Performance of a New Tetra Recart® Package Storing Oxygen Sensitive Food

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June 2022

Popular Science Summary

Since the 19th century, canning has been a widely used preservation method for foods. Originally, canned foods were stored in metal cans and glass jars since they have excellent ability to prolong the shelf life and preserve nutrients in the food, but did you know that the only part of these packages that comes from a renewable resource is the paper label*? With increasing interest for sustainability, Tetra Pak® launched the Tetra Recart® solution, which is a modern, carton-based alternative to metal cans and glass jars. With 81%* lower carbon emissions and 40%* less energy requirements, Tetra Recart® is expected to be a more environmentally friendly packaging solution. However, Tetra Recart® has a challenge, which is to effectively protect the food from oxygen.

In Europe, more than every fourth household are single households and this number have increased steadily since the 20th century**. This have led to an increased demand of portion sized food packages to avoid food waste in the smaller households. Therefore, Tetra Pak® is now releasing a smaller Tetra Recart® package with a volume of only 100 mL. To make sure that the food, especially food sensitive to oxygen, is safe and attractive to eat after storage in this package, we were asked to investigate this. We choose to focus on the breakdown of vitamin C, colour changes of the product and how the sensory characteristics, such as taste and texture, changed over time. All these factors are affected in a negative way when oxygen is present. Vitamin C is very crucial for the body and health and therefore, it is important to make sure that not all vitamin C is broken down when the food is supposed to be eaten. The colour of food is also very important to control since the food must look appealing. For example, no one wants to eat a brown pineapple. Last but not least, the taste is highly important because everyone wants to eat tasty and delicious food.

From our study, we could see that the food kept its quality over time and that the small package did not cause any unwanted or unexpected changes of the food. Though, it was seen that the temperature that the package was stored in had a large impact on the food quality, where storage in room temperature (25°C) was better than warmer storage. However, the storage temperature does also affect the quality of food packed in metal cans and glass jars. Based on the results, it was concluded that the shelf life will not be affected when stored in Tetra Recart at 25°C, independent of the size of the package. Therefore, the new, smaller Tetra Recart package might be suitable even for oxygen sensitive foods.

* Tetra Pak, n.d. *Tetra Recart Sustainability Infographics*. [Online] Available at: https://www.tetrapak.com/content/dam/tetrapak/media-box/global/en/packaging/package-type/tetra-recart/documents/Tetra_Recart%20Sustainability%20Infographic.pdf [Accessed 19 May 2022]

** Höjer, H., 2014. *Ensamboendet ökar i hela världen och Sverige ligger i topp*. [Online] Available at: https://fof.se/artikel/2014/10/ensamboendet-okar-i-hela-varlden-och-sverige-ligger-i-topp/ [Accessed 19 May 2022]

Abstract

Tetra Recart® is an innovative, carton-based packaging alternative for canned foods. Tetra Pak® has seen an increasing demand of a smaller Tetra Recart® package size. Therefore, the Tetra Recart® package family has been extended with a new, smaller package volume of 100 mL. Package dimensions may impact the shelf life of the packed food, where a smaller package (with headspace) is suspected to have a shorter shelf life compared to larger packages due to larger oxygen-to-food ratio in the smaller sized package. Therefore, verifying shelf life of oxygen sensitive foods in the new, smaller Tetra Recart® package is critical.

In this thesis, two different package sizes (100 mL and 200 mL) were filled with three different food variants (corn in brine, natural mango puree and mango pure enriched with ascorbic acid). Two different storage conditions (25°C and 35°C) were investigated. To evaluate the changes of food quality properties during storage, experimental measurements, and evaluation of ascorbic acid degradation, browning and oxygen changes was performed. Additionally, kinetic modelling of ascorbic acid and browning, as well as a sensory evaluation, were performed.

It was concluded that food quality properties of oxygen sensitive foods were equally maintained during storage in the different package sizes. This means that the larger oxygen-to-food ratio does not seem to impact the shelf life negatively. Though, it was concluded that storage temperature had a large impact on the quality of the packed food. Therefore, oxygen sensitive foods packed in Tetra Recart® of different sizes will most likely have similar shelf lives at the same storage temperature. This indicates that smaller package sizes, that are oxygen critical due to the large oxygen-to-food ratio, may still be suitable for oxygen sensitive foods.

Keywords: Shelf Life, Ascorbic Acid, Vitamin C, Browning, Headspace, Tetra Recart®

Sammanfattning

Tetra Recart® är ett innovativt, kartong-baserat förpackningsalternativ till konserverad mat. Tetra Pak® har sett en ökad efterfrågan på en mindre förpackningsvolym av Tetra Recart®, vilket har gjort att Tetra Recart® utvidgat förpackningsportfolion med en ny, mindre förpackning på 100 mL. Förpackningars dimensioner kan påverka hållbarhetstiden för det förpackade livsmedlet, där mindre förpackningar (med ”headspace”) generellt sett har kortare hållbarhet jämfört med större förpackningar, på grund av större syre-mat förhållande i mindre förpackningar. På grund av detta är det viktigt att verifiera hållbarheten på syrekänslig mat i den nya, mindre Tetra Recart® förpackningen.

I denna uppsats fylldes två olika förpackningsstorlekar (100 mL och 200 mL) med tre olika livsmedelsvarianter (majs i lag, naturlig mangopuré och mangopuré berikad med askorbinsyra). Två olika lagringsförhållanden (25°C och 35°C) studerades. För att utvärdera förändringar av livsmedlenas kvalitetsparametrar under förvaringen utfördes experimentella mätningar, samt utvärdering av, askorbinsyranedbrytninen, färgförändringar och syreförändringar. Dessutom utfördes kinetisk modellering av askorbinsyranedbrytningen och färgförändringarna, samt en sensorisk utvärdering.

Slutsatsen som drogs var att livsmedelskvaliteten hos syrekänsliga livsmedel bibehölls lika bra under lagring i de olika förpackningsstorlekarna. Detta betyder att det större förhållandet mellan syre och livsmedel inte påverkar hållbarheten negativt. Dock hade lagringstemperaturen stor inverkan på kvaliteten på den förpackade maten. Därför kommer syrekänsliga livsmedel förpackade i Tetra Recart® förpackningar med olika storlekar med största sannolikhet att ha liknande hållbarhetstid vid samma lagringstemperatur. Detta indikerar att mindre förpackningsstorlekar, som är syrekritiska på grund av det stora syre-till-mat förhållandet, fortfarande kan vara lämpliga för syrekänsliga livsmedel.

Nyckelord: Hållbarhetstid, Askorbinsyra, C-vitamin, Tetra Recart®

Acknowledgements

This thesis was conducted at Tetra Pak Packaging Solutions AB and the Faculty of Engineering, Lund University from the 17th of January 2022 until the 17th of June 2022.

First, we would like to thank Katarina Flemmer Karlsson who was our supervisor at Tetra Pak®. We are very thankful for her support and guidance throughout our thesis. She was a great source of inspiration, had wise inputs and was always very enthusiastic. During stressful periods, she always cheered us up and had a positive attitude that was contagious.

We would also like to thank Federico Goméz Galindo at the Department of Food Technology, Engineering and Nutrition at Lund University. Thank you for being our academic supervisor, for all the important guidance and feedback throughout the thesis.

We would also like to express our gratitude to the team at Tetra Recart® Food Development Centre at Tetra Pak®. Thank you for letting us borrow equipment and the food laboratory during our experimental work. And a special thank you to Fanny Cedergårdh, Food Technologist at Tetra Pak®, for all help and wise inputs. Especially for preparing us before the filling week and helping us during the filling week. And a warmest thank you to Magnus Cavallin for the tasty cardamom buns.

To Paola Andersson, Rose-Marie Janusz and Carmela Wetter at Tetra Pak®, thank you for giving us instructions on how to use different equipment and for letting us borrow the equipment. And thank you to Martin Gunnarsson for performing the OTR measurements and showing us around in the lab and teaching us about what you work with.

A large thank you to everyone else at Tetra Pak® who have been involved in this thesis project. Peter Larsson, Fredrik Nilsson, Hanna Eliasson, Per Alex, Kristina Helstad, Rickard Preiss, Caroline Malm, Jesper Törnquist and Per Bierlein, we are grateful for those of you who had meetings with us and for everyone who helped us get though our filling week. A special thanks to Camilla Petersson for being a good support at the workspace next to us.

We are grateful to our examiner Andreas Håkansson and opponent Abraham Zamudio for valuable feedback on our report and presentation.

Finally, we would like to thank our families for the support during this master thesis. And especially for being there and supporting us at our presentation.

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List of Abbreviations

ANOVA – Analysis of variance

CB – Corn in brine

CO_2 – Carbon dioxide

DHA – L-dehydroascorbic acid

DKG – 2,3-diketogulonic acid

D.O. – Dissolved oxygen

MN – Mango natural

MA – Mango additive

N_2 – Nitrogen

O_2 – Oxygen

OTR – Oxygen transmission rate

RMSE – Root-mean-square error

TRC – Tetra Recart®

TRC100 – Tetra Recart® 100 mini (100 ml)

TRC200 – Tetra Recart® 200 mini (200 ml)

μ – Average

σ – Standard deviation

1 Introduction

There are many different types of food packaging solutions available on the market. Examples of common types of packages are metal cans, paperboard cartons, plastic bottles, or glass bottles. The role of the food package is to protect the food from outside factors that can impact the integrity, safety, and quality of the food. Examples of such outside factors, also called extrinsic parameters, are microorganisms, light, gases, water and mechanical stresses such as compression forces and vibrations. For some of these outside factors, transfer could occur to the inside environment of the package. The package must therefore be made from materials that can minimise these transfers. Food deterioration of packed foods often increases if the integrity of the package is breached. Appropriate package is therefore of large importance to extend the shelf life of the food. Intrinsic factors, i.e., factors connected to the food itself, can also influence deteriorative reactions in food. Examples of intrinsic factors are oxygen (O_2) content, food formulation and presence of preservatives. The shelf life of a food can be extended by altering the food formulation, the package, the storage conditions and by altering processing parameters (Robertson, 2016; Robertson, 2012)

Canning is an example of a widely used preservation method in the food industry. The term canning means that the food is stored and sterilised in a hermetically sealed container, which prolongs the shelf- life of the food. Several different types of foods can be canned, for example vegetables, fruits, and meat. Most of the nutrients in the food are preserved during canning, which makes it a suitable preservation method. Originally, canned foods were stored in metal cans, for example tin or aluminium (Britannica, n.d). However, new, more sustainable solutions for canned foods have been developed, where one example is Tetra Recart® (TRC) by Tetra Pak. TRC is a carton package solution that is used for different types of foods, for example soups, tomatoes, vegetables, pet foods and sauces. The filled cartons are sterilised with steam at high pressures, which is an in-container sterilisation process called retorting. This makes the TRC packages aseptic, which enables ambient storage and distribution (Tetra Pak (1), n.d). The TRC package is built up from different types of material layers where most of the packaging material comes from a renewable resource (Tetra Pak (2), n.d; Tetra Pak (3), n.d). The material layers consist of polypropylene, adhesive, water-based ink, paperboard with clay coat and aluminium foil, which is illustrated in Figure 1 (Tetra Pak (4), n.d). Polypropylene gives a protective layer with barrier properties against moisture (Robertson, 2016) and works as an adhesive. The paperboard gives the package more stiffness and makes it more robust. The aluminium foil provides barrier properties against light, moisture, and gases (Lamberti & Escher, 2007; Tetra Pak (4), n.d).

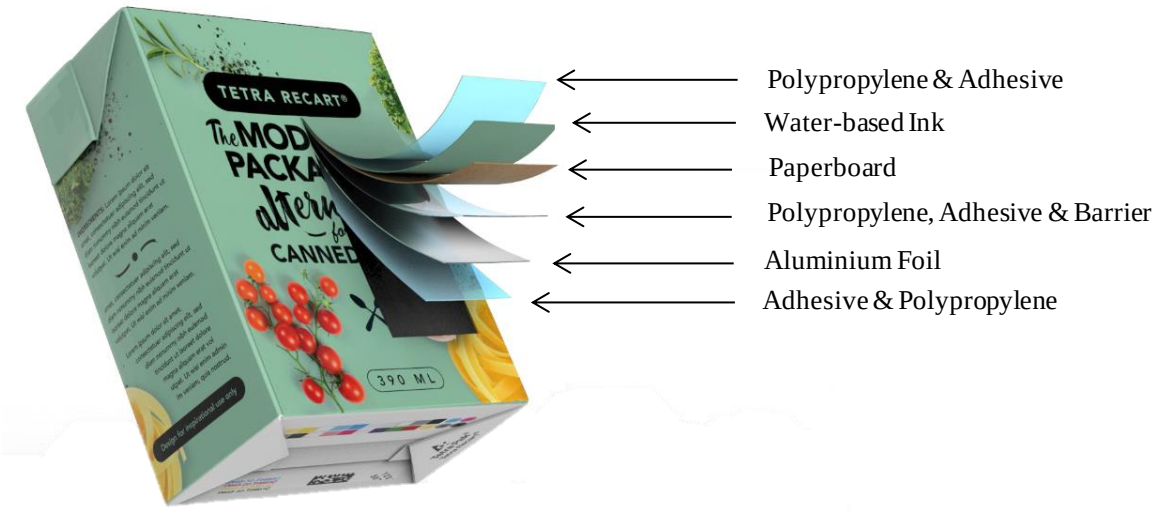

Figure 1. Material layers of TRC package. Modified from (Tetra Pak (4), n.d).

TRC packages are approximately 30% more space efficient compared to its competitor packages such as metal cans and glass cans. TRC packages are also lighter in weight compared to metal cans and glass bottles. The higher space efficiency and lower weight of the packages makes transportation of both empty and filled packages more efficient. TRC packages are also predicted to have lower carbon emissions over its lifetime compared to both metal and glass cans (Tetra Pak (2), n.d; Marwardt & Wellenreuther, 2017).

The TRC packages are available in five sizes ranging from 200 mL to 500 mL (Tetra Pak (2), n.d). Tetra Pak® has seen an increasing demand of a smaller food package size. Therefore, the TRC package family has been extended with a new, smaller package volume of 100 ml, called "TRC 100 mini" (TRC100). The new package size is intended for packaging of, for example, baby food, vegetables and pet food. The TRC package, being a flexible package, does allow some oxygen to permeate through the package, mainly through the longitudinal and transversal seals, which might shorten the shelf life of the packed food. The package dimensions may also impact the shelf life of the packed food, where a smaller package (with headspace) generally has a shorter shelf life compared to larger packages due to larger oxygen-to-food ratio in the smaller sized package. This is because the headspace-to-food ratio and the surface area-to-food ratio are larger for the smaller sized package (Robertson, 2016). Therefore, verifying shelf life of oxygen sensitive foods in the TRC packages of a new, smaller size is critical. As a part of the verification of the new package size, this thesis is a shelf life study of oxygen sensitive foods stored in TRC100 package. To do that, it was decided to study corn in brine (vegetable) and mango puree (baby food) in this thesis. The reason for performing this study is that this smaller package volume is recently developed, and food studies have not yet been performed. Simulations has predicted that the packaging will maintain the food quality, in a similar way as the TRC 200 mini (TRC200) package.

2 Objectives

The aim of this master thesis was to verify that the food quality properties of different foods (mango puree and corn in brine) were maintained during storage in a TRC100 package, i.e., to verify the package's ability to protect the packed food. The aim was also to evaluate if the storage temperature and package volume affects the quality of the packed food.

2.1 Specific Objectives

The purpose of this study was to give an in-depth evaluation of selected factors that are critical for shelf life. To meet the aim and purpose of this master thesis, the following specific objectives were defined as:

- To evaluate how the headspace volume and package volume affects the shelf life
- To evaluate ascorbic acid degradation, browning and total oxygen
- To evaluate kinetics of ascorbic acid degradation and browning
- To evaluate sensory changes over time
- To compare the food quality changes during storage at different temperatures

3 Theoretical Background

3.1 Shelf Life

According to Regulation (EU) No. 1169/2011 in the European Union, shelf life of foods is referred to as "date of minimum durability of a food", which is defined as "the date until which the food retains its specific properties when properly stored." (European Parliament, Council of the European Union, 2011). In practice, this means that shelf life is limited by food deterioration processes causing quality changes and consumer unacceptability. Food quality changes occurring during shelf life can be either microbial, chemical, biochemical, or physical (Moschpoulou, et al., 2019). These quality changes could limit the shelf life of the food by for example impacting the nutritional quality or sensory quality of the food**.** In general, changes in sensory quality are most often the limiting factor for shelf life of foods (Giménez & Ares, 2019). However, products that are shelf stable and stored at ambient temperatures, often has a shelf life that is limited by the chemical and biochemical deterioration reactions, e.g., oxidation reactions and non-enzymatic browning (Calligaris, et al., 2019). This thesis will focus on chemical and biochemical deterioration of food in TRC packages.

There are a lot of factors influencing which food quality change that is the most dominant. i.e., limiting factor of the shelf life, and these factors can be product factors or, alternatively, external factors. Examples of product factors are the raw material composition, product formulation, and availability of oxygen whilst examples of external factors are processing conditions, packaging material and conditions within the package system, temperature, and light exposure (Man, 2016).

3.1.1 Factors Limiting the Shelf Life of Packed Products

A common way to extend shelf life and maintain the quality, as well as the safety of packed foods is to modify the atmosphere in the package. This is a method commonly used to preserve packed fruits and vegetables due to their oxygen sensitivity (Opara, et al., 2019). Generally, O_2 has been seen to increase the rate of deteriorative reactions that decrease the nutritional quality of the food as well as pigment oxidation reactions and browning reactions (Robertson, 2016). Therefore, the atmosphere in the package can be modified by adding for example nitrogen gas (N_2), carbon dioxide gas (CO_2), or gas mixtures. N_2 is an inert gas that has low solubility in most foods and can be used to replace atmospheric air in a package to reduce the O_2 content (Robertson, 2012). CO_2 is often used because of its bacteriostatic and fungistatic properties (Opara, et al., 2019). Physiological processes, biochemical processes and senescence of fruit and vegetables has also been seen to be reduced when having modified packaging consisting of a gas mixture with high CO_2 content (Opara, et al., 2019). In general, when replacing the air with other gases, the deterioration processes in the food is slowed down and the growth of pathogenic or spoiling microorganisms is limited, which extend the shelf life of the packed food (Majou, 2019).The modified atmosphere might be a way to limit the oxygen inside the package, however it is also important to prevent the oxygen supply into the package by having sufficient oxygen barrier properties of the packaging material. In flexible packages, for example carton packages, the supply of oxygen into the package often depends on the packaging material permeability but also on the seals or the closure (Robertson, 2016). To minimise oxygen transmission in flexible packages,

aluminium foil is usually used because if its sufficient oxygen barrier properties. The thickness of the aluminium layer affects the barrier performance, where a thicker layer generates an improved barrier (Morris, 2017). Furthermore, the storage temperature influences the transfer of gas into the food, but also the permeability of the oxygen barrier in the packaging material. Additionally, the rate of deterioration processes and microbial growth increases with increasing temperature (Opara, et al., 2019).

