxide.

Not many studies from literature have reported the degradation of hexanal or the chemical reaction of this degradation. It has been clearly shown from the results that over the storage period, hexanal concentration began to decrease after reaching a maximum point. It has been suggested that during the more advanced stages of oxidation the saturated aldehydes accumulate and the unsaturated aldehydes are further oxidised to lower aldehydes. And also, at elevated temperatures, saturated aldehydes are further oxidised into dibasic acids (E Frankel, 2005). There was a lag period where hexanal concentration continued to increase with limited oxygen in the system. It was suggested that degradation of hexanal may involve a complex reaction and mechanism; more work is needed to justify this.

It can be noted that carbon dioxide and carbon monoxide were produced during the reaction as the storage time progressed. Figure 6.4-6 showed the chromatogram of the initial concentration of headspace analysis of oxygen in week 0 of storage period. The first peak in the figure is the air at retention time of 0.8 min and the second peak is the oxygen at the retention time of 3.7 min; there are no other significant peaks in between.

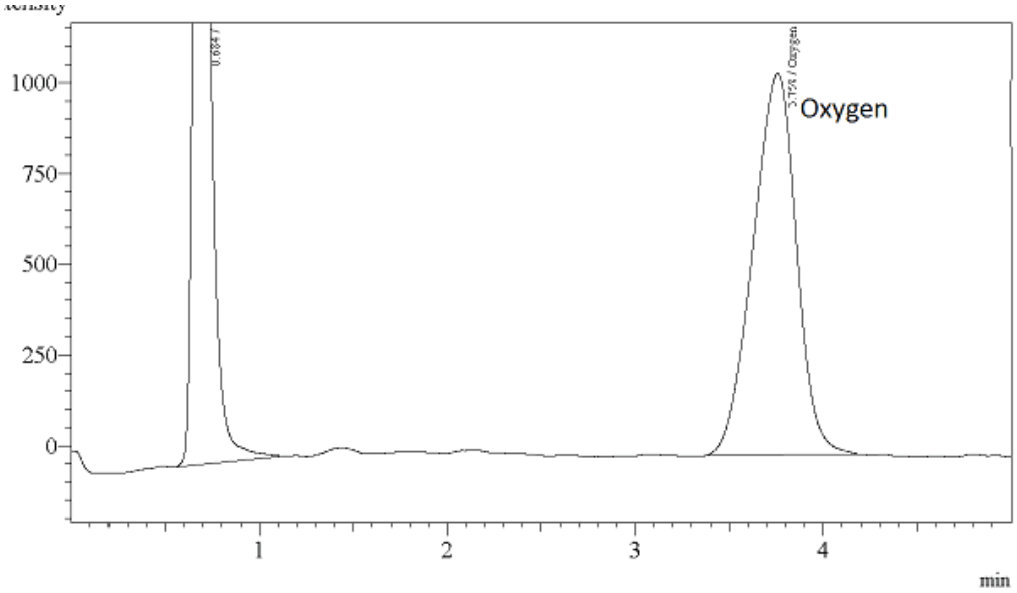


Figure 6.4-6: Chromatogram in week 0 of the storage period showing only the oxygen peak.

As the storage period progresses, two distinct peaks appeared in the chromatogram, a carbon dioxide peak at the retention time of 1.4 min and a carbon monoxide peak at 2.3 min (Figure 6.4-7). This suggests that there is some kind of degradation reaction which produces carbon dioxide as the end product, causing the hexanal concentration to decrease over time. Further research is required to draw a useful conclusion.

It should be noted that the areas of individual peaks cannot be compared. For example, from Figure 6.4-7 the area of carbon monoxide peak is bigger than the peak of carbon dioxide; however, this does not necessarily mean that the concentration of carbon monoxide is higher than the concentration of carbon dioxide. The oxygen measurement was done using gas chromatography with a thermal conductivity detector. Different compounds have different thermal conductivities, and, therefore, they can only be compared with the area of the same compound. It can also be seen

from the figure that the carbon dioxide peak is negative; this is because carbon dioxide has a thermal conductivity higher than the carrier gas (nitrogen gas).

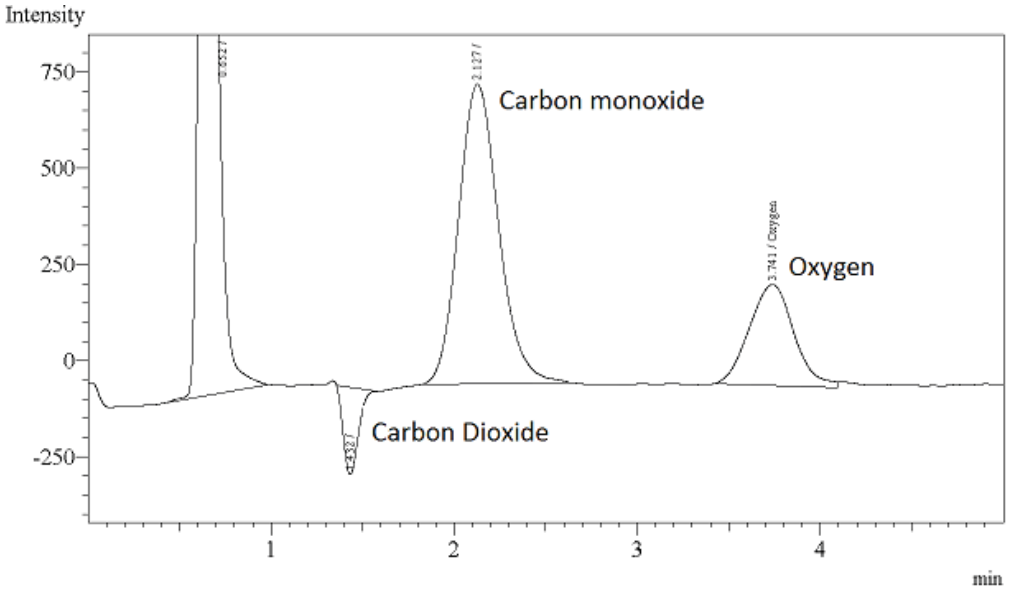


Figure 6.4-7: Chromatogram showing the carbon dioxide peak, carbon monoxide peak and oxygen peak in week 5 at a storage temperature of 57°C.

6.4.2 Additional Secondary and tertiary oxidation Products

Initially only a hexanal peak was evident from the chromatogram of the GC, but as the storage trials proceeded, more peaks were discovered. A mass spectrometer was able to identify the possible compounds presented in the powder from its ion fragments. Compounds such as pentanal, butanal, pentane, hexane and pentanol were identified by the mass spectrometer. These compounds are typical secondary or tertiary oxidation products, such as aldehydes, hydrocarbons and alcohols. Only qualitative analysis was undertaken to identify these unknown peaks; quantitative analysis was made only for hexanal.

6.5 Sensory

The method for sensory analysis can be found in chapter 5 of the method development section. Figure 6.5-1 and Figure 6.5-2 show the sensory score for Powder A and Powder B respectively over the storage period. It is expected that there would be an increase in the sensory score as the storage period increases; this is due to the build up of off flavours in the powder. Also, the sensory score is expected to be higher with a higher storage temperature over the storage period. The sensory data will be used to establish an acceptable threshold limit of hexanal concentration.

Looking at Figure 6.5-1 and Figure 6.5-2, overall, the sensory scores were higher with higher storage temperatures; although, there were a few exceptions in week 4 of Powder B and week 8 of Powder A. A sensory score of 3 (moderate difference) was determined to be unacceptable to the customer; therefore, it has been set that a sensory score at 2.5 will be the threshold limit. All the sensory scores at storage temperature of 37°C were below the threshold limit for both the products. The trend from sensory score at a storage temperature of 57°C for Powder A was not expected, showing

a V shape pattern over the storage period. The point in week three seems to be an outlier, as the powder was only three weeks in the storage time; it was expected that the sensory score would be lower at this time. The sensory data from Powder B at 57°C also seems to be inconsistent, as the results fluctuated over the storage period. This discrepancy could be due to the fact that the powder stored at 57°C has a higher chance to produce caramel flavours due to Maillard reactions. Lea *et al.* (1943) reported that temperatures above 60°C rapidly produced caramel-like flavour from Maillard reactions (Lea et al., 1943). As a result, there is a possibility that the sensory results had picked up this difference with the fresh milk powder. It should be noted that the sensory analysis method is different from the control test. It is to determine if a difference exists between samples and a control sample. So any differences in the flavour between the sample and the control sample would be taken into account, and is not necessary when an oxidative flavour changes. Therefore, it was decided that sensory data for 57°C for both products would not be used for the quantification of a hexanal threshold level.

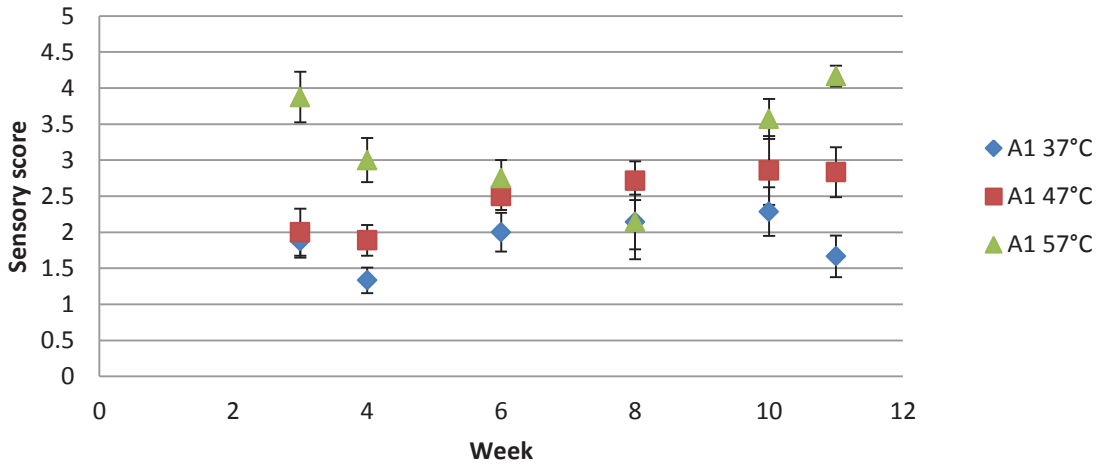


Figure 6.5-1: Sensory score for the first trial of Powder A.

