

**Decreasing barrier while maintaining shelf-life for highly sensitive products - A more recyclable and sustainable packaging for infant formula**

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DIVISION OF PACKAGING LOGISTICS | DEPARTMENT OF DESIGN SCIENCES  
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MASTER THESIS



# Decreasing barrier while maintaining shelf-life for highly sensitive products

A more recyclable and sustainable packaging for infant  
formula

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**LUND**  
UNIVERSITY

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# Abstract

With increasing concerns about environmental impacts of the food that is consumed, so is also the concern about packaging materials. This also comprises the packaging that is currently used to pack infant formula (IF), very often containing aluminum as the barrier material. IF is a very sensitive product that demands for the highest barriers to protect it from lipid oxidation. This implies that the barrier material has to be able to prevent O<sub>2</sub> and moisture, as well as light from entering the package. In this study it was investigated whether one or more of five tested materials is/are able to replace the aluminum package and guarantee the same shelf-life of the product. A combination of accelerated shelf-life testing and the TBARS assay were used to determine the amount of malondialdehyde (MDA) produced during lipid oxidation in the samples stored in the different packaging materials. The results showed that there was no significant increase in oxidation in any of the materials (based on linear regression). No conclusions could be drawn regarding which of the materials could be an alternative to the aluminum-based one. However, a decreasing O<sub>2</sub> trend was visible in the package filled with ambient O<sub>2</sub>. This can be taken as an indication that the powder did consume oxygen during the time of the study.

**Keywords:** infant formula, packaging material, lipid oxidation, TBARS assay, shelf-life

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# List of Acronyms and Abbreviations

$a_w$	water activity
EVOH	ethylene-vinyl alcohol
HDPE	high-density polyethylene
IF	infant formula
LDPE	low-density polyethylene
MA	modified atmosphere
MDA	malondialdehyde
MVTR	moisture vapor transmission rate
OTR	oxygen transmission rate
PA	polyamide
PE	polyethylene
PET	polyethylene terephthalate
PP	polypropylene
RH	relative humidity
SD	standard deviation
SEM	standard error of means
TBA	thiobarbituric acid
UBC	used beverage cartons
UFA	unsaturated fatty acids

# 1 Introduction

*The following chapters (1.1 to 1.5) give an overview of the basic settings for this degree project. Starting off with the Background, an insight is given into the current packaging solutions for infant formula (IF), followed by the Problem Description, determining the factors that demand for a change in packaging. Furthermore, the Goal & Purpose of this Research chapter discusses why this research is of need and the section Scope and Delimitations frames the topic to a specific focus. In the end of this section the Company Description gives an insight into the industry that has an interest in this research.*

## 1.1 Background

Currently IF is often packed in either metal cans or composite cans containing aluminum as a barrier layer (Wang, An & Lee, 2017). This enables the product to be protected in an optimal way, providing barriers to light, moisture and oxygen. These factors all influence the shelf-life of IF, since they can induce lipid oxidation in the powder (Teherany & Sonneveld, 2009). This is due to the IF containing a comparatively high amount of fats, of which especially unsaturated fatty acids (UFA) which are very prone to lipid oxidation. Due to the water content in the IF powder being very low (<5%), meaning that microorganisms cannot grow, the main deteriorative factor is lipid oxidation (Teherany & Sonneveld, 2009).

Also, AR Packaging currently uses composite cans containing an aluminum layer to pack IF.

Aluminum is, based on the mining methods and low recyclability rates when combined with other materials, not always the preferred packaging solution. Moreover, it is a non-renewable resource, making resource depletion an aspect to consider as well (Åhman, 2018). China is one of the biggest producers of aluminum (42.9% of total primary aluminum production in

2013), using more than 5% of the total energy used in China for its production only. Next to this the air is polluted by many compounds, harming the environment close by (Zhang et al., 2016). The recyclability of aluminum composites is a complex process as described by Lopes & Felisberti (2006) by means of a Tetra Pak package. The material is either incinerated with energy recovery; aluminum is recovered by pyrolysis or the plastic-metal mixture used in the material is extruded to be used as a composite again. The latter two steps demand very specific treatment, which might not be available at all waste treatment plants in all countries and result in incineration with energy recovery.

Due to the growing concerns of customers regarding the environmental impact of products it is also in the company's interest to change something in the packaging currently used. Hence, to investigate the possibility of implementing an alternative material without an aluminum layer and provide sufficient barrier properties for the product to have the same quality and shelf-life as with the current composite can.

However, also food products themselves have huge impacts on the environment based on their production or created food waste. According to Ekman Nilsson (2018) food consumption makes up 25% of the greenhouse gas emissions in Sweden. They further state that about 30% of the global greenhouse gas emissions is based on food production (e.g. land use) and consumption, with the highest cause during primary production (i.e. agriculture, aquaculture, fishery). Depending on the product the environmental impact can be bigger for the food product itself (e.g. beef, pork, dairy products) compared to the impact the packaging has. Ekman Nilsson contrasts that the production of 1 L of milk produces the same amount of emissions as the production of 50 plastic bags (~1 kg CO<sub>2</sub>e). Food waste can have several causes. Olsson (2019) states that on one hand the consumer himself might be the cause due to the wrong storage or utilization of the product. Also, wrong amounts, leading to leftovers of the food product and thus food waste, can be a result of the behavior of consumers. Nevertheless, packaging has an immense influence in the shelf-life of a product. With packaging being designed in an inefficient way, food can remain in the package not possible to be emptied by the consumer (Olsson, 2019). The environmental impact packaging can have if it is used in too high or too low amounts can be seen in figure 1.



**Figure 1: Innventia AB model showing environmental impact of under- and overpacking (Tomaszewski, 2014).**

As can be seen, the impact of underpacking is higher compared to that of overpacking. This is because the impact of e.g. the food product itself often is, as stated above, much higher than that of the packaging only. Furthermore, if the package does not provide the right protection (e.g. mechanical strength, barrier properties) for the product, this can also end up in food waste, due to the food getting spoiled or losing its quality in a shorter time. Since about 1/3<sup>rd</sup> of the food produced is wasted (Ekman Nilsson, 2018) it is very essential to create good packaging that can protect the food in an optimal way and help reduce food waste.

It is thus important to understand and foresee all the factors impacting the product's shelf-life as well as the functionality and environmental impact the packaging itself has.

## 1.2 Problem Description

Currently the packaging used for IF by AR Packaging contains an aluminum layer to provide the barrier that is needed for the IF to be safe to consume and have a long shelf-life of up to 24 months (internal company information). With aluminum being no sustainable option in the packaging, alternative materials are needed. So far, no material was however found with the same barrier properties as aluminum, making it very hard to fully replace it. Since

IF is a very sensitive product, a high barrier against factors like oxygen, moisture and light is indispensable.

To be able to evaluate whether other materials are able to replace the aluminum layer in the packaging, the effect of the barrier properties on the shelf-life needs to be further studied.

### 1.3 Goal & Purpose of this Research

The purpose of this research is to evaluate alternative barrier materials to replace aluminum foil in composite can packages that are currently used for IF. At the same time, it is intended to obtain a better insight on how the barrier properties influence the deterioration of the product to be able to provide a packaging that can maintain the same quality and shelf-life of the IF as the current one.

The main goal is to answer or perform the following aspects.

1. How can the Alu-barrier of a composite can package for IF be replaced, while maintaining the same shelf-life and quality of the product?
2. Which factors have the greatest impact on the deterioration of the product?
3. How can the shelf-life of the IF stored in different materials be evaluated based on the findings for question 2?

### 1.4 Scope and Delimitations

The degree project was conducted during a period of 20 weeks, meaning that all research and written parts had to be performed within this time frame. Since the project was performed in cooperation with a company, certain delimitations were given by them as well, which will be explained as follows. Due to the limited time, the focus was laid on changing the material in the body of the packaging only (see figure 10 in section 2.7 *Packaging of Infant Formula*). This means that a change in sealing techniques or in material of the top and bottom were not considered. Due to this as well as to decrease the workload, two membranes were used instead of one membrane and one

base. The materials used for this research were not allowed to be specified in detail and had to be denoted with name codes instead (e.g. *Alu* and *A2*). Furthermore, only the primary packaging in direct contact with the food product was taken into consideration for this research. The degree project had to be adjusted such that it fulfilled the requirements of both the company as well as those of Lund University. The research was conducted both at the company and at Kemicentrum at Lund University and had to be adapted such that all necessary equipment was available. Moreover, it was assumed that the light barrier is given mainly by paperboard (Tu & Apt, 2013) that is present in all materials and therefore light is not taken into consideration as a deteriorative factor causing lipid oxidation in the IF during this research. The study was adjusted such that moisture was assumed to not have an impact on the IF's lipid oxidation (discussed in section 2.1.3 *Deteriorative Factors*). This was done to limit the factors considered in this research to a minimum. The tests were chosen based on feasibility within the time frame and based on the availability of equipment and chemicals in the laboratory. Due to a possible laboratory closure at the university based on the Covid-19 pandemic, different scenarios were established under which the degree project was possible to be further conducted (see section 3.2.7 *Different Scenarios for the study*).

## 1.5 Company Description

The degree project is performed in cooperation with the company AR Packaging. AR Packaging is a company with its origin in Lund, Sweden. The company strives to fulfill its customer's needs regarding carton-based packaging solutions for several target markets (AR Packaging, 2020). The three main sectors comprise food products, health care products and the tobacco industry. They not only provide packages such as clamshells, folding cartons and trays, but also have their company's own packaging solutions: Sealio®, Boardio® and Cekacan®. Next to this, the company also provides machine systems that can be installed by their customer companies and used with individual packaging designs (AR Packaging, 2020).

The packaging used in this study is described in more detail in section 2.7 *Packaging of Infant Formula*.

## 2 Frame of Reference

*This chapter is intended to give the reader all the information needed to understand why and how the research was performed. In the first section the focus is on the IF's composition, production and requirements, followed by an overview of lipid oxidation and methods to measure the extent of oxidation. The chapter ends with information about the food packaging materials commonly used and used in this specific project.*

### 2.1 Infant Formula

IF is intended to be a substitute to breast-milk in case the mother of the child is unable to feed it herself (Jiang & Guo, 2014). IF is based on a milk powder other than human milk (Codex Alimentarius Commission, 2007), which causes the milk to have a different profile in lactose, protein, fat and mineral content and composition compared to human milk (Jiang & Guo, 2014). IF is thus based on milk of animal origin, e.g. cows. Cow milk provides however a lower nutritional profile than human milk and needs to be modified to fulfill the needs of the infants and is therefore mixed with other proven and allowed ingredients. All of these must be gluten-free. 100 ml of prepared IF is to have between 60 and 70 kcal in total energy (Codex Alimentarius Commission, 2007).

An overview of the macronutrient content allowed per 100 kcal of prepared (mixed with water) IF can be seen in table 1.

The Codex Alimentarius Standard CODEX STAN 72-1981 (Codex Alimentarius Commission, 2007) not only determines which composition IF must have to be able to call it IF, but it also states that its processing and packaging are to be designed in a way that they aim to prevent contamination of any means under the given storage conditions in the country the product is sold in.

**Table 1: Macronutrient requirements of IF (Codex Alimentarius Commission, 2007).**

<b>Nutrient</b>	<b>Minimum (g/100 kcal)</b>	<b>Maximum (g/100 kcal)</b>	<b>GUL<sup>1</sup></b>
Protein	1.8	3.0	-
Total fat	4.4	6.0	-
Carbohydrates	9.0	14.0	-

Due to their essential character the fatty acids linoleic acid and  $\alpha$ -linolenic acid have to be included in the IF as well (Codex Alimentarius Commission, 2007), with restrictions as seen in table 2.

**Table 2: Essential fatty acid requirements for IF (Codex Alimentarius Commission, 2007).**

<b>Fatty Acid</b>	<b>Minimum (mg/100 kcal)</b>	<b>Maximum (mg/100 kcal)</b>	<b>GUL</b>
Linoleic Acid	300	-	1400
$\alpha$ -Linolenic Acid	50	N.S. <sup>2</sup>	-

Next to these essential fatty acids also polyunsaturated fatty acids are important for the prevention of allergy development in the children (Guo & Ahmad, 2014). According to USDA (2019) the addition of long-chain polyunsaturated fatty acids can also help to decrease the risk for diabetes type II to develop, since they help to increase the skeletal muscle membrane. Fat in general is the main source of energy used by the human body and therefore very important for a good development of the child, especially in the early months when growth is most extreme.

Very often vitamins are added to IFs, which also can have an impact on the shelf-life of the product (antioxidant effect). Vitamin D for example, a very important vitamin, which milk powders (hence also IF) are usually fortified with, is necessary for a good development of the child regarding bone health as well as a well-functioning absorption of calcium (USDA, 2019). Also, vitamin A, needed for a good vision, immune system development and growth is often added to IF. Vitamin E is one of the antioxidative vitamins

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<sup>1</sup> GUL = guidance upper levels (for nutrients without sufficient information for a science-based risk assessment)

<sup>2</sup> N.S. = not specified

that can be found in IF as well. It is needed due to the antioxidative action in cells, combined with its function in the immune and neuro-system. Vitamin K, C and B can also be added fulfilling different functions (USDA, 2019).

### **2.1.1 Target Group**

The target group is, with infants in the age range of 1 to 12 months, one of the most vulnerable groups (WHO, 2020; Codex Alimentarius Commission, 2007). Since IF is the food they consume as the single and daily food product it is of utmost importance that the safety and quality required is met and that the IF provides all the nutrients needed by the infants.

According to Guo and Ahmad (2014) a balanced nutrition in the first months of an infant's life is very important for a good development according to age and for its further health in life. Oligofructose and oligogalactose for example are only present in human milk and not in cow's milk (Walsh, 2014). The absence of them in the diet of an infant can cause the intestinal flora of the children to differ from those being breast-fed, possibly leading to health problems. To compensate for this, Walsh (2014) states that often galacto-oligosaccharides or inulin are added (have promoting effect for the desired intestinal microbial flora).

### **2.1.2 Manufacturing**

Jiang and Guo (2014) state that powdered IF is produced by either dry blending or wet mixing-spray drying. In which the dry blending process comprises that each ingredient gets prepared individually, treated with heat, dried and in the end mixed with all other ingredients upon dry blending. During the wet mixing-spray drying process on the other hand, ingredients are mixed from the beginning on in liquid state and are then pasteurized or sterilized after which they get spray dried into the powder. Combinations of the two processes do also exist (Jiang & Guo, 2014).

The general manufacturing process of powdered IF can be seen in figure 2.

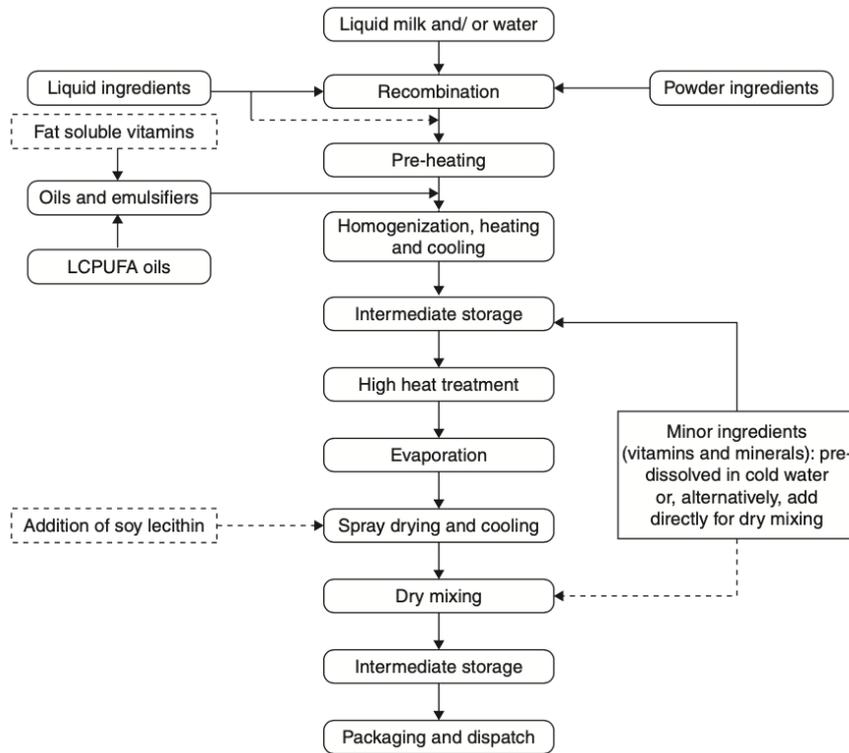
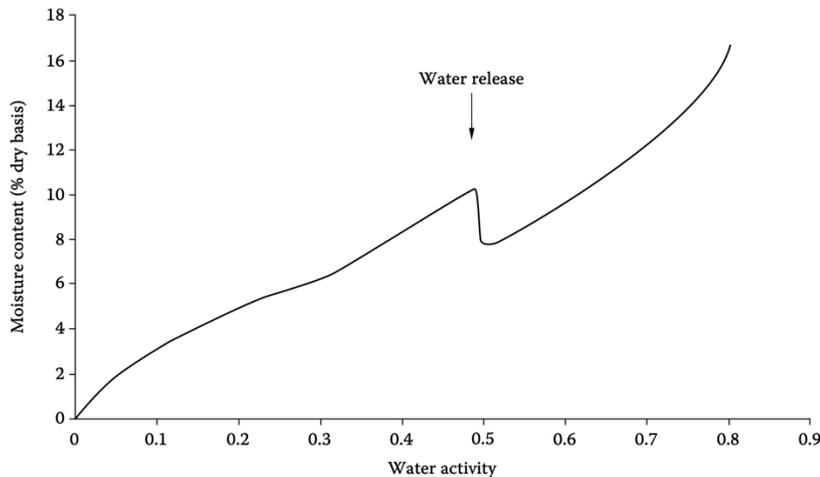


Figure 2: Manufacturing process of powdered IF (Guo & Ahmad, 2014).

### 2.1.3 Deteriorative Factors

During the drying steps mentioned under the manufacturing section the water content of the powder usually goes down to 2.5% to 5%, which is such a low concentration that microbes are not able to grow (Tehrany & Sonneveld, 2009).



**Figure 3: Sorption isotherm of milk powders (generalized) with a critical  $a_w$  of 0.5, which is where lactose starts crystallizing (Therany & Sonneveld, 2009).**

Teherany and Sonneveld (2009) summarize the deteriorative factors for IF as cohesion of powder particles to the spray drying equipment (due to decreased particle size or lipid liquefaction), caking of the powder (loss of quality), Maillard reactions (due to high temperature treatments or storage at moderate temperatures for long times) and lipid oxidation (off-flavors). Furthermore, also water activity ( $a_w$ ; has effects on lipid oxidation), temperature (high temperatures increase the rate of lipid oxidation and can cause Maillard reactions), oxygen content (influence on lipid oxidation) and light exposure (influence on lipid oxidation) have negative effects. Krochta (2006, p. 858) states however that solid foods (including powders) are less sensitive to light exposure compared to liquid foods and since all the packaging materials in this study will provide light protection due to inclusion of paperboard, this factor is not considered a risk for the product to deteriorate.