Common chemical and biochemical deterioration reactions in foods are oxidation-reduction reactions in presence of atmospheric oxygen, fat oxidation, hydrolysis, lipolysis, proteolysis, enzymatic browning, and non-enzymatic browning (Kong & Singh, 2016; Man, 2016). These reactions cause for example formation of off-flavours, loss of nutrients and changes in colour and texture. Environmental conditions highly influence the rate of these reactions, where oxygen availability and temperature are two important factors. By adjusting the storage conditions and packaging material, the deterioration of foods can be slowed down. Other factors that may minimise the deterioration rate is to adjust the food formulation and processing. (Kong & Singh, 2016).

Another factor that affects the shelf life of packed food products is the transmission of light, mostly UV light, through the packaging material. Reducing light transmission is important to extend the shelf life, especially important for food susceptible to oxidation reactions. Light could cause different food quality losses such as colour changes and formation of off-flavours. Different packaging materials have different light transmission properties but in general it can be concluded that the better light barrier in the packaging material, the longer shelf life. Examples of packaging materials that may provide sufficient light barrier properties are some types of paper materials and aluminium foil (Morris, 2017).

3.1.1.1 Mango Puree and Corn

Mango puree and corn were chosen as the studied food in this thesis, due to their oxygen sensitivity (Gonzalez-Aguilar, et al., 2010; Becerra-Sanchez & Taylor, 2021). Mango is very sensitive to sensorial and nutritional changes, where loss of antioxidants and vitamins are the most important from a nutritional perspective (Gonzalez-Aguilar, et al., 2010). A study performed by Kaushik, et al. (2017) showed that the shelf life of heat treated mango pulp was extended when stored in refrigerator (5°C) compared to when stored at 37°C. During refrigeration, the shelf life was limited by changes in sensory attributes and by microbial growth. However, when stored at 37°C, the limiting factor for shelf life was browning reactions (Kaushik, et al., 2017). Sweet corn, on the other hand, is especially sensitive to colour changes and sensory changes, where discolouration and loss of sweetness are the major factors. The fresh corn kernels are also sensitive to desiccation and altered texture, which are, in combination with colour and taste, considered the most important characteristics to preserve (Geetha, et al., 2017; Becerra-Sanchez & Taylor, 2021). For canned corn, the major issue affecting shelf life is browning of the kernels. Though, this might be limited by modifying the atmosphere to reduce the presence of oxygen. Like the mango pulp, the shelf life of corn is extended in chilled conditions, compared to ambient or warm conditions. However, freezing and thawing of corn may cause injuries to the kernels, where colour changes, altered texture and off-odours are the most predominant (Becerra-Sanchez & Taylor, 2021).

3.1.1.2 Degradation of Ascorbic Acid

Ascorbic acid, also known as vitamin C, is naturally found in several different fruits. It has shown to have beneficial effects on human health due to its antioxidant properties. It is considered a good indicator of nutritional quality of fruit products due to its relatively high sensitivity to both heat and oxygen (Stešková, et al., 2006; van Bree, et al., 2012). Oxygen can be present in three different ways in cardboard packages; in the headspace, dissolved in the food product and by transmission through the packaging material (van Bree, et al., 2012). Monitoring of the ascorbic acid concentration is therefore an important aspect during storage in TRC packages.

Ascorbic acid can be degraded both aerobically and anaerobically, depending on the circumstances (Villota & Hawkes, 2007). There are several different factors that influence the rate of ascorbic acid degradation, where pH, temperature, and light intensity, as well as presence of oxygen and metallic ions are the most important factors (Zee, et al., 1991). Herbig & Renard (2017) studied the influence of initial ascorbic acid concentration on the degradation rate and concluded that the rate was not affected by the initial concentration in apple puree. However, there are researchers meaning that higher concentrations improve the stability of ascorbic acid. It has been observed that the aerobic degradation rate is highest in food with pH around 7-8 (Herbig & Renard, 2017), but the anaerobic degradation is highest in pH 4. Additionally, the degradation rate increases with increasing storage temperature, at temperatures above 0 °C. Generally, the break-down of ascorbic acid in different food products follow first order kinetics but may be described as second order in oxygen limited conditions (Villota & Hawkes, 2007). Though, the degradation of ascorbic acid depends on several different factors, as mentioned before, which means that the mechanism is specific for each food system and may be described by different kinetic orders (Nkwocha, et al., 2018). In presence of oxygen, ascorbic acid is easily oxidised to L-dehydroascorbic acid (DHA), which is a reversible reaction. The bioavailability of DHA is shown to be similar to the activity of ascorbic acid, but the antioxidant activity of DHA is significantly lower compared to ascorbic acid. However, the bioavailability is lost when it is degraded further by irreversible hydrolysis to 2,3-diketogulonic acid (DKG) (van Bree, et al., 2012; Jutkus, et al., 2015; Villota & Hawkes, 2007). Additionally, the anaerobic degradation pathway may take place even though oxygen is present. The anaerobic pathway is not fully known, but through the anaerobic pathway, the lactone ring in the ascorbic acid molecule is cleaved and may react further through decarboxylation. Both pathways, aerobic and anaerobic, include releasing of CO_2. (Jutkus, et al., 2015). The anaerobic degradation rate is considered slow in room temperature when excess oxygen is present. Therefore, in presence of oxygen, the anaerobic degradation can be considered negligible. In canned products, the dominating degradation is assumed to be aerobic at the beginning, meaning that the degradation rate is high. Upon storage, the oxygen in the package, mainly the headspace, is consumed by oxidation reactions (Herbig & Renard, 2017; Villota & Hawkes, 2007). After the initial depletion where the oxygen is consumed, Oey, et al. (2006) showed that the degradation reaches a plateau where the degradation rate is significantly lower. Then, the main mechanism for ascorbic acid degradation is assumed to be the anaerobic pathway (Villota & Hawkes, 2007). However, the anaerobic pathway has been seen to be significant only at higher temperatures, above approximately 120°C (Herbig & Renard, 2017). The full degradation mechanism of ascorbic acid can be seen in Figure 2 below.

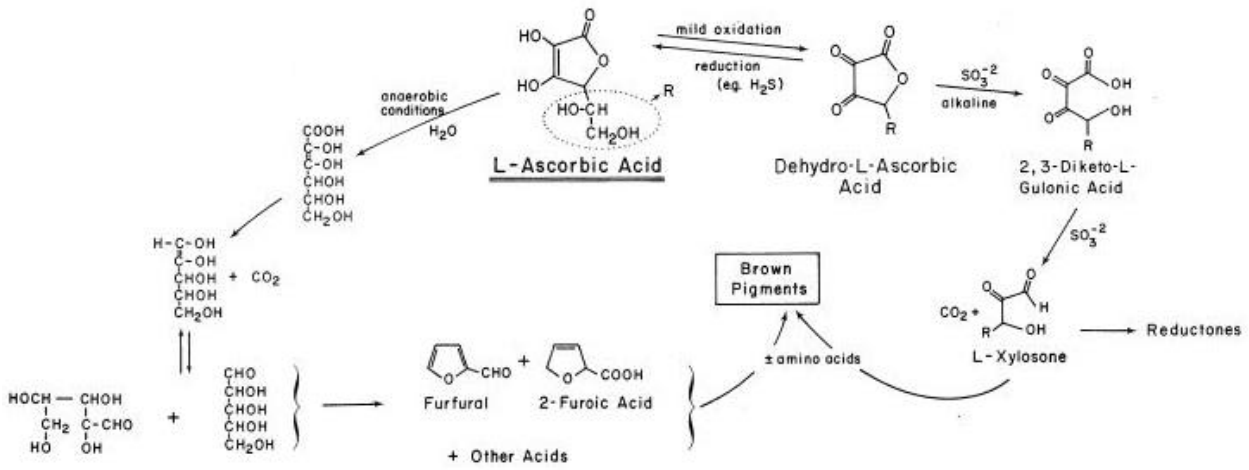

Figure 2. Degradation pathways of ascorbic acid. Modified from (Villota & Hawkes, 2007).

In a storage study conducted by Zee, et al. (1991), it was concluded that different fruits and vegetables showed different ascorbic acid degradation patterns during storage in bins allowing air circulation. Most fruits and vegetables had the greatest ascorbic acid degradation during the first month. One of the studied vegetables was corn, where it was seen that the ascorbic acid degradation was fastest during the first few weeks of storage. In the study, the corn was stored at three different temperatures, 3°C, 8°C and 23°C, where the degradation was fastest at 23°C. However, there was little temperature dependence on the degradation rate of ascorbic acid for the corn (Zee, et al., 1991).

Some naturally present food components might have a stabilising, alternatively destabilising effect and take part of the degradation of ascorbic acid. Therefore, it is important to study kinetic models of ascorbic acid not only in experimental set-ups but also in real food products. Example of food components that have been seen to stabilise ascorbic acid is fructose and glucose. This type of stabilisation has been seen for temperatures between 24°C and 45°C. Another example is the group of phenols that are called flavonols which have also been seen to stabilise and protect ascorbic acid from degradation (Herbig & Renard, 2017). Both sugars and flavonols improve ascorbic acid retention by decreasing the solubility and availability of oxygen in the food products (Giannakourou & Taoukis, 2021). Additionally, Miller & Rice-Evans (1997) showed that the stability of ascorbic acid was higher in fruit juices compared to water, at 37°C. This is probably due to that the polyphenols, that are naturally present if fruits, protect the ascorbic acid from degrading (Miller & Rice-Evans, 1997; Herbig & Renard, 2017). However, high levels of CO_2 in presence of the food have been seen to have a negative effect on the ascorbic acid degradation in fruits and vegetables. The reason behind this is because CO_2 could activate ascorbate peroxidase, which catalyse oxidation of ascorbic acid, resulting in loss of ascorbic acid (Giannakourou & Taoukis, 2021).

Ascorbic acid is commonly added to foods, especially fresh or canned fruits and vegetables, to minimise browning and increase the nutritional value (Stešková, et al., 2006). Because of its antioxidant properties, it prevents browning and other deteriorative oxidation reactions. Other, more stable additives, such as SH-containing compounds, may be added to avoid browning of the

food products. However, ascorbic acid is generally recognised as safe and naturally occurring in most fruits and vegetables (Bharate & Bharate, 2014). In foods and beverages fortified with ascorbic acid it has been seen that the stability of the vitamin is highly affected by temperature, the form of the ascorbic acid as well as the food matrix. The degradation rate of ascorbic acid increases with increasing storage temperature and the rate constant is also affected by the food matrix. One way of protecting the ascorbic acid in food and beverages is by encapsulation where an example is encapsulation by liposomes in liquid foods (Stešková, et al., 2006). To minimise the degradation of ascorbic acid and the related non-enzymatic browning, it is important to keep the headspace volume and the amount of dissolved oxygen as low as possible since ascorbic acid is sensitive to oxygen (van Bree, et al., 2012).

3.1.1.3 Non-Enzymatic Browning

One of the most important factors that affects the quality of foods during heating and long-term storage is the non-enzymatic browning. Non-enzymatic browning is a term for chemical reactions that cause sensory changes, such as colour and taste, without enzymatic activity. The three main chemical reactions that causes non-enzymatic browning is caramelisation, Maillard reaction and ascorbic acid degradation. Caramelisation is a pyrolysis reaction where sugars are oxidised, resulting in a nutty flavour and brown colour. Maillard reaction, on the other hand, is a chemical reaction between a reducing sugar and an amino acid. This is a complex mechanism, resulting in a variety of flavours and odours, as well as a brown colour. Usually, the caramelisation and Maillard reaction require applied heat. Additionally, caramelisation mainly occurs in products with low water content and high sugar content. Maillard reaction, on the other hand, does not occur in food products with high acidity (pH below 4.0). Because of this, neither caramelisation nor Maillard reactions are a big quality issue during ambient storage of fruit and vegetable products (Bharate & Bharate, 2014).

The major type of non-enzymatic browning that is contributing to quality changes in stored fruit products is the degradation of ascorbic acid. As described in *3.1.1.2 Degradation of* one of the products when ascorbic acid is degraded is DKG. This molecule can be degraded to xylosone, as seen in Figure 2. The anaerobic pathway may end up with furfural and 2-furan carboxylic acid as products. In presence of amino acids, all these compounds can form brown pigments, mainly different furans, through Strecker degradation, which causes visible colour changes (Bharate & Bharate, 2014; Jutkus, et al., 2015). Furthermore, Kacem, et al. (1987) studied how different ascorbic acid and amino acid levels affect browning of orange juice. The results showed that high levels of ascorbic acid increased the non-enzymatic browning, while high amino acid levels did not affect the rate of browning reactions. Therefore, the conclusion of the research was that the ascorbic acid content is critical for browning to occur.

3.1.1.4 Enzymatic Browning

Browning in fruit and vegetables may also be caused by enzymatic activities. The most well-known enzyme responsible for enzymatic browning is called polyphenol oxidase and is naturally present in most fruits and vegetables. The mechanism of polyphenol oxidase is based on oxidation of phenolic compounds in the food. When the tissue of the fruit or vegetable is disrupted in any

way, like cut or peeled, the polyphenol oxidase is released and activated, which catalyses a reaction between the phenolic compounds and oxygen. The phenolic compounds are converted by the polyphenol oxidase to melanins, which gives the food product a brown colour (Mesquita & Queiroz, 2013).

Most of the polyphenol oxidase found in fruits and vegetables have highest activity between pH 5.0-7.0 and at 25-37°C. The limiting factor of the enzymatic browning is mainly the dissolved oxygen in the food since it is required for the oxidation reaction to occur. Enzymatic browning can therefore be minimised by controlling a few different parameters. By removing the oxygen present in contact with the food, the browning can be avoided since the enzyme requires oxygen. This can be done by for example using modified atmosphere. Addition of ascorbic acid or citric acid may also minimise the enzymatic browning by decreasing the pH and act as antioxidant. Additionally, ascorbic acid will use up the oxygen present in the product and in that way limit the enzymatic browning (Mesquita & Queiroz, 2013). However, the most widely used method to minimise enzymatic browning in fruit purees is to inactivate the polyphenol oxidase by heat treatment of the food. When exposing the enzymes to temperatures typically between 70-90°C, their catalytic activity is lost (Chutintrasri & Noomhorm, 2006). At those temperatures, the time required to ensure low enzymatic activity is minimum 5 minutes. Additionally, heat treatment eliminates microorganisms, which ensures safe consumption of the food (Mesquita & Queiroz, 2013).

3.1.2 Evaluation of Shelf Life

Storage trials are often part of shelf life studies. The aim of these trials is often to learn about changes that occur in the food during storage and to determine the quality at the end of shelf life of a food product. The trials are often conducted after the food safety has been confirmed. Usually, they are conducted during a period of time that is long enough to reach the point where the food reaches the end of its shelf life and becomes unacceptable to consumers. One example of a storage trial protocol of shelf-stable foods is to store it for 18 months or longer and perform sampling at month 0, 1, 2, 3, 6, 12, 18 and perhaps longer. Measurements or analysis methods used to measure the shelf life progress are for example microbial analysis, chemical analysis, instrumental measurements as well as sensory evaluation (Man, 2016).

The storage trials are usually conducted under conditions that are similar to the intended storage, distribution, consumer usage etc., of that particular product. However, a complete and comprehensive storage trial is rarely done since it is difficult to perform. The storage conditions, based on temperature, humidity, light etc., during storage trials are often fixed, i.e., not varying. Common storage trials conditions that are fixed are frozen conditions at -18°C and approximately 100% relative humidity, chilled conditions at 0-5°C at high relative humidity, 25°C at 75% relative humidity and tropical conditions at 38°C at 90% relative humidity (Man, 2016).

The time period of which a specific product maintains its sensory properties, before becoming unacceptable, is called the sensory shelf life. The sensory shelf life is often determined by using sensory evaluation techniques on food that have been stored for different time periods. One experimental design that is used in sensory shelf life evaluation to get food samples with different storage times is the so called reversed design. The reversed design means that food samples with

different storage times are evaluated at the same occasion. The food samples with different storage times can be achieved by for example storing the food product at desired conditions until the sampling points. At the sampling point, the product sample is moved to storage conditions, for example freezer or fridge, where deterioration processes are slowed down or most desirably stopped. However, it is important that the storage conditions used to slow down the deterioration rate does not impact the sensory characteristics of the food. Freezing is not optimal to slow down the deterioration processes of unprocessed and minimally processed vegetables and fruits (Giménez & Ares, 2019). As mentioned in *3.1.1.1 Mango Puree and Corn*, the reason for this is because freezing may alter the texture of fruits and vegetables (Becerra-Sanchez & Taylor, 2021). However, freezing has also been seen to preserve ascorbic acid in fruits and vegetables in an efficient way (Giannakourou & Taoukis, 2021).

3.1.3 Kinetic Modelling

A common method used to predict quality changes in food is kinetic modelling and identification of kinetic parameters. Kinetic modelling can be applied in evaluation of quality-related chemical changes, such as ascorbic acid and colour. This is done by connecting kinetic models, see Equation 1 below, to experimental data (van Boekel, 2021).

$$\frac{dc}{dt} = k_r \cdot C^{n_t} \tag{1}$$

The equation includes concentration of analysed parameter, *C*, and two kinetic parameters: the rate constant, k_r, and the reaction order, n_t. Reaction orders in food science are assumed to be either zero, first, or second (van Boekel, 2021). Generally, ascorbic acid degradation is described best by first-order reactions, but it depends on the processing of the food (van Bree, et al., 2012). Furthermore, colour changes in foods are usually best described by zero-order kinetics (Wibowo, et al., 2015) or first-order kinetics (Ahmed, et al., 2002). Depending on the reaction order, the kinetic model (Equation 1) looks different. The model associated with each reaction order is displayed in Equation 2-4 below, where Equation 2 represent zero-order (n=0), Equation 3 represent first-order (n=1) and Equation 4 represent second-order (n=2) (Nkwocha, et al., 2018).

$$C = C_0 - k_r t \tag{2}$$

$$\ln(C) = \ln(C_0) - k_r t \tag{3}$$

$$\frac{1}{C} = \frac{1}{C_0} + k_r t \tag{4}$$

In addition to the kinetic model described above, the half time, $t_{1/2}$ of ascorbic acid can be determined. The half time is described as the time it takes for the ascorbic acid to reach 50% of the initial concentration. It can be calculated according to equation 5-7 below, depending on the reaction order, where Equation 5 is applicable for zero-order, Equation 6 for first-order and Equation 7 for second-order (Jutkus, et al., 2015).

$$t_{1/2} = \frac{C_0}{2k_r} \tag{5}$$

$$t_{1/2} = \frac{\ln(2)}{k_r} \tag{6}$$

$$t_{1/2} = \frac{1}{k_r \cdot C_0} \quad (7)$$

At 26.7°C, ascorbic acid in most canned vegetables have a *k*-value around 10-20×10^{-8} min^{-1}, while ascorbic acid in most canned fruits have a *k*-value between 50-70×10^{-8} min (Villota & Hawkes, 2007). In Table 1 below, the kinetic parameter, as well as the half time for ascorbic acid degradation of a few, selected canned fruits and vegetables are summarised.