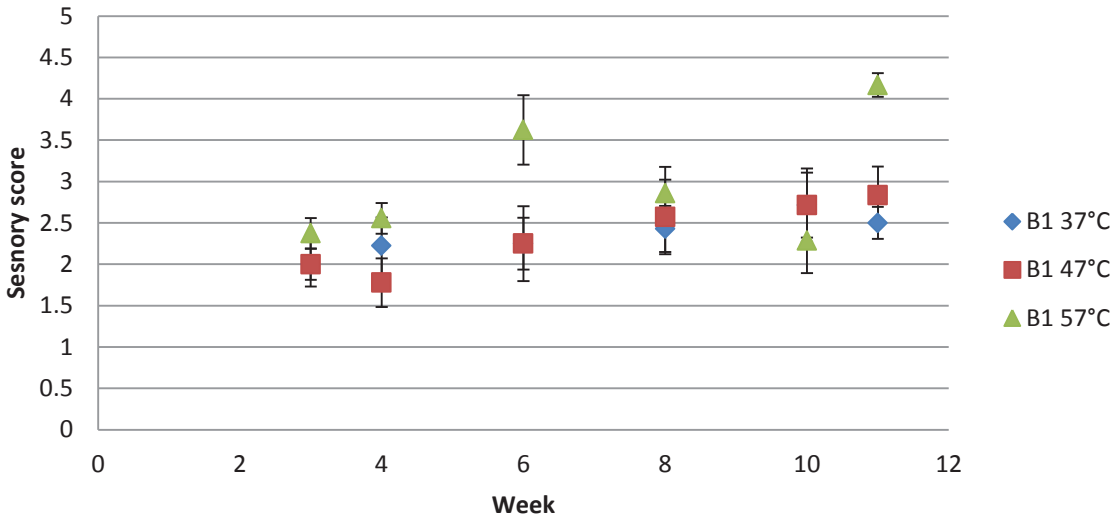


Figure 6.5-2: Sensory score for the first trial of Powder B.

For Powder A, it was found to be unacceptable (sensory score >2.5) at week 6 at the storing temperature of 47°C, and for Powder B it was also found to be unacceptable at week 8 at the storing temperature of 47°C. Table 6.5-1 and Table 6.5-2 below present the sensory score to the corresponding hexanal concentration. The concentration of hexanal was 3.52 mg/kg powder at week 8 at the storage temperature of 47°C for Powder A and 2.25 mg/kg powder for Powder B. Therefore, it can be concluded that the threshold limit of hexanal concentration was below 3.41 mg/kg powder for Powder A and 2.25 mg/kg powder for Powder B. To be more conservative and on the safe side, the threshold limit for Powder A was determined to be at 3.35 mg hexanal/kg powder. For Powder B the threshold limit was determined to be 2 mg hexanal/kg powder (below 2.25 mg hexanal/kg powder).

Further discussion regarding the threshold limit of hexanal concentration and the oxygen concentration prediction is covered in section 6.7.

Table 6.5-1: Sensory score corresponding to the hexanal concentration for the first trial of Powder A.

	Powder A 37°C		Powder A 47°C		Powder A 57°C	
Week	Sensory score	Hexanal Conc. (mg/kg powder)	Sensory score	Hexanal Conc. (mg/kg powder)	Sensory score	Hexanal Conc. (mg/kg powder)
3	1.88 ±0.227	NA	2.00 ±0.327	1.09	3.88 ±0.350	3.38
4	1.30 ±0.177	0.363	1.90 ±0.212	1.54	3.00 ±0.306	6.08
6	2.00 ±0.267	0.481	2.50 ±0.189	3.41	2.75 ±0.250	6.73
8	2.14 ±0.378	0.865	2.71 ±0.267	4.83	2.14 ±0.518	7.31
10	2.29 ±0.336	NA	2.86 ±0.476	5.16	3.57 ±0.278	9.60
11	1.67 ±0.289	1.74	2.83 ±0.348	5.36	4.17 ±0.144	11.0

Table 6.5-2: Sensory score corresponding to the hexanal concentration for the first trial of Powder B

	Powder B 37°C		Powder B Ive 47°C		Powder B Ive 57°C	
Week	Sensory score	Hexanal Conc. (mg/kg powder)	Sensory score	Hexanal Conc. (mg/kg powder)	Sensory score	Hexanal Conc. (mg/kg powder)
3	2.00 ±0.189	NA	2.00 ±0.267	0.607	2.38 ±0.183	1.15
4	2.20 ±0.344	0.384	1.80 ±0.295	0.628	2.50 ±0.186	1.48
6	2.25 ±0.453	0.481	2.25 ±0.313	1.09	3.63 ±0.420	3.02
8	2.38 ±0.500	0.523	2.57 ±0.450	2.25	2.86 ±0.318	2.92
10	2.51 ±0.443	NA	2.71 ±0.393	2.46	2.29 ±0.393	3.10
11	2.50 ±0.194	0.740	2.83 ±0.348	3.15	4.17 ±0.144	2.82

6.5.1 Statistical Sensory analysis

Sensory tests often have a high level of variability between results; therefore, statistical analysis was done to compare the sensory results. The raw data of sensory scores can be seen in the Appendices section Table 9-6. Analysis of a variance (ANOVA) test was used to determine significance differences between the means from the sensory results. Error bars were also shown in both of the figures (Figure 6.5-1 & Figure 6.5-2). Figure 6.5-3 shows the result of statistical analysis of ANOVA between the storage temperatures of the powder in each storage period. For A1 powder, the means (the scores for three storage temperatures) are significantly different in week 3, 4 and 11 where week 4 and week 10 were fairly different (p= 0.1) and week 8 showed no difference in the means. However, for B1 powder, only week 6 and week 11 showed significant differences between the means. The error bars in the figures (Figure 6.5-1 & Figure 6.5-2) also show the same trend as ANOVA analysis.

Powder A1

Week 3

Source	DF	SS	MS	F	P
Factor	2	20.083	10.042	13.39	0.000
Error	21	15.750	0.750		
Total	23	35.833			

Week 4

Source	DF	SS	MS	F	P
Factor	2	12.963	6.481	14.29	0.000
Error	24	10.889	0.454		
Total	26	23.852			

Week 6

Source	DF	SS	MS	F	P
Factor	2	2.333	1.167	2.58	0.100
Error	21	9.500	0.452		
Total	23	11.833			

Week 8

Source	DF	SS	MS	F	P
Factor	2	1.52	0.76	0.59	0.563
Error	18	23.14	1.29		
Total	20	24.67			

Week 10

Source	DF	SS	MS	F	P
Factor	2	5.81	2.90	2.61	0.101
Error	18	20.00	1.11		
Total	20	25.81			

Week 11

Source	DF	SS	MS	F	P
Factor	2	18.778	9.389	15.65	0.000
Error	15	9.000	0.600		
Total	17	27.778			

Powder B1

Week 3

Source	DF	SS	MS	F	P
Factor	2	0.750	0.375	1.00	0.385
Error	21	7.875	0.375		
Total	23	8.625			

Week 4

Source	DF	SS	MS	F	P
Factor	2	2.741	1.370	2.14	0.139
Error	24	15.333	0.639		
Total	26	18.074			

Week 6

Source	DF	SS	MS	F	P
Factor	2	10.08	5.04	3.94	0.035
Error	21	26.88	1.28		
Total	23	36.96			

Week 8

Source	DF	SS	MS	F	P
Factor	2	0.67	0.33	0.23	0.800
Error	18	26.57	1.48		
Total	20	27.24			

Week 10

Source	DF	SS	MS	F	P
Factor	2	0.86	0.43	0.32	0.732
Error	18	24.29	1.35		
Total	20	25.14			

Week 11

Source	DF	SS	MS	F	P
Factor	2	9.333	4.667	9.77	0.002
Error	15	7.167	0.478		
Total	17	16.500			

Figure 6.5-3: ANOVA of sensory scores between three storage temperatures of Powder A1 and Powder B1 in the storage period.

The sensory analysis for Powder A1 showed a better representation than the results for Powder B1. It can be concluded that the temperature differences did influence the rancidity of the powder. However, for Powder B1 the differences in means were not as large as for A1 powder between the storage temperatures. It was interesting to observe that the amount of hexanal produced by B1 powder was significantly less than A1 powder. For example, at week 4, the hexanal produced from A1 powder was 0.363, 1.54 and 6.08 mg/kg powder for storage temperatures of 37°C, 47°C and 57°C respectively. For B1 powder, at week 4, it was 0.384, 0.628 and 1.48 mg/kg powder for storage temperatures of 37°C, 47°C and 57°C respectively. The differences between the amount of hexanal for A1 powder was larger than the differences between B1 powder. The amounts of hexanal concentration between 37°C and 57°C for Powder A1 at week 4 were almost 17 times different compared to only 4 times different for B1 powder between 37°C and 57°C. Therefore, the panellists may find it harder to pick up this difference for B1 1 powder than A1 powder.

The threshold limit for A1 powder was determined to be at week 6; as a result the sensory results proved to be a reliable tool for A1 powder as the means of the sensory scores are significantly different up to week 6 ($p<0.1$). However, for powder B1, the means showed small differences, and the sensory result may not be as a good representation as A1 powder. Improvement can be made to reduce the variation on the sensory results and will be discussed in a later section.

6.5.2 Trial two sensory results

Trial two sensory measurements were conducted but the data was not reliable enough to produce an impact conclusion. The sensory data was only conducted for 3 weeks (3 points), and only 3 or 4 panellists were used for each sensory test. As a result, large uncertainties were introduced to the sensory analysis. Therefore it was concluded that the results from trial 2 could not be used.

6.5.3 Improvements to the sensory analysis

Further improvements can be made in conducting the sensory analysis. It should be noted that the panellists were trained in a relatively short time before the sensory test started. As a result, the sensory data may be influenced. One of the future suggestions is that the panellists should be trained to pick up the oxidative flavour. Oxidative flavour is often associated with cardboard, cappy, fishy, tallowy and metallic flavours. Secondary oxidation products such as aldehydes impart a cardboard flavour strongly (Shipe et al., 1978). The correlation between the sensory score and hexanal concentration may be stronger if the panellists are able to distinguish the oxidative flavour.

6.6 Reaction Kinetics

The oxygen concentration under different storage temperatures can be predicted by Eq. 4.4-8 from Chapter 4 where n=0.5. The fitted profile is compared with the experimental data of oxygen concentration over three storage temperatures in Figure 6.6-1 for Powder A1 and Figure 6.6-2 for Powder B1. The model profiles showed a good fit between the experimental data and the predicted values. The goodness of fit (R^2) was calculated as 0.98 for Powder A1 and 0.96 for Powder B1. Initially n was predicted by fitting using Matlab, and was found to be n= 0.6 for Powder A1 and n= 0.4 for Powder B1. It was discussed in section 4.4.1 that the order of reaction for oxygen consumption was between 0 and 1 due to the availability of oxygen concentration and complex reaction. The R^2 was found to be 0.98 where n=0.6 for Powder A1 and R^2= 0.97 where n=0.4 for Powder B1. In conventional practice in the literature (Theodore P. Labuza & Dugan, 1971), the oxygen consumption was described as a half order reaction rather than n=0.4 or 0.6. By comparing the values of R^2, the number did not change significantly. Therefore, n=0.5 was used in Eq. 4.4-8.