After the drying step, lactose as well as whey proteins, caseins and milk fat are the major components of the milk powder (Thomsen, Lauridsen, Skibsted & Risbo, 2005). The  $a_w$  of milk powder can be as low as 0.2, which is lower than the value that divides the states of glassy and non-glassy lactose (at  $a_w$  of 0.4, or as stated by Therany & Sonneveld, 2009 and shown in figure 3, 0.5). At ambient temperature lactose is present in the non-glassy state. The study of Thomsen et al. (2005) indicates that with lactose crystallizing, not only the  $a_w$  increases, but by this also the risk for lipid oxidation, Maillard

reactions and other chemical deteriorative reactions. They further state that the crystallization of lactose does however not occur below the glass transition temperature ( $T_g$ ), which is found to be at around 40°C for whole milk powder.

Keeping the temperature during the accelerated shelf-life test (ASLT) below 40°C (38°C) in this study could help to prevent the lactose to crystallize. Next to this, by keeping the relative humidity (RH) at a level of 25%, which is much lower than ambient RH (30-40% during winter months), can also decrease the risk of an increase in  $a_w$  (RH is equal to  $a_w$ ; Soponronnarit, Srisubati & Yoovidhya, 1998) and by this increase in deteriorative reactions. It is however only possible to control the RH inside the climate chamber. The RH for the samples stored outside the climate chamber was measured and will be discussed in section 4 *Results & Discussion*.

By controlling the factors mentioned above, Lipid oxidation can be seen as the main deteriorative factor in powdered IF. It can be recognized as soon as enough volatile compounds are formed that are perceivable as a rancid smell.

In fact, already during the manufacturing process lipid oxidation can occur (Cesa, Casadei, Cerreto & Paolicelli, 2015). To prevent or delay further lipid oxidation, a maximum level of  $O_2$  of 2% in the package is suggested by An, Lee & Lee (2018a) and Parry (2012, p. 5) states that this can usually be obtained by flushing the packages with nitrogen.

During lipid oxidation one of the compounds formed is malondialdehyde (MDA). Even though there is no official upper limit for the MDA concentration that is allowed in IF, Martysiak-Żurowska & Stołyhwo (2006) suggest an upper limit of 48 µg/100 ml of prepared milk. Since the IF producer of the one used in this study indicates a safe usage of the powder up to one month after opening, the value obtained during the laboratory tests for an open package after one month could also be taken as reference for an upper limit (more detailed information about the test in section 3.2.8 *Shelf-life Assessment*).

## 2.2 Lipid Oxidation

Lipid oxidation is the main factor for dairy-based powders to deteriorate. Upon oxidation of fats volatile compounds are formed that have a rancid

character which are undesired by the consumer. Furthermore, the compounds formed can be harmful to the consumer, especially being a risk for the vulnerable target group (Frankel, 1980). And next to this oxidation reactions cause the food product to lose some of its nutritive value (Tehrany & Sonneveld, 2009).

Unsaturated fatty acids (UFAs) are most prone to oxidation resulting in very reactive undesired compounds. As soon as lipid oxidation starts, a cascade of reactions proceeds impacting not only lipids, but also structural proteins and enzymes, as well as nucleic acids, vitamins and polysaccharides (Schaich, Shahidi, Zhong & Eskin, 2013). This reaction is, after initiation, typically independent of the oxygen concentration (Robertson, 2005, p. 358).

Lipid oxidation comprises three steps: initiation, propagation and termination. Each of these steps will be explained more in detail as follows.

### 2.2.1 Initiation

Lipid oxidation does not occur by itself but needs an initiating factor to start oxidation reactions. This is because atmospheric oxygen has a triplet spin character, whereas it needs to be in singlet spin state (since the double bonds of the UFAs are in this state) to cause oxidation. If then such an initiator is present, lipid alkyl radicals are formed upon reaction of the initiator with the UFA (Schaich, Shahidi, Zhong & Eskin, 2013). According to Frankel (1980) the initiation step can be based on (initiating factor) thermolysis (i.e. temperature), hydroperoxide decomposition or photolysis (i.e. light). An overview of the basic reaction can be seen in figure 4.

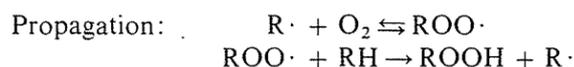


Figure 4: Initiation reaction in lipid oxidation (Frankel, 1980).

### 2.2.2 Propagation

In this stage oxygen binds to the lipid alkyl radicals (not very reactive) to convert them into peroxy radicals (reactive). These peroxy radicals cause the reaction to enter an ongoing oxidation. While forming hydroperoxides when binding to hydrogen on one side, they form new radicals upon this reaction that again will react with other molecules on the other side. This

reaction continues until hydrogen is used up or no radicals are available anymore (Schaich et al., 2013). The basic propagation step can be seen in figure 5.



**Figure 5: Propagation reaction in lipid oxidation (Frankel, 1980).**

### 2.2.3 Termination

Schaich et al. (2013) states that even though it is called termination the reaction is not really terminated. It simply means that the reaction is slowed down, due to many radicals reacting with each other to form non-radical products. There will however always be some radicals left to react further. The reaction speed is decreased since the creation of new radicals is slower than that of nonradical products. There are four main causes for termination to occur: radical recombination, alpha and beta scission reactions of alkoxy radicals, co-oxidation of non-lipids (e.g. proteins) and group eliminations/dismutation (Schaich et al., 2013).

The basic termination step in lipid oxidation is depicted in figure 6.



**Figure 6: Termination reaction in lipid oxidation (Frankel, 1980).**

## 2.3 Measuring Techniques for Evaluation of Oxidation

To determine the extent of lipid oxidation in a fat containing product, there are several different measurements to be used. The most common ones are explained below.

### 2.3.1 Peroxide Value

The peroxide value is a measure for the milliequivalents of peroxide oxygen found in one kilogram of oil and the method most widely used to determine

lipid oxidation. A titration of thiosulfate with the sample mixture determines the amount of iodine released by the peroxides (Patterson, 2011). Another method is to let hydroperoxides oxidize ferrous ions to ferric ions and by colorimetric measurements (at an adsorption of 293 nm) determine the concentration of hydroperoxides present in the sample (Talbot, 2016). The peroxide value however only tells something about the primary oxidation products (peroxides), which are not directly linked to the rancid off-flavors that often are intended to be investigated (Patterson, 2011; ). This fact needs to be considered when evaluating the resulting peroxide values. Another disadvantage of the method is that the fat/oil needs to be extracted from the product to be examined (AOCS, 2020a).

### 2.3.2 TBARS assay

According to Fernández, Pérez-Álvarez and Fernández-López (1997), TBARS is a specification for substances reacting with thiobarbituric acid (TBA), besides malondialdehyde (MDA). Substances reacting with TBA are those that are formed during lipid oxidation. More precisely, they are secondary reaction products, formed during the breakage of hydroperoxides. If detected by the TBARS assay they are an indication that lipid oxidation has occurred and specific TBA-values can suggest the product tested to be rancid (Fernández, Pérez-Álvarez and Fernández-López, 1997).

During the TBARS assay two TBA molecules react with one MDA molecule resulting in a red pigment (see figure 7) that can be measured with spectrophotometry. The measurement will show results at 532 - 535 nm (Fernández, Pérez-Álvarez & Fernández-López, 1997).

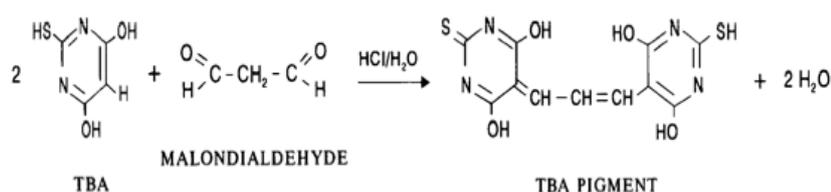


Figure 7: TBA reaction with MDA resulting in red pigment (Fernández, Pérez-Álvarez & Fernández-López, 1997).

### 2.3.3 Anisidine Value

Secondary products formed during oxidation can be measured by a variety of tests, amongst which also the p-Anisidine test. Determining the anisidine

value is a sensitive tool to evaluate the concentration of aldehydic and ketonic compounds that are formed in the fat-containing product due to lipid oxidation and that cause the undesired rancidity (Dubois, Van de Voort, Sedman, Ismail & Ramaswamy, 1996; Talbot, 2016). Even though the method is comparatively time intensive and needs to be performed in a very precise manner it is also very simple. Another downside is that the chemicals used are relatively toxic (Dubois et al., 1996). The value can be obtained by means of measuring the absorbance at 350 nm (AOCS, 2020b). For this however, the fat needs to be extracted from the sample (Yang & Boyle, 2016).

#### **2.3.4 Oxidative Stability Test (Rancimat)**

To perform the Rancimat test, specific equipment is needed. The basic system of the test is that oil is exposed to a hot airflow in the Rancimat equipment and by this oxidation is initiated (Farhoosh, Niazmand, Rezaei & Sarabi, 2008). The outgoing air is then going through a vessel filled with deionized water in which the conductivity is measured. The conductivity changes when volatiles formed during the oxidation process enter the water (AOCS, 2020c). According to Metrohm (2020) the Rancimat evaluates the oxidative stability of the sample (natural oils and fats). And also, fat-containing food products or cosmetics can be examined with this method.

#### **2.3.5 Test used in this Research**

Due to the limited time and availability of equipment during this thesis project it was determined that the TBARS assay was the method most suitable to evaluate the extent of lipid oxidation in the presented IF. The measurement is fast (since no oil needs to be extracted) and considered more meaningful due to its power to determine secondary reaction products that are more correlated to the rancid off-flavors than primary reaction products.

## **2.4 Shelf-life Determination**

*Shelf-life can either be determined from the perspective of the consumer or from the product (Robertson, 2005, p. 331). Having the product in focus, a deterioration in time is followed, such as e.g. quantifying the number of microbes present, decrease in desired compounds as well as the*

*increase/production of undesired compounds. With the consumer in focus the determination of shelf-life is based on the time a consumer would still accept the product vs. the time when the product is regarded as unacceptable. During this study the shelf-life will be determined from the product side. Shelf-life is based on so-called indices of failure (IoFs), which are the quality attributes that determine whether the food product is still acceptable or not. There can be a single IoF or several, depending on the food product (Robertson, 2005, p. 332). IoFs can be attributes such as the emergence of rancid off-flavors (oxidation), loss of color or carbonation, caking in powders or loss of texture due to moisture increase. When the IoFs have been determined an upper limit can be set to have a cutoff point at which the product is considered unacceptable (Robertson, 2005, p. 333). The cutoff point can be taken as the upper limit for instrumental measurements, after which quality goes down.*

#### **2.4.1 Accelerated Shelf-life Tests**

According to Robertson (2005, p. 534) to be able to perform accelerated shelf-life tests (ASLTs) it must be assumed that all reactions occurring in the food product are following kinetic principles. This is, because external factors are altered and controlled in a way to influence the reaction such that it proceeds faster than normal. Elevating external factors such as e.g. temperature will lead to a product that reaches its end of shelf-life much faster than under normal storage conditions. ASLTs are a very useful tool when it comes to predicting the shelf-life of foods in the case of altering e.g. the formulation or packaging, since the evaluation of the effect would, under normal conditions, take a long time (here 24 months). Usually they are used to determine which of the tested products/materials should be further assessed in a study with the time frame of the actual shelf-life.

The common procedure in ASLTs is as follows (Robertson, 2005, pp. 356-257):

1. Determine the quality parameters of the product.
2. Determine the IoFs that will end the product's shelf-life.
3. Select the package the product should be stored in.
4. Determine the extrinsic factors that will be elevated (e.g. temperature).
5. Select the time for the ASLT to last.
6. Select the frequency when the tests should be performed.

7. Calculate the amount of packages that need to be stored at the selected storage condition and include references (controls).
8. Evaluate the date while starting with the ASLT, to be able to adjust the frequency of the tests if needed.
9. Of all the test results determine the rate constant and shelf-life and use appropriate shelf-life plots to estimate the shelf-life of the product under normal storage conditions.

Typically, temperatures that are used during ASLTs are between 30°C - 40°C. This is, because above 40°C reactions often are not representative for those occurring under normal storage conditions anymore (Robertson, 2005). In the case of the IF, it is also necessary to stay below 40°C to try to keep the fat from becoming liquid.

#### **2.4.2 Oxygen Transmission Rate**

The oxygen transmission rate (OTR) is a means of describing how much oxygen (volume) is able to enter a package via the packaging material per square meter of material and day (Kerry & Tyuftin, 2017) as well as other units (e.g. cc/m<sup>2</sup>/day) can be used.

The OTR can be taken as a reference when comparing different packaging materials. Furthermore, due to specific packaging requirements per product the OTR gives an indication if the requirements will be able to be met or not.

In this study the OTR was determined for the flat material. There is a different permeation rate in the flat material compared to entire cans. This is because there is also permeation through seals formed during making the packaging. According to Reinas et al. (2016) the thermoformed seals can cause up to 25% of the total permeation of oxygen and moisture in a package. Due to the limited time frame however only the OTR values of the flat material were determined.

#### **2.4.3 Moisture Vapor Transmission Rate**

The moisture vapor transmission rate (MVTR) is the mass of moisture vapor that is able to penetrate through the packaging material into the package in grams per square meter per day (Kerry & Tyuftin, 2017).

Like the OTR it is measured for the flat material only, however having higher impacts in seals.

#### **2.4.4 Residual Headspace Oxygen**

Andersson (1995) describes that there are three possible parts where oxygen can be found: in the product itself (absorbed oxygen), in the headspace above the product and permeating through the packaging material.

The residual headspace oxygen can be measured by means of e.g. a Dansensor. The apparatus has a needle that can be inserted into the package without opening it and a sensor inside the apparatus measures the amount of O<sub>2</sub>, CO<sub>2</sub> and shows the balance of the two. When the level of O<sub>2</sub> is above 2% the package is considered to not be gas-tight any longer due to faulty seals, long storage times or that there have been problems during application of modified atmosphere (MA) leading to higher O<sub>2</sub> levels in the can. Since the measurement is slightly destructive, it must be performed right before measuring the TBARS assay, so no impact of the ingress in O<sub>2</sub> through the small hole is detectable in the powder.

## **2.5 Packaging System**

A packaging system is made up of three levels (see figure 8): the primary packaging, the secondary packaging and the tertiary packaging. The primary packaging is in direct contact with the product contained, whereas the secondary packaging usually contains a number of primary packages. The tertiary packaging often is a pallet or roll container containing a number of the secondary packages (Hellström & Saghir, 2007).

During this degree project focus will be on the primary packaging only, since only the material of this packaging level changes, albeit not the shape or dimensions of the package that would impact the secondary or tertiary packaging (to a larger extent).

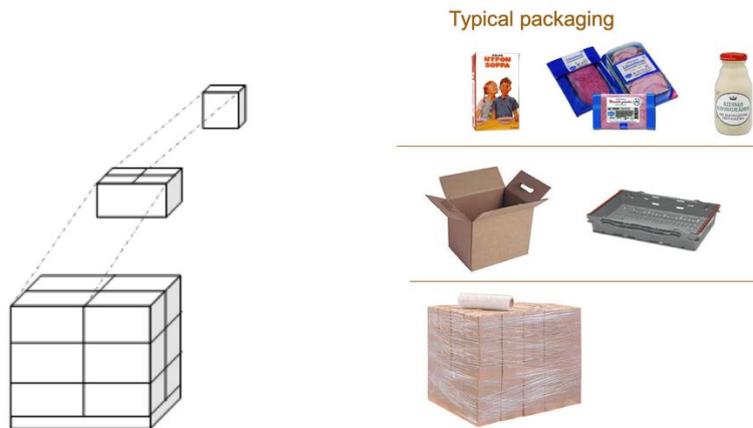


Figure 8: Packaging system with examples of packaging (Pålsson, 2018).

## 2.6 Packaging Functions

Packaging has several functions it needs to fulfill, such as the four most common ones: containment, convenience, protection and communication named by Robertson (2005, p. 2). According to Krochta (2006), containment describes the capability of the package to withstand any changes during all processing steps while containing the product and its integrity. Convenience on the other hand is a factor comprising everything that makes the package more user-friendly, that is e.g. reclosability, several sizes, etc. Protection implies that the product is kept safe during the entire manufacturing after filling as well as distribution. It includes not only physical damage prevention, but also the prevention of contamination or provision of barrier properties. Communication comprises the interaction between the producer and the consumer by means of e.g. the ingredient list, origin of the product, etc. But not only that. Packaging also needs to fulfill requirements given by the entire supply chain, including among others logistical effectiveness, handleability, stackability, etc. (Hellström & Saghir, 2007). Next to these essential packaging functions, there are thus many more. These functions are not further investigated during this study, since the package itself would not change (only the material) and thus also none of the functions evaluated with the current package would deviate.

## 2.7 Packaging of Infant Formula

According to Codex Alimentarius Commission (2007) “the product (IF) shall be packed in containers which will safeguard the hygienic and other qualities of the food”. Furthermore, the packaging can contain nitrogen or carbon dioxide, while it needs to be made sure of that the materials used for the packaging are made of compounds that are allowed to be in food contact and safe for the consumer (Codex Alimentarius Commission, 2007).

Walsh (2014) states that the packaging needs to be able to prevent light exposure, and have a very low moisture, moisture vapor and oxygen permeability. Next to this, it needs to be made sure of that all seals are tight so there is no permeation through them. Furthermore, the package should be rigid enough, so it can be stacked, and the product is protected during all stages in the supply chain.

Powdered IF is currently packed in either plastic pouches or metal and composite fiber cans (An, Lee & Lee, 2018b). They are able to protect the product and prolong the shelf-life due to good gas barriers. These are packed under MA of either nitrogen or carbon dioxide, which can help to protect the product from lipid oxidation and prolong the product’s sensory quality. Often nitrogen is preferred, since it is an inert gas, meaning it will not interact with the food, which carbon dioxide on the other hand will do (Wang, An & Lee, 2017).

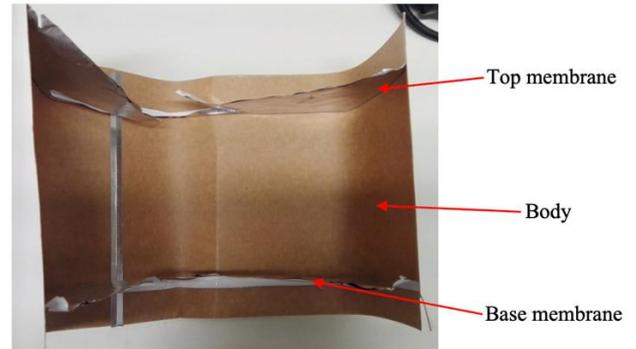
Wang, An and Lee (2017) further describe what also needs to be considered when choosing the right packaging is that the place of production might differ from the place of selling in e.g. altitude. The altitudinal difference can then lead to pressure changes in the package that can cause it to either implode or burst.

For this study a packaging was used like the one seen in figure 9 and 10. It was made of several different individual parts. It has a body that is formed into a sleeve and sealed with a tape. Then it is formed to a specific shape followed by the addition of a membrane. The package is then filled by the product (base filling) and in the end a base (in this case another membrane) is sealed to the body to close it.

This package is a gas-tight carton solution for sensitive products, including powder products such as IF.



**Figure 9: Example of one of the cans made to store the IF.**



**Figure 10: Example of a can cut open lengthwise (no aluminum in body).**

## 2.8 Common Packaging Materials

*There are many different materials available to be used as a packaging material. Which one is chosen is very dependent on the product the package needs to contain. The material used by itself can have an immense impact on the shelf-life of the product due to e.g. the ability to withstand specific forces or providing necessary barrier properties. The most common materials that are used in the packaging industry are discussed below.*

### 2.8.1 Plastics

Plastic is the material used in most packaging of food products. The performance of a plastic is very dependent on the physical and chemical polymer properties. The molecular weight, extent of crystallinity, molecular structure as well as composition determine these properties and are also affecting the polymer density and transition temperature (Robertson, 2005, p. 12).