Table 1. Kinetic parameters for ascorbic acid degradation in canned foods at 26.7°C (Villota & Hawkes, 2007).

Food type		$k_{26.7}$ **[min^{-1}]**	$t_{1/2}$ **[min]**
Canned vegetables	Corn (yellow)	18.8×10^{-8}	3.7×10^{6}
	Peas (sweet)	18.0×10^{-8}	3.9×10^{6}
Canned fruits	Apricots	53.2×10^{-8}	1.3×10^{6}
	Orange juice	68.5×10^{-8}	1.0×10^{6}
	Peaches	56.9×10^{-8}	1.2×10^{6}
	Pineapple	67.6×10^{-8}	1.0×10^{6}

In a study performed by Wibowo, et al. (2015), the *k*-values of ascorbic acid degradation in mango juice, stored in a PET bottle, was determined with first-order models at 42°C. The study showed that the k_{42}-value of ascorbic acid degradation was higher for mango juice with 230 mg/L and 460 mg/L added ascorbic acid (k_{42}=0.19 $week^{-1}$ and k_{42}=0.17 $week^{-1}$ respectively), compared to natural mango juice (k_{42}=0.04 $week^{-1}$) (Wibowo, et al., 2015).

Ahmed, et al. (2002) studied colour degradation in mango puree, with regards to the *b** value (see explanation of *b** value in *5.3.5 Colour Measurement and Pictures*). The *b** value was considered responsible for colour degradation, since it describes yellow colour changes, and the main colour of mango puree is yellow. According to the study, the colour degradation (*b**) of mango puree was best described by first-order kinetic models (Ahmed, et al., 2002).

4 Experimental Overview

The overall methodology approach of this master thesis included three different parts (see Figure 3). All three parts were integrated and critical to evaluate the changes of food quality properties during storage, i.e., the package ability to protect the packed food. The first part was experimental measurements and evaluation of ascorbic acid degradation, browning and total oxygen changes. The purpose of the first part was to evaluate how oxygen (headspace, dissolved oxygen and OTR) affect the shelf life of foods in TRC100. Due to the oxygen sensitivity of ascorbic acid, it makes it a good indicator of shelf life of oxygen sensitive foods. Additionally, it was evaluated if ascorbic acid affects browning since that is one of the main quality issues with processed fruits and vegetables. The second part was kinetic modelling of ascorbic acid and browning. This part was conducted to estimate the ascorbic acid content and colour at the end of shelf life. The last part was performance of a sensory analysis to evaluate if any sensory characteristics changed during the first 12 weeks of storage. The three parts were performed to evaluate if the shelf life of fruits and vegetables stored in TRC100 was different compared to storage in TRC200. All analyses were conducted on packages stored at 25°C and 35°C to evaluate how the storage temperature affects shelf life.

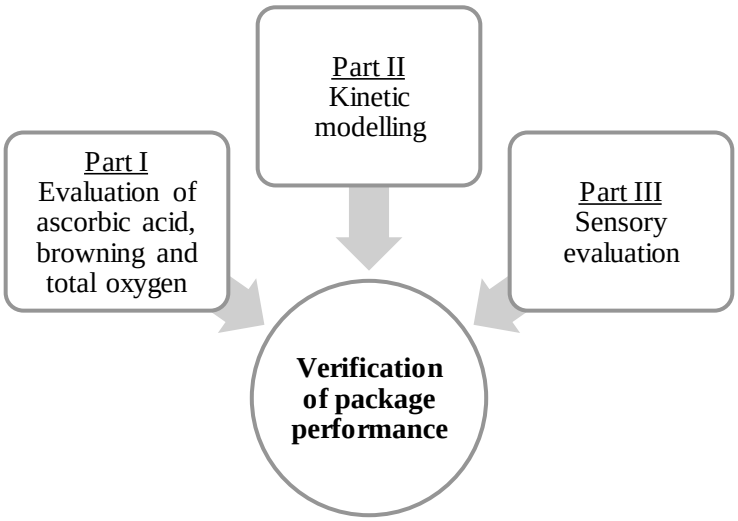

Figure 3. Overall approach of this master thesis, including three integrated parts.

5 Material and Methods

5.1 Raw Materials

The nutritional values of the raw materials, mango puree and corn, used in this study can be seen in Appendix *A. Nutritional Values*, and the brix, i.e., total soluble solids (Zoecklein, et al., 2010), was given to be 19±2°. The mango puree was from the company Les Vergers Boiron (France) and the corn was from the company Magnihill (Sweden). Ascorbic acid from the company Santa Maria (Sweden) and salt from the company JOZO (Denmark) were other raw materials used in this study.

5.2 Sample Preparation and Processing

Three different food variants were made for this study. Two of the variants consisted of mango puree, where one was natural mango puree without any additions (called "MN") and the other one was mango puree with addition of 630 mg/L of ascorbic acid (called "MA"). The addition of ascorbic acid to the mango puree was 630 mg/L in order to achieve an initial ascorbic acid concentration of approximately 800 mg/L, including the naturally present ascorbic acid. The third food variant was corn in brine (called "CB") where the brine contained ascorbic acid and salt. The initial amount of ascorbic acid in the brine was 800 mg/L, together with 15 g salt per litre brine. The reasoning behind the chosen ascorbic acid concentrations of the mango puree, as well as the brine can be seen in Appendix *B. Reasoning Behind the Ascorbic Acid Additions*.

All sample variants that were part of this study are summarised in Table 2. Both TRC100 and TRC200 were studied in this thesis, to be able to evaluate how the package volume affects the package performance. Additionally, the TRC100 packages were stored both at 25°C and 35°C. The reason for this is because the TRC packaging is designed for ambient storage. However, since this study was only for 12 weeks, it was decided to store packages at 35°C as well to increase the rate of deterioration reactions. According to Ashurst (2016), storage at 35°C makes the deterioration reactions occur 3-4 times faster compared to ambient conditions. This means that storage at 35°C for 12 weeks should correspond to storage for up to 1 year at ambient temperature.

Table 2. Summary of the different sample variants.

Food	**Sample abbreviation**	**Added ascorbic acid [mg/L]**	**Headspace content**	**Package type**	**Package volume [mL]**	**Storage temperature [°C]**
Mango Natural	MN/100/25	0	N_2	TRC100	100	25
	MN/100/35			TRC100	100	35
	MN/200/25			TRC200	200	25
Mango Additive	MA/100/25	630	N_2	TRC100	100	25
	MA/100/35			TRC100	100	35
	MA/200/25			TRC200	200	25
Corn in Brine	CB/100/25	800	N_2	TRC100	100	25
	CB/100/35			TRC100	100	35
	CB/200/25			TRC200	200	25

The process procedure from food preparation until storage of filled packages can be seen in Figure 4. Food was first prepared and then filled in package and sealed in a commercial Tetra Recart filling machine called "Tetra Pak R2 0600" (Tetra Pak, Sweden). Nitrogen flush, i.e., replacement of air in package headspace with nitrogen gas, was used in the filling machine. The filled packages where then retorted according to recommendations at Tetra Pak. The MN and MA was retorted at 85°C until the lethality was 6 min and the CB was retorted at 125°C until the lethality was 8 min. After the heat treatment, the packages were dried and put into storage chambers.

Figure 4. Process procedure from food preparation until storage of filled packages.

5.3 Analyses

The analyses that were performed in this study are summarised in Table 3. All measurements were done in triplicates (n=3 different packages) and performed at room temperature if nothing else was specified.

Table 3. Summary of measurements/evaluations, method/equipment, and measurement frequency.

Analysis	Method/Equipment	Frequency
Oxygen concentration in headspace	Gas analyser	Once per week
Headspace volume	Burette and water bath	Every sixth week
Dissolved oxygen concentration	DO-meter	Once per week
Concentration of ascorbic acid	Titration	Once per week
Colour measurements and pictures	Digi-Eye	Once per week
pH	pH-meter	Once per week
Sensory evaluation	Forced, paired preference test	Once after 11 weeks

In addition to the methods presented in Table 3, microbial analysis was performed to verify the product safety and to make sure that the product was safe to consume during the study. Microbial plating was used for the packaged corn in brine, in combination with pH evaluation. Observation of swelling and pH evaluation was used to check for spoilage in the packaged mango puree. The microbial plating was done on petri dishes containing Plate Count Agar (PCA), which is a non-selective growth medium (Emam-Djomeh, et al., 2016). The methods were performed according to Tetra Pak method specifications.

There were some delays in the measurements in the beginning of the study. "week 0" denotes the week where the filled packages were produced and "week 12" denotes twelve weeks of storage, after week 0. All samples were collected from the storage rooms on time, i.e., collected once per week from week 0 until week 12. However, the measurement of the ascorbic acid concentration for the samples collected during week 0 was performed two weeks later than planned and the measurement for the samples collected during week 1 was performed one week later than planned. The measurements of headspace volume, oxygen content in headspace and dissolved oxygen for

the samples taken during week 0 was performed one week later than planned. The packages were stored at 4°C until the measurements were able to be performed to minimise the deterioration reactions. Except for the delays described above, all measurements were performed on time, i.e., the same week as the samples were collected.

5.3.1 Oxygen Concentration in Headspace

The percentage of oxygen in the headspace of a package was measured with a destructive test method. The measurement was performed using an equipment called CheckPoint 3 (Ametek Mocon, US) equipped with a ceramic solid-state sensor and other materials used were needles, sample gas filters and septums, see Figure 5 (Ametek Mocon, n.d). The measurement was performed according to a Tetra Pak method specification.

Figure 5. Checkpoint 3 with connected needle and filter. Septums to the right.

A needle and a sample gas filter were connected to a sensor on the equipment. A septum was placed at the top corner of a package and then a needle was inserted in the package through the septum. The test was then started by pressing a button and a sample of gas was drawn into the needle. After a few seconds, the O_2 and CO_2 percentage of the gas was displayed. The sensor had an accuracy of ±0.1% and ±2% of readout (Ametek Mocon, n.d).

5.3.2 Headspace Volume

The gas volume in the headspace of a package, also called headspace volume, was measured with a destructive test method. The material needed for the headspace volume measurement was a burette, pileus ball and water bath. The purpose of performing this measurement was to determine the headspace volume, to be able to convert the oxygen concentration in headspace from a percentage into a volume.

The measurement was performed by placing a burette, with a cone-shaped bottom, in a water bath (see Figure 6). A pileus ball was used to fill the burette with water. The TRC packaging was opened with a scissor under the water-filled burette so that the headspace gas could bubble up into the burette. Then, the reduced water volume in the burette corresponded to the headspace gas volume.

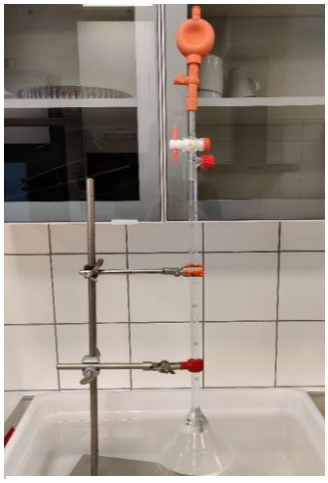

Figure 6. Water bath, burette and pileus ball used for headspace volume measurements

5.3.3 Dissolved Oxygen Concentration

An optical dissolved oxygen sensor, FDO 925 (WTW, Germany), was used together with a dissolved oxygen meter, Oxi 3310 (WTW, Germany), to measure the dissolved oxygen in the food samples (see Figure 7). The sensor had a measuring range from 0-20 mg/L and an accuracy of 1.5% of the measured value at 20°C. To be able to measure the dissolved oxygen in a sample, the sensor should be immersed in the sample, with a minimum immersion depth of 6 cm (WTW, n.d). When the sensor is immersed in the sample, the dissolved oxygen passes through the membrane of the sensor. Inside the sensor, there is a dye that interacts with the oxygen, causing a reduction of the intensity of the luminescence of the dye. The reduction is detected by a photodetector inside the sensor, which is converted to a dissolved oxygen concentration, based on the fact that the intensity reduction is proportional to the oxygen concentration (Ramamoorthy, et al., 2003). The measurement was performed according to a Tetra Pak method specification.

Figure 7. Oxi 3310 D.O. meter with a connected FDO 925 sensor.

The dissolved oxygen concentration, together with headspace volume and oxygen concentration in headspace, described in chapter *5.3.1 Oxygen Concentration in Headspace* and *5.3.3 Dissolved Oxygen Concentration*, can be used to calculate the total oxygen in the package. The Equation 8 used can be seen below,

$$V_{O_2} = x_{O_2\ in\ HS} \cdot V_{HS} + DO \cdot V_{product} \cdot \frac{1}{\rho_{O_2}} \tag{8}$$

where V_{O_2}= total oxygen content [mL], $x_{O_2\ in\ HS}$ = oxygen concentration in headspace [%], V_{HS} = headspace volume [ml], DO = dissolved oxygen [mg/L], $V_{product}$ = volume of filling product [L], ρ_{O_2}= density of oxygen [mg/mL]. The density of oxygen gas was calculated with the ideal gas law and was calculated to be 1.31 mg/mL (at 25°C and 1 atm).

The oxygen consumption from timepoint t_0 [day] to timepoint t [day] can be calculated with Equation 9 below,

$$\Delta O_2 = V_{O_2,t} - V_{O_2,t_0} + OTR \cdot (t - t_0) \tag{9}$$

where ΔO_2 = oxygen consumption from t_0 to t [mL], V_{O_2,t_0}= total oxygen content at t_0 [mL], $V_{O_2,t}$ = total oxygen content at t [mL], OTR = oxygen transmission rate [mL/package/day].

5.3.4 Concentration of Ascorbic Acid

Ascorbic acid concentration was determined with a direct iodimetric titration method, using linear titration. The method is based on changes in voltage in the sample and the principle behind the method is described as follows. When adding iodine to a solution with ascorbic acid, the iodine is converted to colourless iodide (I^-) according to the reaction below. At the same time, hydrogen ions (H^+) are formed in equal quantity, causing a change in voltage of the solution. During the titration, the ascorbic acid is depleted, which disables the reaction to continue. This means that the iodine concentration will increase, which causes the colour of the solution to switch from clear to blue. The point at where the ascorbic acid is fully consumed, and the colour change takes place is the equivalence point. When the amount of added iodine at the equivalence point is known, the concentration of ascorbic acid in the sample can be calculated (Kerr, et al., 2019).

$$I_2(aq) + C_6H_8O_6(aq) \rightarrow 2H^+(aq) + 2I^-(aq) + C_6H_6O_6(aq)$$

The titration equipment that was used was TitroLine 7000 (SI Analytic, Germany) with a Pt62 electrode (SI Analytics, Germany), see Figure 8. Chemicals that were used were iodine solution 0.05 M (BDH Chemicals, United Kingdom) which was used as reagent, sodium thiosulfate solution 0.1 M (Merck KGaA, Germany), oxalic acid solution 5% (Merck KGaA, Germany) and distilled water. The settings that were used during the titrations were the following: reagent step size of 0.010 mL or 0.020 mL and reagent pretitration of 0.100 mL or 0.400 mL depending on the concentration ascorbic acid in the sample. The measurements were performed according to a Tetra Pak method specification.

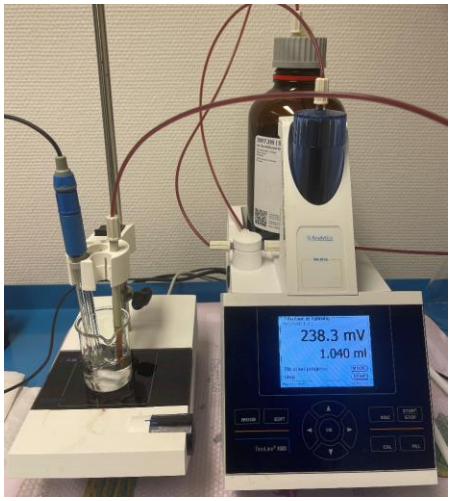

Figure 8. Titration equipment TitroLine 7000 equipped with a Pt62 electrode.

The equipment was first prepared by rinsing the burette inside the titration equipment with distilled water two times and after that the burette was rinsed with iodine solution (0.05 M) three times. First, the calibration was performed by running measurements of samples containing 2 g sodium thiosulfate solution (0.1 M) mixed with approximately 20 g of distilled water. The sample preparation for the product measurements were done by mixing 20 g of mango puree alternatively corn brine together with at least 20 g of oxalic acid solution (5%). The role of the oxalic acid solution is to prevent the ascorbic acid from being oxidised by atmospheric oxygen before the

analysis is performed (Xing, et al., 2021). Therefore, it was of importance to add the oxalic acid to the sample directly after opening a packaging containing the food sample. The exact weight of the sodium thiosulfate solution and food sample (mango puree alternatively corn brine) was weighed and noted down and then inserted into the TitroLine 7000 equipment.

The titration was performed by placing a magnetic stirrer in the beaker containing the sample to be measured. The beaker was placed on the stirrer plate of the TitroLine 7000 equipment. The Pt62 electrode and burette tip was lowered so that the tips were underneath the sample surface, see Figure 8. The exact weight of sodium thiosulfate solution alternatively food sample, depending on if the measurement was a calibration measurement or a product measurement, was inserted into the machine and then the titration was started. During the measurements, the samples were stirred with magnetic stirrers. Both the burette tip and the Pt62 electrode was rinsed with distilled water in between each measurement.

5.3.5 Colour Measurement and Pictures

Colour measurements were done by using an equipment from Cromocol (Sweden) called "Digi-Eye – DigiPix System S/N DE00296" (see Figure 9) and a computer programme called "Digi Production" (Cromocol, Sweden). The equipment was also used to take pictures of all samples that were analysed for colour changes. The measurements were performed according to a Tetra Pak method specification. The colour measurement of the corn was performed on mixed corn due to the inhomogeneous colour of the whole corn kernels.

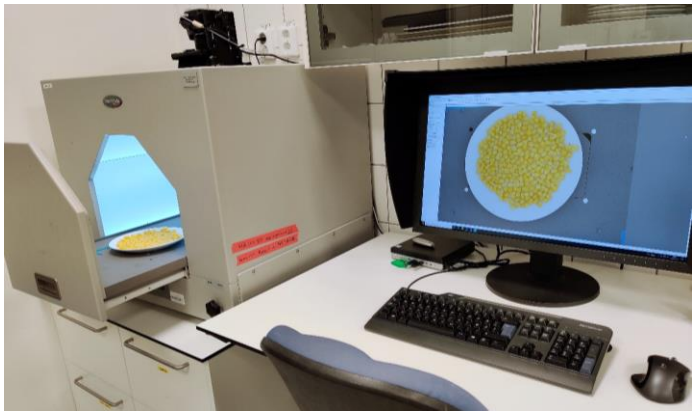

Figure 9. Digi-Eye – DigiPix System S/N DE00296 and computer with Digi Production application.