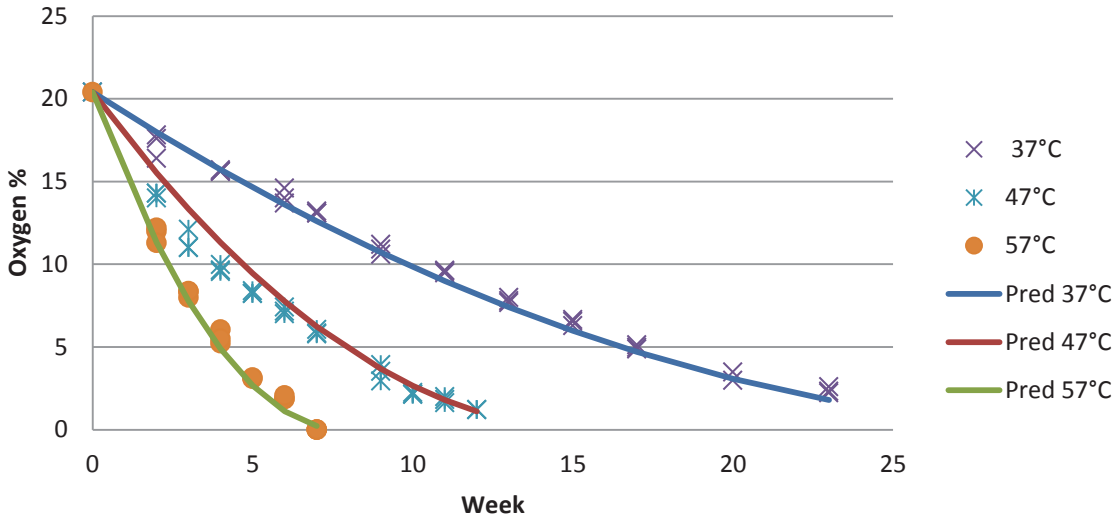


Figure 6.6-1: Fitted profile of the half order kinetic model compared with the experimental data of the oxygen consumption from headspace analysis of Powder A1 (first trial).

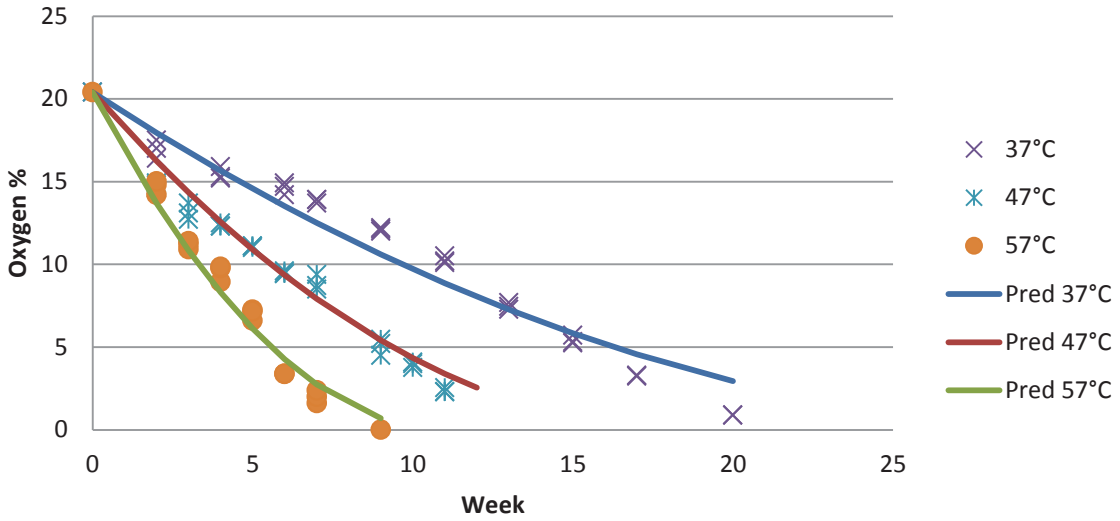


Figure 6.6-2: Fitted profile of the half order kinetic model compared with the experimental data of the oxygen consumption from headspace analysis of Powder B1 (first trial).

Using a half order model reaction, the Arrhenius rate constant, k_0, and activation energy, E, were found to be 4.9×10^{9} $\%^{0.5}$ $week^{-1}$ and 60.9 kJ mol^{-1} respectively for Powder A1 and 1.34×10^{7} $\%^{0.5}$ $week^{-1}$ and 45.60 kJ mol^{-1} respectively for Powder B1.

Figure 6.6-3 and Figure 6.6-4 show the fitted profile of the half order kinetic model of the second trial powder. For the second trial, R^2 was found to be 0.99 for Powder A2 and R^2= 0.98 for Powder B2. The Arrhenius rate constant, k_0, and activation energy, E, were found to be 5.4×10^{9} $\%^{0.5}$ $week^{-1}$ and 60.9 kJ mol^{-1} respectively for Powder A2 and 6.06×10^{8} $\%^{0.5}$ $week^{-1}$ and 54.3 kJ mol^{-1} respectively for Powder B2. The difference between the Arrhenius rate constant of the first trial Powder A and the second trial was 10% with the same activation energy. However, there was a big difference between

the first trial and second trial Powder B. The Arrhenius rate constant for Powder B2 was found to be 45 times higher than B1. The results for these differences were not surprising. The results between Powder A1 and A2 have been fairly consistent from the analysis of oxygen concentration, hexanal concentration and the kinetic parameters, whereas the results between Powder B1 and B2 were relatively different. Therefore, it was decided that the results of Powder B1 would be used rather than trial 2 for the prediction. This is because the trial 1 powder had the latest date of manufacture (28th October 2014 as opposed to 13th March 2014) when the storage began.

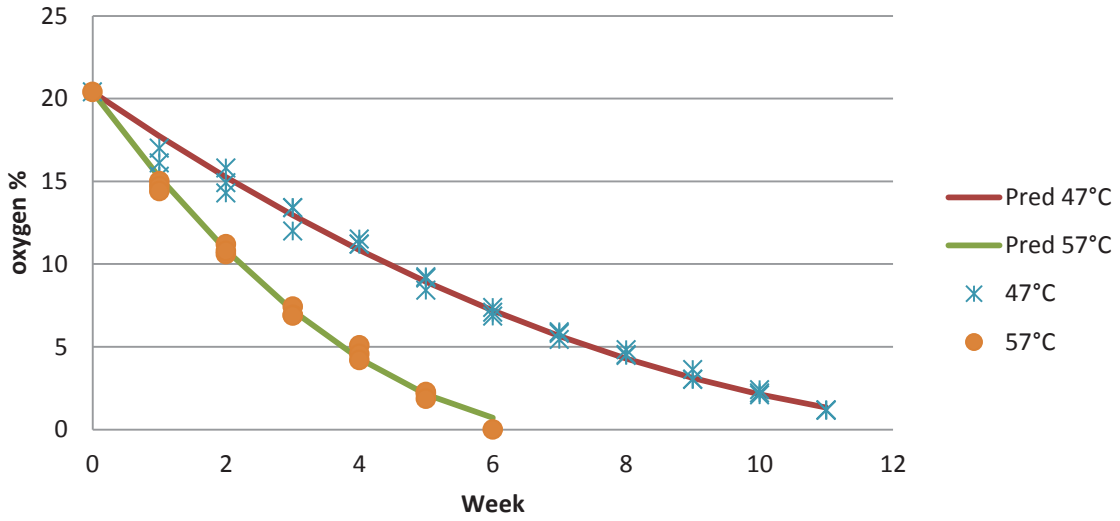


Figure 6.6-3: Fitted profile of the half order kinetic model compared with the experimental data of the oxygen consumption from headspace analysis of Powder A (second trial).

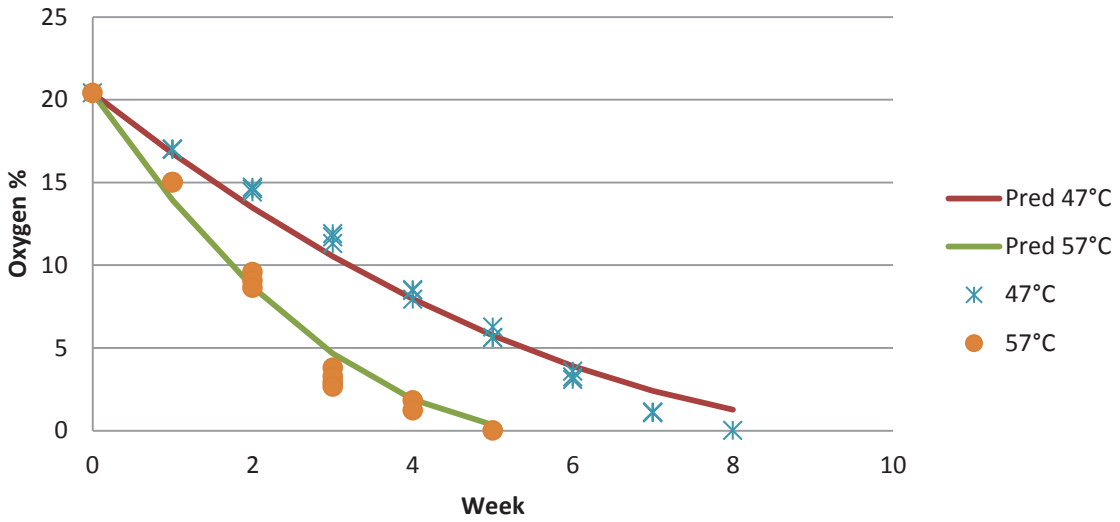


Figure 6.6-4: Fitted profile of the half order kinetic model compared with the experimental data of the oxygen consumption from headspace analysis of Powder B (second trial).

6.6.1 Prediction of Oxygen Consumption

Prediction of oxygen consumption under different storage temperatures can be made from the kinetic parameters obtained from fitting the data. Figure 6.6-5 displays the prediction of oxygen consumption under 25°C storage temperature from the kinetic data obtained from trial 1 and trial 2 results. Looking at the figure, the two predictions obtained from two trials are similar.