A polymer can be characterized as either many repeating units (monomers) or a long chain. They can be classified as homopolymers (consisting of the same repeating unit) or heteropolymers (consisting of differing units) (Robertson, 2005).

#### 2.8.1.1 Polyethylene (PE)/Polypropylene (PP)

Plastics originating from either ethylene or propylene are called polyolefins (oil-forming plastics). This group of polymers is very important in the thermoplastic industry (Robertson, 2005, p. 20). They include low and very low, as well as linear, medium and high-density PEs and propylenes.

##### 2.8.1.1.1 Low Density Polyethylene (LDPE)

Robertson (2005, p. 21) states that this polymer is used in blow molding and film applications for food packaging and one of the most common materials. It can either be petroleum or bio based. Its character to preferably form branches prevents the polymer to pack closely, leading to its low density and comparatively low molecular weight. The material is tough and can be used in blow extrusion to make e.g. tubular films (translucent appearance), as well as it can be used in extrusion applications using slit dies or chill-roll casts which can produce clear films. Performing well upon applied forces (good tensile, burst and tear strength and impact resistance) even at very low temperatures (-60°C) makes it useful in a wide range of applications. While preventing moisture and moisture vapor to permeate into the package very well, its ability in keeping oxygen and other gases out is rather low. It is however very stable against acids, alkalis and inorganic solutions. Oils and greases on the other hand are substances that the material is sensitive to and that can cause the so-called environmental stress cracking (ESC).

##### 2.8.1.1.2 High Density Polyethylene (HDPE)

HDPE is in comparison to LDPE a linear polymer (only few short-branched chains), with nonpolar, thermoplastic properties (Robertson, 2005, p. 23). The material can be classified into the similar strength group as LDPE, performing well at low temperatures. However, the bursting and tensile strengths are, compared to LDPE, higher, while the tear and impact strengths are lower than those of LDPE. With LDPE being weak against oils and greases HDPE has advantageous properties, performing well against chemical substances as well (Robertson, 2005, p. 24). While offering a very good moisture barrier and better gas barrier than LDPE, its appearance is rather opaque and by this not applicable for all situations. The heat-sealing ability of HDPE is, compared to LDPE, rather low. HDPE is often used in blow molding of bottles, which however is competed by, and more and more replaced by, polyethylene terephthalate (PET), having better barrier properties.

#### 2.8.1.1.3 Polypropylene (PP)

According to Robertson (2005, p. 25) PP is a linear polymer with lower density than PEs, however, a higher softening point. PP provides a good barrier against moisture vapor transmission, however only a medium barrier for gas permeation. Regarding its stability against chemical substances, it can be said that it is comparatively resistant, also against oils and greases. Next to this, it is also very temperature resistant (high temperatures), which makes it a good choice in e.g. microwavable packaging and comes with high clarity. These factors make the material perfect for reverse printing. PP is usually used in blow or injection molding applications, often to produce closures for PET, glass and HDPE bottles. At low temperatures (<0°C) the material becomes however brittle (Robertson, 2005, pp. 25-26). Nevertheless, it is necessary to add antioxidants into all PP compounds due to its low oxidative resistance at higher temperatures. PP is a versatile polymer that commonly is used in thermoformed sheets and films and bags (cast formed) and often used for products that need to be sterilized in an autoclave at high temperatures (company internal information). When packing sharp or puncturing products PP might not be the best choice or should be supported by another material to perfectly contain and protect the product. (Robertson, 2005, pp. 25-26)

#### 2.8.1.1.4 Oriented Polypropylene (OPP)

By orienting PP in different ways, a wide range of properties can be obtained (Robertson, 2005, p. 26). OPP often is recognized by its stiff appearance with crinkling noises. It comes with no considerable gas barrier, however, often is coated with another material to make up for this downside.

#### 2.8.1.1.5 Ethylene-Vinyl Alcohol (EVOH)

EVOH has a very high gas barrier, however its barrier to moisture transfer is very low (Robertson, 2005, p. 28). The application of EVOH in many packaging solutions replaced many glass or metal containers on the market. EVOH comes with a very high crystallinity, making their properties very dependent on the copolymer used in the packaging. In general, a high fraction of ethylene in the packaging leads to lower gas barriers but better moisture barriers and vice versa. In the right combination of ethylene and VOH the material is often used in MA packed products, since it is very good in keeping the atmosphere at the same level for a long time (Robertson, 2005, p. 29). EVOH is also resistant to organic vapors and oils. However, the more polar the substance, the lower the resistance. Usually, EVOH gets included in a multilayer material where both sides are supported by a material with good moisture barrier. Common packaging containing EVOH are bottles, bowls,

tubes and trays, flexible films or paperboard cartons for the beverage industry.

#### *2.8.1.2 Polyesters*

Polyesters are based on carbon-oxygen-carbon links in which one of the carbons is included in a carbonyl group (Robertson, 2005, p. 35).

##### *2.8.1.2.1 Polyethylene Terephthalate (PET)*

Robertson (2005, p. 35) states that PET is a linear polymer with transparent thermoplastic characteristics. It is a stiff and strong material, can however be oriented upon stretching during extrusion and molding applications, increasing the stiffness and strength even more. In bottles and films mostly, amorphous PET is used (transparent), whereas in many containers crystalline PET is used instead (opaque white). PET films have high tensile strength and are stable against chemical exposure. They are elastic, usable over a wide range of temperatures and a light-weight option (Robertson, 2005, p. 36). This is why PET is often used in pouches used for boiling the food in the package. To improve the barrier properties, PET is often combined with a copolymer such as LDPE or even metallized. Robertson further notes that PET bottles used for carbonated beverages are prone to ESC, starting at the outside of the bottle and proceeding inward.

##### *2.8.1.3 Polyamides (PA)*

PAs can be seen as linear thermoplastics with amide linkages that provide mechanical strength as well as specific barrier properties (Robertson, 2005, p. 39). PA films with more than six carbon chains tend to have higher moisture vapor transfer resistance. The longer the aliphatic chain in the polymer, the more the melting point is reduced, as well as the moisture absorption and tensile strength, whereas the elongation and impact strength increase (Robertson, 2005, p. 41). PA films have high stability against high temperatures, are still flexible at low temperatures and are stable against alkalis and weak acids. Stronger chemicals however have negative impacts on the PA. There is no moisture barrier, due to the PA being polar itself. Also, the gas barrier is considerably low and needs to be compensated for by copolymers (Robertson, 2005, p. 42). However, in packages for cheeses the lower gas barrier can be desired to give the cheese room to breathe (company internal information).

An overview of the OTR and MVTR (WVTR) values of the most common plastic polymers can be seen in table 3. Note however, that there is no information about the thickness of the materials, which can lead to different values when used in very thin films.

**Table 3: OTR and MVTR (WVTR) values of most common plastic films (Robertson, 2005, p. 118).**

Polymer (25 $\mu\text{m}$ Thick)	OTR	WVTR	
	(23°C/0% RH) $\text{mL m}^{-2} \text{ day}^{-1}$	(38°C/90% RH) $\text{g m}^{-2} \text{ day}^{-1}$	(25°C/75% RH) $\text{g m}^{-2} \text{ day}^{-1}$
Low density polyethylene	7400	12.50	4.00
High density polyethylene	1600	3.70	1.45
Polypropylene (cast)	3040	8.20	3.30
Polypropylene (oriented)	1550	5.00	1.35
Polypropylene (acrylic-coated)	1200	4.60	1.80
Polypropylene (oriented and metallized)	35	1.00	
Poly(vinyl chloride) (rigid)	120	32.00	12.00
Poly(vinyl chloride) (oriented)	27	17.50	7.00
Poly(vinyl chloride) (plasticized)	190–3100	85.00	32.70
PVdC/PVC copolymer	1.25–14.5	0.6–3.20	0.25
Poly(ethylene terephthalate)	55	20.00	7.00
Poly(ethylene terephthalate) (PVdC/PVC copolymer-coated)	8.00	8.50	3.40
Poly(ethylene terephthalate) (metallized)	0.65	1.00	0.40
Polyamide (nylon) 6	40	280	80–110
Polyamide (nylon) 6 (oriented)	18	130	28
Polyamide (nylon) 6,6	35	90	15–30
EVOH copolymer (32% ethylene)	0.2	80.0	32.0
Cellulose film MXXT/A (PVdC/PVC copolymer coated)	8.75	8.60	3.40

## 2.8.2 Metals

In general, in food packaging the following four metals are used: aluminum, steel and chromium. Of these, chromium and steel, as well as steel are often used as composite materials (i.e. chromium-coated steel). The focus will however be on aluminum and metallized films.

### 2.8.2.1 Aluminum

Aluminum is made of alumina or aluminum oxide, which in nature can be found as corundum, gibbsite, diaspore and bauxite. Of these, bauxite is the major natural resource. It must however be purified to obtain aluminum, since it also contains silica, titanium oxide and other iron oxides. The procedure commonly used to purify bauxite is called the Bayer process, in which alumina is converted to  $\text{Al}(\text{OH})_3$  by means of heating it to  $175^\circ\text{C}$  in  $\text{NaOH}$ . Insoluble compounds are filtered off. When heating the precipitate to

980°C the solids decompose into alumina (Robertson, 2005, p. 195). Most often aluminum is used in cans as a packaging material, can however also be part of laminates or used as aluminum foil (Robertson, 2005, p. 209).

#### *2.8.2.2 Metallized films*

Metallized films are often preferred due to their good barrier properties. When the polymers used for a packaging have rather low barrier properties, while a high barrier is needed (e.g. very sensitive products), a metallized film (thin metal layer vacuum deposited) can decrease the permeability and guarantee the barrier that is needed (Revell, 1991; company internal information).

### **2.8.3 Paper**

Paper can be divided into two groups: fine papers and coarse papers. The first is usually made of bleached pulp and used as writing paper, for books and the like. Coarse paper is however made of unbleached Kraft softwood pulp and used in paper packaging. Paper material has a machine direction (MD) and a cross direction (CD), which always has to be indicated when performing tests. One can say that variation in paper properties is increased in the CD compared to the MD (Robertson, 2005, p. 181).

#### *2.8.3.1 Paperboard*

Paperboard and other paper materials basically do not provide any barrier to gases or water vapor. They are commonly combined with other materials to be able to provide a barrier to the product packed inside. This however leads to a more complex recycling process, since the paper material has to be separated from any of the other materials so it can be reused for new paper material. Starting from a grammage of 250 gsm ( $\text{g/m}^2$ ) upwards paper is called paperboard. The material can either be single-ply or multi-ply, with the latter being separated by so-called web plies (mainly used for rigid boxes or beverage cartons). Since the material is either not in direct food contact anyway or laminated the pulp used can be recycled material (Robertson, 2005, pp. 178-182). This is however not accepted by all companies, due to the possibility of mineral oil contamination by printing inks (company internal information). When combined with aluminum foil or EVOH or SiO<sub>x</sub>-coated PET the material can provide necessary barrier and extend the shelf-life of the food contained (Robertson, 2005, p. 185). According to Tu & Apt (2013) paperboard has the ability to block light to a sufficient extent (the thicker the better the protection), so it cannot cause lipid oxidation. Since

solid foods are less prone to oxidize due to light (2.1.3 *Deteriorative Factors*) it is assumed that the paperboard used during this study can provide sufficient light barrier.

## 2.9 Barrier Properties

*According to Robertson (2005, p. 98) the barrier properties observed for plastics can be summarized by the ability to withstand diffusion and sorption of compounds (e.g. gases and aroma or flavor compounds). Robertson states two different phenomena: the pore effect, where gases and vapors go through micropores or fractures in the material, and the solubility-diffusion effect, where gases or vapors dissolve on one side of the polymer to diffuse through it and evaporate on the other side (true permeability). A good barrier implies a low permeability of substances.*

### 2.9.1 Transmission Rates

Very often material that is used as packaging material is not homogenous but consists of a variety of materials (e.g. coated or laminated materials). An equation to calculate the transmission rate can be seen below (Robertson, 2005, p. 117).

$$TR = \frac{Q}{At} \quad (1)$$

Where

Q is the amount of permeant passing through the polymer

A is the area

t is the time

In general, it can be said that doubling a material will lead to halving the permeability of a substance (Robertson, 2005, p. 117). One can divide the transmission rate for different compounds, in this case the focus will be on the OTR and the MVTR. The equation however does not include the thickness of the material nor the pressure or concentration of the permeant, even though these parameters are very important to be considered.

Common OTRs and MVTRs (WVTR) can be seen in table 3 in section 2.8 *Common Packaging Materials*. Albeit, note that the RH and temperature used to determine them might not be the most suitable for all applications.

## 2.10 Barrier Materials

Figure 11 shows an overview of OTR and MVTR values necessary for different food groups as well as it presents some materials offering such barriers.

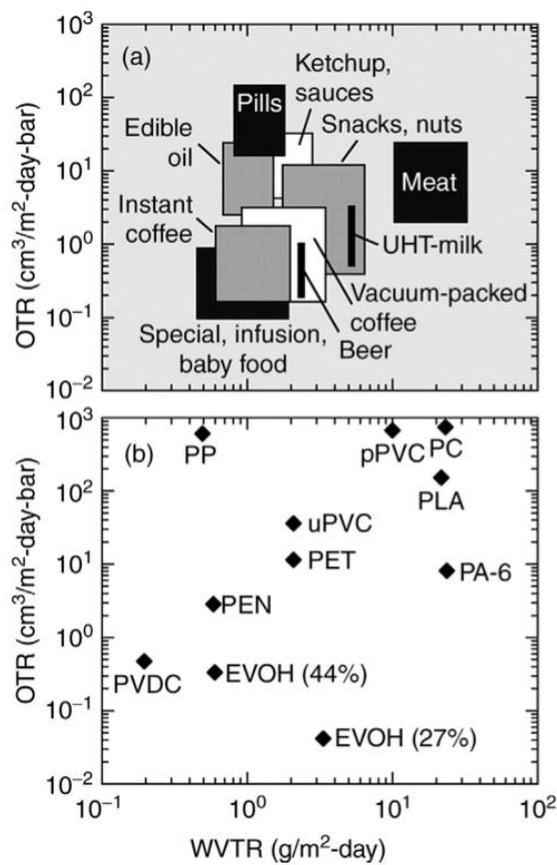


Figure 11: OTR and WVTR values for different food products and packaging materials (Soltani & Spontak, 2017).

It can be seen that IF requires an oxygen barrier with OTR values and a moisture barrier with MVTR values below 1. With this respect, the lower the moisture and oxygen transmission the better.

Other typical materials used to provide barriers in the packaging industry can be seen in figure 12.

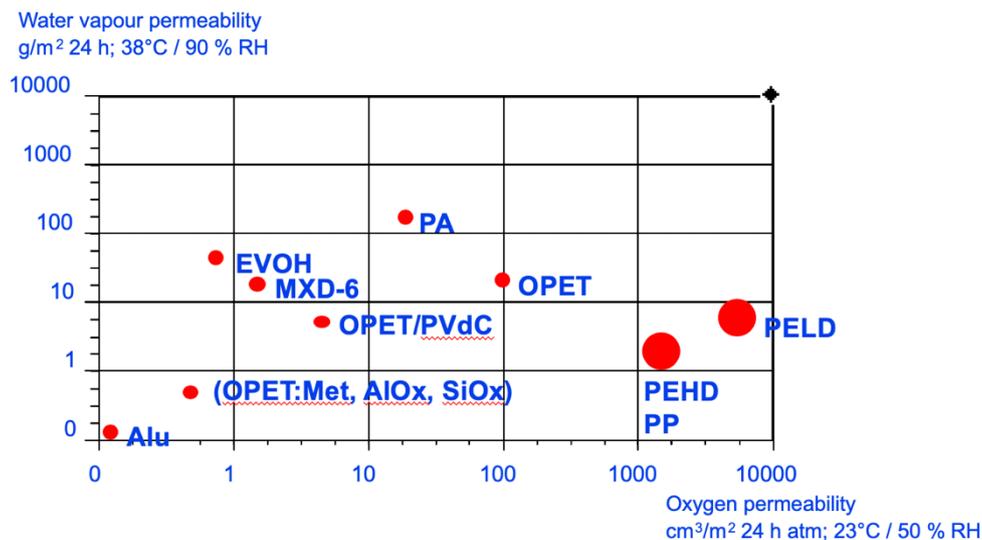


Figure 12: Barrier properties of different materials (with permission from AR Packaging); with barrier thicknesses typical in flexible packaging.

### 2.10.1 Aluminum

Aluminum foil is usually made in thicknesses of 4-150  $\mu\text{m}$  and either used as such or as a laminate with paperboard for e.g. folding cartons. The foil is impermeable to gases and water vapor if its thickness exceeds 15  $\mu\text{m}$ . At lower thicknesses it is permeable by means of pinholes. As an example: a film of 12 $\mu\text{m}$  thickness has an MVTR of less than 0.01  $\text{g}/\text{m}^2/\text{day}$ , whereas a film of 8-9 $\mu\text{m}$  thickness has an MVTR of 0.07 to 0.1  $\text{g}/\text{m}^2/\text{day}$ . These barrier properties are much better than those of e.g. polymers. Since aluminum foil is often combined with polymers, the effect of pinholes is usually very low. Aluminum can also function as a light barrier. (Robertson, 2005, p. 209)

As can be seen in figure 12, the OTR and MVTR of a 6.35 $\mu\text{m}$  thick aluminum foil (coated with PE on both sides) both are at around 0.1  $\text{cm}^3/\text{m}^2/\text{day}$  and  $\text{g}/\text{m}^2/\text{day}$  respectively.

Due to its high barrier properties aluminum is the material most commonly used in laminate materials such as in the packaging for IF.

### 2.10.2 OPET/OPET:Met

According to Sargeant (2006) OPET (12 $\mu$ m) has an OTR value of 6 cm<sup>3</sup>/100 in<sup>2</sup>/day (about 93 cm<sup>3</sup>/m<sup>2</sup>/day) and an MVTR value of 1.9 g/100 in<sup>2</sup>/day (about 29.5 g/m<sup>2</sup>/day). When the barrier necessary for the packaging needs to be even higher, often the plastic is coated with a metal film (vacuum deposited aluminum layer usually with a thickness of 100-300 Å (company internal information), resulting in OPET:Met. The resulting OTR and MVTR values (at 12 $\mu$ m) are 0.1 cm<sup>3</sup>/100 in<sup>2</sup>/day (about 1.55 cm<sup>3</sup>/m<sup>2</sup>/day) and 0.1 g/100 in<sup>2</sup>/day (about 1.55 g/m<sup>2</sup>/day) respectively.

### 2.10.3 AlO<sub>x</sub>

Hirvikorpi, Vähä-Nissi, Mustonen, Iiskola & Karppinen (2010) state in their study that Al<sub>2</sub>O<sub>3</sub> (<25 nm thick) was found to have an MVTR of 0.001 g/m<sup>2</sup>/day when combined with polymers. They note OTR values for polymers coated with Al<sub>2</sub>O<sub>3</sub> at different thicknesses. PP will then have an OTR of 170 cm<sup>3</sup>/m<sup>2</sup>/105 Pa/day at a coating thickness of 25 nm, whereas it will be 109 cm<sup>3</sup>/m<sup>2</sup>/105 Pa/day at a coating thickness of 50 nm.