The aperture of the camera was set to 6.3 and the shutter speed was set to 1/25. The equipment was first calibrated by using a white uniformity board and a colour palate board. Samples were prepared by pouring the food sample (mango puree alternatively mixed corn) on a plate and then placing the plate inside the image acquisition chamber. Pictures were captured and the colour was analysed with the CIELAB colour system. This colour system is three-dimensional and consists of three colour axes: L^*, a^* and b^*. The L^* value represents lightness and ranges from 0 to 100, where 0 indicates black and 100 indicates white. A negative (-) a* value indicates green whilst a positive (+) value indicates red. A negative (-) b^* value indicates blue whilst a positive (+) b^* value indicates yellow (Ly, et al., 2020). ΔE^*, which is the total colour difference during storage, can be determined with Equation 10,

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (10)$$

where $\Delta L^* = L^* - L_0^*$, $\Delta a^* = a^* - a_0^*$ and $\Delta b^* = b^* - b_0^*$. L_0^*, a_0^* and b_0^* represents the colour coordinates of the food samples at time point zero.

If ΔE^* has a value between 0 and 0.5 it can be interpreted as "not noticeable", a value between 0.5 and 1.5 as "slightly noticeable", a value between 1.5 and 3.0 as "noticeable", a value between 3.0 and 6.0 as "well visible" and a value greater than 6.0 as "great" (Wibowo, et al., 2015).

5.3.6 pH Measurement

The pH was measured using a pH-meter (inoLab pH Level 1, WTW, Germany). The equipment was calibrated before usage and the measurement was performed according to Tetra Pak method specifications.

5.3.7 Sensory Evaluation

To evaluate if the sensory attributes of the foods were affected during storage, a paired preference test, which is a type of affective test, was performed. This test was done to evaluate if there were any difference in sensory attributes after 0 and 11 weeks of storage at 25°C in TRC100. The test was performed with 48 untrained panellists. To ensure reliable results, it was not recommended to have less than 20 panellists (Lawless & Heymann, 2010). The untrained panellists were presented with two samples, in randomised order, and asked to indicate which sample that was preferred. The panellists were forced to make a preference choice, i.e., no "no preference" option was included. No communication was allowed during the test. The ballot used for the sensory evaluation can be found in Appendix *C. Sensory Evaluation*.

The results from the test were evaluated with table 13.2 in Lawless & Heymann (2010), which was based on binominal distribution and a two-tailed *Z*-score of 1.96 for a significance level of 95% (α=0.05). Knowing the total number of panellists, the table indicated how many panellists that needed to indicate a preference of one of the samples to give a significant preference difference.

5.3.8 Kinetic Modelling

The reaction order of the ascorbic acid degradation was determined by plotting the experimental data in a linearised form, according to the method described by van Boekel (2021). If C/C_0 is plotted against time and gives a straight line, it can be assumed to be a zero-order reaction. First-order reaction is assumed when $\log(C/C_0)$ versus time gives a straight line. If C_0/C versus time gives a straight line, it is assumed to be a second-order reaction (van Boekel, 2021). To evaluate which reaction order that describes the reaction best, a regression analysis was performed to determine "goodness of fit". This was done by determining the coefficient of determination (R^2) and root mean sum of errors (*RMSE*) (Nkwocha, et al., 2018). According to Nkwocha, et al. (2018), the model with R^2 closest to 1.0 and the lowest *RMSE* can be considered the best.

When the reaction order was determined, a kinetic model could be applied according to the theory presented in *3.1.3 Kinetic Modelling*. From the model, it was possible to extrapolate the curve and estimate the values at end of shelf life.

5.3.9 Statistical Evaluation

The results were statistically evaluated using one-way analysis of variance (ANOVA) to control significant differences and Dixon's test (*Q*-test) to check for outliers.

5.3.9.1 ANOVA

One-way ANOVA was used for comparison of the mean values of different variants. It assumes that the data is normally distributed and that the variance between the groups are unequal. To check if the data was normally distributed and if the variance was equal, Kolmogorov-Smirnov test and Bartlett's test were used. Additionally, to perform ANOVA, it required that the measurements were independent. The null hypothesis used, stated that the mean values of all groups are the same, i.e., H_0: $\mu_1=\mu_2$. If the ANOVA test generated a *P*-value below 0.05, the null hypothesis was rejected, which means that there was a difference between some variants. However, it did not determine which variants that differed. To check this, Tukey's post hoc analysis was used (Smalheiser, 2017). All these tests were performed in Minitab (LLC, Pennsylvania) with a significance level of 95%.

5.3.9.2 Dixon's Test (Q-Test)

When one value within a set of results from a measurement differs unreasonably much, it might be considered an outlier. To control if the deviating value can be considered an outlier, the most commonly used method is "Grubbs' test". This test is an ISO recommended test, which compares the difference between the mean value and the suspected outlier with the standard deviation. However, when checking for outliers in a small sample size ($3\leq n\leq 7$), a common and simple method to use is "Dixon's test". Dixon's test, also called *Q*-test, compares the difference between the suspected outlier and the nearest value with the range of the whole sample. Both the previously mentioned methods assume normally distributed data (Miller & Miller, 2005). Dixon's test was the method used to check for outliers in this thesis and it was calculated with Equation 11,

$$Q = \frac{|suspected\ value - nearest\ value|}{largest\ value - smallest\ value} \tag{11}$$

The calculated *Q* value was compared with a critical *Q* value (Q_{crit}), see Table D1 in Appendix *D. Dixon's Test (Q-Test)*. The chosen significance level was 95%. When the calculated *Q* value exceeded the Q_{crit} value, the suspected value could be considered an outlier. This means that it could be rejected from the data set (Miller & Miller, 2005).

6 Results and Discussion

6.1 Ascorbic Acid, Browning, and Total Oxygen

6.1.1 Changes of Oxygen Content

The headspace volume of the packages was assumed to be stable over time and the average headspace volume of each variant is displayed in Table 4. The CB/100 had a smaller headspace volume compared to the rest of the variants, which might be due to that there were problems when filling the product, resulting in large weight variations between different packages. Except for this, the difference in headspace volume of the different variants was small, and the results mean that the headspace-to-food ratio was larger for TRC100 compared to TRC200. This was in accordance with Robertson (2016) which stated that, generally, there is a larger headspace-to-food ratio in smaller sized packages, see Chapter *1 Introduction*.

Table 4. Headspace volume [mL] presented as $\mu \pm 2\sigma$.

	MN/100	**MN/200**	**MA/100**	**MA/200**	**CB/100**	**CB/200**
Headspace volume [mL]	16±2	18±3	16±2	20±2	11±3	20±3

The oxygen content in headspace, dissolved oxygen in the products and the total oxygen content remained stable for all variants during the storage period of 12 weeks (see Figure E1 in Appendix *E. Total Oxygen Content Over Time*). For the packages containing MN and MA, the oxygen content in headspace was larger in the TRC100 packages, compared to TRC200. The volume of dissolved oxygen was around 0.05 mL for all MN and MA variants, independent of package size or storage temperature. Because of the varying oxygen content in headspace in the packages with MN and MA, the total oxygen volume was higher for the TRC100 packages, compared to TRC200. However, the oxygen content in headspace as well as dissolved oxygen in the packages containing CB did not differ between package size. The total oxygen content in the packages with CB was therefore approximately the same for both package sizes. An overview of the oxygen content in each package variant can be seen in Table 5. The standard deviation of the oxygen in headspace and dissolved oxygen was relatively large, compared to the average. Since there was a low total oxygen content in the packages, small variations in the oxygen measurements might lead to large percentage changes in the oxygen content. In comparison, if no nitrogen gas would have been added to the headspace, the amount of oxygen in headspace would have been approximately 3.8 mL, if assuming that the air consists of 21% oxygen. This means that the small changes in total oxygen most likely were negliable and did probably not affect the results, compared to if no modified atmosphere would have been used. This also means that the oxygen content in the packages was relatively low, which was of importance to minimise oxidation reactions in the food (Robertson, 2016).

The total oxygen content was important to determine in order to evaluate how the oxygen affects the shelf life of food stored in the new TRC package size. This is because oxygen may cause oxidation reactions in the food, e.g. enzymatic browning, non-enzymatic browning and loss of nutrients, and this might shorten the shelf life of the food (Kong & Singh, 2016; Robertson, 2016;

Man, 2016). As seen in Table 5, the total oxygen content was higher or the same in TRC100 as in TRC200 for the MN and MA variants, as well as the CB variants, respectively. Since the amount of food in TRC100 is smaller than in TRC200, it means that the oxygen-to-food ratio is larger for the TRC100, compared to TRC200, which agrees with Robertson (2016), see Chapter *1 Introduction*. According to Kong & Singh (2016), Robertson (2016) and Man (2016), the larger oxygen-to-food ratio may lead to more oxidation reactions in the food packed in the small package, i.e., TRC100.

Table 5. O_2 content in headspace [mL], dissolved oxygen in product [mL] and total oxygen content [mL] for all MN, MA and CB variants. All values are presented as $\mu \pm 2\sigma$

Variant	O_2 content in headspace [mL]	Dissolved oxygen [mL]	Total oxygen content [mL]
MN/100/25	0.35±0.25	0.043±0.040	0.39±0.23
MN/100/35	0.31±0.17	0.045±0.029	0.35±0.16
MN/200/25	0.11±0.11	0.052±0.046	0.16±0.12
MA/100/25	0.26±0.26	0.053±0.030	0.31±0.24
MA/100/35	0.32±0.24	0.054±0.034	0.37±0.22
MA/200/25	0.091±0.12	0.055±0.047	0.15±0.14
CB/100/25	0.22±0.17	0.094±0.016	0.31±0.18
CB/100/35	0.22±0.20	0.094±0.018	0.31±0.21
CB/200/25	0.20±0.20	0.14±0.03	0.34±0.20

During the production process of the packages filled with MN and MA, it was noticed that air bubbles got incorporated into the puree. Air bubbles in the product were also noticed during the measurements each week. This might be one possible source of error connected to the total oxygen content. This is because the air bubbles may contain oxygen and possibly could participate in different reaction processes. However, the air bubbles might not have been detectable with the dissolved oxygen measurement or the content of oxygen in headspace. Even though the air bubbles might not have been detectable, they could still have affected the ascorbic acid degradation and browning of the food. Another source of error could be that the dissolved oxygen meter might not be optimal for measurement on purees.

The change in oxygen content over time was calculated using Equation 9 *(*see *5.3.3 Dissolved Oxygen Concentration)*. The change in oxygen content from timepoint t_0 (week 0) to timepoint t includes the consumption of dissolved oxygen and oxygen in headspace, as well as permeation of oxygen through the package. The results for the different sample variants of MN, MA and CB can be seen in Figure 10. A positive value of the change in oxygen content, ΔO_2 [mL], means that the permeation of oxygen through the package was larger than the consumption of dissolved oxygen and oxygen in headspace and a negative value means that the oxygen permeation was smaller than the oxygen consumption. The changes in oxygen content were larger for the TRC100 variants compared to TRC200 variants. This indicates that the TRC100 variants have a larger ratio between the permeation of oxygen through the package, and the consumption of dissolved oxygen and oxygen in headspace, compared to the TRC200 variants. However, the changes in oxygen content

over time were small for all MN, MA and CB variants. The reason for this is probably due to that the total oxygen content in the packages was low. It was not possible to determine if the changes in oxygen content were significant since there were large variations in the data over time, see Figure 10. Since there was a low total oxygen content in the packages, small variations in the oxygen measurements might lead to large percental changes in the oxygen content. Similar to Table 5, the standard deviations (error bars) were large in comparison to the values.

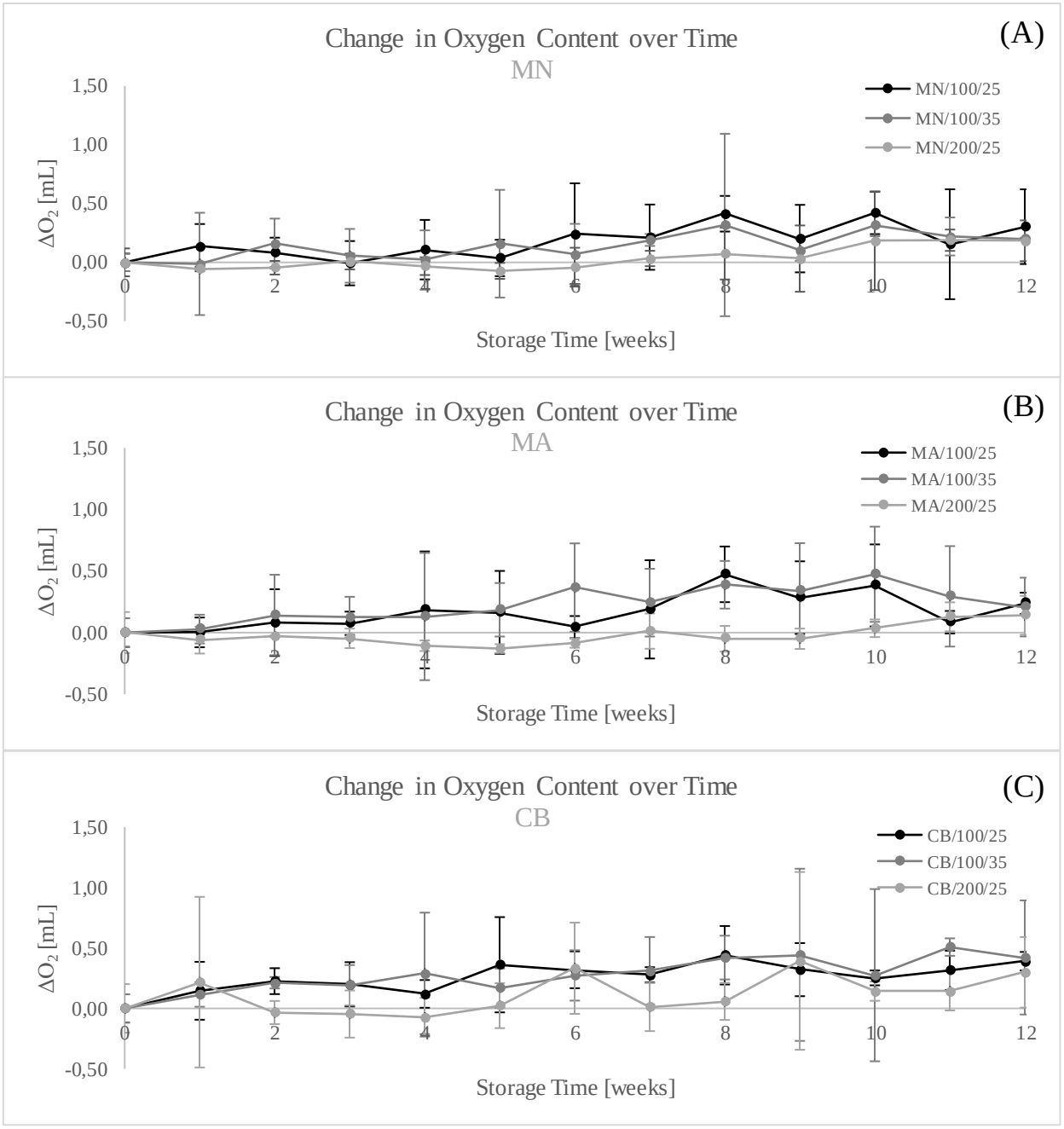

Figure 10. Change in oxygen content, ΔO_2 [mL], against time [weeks], (A) MN variants, (B) MA variants and (C) CB variants.

6.1.2 Degradation of Ascorbic Acid

The ascorbic acid content in the food was measured every week for all variants and Figure 11 shows the results from these measurements. The initial ascorbic acid concentration of MN was 130±10 mg/L, MA had 800±10 mg/L and CB had 350±20 mg/L. The ascorbic acid content remained stable for both MN variants and CB variants over the 12 weeks (P>0.05), while the ascorbic acid degraded over time in MA variants (P<0.05). As can be seen in Figure 11, the error bars were generally small. However, for some weeks, the error bars were larger for some variants. The reason for this might be damaged packaging material, e.g. aluminium cracks, where the barrier properties of the package might have been impaired.

The results means that a higher initial concentration of ascorbic acid in the mango puree generated a higher degradation rate. However, according to a study on apple puree by Herbig & Renard (2017), the initial ascorbic acid concentration do not have an impact on the degradation rate. They also mentioned that some researchers states that a higher initial ascorbic acid concentration can improve the stability of the ascorbic acid in food. This means that the theory states the opposite compared to the results presented above for the MN and MA variants. The reason for these differences could be that the food matrix in this thesis was different compared to the food matrices in the presented theory. In a study by Stešková, et al. (2006), it is stated that food matrices, storage temperatures and the form of ascorbic acid (natural or fortified) can have a large impact on the stability of the ascorbic acid. This means, according to the results, that the naturally present ascorbic acid in MN might be incorporated and protected better by the food matrix compared to the added ascorbic acid in MA. Additionally, sugars, flavonols and polyphenols present in the food, might stabilise the ascorbic acid (Herbig & Renard, 2017; Giannakourou & Taoukis, 2021; Miller & Rice-Evans, 1997). The MN variants have more sugar, flavonols and polyphenols per unit of ascorbic acid compared to the MA variants, which means that it is possible that these compounds protected the ascorbic acid from degradation more efficiently in MN.

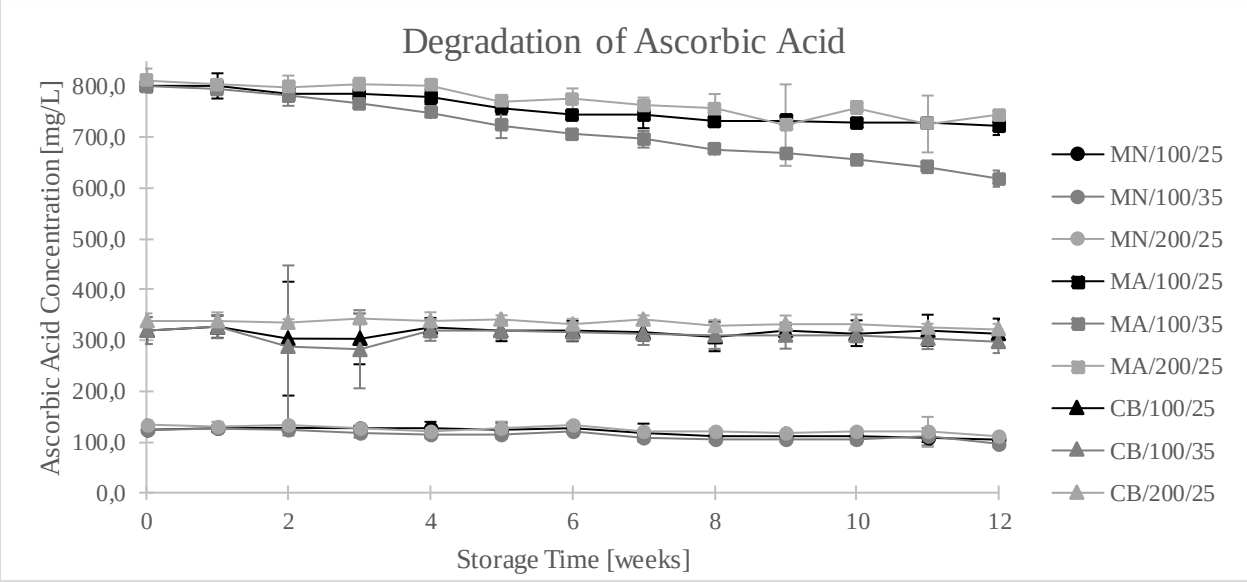

Figure 11. Ascorbic acid concentration [mg/L] against time [weeks] for all variants of MN, MA and CB. Error bars are given with a significance level of 95%.