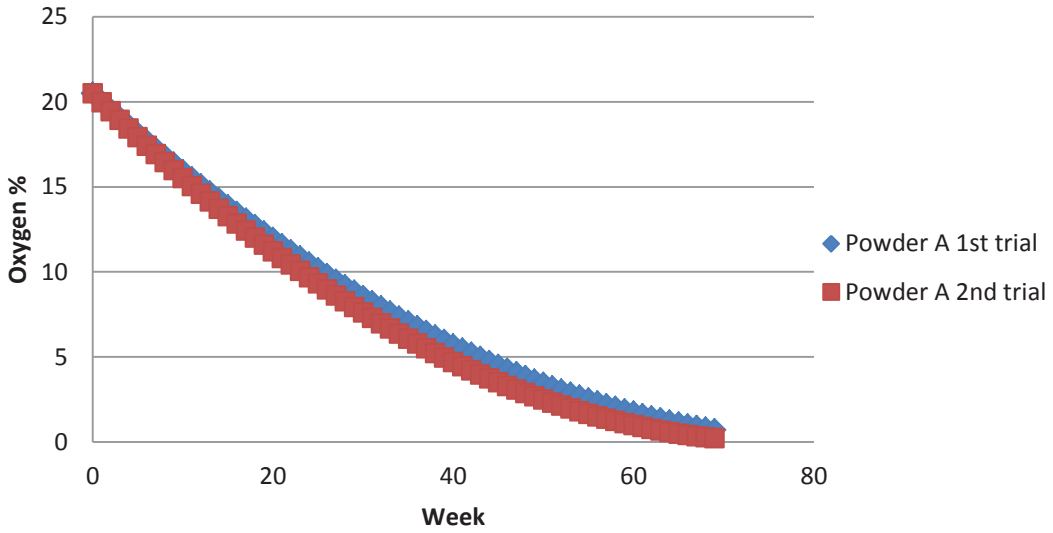


Figure 6.6-5: Prediction of headspace oxygen consumption under storage temperature of 25°C for Powder A1 and A2 kinetic data.

Figure 6.6-6 showed the predictions of oxygen concentration of Powder B under 25°C storage temperature. Trial 1 and trial 2 data have different predictions, due to the different fitted kinetic parameters obtained experimentally.

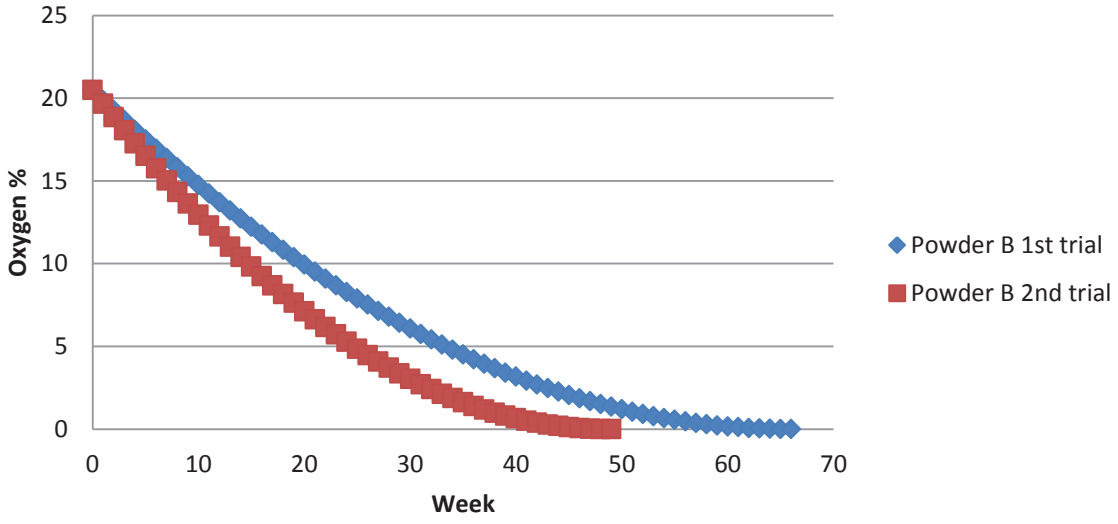


Figure 6.6-6: Prediction of headspace oxygen consumption under storage temperature of 25°C Powder B1 and B2 kinetic data.

New kinetic parameters for Powder A product were obtained by fitting all the data from both trials together. The new Arrhenius rate constant, k_0, and activation energy, E, were found to be 7.8×10^9 % $^{0.5}$ week^{-1} and 62.0 kJ mol^{-1} respectively with R^2 of 0.98.

6.7 Relationship between Oxygen and Hexanal

When the results of oxygen concentration and hexanal concentration were plotted against each other, correlation was discovered. It was anticipated that the consumption of oxygen and the formation of hexanal were related. Figure 6.7-1 and Figure 6.7-2 show the relationship between the formation of hexanal and the depletion of oxygen concentration for Powder A and Powder B respectively. The plots were fitted exponentially to give R^2 of 0.905 for Powder A and R^2 of 0.918 for Powder B. The plots do not start at oxygen consumed = 0 mol/m^3 but at value close to 2 mol/m^3. There was a lag time when oxygen was consumed first to produce lipid hydroperoxides; as lipid hydroperoxides built up, hexanal was formed. Therefore, hexanal concentration started to build up when the powder consumed around 2 mol/m^3 of oxygen. Hexanal concentration increased exponentially towards the end of the storage trial, with the concentration of hexanal increasing continuously without any oxygen in the system. This is also due to the lag phase where all the oxygen in the system had been used for the formation of lipid hydroperoxides, and during this period lipid hydroperoxides were also degraded to form hexanal. It should be noted that Figure 6.7-1 included all the experimental data of Powder A from trial 1 and trial 2 whereas Figure 6.7-2 only included trial one Powder B experimental data. As mentioned earlier, trial 1 and trial 2, data from Powder A were very similar and they were combined into one set of experimental results; however, trial 2 of Powder B data was questionable. Therefore, only trial 1 data was used. The plots are useful to predict the production of hexanal from change in oxygen content. From the plots, prediction of hexanal production from Powder A would be:

$$Hexanal\ Conc. = 0.1616e^{0.1943\times oxygen\ conc.\text{consumed}} \qquad \text{Eq. 6.7-1}$$

For Powder B:

$$Hexanal\ Conc. = 0.2047e^{0.1466\times oxygen\ conc.\ \text{consumed}} \qquad \text{Eq. 6.7-2}$$

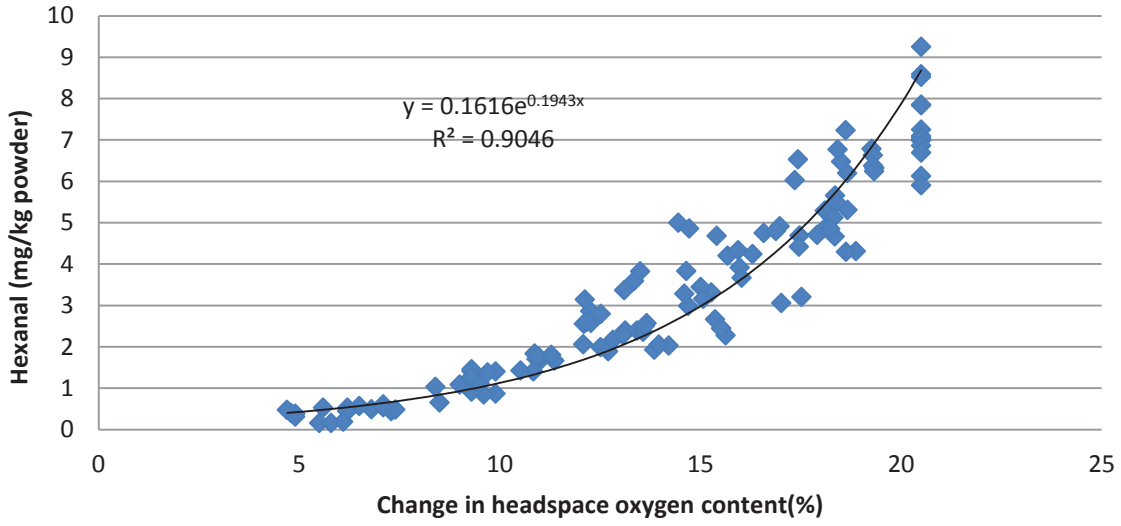


Figure 6.7-1: The relationship between the production of hexanal and the consumption of oxygen, expressed as a change in oxygen content for Powder A (Trial 1+Trial 2).

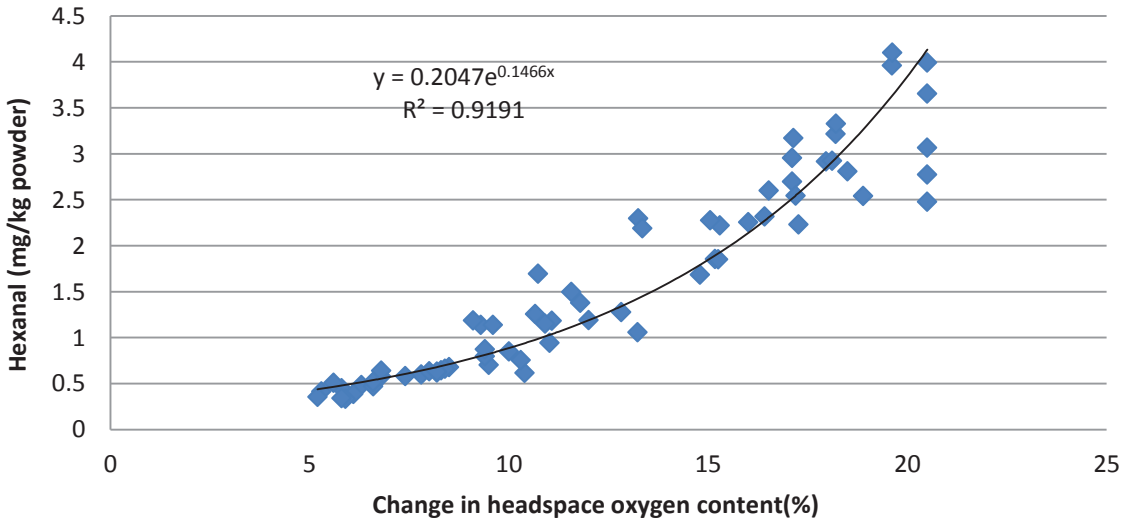


Figure 6.7-2: The relationship between the production of hexanal and the consumption of oxygen, expressed as a change in oxygen content for Powder B (Trial 1 only).

6.8 Application of the kinetic model

Up to this point, predictions of the oxygen concentration and hence the formation of the hexanal from the powder can be made. Threshold limits of hexanal for both products were found from section 6.5, and was determined to be 3.35 mg hexanal/kg powder for Powder A and 2 mg hexanal/kg powder for Powder B. The time for the powder to become rancid during the storage under normal atmosphere environment can be calculated from the model. Using eq. 6.7-1 & eq. 6.7-2, the oxygen consumption (change in oxygen content) required to produce a hexanal threshold limit of 3.35 mg hexanal/kg powder was calculated to be 15.6% oxygen concentration consumed for Powder A and 15.5% oxygen concentration consumed for Powder B, with a threshold limit of 2 mg hexanal/kg powder. Both products have similar oxygen consumption limits; it is concluded that powder that has a change in oxygen content of 15% for both products would be unacceptable.