### 2.10.4 SiO<sub>x</sub>

According to Grüniger and von Rohr (2004) amorphous SiO<sub>x</sub> is more and more used as an oxygen/gas barrier layer in packaging solutions. It is mostly vacuum deposited on oriented films like OPET or OPP (company internal information). Usually, films used are about 10-100 nm thick, which decrease the ingress of gas immensely. In the case of oxygen, SiO<sub>x</sub> can present a barrier improvement factor (BIF) of 20-100. Hedenqvist and Johansson (2003) state that the effect of a low permeability to gases, which SiO<sub>x</sub> creates, is however not yet fully understood. It could be due to the solubility or the diffusion ability of oxygen and other gases. Both studies (Grüniger & Rohr, 2004; Hedenqvist & Johansson, 2003) note that in case of higher gas permeability this is due to material defects mainly. As can be seen in figure 12, both the OTR and MVTR values of SiO<sub>x</sub> lie somewhere below 1 cm<sup>3</sup>/m<sup>2</sup>/day and g/m<sup>2</sup>/day respectively.

### 2.10.5 MXD-6

MXD-6, which is a young polymer, is made from meta-xylylene diamine (MXDA) and adipic acid by means of a poly-condensation (Watanabe, 1987).

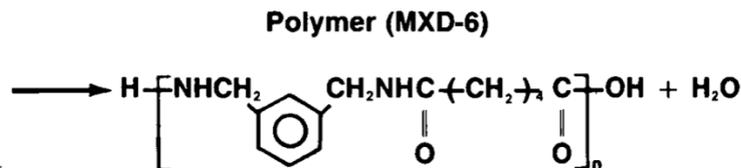


Figure 13: Chemical structure of MXD-6 (Watanabe, 1987).

According to Watanabe (1987) MXD-6 has a water absorption of 2.5% at 23°C and a RH of 65%. The OTR is at 0.06 cc.mm/(m<sup>2</sup>/day/atm) at 20°C and 0% RH. The common thickness of a film is between 5-15µm (company internal information).

### 2.10.6 EVOH

KURARAY (n.d.) is a manufacturer of EVOH (EVAL™) films that states the barrier property against gases is very dependent on the thickness of the EVOH layer. If the thickness of the layer is doubled, the permeability is halved. The material is however very poor in being a barrier against moisture, since it is hygroscopic (takes up water). Compared to HDPE, which has an MVTR value of 5 g.30µm/m<sup>2</sup>/day at 40°C and 90% RH, the EVOH films ranged from 20 to 85 g.30µm/m<sup>2</sup>/day under the same conditions. This means that EVOH in general is only used to decrease the gas permeability and not the moisture permeability. This also implies that the EVOH needs to be kept dry by means of surrounding layers with good MVTR barriers (company internal information).

### 2.10.7 PA

According to Lange & Wyser (2003) PA has an oxygen permeability of 0.1 to 1 cm<sup>3</sup>.mm/(m<sup>2</sup>/day/atm) at 23°C and 50% vs. 0% RH respectively. The water vapor permeability is at 0.5-10 g.mm/(m<sup>2</sup>/day) at 23°C and 85% RH. For further information see section 2.8.1.3 *Polyamides (PA)*.

### 2.10.8 PEHD

PEHD is another description of high-density polyethylene. It has an OTR value of 1600 ml/m<sup>2</sup>/day at 23°C and 75% RH and an MVTR value of 1.45 g/m<sup>2</sup>/day at 25°C & 75% RH, as can be seen in table 3 in section 2.8 *Common Packaging Materials*.

### 2.10.9 PP

As can be seen in table 3 in section 2.8 *Common Packaging Materials*, PP on its own does not provide an oxygen barrier (OTR of 3040 ml/m<sup>2</sup>/day at 23°C and 0% RH) as well as a rather low moisture barrier (MVTR of 3.3 g/m<sup>2</sup>/day at 25°C and 75% RH).

### 2.10.10 PELD

PELD is another term to name low-density polyethylene (LDPE). Its OTR and MVTR values are 7400 ml/m<sup>2</sup>/day at 23°C and 75% RH and 4.00 g/m<sup>2</sup>/day at 25°C and 75% RH respectively (table 3 in section 2.8 *Common Packaging Materials*).

## 2.11 Processing of Polymers

### 2.11.1 Oriented films

The most common films that can be oriented films are made of OPP, OPET and OPA. They are very thin and clear and provide high strength. The method is basically orienting the mechanical properties of the material and leads to improved barrier properties due to the crystallization of the polymers. The films can be used as such or be included into laminates (Halldén Björklund, 2019). The process of making an oriented film can be seen in figure 14. The resin is inserted into an extruder that leads the material through a so-called T-die. Here, the material is becoming flat and stretched between several rollers in machine direction. In the next section of the machine the material is stretched in transverse direction and rolled onto a winder (tenter frame process).

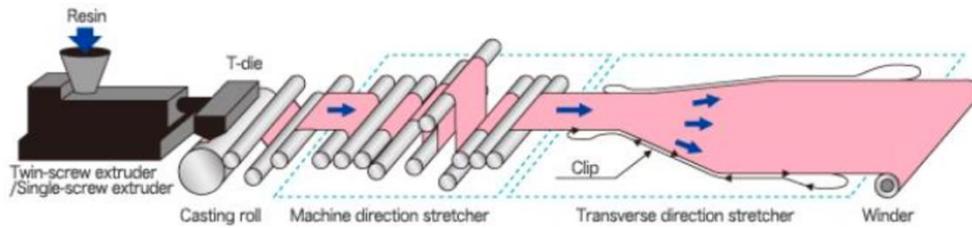


Figure 14: Oriented films processing line (Halldén Björklund, 2019).

#### 2.11.1.1.1 Extrusion

There are different kinds of extrusion, of which the following will be explained: co-extrusion and tandem extrusion.

##### *Co-extrusion:*

Co-extrusion means that two molten polymers will be extruded together to be combined in one film. The procedure is as follows (see figure 15 from left to right): The material (paper) gets unwinded and coated by a polymer (extrusion coating). Another material (aluminum foil) is added (extrusion lamination) after which two polymers get co-extruded onto the material. By means of tandem extrusion the next material (PE-film) is adhered to the layers.

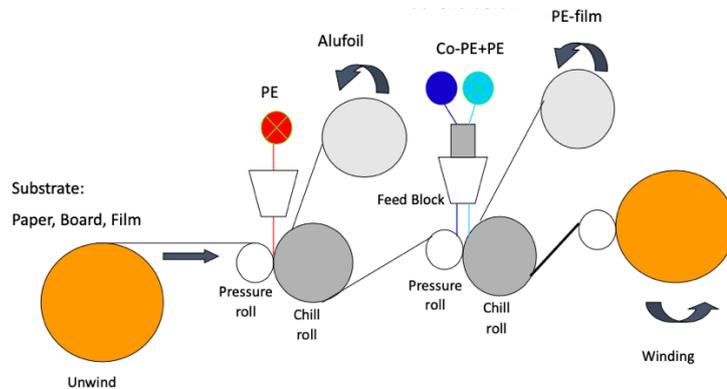


Figure 15: Co-extrusion process (Halldén Björklund, 2019).

##### *Tandem extrusion:*

Following the same system as co-extrusion, a number of materials can be added to the already combined materials, resulting in a multilayered material.

## 2.12 Sealing Techniques

The sealing technique used by AR Packaging is the so-called induction sealing. For induction to be able to work a conducting material (usually a metal) is needed in at least one of the components to be sealed. This is one reason why aluminum is part of the can used for IF. Upon induction heating the aluminum creates heat as soon as it is exposed to a field with alternating electrical current (Matsen, Mittlieder, Privett & Dabelstein, 2003). Induction sealing is often used to seal packages with polymer laminated paperboards along the longitudinal side. This heat causes the polymer layer of the packaging material on the two sides to be sealed to melt and form a tight seam (Robertson, 2005, p. 282). This technique is also used by companies like Tetra Pak in their aluminum containing packages. The frequency of the electrical current determines how much heat is created. Usually, lower frequencies enable an evenly distributed heat field that makes a tight seal (Robertson, 2005, p. 282). An example of an induction sealing set-up can be seen in figure 16.

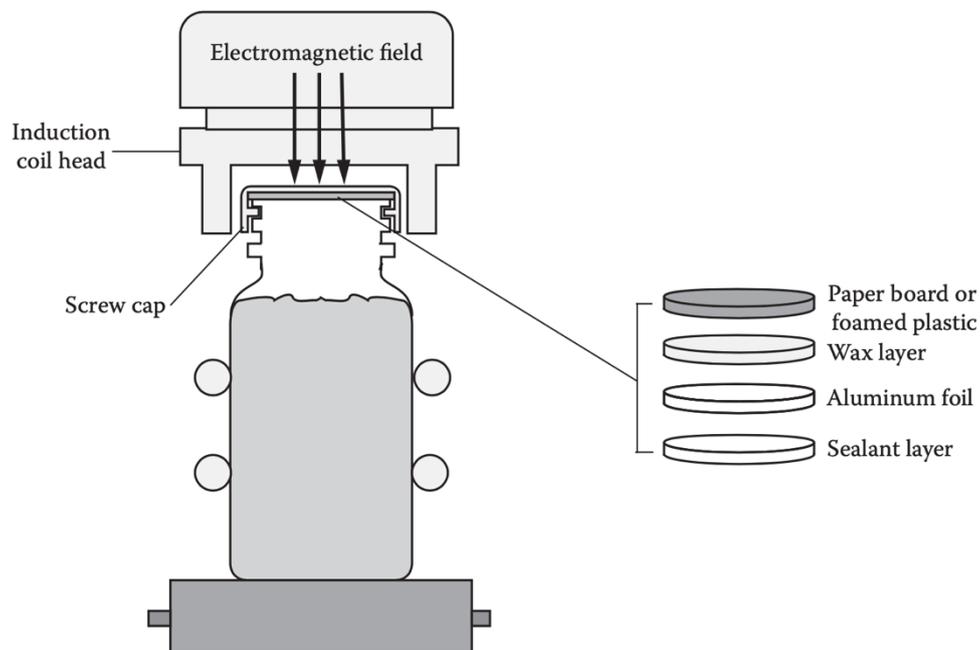


Figure 16: Example of a set-up for induction sealing (Robertson, 2005, p. 282).

## 2.13 Recyclability of Materials

Currently the packaging is difficult to be fully recycled, due to its special structure. There are however several initiatives for recycling of the so-called used beverage cartons (UBC), consisting of exactly the same material, however varying in amounts of each material. According to Ozer, Yuksel, Comert & Guler (2013) the special recycling of UBCs can help to decrease loss in energy, waste production, and use of landfills, while it also means that less raw material has to be used due to better recycling. Ozer et al. (2013) further state that the secondary production (recycling) of aluminum only uses 5% of the energy that primary aluminum production consumes.

Beverage cartons consist in general to about 75% of paperboard, 21% of polymers and 4% of aluminum, according to ACE (2020), one of the organizations that implemented initiatives for the recycling of UBCs. By means of re-pulping (separation of paperboard from layers not consisting of fibers) paperboard used in these beverage cartons can be fully recycled. How the polymer and aluminum fractions are recycled is very dependent on the country the beverage cartons are recycled in (Elopak, 2020). The recycling of UBCs can be seen in figure 17.



Figure 17: Recycling of UBCs (ACE, 2020).

Even though the composite cans used to pack IF are not containing liquid beverage products the material structure is very similar.

However, when changing the material to a non-aluminum material the recycling of the material is very dependent on the recycling system present in each country. Recycling in the sense of re-using the material is a more favorable option compared to incineration, which would mean that in this case the change in material could actually decrease the recyclability of the package. An option to prevent this from happening is to tailor the packaging material such that the best recycling is achieved in the country it is used in. (Information from AR Packaging)

## 3 Materials & Methods

*This chapter gives an overview of the materials used during the degree project, as well as which methods were used to perform the research.*

### 3.1 Materials used

#### 3.1.1 Infant Formula

The IF used during this degree project was a sample batch from one of the customer companies of AR Packaging. The nutritional declaration was as shown in table 4.

**Table 4: Nutritional declaration of sample IF.**

	<b>Per 100 g of powder</b>	<b>Per 100 ml prepared milk</b>
<b>Energy</b>	1970 kJ (469 kcal)	262 kJ (62 kcal)
<b>Fat</b>	20 g	2.6 g
<b>Of which</b>		
<b>Saturated fat</b>	6.4 g	0.9 g
<b>Mono-unsaturated fat</b>	8.3 g	1.1 g
<b>Poly-unsaturated fat</b>	3.8 g	0.5 g
<b>Carbohydrates</b>	63 g	8.3 g
<b>Of which</b>		
<b>Sugar</b>	43 g	5.8 g
<b>Protein</b>	10 g	1.3 g
<b>Salt</b>	0.45 g	0.06 g
<b>Vitamin A</b>	459 µg	61,0 µg
<b>Vitamin D</b>	12 µg	1.6 µg
<b>Vitamin E</b>	6.2 mg	0.82 mg
<b>Vitamin K</b>	33 µg	4.4 µg

<b>Vitamin C</b>	80 mg	11 mg
<b>Thiamine</b>	0.49 mg	0.065 mg
<b>Riboflavin</b>	0.87 mg	0.12 mg
<b>Folate</b>	54.9 µg	7.30 µg
<b>Vitamin B12</b>	1.4 µg	0.19 µg
<b>Biotin</b>	14 µg	1.9 µg
<b>Pantothenic acid</b>	2.9 mg	0.39 mg
<b>Potassium</b>	624 mg	83.0 mg
<b>Chloride</b>	380 mg	50.5 mg
<b>Calcium</b>	813 mg	108 mg
<b>Phosphor</b>	451 mg	60.0 mg
<b>Magnesium</b>	50.0 mg	6.65 mg
<b>Iron</b>	9.0 mg	1.2 mg
<b>Zinc</b>	5.0 mg	0.67 mg
<b>Copper</b>	420 µg	56 µg
<b>Selenium</b>	20 µg	2.7 µg
<b>Iodine</b>	70.0 µg	9.30 µg

### 3.1.2 Packaging Materials

The materials that were used to make the composite cans for the further research are denoted by a number and letter code (e.g. *A1*, *A2*, *A3*). The structure of the reference materials is, as well as the barrier properties of all materials used, shown below. Materials considered for replacement of the aluminum-based material were *A1*, *A2*, *A3*, *B* and *C*. *Alu* and *Low* are taken as reference materials.

The OTR and MVTR values were measured by an external company and are shown in table 5.

*Alu* is the high barrier reference material and is made of paperboard, PE, aluminum (*Al*) and another PE layer. It is depicted in figure 18.

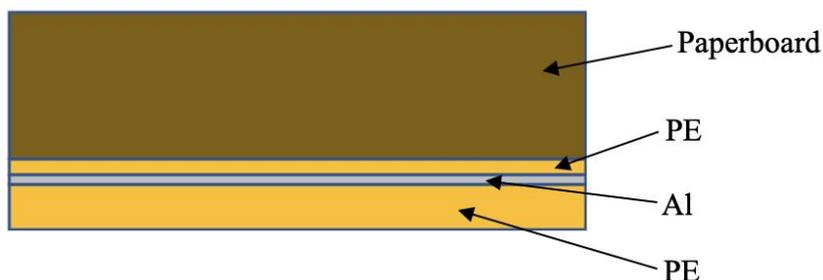


Figure 18: Material layers of high barrier reference material.

This material is the current packaging material of the composite cans and is, due to its high barrier properties, taken as reference material. Also *Low* is taken as a reference material due to its low barrier properties.

Table 5: Barrier properties of the different materials.

Material	OTR (ml/m <sup>2</sup> /day/atm/23°C/50% RH)	MVTR (g/m <sup>2</sup> /day/atm/25°C/75% RH)
<i>Alu</i>	<0.05	<0.05
<i>Low</i>	1500	2.2
<i>A1</i>	0.05	0.55
<i>A2</i>	1.5	1.4
<i>A3</i>	0.5	1.0
<i>B</i>	0.15	0.11
<i>C</i>	0.05	0.03

### 3.1.3 Chemical Agents

For the TBARS assay the following chemicals were used in the stock solution: trichloroacetic acid (Sigma-Aldrich), 2-thiobarbituric acid (Sigma-Aldrich) and hydrochloric acid 1 mol/l (VWR). As active substance 1, 1, 3, 3 – tetramethoxypropane (Aldrich) was used.

For the application of MA common nitrogen gas was used.

The colorant used for the dye penetration inspection was an alcohol-based dye.

It was made sure that all chemicals were prepared with a maximum of three days before performing the laboratory experiments to ensure that all solutions were fresh.

#### **3.1.4 Equipment**

For the production of the packaging a common packaging line machine was used. For the evaluation of the integrity of the cans a microscope was used. Some of the cans were stored in a climate chamber (at 38°C and 25%RH). The residual headspace O<sub>2</sub> was measured by the Dansensor (CheckPoint II). For the TBARS assay common laboratory equipment was used (heating plate with integrated magnetic stirrer, water bath, vortex). Next to this a centrifuge (Beckman Coulter, Allegra X-15R Centrifuge) and spectrophotometer (Varian, Cary 50 Bio) were used.

### **3.2 Methods**

An overview of the experiments performed can be found in graph 4 in Appendix B next to the timeline depicted in graphs 5 and 6 in Appendix B.

#### **3.2.1 Literature Research**

To obtain information about how to execute research and how to set-up the experiments a literature research was performed. Media used were search engines such as Google Scholar, Scopus, LUBcat (University's online library) as well as databases such as the USDA's Food Data Central. Furthermore, information was also provided by the company itself (i.e. staff) and reliable websites (e.g. AR Packaging, FAO).

#### **3.2.2 Making Packaging**

The packaging was made as described in section 2.7 *Packaging of Infant Formula*. Instead of having a base and membrane inserted, two membranes were applied to the sleeve.

### 3.2.3 Modified Atmosphere

To obtain MA, the packages were flushed by nitrogen and then closed. This ensured that the oxygen level was set below the upper limit of 2%.

### 3.2.4 OTR & MVTR determination

Both OTR and MVTR of the flat material were determined by an external laboratory.

### 3.2.5 Tight Can Tests

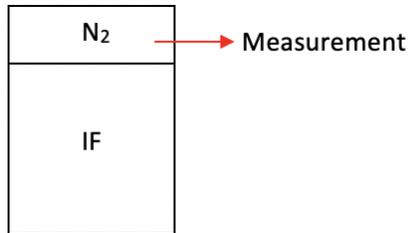
#### 3.2.5.1 Dye-penetrant inspection

For this integrity test an alcohol-based penetrant was used. The penetrating dye (liquid) is applied to e.g. a seal formed during production, to test if the seal is tight or not. To be able to observe possible changes the dye needs to get enough time to detect leaks in the seal/material. In case the dye was able to penetrate through seals or material the package can be regarded as leaking. It is however very important to use the right solvent to not damage the material based on the e.g. corrosive character of the dye (Barcan, 2009).

#### 3.2.5.2 Determination of Residual Headspace Oxygen

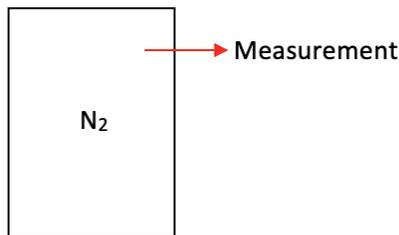
Determining the residual headspace oxygen is a useful tool in determining several things for this research: (1) is MA present; (2) how much O<sub>2</sub> entered the package (increase in headspace O<sub>2</sub>); (3) how much O<sub>2</sub> was consumed by the product (decrease in headspace O<sub>2</sub>).