For the MA variants, the degradation was similar for MA/100/25 and MA/200/25, whilst MA/100/35 degraded more rapidly. Therefore, it can be concluded that the storage temperature affects the degradation rate for the MA variants, where a higher temperature increase the degradation rate (see Figure 11). This agrees with the theory, which states that ascorbic acid is heat sensitive and therefore degrades faster in higher temperatures, compared to storage at lower temperatures (Villota & Hawkes, 2007; Stešková, et al., 2006). However, the storage temperature did not seem to affect the MN and CB variants, which were the variants that did not have a significant degradation of ascorbic acid. The low temperature dependence for the MN variants might be due to that the food matrix may have an impact on the stability of the ascorbic acid, as have been presented previously (Stešková, et al., 2006). It also seems reasonable that the storage temperature did not affect the degradation for the CB variants since similar results were seen in a study by Zee et al. (1991). However, generally, a higher storage temperature should increase the degradation rate of ascorbic acid (Stešková, et al., 2006; Zee, et al., 1991; van Bree, et al., 2012).

An ANOVA was performed to control if the storage temperature affected the degradation of ascorbic acid significantly, for MN, MA and CB variants packed in TRC100. The significance was checked between MN/100/25 and MN/100/35, between MA/100/25 and MA/100/35 and between CB/100/25 and CB/100/35 after 12 weeks of storage. The *P*-value for MN and CB were higher than α=0.05, see Table 6, which means that there was no significant difference in the ascorbic acid degradation between the different storage temperatures (25°C and 35°C). However, MA had a *P*-value smaller than α=0.05, see Table 6, which means that there was a significant difference in the ascorbic acid degradation between the different storage temperatures (25°C and 35°C) at a higher initial concentration of ascorbic acid.

Table 6. Generated *P*-value from ANOVA comparing consumed ascorbic acid after 12 weeks of storage at different temperatures (25°C and 35°C).

Variants	***P*-value**
MN/100/25	0.055
MN/100/35	
MA/100/25	0.00012
MA/100/35	
CB/100/25	0.12
CB/100/35	

An ANOVA was performed on the amount of consumed ascorbic acid after 12 weeks of storage to control if the size of the package affects the degradation for MN, MA and CB. The significance was checked between MN/100/25 and MN/200/25, between MA/100/25 and MA/200/25 and between CB/100/25 and CB/200/25 after 12 weeks of storage. The results from the ANOVA are displayed in Table 7 and as can be seen, the *P*-value for MN, MA and CB were all higher than α=0.05 which means that there was no significant difference between the package sizes, independent of food variant. This indicates that the size of the package did not significantly affect the degradation of ascorbic acid, i.e., the package ability to protect the packed food. Therefore, it can be concluded that the larger oxygen-to-food ratio in TRC100 does not significantly shorten the shelf life, with regards to ascorbic acid degradation, compared to when stored in TRC200.

Table 7. Generated *P*-value from ANOVA comparing consumed ascorbic acid after 12 weeks of storage in different package sizes (100 mL and 200 mL).

Variants	*P*-value
MN/100/25	0.61
MN/200/25	
MA/100/25	0.26
MA/200/25	
CB/100/25	0.44
CB/200/25	

A source of error for the ascorbic acid measurements of the CB variants was that only the brine was used for the titration and not the corn kernels. This was because the titration method was limited to liquid samples. It was therefore assumed that the ascorbic acid was equally distributed between the corn kernels and the brine.

The titration method was chosen due to the availability of equipment and simplicity of the method. Even though the method had good reproducibility, external factors may have affected the results. One example of external factors that may have influenced the results was that the tubes connected to the burette tip occasionally incorporated air bubbles, which might have resulted in faulty additions of iodine. Another source of error was that the iodine concentration may decreased over time, due to the light sensitivity, and that a new iodine solution was used from week 7. However, the equipment was calibrated before each measurement, to adjust the results based on the specific concentration. To achieve more reliable results, it is recommended to use HPLC analysis instead.

6.1.2.1 Correlation with CO_2 Production

An observation made during the measurements of the MA variants was that the CO_2 content in the headspace increased while the ascorbic acid content decreased. This could not be observed for the MN and CB variants due to the stability of ascorbic acid in those samples. The relation between CO_2 in the headspace and the ascorbic acid concentration of the MA/100/25, MA/100/35 and MA/200/25 can be seen in Figure F1 in Appendix *F. Correlation Between Ascorbic Acid and CO_2*.

The produced CO_2 over time was plotted against the consumed ascorbic acid over time for the variant MA/100/35 (see Figure 12). The correlation between the produced CO_2 and the consumed ascorbic acid could best be described by a logarithmic correlation (R^2=0.84 and *RMSE*=0.056) where CO_2 logarithmically increased with degradation of ascorbic acid. A similar correlation was applied to MA/100/25 (R^2=0.59 and *RMSE*=0.032) and MA/200/25 (R^2=0.70 and *RMSE*=0.062), see Appendix *F. Correlation Between Ascorbic Acid and CO_2*. However, the applied correlation for MA/100/25 and MA/200/25 did not describe the data accurately and this could be seen by the lower R^2. Though, it can be concluded that an increase in the degradation of ascorbic acid does increase the amount of CO_2 in the headspace. No correlation was found for the MN and CB variants since the ascorbic acid content and the CO_2 content were stable during the storage time. This indicates that the anaerobic degradation rate was slower when the initial ascorbic acid concentration was lower.

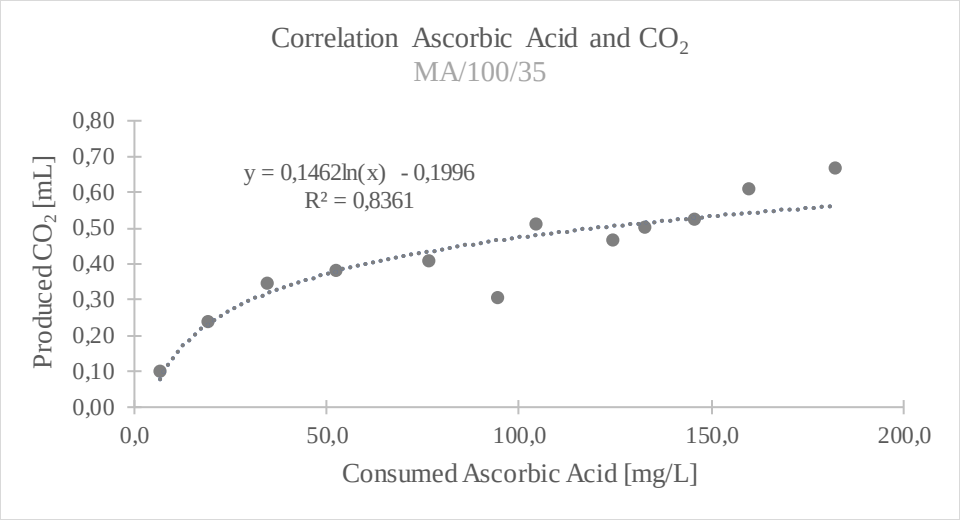

Figure 12. Produced CO_2 [mL] against consumed ascorbic acid [mg/L] for the variant MA/100/35.

The observed relation in MA/100/35 confirms that CO_2 was produced during the ascorbic acid degradation. According to Jutkus, et al. (2015), CO_2 is produced in both the aerobic and anaerobic degradation pathways of ascorbic acid, which means that the correlation could not be used to evaluate which pathway that was predominant. Though, since the amount of oxygen that was present in the package was low and did not change significantly over time, it was assumed that the main degradation pathway was anaerobic. This agrees with a study by Villota & Hawkes (2007), which states that the degradation rate of ascorbic acid in canned products is dominated by the anaerobic pathway upon longer storage. Though, the initial degradation of ascorbic acid often follows the aerobic pathway before it reaches a plateau where the anaerobic pathway becomes dominating (Herbig & Renard, 2017; Villota & Hawkes, 2007; Oey, et al., 2006). However, as seen in Figure 11, the degradation of the ascorbic acid did not show two different degradation behaviours during the storage period. This indicates that the ascorbic acid degradation followed the anaerobic pathway for all variants due to the low levels of oxygen in the packages. Since the same ascorbic acid degradation behaviour and correlation between CO_2 and ascorbic acid as well as a stable oxygen content was seen for all MA variants, it could be assumed that it was anaerobic conditions in both TRC100 and TRC200. This means that the anaerobic degradation pathway was assumed to be predominant independent of the package size. This does also indicate that the larger oxygen-to-food ratio in the small package did not affect the performance of the package, with regards to ascorbic acid degradation.

It is possible that the measurement of CO_2 could be used as an indicator of the ascorbic acid degradation in a TRC package. If CO_2 could be an indicator for the ascorbic acid degradation, it would facilitate the analysis of ascorbic acid concentration since the CO_2 analysis is simpler and faster. However, the correlation presented in Figure 12 might only be applicable for that specific food product, storage condition and initial concentration of ascorbic acid. Since the correlations were not optimally described by the logarithmic model (R^2<1), the role of CO_2 as an indicator of ascorbic acid degradation would have to be studied further. One disadvantage with the production of CO_2 during ascorbic acid degradation is that the degradation of ascorbic acid in fruits and vegetables might be increased in high levels of CO_2, according to Giannakourou & Taoukis (2021).

CO_2 is produced by both degradation pathways (Jutkus, et al., 2015), so the only way to minimise the CO_2 production is by decreasing the ascorbic acid content. However, the addition of ascorbic acid is shown to be crucial to minimise browning of the food (Stešková, et al., 2006; Bharate & Bharate, 2014; Mesquita & Queiroz, 2013). Therefore, it is of importance to not add too much ascorbic acid, but still have enough to minimise browning during the whole shelf life.

6.1.3 Colour Changes

The colour of all variants was first evaluated visually. Below, pictures from week 0 and week 12 of selected variants (MN/100/35, MA/100/35 and CB/100/35) are displayed and the rest can be found in Appendix *G. Colour*. Visually, the colour did only change slightly for the MN and MA variants where the colour after 12 weeks could be perceived as slightly darker, see Figure 13. For the corn variants, a colour change between week 0 and 12 was harder to perceive because of the less homogenic colour of the corn kernels, see Figure 13.

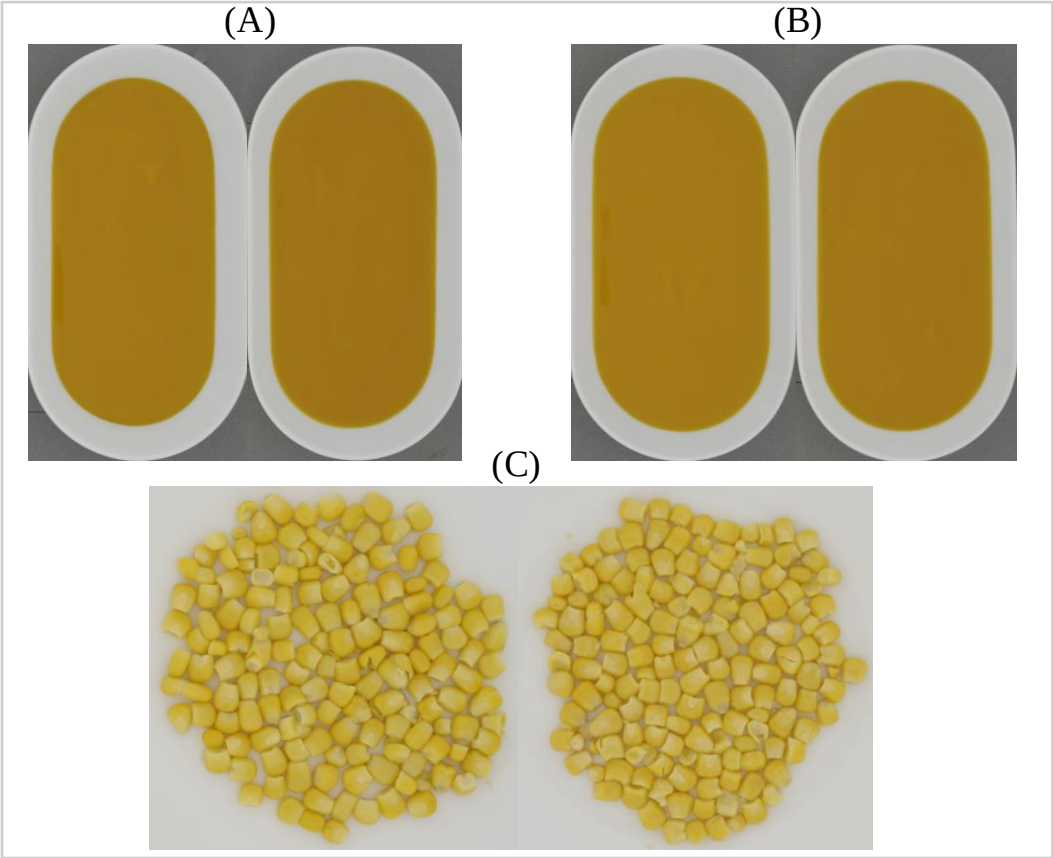

Figure 13. (A) MN/100/35 week 0 (left) and 12 (right), (B) MA/100/35 week 0 (left) and 12 (right) and (C) CB/100/35 week 0 (left) and 12 (right).

In addition to visual evaluation, the L^*, a^* an b^* values were measured to evaluate the colour of the food. Since the three parameters combined gives the true colour of the samples, ΔE^* was calculated for all variants, after 12 weeks of storage, to evaluate the overall colour change. The calculated ΔE^* values are presented in Table 8. As can be seen in the table, all MN, MA and CB variants had an average ΔE^* value between 2.3 and 3.4. This means that all variants fell into the category of a noticeable colour change ($1.5<\Delta E^*<3.0$) and the category of a well visible colour

change ($3.0<\Delta E^*<6.0$) (Wibowo, et al., 2015). Though, as mentioned before, the visual colour change was small. No visual colour change was considered as well visible, which means that the categorisation based on the ΔE^* did not represent the visual colour change accurately. Therefore, the categorisation of ΔE^* might not be fully applicable for mango puree and corn but might give a comparable indication of the colour changes of the variants.

Table 8. Average ΔE^* of week 12 for all variants ($\mu \pm 2\sigma$) and visibility of the colour change.

Variant	ΔE^* of week 12	Visibility of the colour change
MN/100/25	2.9±0.9	Noticeable
MN/100/35	3.4±0.4	Well visible
MN/200/25	3.0±0.4	Well visible
MA/100/25	2.5±0.3	Noticeable
MA/100/35	2.3±0.6	Noticeable
MA/200/25	3.0±0.1	Well visible
CB/100/25	3.1±0.7	Well visible
CB/100/35	2.9±1.0	Noticeable
CB/200/25	3.1±1.0	Well visible

There was significant difference ($P<0.05$) in ΔE^* between the MN/100/35 and MA/100/35 samples, but not between the MN and MA samples stored at 25°C ($P>0.05$). This means that the colour change, i.e., browning, was more pronounced for the MN variant, compared to the MA variant, when stored at 35°C. Based on these results, it can be concluded that the addition of ascorbic acid does not affect the colour change during short storage (12 weeks) at ambient storage. However, according to Ashurst (2016), 12 weeks of storage at 35°C should represent approximately 1 year of storage at ambient temperature, which means that during longer storage (>1 year), the colour change will be larger, and addition of ascorbic acid is assumed to suppress browning. These results agree with the theory about the antioxidant activity of ascorbic acid, and it can therefore be concluded that it is recommended to add ascorbic acid to minimise browning in oxygen sensitive foods (Bharate & Bharate, 2014; Stešková, et al., 2006). Since the MA variants showed less colour change but higher ascorbic acid degradation than the MN variants, it was assumed that the browning was mainly caused by oxidation reactions, and not ascorbic acid degradation, in TRC packages. This was also probable since the brown pigments from ascorbic acid degradation are formed together with amino acids (Bharate & Bharate, 2014; Jutkus, et al., 2015), but the amino acid content in the mango puree was low (Martin & Servera AB, n.d). Maillard and caramelisation reactions require applied heat to occur (Bharate & Bharate, 2014) and since no heat was applied to the samples, the Maillard reaction and caramelisation was probably not either responsible for the browning. This indicates that the browning that occurred was enzymatic. However, the food in this thesis had been heat treated and therefore the enzymes responsible for browning should have been inactivated, according to Chutintrasri & Noomhorm (2006). Enzymatic browning also requires presence of oxygen (Mesquita & Queiroz, 2013) but the oxygen content in the packages were stable at a low level for all variants. Based on this, it was difficult to determine which type of browning that was predominant in the samples studied in this thesis.

There were no significant differences in ΔE^* ($P>0.05$) between MN/100/25 and MN/200/25, as well as between CB/100/25 and CB/200/25. The colour change of MA/100/25 was however significantly smaller ($P<0.05$) than the colour change of MA/200/25. This indicates that a smaller package size does not increase the browning of the packed food. Furthermore, based on the results, the storage temperature does not affect the rate of browning significantly ($P>0.05$) for any of the variants. It can therefore be concluded that neither the package size nor the storage temperature affects browning, which means that the factor affecting browning the most in TRC100 is the addition of ascorbic acid, which suppress browning slightly.

To identify how the different parameters changed, an ANOVA was performed to evaluate if there was any significant change in the specific colour parameters between week 0 and week 12. The results of the ANOVA are presented in Appendix *G. Colour* where it can be concluded that the parameters that changed significantly ($\alpha=0.05$) during the storage time was the a^* and b^* value for the MN and MA variants. This means that the mango puree changed significantly in the green-red colour and yellow-blue colour (Ly, et al., 2020). Only two out of six mango variants also showed a significant change in the L^* value (lightness). Therefore, the change in L^* value over time for the MN and MA variants will be considered negligible. Based on these results, a^* and b^* values are the only parameters that will be discussed further on for MN and MA. This is because the L^* parameter does not seem to change as fast and cause as much browning as a^* and b^* during storage of mango puree in TRC100 and TRC200. According to Ahmed et al. (2002) b^* is mainly responsible for colour degradation in mango puree and therefore the results are reasonable.