Figure 6.8-1 and Figure 6.8-2 represent the prediction of oxygen consumption over the storage period under storage temperature of 25°C for Powder A and Powder B respectively. Based on the threshold limit from above, the time for powder to become rancid under storage can be found using Figure 6.8-1 and Figure 6.8-2. The maximum storage time for Powder A was found to be 40 weeks, and 31 weeks for Powder B. These are the storage times that are predicted for the powder to become unacceptable under a storage temperature of 25°C. Storage time would vary as the storage temperature changes.

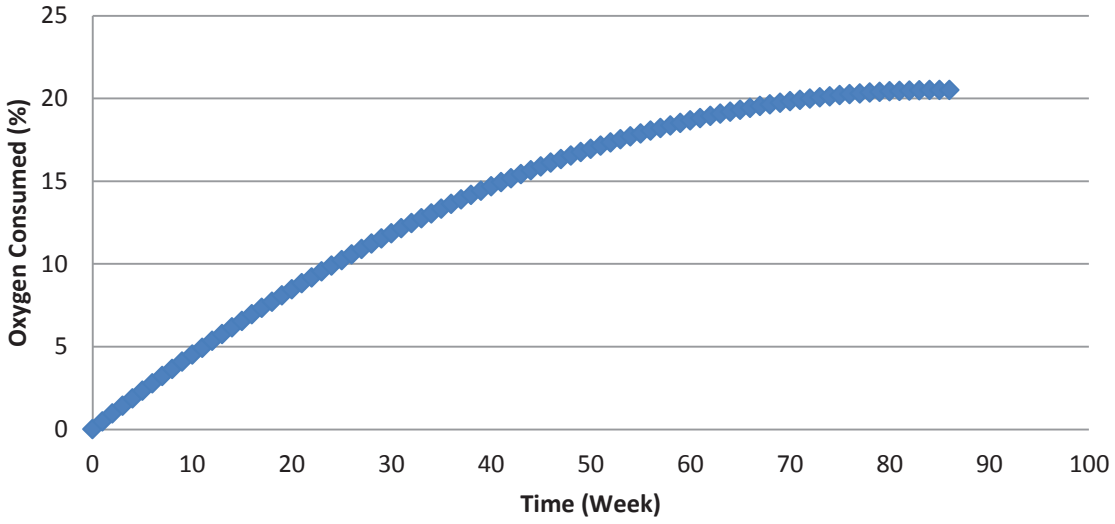


Figure 6.8-1: Predicted oxygen consumption as a function of time for Powder A at 25°C and 20.9% O_2.

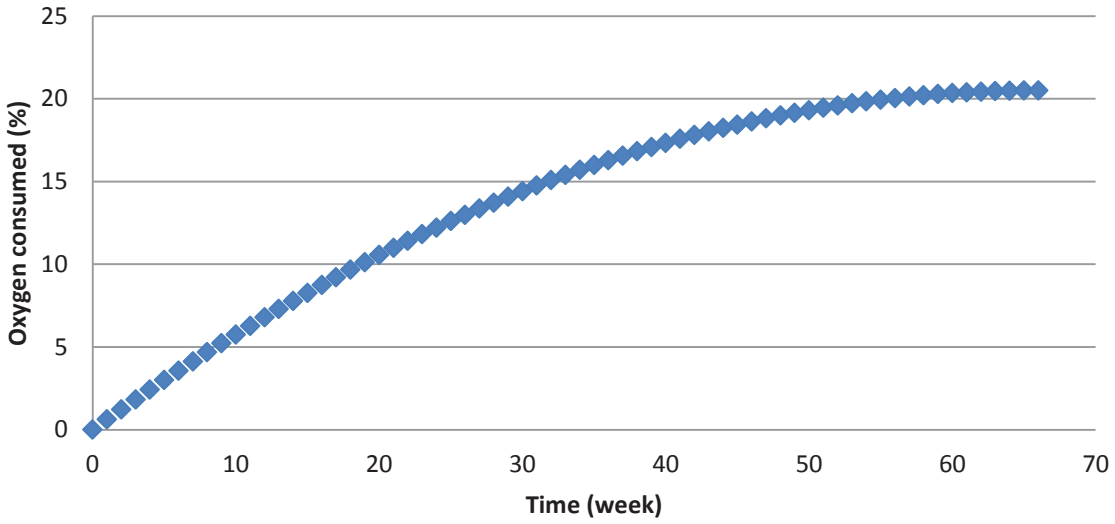


Figure 6.8-2: Predicted oxygen consumption as a function of time for Powder B at 25°C and 20.9% O_2.

6.8.1 Usefulness of the model

Although the secondary oxidation product hexanal is an indicator for when the powder has become rancid, the compound is easily vaporised due to its volatility. For example, if the powder stored in the bag is transferred to the cans, hexanal measurement would not be precise and accurate as some of the hexanal would be vaporised, but at the same time, the rancid flavour would still be present in the powder. However, oxygen measurement is simple and can be easily monitored over the storage time. Prediction of hexanal production can be made by determining the consumption of oxygen in the powder. Prediction can also be made for the powder stored in the bag which has been transferred into the can under a modified atmosphere, as long as the consumption of oxygen by the powder can be measured. It can be used to measure the accumulation of the consumption of oxygen of the milk powder during storage in the bag, and when transferring to the can under a modified atmosphere, and therefore determine its maximum storage time.

Multiple results can be created by using a data table produced by Excel ("What if Analysis" function). The data table can display the concentration of oxygen consumed versus storage time under different initial oxygen concentration or the concentration of oxygen consumed versus storage time under different storage temperatures (Appendix 9.4 for data table). The table can be used to determine the storage time under certain storage conditions such as the temperature and the initial oxygen storage concentrations. For example, Table 6.8-1 and Table 6.8-2 below show the maximum storage time allowed for Powder A and Powder B under certain storage temperatures.

Table 6.8-1: Maximum storage time (in weeks) under certain storage temperature and initial storage oxygen concentration for Powder A.

		Initial Oxygen Concentration (%)			
		5	10	15	20.5
Storage Temperature °C	20			112	63
	21			103	58
	22			94	53
	23			86	48
	24			79	44
	25			73	41
	26			67	38
	27			62	35
	28			57	32
	29			52	29
	30			48	27
	31			44	25
	32			41	23
	33			38	21
	34			35	19
	35			32	18

Table 6.8-2: Maximum storage time (in weeks) under certain storage temperature and initial storage oxygen concentration for Powder B.

		Initial oxygen concentration (%)			
		5	10	15	20.5
Storage Temperature °C	20			77	43
	21			72	41
	22			68	38
	23			64	36
	24			60	34
	25			56	32
	26			53	30
	27			50	28
	28			47	26
	29			44	25
	30			42	23
	31			39	22
	32			37	21
	33			35	20
	34			33	19
	35			31	17

6.8.2 Model Limitation

The above table (Table 6.8-1 & 6.8-2) showed that as long as the initial oxygen concentrations are below 10%, then it would possibly have an "infinite" storage time. However, this is not true and would not happen in the real world situation. As discussed in Section 4.4.1, at low oxygen concentration the rate of oxygen consumption is dependent on the concentration of oxygen, and therefore the reaction kinetics would tend to shift towards a reaction order of 1 as oppose to 0.5. There is a possibility that the model cannot predict the consumption of oxygen accurately under low oxygen concentrations based on the current experimental data; therefore, the model needs to be validated for low oxygen conditions.

7 Conclusions and Suggestions for Future Work

7.1 Conclusions

The development of rancidity of the goat milk infant formula powder was a concern for New Image Group. Two types of rancidity, lipolytic rancidity and oxidative rancidity, can occur in typical milk powder. It was found from experiments that lipolytic rancidity was not significant and it was concluded that oxidative rancidity would be the main concern.

Oxidative rancidity results from a complex lipid oxidation process, occurring in three stages: an initiation stage, a propagation stage and a termination stage. A current test for oxidative rancidity, PV, was not reliable and it was difficult to provide a specific guideline to rancidity. Oxygen was found to be a useful parameter to monitor lipid oxidation. Oxygen is consumed in the lipid oxidation and is simple and easy to measure throughout the process. The headspace oxygen of goat milk infant formula powder was measured using gas chromatography over the storage period. A simple mathematical model of oxygen concentration was generated from the storage trial. The model of the rate of oxygen consumption was predicted and followed half order reaction. The Arrhenius rate constant, k_0, and activation energy, E, were found to be 7.8×10^{9} $\%^{0.5}$ $week^{-1}$ and 62.0 kJ mol^{-1} for Powder A and 1.34×10^{7} $\%^{0.5}$ $week^{-1}$ and 45.60 kJ mol^{-1} for Powder B. Using this model, oxygen concentration can be predicted under different storage temperatures.

It was reported that secondary oxidation products such as hexanal were responsible for the off flavour in milk powder. Hexanal concentration by headspace analysis was quantified by a gas chromatography-mass spectrometer over the storage period. The sensory analysis was carried out to run parallel with the storage trial. From the sensory analysis, the threshold limit of hexanal for Powder A was determined to be 3.35 mg hexanal/kg powder, and 2 mg hexanal/kg powder for Powder B. It was observed that the hexanal concentration continued to increase in the absence of oxygen in the system, reaching to a maximum point then falling afterwards. Unknown peaks from GC were discovered over the storage time, meaning that other chemical compounds were produced. Gas chromatography coupled with the mass spectrometer identified these compounds to be aldehydes, hydrocarbon and alcohol groups such as pentanal, butanal, hexane, pentane and pentanol. These compounds are common secondary oxidation products.

It was discovered that oxygen and hexanal were highly correlated with R^2 of 0.905 for Powder A and R^2 of 0.918 for Powder B when fitted exponentially. The fitted equation can be used to predict the

hexanal production from oxygen concentration. Oxygen consumption prediction was done under a storage temperature of 25°C. It was predicted that Powder A would become unacceptable at a storage times of 40 weeks and 31 weeks for Powder B.

It can be concluded that the accelerated storage trial was able to produce a simple mathematical model to predict the consumption of oxygen of the infant formula powder. The results from trial 1 and trial 2 Powder A powders showed a similar and reproducible result using the accelerated method. However, trial 1 and trial 2 Powder B displayed very different results. This is due to the differences in the manufacturing date of the two batches of Powder B used on the two trials, whereas the two trials of Powder A powder were very similar in age.

Data tables were produced to summarise the results in a more simple way, showing all the scenarios of storage conditions such as storage temperature and initial oxygen concentration.