The following assumptions were made: (1) can be answered by measuring the residual headspace oxygen right after filling (with nitrogen) and closing the package. In this case an O<sub>2</sub> concentrations of less than 2% means that MA is present. The same measurement is performed again right before the powder samples are measured by the TBARS assay. This gives an indication which packaging material is able to maintain the MA and which not. See figure 19.



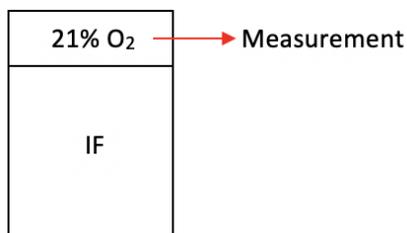
**Figure 19: Schematic overview of residual headspace measurement with MA and product.**

(2) is measured by means of comparing the  $O_2$  concentration in the package in time, when filled with powder and MA and when only containing  $N_2$ . This is, since option (1) does not show how much oxygen is consumed by the powder. Hence, packages containing only  $N_2$  are taken as a reference as well. The frequency of the residual headspace oxygen testing for this study is set to 3 weeks for most samples. See figures 19 and 20 for measurement technique (2).



**Figure 20: Schematic overview of residual headspace measurement with MA only.**

(3) can be answered by measuring samples that were filled at ambient  $O_2$  level. By this, it is known what the begin  $O_2$  concentration is (21%). A decrease in  $O_2$  will then give an indication on how much  $O_2$  is consumed by the IF powder. The material used was with an aluminum layer to ensure the  $O_2$  ingress due to a low barrier was excluded as factor. See figure 21 for measurement technique (3).



**Figure 21: Schematic overview of residual headspace measurement with ambient oxygen and product.**

For this experiment samples were tested in duplicate (two cans of the same material) with measurement time points after every 3 weeks for a total of 9 weeks for the samples filled with IF and 6 weeks for those filled with N<sub>2</sub>.

### **3.2.6 ASLT (accelerated shelf-life testing)**

To be able to mimic the shelf-life of the IF (up to 24 months) within a shorter period of time, the product was stored in the cans made with the different materials (see *3.1.2 Packaging materials*) at elevated temperature (38°C) in a climate chamber. Storing another batch at room temperature (23°C) enables to obtain data that can be used to compare the fastened reaction with that occurring under normal storage conditions.

To make sure that moisture ingress had no influence on the lipid oxidation the RH was set to 25% in the climate chamber. By this, lactose crystallization was assumed to not be influenced (see section *2.1.3 Deteriorative Factors*). The RH was however non-controllable outside the climate chamber but monitored by a measuring device. The results are discussed in section *4 Results & Discussion*.

### **3.2.7 Different Scenarios for the study**

Due to the Covid-19 Pandemic in 2020 it was not clear how the degree project was able to be performed. Therefore, different scenarios based on which the project could be conducted were established.

#### *Scenario 1*

The laboratory stays open and no changes to the original experimental plan have to be made. The study continues as planned. The occurrence of this scenario is very unsure.

#### *Scenario 2*

The entry to the laboratory will be restricted to specific staff (excluding students). A member of the staff (e.g. professor) is conducting the experiments instead and reporting the results. This would imply that the person gets instructed very carefully on what to do. In this case it is assumed that the study can be continued within the same time frame as planned. The likelihood of someone else performing the experiments is however very low.

#### *Scenario 3*

The laboratory at university closes completely and the experiments have to be done by an external laboratory. The laboratory will have different equipment and will conduct the experiments in a different way than planned originally. This will make it very unreliable to compare the first results with those obtained from the external laboratory. Also, the probability of delaying the study in this case is very high, due to time of shipment and the laboratory's own schedule. The likelihood of this scenario to happen is rather low.

#### *Scenario 4*

The laboratory at university is closing completely. The data collected until then is assumed to be enough to evaluate the suitability of the packaging materials for the IF. This means, no further experiments will be conducted. The study will need to be shifted to a more theoretical focus supported by initial data. The likelihood of this scenario to happen is comparatively high.

### **3.2.8 Shelf-life Assessment**

By help of the TBARS assay the level of oxidation is possible to be monitored at different time points (see table 8 in Appendix A.1). The assay was performed following the protocol of Buege & Aust (1978, p. 306) with some adjustments. Instead of 1 ml of biological sample, 1 ml of milk emulsion (IF powder mixed with water as indicated on the package) was used and combined with 2 ml of stock solution. The samples mixed with stock solution were heated for 10 minutes at 90°C and cooled down under running tap water. They were then centrifuged at 3600g for 10 minutes. For the

determination of MDA concentration in each sample a standard curve was prepared instead of using an extinction coefficient. The standard curve was prepared with 1, 1, 3, 3 – tetramethoxypropane (TMP) as the active substance (precursor of MDA), since the compound is more stable than MDA. The TMP concentrations used and the full laboratory protocol are shown in Appendix A.2. The absorbance of all samples was measured with a spectrophotometer at 535 nm (as blank 1 ml of the 0 ppm TMP standard curve sample combined with 2 ml of stock solution was used).

The obtained absorption values were then converted into MDA concentrations by help of the standard curve. The concentrations were then stated in  $\mu\text{g MDA}/100\text{ ml}$  of prepared milk sample.

Samples were measured with duplicates for each material (two cans of the same material) where another duplicate of each can was taken in the laboratory. This means that the final results were based on quadruplets. Samples were tested for a total time of 9 weeks.

### **3.2.9 Data Analysis**

Data was collected in a handwritten notebook and later transferred to Excel (version 16.34). All calculations were conducted in Excel. In the end, data was compared statistically by use of linear regression. Data was considered significantly different from zero lipid oxidation if the p-value was lower than 0.05. The statistics were performed with the data analysis tool in Excel.

## 4 Results & Discussion

The scenario that was chosen for this study was *scenario 1*, in which no changes to the original planning had to be made. This implies that all the experiments as described under *3 Materials & Methods* could be conducted as shown in graph 6 in Appendix B.

The OTR and MVTR values measured by an external laboratory that can be found under section *3.1.2 Packaging Materials* indicate that from the materials used the following are in line with the barrier requirements for IF (OTR & MVTR  $\leq 1$ , figure 11 section *2.10 Barrier Materials*): *Alu, A1, A3, B and C*. *Low*, the low barrier reference material shows a very high OTR (1500 ml/m<sup>2</sup>/day). This was known. The material was still used since it was expected that the lipid oxidation of the powder stored in this material would be comparatively higher, making it a good reference.

Before discussing the results, it should be said that it got detected, after performing the experiments, that the product used did not comply with the regulations stated by Codex Alimentarius in section *2.1 Infant Formula*. As a matter of fact, the product is intended to replace common cow's milk instead of mother's milk as well as it is intended for children from one year onward instead of infants (0-12 months). While IF is to have between 4.4 to 6g of total fat per 100kcal, the used product has only 4.3g of total fat per 100kcal. Next to this, the vitamin E content is high (6.2mg per 100g) helping to prevent the product from oxidizing. This means that the results found are not fully relatable to the data found in literature (for IF) and need to be considered with care. This fact occurred by accident. After ordering IF from the customer company and the delivery of the actual product it did not get checked again if the product was what had been ordered. In general, the product is still assumed to require the same kind of barrier properties, since the ingredients only vary in composition but are still sensitive for deterioration.

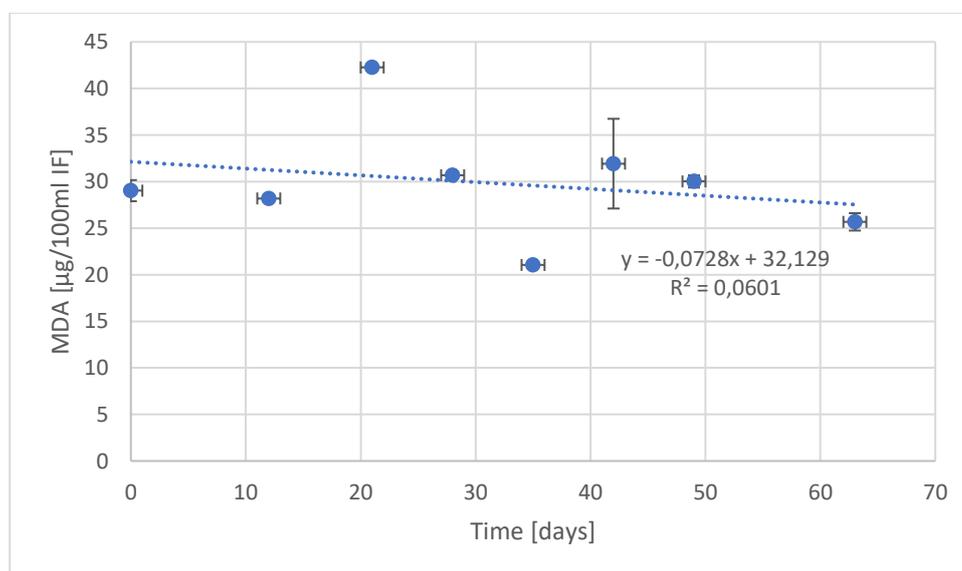
## 4.1 Relative Humidity & Temperature

Graph 7 in Appendix C.1 shows the RH (%) and temperature (°C) profile that was measured during the time frame of the running experiment for the cans stored outside the climate chamber.

The temperature was on average 23.5°C with a standard deviation (SD) of  $\pm 0.6^\circ\text{C}$ . The average of the RH was 21.0% with an SD of  $\pm 5.2\%$ . The resulting RH seems too low, after estimating the common RH during this season of the year to  $\pm 35.0\%$ . Since it cannot be proven however that the measurement was right, the result of the RH measurement cannot be taken as an explanation for unexpected oxidation results that could have occurred due to an increased moisture level possibly leading to lactose crystallization.

## 4.2 TBARS assay

Graph 1 depicts the results of an open package assessed by the TBARS assay. The sample was stored in the original aluminum-based material, however after opening, the IF powder was constantly exposed to the ambient O<sub>2</sub> level (21%).



**Graph 1: MDA concentration in open pack in time with error bars based on the SEM.**

It can be seen that there is an increase in MDA after 21 days and a drastic decrease after 35 days. All other measurement points show MDA concentrations of approximately the same level (~30µg MDA/100ml IF).

Before performing linear regression to see if there has been a significant increase in lipid oxidation, outliers were detected. If the measured value  $\pm$ SEM (standard error of means) was more than 2 SEMs away from the trendline obtained from all measured data points for that material (i.e. open package) it was regarded an outlier. The SEM is calculated by dividing the standard deviation of the data point by the square root of the number of observations in the data set. It could then be assumed that the measured data point was not part of the actual data series.

For the open package the data points after 12, 21 and 35 days were determined to be outliers and removed for further evaluation. The subsequent linear regression showed that there was no significant increase in lipid oxidation for the sample stored in the open package (see table 19 in Appendix D).

It was estimated how long it would take for O<sub>2</sub> to diffuse through the powder in the opened can, to indicate whether the method of obtaining the sample (scooping from the top) for the TBARS assay could have had any effects on the variation in the results. O<sub>2</sub> has a diffusion coefficient of about 0.176 cm<sup>2</sup>/s in air at 25°C (Wikipedia, 2020a). Estimating that the powder particles occupy about 50% of the volume one can assume the powder will double the diffusion time due to resistance. With the height of the powder in the can being ~8.7 cm and by use of equation 2 (Wikipedia, 2020b), this leads to the estimation that it takes about 7.2 minutes for O<sub>2</sub> to diffuse through the entire can.

$$D = \frac{x^2}{2t} \tag{2}$$

With

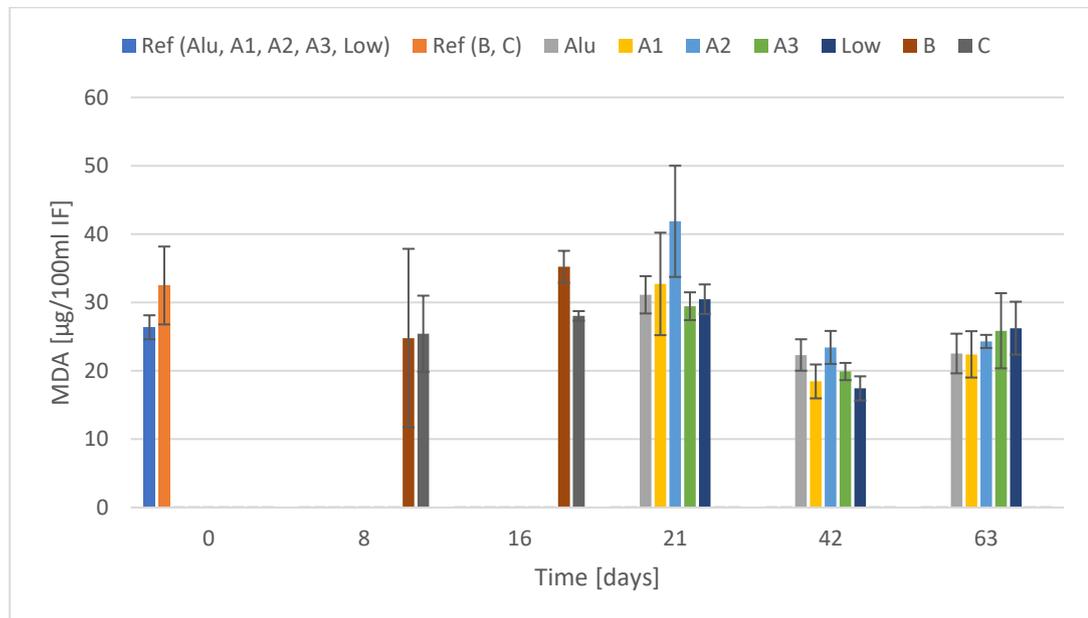
D = diffusion coefficient (cm<sup>2</sup>/s)

x<sup>2</sup> = distance (cm<sup>2</sup>)

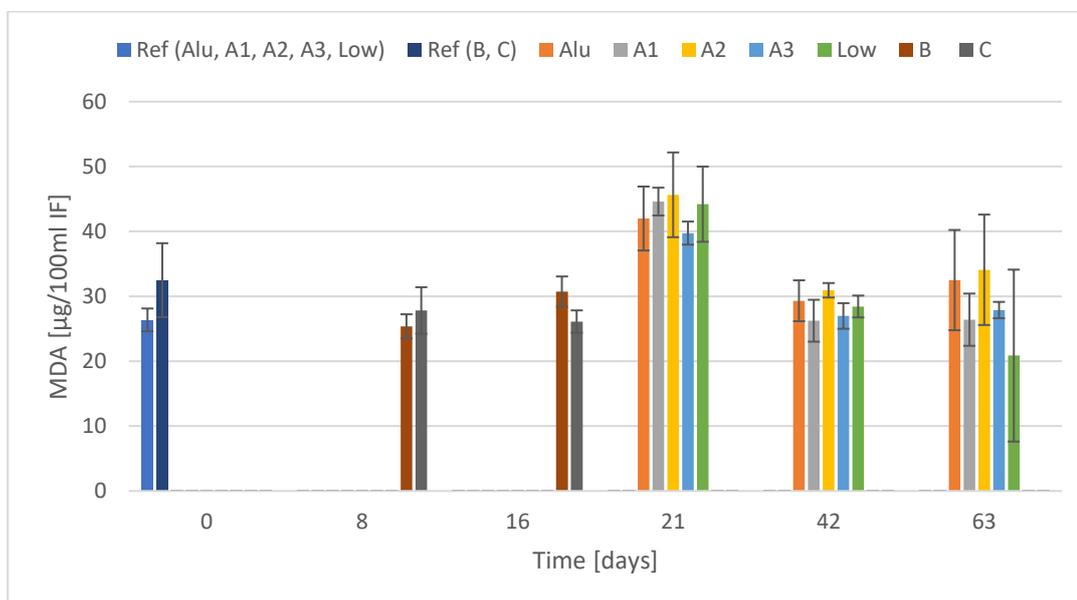
t = time (s)

This estimation shows that the way the laboratory sample was taken from the can should not have had any impact on the MDA concentration found in the sample. At all time points of the measurement in the laboratory the time passed exceeded the time that O<sub>2</sub> needed to diffuse through the entire can, meaning that every powder particle is assumed to have had the same exposure to O<sub>2</sub> in the open can.

Graph 2 and 3 depict the results of the TBARS assay at different time points with all the materials (named under section 3.1.2 *Packaging Materials*) tested combined, for 23°C and 38°C respectively. The error bars are based on the SEM.



**Graph 2: Overview of MDA concentration for each material at each measurement time point stored at 23°C with error bars of SEM.**



**Graph 3: Overview of MDA concentration for each material at each measurement time point stored at 38°C with error bars of SEM.**

At timepoint 0 days a reference can was taken for the materials *Alu*, *A1*, *A2*, *A3* and *Low*. A new reference sample was taken for materials *B* and *C* due to their delayed arrival and later starting date. The exact resulting values of the TBARS assay can be found in table 9 and 10 in Appendix C.2.

It can be seen that the MDA concentration for both temperatures for samples *Alu*, *A1*, *A2*, *A3* and *Low* shows an increase after 21 days, followed by a decrease in MDA concentration after 42 days. The measurement after 63 days shows about the same levels of MDA as after 42 days. For samples *B* and *C*, the MDA concentration shows a decrease after 8 days and a slight increase for *B* after 16 days (23°C & 38°C), while a further decrease for *C* after 16 days (38°C) and slight increase at 23°C.

It can be seen that the SEMs of the samples are varying a lot. This indicates that the method (TBARS assay) showed variations for measurements within one day. And also, the data points at 23°C and 38°C cannot fully be correlated due to the variation. This might give reason to assume that the method is not sensitive enough to detect MDA levels very precisely in the sample at those small magnitudes. Another factor that could have contributed to the variation is measurement faults that could have happened during e.g. weighing, transfer steps (pipetting) as well as equipment that was not well calibrated.

Also, the reagents could have been rather unstable, or the standard curve might not have been precise enough (defective pipettes) leading to variation in results as well. Furthermore, it was noted that a chemical change had to be made due to the first product being empty, meaning that the date of manufacture of the chemical was differing. The older chemical might already have been too old and might have had impurities originating from possible cross-contamination due to long usage.

Even though there is a lot of variation in the results the values fall in the same range of MDA concentration ( $\sim 48\mu\text{g}/100\text{ml}$  IF) that was suggested in literature as an upper limit for IF (note: the product used is however no IF).

The graphs also show that *A2* gives, for all measurement points at  $38^\circ\text{C}$ , the highest MDA concentrations. This is also true for days 21 and 42 at  $23^\circ\text{C}$ .

There is however no clear trend visible between the different materials that could indicate which of them was more suitable to pack the product.

Even though it was expected that *Low* would show higher trends in lipid oxidation compared to the other materials due to its low oxygen barrier, it gave for all measurements about the same results as the other materials if not lower MDA concentrations ( $38^\circ\text{C}$ , day 63).

With *Alu*, *B* and *C* having the best oxygen barriers, it was expected that they would show the lowest MDA concentrations. The results show however no significant differences for both  $23^\circ\text{C}$  and  $38^\circ\text{C}$  (error bars are overlapping).

One could assume that the results at  $38^\circ\text{C}$  on day 21 were significantly different from those on days 8, 16 (*B* and *C*) and 42, since the error bars are not overlapping. However, when assessed as described below it appeared that day 21 was not part of the data series for materials *A1* and *A3*. Since the values decrease after day 21 for all the materials it is however unlikely that it is reliable to say there is a significant difference, rather than that the measurement has been faulty.