As can be seen in Appendix *G. Colour*, the three CB variants showed a significant change ($\alpha=0.05$) in the L^* value between week 0 and week 12. Two out of three CB variants showed a significant change of the a^* value and none of the CB variants showed a significant change in the b^* value. This means that the corn changed significantly in lightness and green-red colour, but no change in the yellow-blue colour could be seen. However, these results are not reliable due to several sources of error. One source of error for the colour measurements of the CB variants is that the corn was mixed before measuring the colour, which resulted in a non-homogenous paste that might have generated large variations in the results. Additionally, it is likely that the mixture does not represent the true colour of the corn kernels since the colour of the inside and outside of the corn kernel is different. Due to the previously presented sources of error, the colour parameters will not be discussed further for CB.

6.2 Kinetic Modelling

Kinetic models was applied to the ascorbic acid degradations and the colour changes to determine the degradation rates at the different storage temperatures. The temperature in the storage rooms varied during the 12 weeks of storage. The 25°C storage room had an average temperature of 26.1±4.0 and the 35°C storage room had an average temperature of 35.0±1.2, see Appendix *I. Temperature in Storage Rooms*.

6.2.1 Ascorbic Acid

Since MA was the only food variant that showed degradation of ascorbic acid, it was only possible to perform kinetic modelling of those samples. It was seen that the degradation of ascorbic acid was well described by both zero-order kinetics and first-order kinetics (independent of storage temperature and package volume). The rate constant as well as the *RMSE* values calculated for zero-order kinetics and first-order kinetics were similar for all MA variants and can be seen in Appendix *H. Kinetics*. However, the kinetic order that described the degradation best was the first-order kinetic model (highest R^2 and lowest *RMSE* values). This is true according to Nkwocha et al. (2018), Villota & Hawkes (2007) and van Bree et al. (2012), who stated that ascorbic acid degradation generally is described by first order reactions, but that it depends on the processing of the food. The first-order kinetic models of MA/100/25, MA/100/35 and MA/200/25 are displayed in Figure 14.

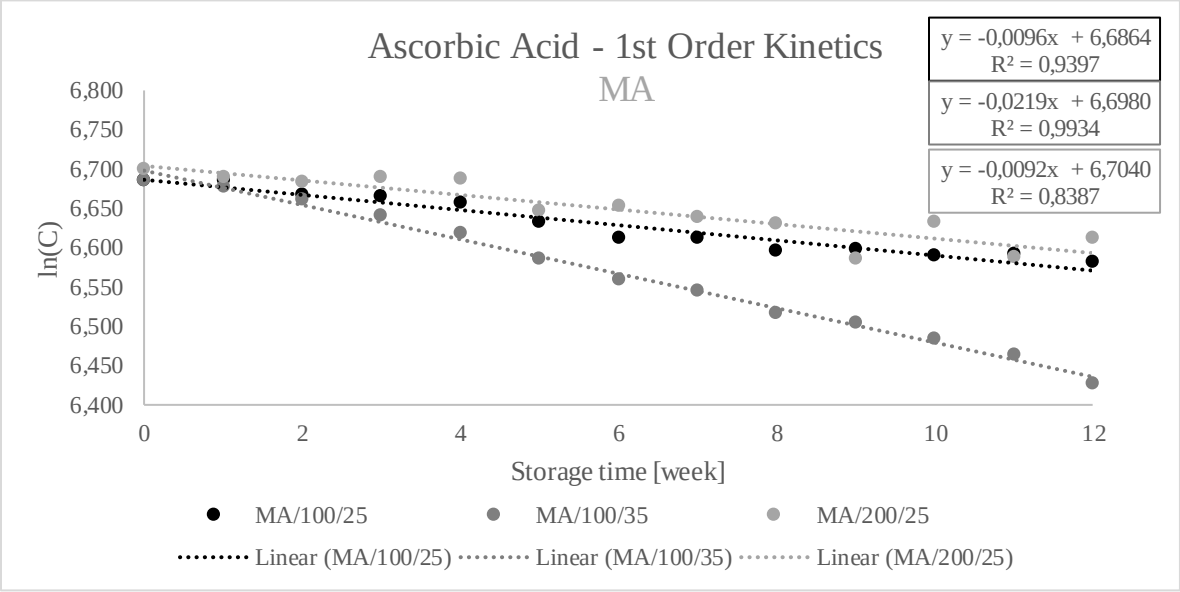

Figure 14. Natural logarithmic concentration, ln(C), of ascorbic acid against storage time [weeks] for the MA variants.

When using the kinetic models to estimate the ascorbic acid levels at end-of-shelf life (2 years), it can be concluded that the ascorbic acid content of MA/100/25 and MA/200/25 will be approximately 300 mg/L at the end of shelf life (2 years), which represents approximately 40% of the initial concentration. After 2 years of storage, MA/100/35 will have an ascorbic acid content of approximately 80 mg/L, representing approximately 10% of the initial concentration. This

means that, if the ascorbic acid degradation follows the kinetic model, all ascorbic acid will not be consumed within the shelf life and a lower initial concentration (<800 mg/L) is probably adequate to maintain the antioxidant properties over the whole shelf life. Furthermore, the measurements of ascorbic acid in MN and CB indicates that the ascorbic acid degradation rate will decrease, and that the ascorbic acid concentration will stabilise at lower concentrations. Because of time limitations, this was not possible to investigate, but to confirm the full degradation model, it is recommended to study the ascorbic acid content during the whole shelf life.

As seen in the Figure 14 and Table 9, the degradation rate of MA/100/25 (0.0096 $week^{-1}$) and MA/200/25 (0.0092 $week^{-1}$) was similar, but the degradation rate of MA/100/35 was approximately two times higher (0.022 $week^{-1}$). See a summary of the degradation rates for the MA variants in Table 9. This indicates that the degradation rate is highly affected by the storage temperature, which is in accordance with Zee, et al. (1991), and Villota & Hawkes (2007), but not affected by the package volume. However, neither the storage temperature nor the package volume seemed to affect the ascorbic acid degradation in MN and CB. Possible reasons for this have been discussed in Chapter *6.1.2 Degradation of Ascorbic Acid* such as initial ascorbic acid concentration, the food matrix, the form of ascorbic acid (natural or fortified), food composition (presence of sugars, flavonols and polyphenols).

Table 9. Rate constant, k, [min^{-1} and $week^{-1}$] and half time, $t_{1/2}$, [min^{-1} and week-1] for the MA variants.

Variant	k **[$week^{-1}$]**	k **[min^{-1}]**	$t_{1/2}$ **[week]**	$t_{1/2}$ **[min]**
MA/100/25	$k_{26.1}$=0.0096	$k_{26.1}$=95×10^{-8}	72	0.73×10^{6}
MA/100/35	$k_{35.0}$=0.022	$k_{35.0}$=220×10^{-8}	32	0.32×10^{6}
MA/200/25	$k_{26.1}$=0.0092	$k_{26.1}$=91×10^{-8}	75	0.76×10^{6}

As have been presented in *3.1.3 Kinetic Modelling*, most canned fruits have a $k_{26.7}$-value around 50-70×10^{-8} min^{-1} and a $t_{1/2}$-value around 1×10^{6} at 26.7°C (Villota & Hawkes, 2007). As seen in Table 9, the MA variants had a k-value between 91-220×10^{-8} min^{-1} and a $t_{1/2}$-value between 0.32-0.76×10^{6} min, depending on storage temperature. This means that the degradation rate of ascorbic acid in the MA variants was faster compared to the values for canned fruits, presented by Villota & Hawkes (2007). However, the k-values and $t_{1/2}$-values for MA/100/25 and MA/200/25 are relatively close to the ranges stated by Villota & Hawkes (2007). It is reasonable that the values presented by Villota & Hawkes (2007) differs slightly from the results of this study because the food that was studied in the literature was not purees. This means that the food matrices were different which could impact the degradation rate (Stešková, et al., 2006). Also, the values given by Villota & Hawkes (2007) was for canned food in metal cans whilst this study has been performed on carton packages. As have been mentioned in *3.1.1 Factors Limiting the Shelf Life of Packed Products*, Morris (2017) states that a thicker aluminium layer in packages improves the package barrier properties. This indicates that carton packages, with a thin aluminium layer in the packaging material, have a higher oxygen transmission rate compared to metal cans, which might be another explanation for the higher degradation rate in the TRC packages compared to the values presented by Villota & Hawkes (2007). Another reason for the difference could be the initial concentration of ascorbic acid in the products, since, as seen in the results, a higher initial ascorbic acid concentration may give a higher degradation rate.

A study performed by Wibowo et al. (2015), presented in *3.1.3 Kinetic Modelling*, showed that the *k*-value of ascorbic acid degradation at 42°C was higher for mango juice with 230 mg/L and 460 mg/L added ascorbic acid (k=0.19 week^{-1} and k=0.17 week^{-1} respectively), compared to natural mango juice (k=0.04 week^{-1}). These degradation rates are higher than the degradation rates determined in this thesis, which is reasonable because the storage temperature of the mango juice was higher than in this thesis. Furthermore, the study showed that the degradation rate was lower for the natural mango juice compared to mango juice with addition of ascorbic (Wibowo, et al., 2015). This was the case in this thesis as well which once again indicates that the degradation rate in some food matrices might be concentration dependent.

6.2.2 Colour

The colour changes for the MN and MA variants were checked if best described by zero-, first- or second-order kinetic modelling. The colour parameters that were checked for the MN and MA variants were a^*, b^* (see explanation in *6.1.3 Colour Changes*) as well as the total colour change ΔE^*. The calculated R^2 values and *RMSE* values for zero-, first- and second-order kinetics for all MN and MA variants can be seen in Appendix *H. Kinetics*. It was seen that a^* had extremely low values of R^2 ($R^2 << 1$) and high values of *RMSE* for all MN and MA variants, compared to the R^2 and *RMSE* values for b^*. It can therefore be concluded that the data for a^* was not well described by any of the kinetic orders, probably due to small changes. The colour parameter a^* will therefore not be studied further in this report. No kinetic model described ΔE^* for the MA variants accurately. This was probably also due to small colour changes and therefore, ΔE^* for MA was not studied further either. However, the changes in b^* for MN and MA were best described by first-order kinetics and ΔE^* for MN was best described by zero-order kinetics since these orders gave the highest R^2 values combined with the lowest *RMSE* values (independent of storage temperature and package volume). These results are in accordance with the theory presented in *3.1.3 Kinetic Modelling*, which states that the colour changes in foods are usually best described by either zero-order kinetics or first-order-kinetics (Ahmed, et al., 2002; Wibowo, et al., 2015). The first-order kinetic models of b^* for the MN and MA variants are displayed in part (A) and (B) of Figure 15. ΔE^* is displayed for the MN variants in part (C) of Figure 15. It must be noted that even though b^* and ΔE^* was best described by first-order kinetics and zero-order kinetics, respectively, the R^2 values were not close to a value of 1. This indicates that these kinetic models may not accurately describe the colour changes but it might give an indication.

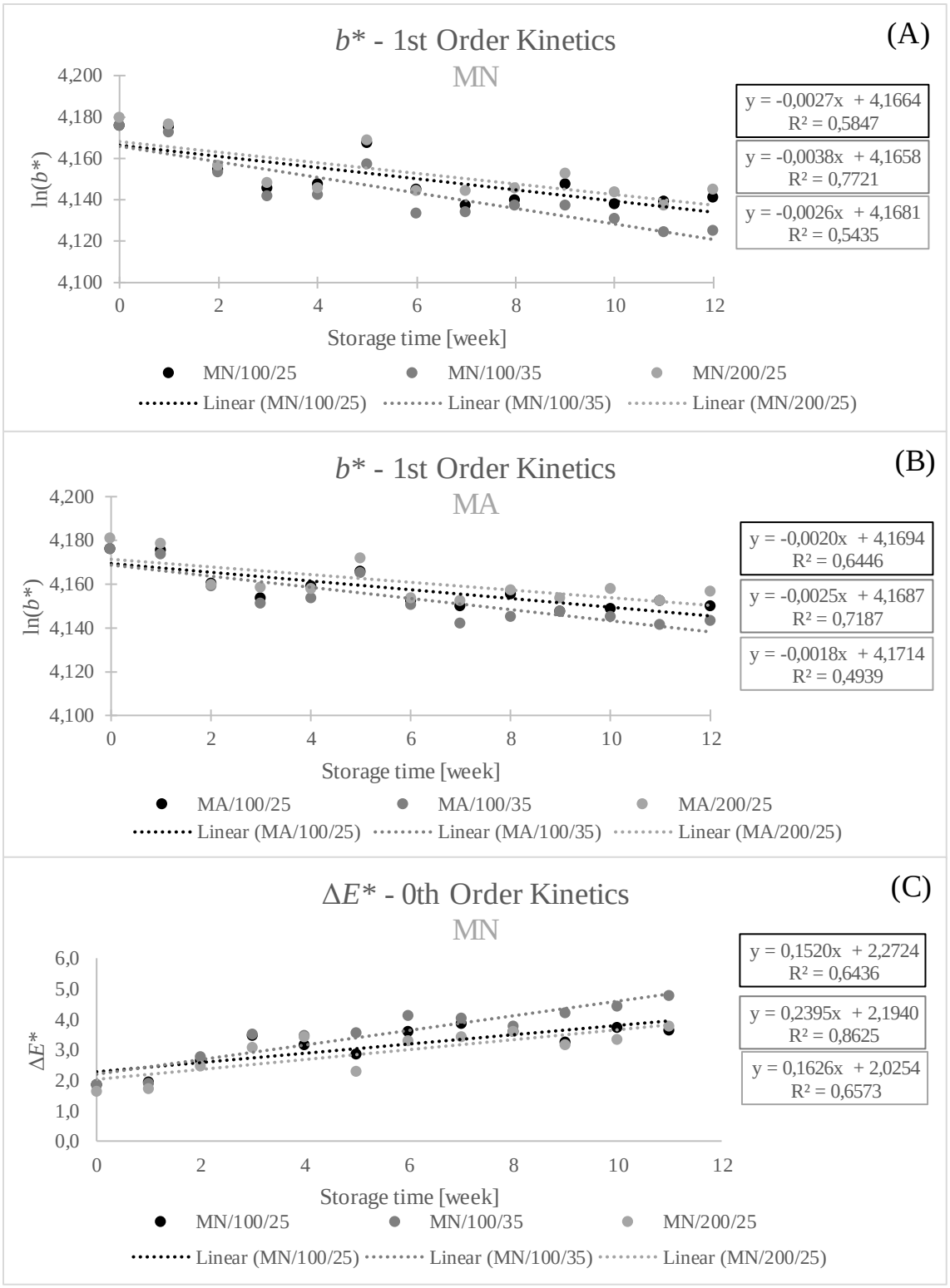

Figure 15. (A) First-order kinetic model for MN variants, logarithm of colour parameter *b** against storage time [weeks]. (B) First order kinetic model for all MA variants, logarithm of colour parameter *b** against storage time [weeks]. (C) Zero-order kinetic model for all MN variants, Δ*E** against storage time [weeks].

As seen in part (A) of Figure 15, the kinetic model for the colour change in MN/100/25 and MN/200/25 were similar, with a slope of 0.0027 week^{-1} and 0.0026 week^{-1}, respectively. Additionally, it was found that the rate of colour change, with regards to *b**, were faster for MN/100/35 (0.0038 week^{-1}) compared to MN/100/25 and MN/200/25. The same trends were found for the MA variants, see part (B) of Figure 15. This means that the colour change, i.e., browning, occurs faster in higher temperatures, which is in accordance with a study by Kong & Singh (2016), but is not dependent on package size. Another trend that was found was that the slope was larger, i.e., higher rate of colour change, for the MN variants compared to the MA variants, which indicates that addition of ascorbic acid suppresses browning and is in accordance with Mesquita & Queiroz (2013).

The graph of ΔE^* for the MN variants, see part (C) of Figure 15, showed similar trends as the figures above. The slopes of the total colour change for MN/100/25 and MN/200/25 are similar, 0.15 week^{-1} and 0.16 week^{-1}, respectively. Furthermore, the rate of the total colour change was faster for MN/100/35 (0.24 week^{-1}) compared to MN/100/25 and MN/200/25. Again, this indicates that the colour change is faster in higher temperatures but is not dependent of package size.

In Table 10 it is displayed how the colour of all variants might look after 2 years of storage in TRC100 and TRC200. As can be seen in the table, there is only a small difference between the *b** values for MN/100/25 and MN/200/25 as well as for MA/100/25 and MA/200/25. The differences were also difficult to detect visually. Therefore, the package size does not seem to have an impact on the colour changes. However, the visual colour difference between MN/100/25 and MN/100/35, as well as between MA/100/25 and MA/100/35 was larger. This means that higher temperature increases the browning reactions, which agrees with Kong & Singh (2016). Additionally, it was observed that the colour changed more for the MN variants, compared to the MA variants. This indicates that ascorbic acid suppress browning, which agrees with previously presented theory in *3.1.1.2 Degradation of Ascorbic Acid* (Bharate & Bharate, 2014; Mesquita & Queiroz, 2013; Stešková, et al., 2006) and results presented in *6.1.3 Colour Changes*. However, the kinetic models above assumes that only the colour parameter *b** changes for the MN and MA variants and not the parameters *a** and *L**. As previously mentioned, *a** also had a significant change between 0 and 12 weeks of storge but the colour change could not be described by a kinetic model. *L** did not have a significant change between 0 and 12 weeks of storage. Even though, *b** was the only colour parameter considered, *a** and *L** might also change considerably over a storage period of 2 years. The colours in Table 10 should therefore only be seen as a rough indication of what the colours might look like after 2 years storage.

Table 10. Measured colour of the MN and MA variants at 0 weeks of storage as well as an indication of what the colour might look like after 2 years of storage.

MN/100 **0 weeks of storage**	**MN/100/25** **2 years of storage**	**MN/100/35** **2 years of storage**	**MN/200/25** **2 years of storage**
*b** = 65.1	*b** = 48.7	*b** = 43.4	*b** = 49.3
MA/100 **0 weeks of storage**	**MA/100/25** **2 years of storage**	**MA/100/35** **2 years of storage**	**MA/200/25** **2 years of storage**
*b** = 65.1	*b** = 52.5	*b** = 49.8	*b** = 53.7

6.3 Microbial and Sensory Evaluation

The microbial evaluation was done in order to ensure that the food was safe to consume before the sensory evaluation was performed. The microbial plating that was done for the CB showed only one colony at one out of three plates after the incubation period. The colony was however outside of the streaking line and was most likely a contamination. The pH evaluation of the CB, which was done in combination with the plating, showed that there were no changes in pH. During the whole storage time, the pH value of the CB was stable at 6.3±0.1. The CB was therefore considered as safe for consumption. The results of the swelling observation and pH evaluation that was used to check for spoilage in the MN and MA showed that no swelling had occurred after incubation and that there were no changes in pH. During the whole storage time, the pH value of both MN and MA were stable at 4.0±0.1 and 3.9±0.1, respectively. This indicates that no acidification or microbial growth have occurred, and the MN and MA were therefore considered as safe for consumption.