7.2 Suggestions for Future Work

The current work measured hexanal as an indicator of secondary oxidation products and sensory analysis and were performed to correlate with hexanal concentration. However, other aldehydes such as pentanal and butanal were also secondary oxidation products that can contribute to the off flavour of the milk powder, although hexanal has the lowest flavour threshold limit. It was discussed that these aldehydes were identified and present in the powder. It is recommended that quantification of these aldehydes can also be done to provide a full analysis on sensory. Also, as suggested in the discussion, sensory panellists can be trained to pick up the specific oxidative rancidity flavour, making the sensory analysis more reliable.

Improvement can be made on the measurement of PV. PV was sent to an external laboratory for measurement due to time constraints and the limited resources. It would be ideal to measure PV internally, ideally to have the same person measure the result and with a routine time to give a better consistency, introducing less noise.

Further work can be done on storing milk powder at a different starting point with different initial oxygen concentrations. Comparison can be made to see whether there will be differences in the rate of consumption of oxygen. Model validation is needed to determine the order of reaction under low oxygen concentration. Also, it would be interesting to observe the production of hexanal if the powder is stored under a modified atmosphere.

For research purposes, future work can be done on the reaction and the mechanism of the degradation of hexanal. It is not clear why hexanal undergoes breakdown in the absence of oxygen. It was found that carbon dioxide was present in the system.

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Nomenclature

A	Area	m^2
E	Activation energy	kJ/mol
k	Rate constant	$Week^{-1}$
k_0	Arrhenius constant	$Week^{-1}$
P	Pressure	Pa
PV	Peroxide value	mEq /kg fat
P_b	Bulk density	kg/m^3
P_p	True density	kg/m^3
R	Universal gas constant	J/mol/k
V	Volume	m^3
V_p	Volume of the product	m^3
V_t	Total volume of the package	m^3
W	Weight	kg
X	Thickness	m

9 Appendices

9.1 Raw data

Table 9-1: Raw data for trial one Powder A and Powder B stored under 37°C.

Powder B 37°C				Powder A 37°C			
Date	Hexanal (mg/kg powder)	Week	Oxygen (%)	Date	Hexanal (mg/kg powder)	Week	Oxygen (%)
0	0	0	20.4	0	0	0	20.4
2	0.182	2	17	2	0.208	2	16.4
2	0.203	2	16.4	2	0.170	2	17.8
2	0.221	2	17.5	4	0.380	2	17.6
4	0.415	4	15.2	4	0.312	4	15.6
4	0.354	4	15.9	4	0.398	4	15.6
6	0.451	4	15.3	6	0.491	4	15.7
6	0.509	6	14.7	6	0.563	6	14.6
6	0.484	6	14.9	7	0.440	6	13.7
7	0.469	6	14.2	7	0.485	6	14
7	0.579	7	13.9	9	0.870	7	13.2
7	0.520	7	13.7	9	0.845	7	13.1
9	0.644	7	13.9	9	0.916	7	13.1
9	0.678	9	12.2	9	0.828	9	10.6
9	0.661	9	12	11	1.679	9	10.9
11	0.617	9	12.1	11	1.705	9	11.2
11	0.849	11	10.1	11	1.837	11	9.48
11	0.755	11	10.5	13	1.989	11	9.58
13	1.059	11	10.2	13	1.891	11	9.63
13	1.277	13	7.27	13	2.170	13	7.99
15	1.684	13	7.68	15	2.027	13	7.8
15	1.855	13	7.45	15	1.930	13	7.68
15	1.852	15	5.7	15	2.050	15	6.29
17	2.696	15	5.33	17	2.278	15	6.65
17	2.232	15	5.25	17	2.662	15	6.54
17	2.545	17	3.39	17	2.442	17	4.87
20	3.961	17	3.23	20	3.206	17	5.13
20	4.381	17	3.3	20	3.060	17	4.98
20	4.1	20	0.89	20	3.11	20	2.98
23	7.17	20	0.88	23	5.13	20	3.48
23	7.59	22	0	23	4.70	23	2.23
23	7.22			23	4.92	23	2.59
26	10.17			26	5.53	23	2.33
26	10.49			26	5.46		

Table 9-2: Raw data for trial one Powder A and Powder B stored under 47°C.

Powder B 47°C				Powder A 47°C			
Week	Hexanal (mg/kg powder)	Week	Oxygen (%)	Week	Hexanal (mg/kg powder)	Week	Oxygen (%)
0	0	0	20.4	0	0	0	20.4
2	0.449	2	14.8	2	0.45	2	14.3
2	0.433	2	14.6	2	0.53	2	14.3
2	0.320	2	14.9	3	1.03	2	14
3	0.601	3	12.7	3	1.12	3	12.1
3	0.641	3	13.7	3	1.11	3	11
3	0.581	3	13.1	4	1.41	3	11
4	0.621	4	12.3	4	1.78	4	9.66
4	0.633	4	12.5	4	1.43	4	9.55
4	0.630	4	12.3	5	2.58	4	9.98
5	0.704	5	11	5	2.56	5	8.23
5	0.799	5	11.1	5	2.87	5	8.4
5	0.876	5	11.1	6	3.37	5	8.24
6	1.183	6	9.42	6	3.59	6	7.4
6	1.152	6	9.59	6	3.82	6	7.15
6	0.942	6	9.48	7	4.99	6	7
7	1.38	7	8.71	7	3.83	7	6.05
7	1.19	7	8.5	7	4.85	7	5.85
9	2.25	7	9.39	9	4.75	7	5.78
9	2.22	9	4.49	9	4.91	9	3.92
9	2.28	9	5.21	10	5.17	9	3.52
10	2.32	9	5.45	10	4.66	9	2.94
10	2.60	10	4.08	10	5.65	10	2.24
11	2.92	10	3.98	11	5.31	10	2.15
11	3.22	10	3.75	11	6.48	10	2.14
11	3.33	11	2.54	11	4.31	11	1.83
12	3.99	11	2.3	12	5.35	11	1.99
12	3.66	11	2.29	12	6.79	11	1.62
14	4.59	12	0	12	6.63	12	1.23
14	5.00			14	8.44	12	1.2
14	4.79			14	5.78	13	0
17	3.95			14	7.30		
17	4.25			17	7.04		
				17	8.56		

Table 9-3: Raw data for trial one Powder A and Powder B stored under 57°C.

Powder B 57°C				**Powder A 57°C**			
Week	Hexanal (mg/kg powder)	Week	Oxygen (%)	Week	Hexanal (mg/kg powder)	Week	Oxygen (%)
0	0	0	20.4	0	0	0	20.4
2	0.83	2	14.8	2	1.33	2	12
2	0.75	2	14.2	2	1.38	2	12.2
2	0.83	2	15	2	1.64	2	11.3
3	1.14	3	11.2	3	2.60	3	8.33
3	1.14	3	10.9	3	2.80	3	7.98
3	1.19	3	11.4	3	3.15	3	8.38
4	1.50	4	8.93	4	3.31	4	6.05
4	1.69	4	9.77	4	3.45	4	5.23
4	1.26	4	9.84	5	5.68	4	5.49
5	2.30	5	6.59	5	6.53	5	3.07
5	2.19	5	7.25	5	6.02	5	3.15
6	3.17	5	7.15	6	6.20	6	1.84
6	2.92	6	3.36	6	7.23	6	1.88
6	2.96	6	3.39	6	6.77	6	2.08
7	2.54	7	1.61	7	8.58	7	0
7	2.81	7	2	7	9.25	7	0
7	2.93	7	2.39	7	8.53	7	0
7	2.48	9	0	9	7.85	9	0
9	2.77	9	0	9	7.84	9	0
9	3.07	9	0	10	9.75	9	0
10	2.97			10	9.56		
10	3.14			10	9.48		
10	3.19			11	10.94		
11	2.79			11	11.19		
11	2.87			11	11.02		
11	2.81			12	9.39		
12	2.78			12	9.48		
12	3.01			12	9.67		
13	2.68			13	6.83		
13	2.96			13	5.94		
13	2.78			13	4.98		
14	3.31			14	7.42		
14	2.94			14	7.37		
17	2.77			17	5.78		
17	2.92			17	5.46		

Table 9-4: Raw data for trial two Powder A and Powder B stored under 47°C.

Powder B 47°C				Powder A 47°C			
Date	Hexanal (mg/kg powder)	Week	Oxygen (%)	Date	Hexanal (mg/kg powder)	Week	Oxygen (%)
1	0.234	0	20.6	1	0.287	0	20.6
1	0.198	1	17	1	0.280	1	15.3
2	0.386	1	17	1	0.237	1	16.1
2	0.336	1	17	2	0.448	1	17
2	0.341	2	14.4	2	0.524	2	14.3
3	0.402	2	14.6	2	0.475	2	14.9
3	0.433	2	14.7	3	0.652	2	15.8
3	0.394	3	11.7	3	0.608	3	12
4	1.04	3	11.3	3	0.536	3	13.4
4	0.84	3	11.9	4	1.08	3	13.4
4	1.00	4	8.52	4	1.41	4	11.5
5	1.26	4	7.94	4	1.45	4	11.2
5	1.40	4	8.45	5	2.06	4	11.2
5	1.22	5	6.24	5	1.66	5	8.42
6	2.35	5	5.61	5	1.81	5	9.14
6	2.46	5	5.61	6	2.57	5	9.22
6	2.21	6	3.59	6	2.39	6	6.84
7	3.82	6	3.07	6	2.39	6	7.37
7	3.22	6	3.23	7	3.16	6	7.08
7	3.31	7	1.1	7	2.98	7	5.43
8	5.02	7	1.11	7	3.28	7	5.8
8	5.31	7	1.06	8	4.20	7	5.9
8	5.07	8	0	8	3.91	8	4.82
9	7.75	8		8	3.67	8	4.52
9	7.19	8		9	4.80	8	4.47
9	6.66			9	4.69	9	3.62
10	9.75			9	4.42	9	3.03
10	10.2			10	5.43	9	3.04
10	11.3			10	5.48	10	2.2
11	13.3			10	5.29	10	2.1
11	13.8			11	6.32	10	2.4
11	15.0			11	6.38	11	1.16
12	14.3			11	6.24	11	1.19
12	13.4			12	7.24	11	1.17
12	16.2			12	7.09	12	0
13	15.7			12	6.99		
13	15.8			13	8.39		
13	16.7			13	7.33		
14	18.2			13	8.50		
14	18.2			14	6.47		
14	16.4			14	7.04		
15	15.6			14	8.17		
15	14.7			15	10.1		
15	14.5			15	10.3		
16	16.0			15	10.2		
16	14.3			16	8.28		
				16	8.76		

Table 9-5: Raw data for trial two Powder A and Powder B stored under 57°C.