Each of the materials was further evaluated individually regarding the extent of lipid oxidation. By means of linear regression it can be said that none of the materials shows a significant increase in lipid oxidation (all p-values were  $>0.05$  or too little reliable data was available after excluding outliers). The resulting tables can be found in Appendix D. The linear regression was

performed after taking out outliers based on the same conditions as discussed before (for the open package).

This result can also explain why *Low* did not show higher MDA concentrations compared to the other materials, since also here, no significant increase in lipid oxidation was observed.

The result from the linear regression analysis could be an indication that the powdered IF used for these experiments is very stable and the time during which these experiments were performed was not sufficient to observe significant lipid oxidation. This is also supported by the fact that the shelf-life after opening for this product was set at one month (by producer). The experimental period did however not exceed the 4 weeks very much (only 1.25 months) and added antioxidants (vitamin A, C, E and selenium) might have slowed down the lipid oxidation reaction in the IF. The result obtained after one month in the open package was initially intended to be taken as an upper limit for the MDA concentration allowed in IF, however, as can be seen in table 19 in Appendix D, no significant increase in oxidation was observable in the open package. Taking the result after 28 days as a reference value seems therefore unreliable. Moreover, as discussed before the amount of fat is comparatively low with only 12.1 g of unsaturated fatty acids per 100g of IF, of which only 3.8g were poly-unsaturated fatty acids (PUFA) that are most prone to oxidation. However, it is also possible that the method (TBARS assay) is not sensitive enough to measure differences in the samples in those small magnitudes (absorbances of ~0.04) in a reliable and precise manner as mentioned before.

Since the results of the linear regression for all materials were similar, no predictions regarding the shelf-life the IF would have in each packaging could be made. This is, because reaction rates would have been necessary to be known that would forecast the behavior of the lipid oxidation. In table 28 in Appendix E it can be seen that there were barely any positive slopes (showing an increase in lipid oxidation). If then also the outliers were taken out, no useful reaction rates were obtained. It was also regarded unreliable to compare the different materials statistically with each other based on these results.

After performing the TBARS assay the packages were also tested regarding the tightness in the sealing areas with the dye-penetrant method (applied at seals). The materials that showed leakages are listed in table 29 in Appendix

F. The results show that the most leakages were found in material *Low*. Most of the packages of material *Low* that were leaking were leaking through the body. This is an indication that the material is not suitable to pack the IF and was expected, since the low barrier properties were known. Next to this also *A1*, *A2*, *B* and *C* are found to be leaking. The leaks were however at the tape, indicating that the sealing parameters have not been optimized in regard to the new materials not containing any aluminum in the body. Furthermore, also some *Alu* packages (including the Ref packages) were showing leakages, however mainly at the tape. This could indicate that something went wrong during sealing as well, since the *Alu* material is the currently used material and was tested extensively to guarantee no leakages. This can possibly have been caused by the fact that different equipment (sealing machines) were used compared to the one used for commercially sold packages. The manual usage can have led to problems that were then leading to higher O<sub>2</sub> levels. This again can then have had impact on the variation seen in the results of the TBARS assay.

### 4.3 Residual Headspace Oxygen

The (partial) results from the residual headspace O<sub>2</sub> measurement can be seen in table 6. The full table is depicted in Appendix G. It should be noted however that sample *A2* (23°C) at 42 days showed tearing signs on the membrane of the can before the measurement was performed, which could have led to an increase in O<sub>2</sub> in the package. Also, the results obtained for all samples after 63 days (for samples *B* & *C* that is the results after 8 days) seemed not reliable since the equipment showed off-values and are therefore denoted with an e for error.

**Table 6: Residual O<sub>2</sub> concentrations (%) measured in the headspace of each can (partial table). Each material was measured in duplicate (values above 2% O<sub>2</sub> were highlighted in yellow).**

Sample	Time [days]	1.Residual O <sub>2</sub> [%]	2.Residual O <sub>2</sub> [%]
<i>Alu</i> (23°C)	0	0.1	1.0
	21	0.5	0.6
<i>Alu</i> (38°C)	21	0.2	0.5
	42	0.1	0.0
<i>A1</i> (23°C)	21	0.2	0.3
<i>A2</i> (38°C)	42	8.1	0.7
<i>A3</i> (23°C)	21	2.4	0.4

<i>Low</i> (23°C)	21	19.8	20.7
<i>Low</i> (38°C)	21	20.4	20.4
<i>Alu</i> (ambient O <sub>2</sub> , 23°C)	21	19.9	20.0
<i>B</i> (23°C)	16	1.8	2.5
<i>C</i> (38°C)	16	2.3	1.4

The samples surpassing the threshold value of 2% O<sub>2</sub> in the headspace that was necessary for a can to be considered being under MA are highlighted (yellow). At time 0 one reference was taken for all the cans (aluminum-based). The duplicates were two different cans of the same material.

The results show that materials *Alu* and *A1* have O<sub>2</sub> levels below 2%. Material *B* gave one result above 2%. This can be an indication that these materials are able to maintain MA for long times and have low OTRs. This is in line with the values found in section 3.1.2 *Packaging Materials* for the OTRs (*Alu*: <0.05, *A1*: 0.05, *B*: 0.15). The higher value for *B* could be explained by possible problems during the process of sealing.

Material *A3* showed only one measurement above 2% and one more at exactly 2%. All other values are below the threshold. This could be e.g. due to the fact that something went wrong during the sealing processes, leaving small pores for O<sub>2</sub> to penetrate. It could also indicate that the material might not have the barrier properties necessary for IF or that it is less suitable for the sealing techniques applied during this study.

Material *A2* showed several measurement points with O<sub>2</sub>-values above 2%. The determined O<sub>2</sub> barrier (OTR value of 1.5) combined with these values could give an indication that the material is not the most suitable to store powdered IF for long times and protecting it from O<sub>2</sub> ingress. This is supported by the result shown for the TBARS assay, where *A2* was at each time point at 38°C the material giving the highest MDA concentration.

For material *Low* it was known that the OTR value was very high (1500) and O<sub>2</sub>-values surpassing the 2% threshold were expected. Hence, the result of O<sub>2</sub> levels around ambient O<sub>2</sub> (~21%) is in line with expectations.

For the sample stored in the aluminum-based packaging but filled with ambient O<sub>2</sub> instead of MA it was expected to see a decreasing trend (with a starting point of ~21% at time 0). The results for the sample *Alu* (*ambient*

$O_2$ ) are in line with that assumption. This could be an indication that the  $O_2$  that was filled into the headspace slowly was consumed by the powder. It can also be seen that this trend was slightly faster in the samples stored at elevated temperature compared to the ones stored at 23°C. This again is what was expected, since reactions occur more rapidly at higher temperatures.

Material *C* showed  $O_2$ -values above 2% at both temperatures. This is surprising, since the OTR value of 0.05 supported the expectation of low values and, next to *Alu* provided the best  $O_2$  barrier. Yet again, the sealing process might have caused pores or channels in the seal area to be created that let in  $O_2$  or the package might have had an  $O_2$  level higher than 2% from beginning on.

The experimental set-up limited the possibility to follow the residual headspace  $O_2$  concentration in time for an individual can. This was due to the reason that the measurement is a destructive method, puncturing the package. While following an individual can would have given a deeper insight on the process of oxygen consumption within a specific can, it would also have caused  $O_2$  to enter the package and altered the results.

Table 7 depicts the results for the residual headspace measurements done for the packages filled with nitrogen ( $N_2$ ) only.

**Table 7: Residual headspace oxygen (%) at different storage temperatures for the different materials filled with  $N_2$  only; values exceeding the 2%  $O_2$  level are highlighted in yellow.**

<i>Material</i>	<i>21 days</i>	
	$O_2$ [%] at 38°C	$O_2$ [%] at 23°C
<i>AluA</i>	0.1	0.1
<i>AluB</i>	0.1	0.1
<i>A1A</i>	0.2	0.2
<i>A1B</i>	0.1	0.1
<i>A2A</i>	0.2	1
<i>A2B</i>	0.9	0.1
<i>A3A</i>	0.6	0.5
<i>A3B</i>	0.2	1.4
<i>LowA</i>	17.9	10.5
<i>LowB</i>	19.7	10.2

There were only two days the measurement was performed, day 21 and day 42. On day 42 the equipment showed no reliable results and the data was therefore excluded from the table. The results from day 21 show that materials *Alu*, *A1*, *A2* and *A3* all have O<sub>2</sub> levels below 2%. *Low* was the only material to show values above 2%, which again was expected due to the high OTR of 1500 ml/m<sup>2</sup>/day. Materials *B* and *C* were not included for these measurements due to their delayed arrival. The results are thus in line with expectations for all materials tested.

The previously mentioned contribution to variation in O<sub>2</sub> content due to defects or pores created during sealing seem not true for all of the packages, since not in all cases of the leaking ones the O<sub>2</sub> value exceeded 2%. Therefore, it is hard to correlate which of the results was influenced by this and which not.

#### 4.4 Overall

Since the results are inconclusive regarding which material would be an alternative to the aluminum-based package it is advised to prolong this type of study. This is because it is expected that it takes a long time for the product to oxidize under ambient conditions (IF is too stable). Although the elevated temperature is expected to speed up reactions, it is not the same condition under which a consumer would store the product and might give different results than at ambient temperatures. Prolonging the study to the indicated shelf-life of the product (24 months if unopened) at ambient temperature, would probably give more reliable results.

Furthermore, it is difficult to control all factors influencing deterioration of the powdered IF. Even though it was aimed to control as many external factors as possible in this study it cannot be guaranteed that e.g. moisture had no effect at all on the oxidation behavior observed. This insecurity makes it hard to correlate results with causes.

Another factor to consider is that the fat in the IF is likely to have been in liquid state in the samples stored at 38°C. This makes it harder to compare the results obtained at 38°C (fat in liquid state) to those at 23°C (fat in solid state), since the oxidation behavior might be different in the two states.

Also, there are no officially determined upper limits of how much MDA is allowed in powdered IF. This makes results very case-dependent and it is hard to draw general conclusions from the obtained data regarding shelf-life of the product.

## 5 Conclusion & Recommendations

A replacement of the current aluminum-containing packaging for IF should be possible with an alternative material providing barrier properties with OTR values of less or equal to  $1 \text{ cm}^3/\text{m}^2/\text{day}$  and MVTR values of less or equal to  $1 \text{ g}/\text{m}^2/\text{day}$ . The materials that fulfilled these requirements with their barrier properties were *A1*, *A3*, *B* and *C*. However, the sealing parameters need to be optimized to guarantee packages without any leaks for further studies. The factors that were identified to contribute most to the deterioration of powdered IF were oxygen, light, moisture and high temperatures, leading to e.g. Maillard reaction and lipid oxidation.

The TBARS assay gave results without any significant increase in oxidation in any of the materials. Moreover, only few of the reaction rates had a positive trend showing a lot of variation within the data set. Based on the results no conclusions can be drawn regarding which of the tested materials is able to replace the aluminum-packaging. However, it can be concluded that the TBARS assay is one of several options to evaluate the lipid oxidation in powdered IF. Nevertheless, due to variations in the obtained results that had no clear cause, it is recommended to further evaluate the reliability of the method and combine it with other ones, e.g. determination of the peroxide value, to be able to compare the results. This also implies the extraction of the fat, which could also decrease the impact of other compounds on the variation in the results. There is however only little data about the reproducibility and precision of any of the methods in literature, making it hard to determine which one is the best.

Further research is recommended, with adaptations in the experimental set-up to be able to follow the oxidation rate and  $\text{O}_2$  concentration in each individual can more precisely and be able to draw more reliable conclusions (the same can for each measurement point). Furthermore, it is suggested to prolong the experiment duration, to obtain more data points and hence more reliable results, and in addition to follow the lipid oxidation for a longer time and see its behavior in this kind of product. However, the product used was too stable to show any significant increases in lipid oxidation and a less stable

product is advised to be used in future studies to see the effect the packaging has in a shorter time.

It is also recommended to include factors such as moisture in future research, since it is hard to control it. This could among other things include measuring the  $a_w$  of the product and follow the moisture absorption in the powder. Also, to be able to predict the shelf-life mathematically it is advised to have a set-up with not just two but several different temperatures.

Another aspect to consider is sensory studies that could help determine an upper limit of MDA in the product based on perceivable rancidity.

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# Appendix A

## A.1

**Table 8: Sample code regarding sampling time.**

<b>Description</b>	<b>Weeks</b>
T0	0
T1	3
T2	6
T3	9

## A.2

### **Laboratory Protocol**

*Chemicals:*

15% w/v trichloroacetic acid (TCA)  
0.375% w/v thiobarbituric acid (TBA)  
0.25M hydrochloric acid (HCl)  
1, 1, 3, 3 – tetramethoxypropane (TMP)  
Deionized water

*Equipment:*

Magnetic stirrer – heated (& magnets)  
Glass bottle  
Glass beakers (different sizes)  
Pipet  
Water bath (90°)  
Eppendorf centrifuge tubes (15 ml)  
Cuvettes

Centrifuge  
Spectrophotometer

*Make stock solution:*

→ used to react with MDA in oxidized product

We have:

Trichloroacetic acid (TCA) → 15% w/v

Thiobarbituric acid (TBA) → 0.375% w/v

1N/M hydrochloric acid (HCl) → 0.25N/M

Weigh/measure all the ingredients and combine in a bottle. **Work under fume hood!** Mildly heat (~50°C) the solution to help TBA dissolve. When everything is dissolved the TCA-TBA-HCl is ready to be used as stock solution.

*Prepare standard curve solution:*

→ to make a standard curve with known concentrations prepare samples with different concentrations of TMP (more stable than MDA)

We have:

1, 1, 3, 3 – tetramethoxypropane (TMP)

**Solution A:**

→ 82.3µl of TMP and fill up with deionized water to 50 ml (10000µM TMP)

**Solution B:**

→ 0.5ml of **solution A** filled up to 50 ml with deionized water (100µM TMP)

Prepare dilutions from 1 to 6 ppm of TMP (total of 5 ml):

	0 ppm TMP	1 ppm TMP	2 ppm TMP	3 ppm TMP	4 ppm TMP	5 ppm TMP	6 ppm TMP
Solution B (ml)	0	0.3	0.6	0.9	1.2	1.5	1.8
H <sub>2</sub> O (ml)	5	4.7	4.4	4.1	3.8	3.5	3.2
µM	0	6	12	18	24	30	36

**Box 1:**

→ take 1 ml of standard solution and add to 2 ml of stock solution in 15 ml Eppendorf tubes with screw caps

- Vortex
- Heat for 10 minutes at 90°C
- Cool down under running tap water
- Centrifuge at 3600 g for 10
- Measure absorbance of supernatant at 535 nm

*Standard curve:*

→ Plot absorbance (y-axis) against concentration TMP/MDA (x-axis) to obtain a straight line

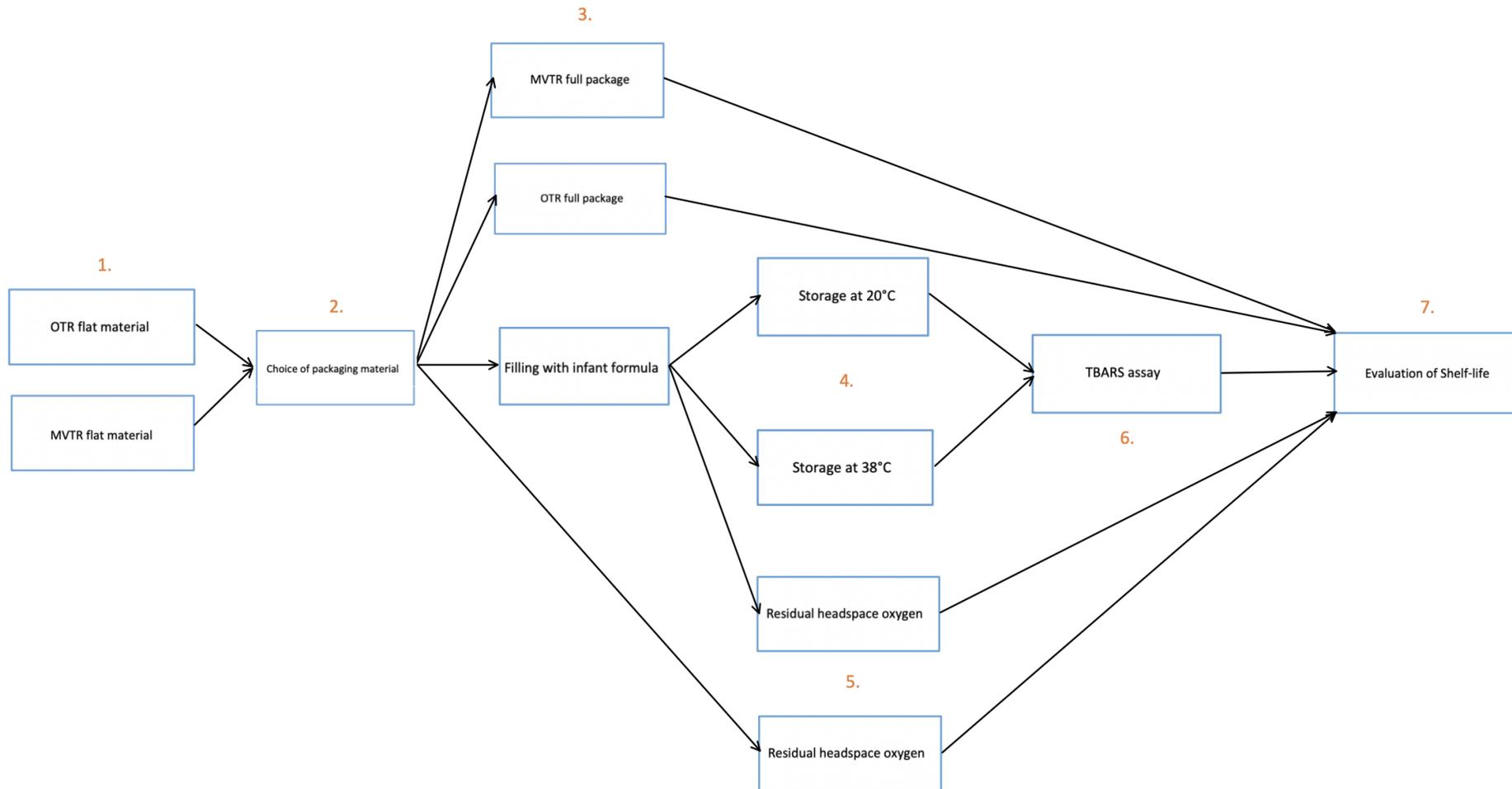
→ from line equation ( $y=mx+b$ ) you can get the unknown concentration of the samples