The results of the paired preference test are presented in Table 11. The table shows the number of persons that preferred each sample. With the help of table 13.2 in Lawless & Heymann (2010), it was seen that at least 32 panellists needed to indicate preference for one sample to be significantly preferred over the other sample, at a significance level of 95%. Since none of the samples had 32 votes or more, it can be concluded that no specific sample of each part was preferred over the other, i.e., the samples that had been stored for 0 weeks was equally preferred as the samples stored for 11 weeks. This means that storage for 11 weeks in a TRC100 does not affect the sensory attributes in a negative way, according to the results, and the sensory shelf life seems to be far longer than 11 weeks. The sensory evaluation was performed on TRC100, however, the TRC200 is assumed to not affect the sensory attributes during storage since it is already tested and commercialised. Based on this, it can be concluded that during short storage, i.e., 11 weeks, the performance of TRC100 is similar to TRC200. However, a sensory evaluation should preferably be performed after longer storage, e.g., after 6 months, 1 year and 2 years, to evaluate changes in sensory attributes in food stored in TRC100 over time.

Table 11. Number of participants that preferred each sample in the paired preference test.

	Part 1		**Part 2**		**Part 3**	
Sample ID	387	456	734	628	912	894
Variant	MN/100/25		MA/100/25		CB/100/25	
Storage Time	0 weeks	11 weeks	0 weeks	11 weeks	0 weeks	11 weeks
Votes	24	24	21	27	23	25
Total	48		48		48	

One source of error for the sensory evaluation could be that the 0 week samples of MN/100 and MA/100 were stored in freezer until the evaluation was performed. The 0 weeks samples of CB/100 were stored in fridge until evaluation was performed since the texture of the corn could be impacted during freezing and thawing (Becerra-Sanchez & Taylor, 2021). The storage in fridge and freezer was done since it was assumed that the degradation reactions would be minimised (Giménez & Ares, 2019). Optimally, a new batch should have been prepared for the 0 week

samples just before the sensory evaluation was performed, but it was not possible due to time limitations. The serving order could also have impacted the results. The mango purees were tasted before the corn which might have given some acidity and sweetness left in the mouth when tasting the corn. However, if the corn would have been served before the mango purees, there might have been saltiness left in the mouth when tasting the mango purees. This problem could maybe have been solved by serving a plain wafer in addition to the water that was used to rinse the taste pallet in between the samples.

An alternative sensory evaluation method that could have been used is a triangle test (Lawless & Heymann, 2010). In a triangle test it would have been possible to compare TRC100 and TRC200 for all variants after 11 weeks of storage. The test would have given information if the samples stored for 11 weeks in TRC100, and the samples stored for 11 weeks in TRC200 could have been distinguished from one another.

6.4 Conclusions

When investigating the headspace of the packages, it was concluded that the headspace-to-food and oxygen-to-food ratio was larger in TRC100, compared to TRC200. The measurements showed that the oxygen content in headspace, dissolved oxygen in the products and the total oxygen content remained stable for all variants during the storage period of 12 weeks. The changes in oxygen content were larger for the TRC100 variants compared to TRC200 variants. This indicates that the TRC100 variants had a larger ratio between the permeation of oxygen through the package and the consumption of dissolved oxygen and oxygen in headspace, compared to the TRC200 variants. However, the changes in oxygen content over time were small for all food variants. The reason for this is probably due to the low total oxygen content in the packages.

Based on the results from the ascorbic acid measurements, it could be concluded that the package size does not impact the shelf life of packed food significantly. Since a relation between CO_2 production and ascorbic acid degradation was found, it was assumed that the ascorbic acid was mainly degraded anaerobically. Therefore, the larger oxygen-to-food ratio in TRC100 does not impact the ascorbic acid degradation, compared to TRC200. When looking at kinetic modelling of the ascorbic acid degradation, it was best described by a first-order kinetic model. From the kinetic model, it could be seen that the storage temperature had a big impact on the degradation rate when the levels of ascorbic acid was high (>500 mg/L). The degradation rate was faster in MA/100/35 and the ascorbic acid concentration would reach a level of 80 mg/L after 2 years of storage compared to approximately 300 mg/L in MA/100/25 after 2 years of storage.

For the browning in the MN and MA variants, it was seen that the a^* and b^* values changed significantly over the storage time for both TRC100 and TRC200. The b^* value could be described by first-order kinetic models, which indicates that the colour was estimated to visually change significantly. Though, no significant difference in colour change was determined between the two package sizes. It could also be concluded that the rate of colour change increased with increasing storage temperature. However, a larger number of storage temperatures would need to be further studied to obtain a more accurate fit to the kinetic-models. Additionally, it was determined that addition of ascorbic acid seemed to suppress browning, i.e., slow down the browning reactions. When looking at ΔE^*, it could be concluded that all food variants showed a noticeable or well visible colour change after 12 weeks of storage. However, the colour of the corn was not investigated further because of several sources of error.

The results from the sensory analysis, performed after 11 weeks of storage, showed that there was no significant difference in sensory attributes of the food after 11 weeks of storage in TRC100, compared to the food stored for 0 weeks. Since storage in TRC200 was assumed to not affect the sensory attributes over time, it could be concluded that TRC100 performs in a similar way as TRC200 during short storage, i.e., 11 weeks. Therefore, it could be concluded that a smaller package size does not impact the sensory attributes in a negative way. However, a sensory evaluation should preferably be performed after storage for e.g., 6 months, 1 year and 2 years to evaluate changes in sensory attributes in food stored in TRC100 over the whole shelf life.

To conclude, the food quality properties of different oxygen sensitive foods (mango puree and corn in brine) were equally maintained during storage in a TRC100 package as in a TRC200 package. This means that the two package sizes have similar ability to protect the packed food, i.e., the larger oxygen-to-food ratio does not seem to impact the shelf life in a negative way. It can also be concluded that storage temperature had a large impact on the quality of the packed food compared to the package volume. Therefore, based on the results, oxygen sensitive foods packed in TRC100 and TRC200 will most likely have similar shelf lives at the same storage temperature. This indicates that smaller package sizes, that are oxygen critical due to the large oxygen-to-food ratio, may still be suitable for oxygen sensitive foods.

6.5 Future Research

It would be preferable to study the food products over the entire shelf life of 2 years. It would also be preferable to perform the measurements in more than triplicates to be able to do a better statistical evaluation and get better accuracy of the results. One source of error connected to the production process of the filled packages could be that it does not completely represent the commercial production process since the food was prepared manually prior to filling. Therefore, it would be interesting to perform the study on packages filled in commercial production processes.

It would also be of interest to store the filled packages in more than two storage temperatures. This would enable evaluation of the temperature dependence of for example colour changes and ascorbic acid degradation in TRC100 with the help of Arrhenius equation.

In the results it was seen that is possible that degradation of ascorbic acid was mainly through the anaerobic pathway and that the measurement of CO_2 could be used as an indicator of the ascorbic acid degradation in a TRC package. If CO_2 could be an indicator for ascorbic acid degradation it would facilitate the analysis of ascorbic acid concentration since the CO_2 method is simpler and faster. However, the correlation between the CO_2 production and the degradation of ascorbic acid might be dependent on the food matrix, storage conditions and initial concentration of ascorbic acid. The role of CO_2 as an indicator of ascorbic acid degradation would be an interesting research field to be studied further.

Another field that could be interesting to investigate further is how the food stored in TRC100 packages are affected by the retorting process. This have not been studied before and was out of the scope of this thesis. However, it is known that the nutritional values and deterioration reactions are affected by heat treatment, which makes this an important aspect of how the food is affected by storage in TRC100. This could be of importance to know how much ascorbic acid that is lost during retorting, to determine the amount of ascorbic acid that has to be added to minimise, e.g., browning.

It would also be of interest to investigate what factors during processing and storage that are the most critical for shelf life of fruit and vegetables in TR100 compared to TRC200. This would require samples with, for example, different ascorbic acid concentrations, a diversity of fruit and vegetable products, as well as different types of heat treatments (temperature and time). With these results it may be possible to determine a regression model to predict the shelf life in TRC100 and TRC200.

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Appendix

A. Nutritional Values

The nutritional values of the raw materials, i.e., mango puree and corn, is presented in Table A1.

Table A1. Nutritional values of mango puree (Martin & Servera AB, n.d.) and corn (Magnihill AB, n.d.).

	Mango puree nutritional value (per 100 g)	**Corn nutritional value (per 100 g)**
Energy	83 kcal	100 kcal
Fat	0.36 g (whereof 0.156 g saturated)	1.5 g (whereof 0.7 g saturated)
Carbohydrate	18.2 g (whereof 13.9 g sugars)	16 g (whereof 3.6 g sugars)
Protein	0.8 g	3.1 g
Fibre	1.8 g	-
Ascorbic Acid	17.2 mg	-
Iron	0.34 mg	-
Calcium	12.8 mg	-
Vitamin A	0.8 µg	-
Salt	<0.01 g	<0.01 g

B. Reasoning Behind the Ascorbic Acid Additions

B1. Mango Puree

When investigating the market, it was concluded that most of the fruit purees for babies had a guaranteed ascorbic acid level of 350 mg/L. Therefore, the goal was to add ascorbic acid so that the concentration was not below this value at the end of shelf-life. To be able to estimate this, a few assumptions was made and described here. It was assumed that 15% of the ascorbic acid would be degraded during the heat treatment, based on previous experience at Tetra Pak. During storage, it was assumed that the ascorbic acid would degrade with a rate of 0.003 mg/g food/week. This assumption was based on previous studies performed by Tetra Pak. Based on these assumptions and the knowledge that the mango puree contained approximately 170 mg/L ascorbic acid, the total initial ascorbic acid concentration was decided to be 800 mg/L. This means that 630 mg ascorbic acid was added per litre of mango puree.

B2. Corn

The goal was to have a little (approximately 100 g/L) ascorbic acid left at the end of shelf-life to ensure that measurements could be performed for the whole shelf-life. The same assumptions were made for the brine as for the mango puree. However, the degradation during heat treatment was assumed to be 50%, due to harsher heat treatment. Based on these assumptions, the initial concentration of ascorbic acid was decided to be 800 mg/L.

C. Sensory Evaluation

Below is the ballot for the sensory evaluation. Half of the panellists answered the ballot below and the other half answered a similar ballot that had reversed serving order.

Sensory Evaluation – Mango Puree and Corn

Paired Preference Test

Read the following before you start:

This test is supposed to be performed individually and no discussion is allowed. Also, be kind to do not discuss the test out loud after you are done since there may be people near you that are waiting to perform the test.

The test is divided into 3 parts, part 1, 2 and 3. Perform part 1 first, part 2 secondly and part 3 last. Read the instructions before tasting the samples.

Please turn to the next page and start the test.

NB! Do not perform the test if you are allergic to corn or mango.

Part 1 – Mango N

Please rinse your mouth by drinking a sip of water before starting.

You will now taste the two samples that are placed in part 1 on the plate. Please taste the two samples in the order presented below, from left to right. Rinse your mouth between the samples. Please consume at least half of the sample provided.

If you have any questions, please ask the hosts.

Circle the number of the sample you prefer
(NB! You must make a choice)

387 456

When you are done with part 1, turn to the next page to perform part 2

Part 2 – Mango A

Please rinse your mouth by drinking a sip of water before starting.

You will now taste the two samples that are placed in part 2 on the plate. Please taste the two samples in the order presented below, from left to right. Rinse your mouth between the samples. Please consume at least half of the sample provided.

If you have any questions, please ask the hosts.

Circle the number of the sample you <u>prefer</u>
(NB! You must make a choice)

734 628

When you are done with part 2, turn to the next page to perform part 3

Part 3 - Corn

Please rinse your mouth by drinking a sip of water before starting.

You will now taste the two samples that are placed in part 3 on the plate. Please taste the two samples in the order presented below, from left to right. Rinse your mouth between the samples. Please consume at least half of the sample provided.

If you have any questions, please ask the hosts.

Circle the number of the sample you <u>prefer</u>
(NB! You must make a choice)

894 912

Thank you for your participation!

Please hand in your ballot and take a piece of cake before leaving.

D. Dixon's Test (*Q*-Test)

The critical *Q* value (Q_{crit}), used for Dixon's test, is presented in Table D1 below. The given values apply to a two-tailed test with a significance level of 95%.

D1. Critical values of Dixon's *Q*-parameter (Q_{crit}) at different sample sizes (*N*). Q_{crit} is given for a two-tailed test at 95% confidence. Modified from (Rorabacher, 1991).

N	$\boldsymbol{Q_{crit}}$ **(α=0.05)**
3	0.970
4	0.829
5	0.710
6	0.625
7	0.568
8	0.526
9	0.493
10	0.466

E. Total Oxygen Content Over Time

The changes in total oxygen content over time for all variants of MN, MA and CB can be seen in Figure E1.

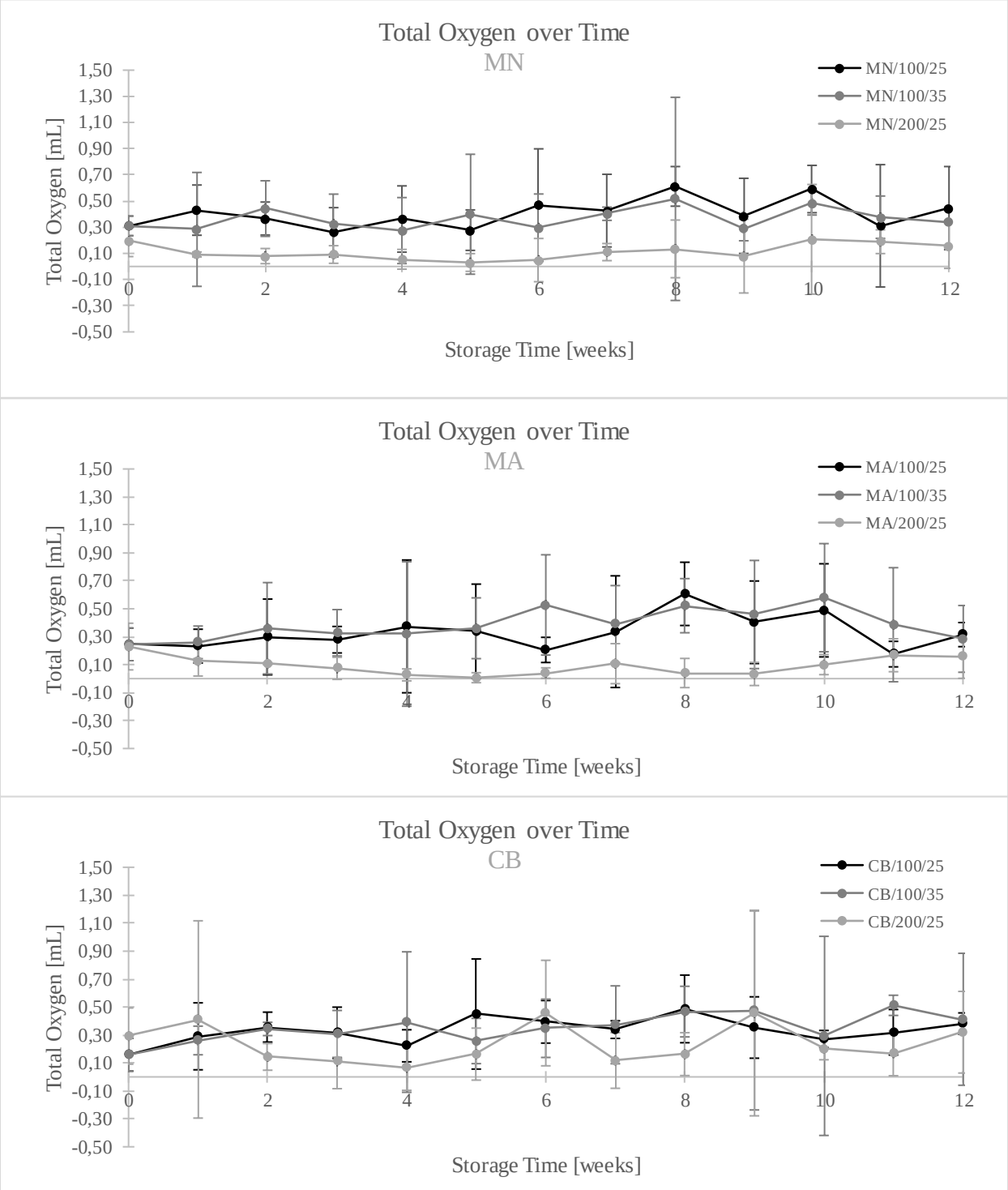

Figure E1. Change in total oxygen content [mL] over time [weeks], (A) MN variants, (B) MA variants and (C) CB variants.

F. Correlation Between Ascorbic Acid and CO_2

The relation between CO_2 in the headspace and the ascorbic acid concentration of the MA/100/25, MA/100/35 and MA/200/25 can be seen in Figure F1.

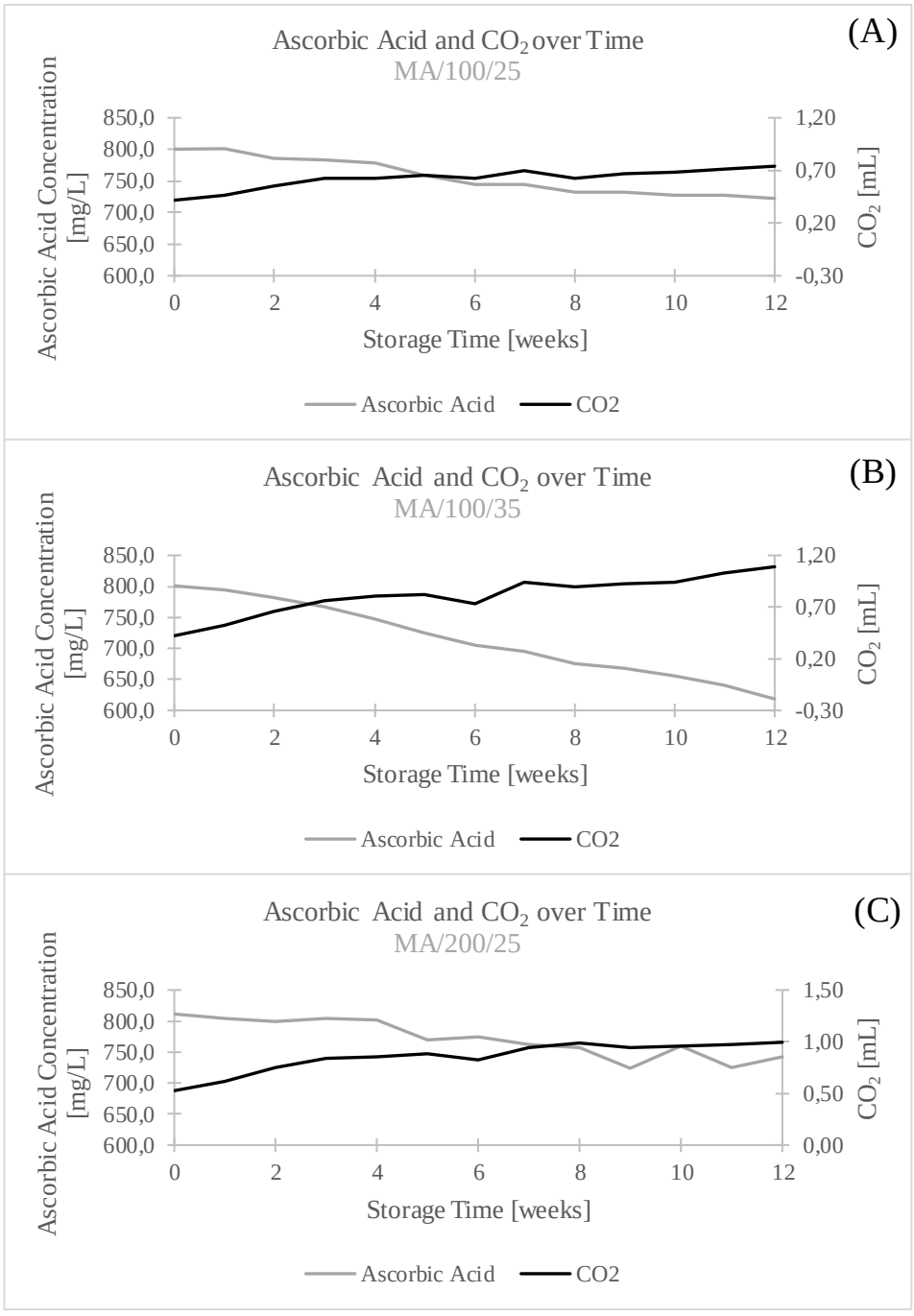

Figure F1. Change in ascorbic acid concentration [mg/L] and CO_2 content [mL] over time [weeks], (A) MA/100/25, (B) MA/100/35 and (C) MA/200/25.