Powder B 57°C				Powder A 57°C			
Date	Hexanal (mg/kg powder)	Week	Oxygen (%)	Date	Hexanal (mg/kg powder)	Week	Oxygen (%)
1	0.553	0	20.6	1	0.156	0	20.6
1	0.552	1	17	1	0.160	1	15
1	0.531	1	16.8	1	0.184	1	14.7
2	0.898	1	18.2	2	1.27	1	14.4
2	0.825	2	9.57	2	1.40	2	11.2
3	1.59	2	9.05	2	1.38	2	10.6
3	1.27	2	8.62	3	2.28	2	10.8
3	1.37	3	3.8	3	2.36	3	7.43
4	8.19	3	2.93	3	2.50	3	6.93
4	6.24	3	3.27	4	4.68	3	6.89
4	6.19	3	2.65	4	4.33	4	5.09
5	12.8	4	1.83	4	4.24	4	4.56
5	12.0	4	1.23	5	5.12	4	4.2
5	11.2	5	0	5	4.85	5	2.18
6	14.9	6	0	5	4.29	5	2.26
6	15.8			6	7.00	5	1.87
6	15.1			6	6.12	6	0
7	15.2			6	5.90		
7	11.9			7	7.05		
7	11.7			7	6.86		
8	17.6			7	6.69		
8	18.3			8	8.15		
8	17.5			8	7.72		
9	15.4			8	7.48		
9	15.8			9	7.76		
9	15.3			9	7.65		
10	28.4			9	7.91		
10	25.8			10	9.65		
10	29.4			10	8.64		
11	26.6			10	8.79		
11	25.1			11	7.47		
11	25.5			11	7.67		
12	21.5			11	7.50		
12	19.9			12	7.42		
12	20.3			12	8.63		
13	20.1			12	7.61		
13	20.8			13	7.99		
13	22.3			13	8.97		
14	13.6			13	8.55		
14	16.4			14	7.69		
14	15.2			14	7.09		
15	16.7			14	7.36		
15	17.3			15	7.44		
15	18.1			15	7.02		

				15	7.27	

Table 9-6: Sensory scores raw data for both A1 & B1.

Week 3	**B 37**	**B 47**	**B 57**	**A 37**	**A 47**	**A 57**
	1	1	2	1	1	2
	2	1	2	1	1	3
	2	2	2	2	1	4
	2	2	2	2	2	4
	2	2	2	2	2	4
	2	2	3	2	3	4
	2	3	3	2	3	5
	3	3	3	3	3	5
Avg	2	2	2.375	1.875	2	3.875
SD	0.535	0.756	0.518	0.641	0.926	0.991
SE	0.189	0.267	0.183	0.227	0.327	0.350
Week 4	**B 37**	**B 47**	**B 57**	**A 37**	**A 47**	**A 57**
	1	1	2	1	1	2
	1	1	2	1	1	2
	2	1	2	1	2	2
	2	1	2	1	2	3
	2	2	3	1	2	3
	2	2	3	1	2	3
	3	2	3	2	2	4
	3	3	3	2	2	4
	4	3	3	2	3	4
Avg	2.22	1.78	2.56	1.33	1.89	3.00
SD	0.97	0.83	0.53	0.50	0.60	0.87
SE	0.34	0.29	0.19	0.18	0.21	0.31
Week 6	**B 37**	**B 47**	**B 57**	**A 37**	**A 47**	**A 57**
	1	1	2	1	2	2
	1	2	2	1	2	2
	1	2	3	2	2	2
	2	2	4	2	2	3
	2	2	4	2	3	3
	3	2	4	2	3	3
	4	3	5	3	3	3
	4	4	5	3	3	4
Avg	2.25	2.25	3.63	2.00	2.50	2.75
SD	1.28	0.89	1.19	0.76	0.53	0.71
SE	0.45	0.31	0.42	0.27	0.19	0.25
Week 8	**B 37**	**B 47**	**B 57**	**A 37**	**A 47**	**A 57**
	1	1	2	1	2	1
	2	1	2	1	2	1
	2	2	2	2	2	1
	3	3	3	2	3	2
	3	3	3	2	3	2
	3	4	4	3	3	3
	3	4	4	4	4	5
Avg	3.00	2.57	2.86	2.14	2.71	2.14
SD	1.41	1.27	0.90	1.07	0.76	1.46
SE	0.50	0.45	0.32	0.38	0.27	0.52
Week 10	**B 37**	**B 47**	**B 57**	**A 37**	**A 47**	**A 57**
	2	1	1	1	1	3
	2	2	1	1	2	3
	2	2	2	2	2	3
	2	3	2	3	3	3

	2	3	3	3	3	4
	4	4	3	3	4	4
	5	4	4	3	5	5
Avg	2.71	2.71	2.29	2.29	2.86	3.57
SD	1.25	1.11	1.11	0.95	1.35	0.79
SE	0.44	0.39	0.39	0.34	0.48	0.28
Week 11	**B 37**	**B 47**	**B 57**	**A 37**	**A 47**	**A 57**
	2	1	4	1	2	4
	2	3	4	1	2	4
	2	3	4	1	2	4
	3	3	4	2	3	4
	3	3	4	2	4	4
	3	4	5	3	4	5
Avg	2.50	2.83	4.17	1.67	2.83	4.17
SD	0.55	0.98	0.41	0.82	0.98	0.41
SE	0.19	0.35	0.14	0.29	0.35	0.14

Key:

	Score
No Difference	1
Slight Difference	2
Moderate Difference	3
Very Different	4
Extremely Different	5

9.2 Matlab®code to predict oxygen from given kinetic parameters and experimental data

9.2.1 Function file

```
function O2= O2pred( parameters, xdata );
% Define variables


k0= parameters(1); %The kinetic rate constant
E=parameters(2);  %Activaiton energy (J.mol-1)


%Import data from the data sheet file
t=xdata(:,1); %The data of time (week)
T=xdata(:,2)+273.15; %Temperature

%First order reaction kinetic
O2= (20.5^(1-n)-k0*exp(-E/8.314./T).*t*(1-n)).^(1/(1-n));
```

9.2.2 Script file

```
   % Read the data from data sheet
expdata=xlsread('experimental data.xlsx','overall','A2:C136');

%Import experimental data
time=expdata(:,1); %Import time from data file
temperature=expdata(:,2); %Import temperatures from data file
O2exp=expdata(:,3); %Import oxygen conc. from data file
params=[7800000000 65000]; %Guess values of the kinetic parameters
O2predicted=O2pred(params,[time  temperature]);  %calculation  the  oxygen
values


%plot graph of the prediction and experimental values of the oxygen conc.
plot(O2predicted, O2exp, 'bo');

%Estimate the kinetic parameters
newparams=lsqcurvefit('O2pred',params,[time temperature],O2exp);
O2new=O2pred(newparams,[time temperature]);

figure=plot(O2exp, O2new,'xr');
xlabel('O2^*e x p e r i m e n t a l');
ylabel('O2^*p r e d i c t e d');

hold on

p=polyfit(O2exp,O2new,1);

SS=O2exp(:,1) - O2new(:,1);
```

```
SS = SS(:,1).^2;

[h,w] = size(SS);
SSum = 0;
   for x = 1:h
       SSum = SSum + SS(x, 1);
```

9.3 Data table

9.3.1 Powder A

Table 9-7: Concentration of oxygen consumption (%) of Powder A with the storage period under different storage temperatures at initial oxygen concentration of 20.5%. With maximum 15% consumption oxygen allowed.

		Time (Week)																								
	Oxygen used (%)	4	8	10	14	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58
Temperature (°C)	20	1.2	2.4	3.0	4.1	5.2	5.8	6.3	6.8	7.3	7.8	8.3	8.8	9.2	9.7	10.1	10.6	11.0	11.4	11.8	12.2	12.6	13.0	13.4	13.8	14.1
	21	1.3	2.6	3.3	4.5	5.7	6.2	6.8	7.4	7.9	8.4	8.9	9.4	9.9	10.4	10.9	11.4	11.8	12.2	12.7	13.1	13.5	13.9	14.3	14.6	
	22	1.5	2.9	3.5	4.9	6.1	6.7	7.3	7.9	8.5	9.1	9.6	10.2	10.7	11.2	11.7	12.2	12.6	13.1	13.5	13.9	14.4	14.8			
	23	1.6	3.1	3.8	5.3	6.6	7.3	7.9	8.6	9.2	9.8	10.3	10.9	11.4	12.0	12.5	13.0	13.5	13.9	14.4	14.8					
	24	1.7	3.4	4.2	5.7	7.2	7.9	8.5	9.2	9.8	10.5	11.1	11.7	12.2	12.8	13.3	13.8	14.3	14.8							
	25	1.9	3.6	4.5	6.2	7.7	8.5	9.2	9.9	10.6	11.2	11.8	12.5	13.0	13.6	14.1	14.7									
	26	2.0	4.0	4.9	6.6	8.3	9.1	9.8	10.6	11.3	12.0	12.6	13.3	13.9	14.5											
	27	2.2	4.3	5.3	7.1	8.9	9.7	10.5	11.3	12.1	12.8	13.5	14.1	14.7												
	28	2.4	4.6	5.7	7.7	9.6	10.4	11.3	12.1	12.9	13.6	14.3	14.9													
	29	2.6	5.0	6.1	8.3	10.2	11.2	12.0	12.9	13.7	14.4															
	30	2.8	5.4	6.6	8.9	10.9	11.9	12.8	13.7	14.5																
	31	3.0	5.8	7.1	9.5	11.7	12.7	13.6	14.5																	
	32	3.3	6.2	7.6	10.2	12.4	13.5	14.4																		
	33	3.5	6.7	8.2	10.9	13.2	14.3																			
	34	3.8	7.2	8.8	11.6	14.0																				
	35	4.1	7.7	9.4	12.3	14.8																				

Table 9-8: Oxygen consumption concentration (%) under various initial oxygen concentrations.