*Sample preparation:*

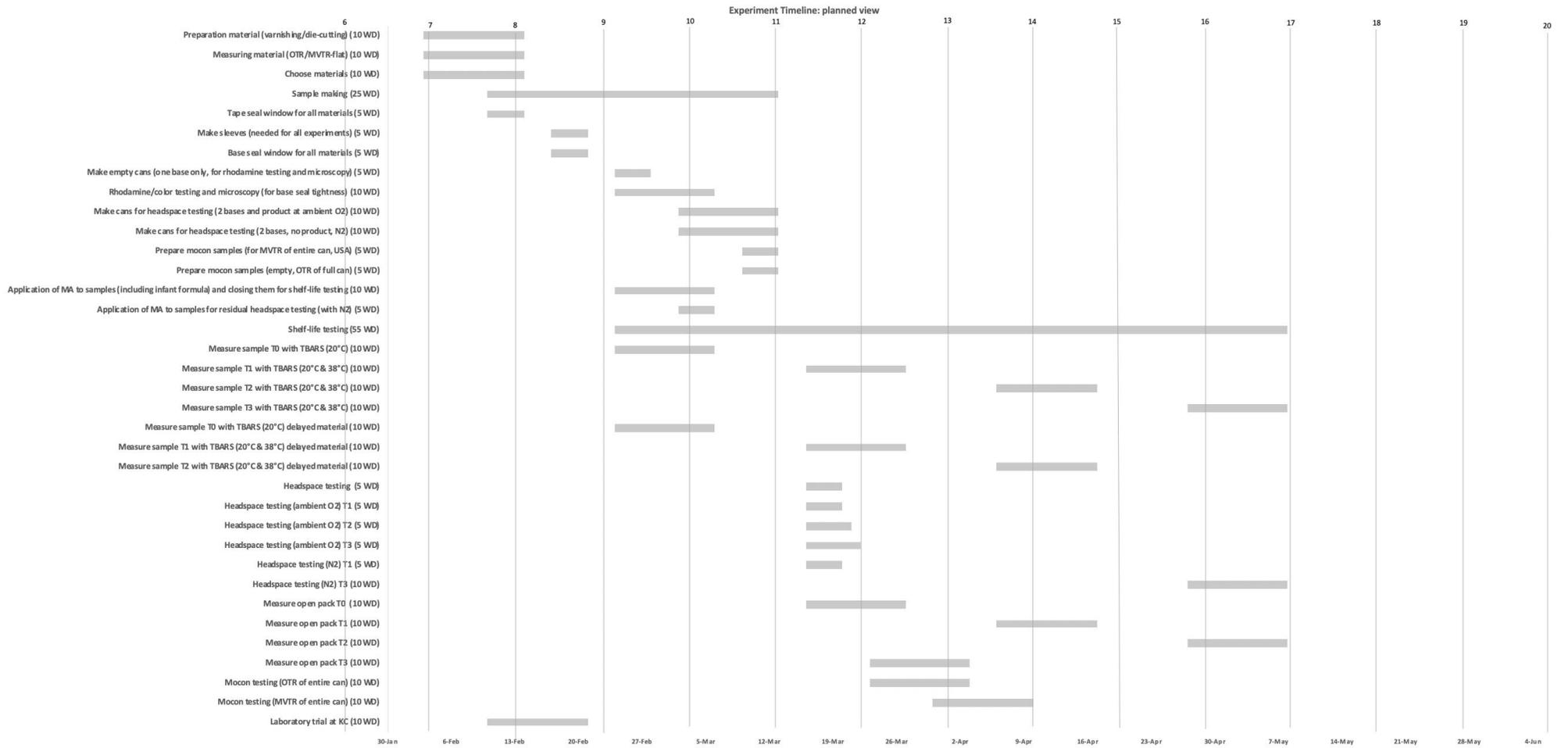
Prepare the IF of each sample as indicated on the package for a volume of 50ml.

→ work as described in **Box 1**, but replace the standard solution by 1 ml of IF sample

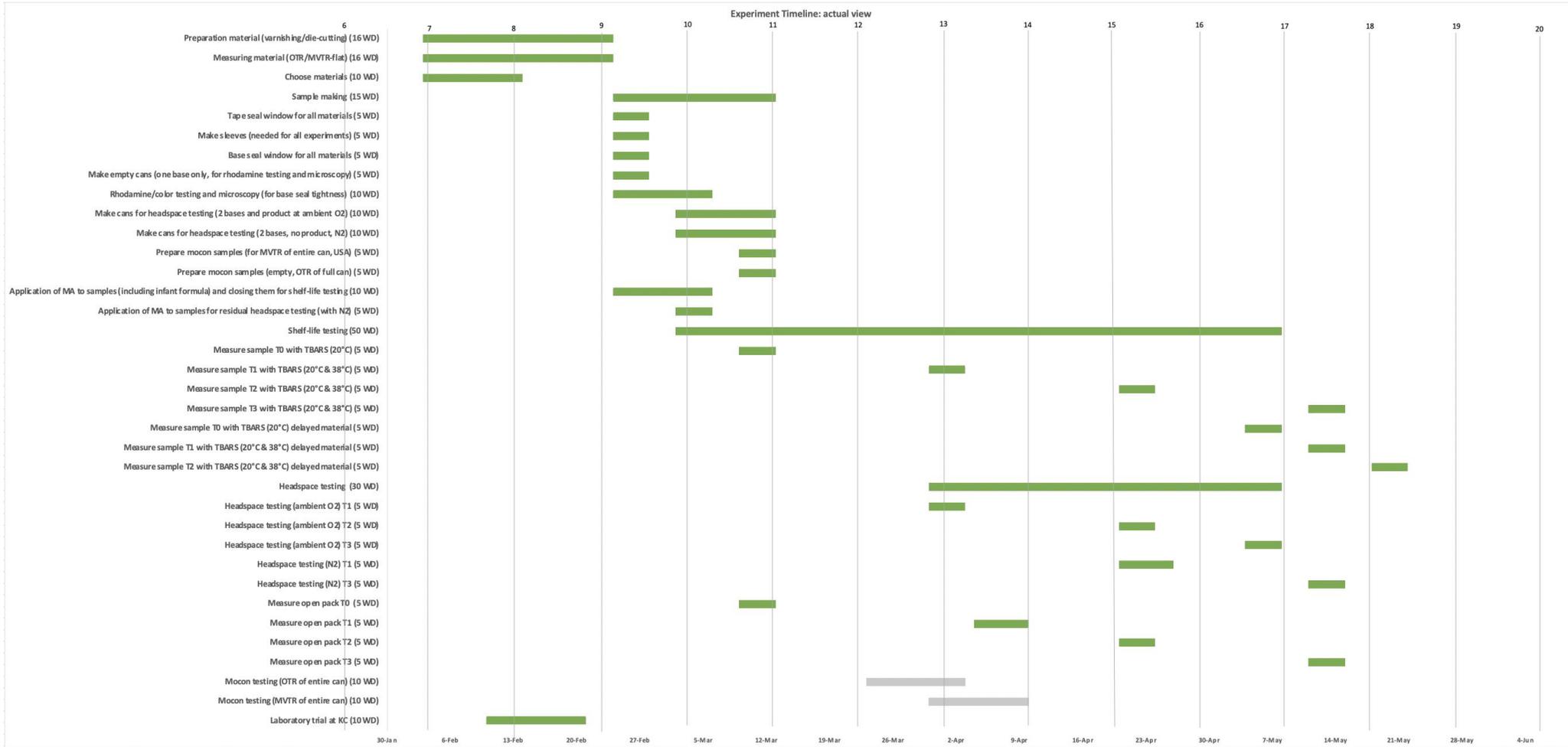
# Appendix B



Graph 4: Experimental plan.



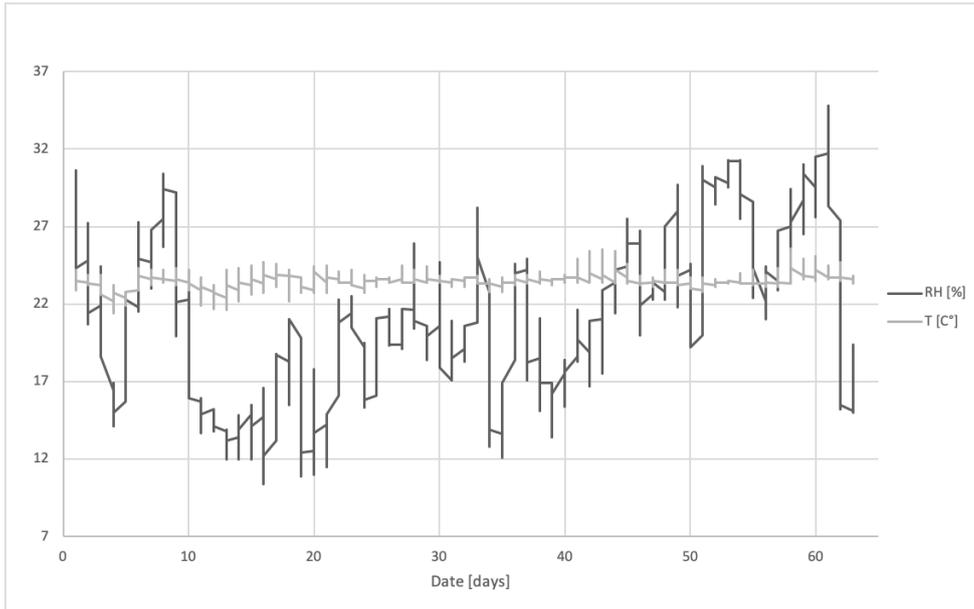
Graph 5: Gantt chart of the planned time frame of the experiments.



Graph 6: Gantt chart of actual time frame of experiments.

# Appendix C

## C.1



**Graph 7: RH and T profile measured for the cans stored outside the climate chamber for the time of the experiment.**

## C.2

**Table 9: Results TBARS assay at 23°C.**

	<b>Alu</b>	<b>A1</b>	<b>A2</b>	<b>A3</b>	<b>Low</b>	<b>Alu (amb. O<sub>2</sub>)</b>	<b>B</b>	<b>C</b>
<b>Time (days)</b>	<b>MDA (µg/100ml IF) ± SEM</b>							
0	26.4 ± 1.8	26.4 ± 1.8	26.4 ± 1.8	26.4 ± 1.8	26.4 ± 1.8	26.4 ± 1.8	32.5 ± 5.7	32.5 ± 5.7
21	31.1 ± 2.7	32.7 ± 7.5	41.9 ± 8.1	29.5 ± 2.0	30.5 ± 2.2	33.8 ± 1.9	24.8 ± 13.0	25.4 ± 5.6
42	22.3 ± 2.3	18.5 ± 2.5	23.4 ± 2.4	19.9 ± 1.3	17.4 ± 1.8	18.1 ± 0.7		
63	22.5 ± 2.9	22.4 ± 3.4	24.3 ± 1.0	25.9 ± 5.5	26.2 ± 3.9	22.5 ± 2.9		

**Table 10: Results TBARS assay at 38°C.**

	<b>Alu</b>	<b>A1</b>	<b>A2</b>	<b>A3</b>	<b>Low</b>	<b>Alu (amb. O<sub>2</sub>)</b>	<b>B</b>	<b>C</b>
<b>Time (days)</b>	<b>MDA (µg/100ml IF) ± SEM</b>							
0	26.4 ± 1.8	26.4 ± 1.8	26.4 ± 1.8	26.4 ± 1.8	26.4 ± 1.8	26.4 ± 1.8	32.5 ± 5.7	32.5 ± 5.7
21	42.0 ± 4.9	44.6 ± 2.1	45.6 ± 6.5	39.7 ± 1.8	44.2 ± 5.8	42.6 ± 3.7	25.4 ± 1.9	27.8 ± 3.6
42	29.3 ± 3.2	26.2 ± 3.2	30.9 ± 1.1	27.0 ± 2.0	28.4 ± 1.7	23.9 ± 1.4		
63	32.5 ± 7.7	26.4 ± 4.0	34.1 ± 8.5	27.9 ± 1.3	20.9 ± 13.3	27.6 ± 2.6		

# Appendix D

**Table 11: Linear regression result for Alu (23°C).**

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	0.63423379
R Square	0.4022525
Adjusted R Squar	0.10337875
Standard Error	3.91294822
Observations	4

ANOVA

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	20.60720921	20.6072092	1.345894373	0.36576621
Residual	2	30.62232762	15.3111638		
Total	3	51.22953683			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	28.628684	3.273807365	8.74476741	0.012825819	14.5426278	42.71474017	14.5426278	42.71474017
X Variable 1	-0.096673	0.083329697	-1.1601269	0.365766211	-0.4552118	0.261865728	-0.4552118	0.261865728

RESIDUAL OUTPUT

<i>Observation</i>	<i>Predicted Y</i>	<i>Residuals</i>	<i>Standard Residuals</i>
1	28.628684	-2.262204908	-0.7080656
2	26.5985505	4.51981112	1.41469173
3	24.5684171	-2.253007516	-0.7051868
4	22.5382836	-0.004598696	-0.0014394

**Table 12: Linear regression result for A1 (23°C).**

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.55476583
R Square	0.30776513
Adjusted R Squar	-0.0383523
Standard Error	6.19804804
Observations	4

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	34.1590524	34.1590524	0.889192803	0.44523417
Residual	2	76.83159895	38.4157995		
Total	3	110.9906513			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	28.9050045	5.185659035	5.5740272	0.030710661	6.59291453	51.21709454	6.59291453	51.21709454
X Variable 1	-0.1244654	0.131992921	-0.9429702	0.445234165	-0.6923851	0.443454312	-0.6923851	0.443454312

RESIDUAL OUTPUT

Observation	Predicted Y	Residuals	Standard Residuals
1	28.9050045	-2.538525479	-0.501617
2	26.2912313	6.422002811	1.26899872
3	23.6774581	-5.228429186	-1.0331465
4	21.0636849	1.344951854	0.26576478

**Table 13: Linear regression result for A2 (23°C).**

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.84405685
R Square	0.71243196
Adjusted R Squar	0.42486392
Standard Error	7.89166309
Observations	3

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	154.2907361	154.290736	2.477437909	0.36032194
Residual	1	62.27834631	62.2783463		
Total	2	216.5690824			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	47.4290056	12.05471449	3.93447772	0.158450362	-105.74066	200.5986759	-105.74066	200.5986759
X Variable 1	-0.4182497	0.265726118	-1.5739879	0.360321942	-3.7946202	2.958120768	-3.7946202	2.958120768

RESIDUAL OUTPUT

Observation	Predicted Y	Residuals	Standard Residuals
1	38.645762	3.221757965	0.57735027
2	29.8625184	-6.44351593	-1.1547005
3	21.0792748	3.221757965	0.57735027

**Table 14: Linear regression result for A3 (23°C).**

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.31525278
R Square	0.09938432
Adjusted R Square	-0.8012314
Standard Error	2.61002883
Observations	3

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	0.75174225	0.75174225	0.110351528	0.79582139
Residual	1	6.812250497	6.8122505		
Total	2	7.563992747			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	27.7615982	2.205876971	12.5852885	0.050478386	-0.2667263	55.78992257	-0.2667263	55.78992257
X Variable 1	-0.0191122	0.057533742	-0.332192	0.795821392	-0.7501478	0.711923253	-0.7501478	0.711923253

RESIDUAL OUTPUT

Observation	Predicted Y	Residuals	Standard Residuals
1	27.7615982	-1.395119093	-0.7559289
2	27.3602409	2.09267864	1.13389342
3	26.5575265	-0.697559547	-0.3779645

**Table 15: Linear regression result for Low (23°C).**

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.21398735
R Square	0.04579059
Adjusted R Square	-0.9084188
Standard Error	3.32910315
Observations	3

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	0.531847348	0.53184735	0.047987983	0.86270973
Residual	1	11.08292778	11.0829278		
Total	2	11.61477512			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	28.1459595	2.813605691	10.0035196	0.063428858	-7.6042904	63.8962095	-7.6042904	63.8962095
X Variable 1	-0.0160757	0.073384538	-0.2190616	0.862709733	-0.9485147	0.916363234	-0.9485147	0.916363234

RESIDUAL OUTPUT

Observation	Predicted Y	Residuals	Standard Residuals
1	28.1459595	-1.779480484	-0.7559289
2	27.8083691	2.669220726	1.13389342
3	27.1331883	-0.889740242	-0.3779645

**Table 16: Linear regression result for B (23°C).**

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.25277807
R Square	0.06389675
Adjusted R Squar	-0.8722065
Standard Error	7.39318675
Observations	3

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	3.73094109	3.73094109	0.068258233	0.8373115
Residual	1	54.65921029	54.6592103		
Total	2	58.39015138			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	29.4635784	6.749025256	4.365605	0.143353144	-56.290918	115.2180751	-56.290918	115.2180751
X Variable 1	0.17072779	0.653471561	0.26126277	0.837311498	-8.1324157	8.473871224	-8.1324157	8.473871224

RESIDUAL OUTPUT

Observation	Predicted Y	Residuals	andard Residuals
1	29.4635784	3.018255851	0.57735027
2	30.8294007	-6.036511702	-1.1547005
3	32.195223	3.018255851	0.57735027

**Table 17: Linear regression result for C (23°C).**

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.62199124
R Square	0.3868731
Adjusted R Squar	-0.2262538
Standard Error	3.95408628
Observations	3

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	9.865303414	9.86530341	0.630983734	0.57264787
Residual	1	15.63479831	15.6347983		
Total	2	25.50010172			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	30.8675853	3.609570416	8.55159526	0.074107994	-14.996355	76.73152599	-14.996355	76.73152599
X Variable 1	-0.2776197	0.349495153	-0.7943448	0.572647872	-4.7183766	4.163137291	-4.7183766	4.163137291

RESIDUAL OUTPUT

Observation	Predicted Y	Residuals	andard Residuals
1	30.8675853	1.614248964	0.57735027
2	28.6466279	-3.228497928	-1.1547005
3	26.4256705	1.614248964	0.57735027

**Table 18: Linear regression result for Alu (amb. O<sub>2</sub>, 23°C).**

SUMMARY OUTPUT

Regression Statistics	
Multiple R	1
R Square	1
Adjusted R Square	65535
Standard Error	0
Observations	2

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	7.345155503	7.3451555	#NUM!	#NUM!
Residual	0	0	65535		
Total	1	7.345155503			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	26.3664791	0	65535	#NUM!	26.3664791	26.36647906	26.3664791	26.36647906
X Variable 1	-0.060838	0	65535	#NUM!	-0.060838	-0.060838002	-0.060838	-0.060838002

RESIDUAL OUTPUT

Observation	Predicted Y	Residuals	Standard Residuals
1	26.3664791	-3.55271E-15	-0.7071068
2	22.5336849	-3.55271E-15	-0.7071068

**Table 19: Linear regression for open package.**

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.32771651
R Square	0.10739811
Adjusted R Square	-0.1901359
Standard Error	2.58042786
Observations	5

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	2.40349677	2.40349677	0.36096085	0.59033242
Residual	3	19.9758239	6.65860796		
Total	4	22.3793206			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	30.6437559	2.27572347	13.4655007	0.00088562	23.4013882	37.8861236	23.4013882	37.8861236
X Variable 1	-0.0323743	0.05388536	-0.6008002	0.59033242	-0.2038616	0.13911292	-0.2038616	0.13911292

RESIDUAL OUTPUT

Observation	Predicted Y	Residuals	Standard Residuals
1	30.6437559	-1.6157884	-0.7230397
2	29.7372746	0.93714116	0.4193558
3	29.284034	2.64451737	1.18337957
4	29.0574137	0.96141873	0.43021963
5	28.604173	-2.9272889	-1.3099153

**Table 20: Linear regression result for Alu (38°C).**

SUMMARY OUTPUT

<i>Regression Statistics</i>							
Multiple R	0.10894603						
R Square	0.01186924						
Adjusted R Square	-0.4821961						
Standard Error	8.24964046						
Observations	4						