The logarithmic correlation between degraded ascorbic acid and produced CO_2 in MA/100/25 can be seen in part (A) in Figure F2. The R_2 and *RMSE* was determined to be 0.77 and 0.031, respectively, for the applied correlation. The logarithmic correlation between degraded ascorbic acid and produced CO_2 in MA/200/25 can be seen in part (B) Figure F2. The R_2 and *RMSE* was determined to be 0.70 and 0.062, respectively, for the applied correlation.

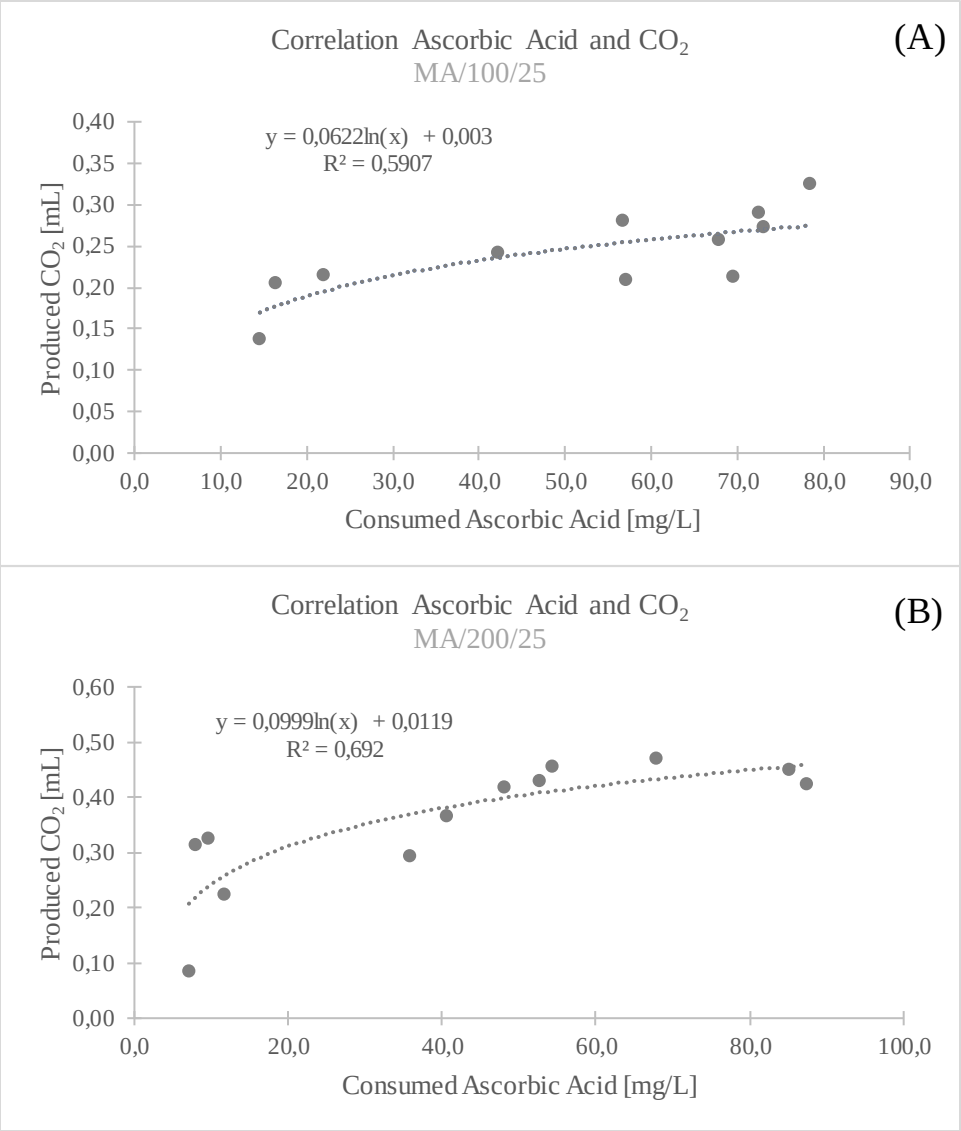

Figure F2. Produced CO2 [mL] against consumed ascorbic acid [mg/L], (A) MA/100/25 and (B) MA/200/25.

G. Colour

Pictures of MN/100/25, MN/200/25, MA/100/25 and MA/200/25 after 0 weeks of storage and after 12 weeks of storage are displayed in Figure G1.

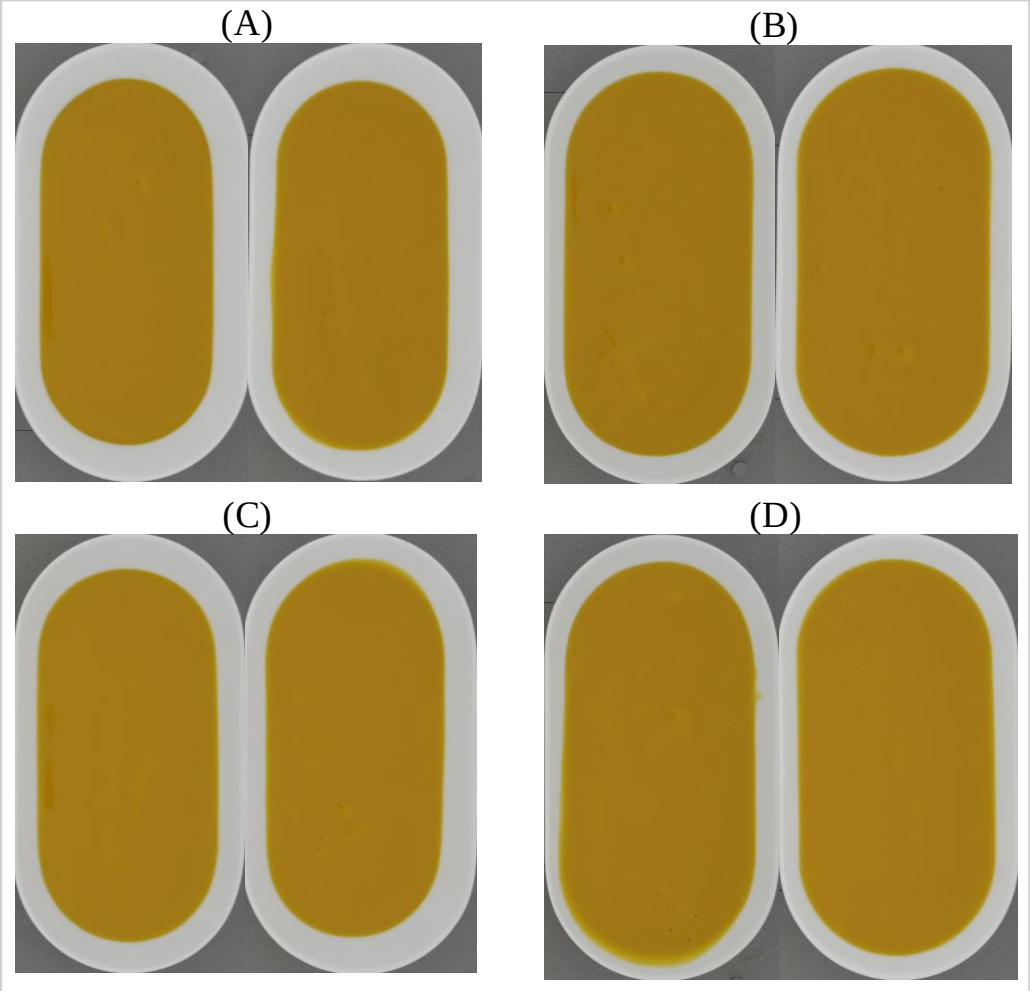

Figure G1. (A) MN/100/25 week 0 (left) and 12 (right), (B) MN/200/25 week 0 (left) and 12 (right), (C) MA/100/25 week 0 (left) and 12 (right) and (D) MA/200/25 week 0 (left) and 12 (right).

Pictures of CB/100/25 and CB/200/25 after 0 weeks of storage and after 12 weeks of storage are displayed in Figure G2.

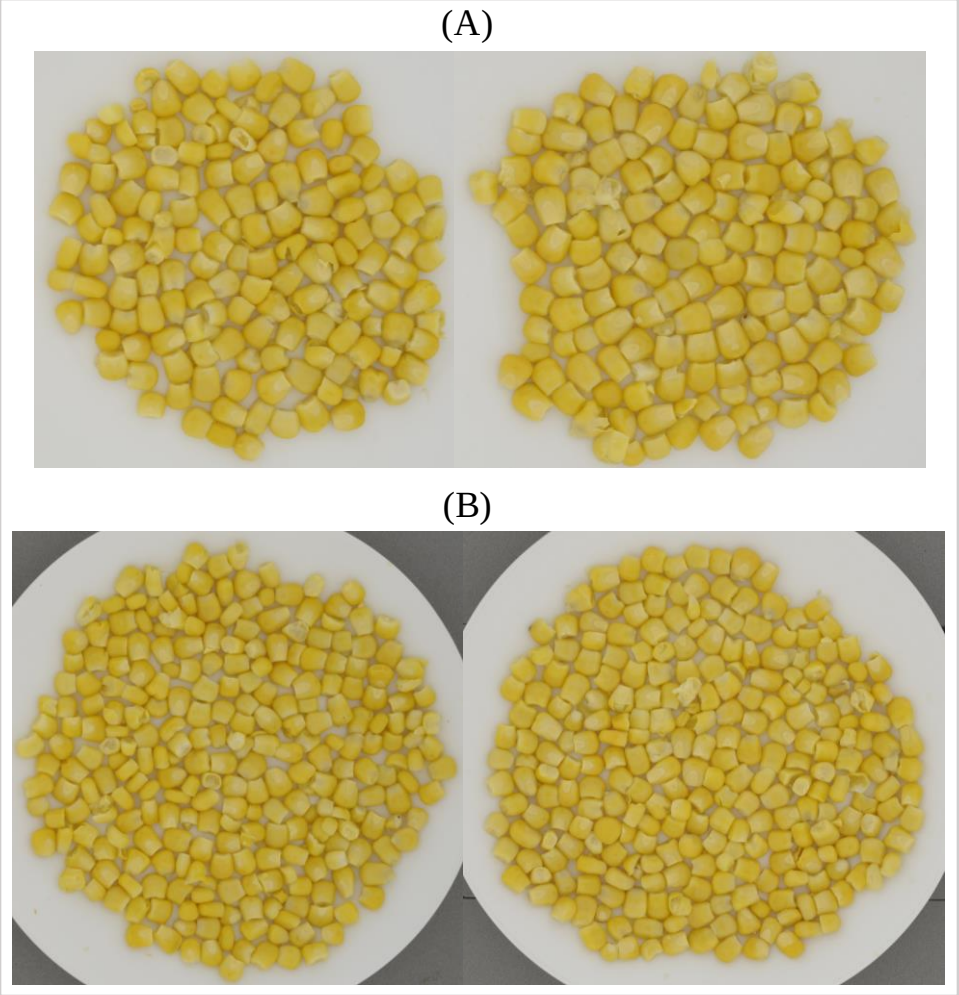

Figure G2. (A) CB/100/25 week 0 (left) and 12 (right) and (B) CB/200/25 week 0 (left) and 12 (right).

The L^*, a^* and b^* values for all variants at beginning of shelf life and after 12 weeks of storage are presented in Table G1. An ANOVA was performed to evaluate if the storage affected the colour parameters significantly and the obtained *P*-values are also presented in Table G1.

Table G1. Obtained *P*-values from ANOVA comparing L^*, a^* and b^* values at beginning of shelf life and after 12 weeks of storage. All data is presented as $\mu \pm 2\sigma$

Variant	Colour parameter	Week 0	Week 12	*P*-value
MN/100/25	L^*	58.0±1.5	59.2±1.3	0.095
	a^*	21.9±0.7	23.1±0.5	0.0082
	b^*	65.1±1.0	62.8±0.3	0.0019
MN/100/35	L^*	58.0±1.5	58.3±0.3	0.54
	a^*	21.9±0.7	23.0±0.2	0.006
	b^*	65.1±1.0	61.9±0.4	5.6×10^{-4}
MN/200/25	L^*	58.1±1.2	59.5±0.7	0.024
	a^*	21.9±0.7	23.4±0.3	0.0030
	b^*	65.3±1.1	63.1±0.2	0.0025
MA/100/25	L^*	59.1±1.4	60.5±0.4	0.032
	a^*	22.8±1.0	24.1±0.3	0.011
	b^*	65.1±0.1	63.5±0.1	9.1×10^{-7}
MA/100/35	L^*	59.1±1.4	59.5±0.5	0.41
	a^*	22.8±1.0	23.7±0.5	0.043
	b^*	65.1±0.1	63.0±0.4	6.8×10^{-5}
MA/200/25	L^*	59.0±2.3	60.5±0.1	0.077
	a^*	22.2±0.76	23.9±0.1	0.0015
	b^*	65.8±1.8	63.9±0.3	0.0017
CB/100/25 (mixed)	L^*	75.5±2.5	78.2±0.4	0.021
	a^*	8.57±0.12	8.91±0.56	0.10
	b^*	47.7±2.2	49.3±1.1	0.096
CB/100/35 (mixed)	L^*	75.5±2.5	77.9±1.1	0.036
	a^*	8.57±0.12	9.60±0.19	8.6×10^{-5}
	b^*	47.7±2.2	48.8±0.4	0.16
CB/200/25 (mixed)	L^*	76.3±2.2	78.6±0.7	0.014
	a^*	8.08±0.72	8.95±0.31	0.016
	b^*	47.9±0.71	59.2±0.8	0.094

H. Kinetics

R_2 and *RMSE* was determined for zero-, first- and second-order kinetics for ascorbic acid degradation in all variants and presented in Table H1.

Table H1. R_2 and *RMSE* for zero-, first- and second-order kinetics for degradation of ascorbic acid for all variants.

Variant	Zero order		First order		Second order	
	R^2	RMSE	R^2	RMSE	R^2	RMSE
MN/100/25	0.82	3.4	0.82	3.5	0.82	3.7
MN/100/35	0.81	3.9	0.80	3.9	0.79	3.9
MN/200/25	0.71	3.6	0.71	3.6	0.71	3.6
MA/100/25	0.94	7.1	0.94	6.8	0.94	6.6
MA/100/35	0.99	5.3	0.99	4.9	0.99	6.0
MA/200/25	0.84	11.4	0.84	11.4	0.83	11.4
CB/100/25	0.0018	7.0	0.0024	7.0	0.0032	7.0
CB/100/35	0.022	12.7	0.017	12.7	0.013	12.7
CB/200/25	0.54	4.5	0.55	4.5	0.55	4.5

R_2 and *RMSE* was determined for zero-, first- and second-order kinetics for colour changes (a^*, b^* and ΔE^*) in all MN and MA variants and presented in Table H2.

Table H2. R_2 and *RMSE* for zero-, first- and second-order kinetics for changes in a^*, b^* and ΔE^* for MN and MA variants.

Parameter	Variant	Zero order		First order		Second order	
		R^2	RMSE	R^2	RMSE	R^2	RMSE
a^*	MN/100/25	0.16	0.51	0.16	0.51	0.17	0.51
	MN/100/35	0.059	0.53	0.062	0.53	0.065	0.53
	MN/200/25	0.21	0.53	0.21	0.53	0.22	0.53
	MA/100/25	0.29	0.55	0.29	0.55	0.30	0.55
	MA/100/35	0.083	0.58	0.086	0.58	0.089	0.58
	MA/200/25	0.20	0.69	0.21	0.69	0.22	0.70
b^*	MN/100/25	0.58	0.54	0.58	0.54	0.59	0.54
	MN/100/35	0.77	0.49	0.77	0.49	0.78	0.48
	MN/200/25	0.54	0.56	0.54	0.56	0.54	0.56
	MA/100/25	0.64	0.36	0.64	0.36	0.64	0.36
	MA/100/35	0.72	0.38	0.72	0.38	0.72	0.45
	MA/200/25	0.49	0.43	0.49	0.43	0.49	0.49
ΔE^*	MN/100/25	0.60	0.40	0.59	0.44	0.57	0.53
	MN/100/35	0.85	0.34	0.78	0.44	0.69	0.68
	MN/200/25	0.61	0.42	0.59	0.46	0.57	0.55
	MA/100/25	0.52	0.54	0.55	0.62	0.52	0.90
	MA/100/35	0.60	0.48	0.56	0.58	0.50	1.0
	MA/200/25	0.25	0.62	0.32	0.67	0.34	0.81

I. Temperature in Storage Rooms

In Table I1, the measured temperatures in the two storage rooms can be seen. The temperatures were measured at least once per week during the whole storage period of 12 weeks. The average temperature in the 25°C storage room was 26.1±4.0°C and in the 35°C storage room it was 35.0±1.2°C.

Table I1. Measured temperatures in the two storage rooms. Average temperature is given as $\mu \pm 2\sigma$.

Date	**Temperature in 25°C storage [°C]**	**Temperature in 35°C storage [°C]**
2022-03-08	27.2	35.0
2022-03-15	26.4	35.1
2022-03-22	25.9	34.9
2022-03-29	24.8	34.1
2022-04-05	25.1	36.3
2022-04-11	31.5	35.8
2022-04-12	29.5	35.0
2022-04-19	24.9	34.6
2022-04-25	24.9	35.0
2022-04-26	24.9	35.3
2022-04-27	24.7	34.2
2022-05-02	24.5	35.0
2022-05-10	25.6	34.7
Average temperature	26.1±4.0	35.0±1.2