		Initial Oxygen (%)									
		2	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20.5**
Time (week)	**0**	0	0	0	0	0	0	0	0	0	0
	1	0.15	0.21	0.26	0.30	0.33	0.36	0.39	0.42	0.45	0.48
	2	0.29	0.41	0.51	0.59	0.66	0.72	0.78	0.83	0.89	0.95
	3	0.42	0.61	0.75	0.87	0.98	1.07	1.16	1.24	1.32	1.41
	4	0.55	0.80	0.99	1.15	1.29	1.42	1.54	1.65	1.75	1.87
	5	0.68	0.99	1.22	1.42	1.60	1.76	1.91	2.04	2.17	2.32
	6	0.80	1.17	1.45	1.69	1.90	2.10	2.27	2.44	2.59	2.77
	7	0.91	1.34	1.68	1.96	2.20	2.43	2.63	2.82	3.00	3.21
	8	1.02	1.51	1.89	2.21	2.49	2.75	2.98	3.20	3.41	3.65
	9	1.12	1.68	2.10	2.46	2.78	3.07	3.33	3.58	3.81	4.08
	10	1.22	1.83	2.31	2.71	3.06	3.38	3.67	3.95	4.20	4.51
	11	1.31	1.99	2.51	2.95	3.34	3.69	4.01	4.31	4.59	4.93
	12	1.39	2.13	2.70	3.18	3.61	3.99	4.34	4.67	4.98	5.34
	13	1.47	2.28	2.89	3.41	3.87	4.29	4.67	5.02	5.36	5.75
	14	1.55	2.41	3.08	3.64	4.13	4.58	4.99	5.37	5.73	6.15
	15	1.61	2.54	3.25	3.86	4.38	4.86	5.30	5.71	6.10	6.55
	16	1.68	2.67	3.43	4.07	4.63	5.14	5.61	6.05	6.46	6.94
	17	1.73	2.79	3.59	4.27	4.87	5.42	5.91	6.38	6.81	7.33
	18	1.79	2.90	3.75	4.48	5.11	5.68	6.21	6.70	7.17	7.71
	19	1.83	3.01	3.91	4.67	5.34	5.95	6.50	7.02	7.51	8.08
	20	1.87	3.11	4.06	4.86	5.57	6.20	6.79	7.34	7.85	8.45
	21	1.91	3.21	4.20	5.05	5.79	6.46	7.07	7.65	8.18	8.82
	22	1.94	3.30	4.34	5.22	6.00	6.70	7.35	7.95	8.51	9.17
	23	1.96	3.38	4.48	5.40	6.21	6.94	7.62	8.24	8.83	9.53
	24	1.98	3.46	4.60	5.57	6.41	7.18	7.88	8.54	9.15	9.87
	25	1.99	3.54	4.73	5.73	6.61	7.41	8.14	8.82	9.46	10.22
	26	2.00	3.61	4.84	5.88	6.80	7.63	8.39	9.10	9.77	10.55
	27	2.00	3.67	4.95	6.03	6.99	7.85	8.64	9.38	10.07	10.88
	28	2.00	3.73	5.06	6.18	7.17	8.06	8.88	9.65	10.36	11.21
	29	1.99	3.78	5.16	6.32	7.34	8.27	9.12	9.91	10.65	11.53
	30	1.97	3.83	5.25	6.45	7.51	8.47	9.35	10.17	10.94	11.84
	31	1.95	3.87	5.34	6.58	7.68	8.66	9.57	10.42	11.22	12.15
	32	1.92	3.90	5.42	6.71	7.83	8.86	9.79	10.67	11.49	12.45
	33	1.89	3.93	5.50	6.82	7.99	9.04	10.01	10.91	11.75	12.75
	34	1.85	3.96	5.57	6.93	8.13	9.22	10.22	11.14	12.02	13.04
	35	1.81	3.98	5.64	7.04	8.28	9.39	10.42	11.37	12.27	13.33
	36	1.76	3.99	5.70	7.14	8.41	9.56	10.62	11.60	12.52	13.61
	37	1.71	4.00	5.76	7.24	8.54	9.72	10.81	11.82	12.77	13.88
	38	1.65	4.00	5.80	7.33	8.67	9.88	10.99	12.03	13.01	14.15
	39	1.58	4.00	5.85	7.41	8.79	10.03	11.17	12.24	13.24	14.41
	40	1.51	3.99	5.89	7.49	8.90	10.18	11.35	12.44	13.47	14.67
	41	1.43	3.97	5.92	7.56	9.01	10.32	11.52	12.64	13.69	14.92
	42	1.35	3.95	5.95	7.63	9.11	10.45	11.68	12.83	13.91	15.17
	43	1.26	3.93	5.97	7.69	9.21	10.58	11.84	13.01	14.12	15.41
	44	1.17	3.89	5.98	7.75	9.30	10.70	11.99	13.19	14.32	15.65
	45	1.07	3.86	5.99	7.80	9.38	10.82	12.14	13.37	14.52	15.88
	46	0.97	3.81	6.00	7.84	9.46	10.93	12.28	13.54	14.72	16.10
	47	0.86	3.77	6.00	7.88	9.54	11.04	12.42	13.70	14.90	16.32
	48	0.74	3.71	5.99	7.91	9.61	11.14	12.55	13.86	15.09	16.53

9.3.2 Powder B

Table 9-9: Concentration of oxygen consumption (%) of Powder B with the storage period under different storage temperatures at initial oxygen concentration of 20.5%. With maximum 15% consumption oxygen allowed.

		Time (week)																
	Oxygen used (%)	4	8	10	14	18	20	22	24	26	28	30	32	34	36	38	40	42
Temperature °C	20	1.8	3.5	4.3	5.9	7.4	8.1	8.8	9.5	10.1	10.8	11.4	12.0	12.5	13.1	13.6	14.2	14.6
	21	1.9	3.7	4.6	6.2	7.8	8.5	9.3	10.0	10.7	11.3	12.0	12.6	13.2	13.7	14.3	14.8	
	22	2.0	3.9	4.8	6.6	8.2	9.0	9.8	10.5	11.2	11.9	12.6	13.2	13.8	14.4	14.9		
	23	2.1	4.2	5.1	7.0	8.7	9.5	10.3	11.1	11.8	12.5	13.2	13.8	14.4	15.0			
	24	2.3	4.4	5.4	7.4	9.2	10.0	10.8	11.6	12.4	13.1	13.8	14.4					
	25	2.4	4.7	5.7	7.8	9.7	10.6	11.4	12.2	13.0	13.7	14.4						
	26	2.6	5.0	6.1	8.2	10.2	11.1	12.0	12.8	13.6	14.3	15.0						
	27	2.7	5.2	6.4	8.7	10.7	11.7	12.6	13.4	14.2	15.0							
	28	2.9	5.5	6.8	9.1	11.2	12.2	13.1	14.0	14.8								
	29	3.1	5.9	7.2	9.6	11.8	12.8	13.7	14.6									
	30	3.2	6.2	7.6	10.1	12.4	13.4	14.3										
	31	3.4	6.5	8.0	10.6	12.9	14.0											
	32	3.6	6.9	8.4	11.1	13.5	14.6											
	33	3.8	7.3	8.8	11.7	14.1												
	34	4.0	7.6	9.3	12.2	14.7												
	35	4.3	8.0	9.7	12.8													

Table 9-10: Oxygen consumption concentration (%) under various initial oxygen concentrations.

	Oxygen (%)									
Time (Week	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20.5**
1	0.19	0.27	0.33	0.38	0.43	0.47	0.51	0.54	0.58	0.62
2	0.37	0.53	0.65	0.76	0.85	0.93	1.01	1.08	1.15	1.23
3	0.54	0.78	0.97	1.12	1.26	1.39	1.50	1.61	1.71	1.82
4	0.70	1.02	1.27	1.48	1.66	1.83	1.98	2.12	2.26	2.41
5	0.85	1.26	1.56	1.83	2.05	2.26	2.45	2.63	2.80	2.99
6	1.00	1.48	1.85	2.16	2.44	2.69	2.91	3.13	3.33	3.56
7	1.13	1.69	2.12	2.49	2.81	3.10	3.37	3.62	3.85	4.12
8	1.25	1.90	2.39	2.81	3.17	3.51	3.81	4.09	4.36	4.67
9	1.37	2.09	2.65	3.12	3.53	3.90	4.24	4.56	4.86	5.22
10	1.47	2.28	2.89	3.41	3.87	4.29	4.67	5.02	5.36	5.75
11	1.57	2.45	3.13	3.70	4.21	4.66	5.08	5.47	5.84	6.27
12	1.65	2.62	3.36	3.98	4.53	5.03	5.49	5.92	6.32	6.79
13	1.73	2.77	3.58	4.25	4.85	5.39	5.89	6.35	6.78	7.29
14	1.80	2.92	3.79	4.52	5.16	5.74	6.27	6.77	7.24	7.78
15	1.85	3.06	3.99	4.77	5.46	6.08	6.65	7.18	7.68	8.27
16	1.90	3.19	4.18	5.01	5.74	6.41	7.02	7.59	8.12	8.75
17	1.94	3.31	4.36	5.24	6.02	6.73	7.38	7.98	8.55	9.21
18	1.97	3.42	4.53	5.47	6.29	7.04	7.72	8.36	8.96	9.67
19	1.99	3.52	4.69	5.68	6.55	7.34	8.06	8.74	9.37	10.12
20	2.00	3.61	4.84	5.88	6.80	7.63	8.39	9.10	9.77	10.55
21	2.00	3.69	4.99	6.08	7.04	7.91	8.71	9.46	10.16	10.98
22	1.99	3.76	5.12	6.27	7.27	8.19	9.03	9.81	10.54	11.40
23	1.97	3.82	5.24	6.44	7.50	8.45	9.33	10.14	10.91	11.81
24	1.94	3.88	5.36	6.61	7.71	8.70	9.62	10.47	11.27	12.21
25	1.91	3.92	5.46	6.77	7.91	8.95	9.90	10.79	11.62	12.60
26	1.86	3.95	5.56	6.91	8.11	9.18	10.18	11.10	11.97	12.98
27	1.81	3.98	5.65	7.05	8.29	9.41	10.44	11.40	12.30	13.36
28	1.74	3.99	5.72	7.18	8.47	9.63	10.69	11.69	12.62	13.72
29	1.67	4.00	5.79	7.30	8.63	9.83	10.94	11.97	12.94	14.07
30	1.58	4.00	5.85	7.41	8.79	10.03	11.18	12.24	13.24	14.42
31	1.49	3.98	5.90	7.51	8.93	10.22	11.40	12.50	13.54	14.75
32	1.39	3.96	5.94	7.60	9.07	10.40	11.62	12.75	13.82	15.07
33	1.27	3.93	5.97	7.68	9.20	10.57	11.83	13.00	14.10	15.39
34	1.15	3.89	5.99	7.76	9.32	10.73	12.02	13.23	14.36	15.70
35	1.02	3.84	6.00	7.82	9.43	10.88	12.21	13.45	14.62	15.99
36	0.88	3.78	6.00	7.87	9.53	11.02	12.39	13.67	14.87	16.28
37	0.73	3.71	5.99	7.92	9.62	11.15	12.56	13.87	15.11	16.56
38	0.57	3.63	5.97	7.95	9.70	11.27	12.72	14.07	15.34	16.82
39	0.40	3.54	5.95	7.98	9.77	11.38	12.87	14.26	15.56	17.08
40	0.22	3.44	5.91	7.99	9.83	11.49	13.01	14.43	15.77	17.33
41	0.03	3.33	5.87	8.00	9.88	11.58	13.14	14.60	15.97	17.57