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	1.63496488	1.63496488	0.02402362	0.89105397
Residual	2	136.113135	68.0565677		
Total	3	137.7481			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	31.6830842	6.90214441	4.59032474	0.04432674	1.98555375	61.3806147	1.98555375	61.3806147
X Variable 1	0.02723014	0.1756834	0.15499554	0.89105397	-0.7286745	0.7831348	-0.7286745	0.7831348

RESIDUAL OUTPUT

<i>Observation</i>	<i>Predicted Y</i>	<i>Residuals</i>	<i>Standard Residuals</i>
1	31.6830842	-5.3166052	-0.7893053
2	32.2549172	9.7311596	1.44469179
3	32.8267502	-3.5125037	-0.5214677
4	33.3985832	-0.9020507	-0.1339188

**Table 21: Linear regression results for A1 (38°C).**

SUMMARY OUTPUT

<i>Regression Statistics</i>							
Multiple R	1						
R Square	1						
Adjusted R Square	65535						
Standard Error	0						
Observations	2						

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	0.01269024	0.01269024	#NUM!	#NUM!
Residual	0	0	65535		
Total	1	0.01269024			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	25.9172389	0	65535	#NUM!	25.9172389	25.9172389	25.9172389	25.9172389
X Variable 1	0.00758631	0	65535	#NUM!	0.00758631	0.00758631	0.00758631	0.00758631

RESIDUAL OUTPUT

<i>Observation</i>	<i>Predicted Y</i>	<i>Residuals</i>	<i>Standard Residuals</i>
1	26.2358639	0	65535
2	26.3951764	0	65535

**Table 22: Linear regression result for A2 (38°C).**

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	1
R Square	1
Adjusted R Square	65535
Standard Error	0
Observations	2

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	66.7498413	66.7498413	#NUM!	#NUM!
Residual	0	0	65535		
Total	1	66.7498413			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	51.4130386	0	65535	#NUM!	51.4130386	51.4130386	51.4130386	51.4130386
X Variable 1	-0.2751001	0	65535	#NUM!	-0.2751001	-0.2751001	-0.2751001	-0.2751001

RESIDUAL OUTPUT

<i>Observation</i>	<i>Predicted Y</i>	<i>Residuals</i>	<i>Standard Residuals</i>
1	45.6359355	0	0
2	34.0817292	7.1054E-15	1

**Table 23: Linear regression result for A3 (38°C).**

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	0.95342409
R Square	0.9090175
Adjusted R Square	0.81803499
Standard Error	0.32528754
Observations	3

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	1.05718069	1.05718069	9.99112423	0.1950635
Residual	1	0.10581198	0.10581198		
Total	2	1.16299267			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	26.2795423	0.31345492	83.8383472	0.00759306	22.2967199	30.2623647	22.2967199	30.2623647
X Variable 1	0.0226648	0.00717042	3.16087397	0.1950635	-0.0684441	0.11377366	-0.0684441	0.11377366

RESIDUAL OUTPUT

<i>Observation</i>	<i>Predicted Y</i>	<i>Residuals</i>	<i>Standard Residuals</i>
1	26.2795423	0.08693675	0.37796447
2	27.231464	-0.2608103	-1.1338934
3	27.7074248	0.1738735	0.75592895

**Table 24: Linear regression result for Low (38°C).**

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	0.98005443
R Square	0.96050669
Adjusted R Square	0.92101337
Standard Error	3.34552084
Observations	3

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	272.210143	272.210143	24.3207422	0.12736287
Residual	1	11.1925097	11.1925097		
Total	2	283.402653			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	54.4969207	5.11036749	10.6639925	0.059524	-10.436455	119.430296	-10.436455	119.430296
X Variable 1	-0.5555432	0.11264955	-4.9316065	0.12736287	-1.9868914	0.87580497	-1.9868914	0.87580497

RESIDUAL OUTPUT

<i>Observation</i>	<i>Predicted Y</i>	<i>Residuals</i>	<i>Standard Residuals</i>
1	42.8305129	1.36580316	0.57735027
2	31.1641051	-2.7316063	-1.1547005
3	19.4976972	1.36580316	0.57735027

**Table 25: Linear regression result for B (38°C).**

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	0.9359041
R Square	0.87591649
Adjusted R Square	0.75183297
Standard Error	8.17610443
Observations	3

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	471.890759	471.890759	7.05908829	0.22916997
Residual	1	66.8486836	66.8486836		
Total	2	538.739443			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	3.33788065	7.46372804	0.4472136	0.73227953	-91.497776	98.1735373	-91.497776	98.1735373
X Variable 1	1.92006421	0.72267236	2.65689448	0.22916997	-7.2623588	11.1024872	-7.2623588	11.1024872

RESIDUAL OUTPUT

<i>Observation</i>	<i>Predicted Y</i>	<i>Residuals</i>	<i>Standard Residuals</i>
1	3.33788065	-3.3378807	-0.5773503
2	18.6983943	6.67576131	1.15470054
3	34.058908	-3.3378807	-0.5773503

**Table 26: Linear regression result for C (38°C).**

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	0.83736429
R Square	0.70117895
Adjusted R Square	0.40235789
Standard Error	12.0521482
Observations	3

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	340.836898	340.836898	2.34648442	0.36819075
Residual	1	145.254277	145.254277		
Total	2	486.091175			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	4.92026891	11.0020557	0.4472136	0.73227953	-134.8741	144.714642	-134.8741	144.714642
X Variable 1	1.63180522	1.06526947	1.53182389	0.36819075	-11.903727	15.1673372	-11.903727	15.1673372

RESIDUAL OUTPUT

<i>Observation</i>	<i>Predicted Y</i>	<i>Residuals</i>	<i>Standard Residuals</i>
1	4.92026891	-4.9202689	-0.5773503
2	17.9747106	9.84053782	1.15470054
3	31.0291524	-4.9202689	-0.5773503

**Table 27: Linear regression result for Alu (amb. O<sub>2</sub>, 38°C).**

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	1
R Square	1
Adjusted R Square	65535
Standard Error	0
Observations	2

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	0.77173363	0.77173363	#NUM!	#NUM!
Residual	0	0	65535		
Total	1	0.77173363			

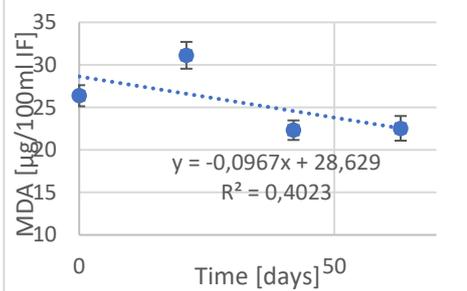
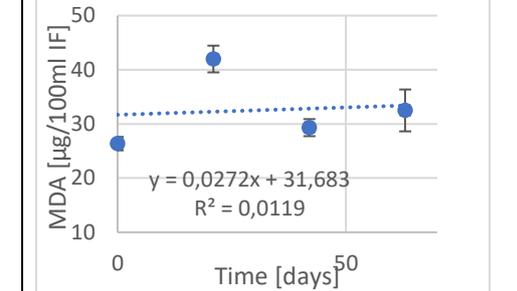
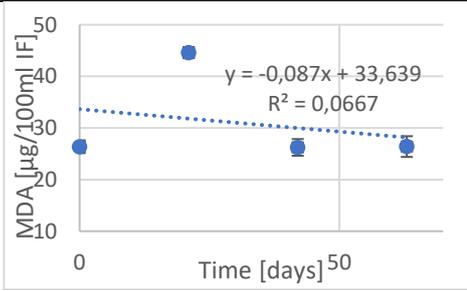
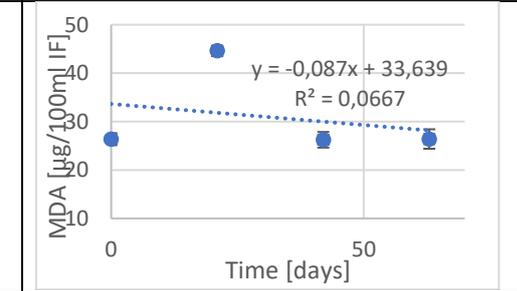
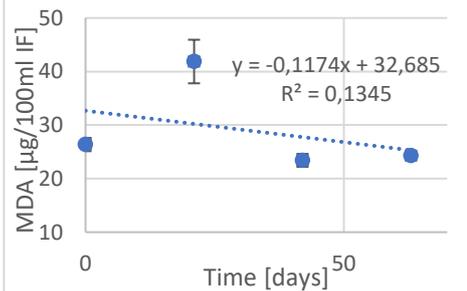
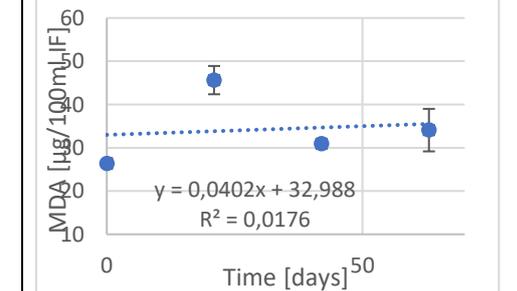
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	26.3664791	0	65535	#NUM!	26.3664791	26.3664791	26.3664791	26.3664791
X Variable 1	0.01972006	0	65535	#NUM!	0.01972006	0.01972006	0.01972006	0.01972006

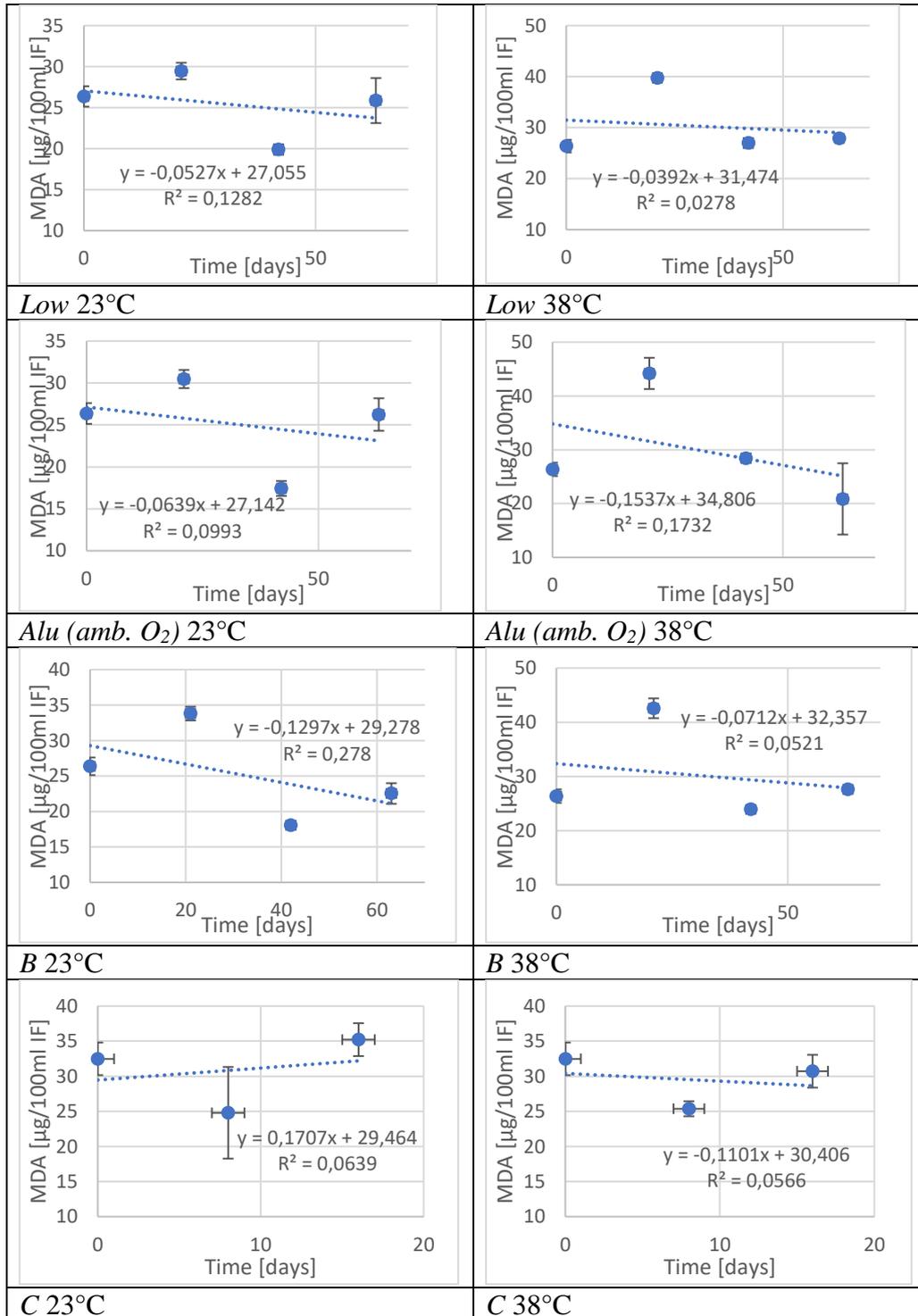
RESIDUAL OUTPUT

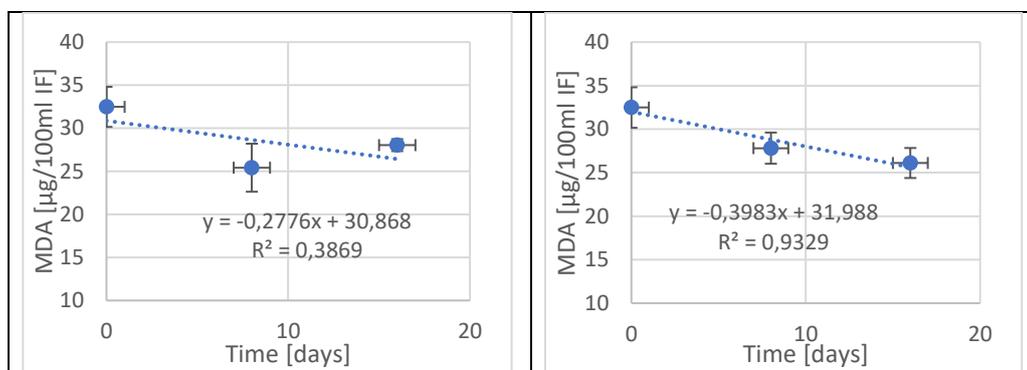
<i>Observation</i>	<i>Predicted Y</i>	<i>Residuals</i>	<i>Standard Residuals</i>
1	26.3664791	0	65535
2	27.6088426	0	65535

# Appendix E

**Table 28: Reaction rates of lipid oxidation in each material for 23°C and 38°C.**

<p><i>Alu</i> 23°C</p>  <p>MDA [<math>\mu\text{g}/100\text{ml IF}</math>]</p> <p>Time [days]</p> <p><math>y = -0,0967x + 28,629</math> <math>R^2 = 0,4023</math></p>	<p><i>Alu</i> 38°C</p>  <p>MDA [<math>\mu\text{g}/100\text{ml IF}</math>]</p> <p>Time [days]</p> <p><math>y = 0,0272x + 31,683</math> <math>R^2 = 0,0119</math></p>
<p><i>Al</i> 23°C</p>  <p>MDA [<math>\mu\text{g}/100\text{ml IF}</math>]</p> <p>Time [days]</p> <p><math>y = -0,087x + 33,639</math> <math>R^2 = 0,0667</math></p>	<p><i>Al</i> 38°C</p>  <p>MDA [<math>\mu\text{g}/100\text{ml IF}</math>]</p> <p>Time [days]</p> <p><math>y = -0,087x + 33,639</math> <math>R^2 = 0,0667</math></p>
<p><i>A2</i> 23°C</p>  <p>MDA [<math>\mu\text{g}/100\text{ml IF}</math>]</p> <p>Time [days]</p> <p><math>y = -0,1174x + 32,685</math> <math>R^2 = 0,1345</math></p>	<p><i>A2</i> 38°C</p>  <p>MDA [<math>\mu\text{g}/100\text{ml IF}</math>]</p> <p>Time [days]</p> <p><math>y = 0,0402x + 32,988</math> <math>R^2 = 0,0176</math></p>
<p><i>A3</i> 23°C</p>	<p><i>A3</i> 38°C</p>





## Appendix F

**Table 29: Leakages detected in different materials after performance of TBARS assay.**

<b>Material</b>	<b>Leakage detected</b>
LowT1A	Yes
LowT1B	Yes
LowT1C	Yes
LowT1D	Yes
LowT2A	Yes
LowT2B	Yes
LowT2C	Yes
LowT3A	Yes
LowT3B	Yes
LowT3C	Yes
LowT3extra	Yes
LowT1NA	Yes
LowT1NB	Yes
LowT1NC	Yes
LowT1ND	Yes
LowT3NA	Yes
LowT3NB	Yes

LowT3NC	Yes
LowT3ND	Yes
A2T1B	Yes
A2T1D	Yes
AluT1RD	Yes
LowT3D	Yes
A2T2C	Yes
A2T3NA	Yes
A2T3ND	Yes
A1T3A	Yes
A1T3B	Yes
A1T3extra	Yes
A1T1NB	Yes
CT1D	Yes
CT2A	Yes
BT1A	Yes
BT1B	Yes
BT1C	Yes
Ref A (B, C)	Yes
Ref B (B, C)	Yes
AluT1ND	Yes

## Appendix G

**Table 30: Residual O<sub>2</sub> concentrations (%) measured in the headspace of each can. Each material was measured in duplicate (values above 2% O<sub>2</sub> were highlighted in yellow); e = error.**

<i>Sample</i>	<i>Time [days]</i>	<i>1.Residual O<sub>2</sub> [%]</i>	<i>2.Residual O<sub>2</sub> [%]</i>
<i>Alu (23°C)</i>	0	0.1	1.0
	21	0.5	0.6
	42	0.6	0.3
	63	e	e

<i>Sample</i>	<i>Time [days]</i>	<i>1.Residual O<sub>2</sub>[%]</i>	<i>2.Residual O<sub>2</sub>[%]</i>
<i>Alu (38°C)</i>	21	0.2	0.5
	42	0.1	0.0
	63	e	e
<i>A1 (23°C)</i>	21	0.2	0.3
	42	0.2	0.1
	63	e	e
<i>A1 (38°C)</i>	21	0.1	0.2
	42	0.0	0.1
	63	e	e
<i>A2 (23°C)</i>	21	0.7	3.6
	42	5.3	4.7
	63	e	e
<i>A2 (38°C)</i>	21	1.9	2.6
	42	8.1	0.7
	63	e	e
<i>A3 (23°C)</i>	21	2.4	0.4
	42	2.0	0.4
	63	e	e
<i>A3 (38°C)</i>	21	1.1	0.8
	42	0.1	0.3
	63	e	e
<i>Low (23°C)</i>	21	19.8	20.7
	42	20.4	20.5
	63	e	e
<i>Low (38°C)</i>	21	20.4	20.4
	42	20.4	20.4
	63	e	e
<i>Alu (ambient O<sub>2</sub>, 23°C)</i>	21	19.9	20.0
	42	19.4	19.5
	63	e	e

<i>Sample</i>	<i>Time [days]</i>	<i>1.Residual O<sub>2</sub>[%]</i>	<i>2.Residual O<sub>2</sub>[%]</i>
<i>Alu (ambient O<sub>2</sub>, 38°C)</i>	21	18.9	18.8
	42	17.4	17.5
	63	e	e
<i>B (23°C)</i>	8	e	e
	16	1.8	2.5
<i>B (38°C)</i>	8	e	e
	16	1.8	0.8
<i>C (23°C)</i>	8	e	e
	16	2.6	2.7
<i>C (38°C)</i>	8	e	e
	16	2.3	1.4